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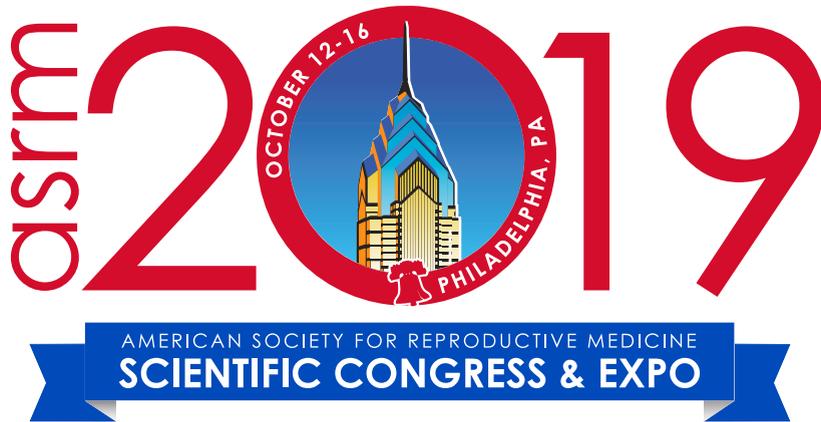
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Oral, Poster, and Video Session Abstracts



*Celebrating 75 Years*  
of  
History and Innovation



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THE AMERICAN  
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REPRODUCTIVE  
MEDICINE



# *Celebrating 75 Years* of History and Innovation

## **ASRM 2019**

**Scientific Abstracts** to be presented at the 75th Scientific Congress of the American Society for Reproductive Medicine, October 12-16, 2019, Philadelphia, Pennsylvania.

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**October 12-16, 2019**  
**Philadelphia, Pennsylvania**

*These abstracts of research studies, published as submitted by the authors, are presented in the ASRM 2019 Congress sessions and are published in the order of their presentation. Abstracts of plenary lectures, symposia and interactive sessions are not included.*

The first six papers are candidates for the ASRM Scientific Congress Prize Paper Awards. Six additional candidates will be presented during the Prize Paper Candidates' session on Tuesday.

### SCIENTIFIC CONGRESS PRIZE PAPER SESSION 1

O-1 Monday, October 14, 2019 10:45 AM

#### ANTIDEPRESSANT MEDICATION EXPOSURE: TIME TO PREGNANCY AND RISK OF PREGNANCY LOSS.

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**OBJECTIVE:** Depression and antidepressant medication use is prevalent in women of reproductive age. Evidence is conflicting regarding antidepressant exposure and miscarriage risk. Therefore, using prospective data on pregnancy and loss from a cohort of women in the Effects of Aspirin in Gestation and Reproduction Trial, we assessed the association between preconception-measured antidepressant exposure and time to pregnancy, pregnancy loss, and live birth.

**DESIGN:** Prospective cohort study of 1228 women with proven fecundity and 1-2 prior pregnancy losses, attempting natural conception while participating in a randomized controlled trial of preconception-initiated low-dose aspirin.

**MATERIALS AND METHODS:** Fluoxetine, sertraline, escitalopram, citalopram, trazadone, nefazodone, etoperidone, and tricyclic antidepressants and related compounds were measured in urine from enrollment and at each conception cycle and pregnancy visit (weeks 4 and 8) via a biochip competitive chemiluminescent immunoassay (Randox Toxicology). Any antidepressant medication use was also assessed via self-report. Cox proportional hazard regression models estimated fecundability odds ratios; log-binomial models estimated pregnancy loss and live birth incidence. Models adjusted for age, body mass index, education level, employment, smoking, alcohol use, marijuana use, and opioid use.

**RESULTS:** Of 1218 women, 183 (15%) had positive detection of antidepressant compounds prior to conception (at enrollment or at the last cycle prior to conception). Antidepressant exposure prior to conception was associated with lower fecundability (FOR: 0.77; 95% CI: 0.61, 0.99) though overall live birth incidence was similar (48% in exposed vs. 56% in non-exposed women; RR: 0.91, 95% CI: 0.77, 1.08). Among 785 hCG pregnancies, there was no association between preconception exposure and pregnancy loss (25% loss in exposed, 24% in non-exposed; RR: 1.04; 95% CI: 0.73, 1.50), and antidepressant exposure at 4 and 8 weeks' gestation also yielded a null finding. Sensitivity analyses including additional women in the positive exposure category based on self-reported antidepressant use yielded similar findings for all outcomes.

**CONCLUSIONS:** Antidepressant medications may lengthen time to pregnancy without impacting live birth rates, and importantly, did not increase risk of pregnancy loss. Given the close prospective follow-up of early pregnancy and loss incidence, including antidepressant exposure assessment both prior to and during early pregnancy, these data help alleviate concerns for miscarriage with use of this important class of medications.

**SUPPORT:** Intramural Research Program, DIPHR, NICHD, NIH.

O-2 Monday, October 14, 2019 11:00 AM

#### EFFECTS OF FOLIC ACID AND ZINC SUPPLEMENTATION IN MEN ON SEMEN QUALITY AND LIVE BIRTH AMONG COUPLES UNDERGOING INFERTILITY TREATMENT: FINDINGS FROM THE FAZST RANDOMIZED TRIAL.

Enrique F. Schisterman, PhD,<sup>a</sup> Lindsey A. Sjaarda, PhD,<sup>b</sup> Traci Clemons, PhD,<sup>c</sup> Douglas T. Carrell, PhD,<sup>d</sup> Neil J. Perkins, PhD,<sup>a</sup> Erica Johnstone, MD,<sup>e</sup> Denise Lamb, BSN,<sup>c</sup> Kayla Chaney, BA,<sup>c</sup> Bradley J. Van Voorhis, MD,<sup>f</sup> Ginny L. Ryan, MD, MA,<sup>g</sup> Karen M. Summers, MPH CHES,<sup>h</sup> James Hotaling, MD,<sup>d</sup> Jared C. Robins, MD,<sup>i</sup> James L. Mills, MD, MS,<sup>a</sup> Pauline Mendola, PhD,<sup>j</sup> Zhen Chen, PhD,<sup>a</sup> C. Matthew Peterson, MD,<sup>c</sup> Sunni L. Mumford, PhD,<sup>k</sup> <sup>a</sup>NICHD, Bethesda, MD; <sup>b</sup>Epidemiology Branch, DIPHR, NICHD, NIH, Bethesda, MD; <sup>c</sup>The Emmes Company LLC, Rockville, MD; <sup>d</sup>University of Utah School of Medicine, Salt Lake City, UT; <sup>e</sup>University of Utah, Salt Lake City, UT; <sup>f</sup>University of Iowa Carver College of Medicine, Iowa City, IA; <sup>g</sup>University of Iowa Hospitals and Clinics, Iowa City, IA; <sup>h</sup>University



of Iowa, Iowa City, IA; <sup>i</sup>Northwestern University, Chicago, IL; <sup>j</sup>National Institutes of Child Health and Human Development, Bethesda, MD; <sup>k</sup>National Institute of Child Health and Human Development, Bethesda, MD.

**OBJECTIVE:** Folic acid and zinc are thought to improve semen quality parameters. We conducted a randomized trial to determine the effect of daily folic acid and zinc supplementation on semen quality and live birth.

**DESIGN:** The Folic Acid and Zinc Supplementation Trial (FAZST) was a multi-center, double-blind, block-randomized, placebo-controlled trial.

**MATERIALS AND METHODS:** Men  $\geq 18$  years old who with partners were planning infertility treatment were block randomized by site and planned infertility treatment strata (IVF, non-IVF at a study site, and non-IVF at an outside clinic) to receive either 5 mg folic acid and 30 mg elemental zinc or placebo for 6 months during infertility treatment. The primary outcomes were live birth and semen quality parameters, analyzed by intention to treat. [ClinicalTrials.gov #NCT01857310](https://clinicaltrials.gov/ct2/show/study/NCT01857310).

**RESULTS:** Between June 3, 2013, and December 30, 2017, 2370 men were recruited and randomized (1185 active, 1185 placebo). Daily supplementation was not associated with live birth (active 399 [34%], placebo 408 [34%], risk difference -0.76, 95% CI: -4.58, 3.06) or with sperm concentration, motility, morphology, or total motile sperm count. Supplementation was associated with increased DNA fragmentation (risk difference 2.5, 95% CI 0.6, 4.4). No effects on pregnancy rate, pregnancy loss, gestational age at delivery, embryo parameters, or other adverse neonatal outcomes were observed, except that preterm birth was higher with supplementation (risk difference 1.94, 95% CI: 0.24, 3.64). Gastrointestinal symptoms were also more common with supplementation.

**CONCLUSIONS:** Use of folic acid and zinc supplementation by men did not improve semen quality and increased DNA fragmentation and gastrointestinal problems. The increase in preterm birth warrants further investigation. The widespread impression that supplements will at least 'do no harm' may be unfounded. The lack of efficacy and potential risks of folic acid and zinc supplementation can now be communicated to couples seeking infertility treatment.

**SUPPORT:** Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health.

O-3 Monday, October 14, 2019 11:15 AM

#### CAN HYSTEOSALPINGO FOAM SONOGRAPHY (HYFOSY) REPLACE HYSTEOSALPINGOGRAPHY (HSG) AS FIRST CHOICE TUBAL PATENCY TEST: A RANDOMIZED COMPARISON (FOAM STUDY)?

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**OBJECTIVE:** Traditionally, tubal patency testing during fertility work-up is performed by hysterosalpingography (HSG). Hysterosalpingo-foam-sonography (HyFoSy) is an alternative technique without radiation exposure and is less expensive than HSG. Globally, there is a shift towards the use of office-based diagnostic methods, such as HyFoSy. Here, we assess whether HyFoSy is as accurate as HSG in evaluating tubal patency and if it leads to comparable pregnancy outcomes.

**DESIGN:** Multicenter prospective comparative study with a randomized non-inferiority design.

**MATERIALS AND METHODS:** Participating women underwent both HyFoSy and HSG, in randomized order, by a physician unaware of the result of the first test (NTR 4746). In case of discordant results for HyFoSy/HSG, women were randomly allocated to either a management strategy based on HyFoSy or one based on HSG. Assuming 7% discordant results, we needed to recruit 1,163 participants (van Rijswijk et al., 2018). Primary outcome is ongoing pregnancy within 12-months after inclusion. Secondary outcomes are concordance between HSG and HyFoSy, pain scores, live birth, time to pregnancy, clinical pregnancy, miscarriage, multiple pregnancy, preterm birth.

**RESULTS:** Between June 2015 and January 2019, a total of 1164 women were scheduled to undergo HSG and HyFoSy. At moment of writing, data on 97% was available. 2.3% of the women did not undergo any tests, 5.0% had HSG only and 0.6% had HyFoSy only. From the women who had both tests, 2.9% had an inconclusive HSG and 8.5% had an inconclusive HyFoSy (RR 2.3, 95%CI 1.6-3.2). In 0.8%, both tests were inconclusive. Among the women with two tests completed, 85% had concordant results (94.7% patent tubes, 3% with unilateral occlusion, 0.9% with bilateral occlusion, 1.4% with other findings). The mean pain score on the 1-10 VAS-scale was 5.4 (95%CI 5.2-5.6) for HSG compared to 3.0 (95%CI 2.9-3.2) for HyFoSy (p-value<0.001). Pain score of HyFoSy was not affected by the order of the tests (p=0.34).

Of the 136 eligible women with discordant results, 108 women gave consent to be randomly allocated to management based on HSG (n=53) or HyFoSy (n=55). At moment of writing, data on the primary outcome were available in 58.5% of the HSG-group versus in 54.6% of the HyFoSy group. Ongoing pregnancy occurred in 30.2% of the women allocated to management based on HSG, and in 27.3% of the women allocated to HyFoSy (RR 1.1, 95% CI 0.6–2.1). By October 2019, 90% of women will have complete one year follow-up.

**CONCLUSIONS:** HyFoSy and HSG have a concordance of 85%, with HyFoSy experienced as significantly less painful and without the need of radiation exposure. In case of a discordant result, management based on the results of HyFoSy or based on the results of HSG lead to similar pregnancy outcomes.

Reference: van Rijswijk J, van Welie N, Dreyer K, van Hooff MHA, de Bruin JP, Verhoeve HR, et al. The FOAM study: is hysterosalpingo foam sonography (HyFoSy) a cost-effective alternative for hysterosalpingography (HSG) in assessing tubal patency in subfertile women? Study protocol for a randomized controlled trial. *BMC women's health*. 2018;18(1):64.

**SUPPORT:** The FOAM study is an investigator initiated study, funded by ZonMw, a Dutch organization for Health Research and Development (project number 837001504). ZonMw funded the whole project. IQ Medical Ventures provided the ExEm FOAM® kits free of charge. The funders had no role in study design, collection, analysis and interpretation of the data.

**O-4 Monday, October 14, 2019 11:30 AM**

#### **MALE MARIJUANA USE AND SPONTANEOUS ABORTION.**

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**OBJECTIVE:** Frequent marijuana use has been associated with poor semen quality. Though evidence is mixed, most studies show deleterious effects. There are no studies of male marijuana use and adverse pregnancy outcomes. We evaluated the association between male marijuana use and spontaneous abortion (SAB).

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** This analysis uses data from 1,413 couples enrolled in Pregnancy Study Online (PRESTO), a North American pre-conception cohort study of pregnancy planners. At baseline (preconception), men and women separately reported demographics, medical history, and lifestyle/behavioral factors, including marijuana use frequency. Women completed bimonthly follow-up surveys for up to 12-months or until conception. Data on SAB were ascertained from follow-up questionnaires completed in early pregnancy (<12 weeks gestation) and late pregnancy (~32 weeks gestation). Additional data were reported on the first positive pregnancy test date, due date, and gestational weeks at loss. Frequency of male marijuana use in the previous 2 months was ascertained at baseline and categorized as follows: no use, <1 time/week, or ≥1 time/week. Cox proportional hazards regression models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association between male baseline marijuana use frequency and SAB. The timescale was gestational

weeks from date of first pregnancy detection. We controlled for age at baseline (male and female), household income, education, race/ethnicity, smoking status, environmental tobacco exposure, alcohol intake, caffeine intake, sugar-sweetened beverage intake, body mass index, exercise, multivitamin use, sleep duration, hours of work per week, history of sexually transmitted infections, depression/anxiety, and frequency of female baseline marijuana use. Additional models controlled for reproductive history, including having impregnated a partner previously, parity (female), history of pregnancy loss (female), and family history of SAB (female).

**RESULTS:** Among the 1,413 couples followed, 1,164 (82.4%) men reported no marijuana use, 132 (9.3%) reported using marijuana <1 time/week, and 117 (8.3%) reported using marijuana ≥1 time/week in the 2 months before baseline. During follow-up, 266 (18.8%) SABs were reported. Compared with no male marijuana use, adjusted HRs for male marijuana use <1 time/week and ≥1 time/week were 1.07 (95% CI: 0.65-1.77) and 2.04 (95% CI: 1.28-3.24), respectively. The association (≥1 time/week vs. none) persisted after adjusting for reproductive history (HR=2.05, 95% CI: 1.29-3.26), and was slightly stronger after restricting to couples where the female partner did not use marijuana (HR=2.19, 95% CI: 1.26-3.80).

**CONCLUSIONS:** Couples with male partners who used marijuana ≥1 time per week during preconception had a greater risk of SAB compared with no male marijuana use. Little association was found for men who used marijuana <1 time per week. Possible mechanisms include an adverse effect of frequent marijuana use on sperm quality.

**O-5 Monday, October 14, 2019 11:45 AM**

#### **BETROOT, WATERMELON AND GINGER JUICE SUPPLEMENTATION MAY INCREASE THE CLINICAL OUTCOMES OF INTRACYTOPLASMIC SPERM INJECTION CYCLES.**



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**OBJECTIVE:** The endometrium is a highly dynamic tissue that undergoes cyclic cellular proliferation, differentiation, and immune cell trafficking in response to circulating ovarian-derived steroids. The goal for the present study was to prove the hypothesis that beetroot, watermelon and ginger juice supplementation would improve the endometrial receptivity and clinical outcomes of intracytoplasmic sperm injection (ICSI) cycles.

**DESIGN:** Prospective randomized study.

**MATERIALS AND METHODS:** This study enrolled 296 female patients undergoing ICSI cycles from Jan/2017 to Jan/2018, in a private university-affiliated IVF center. The sample size calculation suggested that 265 cycles would be enough to demonstrate a 20% effect with 90% power and 5% significance level considering as primary outcome clinical pregnancy rate. Female patients were randomized in a 1:3 ratio to either Control (n=74) or Supplementation Group (n=222). All patients received nutritional orientation before the beginning of the treatment. Participants in the Supplementation Group were instructed to intake a daily dose of homemade juice, prepared with fresh beetroot, watermelon and ginger, from the day of embryo transfer until the day of pregnancy test, while patients in Control Group did not follow the juice protocol. Generalized Linear Models, adjusted for potential confounders (female age, body mass index - BMI, endometrial thickness upon embryo transfer, and number of transferred embryos), followed by Bonferroni post hoc test for the comparison of means between groups, were used to investigate the impact of juice supplementation on the clinical outcomes of ICSI.

**RESULTS:** Similar maternal and paternal ages, maternal BMI, number of follicles, number of retrieved and mature oocytes, fertilization rates, embryo development rates, blastocyst development rates, and number of transferred embryos were observed between the Supplementation and Control groups. Implantation rate (24.2% vs. 17.8%, p<0.001) and clinical pregnancy rate (41.0% vs. 21.6%, p: 0.039) were significantly higher in the Supplementation compared to Control group. A significant difference in miscarriage rate was noted between the Supplementation and Control groups (0.0% vs. 18.0%, p<0.001).

**CONCLUSIONS:** Beetroot contains nitric oxide, which dilates blood vessels allowing a rich supply of oxygenated, nutrient-rich blood to flow to the uterus. Therefore its intake starting on embryo transfer day may improve embryo implantation into the uterus.

Reference: NA.

SUPPORT: None.

### PHASE 3 TRIAL RESULTS: EFFICACY AND SAFETY OF ELAGOLIX IN A SUBSET OF WOMEN WITH UTERINE FIBROIDS AND ADENOMYOSIS.



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**OBJECTIVE:** Adenomyosis is a benign lesion within the myometrium associated with heavy menstrual bleeding (HMB) and dysmenorrhea, and commonly co-exists with uterine fibroids (UF). Adenomyosis is also present in 15-57% of hysterectomy specimens with leiomyoma (Genc M, et al. 2015; Taran FA, et al. 2010). This analysis evaluated the efficacy and safety of elagolix, an oral, gonadotropin-releasing hormone receptor antagonist, with add-back therapy in a subset of women with UF, HMB and co-existing adenomyosis.

**DESIGN:** Data were pooled from two 6-month, randomized, double-blind, placebo-controlled phase 3 studies, Elaris UF-1 and UF-2. Premenopausal women (18-51 years) with ultrasound-confirmed diagnosis of UF and HMB (>80mL menstrual blood loss [MBL]/cycle) were randomized 1:1:2 to placebo, elagolix 300mg twice daily (BID), or elagolix 300mg BID with 1mg estradiol/0.5mg norethindrone acetate (E2/NETA) once daily.

**MATERIALS AND METHODS:** This subset analysis was conducted in women with HMB associated with UF and co-existing adenomyosis diagnosed by ultrasound and/or MRI at baseline (BL). The primary endpoint was the proportion of women with <80mL MBL during the final month and ≥50% reduction in MBL from BL to the final month. MBL and the diagnosis of HMB was assessed with the alkaline hematin method. Adverse events (AEs) were monitored.

**RESULTS:** Of 790 women treated, 16% had ultrasound and/or MRI diagnosed adenomyosis at BL. Pooled data demonstrated that the proportion of responders for the primary endpoint was significantly greater (P<0.001) for elagolix+E2/NETA [76.8% (95% CI, 65.84, 87.82)] compared to placebo [12.1% (95% CI, 0.97, 23.150)]. AEs reported in the adenomyosis subset included hot flushes, night sweats, headache, and nausea.

**CONCLUSIONS:** In women with HMB associated with UF and co-existing adenomyosis at BL, elagolix +E2/NETA significantly reduced MBL versus placebo similar to the all-subject group. AEs reported in this group were similar to the all subject group. These data suggest that further studies investigating the effect of elagolix in women with HMB associated with UF and adenomyosis may be warranted.

References: 1. Genc, M., et al., *Adenomyosis and accompanying gynecological pathologies*. Arch Gynecol Obstet. 2015. 291(4): p. 877-881.

2. Taran, F.A., et al., *Characteristics indicating adenomyosis coexisting with leiomyomas: a case-control study*. Human reproduction (Oxford, England). 2010. 25(5): p. 1177-1182.

**SUPPORT:** This work was funded by AbbVie Inc. AbbVie participated in the study design, research, data collection, analysis and interpretation of data, writing, reviewing, and approving the publication.

#### ART LAB: BASIC

O-7 Monday, October 14, 2019 10:45 AM

### IMPLEMENTATION OF AN ELECTRONIC WHITEBOARD FOR QUALITY MANAGEMENT IN THE IN VITRO FERTILIZATION LABORATORY.



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**OBJECTIVE:** In 2014, we implemented an electronic whiteboard as a quality management tool to assist our embryologists to ensure their adherence to established standards for performing time-sensitive procedures (1). We aimed to test the hypothesis that use of an electronic whiteboard in the IVF laboratory increases the likelihood that critical evaluation procedures are performed within optimum pre-set time ranges.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Retrievals in our IVF clinic between 6/1/12 and 5/31/18 were included. The pre-electronic whiteboard time-period

was 6/1/12 to 4/5/14, during which embryologists strived to adhere to the set optimum evaluation times but without a formal guide. The post-electronic whiteboard time-period was 3/1/15 to 5/31/18. The 13 months after the electronic whiteboard was introduced (4/6/14-2/28/15) were defined as a transition period and were excluded. Optimum pre-set time ranges were 16-18 hours post-insemination or ICSI (HPI) for the pronuclei (PN) check, 65-67 HPI for day 3 evaluations and 114-117 HPI for day 5 evaluations. Log binomial models estimated the risk ratio (RR, 95% confidence interval [CI]) of evaluations occurring within the optimum time ranges. Models were adjusted *a priori* for ICSI.

**RESULTS:** A total of 44,957 oocytes from 6,302 retrievals met inclusion criteria, of which 44.4% underwent ICSI. There were 16,434 oocytes from 2,703 retrievals pre-electronic whiteboard and 28,523 oocytes from 3,599 retrievals post-electronic whiteboard. The proportion of oocytes evaluated at the PN check within the optimum time range was statistically significantly increased after implementation of the electronic whiteboard (89.2% vs 80.8%, RR 1.11 [95% CI 1.10 – 1.12]). The proportion of day 3 and day 5 checks that occurred within the optimum time ranges were also statistically significantly increased after implementation of the electronic whiteboard (day 3: 73.3% vs 57.2%, RR 1.75 [95% CI 1.54 – 1.99]) and (day 5: 74.1% vs 58.8%, RR 1.26 [95% CI 1.24 – 1.29]).

**CONCLUSIONS:** Our findings indicate that use of an electronic whiteboard that posts optimum time ranges for performing critical IVF laboratory procedures tightens the actual evaluation times towards these ranges. Such improved standardization may lead to positive downstream effects on quality assurance analyses and embryo transfer and embryo cryopreservation management decisions. Future studies will investigate whether use of an electronic whiteboard in the IVF laboratory improves overall clinical care.

Reference: 1. Olofsson JI, Banker MR, Sjoblom LP. Quality management systems for your in vitro fertilization clinic's laboratory: Why bother? Journal of Human Reproductive Sciences. 2013;6:3.

**SUPPORT:** None.

O-8 Monday, October 14, 2019 11:00 AM

### THE CLINICAL RESULTS OF PIEZO-ICSI COMPARED TO CONVENTIONAL-ICSI: A SIBLING-OOCYTE STUDY.



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**OBJECTIVE:** Clinically, conventional ICSI (CI) is a common, widely-used method, while there are few reports with respect to Piezo ICSI (PI). PI is effective in degeneration rate and fertilization rate (Kimura, Y & Yanagimachi, R, 1995). It is known that the survival rates of mice oocytes are low after CI; however, degeneration rate improved markedly using PI. PI is an effective technique for cases with fragile oocytes. It has been reported that the survival rate is similarly improved in human oocytes (Hiraoka, K & Kitamura, S, 2015). However, most of the studies reporting on PI are retrospective studies. Here, we prospectively compared the degeneration rates, fertilization rates and embryo development of PI and CI in a sibling study.

**DESIGN:** This is a prospective randomized controlled single-center study, using sibling oocytes, conducted from August 2018 to March 2019. Written informed consent was obtained from all patients involved in this study.

**MATERIALS AND METHODS:** This sibling oocyte study comprised 26 cycles in 26 cases. CI was performed in 149 mature oocytes. CI consists of mechanical penetration of the zona pellucida, breaking the oocyte membrane by aspiration of cytoplasm. PI was performed in 162 mature oocytes. PI consists of breaking the oocyte membrane and zona pellucida by Piezo pulse. P-value of 0.05 or less was considered to be statistically significant. <sup>a</sup>Limitation: The clinical results using vitrified oocytes, artificial oocyte activation, cryptozoospermia, azoospermia cases, and females aged over 40 years old were not included in this study. The pregnancy outcome has not been confirmed in our study.

**RESULTS:** There were no statistically significant differences in the fertilization rates, degeneration rates, cleavage rates, blastocyst formation rates or good quality blastocyst rates (according to the Gardner criteria) between CI and PI (75.8% vs. 78.4% (P=0.592), 7.4% vs. 3.7% (P=0.146), 100% vs. 96.8% (P=0.160), 61.9% vs. 64.0% (P=0.743) and 41.6% vs. 36.8% (P=0.450), respectively).

**CONCLUSIONS:** In conclusion, the present study has demonstrated there was no significant difference in the clinical results of piezo-ICSI and conventional-ICSI. However, this may be attributable to the limited number of cases with fragile oocytes, etc. In our experience, PI is safer and easier to learn and

perform in clinical work in a shorter period, especially for beginners. Further studies are necessary.

Group	Conventional ICSI	Piezo ICSI	P
Patients (N)	26	-	-
No. of ICSI cycles	26	-	-
Female age, years (mean ± SD)	34.8 ± 4.2	-	-
Male age, years (mean ± SD)	36.9 ± 5.4	-	-
No. of oocytes	149	162	-
N(%) of zygotes (2PN)	113 (75.8)	127 (78.4)	0.592
N(%) of degenerated oocytes	11 (7.4)	6 (3.7)	0.146
N(%) of cleaved oocytes	113 (100)	122 (96.8)	0.167
N(%) of blastocysts	70 (61.9)	80 (64.0)	0.477
N(%) of good quality blastocysts	47 (41.6)	46 (36.8)	0.498

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### WOULD AN AUTOMATED SYSTEM DETECTING EMBRYO DEVELOPMENTAL EVENTS SELECT THE SAME EMBRYO AS AN EMBRYOLOGIST USING A MORPHOKINETIC ALGORITHM?.

Raquel Del Gallego, PhD,<sup>a</sup> Lorena Bori Arnal, PhD,<sup>a</sup> Lucía Alegre, PhD,<sup>a</sup> Teija Peura, PhD,<sup>b</sup> Manuel Ugidos, PhD,<sup>c</sup> Marcos Meseguer, PhD<sup>d</sup> <sup>a</sup>IVIRMA Global, Valencia, Spain; <sup>b</sup>Genea Biomedx, Sydney, NSW, Australia; <sup>c</sup>Instituto de Biomedicina de Valencia, Valencia, Spain; <sup>d</sup>IVIRMA Global, Valencia, Spain, Tel Aviv, Israel.



**OBJECTIVE:** The objective of this study is to compare embryo grading and clinical result prediction obtained with a morphokinetic algorithm using an automated system for embryo developmental events annotations vs. manual annotations performed by an embryologist team.

**DESIGN:** Retrospective study including morphokinetic manual and automated data from 1,370 embryos (284 patients) at IVIRMA Valencia clinic. All embryos were normally fertilized embryos cultured up to day 5/6. All were included regardless of their treatment (egg donation or autologous), oocyte origin (fresh or frozen) or patient age (range: 27-44 years).

**MATERIALS AND METHODS:** All embryos were annotated manually by a busy embryologist team in the routine clinical practice using Geri Assess® 1.3 software (IVI). The same videos were retrospectively assessed by the stand-alone Geri Assess® 2.0 software (GA2), including filtration of events falling outside the pre-defined time-ranges, as is done in the full Geri system. Both morphokinetic manual and automated annotations went through an embryo selection algorithm developed by Basile *et al.* (2015) considering the morphokinetic parameters **t3**, **cc2** (t3-t2) and **t5**. Embryos were graded and the accuracy in the prediction was assessed between both groups in terms of embryo outcome, bHCG test, and fetal heartbeat. Data was statistically analyzed with chi-squared and binomial proportion tests.

**RESULTS:** High accordance was found between IVI and GA2 embryo grading through Basile's algorithm. Out of the 1,370 embryos, 1,045 were utilized as transferred or vitrified, showing no statistically significant differences between both groups in all grades: A+, A, B+, B, C+, C, D+ and D; except for No Grade ( $p < 0.05$ ). More ungraded embryos were found in the automated group, as Geri Assess® 2.0 is designed to eliminate events falling outside of pre-defined time-ranges, these annotations being considered anomalous. **t3** was the most unavailable parameter in the GA2 data. Regarding only the 391 KID transferred embryos, bHCG test and fetal heartbeat data also did not show statistically significant results between IVI and GA2. Previous studies in our group have proven a high GA2 detection rate in the great majority of the events, especially in early cleavage divisions, which explains its good performance with the algorithm parameters: **t3**, **cc2** and **t5**.

**CONCLUSIONS:** The results of the study support the use of automated systems for embryo morphokinetic annotations. This non-invasive and objective tool standardizes the annotating process avoiding inter- and intra-grader variability, in addition to facilitating the routine clinical practice. The establishment of automation would need a gradual transition controlled by lab professionals, as yet chaotic embryos or artifacts in the well hinders its performance.

References: Basile N, Vime P, Florensa M, Aparicio Ruiz B, García Velasco JA, Remohí J, Meseguer M. The use of morphokinetics as a predictor of implantation: A multicentric study to define and validate an algorithm for embryo selection. *Hum Reprod* 2015; 30:276–283.

SUPPORT: Predoctoral Generalitat Valenciana and European Social Fund.

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### PROPORTION OF PATIENTS DETECTED WITH SUBCLINICAL HYPOTHYROIDISM IS INDEPENDENT OF TIME OF BLOOD DRAW.

Christine Briton-Jones, PhD, HCLD,<sup>a</sup> Jenna Friedenthal, MD,<sup>b</sup> Sydney Chang, MD,<sup>b</sup> Taraneh Gharib Nazem, MD,<sup>b</sup> Dmitry Gounko, MA,<sup>a</sup> Joseph A. Lee, BA,<sup>a</sup> Alan B. Copperman, MD,<sup>b</sup> <sup>a</sup>Reproductive Medicine Associates of New York, New York, NY; <sup>b</sup>Icahn School of Medicine at Mount Sinai, New York, NY.



**OBJECTIVE:** There are differences in clinical opinion regarding the benefit of treating subclinical hypothyroidism in infertile patients. However, for patients with a thyroid stimulating hormone (TSH) serum level > 2.5mIU/L it is recommended to continue monitoring or administer levothyroxine to reduce TSH serum levels <2.5mIU/L (ASRM guideline document 2015). TSH levels in adults, have a predictable circadian rhythm, with the highest levels produced between 2am and 4am; while the lowest levels occur between 4pm and 8pm. Whether there is a misdiagnosis of subclinical hypothyroidism and underlying normal circadian rhythm due to testing afternoon blood draw is a current clinical concern. Only one study has showed the potential for this misdiagnosis, albeit the study included a small sample size. [1] The objective of this study was to identify any differences in the mean TSH levels obtained from morning compared to afternoon blood draws in patients seeking infertility treatment.

**DESIGN:** Retrospective cohort analysis

**MATERIALS AND METHODS:** This study examined patients having routine TSH levels tested for either cycle day 3 evaluations or as part of a new patient consultation from January 2018 and March 2019. Serum TSH concentrations were obtained via electrochemiluminescence immunoassay Elecsys for use on Cobas e601(Roche) Detection range of 0.005 – 100 mIU/L. Chi Square analysis was used to determine statistical significance with  $p < 0.05$  considered significant.

**RESULTS:** Of the 8345 patients who had routine TSH testing performed, 5028 were drawn in the morning and 3281 were drawn in the afternoon. There was no significant difference in the mean ( $\pm$  SD) TSH levels, 2.10408 (4.30) for am blood draws and 2.10426 (4.31) for pm blood draws. There was also no differences in the in the percentage of TSH results showing >2.5mIU/L in morning 25% compared to afternoon blood draw groups 26%.

**CONCLUSIONS:** This study showed no shift in the mean or in percentage of patients with elevated TSH levels in the morning compared to afternoon blood draw group. This data shows that afternoon blood draws are just as likely to detect elevated TSH levels as blood samples drawn in the morning. The strength of this study is its ability to define risks of misdiagnosis of sub-clinical hypothyroidism due to potential underlying changes in TSH levels for the different times of blood draw using binomial sorting of patient data in a diverse population of patients seeking ART treatment. This study highlights how TSH fluctuations that may occur throughout the day are clinically insignificant and even with ultra-sensitive immunoassays not, detectable in a population of patients undergoing reproductive treatment.

Reference: Roelfsema F. and Veldhuis JD. Thyrotropin secretion patterns in health and disease. *Endocrinology Reviews*. 2013 34(5): 619-57.

SUPPORT: None.

O-11 Monday, October 14, 2019 11:45 AM

### PRELIMINARY RESULTS FROM THE FIRST REGISTERED PILOT TRIAL WITH MATERNAL SPINDLE TRANSFER TO OVERCOME INFERTILITY.

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**OBJECTIVE:** Oocyte cytoplasmic dysfunction is a major contributor to impaired embryo development. Since maternal spindle transfer (MST) allows replacement of the entire cytoplasm of a poor quality oocyte, it holds a great promise to enhance oocyte quality. Our previous studies using mice and human oocytes donated for research have shown the technical feasibility of MST to overcome embryo development arrest. This registered pilot trial represents the next step forward in the validation of MST as a potential methodology for addressing certain infertility problems, some of which are refractory to current clinical strategies.

**DESIGN:** This study includes data from an ongoing prospective pilot trial (ISRCTN 11455145) comprising 25 patients. Nine patients have already been recruited based on their history of several previous failed *in-vitro* fertilization (IVF) attempts, characterized by embryo developmental arrest. Female age over 40 y/o and severe male factor were considered as exclusion criteria. Procedures were authorized by the National Authority (437/23.9.2016) and approved by the Hospital's IRB. Informed consents were obtained to conduct procedures and follow-up children born.

**MATERIALS AND METHODS:** Meiotic spindles from patients' oocytes were isolated under polarized light microscopy within minimal amounts of cytoplasm. Spindles were transferred to previously enucleated donor oocytes, inseminated by ICSI and cultured in a time-lapse incubator. Blastocysts of good morphology were biopsied and screened for aneuploidy. Additionally, mitochondrial DNA (*mtDNA*) carryover levels were assessed. Euploid embryos were transferred and DNA samples were obtained either from amniotic fluid or at birth, to confirm the origin of the mitochondrial and nuclear genomes.

**RESULTS:** A total of 9 MST cycles were performed in patients with an average age of 37.2 y/o (range 32-40) and a mean number of previous failed IVF attempts of 4.9 (range 3-11). The mean number of MII oocytes used for MST per patient was 4.9 (range 1-10). MST was applied successfully in 39 out of the 44 oocytes (88.6%). Of these, 76.9% (30/39) fertilized and 20 developed into good quality blastocysts (66.7%), which were biopsied and vitrified. Genetic analysis revealed 35% (7/20) of the embryos to be euploid and mtDNA carryover levels <1%. Two MST blastocysts were warmed and transferred, resulting in two pregnancies. The first patient (aged 32 and with 4 previous failed IVF attempts) delivered a healthy boy at 39 weeks (51cm and 2.960kg). Analysis of DNA fingerprints from biopsied cells, amniotic fluid and samples collected after birth (blood, urine, saliva, cord blood, placenta) confirmed the parentage of the child and the origin of the donated mtDNA. The second pregnancy is currently at 14 weeks of gestation.

**CONCLUSIONS:** These results indicate that MST-derived embryos are able to implant and sustain a healthy pregnancy to term. Given the difficult reproductive history of the patients, this initial data is encouraging. However, carefully controlled trials will be required to determine whether MST is truly beneficial in the context of assisted reproductive treatment.

**SUPPORT:** Institutional funding

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### EFFECT OF SPERM SELECTION USING MACS TECHNOLOGY IN THE PGT-A PROGRAM COMPARED WITH STANDARD SPERM PREPARATION.



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**OBJECTIVE:** To compare the blastocysts available for biopsy, euploid rates and reproductive outcomes in embryos incubated in a time-lapse system, when MACS technology for sperm selection was applied or not.

**DESIGN:** Secondary analysis from a prospective trial.

**MATERIALS AND METHODS:** 500 patients were distributed in two groups: MACS n=245 (Swim-up + MACS) vs. Control group n=255 before ICSI treatment. With the use of Time-lapse technology, we did a complete embryo follow-up until blastocyst biopsy with further morphokinetic analysis.

**RESULTS:** 1600 blastocyst were biopsied 716 (42.7%) in the group of MACS and 884 (52.0%) in the control group reaching statistical significance with a p valor < 0.001.

The percentage of euploid blastocysts in each group was 42.2% in MACS group and 38.1% in control group without statistical significance.

Similar results were obtained in all the parameters compared between groups, number of embryo transferred with 0.85 (CI95%0.6-1.1) vs 1.0 (CI95%0.5-1.5) and implantation rates of 38.6%(CI95%18.2-59.1)

vs. 55.6%(CI95% 30.1-81.0) in MACS group compared with control group.

**CONCLUSIONS:** We observed an inverse and significant correlation, related to the MACS technology application, in the proportion of blastocysts available for biopsy, but this effect was not detected when we analyzed the number of euploid blastocysts in each group. Similar data was obtained in outcome reproductive results. All these results suggest that MACS technology does not have any effect on PGT-A outcome when performed. Our results are supported by a consistent sample size.

**Reference:** None

**SUPPORT:** None.

### ART OFFSPRING

**O-13 Monday, October 14, 2019 10:45 AM**

### THIRD GRADE ACADEMIC ACHIEVEMENT AMONG CHILDREN CONCEIVED WITH IVF: A POPULATION-BASED STUDY IN TEXAS.



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**OBJECTIVE:** To evaluate public school standardized testing results at the end of third grade among IVF versus non-IVF children.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The Texas Education Agency provided a stand-alone de-identified dataset of children ages 8-9 years who took the 3<sup>rd</sup> grade tests between 2014-18, indexed by IVF (Y/N), maternal race (White, Black, Hispanic, Asian), maternal age (18-29, 30-34, 35-39, 40+ years), plurality (singleton, twin), gestation for singletons (preterm, term), sex (M, F), and for IVF infants, embryo state (fresh, thawed). IVF children were matched by first and last names, and birthdate. Analyses were done using generalized linear modeling. Children who were economically disadvantaged or received special education were excluded since they were disproportionately greater in the non-IVF group. We limited the analysis to the standardized scores of the State of Texas Assessments of Academic Readiness (STAAR) test taken in English (99%) since the IVF group used only the English test. Test scores are reported as means ± SE.

**RESULTS:** After exclusions, there were 5,645 IVF and 10,246 non-IVF children with Reading scores and 5,649 IVF and 10,272 non-IVF children with Mathematics scores. IVF children scored higher in Reading (singletons (IVF, N=2,663), 1,554 ± 2 vs 1,531 ± 1 (non-IVF, N=9,664), 24 point difference, p<0.0001; twins (IVF, N=2,982), 1,544 ± 2 vs 1,507 ± 5 (non-IVF, N=582), 36 point difference, p<0.0001), as well as in Mathematics (singletons (IVF, N=2,659), 1,578 ± 3 vs 1,556 ± 1 (non-IVF, N=9,692), 22 point difference, p<0.0001; twins (IVF, N=2,990), 1,565 ± 2 vs 1,527 ± 5 (non-IVF, N=580), 38 point difference, p<0.0001). Children of mothers ages 30 and older scored higher than children of mothers ages 18-29 among non-IVF children, but were similar for IVF. Preterm and term singletons scored comparably among IVF children. Within the IVF group, there were no differences by fresh vs thawed embryo state.

**CONCLUSIONS:** These results indicate that IVF-conceived children have an academic achievement in third grade that is at least as good as or better than those conceived spontaneously. We were not able to adjust further for socioeconomic status, which may explain some of the observed differences. In the IVF group there was no difference in test results in children born from fresh vs. thawed embryos.

**SUPPORT:** NIH Grant R01 HD84377, Assisted Reproductive Technology and Child Health: Risk of Birth Defects, Mortality, and Effect on Grade School Performance.

### SEX DIFFERENCES IN BIRTH OUTCOMES FOR MASSACHUSETTS INFANTS FOLLOWING



ART. Sunah S. Hwang, MD MPH,<sup>a</sup> Dmitry Dukhovny, MD MPH,<sup>b</sup> Daksha Gopal, MPH,<sup>b</sup> Howard Cabral, PhD, MPH,<sup>c</sup> Hafsatou Diop, MD, MPH,<sup>d</sup> Judy E. Stern, PhD<sup>c</sup> <sup>a</sup>University of Colorado School of Medicine, Aurora, CO; <sup>b</sup>Affiliation not provided; <sup>c</sup>Boston University, Boston, MA; <sup>d</sup>MDPH, Boston, MA; <sup>e</sup>Dartmouth-Hitchcock, Lebanon, OR.

**OBJECTIVE:** Sex differences in child and adult health outcomes have been demonstrated. While prior studies have shown adverse birth outcomes for infants conceived by assisted reproductive technology (ART), data on whether outcomes differ by infant sex are lacking. Our objective in this study was to determine the presence and magnitude of sex differences in neonatal health outcomes among infants conceived by ART.

**DESIGN:** Retrospective observational cohort analysis of singletons born in Massachusetts between July 1, 2004 and December 31, 2013 who were conceived by ART.

**MATERIALS AND METHODS:** We linked the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS), a clinical database of treatment information on all ART cycles and the Pregnancy to Early Life Longitudinal (PELL) data system, which links birth certificates to hospital discharge records for mothers and infants in Massachusetts. The analysis was limited to singleton, live births to women  $\geq 18$  years, conceived by ART. Birth outcomes for ART deliveries were compared between male and female infants using chi-square tests. Health outcomes were obtained from PELL. Multivariable logistic regression was used to model the potential association between infant sex and adverse health outcomes, controlling for maternal age, race/ethnicity, education, insurance, chronic and gestational diabetes, hypertension, parity, and gestational age (results displayed as adjusted odds ratio; 95% confidence interval).

**RESULTS:** A total of 16,034 singleton live births conceived by ART were included: 7,737 female and 8,297 male. In the adjusted analysis, compared to female infants, male infants had greater odds of being preterm (1.19; 1.07-1.32), having birth defects (1.31; 1.05-1.63), experiencing respiratory (1.37; 1.24-1.51) and neurologic (1.29; 1.09-1.53) conditions, and prolonged hospital stay (1.25; 1.09-1.43). Despite the higher odds of preterm birth, male infants had lower odds of being low birthweight (0.8; 0.7-0.92) compared to their female counterparts.

**CONCLUSIONS:** Sex differences in birth outcomes of infants conceived by ART exist. Further studies are needed to elucidate the biologic mechanisms underlying the relationship between infant sex and these adverse health outcomes among infants conceived by ART.

**SUPPORT:** NIH R01HD067270.

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### CONCEPTION BY INFERTILITY TREATMENT AND NEWBORN DNA METHYLATION.



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**OBJECTIVE:** To determine whether newborns conceived by infertility treatment have different DNA methylation patterns from newborns not conceived by treatment.

**DESIGN:** The Upstate KIDS Study recruited women and their newborns (2008-2010), oversampling on infertility treatment exposure.

**MATERIALS AND METHODS:** Mothers reported on use of infertility treatment and the specific type (assisted reproductive technologies (ART) or ovulation induction (OI) / intrauterine insemination (IUI)) at 4 months postpartum. Maternal report of ART use was previously verified by linkage to SART-CORS. Mothers provided permission to use archived newborn dried blood spots collected by Newborn Screening. DNA methylation was measured using the Infinium EPIC microarray. Samples from 855 newborns were used in analysis. Singletons (n=688) and unrelated twins (n=167) were included to maintain independent samples. Quantile normalization was applied for probe type normalization and robust linear regression used to model the associations between 837,933 CpGs and any infertility treatment as well as by type (i.e., ART, OI/IUI, none). Bonferroni significance of  $p < 6 \times 10^{-8}$  was used to account for multiple testing. Analyses were adjusted for maternal age, race, education, pregnancy smoking, private insurance, estimated cell type and batch effects.

**RESULTS:** Newborns conceived with infertility treatment (n=335, 39%) had higher methylation at one CpG in *C14orf166B* (cg21616682,  $p=4.74 \times 10^{-8}$ ) compared to newborns not conceived with treatment (n=514). When the specific techniques were examined, no genome-wide associations were found for conception by OI/IUI (n=177, 20%). However, ART conceived newborns (n=158, 19%) had hypomethylation (ranging from 1.5 to 5.3%) at four CpGs in several gene regions (Table 1). Additional adjustment for plurality, infant sex, gestational age and birthweight did not meaningfully alter the findings.

**CONCLUSIONS:** In one of the largest studies examining differences in newborn DNA methylation by conception with infertility treatment, several genes were identified, with biological evidence linking them to infertility. For instance, *SYCE1* encodes for a synaptonemal complex protein, which is necessary for meiosis, and whose mutations were previously found in association with male and female infertility. Ongoing child follow-up will validate whether methylation differences persist.

TABLE 1. Newborn DNA methylation differences between conception by ART versus no treatment

CpG	Beta	SE	p-value	Chromosome	Position	Gene
cg17676129	-0.01747	0.002484	2.02E-12	10	135382545	<i>SYCE1</i>
cg24413339	-0.01587	0.002703	4.35E-09	10	135237754	<i>SPRN</i>
cg01050010	-0.04998	0.008442	3.21E-09	17	31149877	<i>MYO1D</i>
cg27119318	-0.05358	0.009847	5.28E-08	21	40759574	<i>WRB</i>

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### BABIES BORN FOLLOWING ADMINISTRATION OF NOLASIBAN BEFORE EMBRYO TRANSFER (ET) AFTER IVF: NEONATAL AND INFANT DEVELOPMENT OUTCOMES FROM A DOUBLE-BLIND, PLACEBO-CONTROLLED, CLINICAL TRIAL.



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**OBJECTIVE:** Nolasiban, an oral oxytocin antagonist, has been shown to increase live birth rate when administered prior to ET (Visnova 2018). The objective of this study was to assess neonatal and infant development outcomes after administration of nolasiban or placebo at the time of ET.

**DESIGN:** Multinational, double-blind, randomized, parallel group, placebo-controlled, Phase 3 trial assessing a single oral 900 mg dose of nolasiban or placebo (1:1), administered about 4 hours before ET following IVF. Neonatal outcomes were assessed up to 28 days after birth and infant development assessed using the Ages and Stages Questionnaire-3<sup>®</sup> (ASQ-3) completed at 6 months after birth.

**MATERIALS AND METHODS:** 778 subjects were recruited from 41 fertility clinics in Europe from Mar–Oct 2017. Eligibility criteria included age  $\leq 36$  years,  $\leq 1$  failed ART cycle, use of a GnRH antagonist,  $< 1.5$  ng/mL serum progesterone on the day of hCG, and luteal support with vaginal micronized progesterone. One good quality embryo was transferred on either D3 (n=388) or D5 (n=390). Neonatal and infant development outcomes were summarized for each treatment group using descriptive statistics for the pooled D3/D5 population. No hypothesis testing was planned.

**RESULTS:** There were 108 deliveries (1 set of twins) resulting in 109 infants in the placebo group and 131 deliveries (5 sets of twins) resulting in 136 infants in the nolasiban group. At birth, mean  $\pm$  SD 5 min Apgar-scores (placebo  $9.65 \pm 0.76$ ; nolasiban  $9.61 \pm 0.84$ ), weight (placebo  $3174 \pm 517$  g; nolasiban  $3137 \pm 690$  g), height (placebo  $51 \pm 4$  cm; nolasiban  $50 \pm 5$  cm) and head circumference (placebo  $34 \pm 2$  cm; nolasiban  $34 \pm 2$  cm) were very similar between the treatment groups. There were 4 (3.7%) congenital abnormalities in the placebo group and 5 (3.7%) in the nolasiban group.

At 28 days after birth, weight, height and head circumference continued to be similar between groups. 9 (8.3%) infants had been admitted to intensive care in the placebo group and 9 (6.6%) in the nolasiban group. Neonatal morbidities were reported in 29 (26.6%) infants in the placebo group and 26 (19.1%) in the nolasiban group. The most common neonatal morbidities were jaundice (20 (18.3%) placebo and 18 (13.2%) nolasiban) and respiratory distress syndrome (10 (9.2%) placebo and 11 (8.1%) nolasiban).

At 6-months after birth for those subjects with follow-up data, mean  $\pm$  SD total ASQ-3 scores were  $208.7 \pm 38.8$  in the placebo group and  $208.5 \pm 44.7$  in the nolasiban group. The No. (%) of infants with an ASQ-3 score below the normal range in  $\geq 1$  domain was 33 (37.5%) in the placebo group and 43 (41.7%) in the nolasiban group.

**CONCLUSIONS:** The neonatal and infant developmental outcomes were similar between the nolasiban and placebo groups.

**References:** Visnova H, Tournaye H, Humberstone A, Terrill P, Macgregor L, Loumaye E. A placebo-controlled, randomized, double-blind, phase 3 study assessing ongoing pregnancy rates after single oral administration of a novel oxytocin receptor antagonist, nolasiban, prior to single embryo transfer. *Fertil. Steril.* 2018 110 (4 Suppl.) e45.

**SUPPORT:** The trial was funded by ObsEva SA.

O-17 Monday, October 14, 2019 11:45 AM

### **SIMILAR SUCCESS RATES WITH FROZEN OOCYTES BUT INCREASED RATE OF LARGE FOR GESTATIONAL AGE (LGA) INFANTS COMPARED TO FRESH OOCYTES.**



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**OBJECTIVE:** Evaluate pregnancy and perinatal outcomes of embryos derived from fresh and frozen oocytes in autologous cycles.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The SART database was used to identify autologous oocyte cycles that resulted in an embryo transfer during 2014 and 2015. Generalized linear regression models were used to compare pregnancy and perinatal outcomes of fresh versus frozen oocytes. Models were adjusted for the following factors: maternal age, BMI, current smoking status, parity, infertility diagnosis, prior IVF attempt, ICSI, assisted hatching, number of embryos transferred, multiple gestation and fetal heart reduction. Live birth rate was the primary outcome. Secondary outcomes include miscarriage rate and birth weight.

**RESULTS:** The mean maternal age in autologous oocyte cycles (N=139,734) was 35 years (SD ±3.6). There was no significant difference in live birth rates when comparing embryos derived from fresh and frozen oocytes in autologous cycles (25.7% versus 23.9%, aRR 0.94, 95% CI 0.8-1.1). No significant differences were noted in biochemical pregnancy losses (5.8% versus 6.9%, aRR 1.3, 95% CI 0.94-1.79) or clinical miscarriages (10.9% versus 11.2%, aRR 1.04, 95%CI 0.82-1.33) in embryos derived from fresh and frozen autologous oocytes. Increased risk for large for gestational age infants (4.5% versus 12.5%, aRR 2.69, 95% CI 1.66-4.33) was seen in embryos derived from frozen oocytes. No significant difference was noted in low birth weight infants between the two groups.

**CONCLUSIONS:** Frozen oocytes have similar success rates as fresh oocytes in autologous cycles that resulted in embryo transfer. However, an increased rate of large for gestational age infants was seen in embryos derived from frozen oocyte. This finding warrants further study.

O-18 Monday, October 14, 2019 12:00 PM

### **PREVALENCE OF CLINICALLY SIGNIFICANT CONGENITAL HEART DEFECTS IN LOW-RISK IVF PREGNANCIES.**



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**OBJECTIVE:** Current research has shown increased prevalence of congenital heart defects (CHD) among *in vitro* fertilization (IVF) pregnancies compared to spontaneous pregnancies. We describe the prevalence and characteristics of CHD in IVF pregnancies at a high-volume fetal echocardiography center and outline a low-risk subset of patients for whom echo may not be clinically indicated.

**DESIGN:** Historical Prospective Observational Study.

**MATERIALS AND METHODS:** All fetal echocardiograms for singleton and dichorionic twin pregnancies performed January 1, 2004 to December 31, 2018 at a large tertiary care center utilizing gray scale, color Doppler, and spectral Doppler were reviewed and categorized by gestational age (GA), indications for fetal echo, and presence of structural CHD. All initial diagnoses were made by experienced sonographers and a maternal-fetal medicine specialist, recorded on videotape, and confirmed by a pediatric cardiologist. Neonatal echocardiographic examinations were performed to

confirm diagnoses in cases with prenatal diagnoses of CHD. Prevalence and 95% confidence intervals (CI) calculated utilizing standard statistical methods. Clinical outcomes were available for cases of CHD after 2011.

**RESULTS:** 18,879 fetal echocardiograms were completed during the study period. Of those, 3,893 echocardiograms were performed with only indication being IVF gestation. Patients with previous child with CHD, family history of CHD, medication exposure, diabetes, non-cardiac anomaly, anomaly in previous pregnancy, other abnormality noted on ultrasound, or monozygotic twins were excluded. Mean GA at time of echo for IVF only group was 22.2 ± 1.4 weeks. Prevalence of CHD summarized in Table 1. 25 cases were diagnosed with CHD after 2011. 22 were isolated ventricular septal defects (VSD), 10 CHD were not resolved at time of pediatric cardiology follow-up by 16 months, and 3 were clinically significant requiring intervention or cardiology follow-up after 2 years of age. Prevalence of clinically significant CHD in IVF only pregnancies was 0.15% (95%CI [0 - 0.40%]).

**CONCLUSIONS:** (1) In this low risk IVF cohort, the prevalence of clinically significant CHD is similar to population risk previously reported. (2) A large proportion of CHD in this population are VSD and most spontaneously resolve.

**SUPPORT:** None.

TABLE 1. Prevalence of CHD in IVF Pregnancies

Group	#	# CHD	% with CHD	95% CI
All IVF Pregnancies	4242	76	1.79	1.39 - 2.19
IVF Only Ind.	3893	33	0.85	0.56 - 1.14
IVF Only (2012-18)	2040	25	1.23	0.75 - 1.70
IVF Only (2012-18) CHD Not Resolved	2040	10	0.49	0 - 1.10
IVF Only (2012-18) with Clinically Significant CHD	2040	3*	0.15	0 - 0.40

\* Cases were Coarct/VSD, Pulmonary Stenosis, and asymptomatic vascular ring.

### **CONTRACEPTION AND COMPLEX FAMILY PLANNING**

O-19 Monday, October 14, 2019 10:45 AM

### **TOPICAL LIDOCAINE-PRILOCAINE CREAM VERSUS LIDOCAINE 1% SUBCUTANEOUS INFILTRATION DURING NEXPLANON INSERTION: A RANDOMIZED CONTROLLED STUDY.**



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**OBJECTIVE:** Adequate anesthesia is an important procedural step when inserting contraceptive implants. Subcutaneous injection of lidocaine 1% is a widely used anesthetic method in implant insertion. However, lidocaine injection may be painful due to the penetration of the skin by the needle, and there is a theoretical risk of needle stick injury. This may also cause bleeding or edema which may mislead the intact subdermal insertion of the implant. Lidocaine-prilocaine (LP) cream is an oil/water emulsion in which the oil phase is a eutectic mixture of two anesthetics: lidocaine 2.5% and prilocaine 2.5% in a ratio of 1:1 by weight. Our objective is to compare the anesthetic effect of LP cream versus lidocaine subcutaneous infiltration during insertion of Nexplanon.

**DESIGN:** Randomized, open-label controlled study (Clinicaltrials.gov: NCT03187392).

**MATERIALS AND METHODS:** Reproductive-aged parous women requesting Nexplanon insertion for contraception were counseled to participate. Eligible women based on WHO guidelines were recruited and randomized (1:1) to LP cream vs. lidocaine 1% subcutaneous infiltration. In the cream group, 5 mg was applied on the insertion site, and Nexplanon rod was inserted after 5 minutes later. In the injection group, 2 ml of 1% lidocaine was slowly injected through a 24 G needle at the Nexplanon insertion

site of skin with the depth of 2-3 mm, until at least 5 mm of wheel was observed, then the needle was further advanced under the skin in the direction of Nexplanon insertion and the remaining lidocaine was injected subcutaneously. Nexplanon rod was inserted within 3 minutes afterward. The main study outcomes were the participant's self-rated pain perception utilizing a 10-cm Visual Analogue Scale (VAS) during Nexplanon insertion and 15 minutes post-procedure. A 2-cm difference in VAS score between both arms was considered a clinically significant difference. The secondary outcomes included ease of insertion score, complications of the procedure and patient's satisfaction using a five-point Likert scale. Student's t-test and Chi-square test were used for the analysis of the outcomes.

**RESULTS:** Two hundred sixty women were enrolled and randomized to LP cream arm (n=130) or lidocaine 1% subcutaneous infiltration (n=130). LP cream group reported significantly lower pain scores during Nexplanon insertion (mean±SD: 2.55±0.98 vs. 5.57±1.64, p<0.001) and 15 minutes post-insertion (mean±SD: 2.22±0.89 vs. 4.32±1.27, p<0.001). The ease score of insertion was significantly higher in the LP group (mean±SD: 8.23±0.84 vs. 6.49±0.66, p=0.001). Fifty women (38.5%) in the lidocaine infiltration group suffer from bruising vs. 19 women (14.6%) in the LP group (p=0.001). Additionally, 45 women (34.6%) had bleeding from the insertion site in the lidocaine infiltration group vs. 25 (19.2%) in the LP group (p=0.002). No difference between the patient's satisfaction levels in both groups (p=0.54).

**CONCLUSIONS:** Topical application of lidocaine-prilocaine cream before Nexplanon insertion significantly reduces the induced pain with subsequent easier insertions and less rate of procedure-related complications

**SUPPORT:** None.

**O-20 Monday, October 14, 2019 11:00 AM**

#### **A RANDOMIZED CLINICAL TRIAL BETWEEN ULTRASOUND-GUIDED AND UTERINE SOUND-SPARING APPROACH FOR COPPER INTRAUTERINE DEVICE INSERTION.**

Mohammed Khairy Ali, MD,<sup>a</sup> Ahmed M. Abbas, MD,<sup>a</sup> Asmaa Ramadan, MBBCh,<sup>b</sup> Ahmed M. Abdelmagied, MD,<sup>a</sup> Mostafa Nasr Ibrahim, MD,<sup>a</sup> Ahmed Abu-Elhassan, MD<sup>a</sup> <sup>a</sup>Department of Obstetrics and Gynecology, Faculty of Medicine, Assiut University, Assiut, Egypt; <sup>b</sup>Department of Obstetrics and Gynecology, Qous Central Hospital, Qena, Egypt.



**OBJECTIVE:** The intrauterine device (IUD) is a safe, reliable and long-acting reversible contraceptive method. Pain during insertion may be a barrier to choose IUD use. The trans-abdominal ultrasound guided IUD insertion (TAS-guided IUD insertion) effectively decreases the insertion pain; however, the full bladder during insertion and the need of two investigators may decrease patient's satisfaction. In "Uterine Sounding Sparing Approach" (USSA); the sonographer performs a transvaginal ultrasound before IUD insertion to evaluate the uterine position and length without using uterine sounding; this method may increase patient's satisfaction and acceptance toward IUD use. Our objective was to compare the satisfaction score between both approaches during copper IUD insertion.

**DESIGN:** Randomized Open-label controlled Trial (Clinical Trials. Gov: NCT03383432).

**MATERIALS AND METHODS:** Reproductive-aged women requesting Copper IUD insertion for birth control were counseled to participate. The eligible women were randomized into two groups; group I (TAS-guided IUD insertion) and group II: USSA. The primary outcome was to measure the satisfaction score of both methods. Other outcomes included the easiness score (ES), the difference in pain scores during IUD insertion (measured by Visual analog scale), the duration of insertion in minutes and the successful device placement after one week, evaluated by transvaginal ultrasound (TVS). The outcome variables were analyzed using independent sample T-test.

**RESULTS:** Sixty women were analyzed in both groups (30 women in the arm). The baseline demographic data was homogeneous in both groups without statistically significant differences. The mean satisfaction score was significantly higher in the USSA group than TAS-guided IUD insertion group (6.7±0.90 Vs. 5.0±0.74, p= 0.0001; respectively). The IUD inserted easier in USSA group than TAS-guided IUD insertion group (p= 0.001). Also; the pain during IUD insertion was significantly lower in the USSA group (5.3±0.98 Vs. 6.9±0.75, p= 0.0001; respectively). Moreover; significant shorter duration of insertion (3.67±0.71 Vs. 4.87±0.77 minutes; p= 0.001) was reported in USSA group. At the one week follow-up; TVS showed that all IUDs were in place in all women without statistically significant difference (p= 0.591).

**CONCLUSIONS:** USSA is associated with higher satisfaction and less pain during insertion than TAS-guided IUD insertion approach. Also, this approach is effective, easy and needs less time for IUD insertion.

**References:** Abbas A, Ali MK, Abdalmegeed OS, Yosef AH, Abdelkader AM, Shaaban OM. Evaluation of a novel uterine sound sparing approach for copper intrauterine device insertion. *Fertil. Steril.* 2017;108(3):e123.

**SUPPORT:** None.

**O-21 Monday, October 14, 2019 11:15 AM**

#### **EFFECT OF CELL-PHONE ASSISTED POSTPARTUM COUNSELING ON THE USE OF LONG-ACTING REVERSIBLE CONTRACEPTIVES: A RANDOMIZED CONTROLLED TRIAL.**

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**OBJECTIVE:** The use of long-acting reversible contraceptive (LARC) methods remains substantially lower than less effective methods such as pills or condoms. Our objective is to assess the effect of adding cell-phone to the postpartum family planning counseling and service on the intake of postpartum women to LARC and the overall contraceptive performance.

**DESIGN:** Randomized Open-label controlled Trial (Clinical Trials. Gov: NCT03135288).

**MATERIALS AND METHODS:** All women delivered a live birth greater than 28 weeks' gestation and requested birth spacing for more than one year were counseled for participation. Eligible women were recruited and randomized (1:1) to Cell-phone assisted (study group) who received a reminder of their postpartum family planning visit five weeks after delivery and a phone call 48 hours before the scheduled visit. They were received two follow-up phone calls to answer any queries and to remind them of the follow-up visits after LARC use. They also provided with a cell phone number working seven days a week from 8 AM to 8 PM to answer any query or questions regarding her family planning program. The control group received the standard postpartum family planning counseling without any phone assistance. A follow-up visit was scheduled at six months to assess the study outcomes. The primary outcome was the rate of initiation of LARC method in the first six months after delivery. The secondary outcomes included the rate of continuation of the LARC method, initiation of another method, and rate of an unplanned pregnancy. Unpaired t and Chi-square tests were used for the analysis of the outcomes.

**RESULTS:** Eight hundred and sixty-four women were enrolled and randomized (432 women in each group). Both groups were similar regarding age, parity, BMI, educational level, residence and marriage period. The rate of initiation of LARC method was significantly higher in the cell-phone group (30.3% versus 8.4%; p > 0.001).

Similarly, the rate of continuation was significantly higher in the cell-phone group (95.1% versus 82.9%; p > 0.001). Three hundred thirty-one (76.6%) of cell-phone group had started any contraceptive method during the first six months as compared 188 (43.5%) women in the control group (p<0.001). There were no cases of unplanned pregnancy in the cell-phone group compared with ten cases in the control group (p=0.009).

**CONCLUSIONS:** Adding cell-phone to the postpartum family planning counseling and service can improve the intake of postpartum women to LARC methods and the overall contraceptive performance with a subsequent decrease in the rate of an unplanned pregnancy.

**SUPPORT:** A fund No. (2016-23) received from The Institutional Grants' office.

**O-22 Monday, October 14, 2019 11:30 AM**

#### **INFLUENCE OF GENETIC VARIANTS ON WEIGHT GAIN AMONG ETONOGESTREL CONTRACEPTIVE IMPLANT USERS.**

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**OBJECTIVE:** To identify genetic variants that are associated with weight gain related to etonogestrel (ENG) contraceptive implant use.

**DESIGN:** We conducted a prospective, candidate gene study to test for associations between genetic variants and both subjective weight gain and objective weight changes in ENG implant users.

**MATERIALS AND METHODS:** We recruited healthy, reproductive-aged women using ENG implants for 12-36 months. We asked participants about subjective weight gain (yes/no) during contraceptive implant use and performed medical record review to calculate objective weight changes from implant insertion to study enrollment. We genotyped each participant for 120 single nucleotide polymorphisms (SNPs) in 14 genes involved in progesterin metabolism, regulation, and function. To identify genetic variants associated with subjective weight gain and objective weight changes, we performed backwards stepwise multivariable logistic regression and generalized linear modeling, respectively, adjusting for age, body-mass index (BMI), and self-reported race/ethnicity.

**RESULTS:** We enrolled 350 ethnically-diverse participants. Median BMI was 26.0 kg/m<sup>2</sup> (range 18.5-52.0) and 41.4% reported experiencing subjective weight gain during contraceptive implant use. Weight at time of implant insertion was available for 276 participants with a mean weight change of +3.8 kg ( $\pm$ 7.2). We found two genetic variants significantly associated with subjective weight gain. Carriers of *CYP3A4\*1G* and participants homozygous for the *ESR1* rs9322335 variant were both more likely to report weight gain (aOR 2.32, p=0.001 and aOR 2.41, p=0.001, respectively). Frequencies of these genetic variants were 33.3% and 68.1%, respectively. We found two genetic variants associated with objective weight changes. Participants homozygous for the *ESR1* rs9340799 variant gained 10 kg more weight, on average, compared to all other participants ( $\beta$  = 10.09, p=0.002). Carriers of the *CYP2C19* rs7088784 variant lost 3.9 kg more weight, on average, compared to all other participants ( $\beta$  = -3.88, p=0.001). Frequencies of these genetic variants were 8.3% and 22.5%, respectively. Higher BMI was the only variable significantly associated with both outcomes: aOR 1.10 (p=1.6x10<sup>-5</sup>) and  $\beta$  = 0.56 (p=6.3x10<sup>-13</sup>).

**CONCLUSIONS:** We identified two genetic variants in the *ESR1* (estrogen receptor 1) gene that were associated with subjective and objective weight gain in ENG contraceptive implant users. Neither SNP (rs9322335 or rs9340799) has known associations with obesity or metabolic syndrome, and thus, this may be a progesterin-dependent effect. Additional genetic research is needed to facilitate advances in individualized counseling on the risk of weight gain with exogenous steroid hormones.

**SUPPORT:** This work was primarily supported by the Society of Family Planning Research Fund [grant number SFPRF17-3]. This work was also supported by NIH/NCATS Colorado CTSA Grant Number UL1 TR001082. Dr. Lazowitz's time is also supported by the NICHD K12 Women's Reproductive Health Research Scholar Program (grant number 5K12HD001271-18). Contents are the authors' sole responsibility and do not necessarily represent official NIH views.

O-23 Monday, October 14, 2019 11:45 AM

#### **EFFICACY AND SAFETY OF A MULTIPURPOSE VAGINAL pH-REGULATOR: RESULTS FROM THE PHASE 3, AMPOWER CONTRACEPTIVE CLINICAL TRIAL.**

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**OBJECTIVE:** Amphora<sup>®</sup> (formerly known as Acidform), a multipurpose vaginal pH-regulator (MVP-R), is a novel, non-hormonal, woman-controlled, on-demand, water-based, petroleum-free vaginal gel being investigated for prevention of pregnancy and sexually transmitted infections. Here we present primary results from the confirmatory phase 3 contraception trial, AMPOWER.

**DESIGN:** This was a single-arm, open-label study conducted at 112 sites within the US ([ClinicalTrials.gov](http://ClinicalTrials.gov) number NCT03243305).

**MATERIALS AND METHODS:** All sites obtained IRB approval and all women provided informed consent. Eligibility criteria included healthy, monogamous, sexually active women aged 18-35 years who had normal cyclic menses of length 21-35 days, reported having intercourse  $\geq$  3 times per cycle, and were willing to use the study drug as the only method of contraception over the course of the study. Women were instructed to administer a single prefilled applicator of study drug intravaginally immediately before or up to 1 hour before each episode of vaginal intercourse. Women used eDiaries to record timing of product administration, coital information, and side effects. The primary efficacy analysis was the cumulative pregnancy percentage over 7 cycles with typical-use calculated by the Kaplan-Meier method.

**RESULTS:** A total of 1384 women were included in the Intent-to-Treat (ITT) population, 1182 were included in the primary efficacy analysis (modified ITT [mITT]), and 1330 used at least 1 application of study product and were included in the Safety population. In the ITT population, select baseline characteristics were as follows: mean age, 27.7 years (standard deviation [SD], 4.5); mean body mass index, 28.7 kg/m<sup>2</sup> (SD, 8.1); Caucasian, 69.0% (955/1384); and non-Hispanic or non-Latino origin, 58.2% (805/1384). The mean number of prior pregnancies was 2.5 (SD, 1.8) and the most common contraceptive methods used immediately prior to enrollment were reported to be male condom (56.9% [787/1384], withdrawal method (14.2% [196/1384]), and rhythm method (5.1%, 70/1384). Fewer than 2% of study participants discontinued due to adverse events (AEs) (1.7% [23/1384]). For the primary efficacy analysis in the mITT population, the 7-cycle cumulative pregnancy percentage with typical-use was 13.9% (95% confidence interval [CI]; 10.0%, 17.8%), which met the pre-specified primary endpoint of having the upper bound 95% CI  $\leq$  21%. The most common AEs ( $>$ 2.0%) were vulvovaginal burning sensation (20.0%, 266/1330), vulvovaginal pruritus (11.2%, 149/1330), urinary tract infection (5.7%, 76/1330), vulvovaginal pain (3.8%, 51/1330), vulvovaginal mycotic infection (2.9%, 38/1330), bacterial vaginosis (2.8% [37/1330]), and nasopharyngitis (2.6% [35/1330]). Fourteen women (1.1%) experienced a serious AE with only 1 event (cystitis, 0.1%) considered treatment related.

**CONCLUSIONS:** In this large phase 3 study, the MVP-R, Amphora, was found to be safe and effective in preventing pregnancy. Amphora provides women with an important new non-hormonal, woman-controlled contraceptive option.

**SUPPORT:** Evoform Inc.

O-24 Monday, October 14, 2019 12:00 PM

#### **BLEEDING PATTERNS AND ENDOMETRIAL SAFETY WITH A 1-YEAR, SEGESTERONE ACETATE/ETHINYL ESTRADIOL CONTRACEPTIVE VAGINAL SYSTEM.**

David F. Archer, MD,<sup>a</sup> Kurt T. Barnhart, MD, MSCE,<sup>b</sup> Anita L. Nelson, MD,<sup>c</sup> Mitchell D. Creinin, MD,<sup>d</sup> Jeffrey T. Jensen, MD, MPH,<sup>e</sup> Sebastian Mirkin, MD,<sup>f</sup> Ruth B. Merkatz, PhD<sup>g</sup> <sup>a</sup>Eastern Virginia Medical School, Norfolk, VA; <sup>b</sup>University of Pennsylvania, Perelman School Of Medicine, Philadelphia, PA; <sup>c</sup>Essential Access Health, Los Angeles, CA; <sup>d</sup>University of California - Davis, Sacramento, CA; <sup>e</sup>Oregon Health & Science University, Portland, OR; <sup>f</sup>TherapeuticsMD, Boca Raton, FL; <sup>g</sup>Population Council, New York, NY.

**OBJECTIVE:** We analyzed bleeding patterns and endometrial safety of a contraceptive vaginal system (CVS) releasing a daily mean of segesterone acetate (SA) 0.15 mg and ethinyl estradiol (EE) 0.013 mg for up to 13 cycles of use.

**DESIGN:** Two multicenter, single-arm, open-label, pivotal, phase 3 studies of the SA/EE CVS conducted at 20 US and 7 international sites (3 in Europe, 3 in Latin America, 1 in Australia).

**MATERIALS AND METHODS:** Participants initiated CVS use on day 2-5 of their menstrual cycle, followed a 21/7-day in/out schedule of CVS use for up to 13 cycles, and recorded vaginal bleeding in paper diaries. We summarized scheduled (occurring on cycle days 22-28) and unscheduled bleeding/spotting (occurring on cycle days 1 to 21) by 28-day cycles. We performed multiple logistic regression analyses to identify factors associated with unscheduled bleeding/spotting from the first 4 cycles of CVS use. Women could also participate in an endometrial safety sub-study at 5 sites. Three blinded pathologists examined histology of endometrial biopsies obtained at screening, at cycle 6 (first 25 women reaching 6 cycles), and at end of study (cycle 12/13) or early study termination in the remaining women. We excluded women with endometrial hyperplasia or cancer at baseline and evaluated histologic changes in women with both screening and follow-up biopsies.

**RESULTS:** We analyzed bleeding data from the 2070 of 2308 participants in the two phase 3 studies who had daily bleeding diary data for cycle control analysis. Most women (97.9%) documented scheduled bleeding/spotting (during ring removal days) with a mean of 4.6-5.2 scheduled bleeding/spotting days per cycle. The proportion of women reporting any unscheduled bleeding/spotting occurred in 13.2%-21.7% of women per cycle with a mean of 3.4-5.1 days per cycle for those who had unscheduled bleeding. Absence of scheduled bleeding/spotting was 5%-8% of women per cycle; absence of any bleeding/spotting (complete amenorrhea) was 2.6%-4.9% of women per cycle. A low percentage of women (1.7%) discontinued early due to unacceptable bleeding.

Of the 156 women in the endometrial safety sub-study, 83 had follow-up biopsies. Pathologists reported no cases of endometrial hyperplasia or carcinoma at cycle 6 (n=24), cycles 12/13 (n=30) or other end of therapy times (n=29). The most frequent histologic diagnoses were atrophic/inactive or secretory; atrophic/inactive (cycle 6: 29%, cycles 12/13: 27%, and other end times: 28%, respectively), secretory (29%, 37%, and 45%, respectively), proliferative (17%, 7%, and 21%, respectively), mixed (17%, 10% and 3%, respectively), menstrual (4%, 7%, and 0%, respectively), or insufficient/no tissue (0%, 10%, and 3%, respectively).

**CONCLUSIONS:** Women using the SA/EE CVS (FDA approved in August 2018) for up to 13 cycles experienced good cycle control with few bleeding discontinuations, and did not have any unexpected endometrial histology safety findings.

**SUPPORT:** The Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health (NICHD; Contract Number HHSN27500403372) funded and conducted the US study; the US Agency for International Development (USAID; Grant Number GPO-A-00-04-00019-00) funded the international study, which was conducted by the Population Council; the World Health Organization (WHO) Reproductive Health Research Department funded two international study sites.

## CRYOPRESERVATION AND FROZEN EMBRYO TRANSFER

O-25 Monday, October 14, 2019 10:45 AM

### “UNIVERSAL WARMING PROTOCOL” FOR A TRANSNATIONAL EGG DONATION PROGRAM WITH VITRIFIED OOCYTES: A RETROSPECTIVE MULTI-CENTRE STUDY.

Lodovico Lodovico Parmegiani, PhD, Maria Giulia Minasi, M Sc, Alessandra Arnone, M Sc, Valentina Casciani, PhD, Graciela Estela Cognigni, MD, Rita Viñoles, MD, Maria Teresa Varricchio, MD, Luis Alberto Quintero, MD, Ermanno Greco, MD, Marco Filicori, MD, GynePro Medical Centers- Nexct-Clinics International, Bologna, Italy.

**OBJECTIVE:** We have previously demonstrated that it is possible to warm vitrified human oocytes using a “universal warming protocol” based on subsequent steps with 1M and 0.5 M of ECCP regardless of the warming kit brand; this study investigated the clinical efficiency of this protocol on shipped oocytes in a transnational donor program.

**DESIGN:** Retrospective multi-center observational study on a cohort of 238 patients enrolled in egg donation programs from 02 March 2017 to 19 September 2018. Primary endpoint was the survival rate (n° oocytes surviving/ n° oocytes warmed). Secondary endpoints were fertilization rate (n° fertilized oocytes / n° injected oocytes), blastulation rate (n°blastocysts obtained / n° fertilized oocytes), implantation rate (n°implanted embryos / n° of transferred embryos) and live birth rate (n° of pregnancies giving births / n° of embryo transfer).

**MATERIALS AND METHODS:** Donated oocytes vitrified in Spain, warmed in 2 centers in Italy where ICSI and embryo transfer (ET) were performed. Number of oocytes 1898, ET 238. Vitrification with Vitrification Kit (Kitazato, Japan); warming with two different kits: Kitazato Warming Kit and Vit Ki®-Thaw (Irvine-Fujifilm, US). Warmed oocytes assigned to 2 groups: KK (Kitazato/Kitazato) 939, and KI (Kitazato/Irvine-Fujifilm) 959. Vitrification with Cryotop (Kitazato); embryo culture with Embryoscope (Vitrolife, Sweden). ET at blastocyst stage.

**RESULTS:** Mean age of donors and recipients was comparable. Survival, fertilization, blastulation and implantation rates were all statistically comparable between the study groups. Survival rate was 84.6% (795/939) in group KK vs 82.1% (787/959) in group KI. Fertilization rate was 75.7% (602/795) vs 80.4% (633/787), and blastulation rate 58.5% (352/602) vs 57.8% (366/633). Implantation rate was 38.3 % (80/209) in group KK vs 45.9% (84/183) in group KI. Live birth rate was 52.5% (62/118) in KK and 45.0% (54/120) in KI.

**CONCLUSIONS:** The proven clinical efficiency of this “universal warming protocol” with ready-to-use warming kits with 1 and 0.5 M of ECCP simplifies vitrified oocyte exchange between AR centers in different countries, overcoming potential regulatory/commercial/availability differences affecting clinical practice.

**References:** Parmegiani L, Tatone C, Cognigni GE, Bernardi S, Troilo E, Arnone A, Maccarini AM, Di Emidio G, Vitti M, Filicori M. warming increases survival of slow-frozen sibling oocytes: a step towards a single warming procedure irrespective of the freezing protocol?Reprod Biomed Online. 2014 May;28(5).

**Parmegiani L**, Beilby KH, Arnone A, Bernardi S, Maccarini AM, Nardi E, Cognigni GE, Filicori M. Testing the efficacy and efficiency of a single “universal warming protocol” for vitrified human embryos: prospective randomized controlled trial and retrospective longitudinal cohort study. *J Assist Reprod Genet.* 2018 Oct;35(10):1887-1895.

**SUPPORT:** None.

O-26 Monday, October 14, 2019 11:00 AM

### MORPHOLOGY STILL MATTERS WHEN SELECTING EUPLOID EMBRYOS: INNER CELL MASS (ICM) AND TROPHECTODERM (TE) ARE PREDICTIVE OF PREGNANCY OUTCOMES.

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**OBJECTIVE:** Morphologic grading of embryos has been an ART standard for nearly 4 decades. More recently, PGT-A has improved embryo selection. Data conflicts regarding whether morphological evaluation improves outcomes of euploid embryo transfers [1, 2, 3]. Our objective was to determine whether morphology is predictive of pregnancy outcomes among single thawed euploid embryo transfers (STEETs).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** We reviewed all STEETs at a university-based ART center from 2014-2018. STEETs were excluded if oocytes were cryopreserved, embryos were created at another facility, embryos were frozen or biopsied twice, PGT-M or -SR was used, or an oocyte donor or gestational carrier was used. Only the first STEET during the study period that did not meet any exclusion criterion from each patient was included. Embryo morphology was graded according to Gardner [4]. Outcomes included implantation rate of all transfers and live birth (LB) rate, excluding 154 ongoing pregnancies and 28 pregnancies with unknown birth outcomes. Statistical analysis included chi-square, one-way ANOVA, and 2 multivariable log-binomial regression models to determine the association of predictors (age, expansion, ICM, TE) with implantation and LB.

**RESULTS:** We reviewed 1323 STEETs (mean age 37y; range 24-46y). Overall, implantation was 69% and LB (n=1141) was 55%. ICM and TE were bivariately associated with both implantation and LB (p<0.01), but age and expansion were not. ICM significantly predicted implantation, but TE did not. Both ICM and TE independently predicted LB (see Table for adjusted predicted probabilities of implantation and LB based on ICM and TE grades at mean levels of all covariates in the models).

**CONCLUSIONS:** Ploidy status is not the sole determinant of embryo competence. ICM and TE are strong predictors of LB and can improve selection among euploid embryos. Poor ICM is the greatest negative morphological predictor of implantation and LB. Our model can serve as a counseling tool for patients banking embryos.

Morphological Parameter	Counts	Probability of Implantation (95% CI)	Probability of LB (95% CI)
ICM-A	181	72%(65 – 79%)	57%(50 – 65%)
ICM-B	1088	70%(67 – 72%)	55%(52 – 59%)
ICM-C	54	37%(24 – 51%)	31%(18 – 44%)
TE-a	37	71%(57 – 85%)	67%(51 – 84%)
TE-b	1149	69%(67 – 72%)	56%(52 – 59%)
TE-c	137	59%(50 – 67%)	43%(34 – 52%)
Combined Effect of ICM+TE:	-	-	-
ICM-A + TE-a	23	75%(61 – 89%)	71%(55 – 87%)
ICM-B + TE-a	14	73%(58 – 87%)	68%(52 – 86%)
ICM-A + TE-b	158	73%(66 – 80%)	59%(51 – 67%)
ICM-B + TE-b	950	71%(68 – 74%)	57%(53 – 60%)
ICM-C + TE-b	41	38%(24 – 52%)	32%(18 – 45%)
ICM-B + TE-c	124	60%(51 – 69%)	44%(35 – 53%)
ICM-C + TE-c	13	32%(20 – 44%)	25%(13 – 36%)

No combinations of ICM-A + TE-c or ICM-C + TE-a were present in the sample so these probabilities are not shown.

References: 1) Chen, M., et al. (2015). "Can Comprehensive Chromosome Screening Technology Improve IVF/ICSI Outcomes? A Meta-Analysis." *PLoS One*10(10): e0140779.

2) Capalbo, A., et al. (2014). "Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts." *Hum Reprod*29(6): 1173-1181.

3) Irani, M., et al. (2017). "Morphologic grading of euploid blastocysts influences implantation and ongoing pregnancy rates." *Fertil Steril*107(3): 664-670.

4) Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertility and Sterility* (2000) 73 (6):1155-1158.

SUPPORT: None.

**O-27** Monday, October 14, 2019 11:15 AM

### CLINICAL FACTORS ASSOCIATED WITH THAW SURVIVAL IN A COHORT OF 6167 VITRIFIED-WARMED, EUPLOID BLASTOCYSTS.

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**OBJECTIVE:** Embryo cryopreservation has become integral to IVF treatment. While an embryo failing to survive vitrification-warming is rare, understanding of factors that predict embryo thaw survival could allow for individualized patient counseling. Prior studies on the predictors of thaw survival have been limited by the use of slow-freeze protocols and unscreened embryos. This study analyzed embryo-related factors associated with euploid embryo thaw survival.

**DESIGN:** Retrospective, case-control.

**MATERIALS AND METHODS:** This single center study included vitrified-warmed euploid embryos from autologous IVF-PGT-A cycles from 2010-2019. Blastocysts that did not survive warming were compared to those that survived. Independent variables: patient age, basal antral follicle count (BAFC), body mass index (BMI), stimulation protocol, cumulative gonadotropin (GND) dose, estradiol (E2) and progesterone (P4) level at surge, embryo development day, oocytes retrieved, fertilization method, cleavage stage embryo cell number/fragmentation, number of trophectoderm biopsies and vitrification-thawing, embryo sex, Gardner morphology. Student's t-test, chi-square, and linear regression (generalized estimating equation models) were used.

**RESULTS:** Of the euploid blastocysts thawed (n=6167), 2.8% (n=175) warmed embryos did not survive. Embryos that did not survive came from women with higher BAFC (OR 0.97, 95% CI 0.95-0.99), E2 levels at surge (p=0.03), and number of oocytes retrieved (p=0.005). Embryos cryopreserved on day 5/6 were more likely to survive than day 7 (OR 4.5, 95% CI 2.5-8.1). Embryos that underwent two trophectoderm biopsies had lower odds of survival (OR 3.2, 95% CI 1.7-5.9) than embryos that had a single biopsy. Repeat vitrification-warming was not associated with thaw survival (OR 0.26, 95% CI 0.04-1.9). While cleavage stage cell count was similar between groups, increased fragmentation was associated with reduced survival (OR 0.97, 95% CI 0.94-0.99). Embryos with expansion grade 4 (OR 4.5, 95% CI 2.5-8.1) and 5 (OR 2.1, 95% CI 1.2-3.7) had higher odds of surviving than fully hatched blastocysts. ICM grade was positive correlated with thaw survival (OR 2.2, 95% CI 1.4-3.4), whereas trophectoderm grade was not. Controlling for relevant confounders, increased BAFC, double trophectoderm biopsy, and fully hatched blastocysts remained associated with reduced thaw survival.

**CONCLUSIONS:** Blastocysts that undergo a second trophectoderm biopsy, and/or are fully hatched prior to vitrification are less likely to survive warming. Embryos from 'high responders' also have reduced odds of thaw survival. These findings may be related to the link between polycystic ovarian syndrome and poor oocyte quality. Repeat trophectoderm biopsy and increased exposure of fully hatched embryos may reduce vitrification-warming tolerance. Providers can use this data to better counsel patients regarding the risk of their embryo(s) not surviving the thaw. At the molecular level, studies comparing the transcriptome of fresh and vitrified-warmed embryos may provide insights to optimize vitrification protocols.

References: 1. Cimadomo, Danilo, et al. "Associations of Blastocyst Features, Trophectoderm Biopsy and Other Laboratory Practice with Post-Warming Behavior and Implantation." *Human Reproduction*, vol. 33, no. 11, 2018, pp. 1992-2001. <https://doi.org/10.1093/humrep/dey291>.

2. Loutradi, Kalliopi E., et al. "Cryopreservation of Human Embryos by Vitrification or Slow Freezing: a Systematic Review and Meta-Analysis." *Fertility and Sterility*, vol. 90, no. 1, 2008, pp. 186-193. <https://doi.org/10.1016/j.fertnstert.2007.06.010>.

3. Pal, L, et al. "Postthaw Blastomere Survival Is Predictive of the Success of Frozen-Thawed Embryo Transfer Cycles." *Fertility and Sterility*, vol. 82, no. 4, 2004, pp. 821-826. <https://doi.org/10.1016/j.fertnstert.2004.02.136>.

SUPPORT: None.

**O-28** Monday, October 14, 2019 11:30 AM

### ANTIOXIDANTS INCREASE BLASTOCYST CRYOSURVIVAL AND VIABILITY POST VITRIFICATION.

Thi T. Truong, Bachelor of Sciences, David Gardner, Ph.D., School of BioSciences, University of Melbourne, Melbourne, VIC, Australia.



**OBJECTIVE:** Cryopreservation is important for the preservation of gametes and embryos and consequently is used extensively in human ART. However, cryopreservation can induce oxidative stress resulting in an increase in reactive oxygen species. A combination of antioxidants has been shown to confer significant benefit to mouse IVF and culture, resulting significant improvements in embryo and fetal development. Here, we have examined the effects of the combined antioxidants as a strategy to reduce cellular stress during cryopreservation and hence improve ART outcomes.

**DESIGN:** A laboratory-based analysis of an animal model.

**MATERIALS AND METHODS:** Pronucleate mouse oocytes were collected and cultured in groups under 20% or 5% oxygen to day 4 blastocysts. Expanded blastocysts were vitrified and warmed in medium with and without antioxidants (10  $\mu$ M Acetyl-L-Carnitine /10  $\mu$ M N-Acetyl-L-Cysteine /5  $\mu$ M  $\alpha$ -Lipoic Acid), cultured for a further 24 h, and cell numbers and apoptotic cells analysed. Histones H3K9ac and H3K27ac acetylation levels (as a mark of epigenetic impact) were quantified in blastocysts, and outgrowths and synchronous embryo transfers were performed on vitrified blastocysts.

**RESULTS:** Combined antioxidants supplemented to vitrification and warming media significantly increased ICM (28.34  $\pm$  1.48 vs. 17.92  $\pm$  1.13; P <0.001) and total cell number (91.86  $\pm$  3.71 vs. 77.61  $\pm$  4.44; P <0.01) compared to controls vitrified with no antioxidants. Furthermore, blastocysts vitrified with antioxidants resulted in similar total cell number and apoptotic rates to non-vitrified controls. Blastocysts vitrified with antioxidants also showed a significant increase in *in vitro* outgrowth area and perimeter (P<0.05). Subsequent synchronous blastocyst transfer, following culture in 20% oxygen, resulted in increased fetal weight (190.19  $\pm$  4.61mg vs. 174.29  $\pm$  5.52 mg; P<0.05), crown rump length (11.09  $\pm$  0.10 vs. 10.76  $\pm$  0.11; P<0.05) and limb development (14.89  $\pm$  0.07 vs. 14.56  $\pm$  0.11; P<0.05) when blastocysts were vitrified and warmed with antioxidants. Embryos cultured at 5% oxygen to the blastocyst stage and vitrified with antioxidants also showed increased crown rump length (11.29  $\pm$  0.08 vs. 10.74  $\pm$  0.12; P<0.001) and ear development (14.90  $\pm$  0.05 vs. 14.64  $\pm$  0.11; P<0.05). Importantly, while vitrification reduced acetylation of histones H3K27ac and H3K9ac in vitrified blastocysts, the inclusion of antioxidants significantly ameliorated this (P<0.05).

**CONCLUSIONS:** Vitrification and warming of blastocysts have detrimental effects on embryo development irrespective of oxygen culture conditions. Combined antioxidants in vitrification media significantly reduced the negative effects, resulting in blastocysts with higher developmental potential *in vitro* and increased viability. Thus, viability of vitrified human embryos may be improved by the inclusion of antioxidants during cryopreservation.

**O-29** Monday, October 14, 2019 11:45 AM

### DIFFERENCES IN OOCYTE SURVIVAL BETWEEN DONOR EGG BANKS AND SATELLITE CLINICS WITHIN THE SAME COMPANY.

Whitney Hewitt, BS,<sup>a</sup> Jennifer L. Patrick, PhD,<sup>a</sup> Lauren Johnson, MD, MSCE,<sup>a</sup> Matrika Johnson, MD,<sup>b</sup> Seth Katz, MD,<sup>a</sup> Joe Whelan, III, MD,<sup>a</sup> Tyl Taylor, PhD,<sup>b</sup> <sup>a</sup>Reproductive Endocrinology Associates of Charlotte, Charlotte, NC; <sup>b</sup>REACH, Charlotte, NC.



**OBJECTIVE:** Vitrification of donor oocytes has become a staple in the IVF community. In fact, there are multiple vendors in multiple locations stimulating donors, freezing oocytes, and offering a limited number of oocytes to recipients across the globe. Although oocytes can come from the same company and follow the same protocols, they can come from different satellite locations, thus exposing receiving clinics to different variables that may impact clinical outcomes. This study has two objectives: to compare clinical outcomes of three different egg bank vendors and compare if there are differences between oocytes originating from the same company's different satellite locations.

DESIGN: Retrospective.

**MATERIALS AND METHODS:** Frozen donor oocytes that were shipped to our clinic and warmed between 2015-2019 were compared based on the company the oocytes were received from and the clinic from which they were received. Clinical outcomes including oocyte survival, fertilization, and usable blastocyst rate (those vitrified or transferred) were compared between different donor egg banks and within the same donor egg bank's satellite locations. All warming protocols were followed according to each company's policies.

**RESULTS:** We performed 139 donor oocyte thaw cycles from three competing egg banks, using a total of 850 frozen donor oocytes from 2015-2019. Bank "A" provided 121 patients with 733 oocytes, Bank "B" provided 8 patients with 63 oocytes, and Bank "C" provided 10 patients with 54 oocytes. Oocyte survival rates post-thaw differed significantly between the different banks, with 630/733 (86.0%), 35/63 (55.6%), and 50/54 (92.6%) surviving from bank "A", "B", and "C", respectively ( $P < 0.0001$ ). Fertilization rates and usable blastocyst rate did not differ between the egg banks.

From bank "A", we compared oocyte survival, fertilization, and usable blastocyst rates between three different satellite clinics within the same egg bank company. A total of 103 donor thaw cycles, with 49 from clinic "A", 31 from clinic "B", and 23 from clinic "C", were performed in our lab from 2015-19. A total of 624 frozen oocytes were thawed, 299, 186, and 139 from clinic "A", "B", and "C", respectively. Oocyte survival post thaw were statistically different between satellite clinic "A" (268/299, 89.6%), clinic "B" (133/186, 71.5%), and clinic "C" (120/139, 86.3%;  $P < 0.0001$ ). Fertilization rates and usable blastocyst rate did not differ between clinics.

**CONCLUSIONS:** Our data suggest that there are differences in oocyte survivability post-thaw amongst the different oocyte banking companies. More importantly, there were differences in oocyte survivability amongst the satellite locations from the same egg bank company. This suggests an impact of the embryologist vitrifying or the stimulation protocol utilized.

References: None.

SUPPORT: None.

**O-30** Monday, October 14, 2019 12:00 PM

### IMPROVED EMBRYOLOGY OUTCOME AND MORPHOKINETIC CHARACTERISTICS OF EMBRYOS DERIVED FROM FROZEN/THAWED OOCYTES INJECTED 3 VERSUS 2 HOURS POST-WARMING.

Christopher Norberg, MS,<sup>a</sup> Qiansheng Zhan, MSc,<sup>a</sup> Richard J. Bodine, MS,<sup>a</sup> Samuel H. Jones, BS,<sup>a</sup> Jason Park, MS,<sup>a</sup> Katherine Larkins, BS,<sup>a</sup> Zhen Ye, MS,<sup>a</sup> Robert Clarke, PhD,<sup>a</sup> Zev Rosenwaks, M.D.,<sup>b</sup> Nikica Zaninovic, Ph.D.,<sup>a</sup> Ronald O. Perلمان and Claudia Cohen Center for Reproductive Medicine, New York, NY; <sup>b</sup>The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY.

**OBJECTIVE:** To assess whether the duration of recovery following the warming of cryopreserved oocytes affects the morphological and morphokinetic properties of the developing embryo.

**DESIGN:** This is a retrospective data analysis examining the effect of the post-warming recovery duration of vitrified oocytes prior to ICSI. Frozen oocytes from 36 patients (July 2018 to March 2019), either autologous or donor, were included in the study. The study examined cycles in which the oocytes were injected within a 2-hour recovery period and compared them to those in which the oocytes were injected within 3 hours or more within the same patient oocyte cohort. All injected oocytes were cultured individually in time-lapse incubators (TLM; EmbryoScope, Vitrolife, Sweden). Embryo transfers occurred on D3 or D5 regardless of the study groups.

**MATERIALS AND METHODS:** Standard oocyte freezing protocol included vitrification 2 hours after retrieval using a Kitazato-based (Japan) vitrification media. Oocyte warming was performed using Kitazato thaw media. ICSI was performed in the standard fashion, and embryos were annotated daily for their developmental hallmarks. Embryo selection for ET or freezing was performed by standard laboratory protocols.

**RESULTS:** No significant differences were observed in 2PN and/or abnormal fertilization rates between the groups. Similarly, on day 3, no significant differences were found in the average cell numbers or in the average number of good ( $\geq 8$  cells,  $< 20\%$  frag.), fair (5-7 cells, any abnormal cleavages), and poor ( $\leq 4$  cells) embryos. Equally, the incidence of abnormal cleavage patterns was similar among the cohort. More embryos were selected for transfer from the 3-hour group than from the 2-hour group (51.1% vs. 48.9%;  $p = 0.78$ ). There were no significant differences in the utilization rate (the number of embryos transferred fresh plus blastocysts frozen) between

the 3- and 2-hour groups (49.6% vs. 49.6%, respectively). The time-lapse morphokinetic data indicated faster-growing embryos in the 3- versus 2-hour group. This difference is apparent in late-stage morphokinetic parameters (t9 to tsB). The 3-hour group produced significantly better quality blastocysts in cycles that were either frozen upfront, fresh D5 ET, or PGT ( $p = 0.03$ ).

**CONCLUSIONS:** The duration of the recovery time post-warming showed no significant differences in overall embryology outcomes prior to blastocyst formation. However, at the BL stage, the 3-hour group demonstrated improvement in morphokinetic parameters and in overall BL quality.

Reference: None.

SUPPORT: Internal.

## ENVIRONMENT AND REPRODUCTION

**O-31** Monday, October 14, 2019 10:45 AM

### EFFECT OF WILDFIRE SMOKE ON PREGNANCY OUTCOMES IN THE NON-HUMAN PRIMATE.

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**OBJECTIVE:** In November 2018, the "Camp Fire" wildfire was deemed the most destructive and deadliest wildfire in California history. The resulting poor air quality and ambient particulate matter in the Northern California region offered a rare opportunity to study the effect of wildfire smoke on conception and live birth rates in the non-human primates (*M. mulatta*) that reside outdoors at the California National Primate Research Center (CNPRC) in nearby Davis, CA.

**DESIGN:** We conducted a pilot prospective cohort study investigating pregnancy outcomes after exposure to ambient smoke from the Camp Fire that burned from 11/8/18-11/22/18 about 160 kilometers away. This cohort was exposed to elevated fine particulate matter as recorded by California Air Resource Board (CARB). The fine particulate matter (PM<sub>2.5</sub> – particles less than 2.5  $\mu\text{m}$  in diameter) measured by CARB indicated a rise above national and state ambient air quality standards (15  $\mu\text{g}/\text{m}^3$ ) for 12 days and nights during the 2018-2019 breeding season reaching levels as high as 185  $\mu\text{g}/\text{m}^3$ . The primary outcome of these data is conception and live birth rates.

**MATERIALS AND METHODS:** Through CNPRC, 66 blood (serum) samples were collected from female macaques in the outdoor colony following exposure to ambient smoke during the 2018-2019 breeding season. The primates have since undergone routine surveillance for conception and birth outcomes. For comparison, data was collected from the 2016 and 2017 breeding seasons.

**RESULTS:** Preliminary results show that out of 66 primates sampled, a total of 44 primates have confirmed pregnancies by physical exam (palpation) and/or positive serum macaque chorionic gonadotropin (mCG,  $n = 11$ ). These primates were exposed to hazardous smoke either prior to conception or during the early portion of their pregnancies. Conception rate of the cohort sampled was 66% compared to an average of 92% and 84% overall in the two breeding seasons prior, without exposure to similar wildfire smoke. Live birth rate is still being collected (x) and will be resulted in June 2019.

**CONCLUSIONS:** Exposure to poor air quality as documented by elevated levels of fine particulate matter resultant from wildfire smoke at a key time prior to conception/early in pregnancy may have an effect on both conception rate and pregnancy outcomes in the non-human primate. This study joins the overall small body of literature that has shown deleterious effects of wildfire smoke exposure in pregnancy. Further research is needed to evaluate the mechanism in which wildfire smoke affects placentation and early pregnancy growth and development.

Breeding Season	Conception Rate	Live Birth Rate
2016-2017	92%	86%
2017-2018	84%	76%
2018-2019	66%	x

SUPPORT: Grant number P30ES023513.

### STRESS AND SUCCESS: DATA FROM A PROSPECTIVE COHORT INVESTIGATING THE IMPACT OF EARLY LIFE STRESS ON IVF OUTCOMES.



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**OBJECTIVE:** Women who experienced early life stress (ELS) have aberrant hypothalamic-pituitary-adrenal and autonomic responses as well as an increased inflammatory response to induced stress when compared to ELS naïve women. The impact of ELS on infertile women is largely unknown. We sought to determine the prevalence of ELS in the infertile population and the impact of this dysregulated stress reactivity on IVF cycle characteristics and outcomes.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Women aged 18-42 were recruited for enrollment prior to initiating an autologous IVF cycle. Patients pursuing third party reproduction or fertility preservation were excluded. Consenting participants provided demographic information and completed the CDC-Kaiser Adverse Childhood Experience Questionnaire. Those who indicated 2+/10 positive responses were considered to be ELS positive. A power analysis indicated that a sample size of 277 subjects would provide at least 80% power with an alpha of 0.05 to detect a 40% relative difference in live birth rates between groups. Continuous variables were compared using Student's t-test or Mann-Whitney U test based on normality, while  $\chi^2$  or Fisher's exact tests were used to compare categorical variables by ELS status. Logistic regression was used to assess for predictors of live birth and early pregnancy failure adjusting for confounders as appropriate.

**RESULTS:** The prevalence of ELS positivity in this infertile cohort was 29.2% (n=83/284). ELS positive women and controls were similar in age, race/ethnicity, and history of anxiety/depression, however higher BMIs were observed in the ELS positive group (mean BMI 27.4 vs 25.6 kg/m<sup>2</sup>, p=.02). There were no differences in infertility diagnosis, pregnancy history, number of prior IVF cycles or ovarian reserve parameters. While live birth rates were similar in the two groups (37% vs 35%; aOR 1.13, 95% CI 0.65-1.95, p=0.658), ELS positive women had significantly higher rates of early pregnancy loss (EPL) per transfer (28% vs 17%, p=.04). This association persisted when the analysis was restricted to patients undergoing their first IVF cycle and excluding cycles in which preimplantation genetic testing was performed. After controlling for BMI and parity, ELS positivity remained significantly associated with EPL (aOR 1.95, 95% CI 1.05-3.62, p=0.03). However, when EPL rates were considered only among those who achieved a pregnancy (positive pregnancy test), no difference was observed between groups.

**CONCLUSIONS:** Early life stress has a longstanding impact on adult health. While IVF cycle parameters and pregnancy rates do not seem to be impacted, infertile women who experienced ELS have significantly higher rates of early pregnancy loss per transfer. Further studies are needed to elucidate the precise mechanisms of these findings to identify risk reduction methods in this unique, potentially vulnerable, subpopulation of patients pursuing fertility services.

**SUPPORT:** Penn Presbyterian George L. and Emily McMichael Harrison Fund for Research in Obstetrics and Gynecology NIH T32HD007440-21.

O-33 Monday, October 14, 2019 11:15 AM

### PESTICIDE RESIDUE INTAKE FROM FRUIT AND VEGETABLE CONSUMPTION AND RISK OF LAPAROSCOPICALLY-CONFIRMED ENDOMETRIOSIS.



Holly Harris, M.P.H., Sc.D.,<sup>a</sup> Kara L. Cushing-Haugen, MS,<sup>a</sup> Yu-Han Chiu, ScD, MD,<sup>b</sup> Jorge E. Chavarro, MD, Sc.D.,<sup>c</sup> Stacey A. Missmer, Sc.D.,<sup>d</sup> <sup>a</sup>Fred Hutchinson Cancer Research Center, Seattle, WA; <sup>b</sup>Harvard T.H. Chan School of Public Health, Boston, MA; <sup>c</sup>Harvard School of Public Health, Boston, MA; <sup>d</sup>Michigan State and Harvard T.H. Chan SPH, Grand Rapids, MI.

**OBJECTIVE:** Dietary factors may influence endometriosis through effects on a variety of factors including smooth muscle contractility, steroid hor-

mones, inflammation, prostaglandin metabolism – and these processes may be influenced by food contaminants including pesticide residues. We examined the association between intake of fruits and vegetables known to have high pesticide residue burden and diagnosis of laparoscopically-confirmed endometriosis.

**DESIGN:** Prospective cohort study using data collected from 58,057 premenopausal women from 1999-2013 as part of the Nurses' Health Study II (NHSII).

**MATERIALS AND METHODS:** Diet was assessed with a validated food frequency questionnaire every four years. A Pesticide Residue Burden Score (PRBS) was assigned to each individual fruit and vegetable using a validated method based on surveillance data from the U.S. Department of Agriculture. Cases were restricted to laparoscopically-confirmed endometriosis. Multivariable Cox proportional hazards models were used to calculate rate ratios (RR) and 95% confidence intervals (CI) for the association between high and low PRBS and endometriosis diagnosis.

**RESULTS:** During 14 years of follow-up (baseline age range 35-52), 1,021 incident cases of laparoscopically-confirmed endometriosis were reported. No association was observed between intake of high-pesticide residue fruits and vegetables and endometriosis diagnosis (RR for 5<sup>th</sup> quintile vs 1<sup>st</sup> quintile=0.92; 95% CI=0.70-1.21; p<sub>trend</sub>=0.32). In addition, no association was observed between intake of low-pesticide residue fruits and vegetables and endometriosis diagnosis (RR for 5<sup>th</sup> quintile vs 1<sup>st</sup> quintile=1.00; 95% CI=0.75-1.33; p<sub>trend</sub>=0.92). When analyses were stratified by fertility status, there was the suggestion that women reporting infertility who had a higher intake of fruits and vegetables with a low-pesticide residue burden had a lower risk of endometriosis (RR for 5<sup>th</sup> quintile vs 1<sup>st</sup> quintile=0.26; 95% CI=0.05-1.35; p<sub>trend</sub>=0.18), however the results were based on small case numbers (n=61) and did not reach statistical significance.

**CONCLUSIONS:** No clear associations were observed between intake of high or low pesticide residue fruits and vegetables and risk of endometriosis diagnosis. This may be due to the age of the NHSII participants during the window of follow-up for which we had PRBS data. If a true association between PRBS and endometriosis exists, it may be impactful only with exposure during adolescence or early adulthood. The suggestion that consuming fruits and vegetables with a low-pesticide residue burden is associated with a lower risk of endometriosis among women with infertility may be due to pathways specific to endometriosis-associated infertility. Further study among those with infertility, within a younger patient population, and quantifying pesticide exposure during adolescence is warranted.

**SUPPORT:** Endometriosis Foundation of America.

O-34 Monday, October 14, 2019 11:30 AM

### BLOOD CADMIUM ASSOCIATED WITH HIGHER TESTOSTERONE AND ANTI-MÜLLERIAN HORMONE IN HEALTHY PREMENOPAUSAL WOMEN.



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**OBJECTIVE:** Cadmium is a toxic metal associated with higher androgen levels and dysregulation of glucose metabolism. Cadmium may therefore be associated with polycystic ovary syndrome (PCOS)-related phenotypes, which are characterized by endocrine and metabolic dysregulation. Our objective was to evaluate associations between blood cadmium concentrations and PCOS-related features in healthy premenopausal women without self-reported PCOS diagnosis.

**DESIGN:** Prospective cohort study of 251 women enrolled in the Bio-Cycle Study.

**MATERIALS AND METHODS:** Participants provided blood samples to measure cadmium concentrations and completed a food frequency questionnaire at baseline. Serum reproductive hormones and metabolic markers, including total testosterone, free androgen index, sex hormone-binding globulin (SHBG), anti-Müllerian hormone (AMH), insulin, and glucose levels, were measured up to 8 times per menstrual cycle for up to 2 cycles. Linear mixed regression models were used to investigate associations between cadmium concentrations (per 0.1 µg/L increase) and changes in log-transformed PCOS-related markers. We also investigated associations between cadmium levels and a mild PCOS-phenotype, defined as a cycle having both total testosterone and AMH in the highest quartile, using Poisson regression

with a robust error variance. Models adjusted for age, percent body fat, race, and smoking. Models were also adjusted for dietary factors that are potentially related to cadmium exposure and PCOS, such as intakes of rice, total grains, and green leafy vegetables.

**RESULTS:** Mean (standard deviation) age and percent body fat were 27.3 (8.2) years and 29.7% (6.0), respectively. Median (interquartile range) cadmium levels were 0.30 (0.19-0.43)  $\mu\text{g/L}$ . Cadmium was associated with higher total testosterone (2.6% difference; 95% confidence interval [CI] 0.7, 4.5;  $P=0.01$ ), SHBG (3.0% difference; 95% CI 0.4, 5.7;  $P=0.03$ ), and AMH (7.0% difference; 95% CI 0.2, 14.2;  $P=0.04$ ), per 0.1  $\mu\text{g/L}$  increase. Our data also suggests that higher cadmium concentrations were associated with 12% higher probability of having a mild PCOS-phenotype with a borderline significance (relative risk 1.12; 95% CI 0.98, 1.29, per 0.1  $\mu\text{g/L}$  increase;  $P=0.09$ ). No associations were found for free androgen index, insulin, and glucose levels. Further adjustment for intakes of rice, total grains, and leafy vegetables did not change these associations.

**CONCLUSIONS:** Among healthy women, cadmium was associated with endocrine features central to PCOS, including total testosterone and AMH. However, we observed no associations with metabolic markers, such as fasting glucose and insulin. Among women without a PCOS diagnosis, these results suggest a potential role of cadmium in the hormonal milieu associated with PCOS.

**O-35** Monday, October 14, 2019 11:45 AM

### THE ASSOCIATION OF URINARY CONCENTRATIONS OF BISPHENOL-A, AND DI-ETHYLHEXYL PHTHALATE METABOLITES WITH THYROID FUNCTION & AUTOIMMUNITY IN WOMEN FROM



**A FERTILITY CENTER: RESULTS FROM THE ENVIRONMENT AND REPRODUCTIVE HEALTH STUDY.** Irene Souter, MD,<sup>a</sup> Lidia Mínguez-Alarcón, PhD,<sup>b</sup> Tim Korevaar, MD, PhD,<sup>b</sup> Jennifer B. Ford, RN,<sup>b</sup> Jorge E. Chavarro, MD, Sc.D.,<sup>c</sup> Russ Hauser, MD, MPH, Sc.D.<sup>b</sup> <sup>a</sup>MGH Fertility Center and Harvard Medical School, Boston, MA; <sup>b</sup>Harvard T.H. Chan School of Public Health, Boston, MA; <sup>c</sup>Harvard School of Public Health, Boston, MA.

**OBJECTIVE:** To evaluate the association of urinary concentrations of bisphenol-A (BPA) and di-ethylhexyl phthalate (DEHP) metabolites with markers of thyroid function and autoimmunity among women seeking fertility treatments.

**DESIGN:** Prospective Cohort Study.

**MATERIALS AND METHODS:** Urine and serum samples were collected from 558 women seeking infertility treatment at an academic institution and participating at the environment and reproductive health (EARTH) study. Urinary BPA and phthalate metabolite concentrations were quantified by isotope dilution tandem mass spectrometry, and the molar sum of four DEHP metabolites was calculated. Biomarkers of thyroid function [thyroid stimulating hormone (TSH), free and total thyroxine (FT<sub>4</sub>, TT<sub>4</sub>), and triiodothyronine (FT<sub>3</sub>, TT<sub>3</sub>), and thyroid autoimmunity [thyroid peroxidase (TPO) and thyroglobulin (Tg) antibodies (Ab)] were quantified in serum using electrochemoluminescence assays.

Linear regression models adjusted for covariates (age, body mass index, diagnosis, specific gravity, BPA for DEHP metabolites and DEHP metabolites for BPA analyses) were used to estimate the relations between urinary BPA and DEHP concentrations, in tertiles, and serum thyroid function and autoimmunity biomarkers.

**RESULTS:** Higher urinary concentrations of DEHP metabolites were associated with lower serum levels of FT<sub>4</sub>, TT<sub>4</sub>, FT<sub>3</sub>, and TT<sub>3</sub> in both adjusted and unadjusted models. The multivariable adjusted means (95% CI) of thyroid function biomarkers for women in the lowest, middle, and highest tertile of urinary DEHP were: 15.6 (15.2, 15.9), 15.3 (14.9, 15.6), and 15.1 (14.7, 15.4) pmol/L for FT<sub>4</sub> (p-trend: 0.06); 101 (97.8, 104), 98.6 (95.9, 101), and 94.8 (91.7, 97.8)\* nmol/L for TT<sub>4</sub> (p-trend 0.01); 4.9 (4.8, 5.0), 4.8 (4.7, 4.9), and 4.7 (4.6, 4.8)\* pmol/L for FT<sub>3</sub> (p-trend: 0.01); and 1.9 (1.9, 2.0), 1.8 (1.8, 1.9),\* and 1.8 (1.7, 1.8)\* nmol/L for TT<sub>3</sub> (p-trend: 0.005); \* p-value <0.05 when comparing that tertile to the lowest tertile of exposure.

DEHP was not related to either TSH [2.0 (0.8, 2.2), 2.2 (2.0, 2.3), and 1.9 (1.8, 2.1) mU/L, for lowest, middle, and highest tertile respectively; p-trend 0.6] or thyroid antibody biomarkers [14.8 (12.9, 17.0), 14.7 (13.1, 16.5), and 14.1 (12.4, 16.1) IU/mL for TPO Ab (p-trend: 0.6); 22.6 (18.8, 27.1), 22.7 (19.5, 26.4), and 19.0 (16.0, 22.6) IU/mL for TgAb (p-trend: 0.2), for lowest, middle, and highest tertile, respectively].

Urinary BPA concentrations were unrelated to thyroid function or thyroid autoimmunity biomarkers.

**CONCLUSIONS:** Urinary DEHP, but not BPA, was inversely related to markers of thyroid function but not of thyroid autoimmunity. Our data suggest that current levels of exposure to certain phthalates negatively impacts thyroid function of reproductive age women through mechanisms that do not involve autoimmunity.

**SUPPORT:** National Institute of Environmental Health Sciences (NIEHS); R01ES022955, R01ES009718, and P30ES000002.

**O-36** Monday, October 14, 2019 12:00 PM

### NON-CHRONIC PRECONCEPTION OPIOID USE AND REPRODUCTIVE OUTCOMES.



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**OBJECTIVE:** In recent decades, prescription opioid use has increased dramatically among reproductive age women. While much is known about the adverse outcomes of opioid abuse during pregnancy, the risk of limited opioid use during the periconception period is unclear. Thus, we examined associations of preconception and early-pregnancy opioid use with fecundability, live birth, and pregnancy loss in a cohort of women from the EAGeR trial.

**DESIGN:** Prospective cohort of 1228 women with 1-2 prior pregnancy losses enrolled in a randomized trial of preconception low-dose aspirin and followed for up to 6 cycles while attempting conception or through pregnancy resolution.

**MATERIALS AND METHODS:** We measured urinary concentrations of opioids by chemiluminescent immunoassay during preconception and, among women who became pregnant, at weeks 4 and 8 of pregnancy. We defined a positive screen as any opioid detected above manufacturer-defined cut points. Women self-reported use of opioid medications during or in the year prior to their last pregnancy and during preconception follow-up cycles. We estimated fecundability odds ratios (FOR) and confidence intervals (CI) with discrete Cox proportional hazard models. We estimated risk ratios (RR) of live birth and pregnancy loss with log binomial models. We adjusted for age, race, BMI, education, smoking, use of alcohol, marijuana, and antidepressants, time since last pregnancy, and gynecological indications for opioid use (e.g. fibroids, cramping).

**RESULTS:** 110 (9%) women screened positive for opioids during the preconception period and 33 (4.8% of 8-week pregnancies) screened positive during week 4 or 8 of pregnancy. 166 (13.6%) women self-reported opioid use during or before their previous pregnancy or during preconception follow-up. Most women screened positive or self-reported use only once. Positive preconception opioid use by screening or self-report was associated with longer time to pregnancy (FOR: 0.75; 95% CI: 0.61, 0.93) and marginally associated with probability of live birth (RR: 0.81; 95% CI: 0.64, 1.01). Positive opioid screening during pregnancy was associated with 2.90 times higher risk of pregnancy loss (95% CI: 1.51, 5.55).

**CONCLUSIONS:** Preconception opioid use was associated with lower fecundability and live birth rate. Use in pregnancy was associated with risk of loss. Opioid use may have adverse reproductive consequences even in non-addicted populations. Further studies are needed to determine the duration of use and specific types of opioids that may be harmful.

**SUPPORT:** This work was supported by the Intramural Research Program, Division of Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development.

### GENETIC COUNSELING

**O-37** Monday, October 14, 2019 10:45 AM

### LESSONS LEARNED FROM EVALUATING DECISIONAL REGRET SURROUNDING PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY.



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TABLE 1. Aneuploidy rates by PA

Paternal age (years)	Cases (n)	Embryos (n)	Average number of embryos per case (n)	Euploid Rate $\pm$ SD (%)	Aneuploid Rate $\pm$ SD (%)	Aneuploidy of Maternal Origin $\pm$ SD (%)	Aneuploidy of Paternal Origin $\pm$ SD (%)	Aneuploidy of Mixed Origin $\pm$ SD (%)
<35	173	1156	6.7	66.3 $\pm$ 1.4	33.7 $\pm$ 1.4	50.6 $\pm$ 3.1	31.1 $\pm$ 2.9	18.3 $\pm$ 2.4
35-37	233	1452	6.2	67.7 $\pm$ 1.2	32.3 $\pm$ 1.2	54.3 $\pm$ 2.8	31.8 $\pm$ 2.6	13.9 $\pm$ 1.9
38-40	287	1972	6.9	66.5 $\pm$ 1.1	33.5 $\pm$ 1.1	57.4 $\pm$ 2.3	24.4 $\pm$ 2.0	18.2 $\pm$ 1.8
41-42	201	1493	7.4	67.6 $\pm$ 1.2	32.4 $\pm$ 1.2	59.2 $\pm$ 2.7	25.0 $\pm$ 2.4	15.8 $\pm$ 2.0
>42	964	7321	7.6	66.4 $\pm$ 0.6	33.6 $\pm$ 0.6	54.1 $\pm$ 1.2	28.7 $\pm$ 1.1	17.2 $\pm$ 0.9
Overall	1858	13394	7.2	66.7 $\pm$ 0.4	33.3 $\pm$ 0.4	54.9 $\pm$ 0.9	28.2 $\pm$ 1.2	16.9 $\pm$ 0.7

**OBJECTIVE:** Patients are often expected to make informed decisions about the use of preimplantation genetic testing for aneuploidy (PGT-A) based upon limited knowledge of its risks and benefits. This study aims to assess whether there are differences in degree of decisional regret between patients who decide to undergo/not undergo PGT-A, and to elucidate whether there are personal beliefs or clinical outcomes that correlate with level of decisional regret.

**DESIGN:** Retrospective cohort survey.

**MATERIALS AND METHODS:** An online survey was distributed to patients who underwent in vitro fertilization (IVF) with or without PGT-A between January 1<sup>st</sup> of 2016 to 2018. The survey consisted of 4 sections: 1) Demographic and Clinical Outcomes, 2) Decision-making factors, 3) Beliefs about PGT-A, and 4) Decision regret scale (DRS). Strength of belief in purported risks and benefits of PGT-A were assessed on a 0-100 scale (0: not true, 100: absolutely true). DRS scores ranged from 0-100, with a validated threshold of >25 indicating moderate to severe regret (MSR). Student's t-test, Wilcoxon Rank-Sum, or Chi square test was applied, as appropriate, to compare baseline characteristics, DRS scores, and MSR rate between those who did or did not complete PGT-A. Multivariate linear regression was used to assess the impact of surveyed factors on DRS scores. Multinomial logistic regression was used to evaluate risk factors for MSR. All patients received evidence-based counseling regarding risks and benefits of PGT-A during a mandatory pre-treatment IVF orientation.

**RESULTS:** At this time, three hundred and thirty-five women completed the eligibility survey. Of the 261 women deemed eligible, 123 women completed the study survey (47%); 66 underwent PGT-A and 57 did not. There were no differences in demographic characteristics between the two groups. In raw analysis, DRS scores were significantly higher in those who did not complete PGT-A, compared to those who did (Median 20 vs 0, IQR 0-30 vs 0-20,  $p=0.02$ ); however, that difference diminished after controlling for live birth outcomes. In the group of patients with no live birth after index IVF cycle, there was no statistically significant difference in DRS scores between those who did and did not complete PGT-A (22 vs 34,  $p=0.15$ ). Participants who completed PGT-A were significantly more likely to believe PGT-A improved the chance of having a healthy baby (88 vs 76,  $p=0.005$ ), and that belief correlated with lower DRS scores regardless of live birth outcomes. MSR was noted in 14 women (21%) who had PGT-A compared to 19 women (33%) who did not ( $p=0.13$ ). Lack of live birth (RRR=0.18,  $p=0.02$ ) and low overall patient satisfaction (RRR=0.98,  $p=0.03$ ) significantly increased risk of MSR.

**CONCLUSIONS:** Decisional regret surrounding PGT-A is largely driven by overall patient satisfaction and live birth outcomes. However, our findings suggest that in the setting of a poor clinical outcome, there is no difference in level of decisional regret between those who do or do not elect for PGT-A. Physicians should feel comfortable counseling patients regarding the risks and benefits of PGT-A, and then allow them to choose.

Reference: Quinn, M., et al. "Decision-Making Surrounding the Use of Preimplantation Genetic Testing for Aneuploidy Reveals Misunderstanding Regarding Its Benefit." *Fertility and Sterility*, vol. 110, no. 4, 2018, pp. 2155-2159.

**SUPPORT:** None.

**O-38** Monday, October 14, 2019 11:00 AM

#### FOCUSING ON PARENTAL ORIGIN OF ANEUPLOIDY: DOES PATERNAL AGE IMPACT ANEUPLOIDY RATES IN EMBRYOS?

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**OBJECTIVE:** While it is known that aneuploidy rates increase with advancing maternal age (MA) due to deterioration of the oocyte's meiotic system,<sup>1</sup> there has been no proven paternal age (PA) association. Some authors have postulated that advancing PA may be associated with increased risks for aneuploidy,<sup>2,3</sup> while other studies have shown no association.<sup>4</sup> In this study, we report the 24-chromosome preimplantation genetic testing for aneuploidy (PGT-A) results for trophectoderm (TE) samples from a series of men who underwent in vitro fertilization (IVF) cycles using oocyte donors, broken down by PA.

**DESIGN:** Retrospective analysis.

**MATERIALS AND METHODS:** All PGT-A cases with TE biopsy and an oocyte donor between July 2010 and April 2019 were included in the analysis. TE and biological parental samples were run on Illumina Cyto12 SNP-based microarrays with informatics to determine parental origin of each chromosome and establish chromosome copy number. Statistical analysis was performed using a two-tailed t-test.

**RESULTS:** Results were obtained on 13,018/13,394 (97.2%) of submitted TE samples. The average PA for this patient cohort was 43.5  $\pm$  6.9 years (range 23-73). Aneuploidy rates are broken down by PA using SART age groups (Table 1). Additional analysis performed for men >50 years showed an aneuploidy rate of 704/2031 (34.7%) which was not statistically different from the other PA groups. Moreover, there was no statistical difference in the paternal aneuploidy rates or aneuploidy of mixed origin between PA groups ( $p>.05$ ).

**CONCLUSIONS:** In this study, we did not observe an increase in aneuploidy rates with advanced PA, adding to existing literature showing a lack of PA effect. SNP microarrays with informatics uniquely allows determination of parental origin of aneuploidy in embryo samples. The difference in overall aneuploidy rates and paternally inherited aneuploidy rates among the PA groups was not statistically significant ( $p>.05$ ). This information can be used to aid in patient counseling by providing reassurance that estimates for aneuploidy rates should be based primarily on the age of the oocyte contributor.

References: 1 Gardner, R. and Sutherland, G. Chromosome Abnormalities and Genetic Counseling; 3rd ed (Aug 28, 2003): 363-368.

2 Buwe et al. Effect of paternal age on the frequency of cytogenetic abnormalities in human spermatozoa. *Cytogenet Genome Res.* 2005;111:213-228.

3 Sartorelli et al. Effect of paternal age on human sperm chromosomes. *Fertil Steril* 2001 Feb;76(6):1119-1123.

4 Kim et al. Effects of paternal age on human embryo development in in vitro fertilization with preimplantation genetic screening. *Clin Exp Reprod Med.* 2019 Mar;46(1):22-29.

**SUPPORT:** Natera, Inc.

**O-39** Monday, October 14, 2019 11:15 AM

#### DO BRCA MUTATIONS IMPACT ANEUPLOIDY RATES IN EMBRYOS? Carrie Chou, MS,<sup>a</sup> Ellen Thomas, MS,<sup>b</sup> Katrina Merrion, MS,<sup>a</sup> Nina Wemmer, MS,<sup>b</sup> <sup>a</sup>Natera, Inc., San Carlos, CA; <sup>b</sup>Affiliation not provided.



**OBJECTIVE:** Analyze chromosome ploidy results in a patient cohort who pursued preimplantation genetic testing for monogenic/single gene defects (PGT-M) for familial BRCA1 or BRCA2 mutations with concurrent 24-chromosome preimplantation genetic testing for aneuploidy (PGT-A). Prior studies suggest BRCA mutations may be associated with diminished ovarian reserve and infertility, and that the effects of BRCA1 mutations may differ from BRCA2.<sup>1,2</sup> Furthermore, mouse models have shown

TABLE 1. PGT-A results in BRCA1/2 carriers

	Total BRCA1/2	All mat BRCA1/2	All pat BRCA1/2	BRCA1 mat	BRCA1 pat	BRCA2 mat	BRCA2 pat	Mat age-matched controls
# of Cycles	123	77	46	50	28	27	18	5147
# of embryos	779	487	292	305	168	182	124	25,945
Mean mat age (range)	34.0 (26-45)	33.9 (26-41)	34.0 (26-45)	33.9 (26-41)	33.6 (26-41)	34.0 (27-41)	34.6 (28-45)	34.0 (26-45)
% aneuploid	44±1.8%	44±2.3%	44±3.9%	48±2.9%	44±3.9%	37±3.6%	45±4.5%	43±0.3%
% euploid	56±1.8%	56±2.3%	56±3.9%	52±2.9%	56±3.9%	63±3.6%	55±4.5%	57±0.3%

evidence that BRCA1 mutations regulate meiotic spindle assembly and check points, which may infer a link between BRCA1 deficiency and aneuploidy.<sup>3</sup>

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: Chart review of all BRCA1 and BRCA2 PGT-M cases referred by in vitro fertilization (IVF) clinics to a lab was completed. PGT-M and PGT-A were performed using SNP microarrays with informatics. A control group of maternal age-matched PGT-A patients was used for comparison. Statistical analysis was done using a two-tailed t-test.

RESULTS: Between Mar 2011 and Sept 2018 a total of 779 embryo biopsies were tested. The overall euploid rate was 56% and mutation positive rate was 50.6%. The euploid rate for controls was 57% (Table 1). A statistically significant difference in the euploid rate of maternal (mat) BRCA1 carriers compared to mat BRCA2 carriers (52% vs 63%; p-value = .02) was observed. There was no difference in the euploid rate between paternal (pat) BRCA1 carriers and pat BRCA2 carriers (56% vs 55%; p-value > .05). Comparison of the control group to all BRCA1/2 cases, to all mat carrier cases, and to all pat carrier cases showed no difference in euploid rates (p-values > .05).

CONCLUSIONS: Overall, we do not see a decrease in euploid rates among all BRCA1/2 carriers compared to age-matched controls. The statistically significant difference between the euploid rates of mat BRCA1 carriers and mat BRCA2 carriers supports the literature suggesting differing mechanisms and potential risk implications for BRCA1 and BRCA2. BRCA1 mutations in females may increase susceptibility to meiotic errors and aneuploidy; this trend was not observed for male BRCA1 carriers. Future studies with larger sample sizes are needed to further assess the aneuploidy risks associated with mat BRCA1 mutations.

References: <sup>1</sup>Oktay K, et al. Biol Reprod. 2015 Jul; 93(3):67, 1-10. <sup>2</sup>Titus S, et al. Sci Transl Med. 2013 Feb; 5 (172): 172ra21. <sup>3</sup>Xiong B, et al. Biol Reprod. 2008 Jul; 79, 718-726.

SUPPORT: Natera, Inc.

TABLE 1. Results

	TP53 (rs1625895) Genotypes			
	CC	TC	TT	P
n	62 (45.6%)	28 (20.6%)	46 (33.8%)	
Age (years)	33.3±2.8	33.3±7.4	33.9±3.0	0.46
AMH (ng/ml)	3.0±3.7 <sup>a</sup>	2.9±4.1	1.2±1.6 <sup>a</sup>	<sup>a</sup> 0.01
AFC (n)	17.8±12.1 <sup>a</sup>	15.8±10.1 <sup>b</sup>	9.4±6.2 <sup>a,b</sup>	<sup>a</sup> <0.0001; <sup>b</sup> 0.01
Total dose of rFSH (IU)	1840±1083 <sup>a</sup>	2036±1101 <sup>b</sup>	2642±1027 <sup>a,b</sup>	<sup>a</sup> <0.0001; <sup>b</sup> 0.007
Follicles (n):Total	15.5±9.9 <sup>a</sup>	13.6±10.6	9.1±5.5 <sup>a</sup>	<sup>a</sup> 0.005
Follicles (n):≥ 18 mm	4.2±2.7 <sup>a</sup>	4.1±2.8	3.2±1.9 <sup>a</sup>	<sup>a</sup> 0.02
Retrieved oocytes:Total	11.0±7.5 <sup>a</sup>	9.3±7.9	6.7±2.4 <sup>a</sup>	<sup>a</sup> 0.01
Retrieved oocytes:Metaphase II	8.2±6.1 <sup>a</sup>	6.8±5.9	4.9±2.6 <sup>a</sup>	<sup>a</sup> 0.01
Fertilization rate	66.5%	67.3%	69.7%	0.66
Implantation rate	36.8% <sup>a</sup>	32.8%	22.0% <sup>a</sup>	<sup>a</sup> 0.01
Pregnancy rate/transfer	50.7%	45.5%	35.3%	0.23
Pregnancy rate/patient	61.3% <sup>a</sup>	53.5%	39.1% <sup>a</sup>	<sup>a</sup> 0.03

Values within rows with the same superscript letter were significantly different.

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**THE TP53 GENE (rs1625895) C > T POLYMORPHISM IS ASSOCIATED WITH OVARIAN RESERVE AND OVARIAN RESPONSE TO RECOMBINANT FSH DURING IVF/ICSI TREATMENT.**



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OBJECTIVE: To investigate a possible association between a TP53 gene polymorphism and ovarian response after IVF/ICSI cycles.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: This study included 136 women submitted to IVF/ICSI cycles. The enrolled individuals met the following inclusion criteria: age ≤37years; normal karyotype; having two ovaries as evinced in ultrasound examination; no history of ovarian surgery, endometriosis, hydrosalpinx, infection, or endocrine disorders. DNA extracted from peripheral blood was sequenced on MiSeq(Illumina) to find single nucleotide polymorphisms (SNPs) in the TP53 gene. SNPs were identified using the TruSeq Custom Amplicon (TSCA) Panel (DesignStudio Illumina). The findings from sequencing were associated with age, anti-Müllerian hormone (AMH) levels, antral follicle counts (AFC), total dose of recombinant FSH (r-FSH), follicle size, number of retrieved oocytes, and clinical outcome of IVF/ICSI cycles.

RESULTS: The TP53 (rs1625895) C>T SNP were identified. Women with the TT genotype had significantly poorer ovarian reserve indicators (lower levels of AMH and AFC), poorer ovarian response to rFSH, and poorer clinical outcomes (implantation rate and clinical pregnancy rate/patient). Table 1 presents a summary of the results.

**CONCLUSIONS:** TP53 (rs1625895) C>T polymorphism was associated with ovarian reserve and apparently affected ovarian response to rFSH and the clinical outcomes of IVF/ICSI cycles. Homozygosity of the T allele was associated with significantly poorer results. The identified SNP might provide an additional tool to test patients for ovarian reserve/response and thus help in the individualization of ovarian stimulation protocols. To the best of our knowledge, this was the first study to associate this SNP and ovarian response to gonadotropins.

**SUPPORT:** Merck Grant for Fertility Innovation (GFI-2014).

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**NON-INVASIVE PRENATAL TESTING HAS ALTERED POSITIVE PREDICTIVE VALUE FOLLOWING TRANSFER OF A EUPLOID BLASTOCYST.**



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**OBJECTIVE:** The positive predictive value (PPV) of non-invasive prenatal testing (NIPT) has been reported to range from 91.3-97.2% in the general population (1,2). However, PPV is dependent upon the prevalence of the disease in the population being tested. Patients who undergo in vitro fertilization (IVF) with preimplantation genetic testing for aneuploidy (PGT-A) and transfer a euploid embryo are presumably a lower risk population when compared to the general population. The objective of this study is to explore the PPV for NIPT following transfer of a euploid blastocyst.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All patients at a single IVF center between 2014 and 2018 who pursued pre-implantation genetic testing for aneuploidy (PGT-A) and underwent transfer of a euploid blastocyst between 2014 and 2018 were contacted to request completion of a medical record release form authorizing release of antenatal records. Records were reviewed, and patients who had documentation of an abnormal NIPT were included in this study. Results of any subsequent prenatal or postnatal diagnostic testing were used to classify each positive NIPT as a “true positive” or a “false positive”. The PPV of NIPT was calculated.

**RESULTS:** A total of 1,202 patients eligible for inclusion were contacted for completion of the medical record release form. Five patients with abnormal NIPT following transfer of a euploid blastocyst were identified. Four of these patients (80%) had subsequent definitive prenatal diagnostic testing which revealed a euploid karyotype concordant with their PGT-A results. One patient, who had a PGT-A result indicating 46,XX but a NIPT positive for Turner syndrome, underwent amniocentesis which confirmed Turner mosaicism (45,X karyotype in 80% of cells). Therefore, the PPV of NIPT in this patient cohort was 20%.

**CONCLUSIONS:** The PPV of NIPT for patients undergoing transfer of a euploid blastocyst is lower than that for the general population. PGT-A may be more likely to yield inaccurate results in the presence of embryonic mosaicism, as illustrated by the true positive NIPT case in this study cohort. PGT-A is an imperfect screening tool and follow-up antenatal screening is advis-

able; however, clinicians and patients should recognize that patients undergoing transfer of a euploid blastocyst are at a relatively lower risk for fetal aneuploidy when compared to the general population and, as a result, the PPV of NIPT is altered in this setting.

**References:** 1. Yu et al. Overall evaluation of the clinical value of prenatal screening for fetal-free DNA in maternal blood. *Medicine* 2017 Jul; 96(27): e7114.

2. Strom et al. Improving the Positive Predictive Value of Non-Invasive Prenatal Screening (NIPS). *PLoS One.* 2017;12(3): e0167130.

**O-42** Monday, October 14, 2019 12:00 PM

**SONOGRAPHIC ABNORMALITIES IN PREGNANCIES CONCEIVED FOLLOWING IVF WITH AND WITHOUT PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY.**



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**OBJECTIVE:** Preimplantation genetic testing for aneuploidy (PGT-A) has been increasingly adopted in IVF clinics across the US. While PGT-A may improve pregnancy rates on a per transfer basis, data demonstrate that patients often hold misconceptions that use of PGT-A will result in a healthy baby. In an effort to improve patient counseling on the benefits and limitations of PGT-A we report the rates and specific types of anomalies detected on anatomy ultrasounds in women who underwent IVF with PGT-A compared to women who conceived following IVF with unscreened embryos.

**DESIGN:** Retrospective cohort at a maternal-fetal medicine referral practice.

**MATERIALS AND METHODS:** All patients with singleton pregnancies who had a mid-trimester anatomy ultrasound between January 1-December 31, 2018 at a single clinic were assessed for inclusion. The charts of patients who conceived with IVF with or without PGT-A were systematically examined. The primary outcome was the rate of anomalies detected on anatomy ultrasound. Nuchal translucency (NT), first trimester and/or serum integrated screening, non-invasive prenatal testing (NIPT), and invasive diagnostic testing results were also extracted as available. Statistical analysis was performed using the student t-test, chi-square, or fisher’s exact test where applicable.

**RESULTS:** Of 4,095 singleton pregnancies during the study period, 433 conceived with IVF, including 278 who had PGT-A and 155 who did not. Rate of low risk nuchal translucency or noninvasive prenatal testing did not differ between patients who did or did not undergo PGT-A. There was a low overall rate of abnormal first trimester and/or serum integrated screen, yet it occurred more commonly in those who had undergone PGT-A (7.6 vs 1.8% p=0.006). Abnormalities of fetal anatomy or placenta were found at similar rates between the two groups.

**CONCLUSIONS:** The rate of abnormal ultrasound findings did not differ in patients who conceived after IVF with PGT-A compared to those who underwent IVF without PGT-A, but there was an increased risk of abnormal analytes on serum screening. Patients should be counseled that standard prenatal screening and ultrasounds are recommended following IVF with PGT-A.

**SUPPORT:** None.

	PGT-A (n=278)	IVF/no PGT-A (n=155)	P-value
Age (mean±SD)	37.3±4.6	38.2±5.1	0.06 <sup>a</sup>
Low risk nuchal translucency (n, %)	187 (of 189), 98.9%	107 (of 107), 100%	0.54 <sup>c</sup>
First trimester screen normal (n, %)	181 (of 196), 92.4%	105 (of 107), 98.1%	0.006 <sup>c</sup>
Low risk noninvasive prenatal testing (n, %)	243 (of 244), 99.6%	126 (of 128), 98.4%	0.27 <sup>c</sup>
Invasive diagnostic testing normal (n, %)	22 (of 22), 100%	11 (of 12), 81.7%	0.35 <sup>c</sup>
Anatomy ultrasound normal (n, %)	230, 86.8%	128, 85.9%	0.80 <sup>b</sup>
Placenta normal by ultrasound (n, %)	191, 72.4%	107, 71.8%	0.91 <sup>b</sup>

<sup>a</sup>t-test.

<sup>b</sup>chi-square.

<sup>c</sup>fisher’s exact.

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### COMPARISON OF EUPLOID RATES VIA PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) AND SUBSEQUENT PREGNANCY OUTCOMES BETWEEN ASIAN AND WHITE



**PATIENTS.** David Huang, MD,<sup>a</sup> Eleni A. Greenwood, MD, MSc,<sup>a</sup> Phil Marsh, BS,<sup>a</sup> Andrew Runge, BS,<sup>a</sup> Marcelle I. Cedars, MD,<sup>b</sup> Mitchell P. Rosen, MD, HCLD<sup>a</sup> <sup>a</sup>University of California San Francisco, San Francisco, CA; <sup>b</sup>University of California San Francisco, Department of Obstetrics and Gynecology, San Francisco, CA.

**OBJECTIVE:** Prior observational studies have suggested Asian ethnicity as a risk factor for poor IVF outcomes. We sought to compare euploid rates by PGT-A between Asian and White patients and their pregnancy outcomes after euploid single embryo transfer (SET).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** We analyzed all day 5 and 6 blastocyst trophectoderm biopsy results via PGT-A from 2010-2019 at a single academic center, with the primary outcome being euploid rate. Euploid rate was determined by dividing the number of euploid blastocysts generated from a single egg retrieval cycle by the total number of blastocysts biopsied in the same cycle. Euploid rates were then compared based on self-reported race of the female partner, focusing on White versus Asian ethnicity. Generalized linear models were employed given clustered nature of the data and to control for oocyte age (STATA v14.2). We further compared pregnancy outcomes by race of the female partner after euploid SET in a subsequent frozen embryo transfer cycle, focusing on live birth or ongoing pregnancy as the outcome.

**RESULTS:** A total of 5,776 blastocyst PGT-A biopsies over 1,291 IVF cycles from 820 White and Asian female patients were identified. Of the blastocyst biopsies analyzed, 3,658 blastocysts were from White female patients and 2,118 blastocysts were from Asian female patients. Overall euploid rates did not vary significantly by female partner race: 43.9% in couples with a White female partner and 43.1% in those with an Asian female partner. After controlling for age of the oocyte, the odds of euploidy in couples with an Asian female partner compared to those with a White female partner were similar (OR 1.02, 95% CI 0.89, 1.17,  $p = 0.75$ ). We also observed no statistically significant differences in ongoing pregnancy or live birth rates between couples with an Asian female partner and those with a White female partner (57.8% vs 54.3%, respectively;  $p = 0.42$ ) following subsequent euploid SET.

**CONCLUSIONS:** We observed no significant differences in euploid rates via PGT-A by female partner race (Asian versus White). We also did not note significant differences in pregnancy outcomes between Asian and White female patients in the setting of frozen euploid SET. These findings suggest that the less successful IVF outcomes among Asians in prior observational studies may be attributed to mechanisms other than poor oocyte/embryo quality or inferior inherent endometrial receptivity.

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### INTERSECTION OF SEXUALLY TRANSMITTED INFECTIONS AND SUBSTANCE USE AMONG LOW-INCOME MINORITY WOMEN: ROUTINE CARE AS A CRITICAL POINT FOR REDUCING REPRODUCTIVE



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**OBJECTIVE:** The objective of this study to determine the relationship between clinically reported substance use on the prevalence of STIs among AYA women seeking routine well-adolescent and gynecological services in a STI prevalent community.

**BACKGROUND:** Previous data suggest that adolescent and young adult (AYA) women with sexually transmitted infections (STIs) report only one sexual partner and low condom use. While concurrency may be a key factor, the impact of substance use on effective sexual decision making around condom use may be critically important.

**MATERIALS AND METHODS:** This analysis utilizes AYA data from the Women's BioHealth Study (WBS), a large human subjects approved cohort study prospectively enrolling mostly African American low-income female patients 13-29 years during routine well AYA and gynecologic visits in which specimens were also collected for *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT) to assess for sexual risk and infection of *Mycoplasma genitalium* (MG) and *Trichomonas vaginalis* (TV). Participants provided demographic, clinical, sexual risk behavior, and biological specimens for *Trichomonas vaginalis* (TV) and *Mycoplasma genitalium* (MG) testing. Additionally, for this analysis, serial electronic medical records (EMR) from visits were reviewed to explore study STI outcomes associated with reported substance use behaviors during clinical visits for women  $\leq 25$  years, a defining point for AYA STI risk.

**RESULTS:** 443 patients with a mean age of 20.8 years (SD 2.7) were reviewed. Thirty-nine percent had a history of marijuana use, 43% had a history of alcohol use, and 3% had a history of substance use other than alcohol or marijuana. AYA  $< 21$  were 1.5 times more likely AYA  $\geq 21$  years to use marijuana (OR: 1.53, 95% CI 1.02 to 2.29,  $P=0.032$ ). Marijuana was a predictor of increased behavioral risk scores (0.53 average difference, 95% CI 0.26 to 0.80,  $P<0.001$ ) and of not always using a condom (OR: 0.54, 95% CI 0.32 to 0.92,  $P=0.024$ ). Participants with a history of marijuana use (OR: 1.68 95% CI 1.11 to 2.56,  $P=0.015$ ) or other substance use (OR: 3.81, 95% CI 1.33 to 10.95,  $P=0.013$ ) were more likely to test positive for an STI.

**CONCLUSIONS:** A history of marijuana or substance use other than alcohol put AYA patients at a greater risk of not using a condom and contracting an STI, which could negatively affect reproductive health. Strategic approaches to addressing substance use disorders alongside STI prevention efforts among AYA served by practices in low income minority, STI-prevalent communities are warranted.

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### ANTI-MÜLLERIAN HORMONE (AMH) TRAJECTORIES IN REPRODUCTIVE AGED AFRICAN-AMERICAN WOMEN: FINDINGS FROM THE STUDY OF OVARIAN AGING AND RESERVE



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**OBJECTIVE:** AMH, a member of the transforming-growth-factor- $\beta$  family, is produced by the granulosa cells of preantral and small antral follicles. It has been widely used as the preferred serum biomarker of ovarian reserve due to its relative stability over the menstrual cycle. Despite its increasing use, there have been very few studies that have followed AMH levels over time in young, healthy, non-Caucasian women. The objective of this study was to determine AMH trends in reproductive aged African American women (AAW).

**DESIGN:** Longitudinal study.

**MATERIALS AND METHODS:** SOAR leveraged an existing cohort of AAW who were recruited from the Detroit, MI community, as part of the Study of the Environment, Lifestyle and Fibroids (SELF). Anthropometric measurements, health information and blood samples were collected from each participant at four time points over a 5-year period. Serum AMH levels were measured using the ultrasensitive picoAMH assay (Ansh Labs, Webster, TX). Summary statistics were derived for the variables of interest, and linear mixed models for logAMH on age and age<sup>2</sup> with random slopes and intercepts were used to estimate trajectories of AMH levels (SAS 9.4 - Cary, NC).

**RESULTS:** A total of 1,692 women were included in the analysis. The majority of the participants completed all four study visits (66.5%). The mean duration between the first and last visit was  $59.0 \pm 3.6$  months. The median AMH values for the four visits were  $5.9 \pm 4.2$ ,  $4.6 \pm 3.9$ ,  $4.1 \pm 3.7$ , and  $3.9 \pm 3.7$  ng/mL, respectively. The mean ages at each study visit were  $29.2 \pm 3.4$ ,  $30.9 \pm 3.4$ ,  $32.5 \pm 3.4$ , and  $34.3 \pm 3.4$  years. At baseline, 59.7% of women were obese, 19.1% were current smokers, and 31% used hormonal contraception within 4 weeks of their visit. In models adjusted for BMI,

smoking status and current hormonal contraception use at baseline, the overall estimated AMH trajectory showed minimal decrease until age 25 years after which time the levels followed a relative linear decline, with accelerated rate after age 30. The correlation between random slopes and random intercepts was -0.93. The rates of AMH decline were similar among women (variance of random slopes: 0.0055) and AMH levels exhibited very little within-individual correlation (intraclass correlation coefficient: 0.17).

**CONCLUSIONS:** This is the first longitudinal study of AMH trajectories over a substantial period of time in reproductive aged AAW, a group that is largely underrepresented in the ovarian reserve literature. In this population, AMH decline with age seems to follow a common pattern with women with higher AMH levels at baseline exhibiting slightly slower rate of decline than women with lower than average initial levels. As reproductive lifespan and outcomes seem to be influenced by race, a better understanding of AMH trajectories and the role of potential modifiable factors on AMH decline in women of different backgrounds will improve physician counseling and empower women to make well-informed reproductive choices.

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**PERSISTENT WIDENING IN RACIAL DISPARITIES BETWEEN BLACK AND WHITE WOMEN UNDERGOING ART OVER THE LAST 10 YEARS.**



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**OBJECTIVE:** To determine if the trends in disparities of outcomes between black, non-Hispanic (BNH) and white women undergoing ART over the last 10 years have changed and to identify possible contributing factors that may have influenced such change.

**DESIGN:** Retrospective, cohort study and comparison of reported outcomes in the SARTCORS database for 2014-2016 with those previously reported in 2004-2006.

**MATERIALS AND METHODS:** Analysis of 2014-2016 SARTCORS for member clinics that performed at least 50 cycles of ART and reported race in more than 95% of cycles. 125,555 cycles using autologous, fresh, non-donor embryo cycles were analyzed of which 16,551 cycles were from BNH women and 109,004 cycles were from white women. Findings from this analysis were compared with previously analyzed cycles reported for 2004-2006 (Fertil Steril 93:626-35, 2010).

**RESULTS:** Reporting of race of 60% of cycles was essentially unchanged over the 10 year period. The proportion of cycles from BNH women increased nominally over the same period. When comparing 2014-16 to 2004-06, a greater proportion of BNH cycles were from older ages ( $\geq 38$ ) and cycles with diminished ovarian reserve (DOR) compared to cycles from white women ( $p < 0.001$ ). The number of cycles with BMI  $\geq 30$  kg/m<sup>2</sup> was greater in cycles from black versus white women ( $p < 0.001$ ). Similar to 2004-06 data, cycles from BNH women were 3 times more likely to be associated with tubal factor and/or uterine factor and SAB rates continued to be greater ( $p < 0.001$ ) compared to cycles from white women. The proportion of live birth (LB) per cycle start remained less for cycles for BNH women compared to white women ( $p < 0.001$ ), as was observed from 2004-06. Race was an independent predictor of LB. Multivariate logistic regression demonstrated that cycles from black women were less likely to have a LB than white women for their initial cycle (OR 0.69;  $p < 0.001$ ). These findings were independent of age, parity, BMI, etiology of infertility, use of ICSI or number of embryos transferred. While similar proportions of black and white cycles were noted in mandated states there was a significant percentage of black cycles less represented among non-mandated states compared to cycles from white women ( $p < 0.001$ ).

**CONCLUSIONS:** While the proportion of cycles from BNH women using ART has incrementally increased, significant disparities have increased compared to white women over the last 10 years. This may be in part due to the increasing proportion of older age BNH women accompanied by DOR, increased BMI and greater SAB rates concomitant with a persistence of tubal and uterine factor. Access to state non-mandated insurance may have a significant impact upon relevant clinical differences between cycles from BNH and white women. Race has continued to be an independent prognostic factor for LB from ART over time. Further analysis of these persistent trends

over time is necessary to address racial disparities in access and outcomes to ART treatment for infertility. Such insight could lead to strategic approaches that could potentially narrow the racial disparity gap and eventually be evaluated for their effectiveness.

**O-47** Monday, October 14, 2019 11:45 AM

**SOUTH ASIAN WOMEN HAVE POORER IVF OUTCOMES DESPITE BEING YOUNGER AND HAVING BETTER OVARIAN RESERVE COMPARED TO CAUCASIANS.**



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**OBJECTIVE:** IVF outcomes in ethnic minorities have been reported previously to be worse compared to Caucasians (C), including those of South Asian (SA) descent (India, Pakistan, Bangladesh, Nepal). Little has been published on ovarian stimulation parameters in SA as compared to C.

**DESIGN:** Retrospective.

**MATERIALS AND METHODS:** A total of 557 cycles were reviewed (176 in SA and 401 in C). Markers of ovarian reserve (AMH, AFC, FSH, E) and cycle outcomes were compared between the two groups. The clinical outcome of those who had a fresh embryo transfer were also compared.

**RESULTS:** SA women were significantly younger (34.3 vs 35.7 yrs,  $P < 0.001$ ), had lower basal E<sub>2</sub> (37.8 vs 42.1 pg/ml,  $P = 0.042$ ), higher AMH (3.54 vs 2.84 ng/ml,  $P = 0.018$ ), lower basal Vit D (31.9 vs 36.6 ng/ml,  $P = 0.001$ ), required less gonadotropins (3374.6 vs 3567 IU,  $P = 0.045$ ), had lower peak Vit D (40.8 vs 45.4,  $P = 0.008$ ), and had lower total blastocyst number (2.64 vs 3.1,  $P = 0.036$ ). For those who had a fresh ET (43 SA and 75 C), the live birth rate was lower in SA (52.7% vs 67.4%,  $P = 0.014$ ). For those undergoing PGT-A, there was a lower incidence of euploid embryos in C (150 cycles in C and 87 in SA) (45.6% in SA vs 35.6% in C,  $P = 0.042$ ).

	SA (N=176)	C (N=401)	P-value
Age	34.3 ± 4.2	35.7 ± 4.1	< 0.0001
AMH	3.5 ± 6.50	2.84 ± 2.91	0.018
AFC	15.1 ± 9.7	14.2 ± 8.8	0.29
Basal E <sub>2</sub>	37.8 ± 21.5	42.1 ± 23.9	0.042
Basal Vit D	31.9 ± 14.03	36.6 ± 13.13	< 0.0001
Gonadotropins (IU)	3375 ± 1068	3567 ± 1054	0.045
Peak Vit D	40.8 ± 14.3	45.4 ± 13.5	0.008
#Blastocysts	2.64 ± 2.2	3.1 ± 2.5	0.036

**CONCLUSIONS:** Despite being significantly younger and having better ovarian reserve, SA women had significant differences in stimulation parameters. For those who had a fresh ET, SA women had a significantly lower live birth rate compared to Caucasians.

**References:** 1.Racial Differences in ART Outcome between White and South Asian Women. Sharara FI, Fouany MR, Sharara YF, Abdo GA. MEFS Journal 2012.

2.Shahine LK, Lamb JD, Lathi RB, Milki AA, Langen E, Westphal LM. Poor prognosis with in vitro fertilization in Indian women compared to Caucasian women despite similar embryo quality. PloS one 2009.

**SUPPORT:** None.

**O-48** Monday, October 14, 2019 12:00 PM

**REGIONAL DISPARITIES IN ASSISTED REPRODUCTIVE TECHNOLOGY ACCESS TO CARE: EMPLOYING MODERN TECHNOLOGY TO CLOSE THE GAP.**



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**OBJECTIVE:** Today, significant disparities still exist for access to assisted reproductive technology (ART) treatments in the United States. Only 60% of women who require ART are able to proceed with treatment. One of the major obstacles for access is the scarcity of fertility specialists in some regions of the US. Furthermore, the physical and financial burden associated with

time off from work and travel within or out of state, creates additional barriers. Telehealth is a well-established tool that alleviates these burdens. While other areas of medicine have welcomed this technology, reproductive medicine has yet to utilize it to its full potential. We implemented a regional telehealth program to close the gap in ART access in the rural Southeastern US. Our aim is to evaluate our telehealth program's ART outcomes and patient satisfaction of those living remotely.

**DESIGN:** Retrospective cohort and cross-sectional survey study.

**MATERIALS AND METHODS:** Patients who utilized the telehealth application for ART services at Augusta University (AU) between September 2015 to November 2018 were identified. The study was approved by AU IRB. Demographic variables were collected using the electronic medical record including age, type of ART cycles, travel distance, number of visits, and treatment outcomes. Patients were electronically mailed a validated questionnaire created via the qualtrics<sup>SM</sup> application. The survey included a patient satisfaction questionnaire as well as travel distance, number of visits, and ART treatment outcome. Data analysis was performed with descriptive statistics methods.

**RESULTS:** A total of 58 patients were identified of which 53% were < 35 years old (y/o), 16% were 35-37 y/o and 31% >38 y/o. 78% of patients had autologous fresh in vitro fertilization (IVF) cycles, 16% frozen embryo transfer, 3% donor oocytes, 1.4% embryo adoption and 1.4% gestational carrier. The overall clinical pregnancy rate was 60.3% (77% <35 y/o and 37% >35 y/o) with an overall live birth rate of 38% (48% <35 y/o and 22% >35 y/o). The cohort's mean number of visits was 2.93 (+/- 0.82). The survey response rate was 27/58 (46%). 56% of responders were <35 y/o and 44% >35 y/o. The mean number of visits for responders was 3 (+/- 0.99) and mean travel distance 171.4 miles (+/- 0.67). All responders underwent transvaginal oocyte retrieval and embryo transfer. For surrogates, the clinical pregnancy rate was 19/27 (70.3%) with a live birth rate of 16/27 (59.3%). 93% of patients reported being highly satisfied with the telehealth service to enhance access to ART. All responders stated they would recommend telehealth use for ART to others.

**CONCLUSIONS:** Our study demonstrates that employing modern telehealth applications improves access to ART care in underserved areas. Fewer office visits maintains high patient satisfaction due to accessibility and cost reduction associated with travel and time off work. Reproductive health providers may consider utilizing telehealth in delivering ART treatment.

**SUPPORT:** None.

## INFERTILITY AND CANCER

**O-49** Monday, October 14, 2019 10:45 AM

### PREGNANCIES IN CANCER SURVIVORS: OVARIAN RESERVE IS A POOR PROGNOSTICATOR.

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**OBJECTIVE:** While cancer therapies negatively impact ovarian reserve, few studies have examined factors related to clinical pregnancy rates in survivors. The objective of this study was to prospectively assess pregnancy rate, time to pregnancy, and corresponding measures of ovarian reserve in a cancer survivor cohort compared to similar-aged controls to determine factors associated with pregnancy in cancer survivors.

**DESIGN:** This is a prospective cohort study of cancer survivors aged 15-39 years who underwent chemotherapy and were ≥1 year post-treatment with no evidence of disease. Comparative controls were post-menarchal females with regular menses (21-35 days). Only survivors and controls at risk for pregnancy, defined by reporting intercourse with a man during the study period, were included in this analysis.

**MATERIALS AND METHODS:** Participants completed annual study visits with reproductive/contraceptive questionnaires, early follicular phase hormones, and ultrasounds. Demographic characteristics and log-transformed measures of ovarian reserve were compared using Pearson  $\chi^2$  analyses, *t*-tests, and multivariable regression models. The risk of pregnancy during study follow-up in survivors and controls was compared using

Kaplan–Meier curves. Post hoc analyses indicated 80% power to observe a 46% decrease in hazard rates of pregnancy for survivors compared to controls.

**RESULTS:** 96 survivors and 79 controls were followed for a mean of 4.7 years. There was no difference in age, BMI, or duration of follow-up between groups. At enrollment, 18 survivors and 17 controls reported a pregnancy conceived prior to study; 12 of the 18 pre-study pregnancies in survivors were conceived after cancer treatment. A similar proportion of survivors and controls reported additional pregnancies 'captured' during the prospective follow-up time (47/96 [49.0%] survivors; 47/79 [59.5%] controls). There was no difference in the survival distributions for the two groups ( $p=0.27$ ). Four survivors conceived with SO/IUI and seven with IVF. Survivors who conceived were older, more likely to be married or cohabitating, but received similar cyclophosphamide equivalent doses of chemotherapy compared to survivors who did not conceive. Anti-Müllerian hormone measured prior to pregnancy in survivors who conceived was lower than in controls who conceived (1561 vs. 2486 pg/mL,  $p=0.02$ ), but similar to survivors who did not conceive ( $p=0.26$ ). Importantly, half of the captured pregnancies in both groups were unplanned (52% in survivors vs. 48% in controls,  $p=0.9$ ). Among planned pregnancies, survivors reported an average of 16 months (median 9 months) to conceive compared to 11 months (median 1 month) for controls ( $p=0.16$ ).

**CONCLUSIONS:** Pregnancy rate and time to pregnancy was similar in cancer survivors compared to controls despite diminished measures of ovarian reserve. These findings suggest that predictions about the fertility potential of cancer survivors cannot be made on the basis of measures of ovarian reserve alone.

**O-50** Monday, October 14, 2019 11:00 AM

### THE POTENTIAL IMPACT OF NEWER CHEMOTHERAPY REGIMENS ON FUTURE FERTILITY IN MEN AND WOMEN TREATED FOR LYMPHOMA.

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**OBJECTIVE:** The treatment of lymphoma is rapidly advancing to include more non-ABVD-based chemotherapy regimens. The fertility risks for men and women who receive non-ABVD regimens like BEACOPP are poorly understood.

**DESIGN:** Systematic review and meta-analysis.

**MATERIALS AND METHODS:** We searched the MEDLINE, PUBMED, and COCHRANE databases, online trial registries and conference proceedings for published manuscripts and abstracts from 1980 to April 2019. Studies were deemed eligible for meta-analysis if they included reproductive-age women or men with lymphomas (Hodgkin's and non-Hodgkin's) and the following were reported: chemotherapy regimen, patient age, duration from chemotherapy to ovarian reserve assessment (Anti-Müllerian Hormone (AMH)) or semen analysis, and rate of either severe oligo- or azoospermia (men). Estimates were pooled using random-effects meta-analysis comparing AMH levels in women, and rates of normospermia in men, with ABVD versus non-ABVD treatment. For the purpose of meta-analysis, normospermia was defined by a lack of either severe oligo- or azoospermia.

**RESULTS:** Data were extracted from 4 studies involving 440 women and from an additional 7 studies involving 400 men. The range of numbers of women and men included in each of the studies was between 30 to 263 and 19 to 141, respectively. The majority of the cancer diagnoses in all 11 studies were Hodgkin's lymphomas. Three studies had follow-up AMH levels 36 months after completion of cancer treatment; one study measured AMH levels 18 months after treatment. Post-treatment AMH levels (pmol/L) were higher when comparing women who underwent ABVD versus non-ABVD, however this difference did not reach statistical significance (13.3 [95% CI: -1.3 - 30] versus 3.5 95% [CI: -1.8-8.8],  $p = 0.22$ ). Duration of follow-up for post-treatment semen analyses ranged from one to seven years after completion of treatment. There was a significant difference in the rate of post-treatment normospermia among men who underwent ABVD regimen 89% [95% CI 70 - 96%] versus non-ABVD regimen 28.4% [95% CI 15 - 47.0],  $p < 0.001$ ).

**CONCLUSIONS:** As lymphoma treatment evolves, fertility preservation physicians need to be aware that lymphomas may increasingly be treated with chemotherapy regimens that appear to have a more negative impact on future fertility in men and may likely impact it in women as well, though more data are needed.

**O-51** Monday, October 14, 2019 11:15 AM

**CANCER TREATMENT IS ASSOCIATED WITH A MEASURABLE DECREASE IN LIVE BIRTHS IN A LARGE, POPULATION-BASED STUDY.**

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**OBJECTIVE:** Research on the impact of cancer treatment on fertility has evolved over time. Initially, studies tracked rates of amenorrhea and, more recently, rates of conception after cancer treatment. The aim of the present study is to define rates of live birth in a large, population-based study of the most common reproductive-age cancers in women.

**DESIGN:** Retrospective cohort study

**MATERIALS AND METHODS:** We performed a retrospective cohort study using the Utah Population Data Base (UPDB) relating first time cancer diagnosed between 1966 and 2014 to subsequent pregnancy in women in Utah aged 18-45 years. UPDB is a comprehensive source of birth, medical and cancer records of the Utah state population. Women from the study group who had live births after cancer diagnosis (n= 17,960) were compared with age matched controls at the time of cancer diagnosis (n= 89,436) and healthy sisters who had never been diagnosed with cancer (n= 15,099). Age-matched controls were the same age as cancer survivors in the year of cancer diagnosis. Both groups were followed from the year of diagnosis until 2014 and pregnancies achieved during this time recorded. We used conditional Poisson regression models, adjusted for birth year, BMI, and ethnicity, to estimate the association between history of cancer and subsequent live birth.

**RESULTS:** Based on Poisson regression modeling, the total number of live births was 15% lower among cancer survivors compared to healthy sisters (p<0.001). When compared to age-matched healthy controls from the general population, cancer survivors had 25% fewer live births (p<0.01). When compared with their healthy sisters, the reduction in live birth rate was 15% for all cancer types, 16% for breast cancer, 17% for central nervous system cancers, and 36% for soft tissue cancers (p<0.001). 3% of cancer survivors who had a live birth utilized fertility treatment, compared to 2% (p=0.13) of healthy controls who achieved live births. In addition, there were more stillbirths among cancer survivors when compared with their healthy sisters (14 per 1000 births versus 11 per 1000 births, p<0.01).

**CONCLUSIONS:** In this large, population-based study in the Western United States, cancer and its treatment were associated with lower live birth rates when comparing women with cancer versus age-matched controls and healthy siblings. Live birth as a metric may reflect not only decreased fertility, but also an increase in adverse pregnancy outcomes such as stillbirth.

**O-52** Monday, October 14, 2019 11:30 AM

**PREGNANCY OUTCOMES AMONG CANCER SURVIVORS: A POPULATION-BASED ANALYSIS.**

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**OBJECTIVE:** The growing field of onco-fertility has brought necessary attention to improving patients' ability to achieve a pregnancy after cancer treatment. However, relatively little is known about frequency of healthy births, particularly with regard to preterm birth, pre-eclampsia, and low birth-weight, among survivors of cancers diagnosed during the young adult years.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The Utah Population Data Base (UPDB) was used to identify female cancer survivors (ages 18-45) who were diagnosed from 1966 to February 2014. The UPDB is a comprehensive source of birth, medical and cancer records of the Utah state population. We identi-

fied pregnancy outcomes of female cancer survivors (n= 17,960) and compared these with age matched healthy women without a cancer diagnosis who were randomly included based on birth certificates (n= 89,436). Cases were matched to controls who were the same age during the age of the cases during the year of their cancer diagnosis. Cases and age-matched controls were then followed from the year of diagnosis until 2014 and pregnancies achieved during this time were recorded. Live birth rates, Apgar scores after delivery, pre-term delivery, low birth weight (defined as birth weight between 1500-2500 grams), prevalence of pre-eclampsia, and children with congenital malformations were determined. Descriptive statistics and chi-square tests were used, were appropriate.

**RESULTS:** Overall, 3128 births to cancer survivors and 19,405 births to healthy controls were included. In comparison to the control group, cancer survivors had significantly lower live birth rates (18% reduction, p<0.001), an increased rate of preterm delivery (17% vs 13%, P< 0.001), and a higher risk of a child with low birth weight (11% vs 8%, p<0.001). The higher prevalence of these outcomes was mostly due to cancer related chemotherapy and radiotherapy. The number of women with pre-eclampsia, children with congenital malformations, and Apgar score (<7) did not differ significantly between groups.

**CONCLUSIONS:** Currently, a significant focus in onco-fertility is on achieving live birth after cancer treatment. A better understanding of how to achieve a healthy pregnancy after cancer is needed. We find that female cancer survivors have a lower live birth rate and higher risk of pregnancy related complications, including preterm delivery and low birth rate than women without a history of cancer. Whether poorer outcomes reflect gamete, endometrial, and/or uterine mechanisms remain to be determined and may shed light on how to ensure healthier reproductive outcomes.

**O-53** Monday, October 14, 2019 11:45 AM

**STIMULATION OF THE OVARIES IN WOMEN WITH BREAST CANCER UNDERGOING FERTILITY PRESERVATION: ALTERNATIVE VERSUS STANDARD STIMULATION PROTOCOLS.**

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**OBJECTIVE:** to evaluate the effectiveness of ovarian stimulation with tamoxifen or letrozole compared to standard ovarian stimulation on the number of oocytes retrieved in women with breast cancer in the course of fertility preservation.

**DESIGN:** Multi-center randomized open-label trial in the Netherlands and Belgium.

**MATERIALS AND METHODS:** Women between 18 and 43 years with breast cancer who opted for banking of oocytes or embryos in the course of fertility preservation were included. We randomly assigned them to one of the three study groups; group 1 ovarian stimulation plus tamoxifen (60 mg per day), group 2 ovarian stimulation plus letrozole (5 mg per day) or group 3 standard ovarian stimulation without additional medication. Primary outcome was the number of oocytes retrieved at follicle aspiration. Secondary outcomes were number of mature oocytes retrieved, number of oocytes or embryos banked and peak E2 levels during ovarian stimulation.

**RESULTS:** Between January 2014 and December 2018, we randomised 162 women with breast cancer. We analysed the primary outcome for 148 (91%) women of which 142 women (88%) underwent ovum pick up. Mean age of the women was 32 years. 51 women underwent ovarian stimulation plus tamoxifen, 51 plus letrozole and 46 standard ovarian stimulation without additional medication. The mean number of oocytes retrieved at follicle aspiration was 12.6 (group 1), versus 14.2 (group 2) versus 13.3 (group 3) (mean difference in number of oocytes: group 1 vs. group 3 -0.675 ;95% CI, -5.6 to 4.3; group 2 vs. group 3 0.871; 95% CI, -4.1 to 5.9). The mean number of oocytes banked was 10.4 (group 1) versus 10.5 (group 2) versus 10.2 (group 3) (mean difference in number of oocytes banked: group 1 vs.

Live birth after first cancer diagnosis	18-25 (N=2537)	26-30 (N=2870)	31-35 (N=3413)	36-40 (N=4162)	>40 (N=4162)	P value
0	1218 (48.0%)	1822 (64%)	2835 (83%)	3995 (96%)	4962 (99.7%)	<0.001
1	545 (22%)	595 (21%)	406 (12%)	144 (4%)	14 (0.3%)	
2	420 (17%)	308 (11%)	142 (4%)	22 (0.5%)	1 (0%)	
≥ 3	354 (14%)	145 (5%)	30 (1%)	1 (0%)	1 (0%)	

group 3 0.243; 95% CI, -4.2 to 4.7; group 2 vs. group 3 0.297; 95% CI, -4.2 to 4.8). Mean number of embryos banked was 5.9 (group 1) versus 4.9 (group 2) versus 5.0 (group 3) (mean difference in number of embryos banked; group 1 vs. group 3 0.857; 95% CI, -3.0 to 4.7; group 2 vs. group 3 -1.33; 95% CI, -4.0 to 3.7).

**CONCLUSIONS:** These results show that the addition of tamoxifen or letrozole to standard ovarian stimulation did not effect the number of oocytes or embryos banked in the course of fertility preservation for women with breast cancer. Whether the addition of tamoxifen or letrozole to standard ovarian stimulation affects the long-term follow up in terms of safety in women with breast cancer, remains to be seen.

**SUPPORT:** The STIM trial was funded by the Pink Ribbon foundation.

**O-54** Monday, October 14, 2019 12:00 PM

**DESCRIBING LIVE BIRTHS AFTER CANCER TREATMENTS: WHEN DO PATIENTS CONCEIVE AND HOW MANY CHILDREN DO THEY HAVE? A POPULATION-BASED STUDY IN THE WESTERN UNITED STATES.**



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**OBJECTIVE:** Oncologists typically advise women to wait for two to five years after cancer treatment before trying to conceive. Age-related fertility concerns can be increased by both this period of waiting and the acceleration of ovarian follicle loss during and after cancer treatment. Little is known about the impact of age on how long it takes women to complete their family building after cancer treatment.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The Utah Population Data Base (UPDB) was used to identify female cancer survivors in Utah state with first time cancer diagnosed between 1966 to 2014. We identified first and last live births of cancer survivors (n= 17,960) in various age groups and reported these relative to the timing of their cancer diagnosis. Descriptive statistics and chi-square testing were used where appropriate.

**RESULTS:** Our population included 17,960 women with first cancer diagnosis at age 18-45 years. These age groups were split into 18-25,

26-30, 31-35, 36-40, > 40 years old with the fraction of patients included in each group as follows: 18-25=14%, 26-30=16%, 31-35=19%, 36-40=23%, >40= 28%. The most common cancer types among the cohort were breast cancer in 23%, gynecologic cancers in 29%, lymphomas in 4%, and leukemia in 2%. A total of 36% of women had no children at the time of their cancer diagnosis. Nulliparity at the time of diagnosis was more common in the 18-25-year-old age group (62%). Approximately 17% of women had children after their diagnosis of cancer and they tended to have children approximately 2-3 years after cancer diagnosis. Women in the 18-25 age group tended to have their first post treatment child further from diagnosis than women who were > 40. Also, women 18-25 years old tend to have their last child 7 years after their cancer diagnosis, whereas women >40 tend to have their last children approximately 2 years after cancer diagnosis. Number of live births after cancer diagnosis was also higher among younger women, as reflected in the table below.

**CONCLUSIONS:** For both oncologists and infertility specialists, it is important to understand the timeline of when women with a history of cancer tend to build their families, and to incorporate this information into counseling about treatment-related infertility risk. Since the choice of when to build a family is highly personal and may vary across regions, more time-to-pregnancy data from other populations should also be collected.

**MALE REPRODUCTION AND UROLOGY: TRAVELING SCHOLARS**

**O-55** Monday, October 14, 2019 10:45 AM

**EVALUATION OF FERTILITY PRESERVATION COUNSELING AND REFERRALS IN US CLINICAL PRACTICES: REVIEW OF ASCO'S QUALITY ONCOLOGY PRACTICE INITIATIVE (QOPI).**



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Men				Female			
Age (years)	Ratio	Percentage	p-value	Age (years)	Ratio	Percentage	p-value
18-21	24/35	68.6%	<0.001*	18-21	19/28	67.9%	<0.001*
22-25	42/64	65.6%		22-25	86/116	74.1%	
26-30	88/167	52.7%		26-30	259/383	67.6%	
31-35	115/274	42.0%		31-35	879/532	60.5%	
36-40	145/410	35.4%		36-40	644/1425	45.2%	
41-45	186/693	26.8%					
46-50	342/1412	24.2%					
Cancer Type	Ratio	Percentage	p-value	Cancer Type	Ratio	Percentage	p-value
Breast	1031/1799	57.3%	<0.05**	GI	462/1686	27.4%	<0.05**
GI	171/394	43.4%		Thoracic	124/423	29.3%	
Hematopoietic	69/100	69.0%	<0.05**	Hematopoietic	144/263	54.8%	
GU	27/70	38.6%		Other	17/63	27.0%	
Thoracic	14/32	43.8%		GU	14/48	29.2%	
Other	8/22	36.4%		Bone/Skin	11/43	25.6%	

\* P-value Chi-Squared for Trend.

\*\* P-value Chi-Squared.

**OBJECTIVE:** Discussions about fertility preservation are essential in reproductive-aged patients with newly diagnosed cancer. Our objective was to identify factors affecting discussion of fertility risks in reproductive-aged patients prior to initiating chemotherapy.

**DESIGN:** The American Society of Clinical Oncology (ASCO) Quality Oncology Practice Initiative (QOPI) is an oncologist-led quality assessment program that surveys 994 oncology practices on a yearly basis.

**MATERIALS AND METHODS:** Each practice in the ASCO QOPI submitted individual patient data from 2015 to 2018. Patients of reproductive age were females 18-40 years and males 18-50 years. Primary outcome was whether fertility risks were discussed prior to chemotherapy. Multivariate logistic regression was performed to identify predictors of fertility preservation counseling, controlling for the below variables.

**RESULTS:** Of 5,887 reproductive age patients, 42.1% discussed the risk of infertility associated with chemotherapy. Females were more likely to be counseled about the risk of infertility (1540/2831, 54.4%) compared with men (942/3055, 30.8%;  $p < 0.001$ ). Type of cancer and other variables appear in Table 1. In regression to assess whether fertility risks associated with chemotherapy were discussed, male sex (OR 0.73; CI:0.60-0.88) and increasing age (0.93; 0.92-0.94) reduced the likelihood of discussion while breast cancer (1.44; 1.19-1.75), hematopoietic cancers (1.55; 1.17-2.05), and receiving care in an academic clinic (1.45; 1.05-2.01) predicted higher rates. States with legislatively-mandated coverage of fertility preservation had significantly higher rates of fertility risk discussion (48.6% vs 39.6%,  $p < 0.001$ ).

**CONCLUSIONS:** Providers are more likely to counsel younger patients and female patients. State laws improve frequency of discussing fertility risk; further research is needed to identify factors that optimize fertility counseling prior to chemotherapy.

Reference: None.

**SUPPORT:** Department of Urology, University of Miami.

O-56 Monday, October 14, 2019 11:00 AM

#### LOWER TOTAL MOTILE COUNT IS ASSOCIATED WITH SMALLER HISTORIC INTERGENERATIONAL FAMILY SIZE: A PEDIGREE ANALYSIS FROM THE UTAH POPULATION DATABASE



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**OBJECTIVE:** Genetic heritability of male factor infertility may contribute to intergenerational variations in family size. We sought to assess the correlation of total motile count and intergenerational family size within the Utah Population Database (UPDB).

**DESIGN:** This is a retrospective, population-based, cohort analysis of men with at least a single measure of total motile count (TMC) within the UPDB and with complete pedigree data.

**MATERIALS AND METHODS:** These men must have at least one generation within their pedigree born in and prior to 1935 for inclusion to reduce the effect of contraception on the results. We identified the average number of generations for each individual overall, as well as the average number of generations and offspring within each generation occurring in and prior to 1935. Linear logistic regression models with clustered sample design were used to assess the relationship between TMC within 5<sup>th</sup> and 25<sup>th</sup> percentile and intergenerational family size in and prior to 1935. Additionally, generalized estimating equations with independence correlation structure and clustered sample design were created to estimate the change in TMC per increase in number of offspring among proband ancestors.

**RESULTS:** We identified 2,182 men with a measure of TMC within the UPDB and complete pedigree information. 541 men (24.8%) were within the 25<sup>th</sup> percentile for TMC while 112 men (5.1%) were within the 5<sup>th</sup> percentile for TMC (including azoospermic men). The average number of generations within each individual's pedigree was 4.2 (SD: 1.1). The average number of generations and offspring within each generation occurring prior to 1935 were 3.6 (SD: 1.0) and 6.5 (SD: 1.6), respectively. We found no significant association between intergenerational size and TMC within the 5<sup>th</sup> percentile (including azoospermic men) (RR = 0.97, 95% CI 0.93-1.01,  $p = 0.18$ ) or the 25<sup>th</sup> percentile (RR = 1.00, 95% CI 0.97-1.03,  $p = 0.98$ ). When TMC was analyzed as a continuous variable, generalized estimating equations suggest that lower TMC is related to smaller intergenerational fam-

ily size. For every additional child in their historical pedigree back to 9 generations, we saw an increase in TMC of 1.88 million ( $p = 0.031$ ).

**CONCLUSIONS:** This is one of the first studies examining the relationship between intergenerational family size and TMC as a marker of male factor infertility. We found a significant association between TMC as markers of male factor infertility and family size, suggesting that lower TMC is related to smaller intergenerational family size. This hypothesis generating data questions an effect of genetic heritability and male factor infertility on intergenerational family size.

O-57 Monday, October 14, 2019 11:15 AM

#### DECISIONAL CONFLICT AND KNOWLEDGE AMONG PATIENTS WITH VARICOCELE SEEKING TREATMENT FOR INFERTILITY.



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**OBJECTIVE:** To measure disease-specific knowledge and decisional conflict in men with varicoceles being counseled for infertility, and to gain insight into decision-making in male versus female-centric treatments for infertility.

**DESIGN:** This was a cross-sectional, observational, survey-based study of patients with clinical varicoceles and infertility.

**MATERIALS AND METHODS:** 84 patients were identified prior to their initial infertility consultation with a fellowship-trained male reproductive surgeon at the University of California, Los Angeles. Following consultation, patients completed a survey instrument measuring disease-specific knowledge, decisional conflict, satisfaction with care, and impression that shared decision-making occurred at the time of consultation. This instrument also queried patients' preferred infertility treatment modality both before and after consultation. Treatment-associated decisional conflict was measured with the validated SURE metric. Patient characteristics and survey responses were compared between those without decisional conflict (SURE score of 4) and those with some degree of decisional conflict (SURE score of 1-3) using Chi-squared (Fisher's exact if needed) and Wilcoxon rank-sum tests.

**RESULTS:** Mean age (SD) of patients and their partners were 36.3 (6.1) years and 34.4 (5.3) years, respectively. 66% of varicoceles were grade 2 or greater. The mean knowledge score was 57%. 45% of patients reported no decisional conflict. Compared to those with decisional conflict, men without decisional conflict scored higher on the infertility knowledge assessment (63% vs 51% correct), were more likely to feel included in the treatment decision (100% vs 83%), and were more likely to feel that they discussed treatment options with their physician in detail (100% vs 82%) (all  $p < 0.01$ ). Prior to consultation, 27% of all patients preferred assisted reproductive technologies (i.e., IVF, IUI) and 2% preferred varicocelectomy as the primary treatment for infertility. Following consultation, 14% and 17% preferred assisted reproductive technologies and varicocelectomy, respectively. The increase in treatment preference for varicocelectomy was greater in men with no decisional conflict (5% to 29%) than those with decisional conflict (0% to 7%) ( $p = 0.03$ ). There was a concomitant decrease in preference for assisted reproductive technologies following consultation (34% to 18% vs 22% to 9%).

**CONCLUSIONS:** Patient knowledge on the etiology and treatment of male infertility is insufficient and associated with decisional conflict. Prior to consultation, men with varicoceles showed preference for assisted reproductive technology over varicocele surgery; this trend reversed after consultation. Men with decisional conflict were much less likely to prefer varicocelectomy, even after consultation. An intervention that improves infertility knowledge may reduce decisional conflict and optimize shared decision-making in the treatment of infertility for men with varicoceles.

O-58 Monday, October 14, 2019 11:30 AM

#### SPERM EXTRACTED FROM MEN WITH OBSTRUCTIVE AZOSPERMIA VIA MINIMALLY-INVASIVE EPIDIDYMAL SPERM ASPIRATION (MIESA) RESULTS IN NON-INFERIOR IVF OUTCOMES COMPARED WITH NORMAL EJACULATED SEMEN IN COUPLES WITH UNEXPLAINED INFERTILITY.



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**OBJECTIVE:** Surgically extracted sperm is generally expected to have inferior IVF outcomes compared to ejaculated sperm. We sought to evaluate sperm quality and IVF outcomes of cryopreserved epididymal sperm samples obtained from patients with obstructive azoospermia (OA) via office-based MIESA. We report sample characteristics and compare fertility outcomes of MIESA patients who underwent IVF with intracytoplasmic sperm injection (ICSI) to a control group of couples who underwent ICSI for unexplained infertility with fresh, normal ejaculated sperm samples.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The MIESA is performed in the office with oral or intravenous sedation using only loupe magnification. Samples are cryopreserved for later IVF/ICSI. Epididymal sperm is extracted in the same manner as an obliterative microsurgical epididymal aspiration (MESA), except without the need for general anesthesia, an operating microscope, or complete epididymal exposure. We analyzed MIESA samples for sperm quality/quantity and compared IVF cycle outcomes to a computer-generated control group of age-matched females who underwent IVF/ICSI for unexplained infertility. All couples with identified female factor infertility were excluded. Chi Square and student t test analysis were used to determine statistical significance.

**RESULTS:** 43 MIESA procedures were performed between December 2013 and July 2018. Causes of OA included vasectomy (35%), failed vasectomy reversal (55%), congenital bilateral absence of the vas deferens (16%), and other (6%). High quality MIESA samples were obtained with a mean retrieved total motile sperm count of 13.7 million which were cryopreserved in a mean of 4.8 vials. Mean semen parameters of the controls were all within normal limits. The mean female partner ages were 34.0 and 33.3 years ( $p=0.45$ ) for the MIESA group and controls, respectively. With the primary embryo transfer, 53.5% of MIESA couples achieved a live birth compared to 48.8% of controls ( $p=0.67$ ). There was no significant difference in the fertilization rate (70.7% vs 78.1%,  $p=0.06$ ) or the blastulation rate (58.9% vs 62.0%,  $p=0.59$ ) between the MIESA and control groups, respectively. The cumulative live birth rate, defined as the combined fresh and subsequent frozen embryo transfers from the same IVF cycle was 79% in the MIESA group compared to 61% in the control group ( $p=0.13$ ) with an average of 1.72 and 1.54 transfers per live birth, respectively.

**CONCLUSIONS:** MIESA provides high-quality cryopreserved sperm samples for men with OA. IVF/ICSI outcomes, including fertilization rate, blastulation rate, and live birth rate, were non-inferior to a comparison group of couples with female age-matched controls with unexplained infertility that underwent IVF/ICSI using fresh, normal ejaculated sperm.

**O-59** Monday, October 14, 2019 11:45 AM

**GONADAL END ORGAN EFFECTS IN MALE TO FEMALE TRANSGENDER PATIENTS ON HORMONAL THERAPY.** Priyanka Bearely, MD, Jaromir Slama, MD, Robert D. Oates, M.D. Boston University School of Medicine, Boston, MA.



**OBJECTIVE:** The objective of this study is to investigate changes in spermatogenesis as a consequence of the quantitative reduction in testosterone production and action and/or possible direct effects of estrogen on seminiferous epithelium. This unique patient population provides this unusual opportunity because of the high volume of individuals undergoing gender confirmation surgery.

**DESIGN:** An IRB-approved retrospective review of 35 neovaginoplasty patients and 21 patients who underwent bilateral orchiectomy as a stand-alone procedure was conducted. Testicular histology of 56 patients (112 testicles) was examined by the investigators, and predominant patterns of spermatogenic disruption were defined. Presently, a prospective, IRB-approved analysis is being conducted on these same two patient populations to correlate testicular histology, intraoperative wet prep analysis to identify fully formed spermatozoa, medication type and dosage, and serum levels of Estradiol, Testosterone (T), Luteinizing hormone (LH), and Follicle Stimulating hormone (FSH) obtained immediately prior to surgery. This is in an effort to refine or define an explanation for the negative effect of these medications on spermatogenesis.

**MATERIALS AND METHODS:** Between January 2017 to September 2018, 35 transgender women underwent neovaginoplasty, and seminiferous tubule histology was retrospectively examined. In addition, in 2017, 21 patients underwent bilateral orchiectomy as a stand-alone procedure. Classification included complete absence of germ cells, spermatocytic maturation arrest (SMA), hypospermatogenesis (mild, moderate, and severe), and

normal histology. As part of the early prospective cohort, 6 patients underwent bilateral orchiectomy, and 2 patients underwent neovaginoplasty. Intraoperative testicular wet prep findings were recorded as number of spermatozoa per high powered field.

**RESULTS:** Retrospectively, of the 35 neovaginoplasty patients, the following histology was seen: 2 with complete absence of germ cells, 3 with mild hypospermatogenesis, 8 with SMA only, and the remaining with a combination of SMA with mild (4), moderate (5), and severe (11) hypospermatogenesis. Of the 21 orchiectomy patients, the following histology was seen: 4 with SMA only, and the remaining with a combination of SMA and mild (4), moderate (6), and severe (7) hypospermatogenesis. In our early prospective data set, 2 out of 8 patients had spermatozoa seen on intraoperative wet prep (T:70, E2:168 ; T:18, E2:120).

**CONCLUSIONS:** Estrogen therapy and testosterone blockers (spironolactone) are routinely used in combination in MTF individuals to suppress testosterone and its androgenic effects while promoting welcome estrogenic bodily changes. The consequent reduction in spermatogenesis is quite variable, as clearly demonstrated by the unexpected results of the retrospective review—meiotic progression was uniformly impaired while a decrease in the total number of germ cells per tubule was not uncommon. We envision our nascent prospective study to allow us to formulate some mechanistic models of biological causality.

**O-60** Monday, October 14, 2019 12:00 PM

**THE UTILITY OF SPERM CRYOPRESERVATION AT THE TIME OF VASECTOMY REVERSAL.** Jessica A. Marinaro, MD,<sup>a</sup> Russell P. Hayden, MD,<sup>b</sup> Paul Shin, MD,<sup>c</sup> Cigdem Tanrikut, MD,<sup>d</sup> <sup>a</sup>MedStar Georgetown University Hospital, Washington, DC; <sup>b</sup>Weill Cornell Medicine, New York, NY; <sup>c</sup>Shady Grove Fertility, Washington DC, DC; <sup>d</sup>Shady Grove Fertility, Rockville, MD.



**OBJECTIVE:** To evaluate the utility of cryopreserving sperm at the time of vasectomy reversal.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** From April 2016 through December 2018, 26 men underwent vasectomy reversal. Sperm cryopreservation is routinely offered at the time of vasectomy reversal at our institution. We sought to assess utilization of cryopreserved sperm by those men with early or late failure.

**RESULTS:** Of 26 patients presenting for vasectomy reversal, 22 elected to cryopreserve sperm (85%); sperm were obtained for freezing from the vaginal fluid (N=3), epididymal fluid (N=7), or via testicular biopsy (N=12). Three patients were lost to follow-up post-operatively. Of the 23 who presented for post-procedure follow-up, 19 either had semen analyses (SAs) with motile sperm or a live birth (83% success rate). There were 2 early failures and 4 late failures; all failures had elected to cryopreserve sperm at the time of initial reversal. Two of the six individuals with vasectomy reversal failure elected to use cryopreserved sperm for IVF-ICSI, both resulting in ongoing clinical intrauterine pregnancies.

**CONCLUSIONS:** Of those patients who experienced vasectomy reversal failure, 1/3 elected to use cryopreserved sperm that had been procured at the time of initial reversal. Cryopreservation of sperm at the time of vasectomy reversal should be routinely offered given potential for early or late failure as a means of avoiding added expense and potential morbidity of future surgical sperm retrieval.

## NUTRITION

**O-61** Monday, October 14, 2019 10:45 AM

**PRECONCEPTION MARIJUANA USE, ANOVULATION, AMH, AND PREGNANCY OUTCOMES.** Sunni L. Mumford, PhD,<sup>a</sup> Kerry S. Flannagan, PhD,<sup>a</sup> Jeannie G. Radoc, BS,<sup>a</sup> Torie C. Plowden, MD,<sup>a</sup> Keewan Kim, PhD,<sup>a</sup> Alexandra C. Purdue-Smithe, PhD,<sup>a</sup> Jessica R. Zolton, DO,<sup>b</sup> Lindsey A. Sjaarda, PhD,<sup>c</sup> Neil J. Perkins, PhD,<sup>a</sup> Josh Freeman, MPH,<sup>a</sup> Zeina Alkhalaf, MPH,<sup>a</sup> Victoria C. Andriessen, BS,<sup>a</sup> Robert M. Silver, MD,<sup>d</sup> Enrique F. Schisterman, PhD,<sup>a</sup> <sup>a</sup>NICHD, Bethesda, MD; <sup>b</sup>Walter Reed National Military Medical Center, Bethesda, MD; <sup>c</sup>Epidemiology Branch, DIPHR, NICHD, NIH, Bethesda, MD; <sup>d</sup>University of Utah, Salt Lake City, UT.



**OBJECTIVE:** Marijuana is the most widely used illicit drug in the US, with legalization further increasing both medical and recreational use. Studies evaluating self-reported use yield mixed results about whether marijuana is harmful in pregnancy. However, there is concern for underreporting due to the stigma of marijuana use as it is not federally legalized. Our aim was to examine associations between preconception marijuana use, via both self-report and urinary tetrahydrocannabinol (THC), and fecundability, live birth, and pregnancy loss. We also evaluated these relationships in the context of ovulatory function and anti-müllerian hormone (AMH).

**DESIGN:** A prospective cohort of 1212 women enrolled in the EAGeR trial, aged 18-40 years, with regular menstrual cycles and a history of 1-2 prior pregnancy losses.

**MATERIALS AND METHODS:** Women were screened for urinary THC up to 2 time points prior to conception using a homogenous enzyme immunoassay (Randox Laboratories) and reported past year marijuana use at baseline. Women were followed for up to 6 cycles while attempting pregnancy. Anovulation was assessed using fertility monitors and, where available in the first 2 cycles of follow-up, supplemented with urinary pregnanediol glucuronide measures. Serum AMH was measured at the baseline visit. Cox proportional hazard regression was used to calculate fecundability odds ratios (FOR), and log-binomial regression was used to estimate risk ratios (RR) for live birth, pregnancy loss, anovulation, and low AMH ( $\leq 1.0$  vs  $>1.0$  ng/ml) adjusting for age, race, BMI, education, smoking, alcohol, and antidepressant use.

**RESULTS:** Of the 33 (2.7%) women who screened positive for THC, only 14 self-reported marijuana use. A total of 62 women (5.1%) screened positive for THC or self-reported use in the year prior. Women positive for urinary THC or with self-reported marijuana use had reduced fecundability (FOR 0.53, 95% CI 0.33, 0.86). No associations were observed with live birth (RR 0.71; 95% CI 0.41, 1.22) or pregnancy loss (RR 0.78; 95% CI 0.28, 2.18). Further, no associations were observed with anovulation (RR 0.94, 95% CI 0.51, 1.73) or with low AMH (RR 1.25, 95% CI 0.71, 2.20).

**CONCLUSIONS:** Women who screened positive for THC during preconception, or self-reported use during the past year, had reduced fecundability, though no associations were observed with live birth or pregnancy loss. Associations with reduced fecundability are not likely to be explained by anovulation or AMH levels, suggesting that other mechanisms may be at play. Further investigations are needed to confirm these observations, determine potential mechanisms and what duration and dose of marijuana may negatively impact fecundability.

**SUPPORT:** Intramural Research Program, Division of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development.

O-62 Monday, October 14, 2019 11:00 AM

### SERUM OMEGA-3 AND OMEGA-6 FATTY ACID CONCENTRATIONS AND FECUNDITY.

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**OBJECTIVE:** Omega fatty acid serum concentrations have been shown to play an important role in reproduction in animal models, with conflicting results in human studies. We sought to determine the association between serum omega-3 and omega-6 fatty acid concentrations and the probability of conceiving, the risk of miscarriage, and ovarian reserve.

**DESIGN:** A nested case control study was conducted of 200 women [fertile (n=50), subfertile, (n=100) and infertile (n=50)] randomly selected from subjects within Time to Conceive (TTC), a prospective, time-to-pregnancy cohort study.

**MATERIALS AND METHODS:** In TTC, women aged 30 – 44 years, trying to conceive <3 months, with no history of infertility provided serum in the first menstrual cycle following enrollment and were subsequently followed for up to one year of pregnancy attempt or through the end of pregnancy. For this nested study, serum from 200 subjects was analyzed for omega-3 and omega-6 fatty acid concentrations by liquid chromatography – mass spectroscopy. Omega concentrations from the fertile (conceived in the first 3 cycles of attempt), subfertile (4-12 cycles of attempt), and infertile groups were compared using bivariate analyses. Subsequently age-adjusted fecundability ratios were calculated using the entire cohort. Bivariate analyses and logistic regression models were used to determine the association between omega fatty acids and pregnancy outcome (live birth versus miscarriage). The association between serum omega fatty acid concentrations and

AMH level was analyzed using Pearson's Correlation.  $P < 0.05$  was considered statistically significant.

**RESULTS:** 200 women provided 1321 cycles for analysis. Mean age was  $33.27 \pm 3.20$  years. Mean omega-3, omega-6, and omega-6:omega-3 ratios did not significantly differ between fertile, subfertile, and infertile groups. Fecundability did not differ significantly by omega-3 or omega-6 fatty acid concentration (Table 1). There was no significant association between any serum omega fatty acid concentration and the age-adjusted odds of miscarriage. None of the serum omega fatty acid concentrations were correlated with AMH.

**CONCLUSIONS:** These data suggest that omega-3 and omega-6 serum levels do not affect fecundity. Future investigation is needed to determine if omega-3 fatty acid supplementation may benefit women planning to conceive naturally.

TABLE 1. Omega fatty acid fecundability ratio (FR)

Omega Fatty Acid	FR (95% Confidence Interval)
Alpha linolenic acid (ALA)	1.10 (0.57, 2.11)
Eicosapentaenoic acid (EPA)	1.12 (0.69, 1.84)
Docosahexaenoic acid (DHA)	1.02 (0.90, 1.13)
Linoleic acid (LA)	0.99 (0.98, 1.00)
Dihomo-gamma linolenic acid (DGLA)	1.02 (0.90, 1.20)
Arachidonic acid (AA)	0.99 (0.89, 1.09)
Omega-6:Omega-3	0.99 (0.92, 1.07)

Reference: N/A.

SUPPORT: N/A.

O-63 Monday, October 14, 2019 11:15 AM

### THE ROLE OF MATERNAL PRECONCEPTION VITAMIN D STATUS IN HUMAN OFFSPRING SEX RATIO.

Alexandra C. Purdue-Smithe, PhD,<sup>a</sup> Keewan Kim, PhD,<sup>a</sup> Carrie J. Nobles, PhD,<sup>a</sup> Enrique F. Schisterman, PhD,<sup>a</sup> Karen C. Schliep, PhD,<sup>b</sup> Neil J. Perkins, PhD,<sup>a</sup> Lindsey A. Sjaarda, PhD,<sup>c</sup> Josh Freeman, MPH,<sup>a</sup> Sonia L. Robinson, PhD,<sup>d</sup> Jeannie G. Radoc, BS,<sup>a</sup> James L. Mills, MD, MS,<sup>a</sup> Robert M. Silver, MD,<sup>b</sup> Sunni L. Mumford, PhD<sup>c</sup> <sup>a</sup>NICHD, Bethesda, MD; <sup>b</sup>University of Utah, Salt Lake City, UT; <sup>c</sup>Epidemiology Branch, DIPHR, NICHD, NIH, Bethesda, MD; <sup>d</sup>National Institutes of Child Health and Human Development, Bethesda, MD; <sup>e</sup>National Institute of Child Health and Human Development, Bethesda, MD.



**OBJECTIVE:** Experimental data suggests that maternal inflammation is specifically detrimental to the implantation or survival of male embryos, which may contribute to sex ratio reduction on the population scale. However, it is currently unknown whether other factors associated with both pregnancy and inflammation, such as vitamin D status, are associated with altered offspring sex ratio. Our objective was to therefore evaluate the association of preconception serum 25-hydroxyvitamin D levels [25(OH)D] and male live birth among reproductive-age women attempting pregnancy.

**DESIGN:** This was a prospective secondary analysis of the Effects of Aspirin in Gestation and Reproduction trial, which included 1,228 reproductive-age women attempting to conceive.

**MATERIALS AND METHODS:** 25(OH)D and high sensitivity C-reactive protein (hsCRP) levels were measured in serum at baseline. Participants were classified as vitamin D sufficient versus insufficient [25(OH)D  $\geq 30$  vs.  $<30$  ng/mL]. Fetal sex was ascertained by medical record abstraction among live births and by chromosomal analysis among clinical pregnancy losses. We estimated unadjusted and adjusted relative risks (RRs) and 95% confidence intervals (CIs) for male live birth and pregnancy with a male fetus according to preconception vitamin D status using generalized estimating equations of log-binomial regression with robust standard errors.

**RESULTS:** Among 1,094 women who completed follow-up, the proportion of male live births was 24% (n=136) and 30% (n=156) in the vitamin D insufficient and sufficient groups, respectively. In multivariable models, women in the vitamin D sufficient group were 25% (RR = 1.25; 95% CI = 1.02, 1.52) more likely to have a live-born male infant compared to the insufficient group. These associations were stronger among women with high versus low levels of preconception hsCRP ( $>1.95$  ng/mL; RR = 1.44;

95% CI = 1.01, 2.05, versus  $\leq 1.95$  ng/mL RR = 1.08; 95% CI = 0.81, 1.43), a marker of systemic low-grade inflammation. In analyses utilizing available karyotype data from clinical pregnancy losses, sufficient versus insufficient vitamin D was also positively associated with pregnancy with a male fetus (RR = 1.21; 95% CI = 1.01, 1.46). Estimates were stronger among women with high versus low levels of hsCRP ( $>1.95$  ng/mL: RR = 1.34; 95% CI = 0.96, 1.88 versus  $\leq 1.95$  ng/mL RR = 1.12; 95% CI = 0.90, 1.39), though not statistically significant.

**CONCLUSIONS:** Our findings that preconception vitamin D status is positively associated with male live birth and pregnancy with a male fetus, particularly among women with elevated inflammation, suggest that sufficient levels of preconception vitamin D may mitigate maternal inflammation that would otherwise be detrimental to the implantation or survival of male conceptuses *in utero*. These findings highlight the importance of vitamin D in reproduction and implicate a novel factor associated with altered offspring sex ratio in humans.

**SUPPORT:** This research was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (National Institutes of Health, Bethesda, MD, USA; contract numbers HHSN267200603423, HHSN267200603424, and HHSN267200603426).

**O-64** Monday, October 14, 2019 11:30 AM

#### CAFFEINATED BEVERAGE INTAKE AND SERUM CAFFEINE METABOLITES AND RISK OF PREGNANCY LOSS.

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**OBJECTIVE:** According to current ACOG recommendations, moderate intake of caffeine ( $<200$  mg/day) during pregnancy does not increase risk of pregnancy loss, though epidemiologic evidence to support this recommendation is controversial. Our objective was to address limitations of prior studies by evaluating associations of both self-reported intake of caffeinated beverages and preconception and early pregnancy serum caffeine biomarkers and risk of pregnancy loss, while accounting for nausea and vomiting, smoking, and alcohol intake in early pregnancy.

**DESIGN:** This was a secondary analysis of the EAGeR trial, which included 1,228 reproductive-age women attempting pregnancy during 2007-2011.

**MATERIALS AND METHODS:** Questionnaires administered at baseline assessed self-reported intake of caffeinated beverages and other demographic and lifestyle variables. During pregnancy, daily questionnaires assessed caffeinated beverage intake, nausea and vomiting, alcohol intake, and smoking. Serum caffeine, paraxanthine, and theobromine were measured at preconception and during the 8<sup>th</sup> week of gestation. HCG-detected losses occurred prior to ultrasound confirmation and clinical pregnancy losses occurred after ultrasound visualization of a gestation sac. We used Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for pregnancy loss according to: 1) baseline caffeinated beverage intake and caffeine biomarkers measured at preconception and the 8<sup>th</sup> week of gestation, adjusted for baseline factors and, 2) time-varying caffeinated beverage intake during pregnancy adjusted for baseline factors and time-varying nausea and vomiting, alcohol intake, and smoking.

**RESULTS:** 67%, 28%, and 9% of women reported any preconception intake of caffeinated sodas, coffee, and tea, respectively. Preconception total caffeinated beverage intake of  $\geq 2$  vs. 0 servings/d was marginally associated with increased risk of any loss (HR = 1.51, 95% CI = 0.98, 2.34), and associations were stronger for hCG-detected losses than for clinical losses. Soda intake was more strongly associated with hCG-detected losses, whereas coffee intake was more strongly associated with clinical losses. Any detectable level of serum caffeine ( $>0.2$  vs.  $\leq 0.2$  ng/mL) at preconception was strongly associated with hCG-detected loss (HR = 4.51; 95% CI: 1.36, 14.91), but biomarkers measured during the 8<sup>th</sup> week of gestation were not associated with loss. Further adjustment for nausea and vomiting and other factors that change during early pregnancy showed that caffeinated beverage intake at levels lower than those corresponding to current medical recommendation was positively associated with risk of loss (0 vs.  $\geq 1$  servings/day HR = 1.73;

95% CI = 1.02, 2.94), particularly among hCG-detected losses (HR = 2.83; 95% CI = 1.08, 7.39).

**CONCLUSIONS:** Our findings suggest that any level of caffeine intake during pregnancy may increase risk of pregnancy loss, particularly in the first 8 weeks' gestation. Women attempting to conceive may benefit from eliminating caffeine intake during preconception and early pregnancy.

**SUPPORT:** This research was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (National Institutes of Health, Bethesda, MD, USA; contract numbers HHSN267200603423, HHSN267200603424, and HHSN267200603426). Jeannie G. Radoc was supported by the NIH Medical Research Scholars Program, a public-private partnership supported jointly by the NIH and generous contributions to the Foundation for the NIH from the Doris Duke Charitable Foundation (DDCF Grant # 2014194), Genentech, Elsevier, and other private donors.

**O-65** Monday, October 14, 2019 11:45 AM

#### LEPTIN IS A MEDIATOR IN THE ASSOCIATION BETWEEN PERCENT BODY FAT AND DECREASED AMH AMONG HEALTHY WOMEN.

Jasmine Aly, MD,<sup>a</sup> Elizabeth A. DeVilbiss, PhD,<sup>b</sup> Sunni L. Mumford, PhD,<sup>b</sup> Micah J. Hill, DO,<sup>a</sup> Alan H. DeCherney, MD,<sup>a</sup> Laura Zalles, MD,<sup>c</sup> Neil J. Perkins, PhD,<sup>b</sup> Robert M. Silver, MD,<sup>d</sup> Enrique F. Schisterman, PhD<sup>b</sup> <sup>a</sup>Program in Reproductive Endocrinology and Gynecology, NICHD, NIH, Bethesda, MD; <sup>b</sup>National Institute of Child Health and Human Development, Epidemiology Branch, DIPHR, NICHD, NIH, Bethesda, MD; <sup>c</sup>Cooper University Hospital, Department of Obstetrics and Gynecology, Camden, NJ; <sup>d</sup>University of Utah, Salt Lake City, UT.



**OBJECTIVE:** While obesity is associated with decreased serum AMH, the mechanism by which this occurs is unknown. Studies have found that increased adipokines produced in the adipose tissue, such as leptin, can directly inhibit ovarian function. A recent study by our group found that increases in leptin were associated with lower serum AMH. Because percent body fat and leptin are closely related there is a need to understand the impact of percent body fat on AMH, and to what extent this relationship is driven by leptin. We hypothesize that increased leptin is associated with decreased AMH and that leptin is a direct mediator of this relationship.

**DESIGN:** Prospective analysis of 259 women aged 18-44 years from western New York State, followed for up to 2 menstrual cycles.

**MATERIALS AND METHODS:** Serum AMH and leptin were measured five to eight times per cycle for one (n = 9) or two (n = 250) cycles per woman. Participant characteristics and mean AMH hormone levels were examined by tertile of average leptin over 2 cycles (First tertile: 4.1-14.2 ng/ml; Second tertile: 14.4-29.8 ng/ml; Third tertile: 29.9-86.8 ng/ml). 248 women participated in a dual energy X-ray absorptiometry (DXA) scan to measure fat and lean mass from which total percent body fat and percent truncal fat were derived. Using the product method, a mediation analysis was performed for percent body fat (exposure), leptin (mediator), and serum AMH (outcome) to determine the extent to which leptin mediates the association between body fat and AMH. Marginal structural models with inverse probability of exposure weights were used to relate body fat to leptin (mediator model) and body fat and leptin to serum AMH at the next visit (outcome model). The mediator model was adjusted for FSH, LH, estrogen, and progesterone, and the outcome model was adjusted for age, smoking status, caloric intake, and physical activity.

**RESULTS:** Overall, we observed an inverse relationship between percent body fat and serum AMH, such that for each 10% increase in body fat there was a 14% decrease in AMH (95% CI -24.5, -2.1). Mediation analysis results showed that the 14% decrease in AMH was mostly explained by leptin (indirect effect -7.7%, 95% CI -11.6, -3.7), though some of the decrease was also due to other non-leptin mediated pathways (direct effect -5.7%, 95% CI -18, 8.3).

**CONCLUSIONS:** Among healthy women, higher body fat and serum leptin were both associated with lower AMH concentrations. The inverse relationship between percent body fat and AMH is largely mediated by leptin.

**SUPPORT:** This research was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (National Institutes of Health, Bethesda, MD, USA; contract numbers HHSN275200403394C HHSN275201100002I, and Task 1 HHSN27500001), and the Program in Reproductive and Adult Endocrinology, NICHD, NIH.

**OMEGA-3 FATTY ACID SUPPLEMENTATION AND FECUNDABILITY.**

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**OBJECTIVE:** Omega-3 fatty acids supplementation in animal models have been shown to alter prostaglandin biosynthetic pathways in the ovary and endometrium, and thereby improve folliculogenesis, oocyte maturation, embryo quality, and implantation. However, little is known about the effects of omega-3 supplementation on human fecundity. We sought to determine the association between omega-3 fatty acid supplementation and fecundability, the probability of natural conception in a given menstrual cycle.

**DESIGN:** Secondary data analysis of Time to Conceive (TTC), a prospective, time to pregnancy cohort study.

**MATERIALS AND METHODS:** In TTC, women aged 30 – 44 years, trying to conceive <3 months, with no history of infertility were followed for up to one year of pregnancy attempt using standardized pregnancy testing. While attempting to conceive, women daily recorded intercourse, menstrual cycle events, and vitamin, supplement, and medication intake using the Cerner Multum Drug Database. For this analysis, supplements and vitamins containing omega 3 [for example: docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), prenatal vitamin formulations including omega 3, and fish oil) were identified. The percentage of days in a given menstrual cycle on which a woman took omega-3 supplements was calculated, and, based on the Akaike Information Criterion, a cut-off value of 20% was used to dichotomize omega-3 use in each cycle. A positive urine pregnancy test was used to define conception. A discrete-time Cox proportional hazards model was used to calculate the fecundability ratio, adjusting for age, obesity, history of prior pregnancy, race, and Vitamin D intake in the cycle.

**RESULTS:** Of 1036 women enrolled in TTC comprising 4,775 cycles, 136 women and 2,265 cycles were missing daily diary data and were excluded. 900 women comprising 2,510 cycles were analyzed. ±3.11 years. Women taking omega-3 supplements were younger, thinner, and more likely to be nulligravid and white compared to women not taking omega-3. After adjusting for age, obesity, previous pregnancy, race, and Vitamin D intake, women taking omega-3 supplements had 1.83 (95% CI 1.42, 2.35) times the probability of conceiving in a given menstrual cycle compared to women not taking omega-3 supplements.

**CONCLUSIONS:** These data suggest omega-3 supplementation significantly increases the probability of a woman conceiving. Randomized controlled trials are needed to further investigate the benefits from omega-3 supplementation for women trying to conceive naturally.

Reference: N/A.

**SUPPORT:** N/A.

**OVARIAN STIMULATION**

O-67 Monday, October 14, 2019 10:45 AM

**A COMPUTERIZED DECISION –SUPPORT SYSTEM FOR DAY TO DAY MANAGEMENT OF OVARIAN STIMULATION CYCLES DURING IN VITRO FERTILIZATION.**

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**OBJECTIVE:** The purpose is to describe a computer algorithm designed for IVF management and to assess accuracy in decision making during ovarian stimulation for IVF when compared to evidence based decisions by the clinical team.

**DESIGN:** Evaluation study of novel software; comparative; quantitative.

**MATERIALS AND METHODS:** Data was in the form of IVF cycles. Our data set included estradiol concentrations (pg/ml); ultrasound measurements of follicle diameters in 2 dimensions in mm; cycle day and dose of recombinant FSH during ovarian stimulation for IVF. In a pilot study we evaluated 5 predictive analytics including classification and regression trees, random forests, support vector machines, logistic regression and neural networks. We then developed a hybrid algorithm for automated prediction of 4 decisions critical to management during ovarian stimulation : (1) Stop the cycle (trigger or cancel) or (2) continue and return for follow-up. If decision was to stop, the algorithm added a modifier regarding trigger or cancellation. If the decision

was to return, the algorithm identified (3) number of days to follow up and (4) dosage adjustment if needed. Database consisted of 2603 total cycles. (1853 autologous and 750 donor) incorporating 7,376 visits. Seventy percent of the cycles were used for training and validation and 30% for challenge. There were 59,706 data points. We compared DSS performance against evidence based decisions by 12 clinicians. Performance was defined as outcome accuracy or agreement between the clinicians' decisions and the DSS when challenged using 556 cycles to which the algorithm was naïve (no prior exposure). Algorithms were written in "R" language for stat analysis and data manipulation and converted to C++.

**RESULTS:** Outcome accuracy of the algorithm, sensitivity and positive predictive value (PPV) for automated prediction are listed in Table 1 for the final trained model on held-out challenge data for the four decisions analyzed.

TABLE 1. Summary of Decisions

DECISIONS	ACCURACY	SENSITIVITY	PPV
(1) Stop cycle: Trigger or cancel	0.92	0.94	0.95
(2) Return for follow-up	0.96	0.98	0.97
(3) Number days to follow-up	0.87	0.89	0.86
(4) Dosage	0.82	0.96	0.67

**CONCLUSIONS:** We describe a first iteration, predictive analytic algorithm for decision support of 4 key management decisions during ovarian stimulation for IVF. Algorithm performance for the decisions to trigger/cancel, return and days to follow-up was highly accurate and in concordance with clinical decisions. Dose changes (increase or decrease) were relatively infrequent clinical decisions in the database resulting in the lowest outcome accuracy of the algorithm. This algorithm offers the possibility of improved clinical and cost efficiencies for IVF management.

**SUPPORT:** None.

O-68 Monday, October 14, 2019 11:00 AM

**DUAL TRIGGERING OF FINAL OOCYTE MATURATION IN POOR OVARIAN RESPONDERS: A PROSPECTIVE RANDOMIZED CONTROLLED TRIAL.**

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**OBJECTIVE:** Women with POR (Bologna criteria) manifest a very low follicular response to controlled ovarian stimulation irrespective of the stimulation protocol utilized. Dual triggering of oocyte maturation was shown to improve follicle collection yield and oocyte maturation in women with predicted normal ovarian response. These benefits have been attributed to the GnRHa-induced FSH surge believed to promote oocyte nuclear maturation and cumulus expansion. The aim of the study is to show whether the co-administration of a GnRH agonist and hCG for final oocyte maturation improve oocyte collection and maturation rates in women with poor ovarian response (POR) compared with hCG alone.

**DESIGN:** This is an ongoing prospective randomized controlled trial seeking to randomize 140 women with POR undergoing IVF/ICSI treatment into receiving a dual trigger for final oocyte maturation compared with conventional hCG, between May 2018 and December 2019.

**MATERIALS AND METHODS:** Women with POR (Bologna criteria) were randomized to receive either a combination of 0.3 mg Triptorelin subcutaneously (Decapeptyl; Ipsen Beaufour; Denmark) and 10,000 IU hCG subcutaneously (Choriomon; IPSA Pharmaceuticals; Switzerland) or 10,000 IU hCG alone. Primary outcomes were oocyte collection and maturation rates. Secondary outcomes were clinical and ongoing pregnancy rates. Chi Square analysis was utilized for categorical data and student t test for continuous variables. A  $p < 0.05$  was considered for statistical significance.

**RESULTS:** Sixty-eight patients have been recruited to this point with a cycle cancellation of 7.35% (5/68). A total of 63 patients were randomly allocated to the dual trigger (n=28) and hCG alone (n=35) groups. Baseline demographic and stimulation characteristics were comparable between the two groups. The total number of oocytes (4 vs. 4.2;  $p=0.65$ ), number of mature oocytes (3.1 vs. 3.2;  $p=0.81$ ), and number of 2PN zygotes (2.6 vs.

2.2;  $p=0.32$ ) were not significantly different between the dual trigger and hCG alone groups. The oocyte collection (62.5% vs. 64.6%;  $p=0.75$ ) and oocyte maturation rates (77.5% vs. 76.2%;  $p=0.82$ ) were also comparable. Per embryo transfer, the clinical pregnancy rate (15.2 vs. 12.6;  $p=0.96$ ) and ongoing pregnancy rate (13.8 vs. 12.6;  $p=0.63$ ) showed no statistical differences.

**CONCLUSIONS:** There was no significant increase in oocyte collection or maturation rates following dual triggering of final oocyte maturation compared with hCG alone in women with POR. POR (Bologna criteria) represents a subgroup of women with a very poor pregnancy prognosis and also a very challenging fertility management. Although the preliminary findings of this trial do not seem to hold promises in favor of an improved outcome with dual triggering of oocyte maturation in this subgroup of women, conclusive evidence are expected only following completion of the recruitment period.

**SUPPORT:** None.

**O-69** Monday, October 14, 2019 11:15 AM

### RISK OF HARM ASSOCIATED WITH THE USE OF LETROZOLE AS A FERTILITY DRUG: A SYSTEMATIC REVIEW AND META-ANALYSIS.

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**OBJECTIVE:** We undertook this systematic review and meta-analysis to find out the Harm of congenital malformations (major and minor) and pregnancy loss (first and second trimester loss, still birth, intrauterine death and termination of pregnancy) associated with the use of letrozole as a fertility agent.

**DESIGN:** Systematic review and Meta-analysis.

**MATERIALS AND METHODS:** Systematic reviews of literature in accordance with the PRISMA HARM; along with a prospective protocol registration in PROSPERO (CRD42017082260) was performed. Literature was searched (1950 to March 2019), combining the Medical Subject Headings and text words for 'Letrozole' and 'pregnancy' (ovulation, pregnancy) or 'fetal' outcome (fetal, neonatal). The qualities of studies were assessed using the Cochrane risk of bias tool for randomised controlled studies (RCTs) and Newcastle Ottawa scale for non-randomised comparative cohort studies (CCS). McMaster tool (McHarm) was used to report on the quality of harms assessment and reporting. Meta-analysis was performed to address zero and rare events and reported by "peto odds ratio" with 95% confidence intervals. Subgroup analysis was performed based on the design of the study (RCTs or CCS) and whether letrozole was used as ovulation induction agent or as an adjuvant in assisted reproductive cycles. Sensitivity analysis was performed based on quality of studies on McHARM score. Strength of evidence was assessed as per the GRADE recommendation.

**RESULTS:** From 769 potential citations, 245 full-text articles and 20 conference abstracts were assessed for eligibility and 46 studies (18 RCTs; 20 CCS) were included. Qualitative synthesis included 35 studies (25 full texts and 10 conference abstracts).

Out of total 4613 babies reported from 44 studies with the use of letrozole as a fertility agent, 94 (2.04%) babies were born with congenital malformations and 21 (0.46%) babies were born with major congenital malformations.

On Meta-analysis, when letrozole was compared with clomiphene there was no significant difference for all congenital malformations on pooling of the data from 14 RCTs and 10 CCS (pOR 0.72; 95% CI 0.48, 1.08;  $I^2 = 0\%$ ;  $p=0.92$ ); major congenital malformations on pooling of data from 5 RCTs and 4 CCS (pOR 0.76; 95% CI 0.42, 1.36;  $I^2 = 18\%$ ;  $p=0.28$ ); and in pregnancy loss on combining 14 RCTs and 6 CCS (pOR 0.72; 95% CI 0.48, 1.08;  $I^2 = 0\%$ ;  $p=0.92$ ). Letrozole when compared with natural conception or other ovulation induction such as gonadotrophins or when used as an adjuvant in assisted reproductive cycles showed no significant difference in the outcomes of interest. GRADE of evidence showed moderate to high quality of evidence, downgrading the evidence to moderate instead of high quality, mainly due to sparse data secondary to very few events.

**CONCLUSIONS:** This large systematic review and meta-analysis, with a moderate to high quality of evidence, shows that when compared with clomiphene or other ovulation induction agents Letrozole is not associated with higher risk of congenital malformations or pregnancy loss.

**Reference:** None.

**SUPPORT:** None.

**O-70** Monday, October 14, 2019 11:30 AM

### PREMATURE LUTEINIZATION IN THE ERA OF PGT-A: EMBRYONIC REPRODUCTIVE POTENTIAL IS NOT AFFECTED BY ELEVATED PROGESTERONE LEVELS DURING OVARIAN HYPERSTIMULATION.

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**OBJECTIVE:** Premature luteinization or early elevation in progesterone (P4) levels is often observed in patients who undergo a GnRH-antagonist protocol for controlled ovarian hyper stimulation (COH). High levels of P4 have been shown to impair endometrial receptivity which might decrease pregnancy rates. Additionally, an increased level of P4 has been theorized to be a marker for suboptimal embryo quality. This study aimed to evaluate the impact of premature luteinization during COH on rates of blastulation and embryo aneuploidy.

**DESIGN:** Retrospective cohort analysis.

**MATERIALS AND METHODS:** The study included patients who underwent IVF stimulation from 2012-2019. Pre-implantation genetic testing for aneuploidy (PGT-A) were performed on blastocysts reaching criteria for TE biopsy, subsequently embryos were vitrified after biopsy. Cohorts were segregated in two groups: Group 1: blastocysts cryopreserved in the presence of normal P4 levels (P4 < 1.5 ng/mL) the day of ovulation trigger; Group 2: blasts originated from oocytes retrieved after exposure to premature luteinization (P4 ≥ 1.5 ng/mL) on the day of trigger. Demographic, COH parameters, blastulation, and euploidy rates were evaluated. IVF outcomes in a subsequent single euploid FET cycle were assessed. T-test,  $\chi^2$ , and multivariate regressions with GEE models were used for data analysis. A sample size of 260 patients per group was calculated to create an 80% power to detect a difference of 10% on clinical pregnancy rates (CPR) ( $\alpha=0.05$ ).

**RESULTS:** A total of 3,659 patients with normal P4 (29,038 blasts) were compared to 331 patients with elevated P4 (3,327 blasts). Significant differences were found in BMI, AMH levels, Estradiol, and P4 levels on the day of hCG trigger and oocytes retrieved between cohorts. No difference was found in maturity rates (78.7%, 79.4%,  $p=0.1$ ), fertilization rates (81.8%, 82.4%,  $p=0.2$ ), cryopreserved blastocysts (76.5%, 75.5%,  $p=0.2$ ), and aneuploidy rates (35.3%, 35%,  $p=0.7$ ). Blastulation rate was higher in Group 1 (71.8%, 69.2%,  $p=0.0002$ ). Furthermore, no differences were found in pregnancy (74.4%, 72.5%,  $p=0.4$ ), clinical pregnancy (82.9%, 82.9%,  $p=0.5$ ), ongoing pregnancy (70.5%, 68.7%,  $p=0.3$ ) and clinical loss rates (9.7%, 14.1%,  $p=0.5$ ) after a FET. After adjusting for age, BMI, AMH, and number of embryos biopsied per cycle, no association was found between elevated P4 levels and the odds of increased aneuploidy (OR=0.90, CI95% 0.7-1.03,  $p=0.15$ ), blastulation rate (OR=0.90, CI95% 0.7-1.05,  $p=0.18$ ), or number of good quality embryos (≥ 4BB) (OR=1.0, CI95% 0.8-1.22,  $p=0.92$ ). Also, no association was found with elevated P4 levels and impaired CPR (OR=0.82, CI95% 0.5-1.2,  $p=0.31$ ) after adjusting for age, BMI, embryo quality, and endometrial thickness within our model.

**CONCLUSIONS:** In an era of PGT-A/FET cycles, premature P4 elevation during IVF stimulation does not represent an obstacle to embryo implantation potential. Our study shows that premature luteinization occurring during COH is not associated with a negative effect on embryonic development, increased aneuploidy rates, or impaired IVF outcomes following subsequent FET.

**Reference:** None.

**SUPPORT:** None.

**O-71** Monday, October 14, 2019 11:45 AM

**DETERMINING CORRELATION BETWEEN BODY MASS INDEX AND MINIMUM REQUIRED HCG DOSE WHEN USING DUAL TRIGGER WITH HCG AND GnRH AGONIST.** Lilli D. Zimmerman, MD,<sup>a</sup> Kolbe Hancock, MD,<sup>a</sup> Niral J. Shah, MD,<sup>b</sup> Chelsea Canon, MD,<sup>b</sup>



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**OBJECTIVE:** The use of dual trigger for final oocyte maturation using GnRH agonists in conjunction with human chorionic gonadotropin (hCG) has been shown to reduce the risk of developing ovarian hyperstimulation syndrome (OHSS), largely by allowing a lower dose of hCG to be used. It has been well established that absorption of hCG varies by body mass index (BMI), yet there have been no studies published correlating BMI with minimum hCG dose requirements to achieve a targeted post-trigger serum b-hCG (post b-hCG) level. In previous studies, optimal oocyte maturity with controlled ovarian hyperstimulation (COH) was shown to occur at a minimum post b-hCG value of 50 mIU/mL. This study aims to establish minimum hCG dose requirements per unit BMI to achieve specific post b-hCG levels.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All charts between 1/2009 and 3/2019 were reviewed to identify patients who had undergone COH stimulation at our institution and had received a dual trigger (leuprolide acetate 2mg or 4mg and variable doses of hCG from 1,000 to 10,000 units). The total dose of hCG administered was normalized by BMI, and post b-hCG levels were analyzed. Direct correlation analysis was used to analyze the minimum hCG dose required to achieve a specific post b-hCG level based on BMI.

**RESULTS:** 4744 IVF cycles met inclusion criteria, derived from 3655 women aged 14-49 years old with a BMI range of 15-54 kg/m<sup>2</sup>. The mean BMI of the cohort was 24.4 kg/m<sup>2</sup>. There was a direct correlation between the post b-hCG level and the dose of hCG per point of BMI that was administered ( $y = 0.69x + 75.27$ ,  $R^2 = 0.56$ ). To achieve a post b-hCG of 50-54 mIU/mL, 84.1 units of hCG per point BMI (BMI x 84.1) are required.

**CONCLUSIONS:** Our findings suggest that a dual trigger with a sliding hCG scale in accordance with BMI can be utilized with accuracy to predict a specific post b-hCG value. Our current study analyzed only patients who received a dual trigger, as this cohort encompassed a wide range of hCG doses, allowing for creation of a dosing scale based on BMI. However, as the absorption and physiologic response to hCG is independent of GnRH agonist administration, these results can be extrapolated for use in hCG-only trigger cycles. This dosing protocol for hCG in dual triggers allows the provider to titrate the desired post b-hCG value in order to achieve optimal oocyte maturation while minimizing side effects and risk of OHSS. This is also applicable for those patients who are not candidates for a pure GnRH agonist trigger and are at risk of OHSS with traditional higher-dose hCG trigger.

**References:** 1. Gunnala V, Melnick A, Irani M, et al. Sliding scale HCG trigger yields equivalent pregnancy outcomes and reduces ovarian hyperstimulation syndrome: Analysis of 10,427 IVF-ICSI cycles. *Medline PLoS ONE*. 2017; 12(4): e0176019.

2. Reichman D and Rosenwaks Z. GnRH Antagonist-Based Protocols for In Vitro Fertilization. *Methods in Molecular Biology*. New York, NY: Springer New York; 2014. pp. 289–304.

3. Kashyap S, Parker K, Cedars M, Rosenwaks Z. Ovarian Hyperstimulation Syndrome Prevention Strategies: Reducing the Human Chorionic Gonadotropin Trigger Dose. *Semin Reprod Med*. 2010; 28: 475–485.

4. Smith V, Osianlis T, Vollenhoven B. Prevention of Ovarian Hyperstimulation Syndrome: A Review. *Obstet Gynecol Int*. 2015; 2015: 514159; p1–10.

5. Nastri CO, Teixeira DM, Moroni RM, Leitão VMS, Martins WP. Ovarian hyperstimulation syndrome: pathophysiology, staging, prediction and prevention. *Ultrasound Obstet Gynecol*. 2015; 45: 377–393.

Post-trigger serum b-hCG range (mIU/mL)	Required dose of HCG (units) per point BMI
15-19	58.4
20-24	59.7
25-29	62.8
30-34	68.7
35-39	71.6
40-44	77.7
45-49	79.5
50-54	84.1
55-59	87.1
60-64	98.4
65-69	105.1
70-74	108.5
75-79	120.4

6. Schmidt DW, Maier DB, Nulsen JC, Benadiva CA. Reducing the dose of human chorionic gonadotropin in high responders does not affect the outcomes of in vitro fertilization. *Fertil Steril*. 2004; 82: 841–846.

7. Haas J, Baum M, Meridor K, Hershko-Klement A, Elizur S, Hourvitz A, et al. Is severe OHSS associated with adverse pregnancy outcomes? Evidence from a case. *Reproductive BioMedicine Online. Reproductive Healthcare Ltd*. 2014; 29: 216–221.

8. Mathur RS, Jenkins JM. Is ovarian hyperstimulation syndrome associated with a poor obstetric outcome? 2000; 107: 943–946.

**SUPPORT:** None.

**O-72** Monday, October 14, 2019 12:00 PM

**EFFECT OF DEHYDROEPIANDROSTERONE (DHEA) SUPPLEMENTATION ON INTRACYTOPLASMIC SPERM INJECTION OUTCOME IN INFERTILE WOMEN WITH ANTICIPATED NORMO-OVARIAN RESPONSE.**



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**OBJECTIVE:** To study the effect of dehydroepiandrosterone (DHEA) supplementation in infertile women with expected normal ovarian response before intracytoplasmic sperm injection (ICSI) procedure.

**DESIGN:** Randomized, double-blind, placebo-controlled study.

**MATERIALS AND METHODS:** All women attended the ART unit for first planned fresh embryo transfer ICSI cycles with expected normo-ovarian response were invited to participate in the study. Women were randomized in a 1:1 ratio to either group I (**DHEA group**) received two capsules of DHEA 25 mg (DHEA<sup>®</sup>, MRM Co., USA) or group II (**placebo group**) received two placebo capsules has the same shape, color and consistency starting eight weeks before the date of controlled ovarian hyperstimulation (COH) and continued throughout the whole stimulation period till the HCG triggering day. The primary outcome of the study was the mean antral follicle count (AFC) after eight weeks of treatment. The secondary outcomes included the duration of gonadotrophins stimulation in days, the dose of gonadotrophins, the number and quality of retrieved oocytes, the endometrial thickness at hCG triggering day, the fertilization rate, implantation rate, clinical pregnancy rate (CBR) and the adverse effects of the medications.

**RESULTS:** We randomly assigned 108 women into both groups (54 in each arm). No significant difference between both groups regarding the baseline demographic characteristics or serum AMH levels. The mean basal AFC after eight weeks of DHEA supplementation was (10.2±4.4 vs. 13.8±5.3, respectively, p<0.001), while no significant difference in the placebo group (10.4±4.5 vs. 10.7±4.6, respectively, p=0.24). No significant difference in the total gonadotropin doses in both groups (p=0.64). DHEA group had statistically significant higher total number of retrieved oocytes (15.3±6.20 vs. 12.9±5.70, p=0.001), and the percentage of good quality oocytes (70.6% vs. 52.3%, p=0.007). No difference between both groups regarding the fertilization rate (62.4% vs. 51.7%, p=0.13), implantation rate (23.1% vs 20.4%, p=0.27), and the clinical pregnancy rate (37.0% vs. 35.2%, p=0.41). Regarding adverse effects, no patients reported major adverse effects during the study period. Only two patients from the DHEA group complained of hot flushes after four weeks of the supplement not interfering with their daily activities.

**CONCLUSIONS:** The use of DHEA in anticipated normal responders eight weeks before ICSI could be valuable in increasing the AFC, the number and quality of the retrieved oocytes relative to placebo, however no improvement in the fertilization, implantation, and clinical pregnancy rates.

**SUPPORT:** None.

**PREIMPLANTATION GENETIC TESTING**

**O-73** Monday, October 14, 2019 10:45 AM

**PGT FOR ANEUPLOIDY IMPROVES PERINATAL OUTCOMES COMPARED WITH FET ALONE: AN ANALYSIS OF THE 2014 AND 2015 SART DATA.**



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James Goldfarb, MD, MBA,<sup>a</sup> Rachel S. Weinerman, MD<sup>a</sup> <sup>a</sup>University Hospitals Fertility Center/Case Western Reserve University, Beachwood, OH; <sup>b</sup>NICHD, Bethesda, MD; <sup>c</sup>National Institute of Child Health and Human Development, Bethesda, MD.

**OBJECTIVE:** Clinical studies have shown a difference in the incidence of preterm delivery (PTD) and low birthweight (LBW) following IVF compared with natural conception. In recent years, frozen embryo transfer (FET) and pre-implantation genetic testing (PGT) have become increasingly common. However, few studies have evaluated the effects of embryo biopsy itself on perinatal outcomes. This study aims to assess the differences in perinatal outcomes of autologous FET using embryos that underwent biopsy for PGT versus those that did not.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The Society for Assisted Reproductive Technology (SART) database was used to identify day 5 FET cycles that did and did not undergo PGT from 2014-2015. Log binomial regression models were used to assess associations between embryo biopsy and pregnancy/perinatal outcomes. A sub-analysis analyzed the effects of PGT for aneuploidy (PGT-A) or PGT for monogenic disorders (PGT-M) on perinatal outcomes versus no biopsy. Models were adjusted for covariates including maternal age, race, BMI, smoking, prior IVF cycles, prior preterm/full-term births and cause of infertility. LBW was the primary outcome.

**RESULTS:** The mean age of the no biopsy patients (N=39,570) and biopsy patients (N=10,367) was 33.9 and 35.6 years, respectively (P<0.01). The mean number of embryos transferred was 1.6 and 1.2 (P<0.01) for non-biopsy and biopsy, respectively. Compared to patients whose embryos were not biopsied, patients whose embryos were biopsied were significantly more likely to have a clinical pregnancy (64.9 vs. 57.7%, adjusted risk ratio (aRR) 1.16, 95% confidence interval (CI) 1.13, 1.19) and live birth (56.2 vs. 46.8%, aRR 1.25, 95% CI 1.21, 1.3). The incidence of multiple gestation was, unsurprisingly, higher in the non-biopsy group (21.4 vs. 12.6%, aRR 0.68, 95% CI 0.60, 0.77). Of the live births (N=18,457 no biopsy, N=5,815 biopsy), the incidence of LBW was significantly lower following transfer of biopsied embryos versus those that were not biopsied (16.5 vs. 23.8%, aRR 0.74, 95% CI 0.66, 0.83). The odds of PTD was also significantly lower in the biopsy group compared to the non-biopsy group (16.0 vs. 21.5%, aRR 0.79, 95% CI 0.71, 0.88). These differences persisted when comparing PGT-A only versus no biopsy (LBW aRR 0.73, 95% CI 0.65, 0.83; PTD aRR 0.79, 95% CI 0.71, 0.89), but not PGT-M versus no biopsy (LBW aRR 1.08, 95% CI 0.73, 1.58; PTD aRR 0.93, 95% CI 0.63, 1.38).

**CONCLUSIONS:** The higher incidence of PTD and LBW in the non-biopsy group compared with the biopsy group can likely be, at least in part, explained by the larger proportion of multiple gestation pregnancies seen in that group. PGT-A, by reducing the number of embryos transferred, also incurs improved perinatal outcomes. Further analysis will assess for the contribution of multiple gestations to the differences in perinatal outcomes. However, it is overall reassuring that embryo biopsy is not associated with any negative effects on perinatal outcomes in FETs, and may potentially be associated with improved outcomes.

**SUPPORT:** None.

**O-74** Monday, October 14, 2019 11:00 AM

**DOES PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) HARM EMBRYOS? NO—A MULTI-CENTER, PROSPECTIVE, BLINDED, NON-SELECTION STUDY EVALUATING THE PREDICTIVE VALUE OF AN ANEUPLOID DIAGNOSIS AND IMPACT OF BIOPSY.**

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**OBJECTIVE:** Two common concerns regarding PGT-A are: 1) trophectoderm (TE) biopsy may have an adverse effect on embryo reproductive potential, and 2) embryos labeled aneuploid may have the potential to implant and deliver and thus may be wrongfully discarded. This study addresses these concerns by 1) comparing implantation rates of the overall study group to a control group, in which biopsy/PGT-A were not utilized, and 2) directly measuring the predictive value (PV) of a diagnosis of embryonic aneuploidy.

**DESIGN:** Prospective, blinded, non-selection study.

**MATERIALS AND METHODS:** All study participants underwent ICSI and blastocyst culture. Usable blastocysts underwent TE biopsy then vitrification. In the next cycle, patients underwent single embryo transfer (SET) of the best embryo selected solely on morphology. PGT-A analysis (targeted amplification-NGS-based) was performed only after the clinical outcome was known. Power analysis yielded a required sample size of 257 to detect a whole chromosome aneuploidy rate of 20% and estimate the PV within 5%. Control group (n=1000) consisted of patients having a cryo-SET not using PGT-A. As relates to the impact of biopsy, the sustained implantation rate (SIR) of the study group was compared to the SIR of the control group using logistic regression. As neither had access to PGT-A results, the groups differed only in that embryos in the study group had undergone TE biopsy. The second goal was to determine PVs of both euploid and aneuploid PGT-A results to correctly prognosticate clinical outcomes.

**RESULTS:** 285 transfers in the non-selection group and 1000 in the control group have known clinical outcomes. The SIRs in the study group (all independent of PGT-A result) were 53%, which is equivalent to that of the controls (54%), demonstrating no detectable detrimental effect of TE biopsy. 50 of the 285 transferred embryos were subsequently analyzed as aneuploid. **The PV of an aneuploid result for failure to deliver was 100% - the SIR was 0/50 (0%).** The PV for a euploid result was 68% (134 of 197). SIRs of embryos labeled mosaic (6 of 9) and segmental aneuploid (7 of 25) are reported but inadequately powered.

**CONCLUSIONS:** These data demonstrate that PGT-A results in no detectable adverse impact on clinical outcomes. The PV of an aneuploid result is sufficiently high that reproductively competent embryos are not being discarded with any demonstrable frequency. The error rate can never be zero, but it must be quite low. This study provides strong evidence regarding the safety of targeted sequencing NextGen PGT-A that may assist clinicians in counseling patients.

**SUPPORT:** Foundation for Embryonic Competence.

**O-75** Monday, October 14, 2019 11:15 AM

**PREIMPLANTATION GENETIC TESTING (PGT) IS ASSOCIATED WITH HIGHER ODDS OF A HEALTHY LIVEBIRTH AMONG DONOR OOCYTE RECIPIENTS IN THE UNITED STATES: A 2013-2015 NATIONAL STUDY.**

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**OBJECTIVE:** Available evidence suggests that PGT is associated with lower odds of live birth among donor oocyte cycles in the US through 2013<sup>1</sup>. However, outcomes reflective of more current practice are lacking.

**DESIGN:** Retrospective cohort of the Society for Assisted Reproductive Technology Database-Clinic Outcomes Reporting System from 2013 to 2015.

**MATERIALS AND METHODS:** Fresh donor oocyte cycles that resulted in embryo transfer (ET) were queried. Thawed oocytes were excluded. The primary outcome was a “good obstetric outcome (GBO),” defined as singleton live birth at ≥37 weeks with birth weight ≥ 2,500g and <

PGT-A Diagnosis	Ongoing Pregnancy / Delivered	Failed or No Pregnancy	Total	Sustained Implantation Rate (%)
Aneuploid	0	50	50	0.0%
<b>Euploid</b>	134	63	197	<b>68.0%</b>

TABLE. Effect of PGT in donor oocyte cycles by ET type: 1st cycle only

Outcome	Effect of PGT within Fresh ET		Effect of PGT within Frozen ET		Interaction P value
	RR (95% CI)	P value	RR (95% CI)	P value	
Good birth outcome	0.99 (0.87, 1.12)	0.892	1.23 (1.11, 1.37)	<0.001	0.010
Live birth	1.04 (0.97, 1.11)	0.310	1.18 (1.10, 1.27)	<0.001	0.007
Term	1.04 (0.93, 1.15)	0.507	1.17 (1.05, 1.29)	0.001	0.093
Clinical pregnancy	1.04 (0.99, 1.10)	0.143	1.16 (1.09, 1.22)	<0.001	0.010
Singleton	1.03 (0.94, 1.12)	0.563	1.17 (1.09, 1.27)	<0.001	0.250

4,000g. Multivariable generalized estimating equation models were fit to analyze the effect of PGT vs no PGT. Models were adjusted with *a priori* covariates: donor age; recipient age, BMI, smoking, parity, prior preterm birth, assisted hatching, single ET, and blast transfer. Interaction effect between transfer type (fresh vs frozen) and PGT was tested. Sensitivity analysis of the first cycle per patient was performed.

**RESULTS:** Of 25,387 included cycles, 2,372 had PGT performed while 23,015 did not. PGT was associated with increased rates of frozen ET (70% vs 41%,  $P<0.001$ ), single ET (67% vs 44%,  $P<0.001$ ) and blast transfer (87% vs 65%,  $P=0.003$ ). Interaction effect between transfer type and PGT was not significant, so the model was fit without an interaction term. Unadjusted rates of live birth and ongoing pregnancy were similar. After adjustment, cycles using PGT significantly increased the probability of a GBO (26.2% vs 23.7%, 1.08 risk ratio (RR), 95% confidence interval (CI) 1.00-1.12,  $P=0.047$ ). PGT was also associated with an 8% increase in probability of a live birth (95% CI 1.03-1.12), 7% increased probability of a term birth (95% CI 1.01-1.14) and 8% increased probability of a singleton (95% CI 1.03-1.14). When only the first cycle was tested ( $n=18,417$ ), there was a significant interaction between PGT and transfer type with superior outcomes for PGT in frozen ETs but no effect in fresh ETs (Table).

**CONCLUSIONS:** PGT, as practiced during the most recently available national data in women using donor oocytes, is associated with improved probability of a healthy live birth.

References: 1. Barad DH, et al. Impact of preimplantation genetic screening on donor oocyte-recipient cycles in the United States. *Am J Obstet Gynecol*. 217(5): 576 e571-578.

O-76 Monday, October 14, 2019 11:30 AM



**PREGNANCY OUTCOMES FOLLOWING IN VITRO FERTILIZATION FROZEN EMBRYO TRANSFER (IVF-FET) WITH OR WITHOUT PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) IN**

**WOMEN WITH RECURRENT PREGNANCY LOSS (RPL): A SART-CORS STUDY.** Shweta Bhatt, MD,<sup>a</sup> Jason Roy, PhD,<sup>b</sup> Sara S. Morelli, MD, PhD,<sup>a</sup> Peter McGovern, MD<sup>c</sup> <sup>a</sup>Rutgers New Jersey Medical School, Newark, NJ; <sup>b</sup>Rutgers - Department of Biostatistics and Epidemiology, Piscataway, NJ; <sup>c</sup>University Reproductive Associates, NJ.

**OBJECTIVE:** Euploid embryo transfer is thought to optimize outcomes in some couples with infertility, but there is insufficient evidence supporting this approach to management of recurrent pregnancy loss; thus, the aim of this study was to assess the pregnancy outcomes in couples with RPL after use of IVF-FET with PGT-A compared to IVF-FET without PGT-A.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This study included data collected by the Society of Assisted Reproductive Technologies Clinical Outcomes Reporting System (SART-CORS) for IVF-FET cycles between years 2010 through 2016. The experimental group included couples with RPL (strictly defined as a history of 3 or more pregnancy losses) undergoing IVF-FET with or without PGT-A. The analysis was restricted to autologous frozen embryo transfer cycles to better compare outcomes with and without PGT-A.

The primary outcome was live birth rate. Secondary outcomes included clinical pregnancy rate and spontaneous abortion rate. Differences were analyzed using generalized estimating equations (GEE) logistic regression models. GEE were used to account for multiple cycles per patient. Covariates included in the model were age, geographic region, race/ethnicity, and indication for assisted reproductive technologies. Analyses were stratified for age less than 35 years versus older than 35 years.

**RESULTS:** 24,007 IVF-FET cycles from the PGT-A group and 43,811 cycles from the control group were included in the analysis (Table 1). The adjusted odds ratio (OR) comparing IVF-FET with PGT-A versus without PGT-A for live birth outcome was 1.30 (95% CI: 1.24, 1.37) for age<35 and 2.01 (95% CI: 1.92, 2.11) for age≥35. For clinical pregnancy, the OR was 1.26 (1.20, 1.33) for age<35 and 1.82 (1.74, 1.91) for age≥35. Finally, for spontaneous abortion, the OR was 0.90 (0.82, 0.98) for age<35 and 0.79 (0.73, 0.86) for age≥35.

**CONCLUSIONS:** This is the largest study to date assessing the utility of PGT-A in women with RPL. PGT-A was associated with improvement in live birth, clinical pregnancy, and spontaneous abortion rates in women with RPL, with a larger difference noted in women with age greater than 35 years. Couples with RPL warrant counseling on all management options to reduce subsequent miscarriage, which may include IVF with PGT-A for euploid embryo selection.

Reference: N/A.  
SUPPORT: None.

O-77 Monday, October 14, 2019 11:45 AM



**CONCURRENT PRE-IMPLANTATION GENETIC TESTING FOR SINGLE GENE DISORDERS AND ANEUPLOIDY SCREENING FROM A SINGLE TROPHOBLAST BIOPSY USING TARGETED NEXT GENERATION SEQUENCING (NGS) WITHOUT WHOLE**

**GENOME AMPLIFICATION (WGA).** Heather Garnsey, BS, MPS,<sup>a</sup> Chaim Jalas, N/A,<sup>a</sup> Yiping Zhan, PhD,<sup>b</sup> Cara Vega, BS,<sup>a</sup> Vaidehi Jobanputra, PhD,<sup>a</sup> Richard Thomas Scott, Jr., MD,<sup>c</sup> Xin Tao, PhD,<sup>a</sup> <sup>a</sup>Foundation for Embryonic Competence, Basking Ridge, NJ; <sup>b</sup>The Foundation for Embryonic Competence, Basking Ridge, NJ; <sup>c</sup>IVI-RMA New Jersey, Basking Ridge, NJ.

TABLE 1. Pregnancy Outcomes Following IVF-FET Cycles With and Without PGT-A

	Live Birth Rate (%)		Clinical Pregnancy Rate (%)		Spontaneous Abortion Rate (%)	
	<35 years	≥35 years	<35 years	≥35 years	<35 years	≥35 years
IVF-FET without PGT-A n=43,811	48.6	35.3	58.4	46.9	9.1	11.1
IVF-FET with PGT-A n=24,007	55.3	52.5	63.9	61.8	8.2	8.9
P Value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

**OBJECTIVE:** NGS provides an unprecedented high-throughput, highly parallel, and base pair resolution data for genetic analysis. In this study, we developed a targeted NGS methodology for simultaneous pre-implantation genetic testing for monogenic/single gene disorders (PGT-M) and aneuploidy (PGT-A) from a single TE biopsy in a single procedure without the need of WGA.

**DESIGN:** Experimental Study

**MATERIALS AND METHODS:** Patients whose TE biopsies underwent PGT-M with clinical validated Taqman genotyping were included. Sequencing primers were designed for 11 different variants, including single nucleotide alterations, deletions, and insertions, and then validated on parental genomic DNA. Abnormal embryos donated for research were thawed, re-biopsied, and lysed. Two re-biopsies from each embryo were pre-amplified with a multiplex primer pool for PGT-M and PGT-A, using a two-step PCR strategy to incorporate sequencing library adapters and indexes. Sequencing was performed on Illumina NextSeq 550 using single 150bp reads. The average read depth was approximately 700X. Reads were aligned to a human reference genome (GRCh37/hg19) with the Burrows-Wheeler Aligner (BWA). The variants were called using Samtools for the PGT-M. Karyotypes were analyzed using an in-house clinical validated bioinformatics workflow. Taqman genotyping was performed on the amplified re-biopsies to further confirm the SGD results.

**RESULTS:** Two TE biopsies of 13 embryos from 7 families, including 9 variants (*CFTR* c.350G>A, *CFTR* c.1521\_1523delCTT, *HEXA* c.1421+1G>C, *HEXA* c.1274\_1277dupTATC, *PAX6* c.76C>G, *TBX5* c.342C>G, *PHEX* c.1180C>T, *HMGCL* c.122G>A, *HMGCL* c.497+4A>G), showed 100% concordant PGT-M diagnoses when compared to previous PGT-M based on Taqman qPCR genotyping. The PGT-A from multiple biopsies of the same embryos also demonstrated consistent karyotypes. For one variant, *MKSI* c.1411dupG, Taqman genotyping assay design was not possible due to the presence of a string of Gs at the mutation site. The targeted NGS provided accurate genotypes for parental DNA and 5 lymphocyte samples. Another X chromosome-linked nonsense mutation (*PCDH19* c.595 G>T) was validated on genomic DNA and 5 fibroblast samples.

**CONCLUSIONS:** This study provides proof of principle that PGT-M and PGT-A can be reliably and consistently performed simultaneously from the same TE biopsy in only one procedure without additional genotyping assays. Selecting euploid blastocysts that are unaffected by the PGT-M may provide the greatest opportunity for a successful outcome.

**O-78** Monday, October 14, 2019 12:00 PM

**MOSAIC EMBRYOS - A COMPREHENSIVE AND POWERED ANALYSIS OF CLINICAL OUTCOMES.**

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cine, Foster City, CA; <sup>b</sup>Zouves Fertility Center, Foster City, CA; <sup>c</sup>Overture Life, Madrid, Spain.

**OBJECTIVE:** To perform the largest analysis of mosaic embryo transfers to date, in order to achieve adequate power of analysis when evaluating which characteristics of mosaicism affect clinical outcomes.

**DESIGN:** Compiled analysis of data from multiple participating clinics.

**MATERIALS AND METHODS:** We collected clinical outcome data (implantation, ongoing pregnancy, birth) for transferred embryos classified as 'mosaic' by Preimplantation Genetic Testing (PGT). The following characteristics of mosaicism were considered: general mosaicism versus control (euploidy), type of aneuploidy involved in the mosaicism, level of mosaicism (using 40% or 50% as cutoffs), mosaic monosomies versus trisomies, and age. Chi-squared or Fisher's test was used to compare groups and evaluate statistical significance.

**RESULTS:** In the adjoining table we present our results from 372 mosaic embryo transfers, with more data presently being collected. This current analysis (powered to 100%) demonstrates that mosaic embryo transfers can result in pregnancies and births, albeit with decreased success rates compared to euploid embryos. Importantly, complex mosaics involving more than two chromosomes should be deprioritized, and higher levels of mosaicism correlate with poor clinical outcome.

**CONCLUSIONS:** This is the largest analysis of mosaic embryo transfers to date, and represents a valuable reference to generate guidelines on mosaic embryo selection and prioritization in the clinic.

**SUPPORT:** Zouves Foundation for Reproductive Medicine.

**REPRODUCTIVE BIOLOGY: HUMAN STUDIES**

**O-79** Monday, October 14, 2019 10:45 AM

**THE RELATIONSHIP BETWEEN CHRONOLOGIC AGE, OVARIAN RESPONSE, AND DNA METHYLATION OF WHITE BLOOD CELLS AND CUMULUS CELLS AMONG INFERTILE WOMEN UNDERGOING IVF.**



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**OBJECTIVE:** Aging is associated with predictable changes in DNA methylation in human somatic cells. An epigenetic clock model has been

General	Mosaic Embryos Transferred (n)	Control Euploid (n)	Mosaic Embryos Implantation (%)	Control Euploid Implantation (%)	P	Mosaic Embryos OP/B* (%)	Control Group OP/B (%)	P			
Mosaic Levels 40	372 ≤40 (n)	1987 >40 (n)	44.6% ≤40 Implantation (%)	61.0% >40 Implantation (%)	<0.0001 <sup>#</sup> P	35.2% ≤40 OP/B (%)	55.3% >40 OP/B (%)	<0.0001 <sup>#</sup> P			
Mosaic Levels 50	256 <50 (n)	116 ≥50 (n)	47.7% <50 Implantation (%)	37.9% ≥50 Implantation (%)	0.0915 P	38.3% <50 OP/B (%)	30.2% ≥50 OP/B (%)	0.161 P			
Age	278 ≤34 (n)	94 >34 (n)	47.5% ≤34 Implantation (%)	36.2% >34 Implantation (%)	0.0715 P	38.8% ≤34 OP/B (%)	26.6% >34 OP/B (%)	0.0347 P			
Losses vs Gains	120 Losses (n)	251 Gains (n)	48.3% Losses Implantation (%)	43.4% Gains Implantation (%)	0.435 P	39.2% Losses OP/B (%)	35.1% Gains OP/B (%)	0.489 P			
	89	53	48.3%	43.4%	0.605	37.1%	35.8%	1.00			
Type	1 or 2 Segmental (n)	Whole Chr (n)	Complex (>2)	Segmental Implantation (%)	1 or 2 Whole Chr Implantation (%)	Complex Implantation (%)	P (Complex vs Rest)	Segm. OP/B (%)	1 or 2 Whole Chr OP/B (%)	Complex OP/B (%)	P (Complex vs Rest)
	116	195	61	54.3%	46.8%	26.2%	<0.0001 <sup>#</sup>	44.0%	37.9 %	19.7%	<0.0001 <sup>#</sup>

\*OP/B = Ongoing Pregnancy/Birth.

<sup>#</sup> These statistically significant findings stem from an analysis that is >80% powered.

described by Horvath based on the methylation status of 353 CpG sites on human DNA (1). This model has been shown to accurately predict the chronological age of individuals. The current study sought to determine whether the age predicted using the Horvath algorithm in white blood cells (WBC) and cumulus cells (CC) is associated with the true age of patients and their response to ovarian stimulation.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Patients undergoing in vitro fertilization (IVF) between July 2017 and December 2018 were recruited under Institutional Review Board approval. On the day of oocyte retrieval, samples of peripheral blood and CC were collected from enrolled patients, and genomic DNA was isolated and stored at  $-80^{\circ}\text{C}$ . DNA from WBC was analyzed using the QIAAsymphony kit (Qiagen, Redwood City, CA, USA). DNA from CC was purified using DNeasy blood and tissue kit (Qiagen, Redwood City, CA, USA). Bisulfite conversion was performed using the Zymo EZ DNA methylation kit (Zymo Research, Irvine, CA, USA). The Illumina 850K DNA methylation EPIC array (San Diego, CA, USA) was then utilized to measure DNA methylation levels. Likelihood ratio tests based on nested linear models were utilized to assess the relationship between predicted age and true age.

**RESULTS:** Methylation data was analyzed for a total of 175 women undergoing IVF (mean age  $35.26 \pm 4.14$  years). The Horvath-predicted age calculation for WBC samples was consistent with the true chronological age of patients ( $p < 0.0001$ ). However, the predicted age from CC was significantly younger than patients' chronological age. The mean predicted age of patients based on methylation-based calculations from CC was  $8.56 \pm 2.07$  years. Poor response to ovarian stimulation during IVF, defined as five or fewer oocytes obtained during oocyte retrieval, did not affect the Horvath-predicted age based on calculations from WBC ( $p = 0.131$ ) or CC ( $p = 0.502$ ).

**CONCLUSIONS:** In women undergoing IVF, the epigenetic algorithm described previously by Horvath accurately predicts age when applied to WBC but not to CC. The methylation-based predicted age obtained from analysis of CC is substantially younger than the true age of patients, suggesting that CC exhibit unique methylation patterns that are distinct from those demonstrated by WBC. A poor response to ovarian stimulation is not associated with predictable changes in CpG methylation sites consistent with aging within WBC or CC. CC may have their own distinct methylation pattern which changes with age and must be clearly delineated since this may have implications for reproductive lifespan. Further studies are also required to determine whether alternative CpG sites can accurately predict chronological age or response to stimulation from CC samples.

**Reference:** 1. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol.* 2013; 14:R115. 10.1186/gb-2013-14-10-r115.

**SUPPORT:** None.

**O-80** Monday, October 14, 2019 11:00 AM

#### THE INCLUSION OF BLASTOMERES INTO THE INNER CELL MASS IN EARLY-STAGE HUMAN EMBRYOS DEPENDS ON THE SEQUENCE OF CELL CLEAVAGES DURING THE FOURTH DIVISION.

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**OBJECTIVE:** In mouse embryos, the fate of the inner cell mass (ICM) is known to be determined during divisions that occur from 8-16 cells. The outer cells give rise mainly to trophoblast (TE). In contrast, cells positioned inside the embryo give rise to ICM. However, there is no information on the order of incorporation of blastomeres into the ICM in human embryos. Refractile bodies (RBs) are some of the dysmorphic phenotypes that are frequently observed in human oocytes. RBs remain present, and almost unchanged in size, at least until embryos reach the blastocyst stage. Thus, our aim was to examine such early developmental stages using time-lapse recorded data, taking advantage of the large RBs within blastomeres as cellular markers.

**DESIGN:** Time series study.

**MATERIALS AND METHODS:** A total of 201 large refractile bodies in fertilized oocytes progressing through normal 2-cell to 8-cell stages were traced until they developed into a blastocyst. Cluster analysis was conducted to group the blastomeres according to the timing of cell division. Simple and

multiple logistic regression analysis were both used to estimate the order in which the cells divided from the second to the fourth division, with the attainment of ICM defined as the endpoint.

**RESULTS:** Following the second division, from 2 cells to 4 cells, the rates of RBs that were distributed to the ICM of blastomeres which cleaved first and second were 20.0% (20/100) and 18.8% (19/101) respectively. During the third division from 4 cells to 8 cells, the rates of RBs that were distributed to the ICM of blastomeres which cleaved first to fourth were 24.1% (13/54), 28.1% (16/57), 10.3% (4/39) and 11.8% (6/51) respectively. During the fourth division from 8 cells to 16 cells, the rates of RBs that were distributed to the ICM of blastomeres which cleaved first to eighth were 35.1% (13/37), 30.8% (8/26), 26.9% (7/26), 30.4% (7/23), 5.3% (1/19), 4.8% (1/21), 4.0% (1/25) and 0% (0/24) respectively. Cluster analysis showed that blastomeres which cleaved earlier tended to reach the ICM and there was a distinct difference between the rates of the second to the fourth divisions. Furthermore, the first 50% of cleaved blastomeres during the fourth division had significantly higher rates of being incorporated in the ICM ( $p < 0.001$ ). Simple logistic regression analysis was used to estimate the order in which the cells cleaved during both the third and fourth division before being included in the ICM, whereas multiple logistic regression analysis was only applied to the fourth cleavage. The third division was thereby removed as a confounding factor and the fourth division was found to be a predictor for ICM (OR:16, CI:4.1-63,  $p < 0.001$ ).

**CONCLUSIONS:** This study found that the cellular composition of the ICM is largely determined at the time of the fourth division. Moreover, it was shown that blastomeres which cleave first to fourth, during the fourth division from 8 cells to 16 cells, gain the ability to be incorporated in the ICM.

**O-81** Monday, October 14, 2019 11:15 AM

#### THE EFFECT OF AGE ON BIOENERGETICS OF HUMAN GRANULOSA CELLS.

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**OBJECTIVE:** Granulosa cells (GCs) support the synchronization of follicle development along with oocyte growth and maturation, being a potential biomarker of oocyte quality and in-vitro fertilization (IVF) outcomes. However, little is known about GC bioenergetics and its impact on female fertility. We aimed to characterize the bioenergetic profile of human GCs and detect the potential impact of aging on the energy metabolism.

**DESIGN:** Observational prospective cohort.

**MATERIALS AND METHODS:** From December 2017 to December 2018, the bioenergetic properties of GCs from 53 egg donors aged  $< 35$  years and 40 infertile patients  $\geq 38$  years were determined. Antagonist protocol was used to carry out controlled ovarian stimulation in all cases and women with diseases that could potentially impair mitochondrial function were excluded. Purified GCs from fresh samples of follicular fluid were seeded in Seahorse XF 24-well microplates. Primary culture was performed for 24 h and followed by a real-time assessment of the oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR) as a proxy for lactate formation on a Seahorse XF24 Extracellular Flux Analyzer (Seahorse Bioscience, Agilent Technologies, Santa Clara, CA, USA). The addition of a set of mitochondrial inhibitors/uncouplers allowed the evaluation of the bioenergetic profile of the samples. Results were normalized according to the protein concentration in each well. Furthermore, adenine nucleotides levels (AMP, ADP and ATP) in GCs were determined by reverse-phase high performance liquid chromatography after extraction with perchloric acid. Statistical analysis was performed using SPSS v24 (SPSS Inc., Chicago, IL, USA) and variables were compared using the ANOVA test, as appropriate.

**RESULTS:** GCs from oocyte donors aged  $< 35$  years showed a higher basal mitochondrial OCR compared to infertile women  $\geq 38$  years ( $12.8 \pm 1.6$  pmol  $\text{O}_2/\text{min}/\text{mg}$  vs.  $11.2 \pm 1.6$ ;  $p = 0.046$ ). Such difference is unlikely to be a result of reduced mitochondrial mass, once the maximum respiratory capacity remained unchanged ( $24.5 \pm 3.3$  pmol  $\text{O}_2/\text{min}/\text{mg}$  vs.  $22.4 \pm 4.3$ ;  $p = 0.226$ ). Thus, the difference in the basal mitochondrial respiration was due to a combined decrease in ATP turnover and the rate of proton leakage. Granulosa cells displayed a very high rate of glycolysis as estimated by the ECAR measurements. However, GCs from older patients showed a substantially lower rate of lactate formation ( $12.9 \pm 1.3$  mpH/min/mg vs.  $10.9 \pm 0.5$ ;  $p = 0.009$ ). Moreover, GCs from younger patients presented higher ATP/ADP

ratio ( $4.45 \pm 0.34$  vs.  $3.37 \pm 0.46$ ;  $p < 0.001$ ) and increased energy charge ( $0.87 \pm 0.01$  vs.  $0.83 \pm 0.02$ ;  $p < 0.001$ ).

**CONCLUSIONS:** The diminished rates of both mitochondrial respiration and glycolytic capacity reflect a marked reduction on the energy metabolism of GCs as women age, which was corroborated by the decreased ATP/ADP ratio and energy charge. Such a detrimental effect of age on GCs bioenergetics are likely to influence overall IVF performance. A new window of opportunity for diagnostic and therapeutic tools may arise from studies focusing on the bioenergetics of granulosa cells, oocytes and embryos.

**SUPPORT:** IVIRMA Madrid.

**O-82** Monday, October 14, 2019 11:30 AM

**REPEATED IMPLANTATION FAILURE PATIENTS DISPLAYS A GREATER DELAY OF THEIR RECEPTIVITY WINDOW DIAGNOSED USING A GENOMIC TEST UNDER HRT TREATMENT COMPARED TO NATURAL CYCLES.**



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**OBJECTIVE:** To identify the receptivity window in patients with repeated implantation failure prepared for frozen embryo transfer under hormone replacement therapy (HRT) treatment or natural cycle.

**DESIGN:** Endometrial biopsies were performed during the implantation window 7-9 days after the LH surge in natural cycle or 5-9 days after progesterone administration under HRT respectively. According to genomic testing result, the transfer strategy was: blastocysts transferred at the specific day where endometrium is identified as 'receptive'; D2/D3 cleavage stage embryos transferred 72/48 hours before the specific cycle day where endometrium is identified as receptive.

**MATERIALS AND METHODS:** 141 RIF patients with several unsuccessful fresh and/or frozen embryo transfers (FRET) were included. The number of previous failed attempts and non-implanted embryos were  $4.5 \pm 2.1$  and  $6.6 \pm 4$  respectively. Genomic testing of endometrial biopsies was performed under natural cycle or HRT. RNAs from biopsies were extracted and mRNA expression levels of specific genes predictive of endometrial receptivity were established using RT-qPCR. Clinical pregnancy was defined by visualization of a gestational sac with a positive fetal heartbeat.

**RESULTS:** Analyses of endometrial receptivity status in 141 RIF patients (age  $37.9 \pm 3.8$  years) revealed a strong inter-patient variability in the occurrence of the receptivity window with mostly a delay between 1 to 3 days. More precisely, biopsies were evaluated under natural cycle ( $n=29$ ), natural cycle with recombinant human chorionic gonadotropin (hCG, Ovitrelle) ( $n=7$ ) HRT ( $n=68$ ) and HRT with GnRH analogue (Decapeptyl) ( $n=37$ ). In patients evaluated under plain natural cycle, the majority were receptive at LH+8 (52%). The remaining 48% displayed receptivity equally at LH+6/+7 (24%) and LH+9 (24%). Under natural cycle with recombinant hCG, 72 % of RIF patients were receptive at hCG+9 while 14% were at hCG+6/+7 and 14 % at hCG+8. In patients under HRT, 38% and 41% were receptive at Pg+7 and Pg+8, respectively, whereas the remaining 21% were receptive before (Pg+5/+6 for 18%) or after (Pg+9 for 3%). Under HRT with GnRH analogue, the majority of RIF patients were receptive at Pg+8 (67%). Others were receptive at Pg+5/+6 (22%), Pg+7 (5%) and Pg+9 (8%). After personalized embryo transfer using the genomic testing strategy, the clinical pregnancy and live birth rates were 36.2 % and 28.4 % respectively.

**CONCLUSIONS:** The acquisition of the endometrial receptivity phenotype is more progressive under HRT compared with natural cycle. The majority of RIF patients displayed a delay in occurrence of their receptivity window revealing a potential cause for the implantation failure. Personalized embryo transfer according to the specific cycle day where endometrium is said receptive improves both clinical pregnancy and LBR in RIF patients under both HRT and natural cycle.

**SUPPORT:** This work was partially supported by a grant from the Ferring Pharmaceutical Company.

**O-83** Monday, October 14, 2019 11:45 AM

**THE ROLE OF VITAMIN D AS A PIECE OF THE UTERINE FACTOR INFERTILITY PUZZLE.**



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**OBJECTIVE:** Previous research has proven that there are vitamin D receptors in the endometrial cavity. This study aims to better understand the role of vitamin D on reproductive outcomes and uterine factor infertility. We sought to investigate the impact of vitamin D deficiency on endometrial thickness in patients with an elevated anti-mullerian hormone (AMH) representative of Polycystic Ovary Syndrome (PCOS).

**DESIGN:** Retrospective chart review at a private multi-location fertility clinic.

**MATERIALS AND METHODS:** A total of 1065 cycles were identified in patients with an AMH  $>5$  ng/ml between August 2016 to March 2019. All patients underwent timed intercourse or intrauterine insemination. Patients received Letrozole or Clomid therapy and were triggered for ovulation induction following the maturation of 1-3 follicles greater than 18 mm. Patients were divided into two groups: vitamin D  $< 30$  ng/ml, in the deficient group, and those with a vitamin D  $\geq 30$  ng/ml, in the sufficient group. The endometrial thickness was compared between the groups. Two sample t-tests and chi-square analysis were used to analyze the data using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

**RESULTS:** Baseline characteristic differences between the two groups, including age, race, BMI, and parity were not significant ( $p > 0.05$ ). The mean AMH levels of the groups were not significantly different ( $p > 0.05$ ). The mean endometrial thickness in patients with an elevated AMH with vitamin D deficiency and sufficiency was 6.968 mm versus 6.345 mm, respectively ( $p = 0.023$ ). There also was a slight negative correlation between vitamin D levels and endometrial thickness. When this data was extrapolated and analyzed in the first phase of the study, pregnancy outcome was compared between the two groups and no difference was noted ( $p > 0.05$ ).

**CONCLUSIONS:** Vitamin D deficiency is extremely prevalent and affects up to 36% of Americans. It has implications for many aspects of physiology and recent research has explored its effects on reproductive biology. It is hypothesized that decreased vitamin D leads to disruption of estrogen signaling and impaired reproductive outcomes. The endometrium has vitamin D receptors and a deficiency is associated with uterine hypoplasia in animal models. In a human model, vitamin D deficiency may be more prevalent in PCOS patients. Women with PCOS have also been shown to have elevated AMH levels  $>5$  ng/ml. Moreover, previous research in a PCOS model has shown a strong correlation between vitamin D deficiency and uterine factors leading to increased miscarriage risk. Therefore, we sought to explore the connection between AMH, vitamin D and endometrial lining.

Interestingly, our study results were statistically significant and showed that higher levels of vitamin D were correlated with a thinner endometrial lining. Thus, we conclude that though vitamin D plays a role in infertility and the endometrium, it is not a factor in determining endometrial thickness. Future studies are needed to determine how vitamin D interacts with the endometrium in PCOS patients leading to poorer reproductive outcomes and can focus on implantation, receptivity and miscarriage.

**SUPPORT:** None.

**O-84** Monday, October 14, 2019 12:00 PM

**DYNAMIC DNA METHYLATION DURING TROPHOBLAST DIFFERENTIATION IN HUMAN PERI-IMPLANTATION STAGE EMBRYOS REVEALED BY SINGLE-CELL WHOLE GENOME BISULFITE SEQUENCING.**



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**OBJECTIVE:** Trophoblast cells play an essential role in the interactions between the fetus and mother. Multipotent trophoblast cells undergo dynamic morphological migration and differentiation around the time of implantation to generate functional placenta, and their coordinated proliferation and differentiation are dependent upon the dynamic expression of a series of genes which are regulated in large part by epigenetic mechanisms. The aim of the

currently study was to characterize DNA methylation dynamics of trophoblast differentiation during human peri-implantation stage embryos by single-cell whole genome bisulfite sequencing (scWGBS).

**DESIGN:** Prospective research study.

**MATERIALS AND METHODS:** Vitrified and warmed day 5 (D5) human blastocysts were cultured to embryonic D8, D10, and D12 according Deglincerti et al., 2016. Cultured embryos were treated with trypsinLE, and three different classes of placental cells were selected based on their size and location, and individually snap-frozen. Cells were categorized as “small” representing cytotrophoblast, “large” representing syncytiotrophoblast, or migratory “migratory trophoblast” (MTB). scWGBS analysis was performed to compare the DNA methylation landscape of these three trophoblast cell lineages at three developmental time points near implantation.

**RESULTS:** We sequenced 96 samples and obtained approximately 10 million 150 bp paired-end reads per sample. In total, we captured approximately 1.2 million CpG sites at 10X coverage. Clustering analysis showed each trophoblast population had a distinct methylome, and the methylome profiles of trophoblast from different developmental stage had the most distinct methylomes. We revealed differentially methylated regions (DMRs) among trophoblast from different stages and lineages, and found that DMRs were largely located at intergenic regions, suggesting that these noncoding regions may play important roles in trophoblast specification. Pathway analysis of the annotated genes from DMRs revealed a number of signaling pathways that are known to be essential for trophoblast development. Finally, by using our previously reported RNA-seq data generated from trophoblast at the same developmental stages, we revealed a weak inverse correlation between gene expression and promoter methylation. Therefore, while CpG methylation plays a role in trophoblast differentiation, it is likely not the only regulatory mechanism involved in this process.

**CONCLUSIONS:** Using the human embryo in vitro extended culture system and scWGBS analysis, we characterized DNA methylation dynamics of implantation stage human early trophoblast cells. This comprehensive analysis provides insight into the critical features of the methylome in trophoblast development and differentiation, and offers meaningful information about the role of epigenetic mechanisms in human embryo implantation.

## REPRODUCTIVE SURGERY AND PROCEDURES

O-85 Monday, October 14, 2019 10:45 AM

### A SURVEY OF MICROSURGERY TRAINING AMONG UROLOGY RESIDENCY PROGRAMS.

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**OBJECTIVE:** The Accreditation council of graduate medical educations (ACGME) establishes surgical minimum numbers of cases for urologic training. Currently there is not a requirement for microsurgery, likely from a belief that residents do not have enough exposure. In an effort to evaluate the availability of microsurgery training among urology residency programs we conducted a **survey** of the programs. We compared microsurgery to male reconstruction, a sub-specialty with surgical minimums.

**DESIGN:** Cross sectional survey.

**MATERIALS AND METHODS:** We obtained a list of the 138 ACGME-accredited urology residencies and contact information from the American Urology Association. We contacted the residency programs by phone or email. For programs that did not reply, we performed a search of the program website. We administered a 3-question survey to assess resident subspecialty training in microsurgery, penile implant and artificial urinary sphincters as a comparison. Additionally, we evaluated whether the residents were trained by a fellowship trained academic faculty member, a private practice fellowship trained physician, or a non fellowship trained physician. Data are reported as frequencies.

**RESULTS:** We obtained data from 134 (97%) programs. A total of 104 (78%) of programs had fellowship-trained physicians for training in microsurgery, 87% for penile implants, and 88% for artificial urinary sphincters. The percentage of fellowship-trained microsurgeons per program did not vary significantly when comparing the different sections of the AUA, however the northeast and southeast sections had the lowest percentage (67% and 68%).

**CONCLUSIONS:** Approximately 80% of urology residency programs have exposure to microsurgery training from a fellowship-trained faculty member. We believe that the lack of a requirement for urologic microsurgery training is unsubstantiated since a majority of programs appear to have

fellowship trained faculty. In order to provide an equal exposure to all graduating urology residents, it is imperative that urology residency programs that lack microsurgery as a specialty identify a faculty member who is fellowship-trained.

**Reference:** None.

**SUPPORT:** None.

O-86 Monday, October 14, 2019 11:00 AM

### OUTCOME OF LAPAROSCOPIC ADHESIOLYSIS IN INFERTILE PATIENTS WITH PELVIC ADHESIONS FOLLOWING CESAREAN DELIVERY: A RANDOMIZED CLINICAL TRIAL.

Adel al shahat Algergawy, M.D.<sup>a</sup> Ayman Shehata Dawood, M.D.,<sup>b</sup> Hesham A. Salem, FA, M.D., M.D.,<sup>c</sup> Ahmed alsayed Alhalwagy, M.D.<sup>d</sup> <sup>a</sup>assistant professor, tanta, Egypt; <sup>b</sup>Lecturer at Tanta University, Tanta, Egypt; <sup>c</sup>Professor at Faculty of Medicine, Tanta University, Tanta, Egypt; <sup>d</sup>assistant professor at Faculty of Medicine, Tanta University, Tanta, Egypt.



**OBJECTIVE:** To evaluate the results of laparoscopic salpingoovariolysis and pelvic adhesiolysis in patients with post cesarean infertility as regards restoration of the fertility and achievement of pregnancy. To identify a group of patients who should primarily be offered laparoscopic salpingoovariolysis and pelvic adhesiolysis and those who should be treated by IVF.

**DESIGN:** Randomized prospective clinical trial.( UMIN000026900.).

**MATERIALS AND METHODS:** 164 patients with secondary infertility diagnosed due to the presence of periadnexal and pelvic adhesions by previous laparoscopy, abnormally placed ovaries by transvaginal U/S and abnormal course of the tubes in HSG, were randomly allocated into two groups : group I (82 cases) treated by laparoscopic adhesiolysis and group II(82 cases) treated for one year by controlled ovarian stimulation and IUI up to 3 trials. Diagnostic work-up of infertility was carried out for the infertile couples denoting normal semen parameters, patent both tubes at HSG, and ovulatory at ovulation testing with normal hormonal profile. The outcomes assessed were feasibility of adhesiolysis, cumulative pregnancy outcome were calculated for each group after one year. In laparoscopy, the utilized technique included mainly bipolar coagulation and unipolar fine needle electrode or unipolar scissors for excision of the adhesions.

**RESULTS:** According to the extent, nature and location of the periadnexal adhesions, the patients were classified into 4 groups: 8 cases inoperable 9.8%, 43 cases with mild type adhesions 52.4%, 26 cases with moderate type adhesions 31.7%, and 5 cases with severe type adhesions 10.9%. The patients were followed up postoperatively for a year. Overall pregnancy outcome was 50 pregnancies 60.97%. For patients with mild adhesions 33 pregnancies were achieved 76.7%, for patients with moderate adhesions 16 pregnancies were achieved 61.5%, and for patients with severe adhesions 1 pregnancy was achieved 20 %.69.4% of pregnant cases occurred in the first 6 months postoperatively. complications were present in (1.57%), cost for laparoscopic adhesiolysis range was (125.7-180.9 \$). while in group (II), over all pregnancy rate after one year was 10.9%(11cases).

**CONCLUSIONS:** laparoscopic adhesiolysis is the method of choice for dealing with mild to moderate periadnexal adhesions after C.S. as the Laparoscopic excision of adhesions offers a reasonable success rate as regards restoration of the fertility and achievement of pregnancy which should be performed before considering IVF. The pregnancy outcome after lysis of severe adhesions is poor. So, such patients are best treated by IVF but laparoscopy may help reposition of the ovaries to facilitate oocyte retrieval and closure of hydrosalpinx that may be present. After adhesiolysis, the patients <30 years of age can be advised to wait the pregnancy for 24 months, but for the patients > 30 years of age it may be necessary to seek IVF earlier due to the declining success of IVF with age. Precautions for prevention or minimization of postoperative adhesion reformation are also important for success of the reconstructive infertility surgery.

O-87 Monday, October 14, 2019 11:15 AM

### RESULTS OF CROMOSOMAL ANALYSIS OF PRODUCTS OF CONCEPTION USING TARGETED DIRECT EMBRYO BIOPSY BY OPERATIVE HYSTEROSCOPY.

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Case	Age	ART or S	Karyotype
#1	33	ED	30% mosaic, XXY
#2	33	FET, IVF	trisomy 22
#3	34	S	trisomy 22
#4	36	FET, IVF	euploidy
#5	37	FET, ED	trisomy 6
#6	38	IVF	trisomy 21
#7	38	S	trisomy 16
#8	39	S	trisomy 18
#9	39	FET, IVF	euploidy
#10	40	S	trisomy 15
#11	40	FET, IVF	euploidy
#12	41	FET, ED	XXY
#13	41	S	trisomy 22
#14	41	S	trisomy 17
#15	42	IVF	trisomy 13
#16	42	IVF	trisomy 16
#17	45	FET, ED	trisomy 8
#18	45	FET, ED	euploidy
#19	51	ED	trisomy 10

ED: egg donation; FET: frozen embryo transfer; S: spontaneous pregnancy.

Humana de Canarias, La Laguna, Spain; <sup>b</sup>Clinica Quisisana, Roma, Italy; <sup>c</sup>Department of Obstetrics and Gynecology, New York University, New York, NY.

**OBJECTIVE:** To evaluate the feasibility and effectiveness of embryo biopsy under direct visualization during operative hysteroscopy to avoid maternal contamination of products of conception (POCs) in cases of early miscarriages. Chromosomal analysis of POCs plays a fundamental role in the evaluation and treatment of recurrent pregnancy loss. With traditional Dilatation and Curettage maternal contamination is around 22%.

**DESIGN:** A series of 20 consecutive operative hysteroscopies was performed in infertile patients with miscarriage between 6+6 and 12 weeks from September 2015 through January 2019 in a private infertility clinic.

**MATERIALS AND METHODS:** Six spontaneous pregnancies plus 14 pregnancies obtained with Assisted Reproductive Technologies (ART): 5 fresh IVF cycles, 3 frozen IVF cycles, 2 fresh egg donation cycles and 4 frozen egg donation cycles. Mean patient age was 39±3.47 years. CRL was 2 to 51 mm. In 80% of cases a heartbeat was seen before miscarriage. Hysteroscopies were performed with a full bladder under transabdominal ultrasound guidance with a Voluson 8 or 6 ultrasound device with a RAB6-D Probe (GE, Austria). A 5 mm compact hysteroscope with an operative channel (Wolf, Germany) and hysteroscopic forceps were used for embryo sampling. Resection of the gestational sac was accomplished with hysteroscopic forceps and scissors. Array Comparative Genomic Hybridization or Next Generation Sequencing were used for chromosomal analysis.

**RESULTS:** Maternal contamination was reported in one case. Of the remaining 19 cases 14 were aneuploidies, 1 was a 45X/46XX mosaic and 4 presented an euploid karyotype (Table). There were no surgical complications. Sixteen of the patients had a sonohysterography performed postoperatively and there was no case of intrauterine adhesions or retained POCs.

**CONCLUSIONS:** Embryo biopsy under direct visualization during operative hysteroscopy is feasible and could be an effective method to limit maternal contamination and furnish targeted biopsies. By offering the ability to separately sample embryo vs. trophoblast this method could illuminate the implications of mosaicism in trophoblast biopsies. This technique may be less disturbing to the endometrium as shown by absence of intrauterine adhesions in postoperative sonohysterography.

**O-88** Monday, October 14, 2019 11:30 AM

**UTERUS TRANSPLANTATION - LIVING DONOR OUTCOME.** Liza Johannesson, MD, PhD, Giuliano Testa, MD Baylor University Medical Center, Dallas, TX.



**OBJECTIVE:** Uterus transplantation is a viable treatment option for women with uterine factor infertility. Most surgeries have utilized uterine grafts from living donors.

**DESIGN:** Clinical study.

**MATERIALS AND METHODS:** Under a IRB approved study protocol 13 living donor uterus transplantations have been performed to date at Baylor University Medical Center Dallas. All donors underwent a thorough evaluation process listed and informed consent was obtained in a private non-coercive environment. A living donor team that included a Nurse Coordinator, a Psychologist, a Living Donor Advocate, Transplant Surgeon, a Gynecologist, and a Fertility Specialist looked after the needs of the donor throughout the evaluation and donation process. The transplant surgeon and gynecologic surgeon decided the best surgical approach and options included abdominal hysterectomy, a robotic hysterectomy, or a laparoscopic hysterectomy. Adverse events related to the surgery were recorded and complications were classified based on the Clavien-Dindo classification. As per the United Network for Organ Sharing (UNOS) guidelines, every donor was followed at three months, six months, one year and two years.

**RESULTS:** The median follow-up interval for the living donor is 291 days with a range of 32-892 days. The median surgical time for donor hysterectomy was 6.5 hours. Median estimated blood loss was 0.80 L (0.4-1.7L) with two donors requiring intraoperative blood transfusion. Median hospital stay was 6 days and only one donor required intensive care unit (ICU). Intraoperative complications were uncommon. Five donors had short-term complications (<30 days after surgery) including gluteal claudication with ambulation that resolved 4 weeks post discharge (grade I), UTI (grade I), anemia requiring 1 unit of pRBC (grade II) and clostridium difficile infection (grade II). Three donors experienced long-term (>30 days) postoperative complications. These included vaginal cuff dehiscence (grade IIIb) and UTI (grade II). The vaginal cuff dehiscence was surgically repaired, and the UTI resolved with oral antibiotics.

Two donors were readmitted to the hospital after their surgery due to acute abdominal pain after intercourse on post-op day 97 diagnosed with vaginal cuff dehiscence (surgically repaired [grade IIIb]) and fecal impaction (post-op 27) requiring digital disimpaction under general anesthesia (grade IIIb). Both donors required 2 days of hospitalization and recovery was uneventful.

The median sick leave of the donors was 28 days (7-42 days). All donors returned to their normal activities after surgery.

Symptoms after vaginal intercourse were present in 8 out of the 13 donors. Most donors complained of temporary pain, tenderness or discomfort during sexual intercourse. One donor experienced severe pain after her first sexual encounter and was diagnosed with vaginal cuff dehiscence.

**CONCLUSIONS:** The follow-up data of our initial 13 living uterus donors indicate that the living donor hysterectomy is associated with low risk of complications. Importantly, all living donors returned to their presurgical social and physical outcome.

Reference: None.

SUPPORT: None.

**O-89** Monday, October 14, 2019 11:45 AM

### PREGNANCY OUTCOMES FOLLOWING HYSTEROSCOPIC CORRECTION OF T-SHAPED UTERI.

Shelby A. Neal, MD,<sup>a</sup> Richard Thomas Scott, Jr., MD,<sup>a</sup> Linnea R. Goodman, MD<sup>b</sup> <sup>a</sup>IVI-RMA New Jersey, Basking Ridge, NJ; <sup>b</sup>University of North Carolina, Raleigh, NC.



**OBJECTIVE:** To evaluate pregnancy outcomes following hysteroscopic correction of T-shaped uteri in patients with poor reproductive histories and T-shaped uterine cavities diagnosed by three-dimensional (3D) ultrasound.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** All patients at a single large IVF center undergoing fertility evaluation between 2016 and 2018 with a T-shaped uterine cavity diagnosed by 3D saline infusion sonohysterogram and a poor reproductive history (defined as ≥ 2 of the following events: clinical miscarriage, failed transfer of a euploid blastocyst, ectopic pregnancy, cycle cancellation secondary to endometrial hypoproliferation) were eligible for hysteroscopic correction and inclusion in the study. Surgery was performed in the early proliferative phase under conscious sedation. With saline as a distention medium, a hysteroscopic tissue morcellator was used to shave the lateral walls of the uterine cavity until both tubal ostia could be visualized simultaneously or healthy vascularized tissue was encountered. Post-operative imaging was performed the next month. All patients were followed for up to 6 treatment cycles. The primary outcome was ongoing pregnancy (presence of a fetal heartbeat at 8 weeks gestation). Secondary outcomes included miscarriage (pregnancy loss following documentation of gestational sac), ectopic pregnancy, and mean number of treatment cycles to achieve an

ongoing pregnancy. Patients who achieved an ongoing pregnancy were compared to those who did not using Student's *t*-test and Fisher's exact test.

**RESULTS:** Sixteen patients (age  $37.4 \pm 5.4$  years, median of 22.5 months attempting conception) with T-shaped uteri were included in this study. Indications for surgery included recurrent pregnancy loss ( $n=3$ ), recurrent implantation failure ( $n=2$ ), recurrent ectopic pregnancy ( $n=1$ ), endometrial hypoproliferation ( $n=3$ ), or a combination of factors ( $n=7$ ). There were no surgical complications. Post-operative imaging revealed expansion of the uterine cavity for 14 (87.5%) patients, as assessed by a single independent reviewer.

Following surgery, a total of 34 treatment cycles were attempted by 15 patients, resulting in 6 (17.6%) ongoing pregnancies, 3 (8.8%) miscarriages and 3 (8.8%) ectopic pregnancies. The cumulative ongoing pregnancy rate was 40.0%, with those who achieved an ongoing pregnancy requiring a mean number of 1.5 treatment cycles. There were no differences in age, body mass index, reproductive history or treatment modalities between patients who achieved an ongoing pregnancy and those who did not.

**CONCLUSIONS:** Patients who underwent hysteroscopic correction of a T-shaped uterus achieved a cumulative ongoing pregnancy rate of 40% over six treatment cycles. Further prospective studies with appropriate control groups are needed in order to ascertain if our findings represent a true improvement in pregnancy outcomes or simply regression to the mean.

**O-90** Monday, October 14, 2019 12:00 PM

**SINGLE EUPLOID FROZEN EMBRYO TRANSFER: EVALUATING IMPACT OF INTERVAL SINCE OPERATIVE HYSTEROSCOPY.**

Allison C. Petrini, MD,<sup>a</sup> Catherine W. Chan, MD,<sup>b</sup> Kelly McCarter, MD,<sup>b</sup> Micha Thompson, BA,<sup>a</sup> Monica Pasternak, MD,<sup>a</sup> Nigel Pereira, MD,<sup>a</sup> Steven Spandorfer, M.D.<sup>a</sup> <sup>a</sup>Ronald O. Perlman and Claudia Cohen Center for Reproductive Medicine, New York, NY; <sup>b</sup>Weill Cornell Medicine, New York, NY.



**OBJECTIVE:** There is limited data on the optimal time between operative hysteroscopy and embryo transfer<sup>1</sup>. Previous studies have demonstrated no difference in pregnancy outcome if the interval between polypectomy<sup>2</sup> or all indications<sup>1</sup> and embryo transfer (ET) is increased, but did not address operative hysteroscopy solely in the ideal group of patients undergoing single euploid embryo transfers. Our aim was to determine whether a difference in pregnancy outcome exists if the time between operative hysteroscopy and single euploid embryo transfer is increased.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Patients undergoing single euploid ET at our center over 3 years were examined for history of hysteroscopy prior to ET. Patients were grouped by surgical pathologic diagnosis and were further stratified into groups based on time between hysteroscopy and ET. They were designated as group 1, 2, or 3 to indicate an interval to next menstrual cycle, within 2 menstrual cycles, or within 3 menstrual cycles, respectively. Treatment outcomes were examined and classified as pregnant or not pregnant and then grouped into ongoing pregnancy or pregnancy loss. Student's and nonparametric *t*-tests, Mann-Whitney U test, and chi-square tests were used as indicated with  $p < 0.05$ .

**RESULTS:** A total of 1123 patients met inclusion criteria; 375 underwent hysteroscopy prior to ET during the study period. 77.7% of cases were operative, and 22.3% were diagnostic. Of operative cases, polyps represented 40% ( $n=98$ ), adhesions 34% ( $n=84$ ), and myomas 11% ( $n=28$ ). There were no differences in the baseline demographics between those who were pregnant and not pregnant, or between those with an ongoing pregnancy versus pregnancy loss. The baseline demographics were also comparable between those who underwent hysteroscopy and those who did not. There was no difference in the pregnancy rate between the groups who underwent ET 1, 2, or 3 menstrual cycles from operative hysteroscopy. In addition, there was no difference in the rate of ongoing pregnancy between groups.

	Group 1 (ET in next menstrual cycle)	Group 2 (ET in 2 menstrual cycles)	Group 3 (ET in 3 menstrual cycles)	<i>p</i>
Pregnancy rate	66.7%	65.7%	69.6%	NS
Mean age (years)	36.3 ( $\pm 4.0$ )	36.7 ( $\pm 4.1$ )	36.5 ( $\pm 4.0$ )	
Mean BMI ( $kg/m^2$ )	24.0 ( $\pm 5.7$ )	24.3 ( $\pm 5.5$ )	24.0 ( $\pm 5.3$ )	
Ongoing pregnancy	52.4%	57.1%	58.9%	NS
Mean age (years)	37.1 ( $\pm 3.7$ )	37.1 ( $\pm 3.6$ )	37.3 ( $\pm 3.6$ )	
Mean BMI ( $kg/m^2$ )	23.6 ( $\pm 4.6$ )	23.5 ( $\pm 4.6$ )	23.5 ( $\pm 4.5$ )	

**CONCLUSIONS:** The time between operative hysteroscopy and euploid frozen embryo transfer did not have an effect on the ability to become pregnant or the chance of having an ongoing pregnancy. Thus, clinicians can advise patients that they may proceed with a frozen transfer as soon as the next menstrual cycle after operative hysteroscopy.

References: 1. Aharon D, et al. Optimal Interval of Time from Operative Hysteroscopy to Embryo Transfer in an In Vitro Fertilization Cycle. *J Minim Invasive Gynecol.* 2018 Oct; pii: S1553-4650(18)31340-2.

2. Pereira N, et al. Does the time interval between hysteroscopic polypectomy and start of in vitro fertilization affect outcomes? *Fertil Steril.* 2016;105:539-54

**SCIENTIFIC CONGRESS PRIZE PAPER SESSION 2**

**O-91** Tuesday, October 15, 2019 10:45 AM

**ARTIFICIAL INTELLIGENCE ASSESSMENT OF TIME-LAPSE IMAGES CAN PREDICT WITH 77% ACCURACY WHETHER A HUMAN EMBRYO CAPABLE OF ACHIEVING A PREGNANCY WILL MISCARRY.**

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**OBJECTIVE:** To determine whether convolutional neural network (CNN) can be used to predict whether an embryo capable of achieving a pregnancy will ultimately miscarry or lead to live birth based on Artificial Intelligence (AI) analysis of time-lapse (TLM) embryo images.

**DESIGN:** Diagnostic efficacy assessment of the capability of an Artificial Intelligence system predicting outcome of blind data from two independent clinics, qualitatively assessed using ROC curves with AUC scores and confusion matrices to quantify sensitivity and specificity (True Positive = TP, True Negative = TN, False Positive = FP, False Negative = FN).

**MATERIALS AND METHODS:** 3412 Time Lapse images of blastocysts with known live birth outcome following a single embryo transfer ("Live Birth",  $n=1756$ , 51%; "Miscarriage",  $n=1656$ , 49%), were used to train a CNN model for image classification using Tensor Flow. These images were all derived from the same brand of time-lapse incubator (Embryoscope<sup>TM</sup>) and the same time post insemination (111.5 hours) to optimise input data normalisation. Images were allocated into Training (63%,  $n=2140$ ), Validation (15.5%,  $n=536$ ) and Test (21.5%,  $n=736$ ) with an even distribution for confounding factors (patient age cohort, clinic, oocyte donation) and outcome.

"Positive" data was labelled as embryos with a Live Birth outcome, "negative" data, as embryos with a Miscarriage outcome.

**RESULTS:** Following training (AUC=0.85; loss=0.3), the AI had a performance that improved on current embryo selection methods within a blind data set (AUC=0.79): True Positive = 358, True Negative = 207, False Positive = 153, False Negative = 18. 565/736 images were correctly predicted with the blind data set (77% accuracy), with a 58% specificity (207/360) and 95% sensitivity (358/376). Amongst embryos classified as High risk of miscarriage, miscarriage rate was 92% (207/225), compared with 30% (153/511,  $p<0.001$ ) when embryos were classified as reduced risk of miscarriage.

**CONCLUSIONS:** This is the first time that such a large data of single embryo transfer embryos from multiple clinics is used to assess AI capabilities

in predicting miscarriage once pregnancy was confirmed. The high accuracy rate achieved suggests that visible embryo characteristics play a predominant role in maintaining pregnancy to live birth, once the biochemical pregnancy is established, compared to other factors, such as, the endometrium, or other non-visible embryo factors. Additional information (i.e. embryo genetic or proteomic information, or endometrial information) may help improve the specificity of miscarriage prediction.

This technology will now be tested prospectively in other clinics to assess whether these results can be generalised and whether this technology can be used to help advance embryo diagnosis and selection, not only in terms of prediction of live birth, but also miscarriage, an outcome associated with considerable emotional distress to patients.

**SUPPORT:** This research was funded by São Paulo Research Foundation (FAPESP), grant number 2017 / 19323-5.

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### CONTRIBUTIONS TO PREMATURITY OF MATERNAL HEALTH CONDITIONS, SUBFERTILITY, AND ASSISTED REPRODUCTIVE TECHNOLOGY (ART).

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**OBJECTIVE:** Previous studies show subfertility and ART to increase rates of prematurity. Our goal was to evaluate health conditions that underlie prematurity and assess whether subfertility/ART influence these.

**DESIGN:** Retrospective cohort

**MATERIALS AND METHODS:** Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) deliveries were linked to Massachusetts birth certificates and hospital stays to identify privately insured singleton first births to women  $\geq 18$  years of age between 2004-2013. Deliveries were classified as ART when they linked to SART CORS, medically assisted reproduction (MAR) when fertility treatment was indicated on the birth certificate, unassisted subfertility (USF) when they had infertility diagnosis in prior hospital records or treatment for fertility in a prior delivery, and fertile if in none of the above groups. Late preterm birth (LPTB: 34-36 weeks) and early preterm birth (EPTB: <34 weeks) deliveries were compared to term deliveries ( $\geq 37$  weeks). Covariates that significantly influenced the outcome of premat-

urity in a binary analysis (mother's age/race/ethnicity, education, chronic diabetes and hypertension, prior uterine surgery, thyroid disease, ectopic pregnancy, leiomyoma, bleeding/menstrual disorders, electrolyte imbalance, psychological disorders, overweight/obesity, prior hospitalizations, gravidity, gestational diabetes, pregnancy hypertension, placental problems, bleeding, and father's age, race and education) were modeled using multinomial logistic regression.

**RESULTS:** There were 155,997 term, 8,210 LPTB, and 2,756 EPTB deliveries. When adjusted for all covariates and compared with fertile, LPTB was increased in the USF (adjusted odds ratio [AOR] 1.32, 95% confidence interval [CI] 1.06-1.65) and ART (AOR 1.43, 95% CI 1.31-1.56) but not MAR (AOR 1.16, 95%CI 0.98-1.67) groups and ETPB was increased in all (AOR 1.67, 95% CI 1.21-2.31, USF; AOR 1.67, 95% CI 1.31-2.12, MAR; AOR 1.40, 95% CI 1.22-1.62, ART). The four strongest effectors of prematurity when adjusted for all parameters were placental problems (AOR 4.01 LPTB; AOR 10.25 EPTB) pregnancy hypertension (AOR 2.14 LPTB; AOR 2.88 EPTB), chronic hypertension (AOR 1.85 LPTB; AOR 2.78 EPTB), and chronic diabetes (AOR 1.71 LPTB; AOR 2.01 EPTB) ( $P < 0.001$  for all). Removing subfertility/ART from the models did not change the AOR of any parameter. Removing placental problems increased LPTB (AOR 1.53) in the ART group and EPTB in UF (AOR 1.79), MAR (AOR 1.71) and ART (AOR 1.67) groups.

**CONCLUSIONS:** Although subfertility/ART increased LPTB and EPTB they did not alter the effect of other parameters. However, placental problems appear to be on the pathway to LPTB and EPTB for subfertile/ART deliveries.

**SUPPORT:** NIH R01HD067270

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### HIGHER INCIDENCE OF POSTPARTUM COMPLICATIONS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME.

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**OBJECTIVE:** To assess the risk of perinatal and postpartum depression and postpartum cardiovascular complications in women with PCOS.

**DESIGN:** Retrospective cohort study using administrative claims from 2000-2016.

**MATERIALS AND METHODS:** We included women aged 18-50 years enrolled continuously in the claims database Optum for a minimum of 6 months prior to conception, their entire pregnancy and at least 6 weeks following delivery. The PCOS cohort and all comorbidities were identified using specific codes from the International Classification of Diseases (ICD). Primary outcomes were incidence of perinatal and postpartum depression (within 3 months after date of delivery). Secondary outcomes included postpartum preeclampsia, postpartum eclampsia (within 6 weeks after the date of delivery), and peripartum cardiomyopathy (within the last month before or first 5 months after date of delivery). We compared outcomes between the PCOS and non-PCOS cohorts using univariate and multivariable logistic regression models adjusting for covariates including age, geographic location, preterm delivery, ART use, multiple births, pre-pregnancy depression, pre-pregnancy diabetes, pre-pregnancy hypertension, gestational diabetes, gestational hypertension, obesity, history of hyperlipidemia, smoking and race.

**RESULTS:** We identified 42,391 unique women with PCOS and 795,480 women without PCOS. Women with PCOS were more likely to have depression (4% vs 3%), diabetes (5% vs 1%), hypertension (6% vs 3%) and obesity (15% vs 5%) compared to women without PCOS ( $p < 0.001$  for all). They had a higher prevalence of gestational diabetes (24% vs 13%), gestational hypertension (14% vs 8%) and antepartum preeclampsia (5% vs 3%) than women without PCOS ( $p < 0.001$ ). In multivariable models, women with PCOS had a significantly higher odds of both perinatal and postpartum depression and postpartum

	PCOS (n=42, 391)	Non-PCOS (n=795,480)	OR	aOR (95% CI)	p-value
Perinatal depression	2, 956 (6.97)	40, 311 (5.07)	1.40 (1.35-1.46)	1.26 (1.21-1.31)	<0.001
Postpartum depression	899 (2.12)	10, 815 (1.36)	1.57 (1.47-1.68)	1.43 (1.33-1.54)	<0.001
Postpartum preeclampsia	390 (0.92)	4, 060 (0.51)	1.81 (1.63-2.01)	1.26 (1.13-1.40)	<0.001
Postpartum eclampsia	85 (0.2)	705 (0.09)	2.27 (1.81-2.84)	1.40 (1.10-1.80)	0.007
Peripartum cardiomyopathy	116 (0.27)	1, 182 (0.15)	1.84 (1.52-2.23)	1.16 (0.95-1.43)	0.146

preeclampsia and eclampsia compared to those without PCOS (Table). Postpartum results remained similar in planned sensitivity analyses in women with at least one year of pre-conception data, when including date of delivery in outcome definition and when varying the definition of perinatal and postpartum depression from the DSM-V criteria of 4 weeks postpartum to a commonly utilized literature length of one year postpartum.

**CONCLUSIONS:** This study demonstrates for the first time that women with PCOS are at higher risk for depression, preeclampsia and eclampsia in the fourth trimester of pregnancy. Our results highlight the need for comprehensive screening and targeted interventions during the postpartum period in this high-risk population.

**SUPPORT:** Snigdha Alur-Gupta is supported by the NIH T32 Training Grant: HD007440

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### DEVELOPMENTAL POTENTIAL OF ANEUPLOID HUMAN EMBRYOS BEYOND IMPLANTATION.

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**OBJECTIVE:** Aneuploidy is one of the major limitations of human reproduction. However, the developmental consequences of specific aneuploidies during the early stages of post-implantation development remain poorly characterized. In this study, we investigated the post-implantation development of human embryos with specific aneuploidies compared to euploid embryos.

**DESIGN:** Experimental study

**MATERIALS AND METHODS:** Aneuploid (n=71) and euploid (n=22) human blastocyst stage embryos were cultured up to day 9 of development, using a novel methodology that allows human embryo development *in vitro* to post-implantation stages<sup>11</sup>. Embryos with specific aneuploidies (trisomy 21 [n=14], trisomy 15 [n=16], trisomy 16 [n=24], monosomy 21 [n=17]), which have a high global incidence and lack strong pre-implantation alterations in development were assessed. Immunofluorescent techniques were used to detect the expression of molecular markers associated with development of embryonic and extra-embryonic lineages on day 9: OCT4+ embryonic epiblast (precursor of the fetus and amnion), GATA6+ extra-embryonic hypoblast (precursor of the yolk sac), and OCT4- GATA6- extra-embryonic trophoblast (precursor of the placenta). Chromosome copy number in post-implantation embryos was determined by targeted next generation sequencing (tNGS).

**RESULTS:** We first analysed the global development of the different aneuploidies, and observed that monosomy 21 embryos had a higher incidence of arrest in culture (p=0.0105), which was specific to the implantation phase of development. The three trisomies analyzed developed similarly up to day 9 in terms of attachment, and preservation of the embryonic and extra-embryonic lineages. However, careful analyses of cell numbers revealed that while trisomy 15 and trisomy 21 embryos developed similarly to euploid embryos, trisomy 16 embryos had a specific hypoproliferation defect of the trophoblast (p<0.004), while the epiblast and primitive endoderm tissues (derived from the inner cell mass) were not affected (p=NS). In addition, analyses of the specific subset of monosomy 21 embryos that did not arrest during culture, unveiled a similar hypoproliferation phenotype of the trophoblast. To test whether this phenotype was due to mosaicism, embryos were dissected into different pieces for tNGS. This revealed 3 non-concordant cases out of a total of 29 embryos analysed. One case was identified as 45,XX,-21 based on trophectoderm biopsy at day 5 and PGT-A by tNGS, but showed 45,XX,-21 and 46,XX on day 9. Remarkably, this embryo developed well up to day 9 *in vitro*, although it displayed a hypoproliferative trophoblast.

**CONCLUSIONS:** Our results show that specific aneuploidies lead to specific developmental phenotypes during the first days of post-implantation development. Culturing human embryos beyond day 7 *in vitro* is a powerful tool to understand how chromosomal alterations influence embryo morphogenesis and to detect cases of mosaicism that cannot be identified by sampling trophectoderm cells on day 5.

**References:** 1. M. N. Shahbazi *et al.*, Self-organization of the human embryo in the absence of maternal tissues. *Nat Cell Biol* **18**, 700-708 (2016).

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### A LIFESTYLE INTERVENTION TARGETING WOMEN WITH OBESITY AND INFERTILITY IMPROVES THEIR FERTILITY OUTCOMES, ESPECIALLY IN WOMEN WITH PCOS: A RANDOMIZED CONTROLLED TRIAL.

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**OBJECTIVE:** To evaluate the impacts of a lifestyle intervention on fertility outcomes in women with obesity seeking fertility treatments, with or without the polycystic ovary syndrome (PCOS).

**DESIGN:** Randomized controlled trial including 127 women with infertility and obesity, with no major infertility factor (female or male).

**MATERIALS AND METHODS:** Women were randomized either in the control group (CG; usual standard of care) or the lifestyle program group (LPG; lifestyle intervention with individual sessions (kinesiologist and nutritionist) and group sessions). A total of 108 women have completed  $\geq 6$  months of the study (51 LPG and 57 CG). Since randomisation was stratified according to the presence of PCOS (PCOS: CG=35, LPG=33, Non PCOS: CG=22, LPG=18), we present results on fertility outcomes at 18 months of follow-up, and anthropometric and lifestyle changes at 6 months, in all women as well as in women with or without PCOS. Student's *t*-tests were used to compare means and chi-squared tests for proportions. P-values  $\leq 0.05$  were considered significant.

**RESULTS:** As compared to the CG, our lifestyle program increased significantly the pregnancy rates for all women (60.8% vs 38.6%, 1.58 fold, p=0.021) or for women with PCOS (57.6% vs 34.3%, 1.68 fold, p=0.005), but this difference was not significant for women without PCOS (66.7% vs 45.5%, 1.47-fold, p=0.18). Our lifestyle program similarly increased the rates of spontaneous pregnancy (All: 33.3% vs 12.3%, 2.7 folds, p=0.009; PCOS: 27.3% vs 5.7%, 4.8 folds, p=0.016; Non PCOS: 44.4% vs 22.7%, 2.0 folds, p=0.145) and live birth (all: 51.0% vs 36.8%, 1.39 fold, p=0.139; PCOS: 54.8% vs 31.4%, 1.75 fold, p=0.05; Non PCOS: 66.7% vs 45.5%, 1.47 fold, p=0.18). Pregnancy rates in women using an assisted reproductive technology (ART, n=63) were increased in the LPG for all women (58.6% vs 47.1%, 1.24 fold, p=0.36) and mildly for women with PCOS (51.9% vs 47.6%, 1.09 fold, p=0.744), although this was not statistically significant. Finally, compared to the CG, the LPG has lost significantly more weight at 6 months (all: 3.43%  $\pm$  4.45 vs 0.89%  $\pm$  3.67, p=0.003; PCOS: 3.66%  $\pm$  4.47 vs 0.93%  $\pm$  4.22, p=0.015), except for non-PCOS women who lost less weight (-2.31%  $\pm$  4.34 vs -0.48%  $\pm$  2.84, p=0.139). Women in the LPG also improved significantly more the quality of their diet (healthy eating index, all: +18.0  $\pm$  13.7 vs +5.3  $\pm$  12.4 on 100, p<0.001; PCOS: +20.0  $\pm$  15.0 vs +4.4  $\pm$  13.0 on 100, p<0.001; Non PCOS: +13.6  $\pm$  10.4 vs +6.5  $\pm$  11.3 on 100, p=0.055).

**CONCLUSIONS:** A lifestyle intervention targeting women with obesity and infertility improves their chances of conceiving, especially spontaneously (with no fertility treatment). Our results suggest that such intervention could benefit women with PCOS even more. It is also possible that lifestyle modifications improve the effectiveness of ART in these women, but to a lower extent. Accordingly, our lifestyle approach could significantly decrease the costs associated with the fertility care of women with obesity and infertility.

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### CONSERVATIVE SURGERY FOR OVARIAN TORSION IN YOUNG WOMEN: PERIOPERATIVE COMPLICATIONS AND NATIONAL TRENDS.

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**OBJECTIVE:** Ovarian torsion represents a gynecologic emergency for which oophorectomy was traditionally performed due to hypothetical risk of thrombotic events or necrosis following detorsion. Mounting evidence, however, has supported ovarian-preserving surgery in young women. This study compared incidence of perioperative complications and analyzed recent populational trends following conservative surgery vs. oophorectomy in young women.

**DESIGN:** A retrospective analysis of the Nationwide Inpatient Sample, a US population-based database, between 2001-2015.

**MATERIALS AND METHODS:** Women <50 years of age who underwent inpatient surgery for ovarian torsion were included. Those with ovarian malignancy were excluded. ICD-9 codes were used to compare those who had conservative surgery (detorsion with or without cystectomy) vs. oophorectomy. Perioperative complications were compared between the two groups after fitting a propensity score-based inverse probability of treatment weighting (IPTW) model to adjust for background differences. Multivariable analyses with a binary logistic regression model were performed to determine independent factors associated with conservative surgery, and temporal trends were assessed.

**RESULTS:** There were 89,801 cases of ovarian torsion during the study period; 20,643 (23.0%, 95% confidence interval (CI) 22.7-23.3) women had conservative surgery, while 69,158 (77.0%) women had oophorectomy. In the IPTW model, conservative surgery was independently associated with a decreased risk of perioperative complications by approximately 30% after controlling for patient demographics, surgical factors, and hospital characteristics (8.3% vs. 11.9%, adjusted-odds ratio 0.700, 95%CI [0.649-0.754], P<0.001). In particular, conservative surgery was not associated with venous thromboembolism (0.3% vs. 0.3%, P=0.561) or sepsis (0.3% vs. 0.3%, P=0.843). Rate of conservative surgery started decreasing steadily at age 14 and then sharply declined following age 35 (P<0.001). On multivariable analysis, younger women, those with higher income, residents of the Northeast, those who had laparoscopic surgery, and those who had surgery at large and urban-teaching hospitals were more likely to undergo conservative surgery (all, P<0.001). Conversely, those with higher comorbidity and morbidly obese women were less likely to have conservative surgery (all, P<0.001). Performance of conservative surgery significantly increased from 18.8% in 2001 to 25.1% in 2015 (33.4% relative increase, P=0.001).

**CONCLUSIONS:** Conservative surgical management of ovarian torsion is not associated with increased perioperative complications. While utilization of conservative surgery for ovarian torsion is increasing in the United States overall, significant variation exists based on patient demographic, surgical, hospital, and geographic factors. This supports continued efforts for ovarian preservation after detorsion in young women given long-term hormonal and fertility benefits.

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### CATCHING UP TO THE MANDATE: A MYSTERY CALLER STUDY OF SOCIETY FOR ASSISTED REPRODUCTIVE TECHNOLOGY (SART) MEMBER CLINICS IN STATES MANDATING FERTILITY PRESERVATION (FP) COVERAGE.



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**OBJECTIVE:** As of April 2019, six U.S. states now mandate private insurers to cover FP for patients facing iatrogenic infertility. In Illinois, this mandate extends to cover Medicaid recipients. We sought to assess how SART clinic representatives address questions relating to insurance coverage for cancer patients seeking FP.

**DESIGN:** 'Mystery caller' telephone survey of SART member clinics in FP coverage-mandated states.

**MATERIALS AND METHODS:** We developed and piloted two telephone scripts of a caller posing as a 30-year-old breast cancer patient interested in FP: one script specific to private and the other to public insurance. Our primary outcome was whether information provided reflected state FP coverage mandates. We called all SART member clinics in FP coverage-mandated states (as of 1/1/2019) and performed a second call for Illinois clinics to assess responses specific to public insurance. Clinics were categorized by state, practice type (university-affiliated vs private practice), and time since enactment of coverage mandate (pre- vs post-1/1/2019). All responses were recorded and coded for analysis; responses to "Does insurance [or Medicaid] ever cover FP for cancer patients?" were categorized as positive (yes, usually, sometimes or it depends) versus negative (no, usually not, and I don't know), and subcategorized as confident (yes, usually, no, and usually not) or not confident (sometimes, it depends, or I don't know). Data are presented as %, and Fisher's exact test ( $p < 0.05$ ) was used to compare responses across clinic characteristics and insurer.

**RESULTS:** We identified 35 SART member clinics in IL, CT, DE, MD, and RI; 3 were excluded due to nonresponse or conflicts. Of 32 clinics, 29 (91%) offered FP for cancer patients. Less than half (39%,  $n=11$ ) were confident that insurance would cover FP, and 7% reported they did not accept any insurance. Only 21% ( $n=6$ , 4 from IL) referenced legislation mandating FP coverage. Neither practice type nor time since enactment of state mandate influenced clinic responses regarding private insurance coverage of FP ( $p > 0.05$ ). In IL, less than half (44%) were confident that private insurance would cover FP. We found that IL clinics were more likely to report any positive confirmation for coverage if the patient reported she had private compared to Medicaid insurance (81% vs. 14%,  $p < 0.005$ ). 87% of IL clinics did not accept Medicaid, and none provided a direct referral. University clinics in IL were more likely than private clinics to accept Medicaid (66.7% vs 0%,  $p < 0.05$ ) and know that Medicaid covered FP (66.7% vs 0%,  $p < 0.05$ ).

**CONCLUSIONS:** In states where FP coverage is mandated, the SART clinic representatives were often unaware of insurance coverage of FP. In IL, clinic staff are especially uninformed of Medicaid coverage of FP. Our findings identify a critical gap in knowledge among SART clinics, as patients may choose to permanently abandon FP due to misinformation regarding financial coverage. This study highlights the need for educational interventions and improved clinic protocols to reflect state mandates. Future research should examine public awareness of coverage mandates.

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### HUMAN SPERM MORPHOLOGY ANALYSIS USING SMARTPHONE MICROSCOPY AND DEEP LEARNING.



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PhD,<sup>b</sup> Hemanth Kandula, BS,<sup>a</sup> Sandeep Kota Sai Pavan, BS,<sup>a</sup> Divyank Yarravarapu, BS,<sup>a</sup> Hadi Shafiee, PhD.<sup>a</sup> <sup>a</sup>Brigham and Women's Hospital, Harvard Medical School, Boston, MA; <sup>b</sup>Massachusetts General Hospital, Harvard Medical School, Boston, MA.

**OBJECTIVE:** Sperm concentration, motility and morphology are among the primary measures of a semen analysis. While at-home methods for sperm concentration and motility evaluations have been developed, owing to the complexity of morphology assessments, automated microscopy-based evaluation of sperm morphology at-home has never been possible. Furthermore, all proposed alternative technologies have either been too expensive or inaccurate. An inexpensive, portable and automated sperm morphology assessment tool for at-home testing can improve access to care especially in resource limited settings.

**DESIGN:** We utilized a smartphone-based microscope that we have developed for sperm concentration and motility analysis<sup>1</sup>. Here, we also adapted a deep-convolutional neural network that made use of a layered learning approach described previously, to sperm images acquired using an inexpensive smartphone device<sup>2</sup>. For evaluation, clinical samples were prepared by the center's trained technicians and were evaluated conventionally under a microscope. These slides were then imaged using our smartphone system and the results of the two methods were tested qualitatively (normal/abnormal), to illustrate the system's applicability as a screening tool.

**MATERIALS AND METHODS:** A smartphone-based microscope setup was built using 3D printing<sup>1</sup>. The total cost of materials for the system was estimated to be \$5. Over 170,000 annotated sperm images were used in developing our network and 7000 individual sperm images from 35 semen samples were used in evaluating the network. An android application processes the image on phone, without the requirement of internet access in <1s and reports the % of morphologically normal sperm that are present in the sample. The system reported measurements and conventionally reported morphology scores were compared to evaluate the performance of the smartphone system.

**RESULTS:** The smartphone system when tested with 35 patient semen samples was able to correctly identify samples based on morphological quality ( $\geq 4\%$  good morphological sperm) with 88.5% accuracy with a 95% confidence interval (CI) ranging between 73.3% to 96.8%. Furthermore, a receiver operator characteristic (ROC) revealed an area under the curve (AUC) of 0.928 (CI: 0.788 to 0.988), which confirmed that the artificial intelligence (AI) algorithm can effectively separate the normal and abnormal morphological quality samples. The sensitivity and specificity of the network was 80% and 92% along with a positive and negative predictive value of 80% and 92%, respectively.

**CONCLUSIONS:** Here, we have reported the first implementation of an automated artificial intelligence-empowered smartphone-based tool for measuring sperm morphological quality. The system is inexpensive, rapid, accurate, and reliable making it suitable at-home screening test. Our future focus is on developing a microfluidic sample handling device that can further sample preparation for at-home use. We have shown that the utilization of AI technologies allows for objective/standardized evaluation of sperm morphology using inexpensive hardware for the first time.

**References:** 1. M. K. Kanakasabapathy, M. Sadasivam, A. Singh, C. Preston, P. Thirumalaraju, M. Venkataraman, C. L. Bormann, M. S. Draz, J. C. Petrozza and H. Shafiee, *Science translational medicine*, 2017, **9**, eaai7863.

2. P. Thirumalaraju, C. Bormann, M. Kanakasabapathy, F. Doshi, I. Souter, I. Dimitriadis and H. Shafiee, *Fertility and sterility*, 2018, **110**, e432.

**SUPPORT:** This work was partially supported by the Brigham Precision Medicine Developmental Award (Brigham Precision Medicine Program, Brigham and Women's Hospital) and R01AI118502, R01AI138800, and R21HD092828 (National Institute of Health).

O-99 Tuesday, October 15, 2019 11:15 AM

### HAS THE MASSACHUSETTS INFERTILITY MANDATE LIVED UP TO ITS PROMISE?

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**OBJECTIVE:** Massachusetts law mandates coverage for 31 conditions or services including infertility. The public perception is that all residents have

coverage for infertility treatment. In fact, the majority do not. Federal pre-emptions and other exemptions in state law restrict fertility coverage even in states with mandates. The goal of this study was to determine the percentage of reproductive age women in Massachusetts with coverage for infertility treatment.

**DESIGN:** Population based cross-sectional study

**MATERIALS AND METHODS:** We obtained de-identified population level data from 3 sources: the State Census Bureau (number of women aged 20-44 living in Massachusetts in 2016); the Center for Health Information and Analysis (CHIA) (number of women self-insured or with public assistance insurance); and the US Department of Defense (number of women with military health insurance).

**RESULTS:** In 2016, 1,142,542 women aged 20-44 lived in Massachusetts. Table 1 shows their health insurance enrollment.

TABLE 1. Population and Coverage

Population	#of Women	% of Women
Census data: age 20-44 residing in MA in 2016	1,142,542	100%
Exempt from Coverage		
Self-insured employer sponsored Plans	498,931	43.7%
Public Assistance Insurance	184,179	16.1%
Military Insurance	5,080	0.4%
No Insurance	39,746	3.5%
Subtotal	727,936	63.7%
Potential Coverage		
Mandate Eligible Insurance	414,606	36.3%

**CONCLUSIONS:** There are notable exemptions to Massachusetts' mandated health benefits statutes. Self-insured policies provided by employers are governed by the Federal Employee Retirement Income Security Act (ERISA) and are not subject to state mandated benefits. Federally-funded plans covering Military and Civilian federal employees as well as MassHealth, which administers the Massachusetts Medicaid program, are also exempt.

As a result, only 36.3% of reproductive aged women in Massachusetts have health insurance subject to the mandate. Moreover, the Massachusetts Division of Insurance permits insurers latitude in applying the law. Thus, women who do not meet certain biological criteria, have exceeded a predetermined number of treatment cycles, or have surpassed a total dollar cap are exempt from coverage. Un-partnered women may not have treatment coverage until they have paid out-of-pocket for 6-12 months of treatment.

Massachusetts is often cited as the model state for health insurance coverage of infertility treatment. Yet, only 36.3% of reproductive aged women are subject to the mandate and even fewer have meaningful access to services when exceptions are considered. Given that Massachusetts' mandate has fallen short of affording women real access to care, future study is warranted with the hope of informing legislative action.

O-100 Tuesday, October 15, 2019 11:30 AM

**RIGHT TO HEALTH: THE SITUATION OF ASSISTED REPRODUCTION TECHNIQUES WITH GAMETES DONATION IN ITALY.**

Giulia Scaravelli, PhD, Roberto De Luca, Master degree, Roberta Spoletini, Master degree, Vincenzo Vigiliano, Master degree. Simone Bolli, high school, Simone Fiaccavento, high school, Lucia Speziale, Master degree, Anna Bertini, Master degree Italian Assisted Reproductive Technology Register, Italian National Institute of Health, Rome, Italy.



**OBJECTIVE:** The postponement of childbearing age in Italy, determine a large number of older women wanting a baby. In these cases and in many others gametes donation could represent an important option for infertility treatments. Only the recent change of the Law 40/2004 in Italy in April 2014 allowed infertile couples to access to gametes donation. The objective is to analyze the number and type of gametes donation cycles collected by The Italian Assisted Reproductive Technology Register (IARTR) since 2014.

**DESIGN:** In this study IARTR analyzed retrospectively data on 16807 gametes donation cycles performed from May 2014 till December 2017 on

14577 patients. 220 Assisted Reproductive Technology (ART) clinics and 179 Intrauterine Insemination (IUI) clinics sent data to the National Italian Register and participated in the study with 102 out of 220 (46.4%) and 3 out of 179 (1.7%) performing donation cycles.

**MATERIALS AND METHODS:** All ART and IUI centres which has performed at least one cycle with gametes donation, that have sent data during the study period were included in the study. Parameters regarding number of patients, number of cycles, treatment indications, age classes, pregnancies and live births rates were statistically analysed using SPSS statistic 25.0.

**RESULTS:** The centers participating in the annual data collection and which performed at least one donation cycle were 105, of which only 12 were public structures. 14577 patients underwent 16807 initiated cycles (4.4% of all ART and IUI cycles performed in the same period) that included 14800 cycles performed with complex ART techniques and 2007 cycles performed with IUI-D. Most cycles (44.6%) were carried out with oocyte donation, 25.8% with sperm donation and 29.6% with cryopreserved embryos obtained after a donation. The main indication for treatment for oocyte donation was the maternal advanced reproductive age (37.9%), while for sperm donation, 94.0% of indications refer to diseases that affect sperm vitality. Almost all the gametes used for the treatments come from abroad (97.8% of the oocytes and 80.7% of semen). The pregnancy rate per transfer carried out in the study period was 37.7% for fresh egg donation, 34% for cryopreserved oocytes, 33.5% for cryopreserved embryos and 38.6% for sperm. The percentage of multiple deliveries was 32.6%, 17.5%, 13.8% and 18.1% respectively. For the semen used in intrauterine insemination the pregnancy rate per cycle started was 20% and the multiple delivery rate was 14.4%. In these 4 years 3857 children were born alive, equal to 7.3% of those born from all ART techniques in the same period.

**CONCLUSIONS:** The Italian situation regarding gametes donation policy, arises the question of equity in access to these procedures. The impossibility of finding donors in our country, dictated by the absence of information campaigns and the lack of possibility of compensation for donors limits the use of these techniques in public structures. Only a few regions have adopted policies to improve donation cycles in public centers.

O-101 Tuesday, October 15, 2019 11:45 AM

**THE PRICE IS RIGHT? ANALYSIS OF PRICE TRANSPARENCY IN ASSISTED REPRODUCTION.**

Katherine Elise McDaniel, MD, Meghan B. Smith, MD, Brittany L. Klooster, MD, Rachel Blair Danis, MD, Kristin Bendikson, M.D., Richard J. Paulson, MD, MS, Jacqueline Ho, MD MS. University of Southern California, Los Angeles, CA.



Price or Discount Information Type	Percentage of Clinics	
	Reporting (n = 375)	Mean Cost Reported
Any Price Listed	78 (20.8%)	N/A
Consultation	33 (8.8%)	\$364.84 (SD \$159.64)
IUI Cycle	26 (6.9%)	\$1,665.07 (SD \$900.10)
IVF Cycle	48 (12.8%)	\$10,334 (SD \$2,980.00)
FET Cycle	24 (6.4%)	\$4,060.00 (SD \$1,017.86)
OC Cycle	33 (8.8%)	\$7,190.00 (SD \$2,694.00)
Any Discount/Financing Listed	249 (66.4%)	N/A
Medication Discounts	69 (18.4%)	N/A
Shared Risk Refund	75 (20.0%)	N/A
Lending Programs	151 (40.2%)	N/A
External Financing or Grants	98 (26.1%)	N/A
Internal Financing (e.g.: Multi-Cycle Discounts)	88 (23.4%)	N/A
Military Discounts	74 (19.7%)	N/A
Cancer Discounts	36 (9.6%)	N/A

**OBJECTIVE:** A recent influx of assisted reproductive technology (ART) and services promote price transparency, with detailed costs of services listed on websites. Given current market trends, we sought to determine how existing clinics perform in terms of price transparency and to what extent they provide information on available discounts and financial assistance.

**DESIGN:** Cross-sectional analysis of Society for Assisted Reproductive Technology (SART) registry clinics.

**MATERIALS AND METHODS:** Clinics were identified through the SART website clinic search function on 4/14/19. Military clinics and those clinics without a website were excluded. Between 4/15/19 and 4/22/19, each clinic's website was queried. Practice location (city, state) and type (private vs. academic vs. other [e.g.: managed care]) were recorded. Prices for consultation, intrauterine insemination (IUI; including monitoring and sperm preparation), in vitro fertilization (IVF; excluding pre-implantation genetic testing), frozen embryo transfer (FET), and oocyte cryopreservation (OC) were recorded. Mean costs were calculated for each reported price.

**RESULTS:** 382 clinics were listed on the SART website and 375 met study inclusion criteria. Table 1 illustrates the number and percentage of clinics that provided costs for services, information on discounts, and available financial assistance on their websites. Only 22.8% (67/293) of private practices and 11.7% (9/77) of academic practices reported the price of one or more services.

**CONCLUSIONS:** Most existing clinics do not report the costs of consultation or of various treatments on their websites. This lack of transparency may actually create barriers to care if costs are lower than anticipated. The majority of clinics provide information on available discounts and/or financing information, the most common being links to lending programs. Full disclosure of cost on clinic websites will not only match new market trends in ART, but also demystify the costs of fertility treatment and potentially democratize fertility care by fostering price competition.

**O-102** 12:00 PM Tuesday, October 15, 2019

#### **EMERGENCY COS IN ONCOFERTILITY PRESERVATION.**

Marouen Braham, Associate professor,<sup>a</sup> Sarah Amari, Medical Degree,<sup>b</sup> Khadija Feriel Kacem Berjeb, Associate professor,<sup>c</sup> Molka Bouricha, resident,<sup>c</sup> Wissal Jaafar, Medical Degree,<sup>a</sup> Manel Hamdoun, Medical Degree,<sup>d</sup> Linda Debbabi, Medical Degree,<sup>c</sup> Olfa Bahri, Sr., Professor,<sup>d</sup> Anis Fadhlou, Associate Professor,<sup>a</sup> Fethi Zhioua, Pr.<sup>a</sup> <sup>1</sup>Aziza Othmana University hospital, Tunis, Tunisia; <sup>2</sup>Gynecology, Obstetric and Reproductive Medicine Department. Aziza Othmana University Hospital, Tunis, Tunisia; <sup>3</sup>Reproductive Medicine Laboratory. Aziza Othmana University Hospital., Tunis, Tunisia; <sup>4</sup>Biochemistry Department. Aziza Othmana University hospital., Tunis, Tunisia.



**OBJECTIVE:** There is often an urgent need to start cancer treatment. Therefore, protocols with alternative timing to start COS have been proposed in fertility preservation. Is random start COS as effective as conventional start COS in fertility preservation?

**DESIGN:** We conducted a retrospective study.

**MATERIALS AND METHODS:** The study included 104 patients recently diagnosed with cancer and in preparation for gonadotoxic therapy, from January 2017 to January 2019.

Patients were evaluated within 24-48h after the referral, clinically, by ultrasound (antral follicular count) and by an AMH dosage. The underlying conditions were mainly: Hodgkin's Lymphoma (46% patients), Breast cancer (30%), Rectal cancer (3%), and various other pathologies (Ovarian, Gastric cancer, T Lymphoma, etc.). AMH levels ranged from 0,2ng/ml to a maximum of 10,5ng/ml. All 104 patients underwent IVF cycles using GnRH antagonist protocol. 65 patients underwent an early-follicular start COS (Group 1), whereas 49 had a random (late follicular or luteal) start (Group2). The addition of Letrozole was compulsory in case of estrogen-sensitive tumors and E2 levels, closely monitored.

Oocyte retrieval was done transvaginally in 65% of cases and was transurethral in 35%. Oocyte or embryo vitrification were proposed to the patients based on marital status and preference.

Our aim was to compare the outcome of random-start versus conventional start COS

**RESULTS:** Our patients' age ranged from 14 to 41 years, with a mean of 26 in both groups. As for status, 73% were single, and 27% married. Mean AMH levels were similar in both groups (2.34+/- 0.7 in Group 1; 2.29+/-

0.9 in Group 2). All patients followed an antagonist protocol. There was no significant difference in the duration of stimulation (10.6+/-2 days in case of early follicular start COS versus 10.13+/- 2 days in random-start COS; p=0.5).

Furthermore, the total number of oocytes retrieved upon pick-up was similar in both groups (8.06+/-3 in Group 1 versus 7.37+/-2 in Group 2; NS). As for the maturity rate, no significant difference was noted (76% oocyte maturity rate in early follicular start COS and 73% in random-start COS).

**CONCLUSIONS:** Random start COS seems as effective as conventional start COS in fertility preservation. The main advantage is that Random-start can minimize delays and allow more patients to undergo fertility preservation, and yet still proceed with cancer treatment within 2 weeks.

#### **ANDROGEN EXCESS**

**O-103** Tuesday, October 15, 2019 10:45 AM

#### **PREGNANCY-RELATED ECONOMIC BURDEN OF POLYCYSTIC OVARY SYNDROME (PCOS).**

Carrie Riestenberg, MD,<sup>a</sup> Anika Jagasia, BA,<sup>b</sup> Ricardo Azziz, MD, MPH.<sup>c</sup> <sup>a</sup>University of California, Los Angeles, Los Angeles, CA; <sup>b</sup>University of Pennsylvania, Philadelphia, PA; <sup>c</sup>University at Albany, SUNY, Albany, NY.



**OBJECTIVE:** PCOS is the most common endocrine abnormality of reproductive-aged women, affecting approximately 6-10% of unselected reproductive-aged women (~4-6 million women in the U.S.)<sup>1</sup> depending on the criteria used (National Institutes of Health (NIH), Rotterdam or the Androgen Excess and PCOS Society). The cost of initial evaluation and treatment of reproductive-aged women with PCOS, excluding those associated with pregnancy, has previously been shown to represent a significant financial burden to our health care (~\$4.36 billion in 2004 dollars, 5.39 billion in current dollars).<sup>2</sup> The goal of the present study was to define, using current definitions and prevalence or incidence data, the minimal excess economic burden of pregnancy in women with PCOS in the U.S.

**DESIGN:** Systematic literature review and economic burden analysis.

**MATERIALS AND METHODS:** We performed a systematic review of the published literature to identify studies evaluating the epidemiology of PCOS in pregnancy and its clinical consequences and costs. We selected the three most consistently reported and prevalent pregnancy related health outcomes associated with PCOS to generate our cost analysis: gestational diabetes (GDM), pregnancy-induced hypertension (PIH) and preeclampsia. We linked published cost data for the aforementioned health consequences to their excess incidence attributable to PCOS in order to calculate overall estimated health care-related economic costs.

**RESULTS:** We estimate that there were 254,463 PCOS-related births in the U.S. in 2017. After accounting for baseline risk, we estimate that an excess 27,177 of these births were complicated by GDM, 16,286 by PIH, and 7,354 by preeclampsia as a result of PCOS. We estimate the mean excess annual cost of pregnancy-related care for women with PCOS in the U.S. due to GDM to be \$53,563,896, PIH to be \$149,831,200 and preeclampsia to be \$84,291,548.

**CONCLUSIONS:** A conservative estimate of the excess cost of pregnancy-related complications attributable to PCOS in the U.S. exceeds \$287 billion in current dollars.

References: 1. National Health Statistics Reports Number 86, May 20, 2015. <https://www.cdc.gov/nchs/data/nhsr/nhsr086.pdf>

2. Azziz R, Marin C, Hoq L, Badamgarav E, Song P. Health care-related economic burden of the polycystic ovary syndrome during the reproductive life span. The Journal of Endocrinology & Metabolism. 2005; 90:4650-4658.

**SUPPORT:** None

**O-104** Tuesday, October 15, 2019 11:00 AM

#### **SEVERE ENDOMETRIOSIS IN RHESUS MACAQUES CONSUMING A WESTERN-STYLE DIET (WSD) AND CHRONICALLY TREATED WITH ANDROGEN.**

Ov D. Slayden, PhD,<sup>a</sup> Cecily V. Bishop, PhD,<sup>b</sup> Emily Mishler, MS,<sup>c</sup> Lauren Drew Martin, DVM,<sup>c</sup> Heather M. Sidener, DVM, DACLAM,<sup>c</sup> Jon D. Hennebold, PhD,<sup>d</sup>



Richard L. Stouffer, PhD<sup>a</sup> Professor, Portland, OR; <sup>b</sup>Affiliation not provided; <sup>c</sup>Oregon National Primate Research Center, Division of Reproductive & Developmental Sciences, Beaverton, OR.

**OBJECTIVE:** To evaluate the effects of chronic mild hyperandrogenemia and/or consumption of a western-style diet (WSD) on the rate of endometriosis in rhesus macaques.

**DESIGN:** 2 by 2 factorial.

**MATERIALS AND METHODS:** Female rhesus macaques were treated beginning at menarche (2.5 y) with implants containing cholesterol or testosterone (T). The T implants elevated serum T levels approximately 5-fold and did not prevent menstrual cyclicity (1). Half of the animals in each group were fed a standard monkey chow diet, and the other half received a WSD resulting in 4 treatment groups: controls (C), T, WSD, and T+WSD (n = 9-10/group). After 3 years of treatment, the animals received multiple reproductive manipulations including laparoscopically-guided TruCut™ needle biopsies of the endometrium, ovarian stimulation, laparoscopic follicle aspiration and caesarian section. Beginning in year 5, the animals were evaluated laparoscopically and by transabdominal ultrasound for the presence of endometriosis. Endometriosis was scored for disease stage based on the ASRM Revised Classification criteria. In macaques, stage I animals present with superficial red/brown lesions and mild adhesions. Stage II is defined by extensive lesions (>1 cm<sup>2</sup> in area in aggregate) and adhesions. Stage III endometriosis includes deep lesions > 2 cm<sup>2</sup> in size that are identifiable by ultrasound and confirmed by laparoscopy. Stage IV animals present with large lesions and ablation of the uterine cul de sac. Endometriotic stromal cells were isolated from one of the animals with advanced disease. The cells were treated *in vitro* with steroid hormones (1 ng/ml: E<sub>2</sub>, E<sub>2</sub> + P, T, DHT E<sub>2</sub>+T and E<sub>2</sub>+DHT) in replicate plates (n=5). The cells were analyzed for expression of steroid receptors (ESR1; PGR, AR), aromatase (CYP19A1), and Ki-67 by quantitative real-time PCR (qPCR). Representative lesions were assessed by immunohistochemistry (IHC).

**RESULTS:** T + WSD animals presented with the highest prevalence (7 out of 10) of severe endometriosis, including 5 animals with Stage IV disease (Table 1). In contrast, 3 of 10 controls possessed only adhesions or Stage I endometriosis. IHC showed strong staining for ESR1, PGR and AR staining. Stromal cells, *in vitro*, expressed minimal ESR1 and PGR regardless of hormonal treatment. However, treatment with T and DHT (with or without E<sub>2</sub>) significantly increased AR and aromatase levels (P<0.05). Moreover, treatment with T and DHT significantly increased Ki-67 staining intensity (P<0.05).

TABLE 1. Frequency and stage of endometriosis (n=10/group).

	Control	T	WSD	WSD + T
Adhesions	1	0	1	1
Stage I	2	1	1	1
Stage II	0	1	0	0
Stage III	0	1	2	0
Stage IV	0	4	5	7
Total Cases	3	4	5	7

**CONCLUSIONS:** These data support the hypothesis that T in the presence of an obesogenic diet increases the risk for advanced endometriosis. Moreover, androgen alone drives cell proliferation in endometriotic cells obtained from chronically treated T+ WSD animals.

References: 1. Bishop CV, Mishler EC, Takahashi DL, Reiter TE, Bond KR, True CA, Slayden OD, Stouffer RL. Chronic hyperandrogenemia in the presence and absence of a western-style diet impairs ovarian and uterine structure/function in young adult rhesus monkeys. Hum Reprod. 2018;33(1):128-39.

**SUPPORT:** NIH P50-HD071835 (RLS/JDH), and NIH P51-OD011092 (ONPRC).

**O-105** Tuesday, October 15, 2019 11:15 AM

**POSTPARTUM WEIGHT RETENTION IN WOMEN WITH PCOS AND CONTROLS.** Iris Tienlynn Lee, MD,<sup>a</sup> Snigdha Alur-Gupta, M.D.,<sup>b</sup> Anuja Dokras, M.D., Ph.D.<sup>a</sup>

<sup>a</sup>UNIVERSITY OF PENNSYLVANIA HEALTH SYSTEM,



PHILADELPHIA, PA; <sup>b</sup>University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.

**OBJECTIVE:** To evaluate whether women with PCOS are more likely to retain weight after delivery compared to women without PCOS.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Women over age 18 who delivered a live, full-term singleton from January 2014-2019 and had a prepregnancy weight, peak pregnancy weight, and at least one weight recorded within a year of their most recent delivery were included. Weights were categorized into four time points: 6weeks, 3 months, 6 months, and 12 months postpartum. ICD codes were used to identify the PCOS cohort through the hospital database. Covariates included in univariate and multivariate models to assess the association between high weight retention (five kilograms or more above prepregnancy weight) and PCOS at the six-week time point included age, parity, race, and prepregnancy BMI; for the three-month time point, age and prepregnancy BMI were included.

**RESULTS:** A total of 7692 women were included (5.6% with PCOS). On average, women with PCOS were older (median age 32 versus 31 years), had fewer prior deliveries (median of 1 versus 2), and were more likely to be White (54.31% versus 36.07%) (p<0.001 for all) compared to controls. Women with PCOS had higher prepregnancy BMI (26.4 vs. 24.7 kg/m<sup>2</sup>, p<0.001) as well as higher prevalence of gestational diabetes (10.72% versus 6.32%, p<0.001) and hypertension (13.02% versus 8.66%, p=0.002) compared to controls. At each of the four postpartum time points, women with PCOS had a higher BMI than controls. However, total weight gain during pregnancy was lower in the PCOS group (12.50 vs. 13.29 kg, p=0.015). The percentage of women who surpassed Institute of Medicine (IOM) guidelines for pregnancy weight gain based on BMI was similar between groups (43.46% PCOS versus 46.85% controls, p=0.158). At six weeks postpartum, the amount of weight retained by women with PCOS (2.95kg, -0.77-6.07kg) was lower than controls (3.96 kg, 0.76-7.32kg). The likelihood of retaining five or more kilograms at this time was lower in the PCOS group (32.91% versus 40.95%, aOR 0.79, 95% CI 0.63-0.99). The proportion of high weight retainers at three (28.17% versus 38.41%), six (34.41% versus 32.16%) and 12 (22.69% versus 28.32%) months was not significantly different between the PCOS group and controls, although approximately 20% of the cohort had an increase in BMI category at the end of 12 months. Prepregnancy BMI category did not differentially affect postpartum weight retention in either group.

**CONCLUSIONS:** Women with PCOS were more likely than controls to be obese prior to pregnancy but had less weight gain in pregnancy. This is the first study to examine postpartum weight loss in women with PCOS up to one year and shows no difference compared to women without PCOS. Early nutritional counseling is needed to prevent pregnancy weight gain above the IOM guidelines. This is offered to obese pregnant patients in our health system, and our study also highlights the need for continued nutritional counseling during the postpartum period given the proportion of women with high weight retention and increase in BMI category 12 months after delivery.

**O-106** Tuesday, October 15, 2019 11:30 AM

**METABOLIC SYNDROME (METS): FECUNDABILITY AND ADVERSE PREGNANCY OUTCOMES WITH OVULATION INDUCTION (OI) IN POLYCYSTIC OVARY SYNDROME (PCOS).** Sushila Arya, MD,<sup>a</sup>

Karl R. Hansen, MD PhD,<sup>a</sup> Richard S. Legro, M.D.,<sup>b</sup> Robert A. Wild, M.D., M.P.H. Ph.D.<sup>a</sup> NICHD's Reproductive Medicine Network, <sup>a</sup>University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma city, OK; <sup>b</sup>Penn State College of Medicine, Hershey, PA.



**OBJECTIVE:** To determine the association of MetS with fecundability and pregnancy complications after OI with clomiphene citrate (CC) or letrozole (L) for infertility with PCOS.

**DESIGN:** Secondary analysis of a randomized clinical trial (RCT) investigating probability of live birth following OI with CC or letrozole in infertile women with PCOS.

**MATERIALS AND METHODS:** 750 couples undergoing OI treatment in The Pregnancy in Polycystic Ovary Syndrome II (PPCOSII) trial. This trial enrolled women at 14 sites in U.S, age 18-40 years with PCOS (anovulation or oligovulation, with either hyperandrogenism or polycystic ovaries on

ultrasound) and at least one patent fallopian tube. Women underwent OI with either CC or letrozole for up to 5 cycles. Male partners were required to have a semen analysis with sperm concentration of at least 14 million/ml. Chi-Square/Fisher exact, Student's t, and logistic regression were utilized as appropriate. A p-value of < 0.05 was considered statistically significant. MetS was defined by International Diabetes Federation criteria. Overweight/obese = HiBMI (>25 kg/M<sup>2</sup>), and Very high BMI (VHBMI) >35kg/M<sup>2</sup>. Pregnancy complications included pre-eclampsia (Pr-E), gestational diabetes (GDM), preterm delivery, large for gestational age (LGA) and intrauterine growth restriction (IUGR).

**RESULTS:** Prevalence of HiBMI was 83.5%, VHBMI 50.8%, and MetS 34.5%. For VHBMI 47% had MetS compared to 21% when BMI < 35Kg/M<sup>2</sup>. The odds for clinical pregnancy (fetal heart rate) were 0.59 (0.38, 0.90) with MetS and 0.60 (0.43, 0.83) with VHBMI in the CC group. Similarly the OR for live birth were reduced, 0.55 (0.34, 0.89) with MetS and 0.61 (0.43, 0.85) with VHBMI. Pregnancy complications occurred in 42.5% with CC and 41.5% with letrozole. In the presence of MetS the OR for GDM was 2.68 (1.22, 5.85) with letrozole, adjusted for VHBMI; and it was 3.31 (1.01, 10.82) with CC. The odds for LGA were higher after adjusting for VHBMI also (both OI agents). VHBMI increased the odds (1.76 [1.25, 2.48]) for Pre-E (CC and letrozole). MetS was not associated with Pre-E in either group.

**CONCLUSIONS:** MetS and VHBMI lowered the odds of fecundity with CC. MetS was associated with higher odds of gestational diabetes and LGA infants after adjusting for VHBMI with both agents. MetS did not confer risk for Pr-E with either agent. VHBMI in contrast was associated with greater odds for Pr-E with either. Incidence of pregnancy complications is high following OI for oligovulation in PCOS, in part because of MetS and/or VHBMI.

**SUPPORT:** The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD): U10 HD077680, U10 HD39005, U10 HD38992, U10 HD27049, U10 HD38998, U10 HD055942, HD055944, U10 HD055936, and U10HD055925. This research made possible by the funding by American Recovery and Reinvestment Act. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NICHD or NIH.

**O-107** Tuesday, October 15, 2019 11:45 AM

**OXIDATIVE CAPACITY IS PRESERVED IN LIPOPOLYSACCHARIDE (LPS) TOLERANT MONONUCLEAR CELLS (MNC) OF OBESE WOMEN WITH POLYCYSTIC OVARY SYNDROME (PCOS).** Frank González, M.D.,<sup>a</sup> Robert V. Considine, Ph.D.,<sup>b</sup> Jiaping Xue, Ph.D.,<sup>a</sup> Anthony J. Acton, Jr., B.S.<sup>b</sup> <sup>a</sup>University of Illinois at Chicago College of Medicine, Chicago, IL; <sup>b</sup>Indiana University School of Medicine, Indianapolis, IN.



**OBJECTIVE:** In PCOS, lipid-induced oxidative stress promotes inflammation.<sup>1</sup> *In vitro* exposure to lipid+LPS suppresses inflammation in LPS tolerant MNC of obese women with PCOS.<sup>2,3</sup> We examined the effect of MNC exposure to lipid alone versus lipid+LPS on IL-6 mRNA and secretion, and on the mRNA and protein of p47<sup>phox</sup>, the key component of the ROS producing enzyme NADPH oxidase, in women with PCOS compared with ovulatory controls.

**DESIGN:** Cross-sectional study.

**MATERIALS AND METHODS:** We studied 20 women with PCOS (10 lean; 10 obese) diagnosed on the basis of oligo-amenorrhea and hyperandrogenemia, and 20 ovulatory controls (10 lean; 10 obese) ages 18-40. MNC isolated from fasting blood samples were cultured with palmitate under pre- (0.4 mM) and post-prandial (0.2 mM) conditions with or without LPS. IL-6 and p47<sup>phox</sup> mRNA was quantified by RT-PCR. IL-6 secretion was measured by ELISA in culture supernatants. p47<sup>phox</sup> protein was quantified by Western blotting. Androgens were measured by RIA from blood samples drawn at 0, 24, 48 and 96 hours after HCG administration. Insulin sensitivity was derived by IS<sub>OGTT</sub>.

**RESULTS:** In response to lipid alone, the change from baseline ( $\Delta$ ) between pre- and post-prandial conditions increased in lean and obese women with PCOS and obese controls, and was significantly different (p<0.04) compared with the decrease in lean controls for IL-6 (mRNA [% $\Delta$ ]: 31 $\pm$ 4, 36 $\pm$ 5, 25 $\pm$ 5 vs. -2 $\pm$ 5; secretion [absolute  $\Delta$ , pg/ml]: 3.4 $\pm$ 1.1, 4.3 $\pm$ 1.9, 2.5 $\pm$ 1.2 vs. -1.8 $\pm$ 0.8) and p47<sup>phox</sup> (mRNA: 27 $\pm$ 4, 31 $\pm$ 6, 22 $\pm$ 6 vs. -2 $\pm$ 6; protein [% $\Delta$ ]: 29 $\pm$ 2, 35 $\pm$ 4, 22 $\pm$ 3 vs. -6 $\pm$ 5). The  $\Delta$ IL-6 response to lipid+LPS increased in lean women with PCOS and obese controls, and was

different (p<0.0001) compared with the decrease in obese women with PCOS and lean controls (mRNA: 7 $\pm$ 1, 6 $\pm$ 1 vs. -12 $\pm$ 2, -11 $\pm$ 1; secretion: 73 $\pm$ 23, 59 $\pm$ 19 vs. -121 $\pm$ 33, -108 $\pm$ 22). However, the  $\Delta$ p47<sup>phox</sup> response to lipid+LPS increased further in lean and obese women with PCOS and obese controls, and was different (p<0.0009) compared with the decrease in lean controls (mRNA: 38 $\pm$ 5, 62 $\pm$ 14, 32 $\pm$ 6 vs. -9 $\pm$ 6; protein: 38 $\pm$ 4, 67 $\pm$ 8, 31 $\pm$ 3 vs. -4 $\pm$ 5). Compared with controls, women with PCOS had a greater (p<0.02) HCG-stimulated area under the curve (AUC) for testosterone (T) (lean: 6004 $\pm$ 676 vs. 3518 $\pm$ 416; obese: 7639 $\pm$ 1135 vs. 3683 $\pm$ 180) and androstenedione (A) (lean: 510 $\pm$ 30 vs. 312 $\pm$ 25; obese: 562 $\pm$ 48 vs. 321 $\pm$ 34). For the combined groups after lipid+LPS,  $\Delta$ p47<sup>phox</sup> was directly correlated with AUC for T (mRNA: r=0.66, p<0.0001; protein: r=0.60, p<0.0001), and A (mRNA: r=0.57, p<0.0005; protein: r=0.60, p<0.0002), and was inversely correlated with IS<sub>OGTT</sub> (mRNA: r=-0.57, p<0.0004; protein: r=-0.58, p<0.0002). In women with PCOS after lipid+LPS,  $\Delta$ IL-6 secretion was inversely correlated with AUC for T (r=-0.47, p<0.05) and directly correlated with IS<sub>OGTT</sub> (r=0.51, p<0.04).

**CONCLUSIONS:** In PCOS, lipid-induced increases in IL-6 and p47<sup>phox</sup> are independent of obesity. Oxidative capacity is preserved in the face of LPS tolerance manifested by a two-fold increase in p47<sup>phox</sup> despite IL-6 suppression when obesity accompanies PCOS. LPS tolerance may be potentiated by hyperandrogenism to limit insulin resistance.

**References:** 1. González F, Sia CL, Abdelhadi OA, Melvin, RM, Garrett TJ. Lipid-induced reactive oxygen species generation is related to ovarian androgen hyperresponsiveness to HCG stimulation in normal weight women with polycystic ovary syndrome. *Reprod Sci.* 2013; 20 (3 Suppl):79A.

2. González F, Considine RV, Acton AJ, Abdelhadi OA. Hallmark evidence of lipopolysaccharide tolerance in obese women with polycystic ovary syndrome: Lipopolysaccharide-induced NF $\kappa$ B suppression is linked to hyperandrogenism in PCOS. *Fertil Steril.* 2018; 110 (3 Suppl):e8-e9.

3. González F, Considine RV, Xue J, Acton AJ. Combined lipid and lipopolysaccharide exposure dysregulates NF $\kappa$ B p105 gene expression in mononuclear cells of obese women with polycystic ovary syndrome. *Reprod Sci.* 2019; 26 (1 Suppl):193A.

**SUPPORT:** NIH grant R01 DK-107605 to F.G.

**O-108** Tuesday, October 15, 2019 12:00 PM

**SCREENING FOR ANDROGEN EXCESS IN WOMEN: ACCURACY OF SELF-REPORTED EXCESS BODY HAIR GROWTH AND MENSTRUAL DYSFUNCTION.** Jessica L. Chan, MD, MSCE,<sup>a</sup>



Marita Pall, MD, PhD,<sup>a</sup> Uche Ezech, MD,<sup>a</sup> Ruchi Mathur, MD,<sup>a</sup> Erica T. Wang, MD, MAS,<sup>a</sup> Margareta D. Pisarska, MD,<sup>a</sup> Ricardo Aziz, MD, MPH.<sup>b</sup> <sup>a</sup>Cedars-Sinai Medical Center, Los Angeles, CA; <sup>b</sup>University at Albany, SUNY, Albany, NY.

**OBJECTIVE:** To test the use of a simple telephone questionnaire to identify women at increased risk for polycystic ovary syndrome (PCOS) and other androgen excess (AE) disorders.

**DESIGN:** Prospective community-based cohort study.

**MATERIALS AND METHODS:** Women 14-45 years of age were recruited by advertisements seeking women either with irregular menstruation and/or excess body hair, or as healthy controls. A brief telephone screening was undertaken using a questionnaire consisting of 3 questions and subjects were asked to self-assess the presence or absence of male-like body hair and menstrual irregularity. Based on this screening, women with self-assessed irregular menses and/or excess body/facial hair were labeled as having possible androgen excess (Poss-AE); those self-assessed with regular menses and no excess body/facial hair were labeled as probable non-AE (Non-AE). All subjects were then examined directly; the evaluation included a health questionnaire, assessment of hirsutism using the modified Ferriman-Gallwey (mFG) score, ultrasound evaluation of the ovaries, and measurement of DHEAS, total and free testosterone, TSH, prolactin and 17-hydroxyprogesterone. A luteal phase progesterone level was performed in eumenorrheic subjects to confirm ovulation. All women evaluated were not on hormonal medications in the prior 3 months.

**RESULTS:** The study was completed in 206/298 (69%) of eligible women in the Poss-AE cohort and in 139/192 (73%) of eligible women in the Non-AE cohort. The Poss-AE cohort was minimally younger, (30 $\pm$ 6 years, vs. 32 $\pm$ 7 years, p=0.003) and had a higher mean BMI (32 $\pm$ 8 kg/m<sup>2</sup> vs. 27 $\pm$ 6 kg/m<sup>2</sup>, p<0.0001) than the Non-AE cohort. The Poss-AE cohort had higher mean free testosterone (5.2 $\pm$ 3.7 vs. 2.4 $\pm$ 1.9 pg/mL, p<0.001), higher total testosterone (40.9 $\pm$ 25.8 vs. 26.5 $\pm$ 14.3 pg/mL, p<0.001) and DHEAS

(241.1±118.6 vs. 193.3±98.9,  $p<0.05$ ) than the Non-AE cohort. Of the Poss-AE and Non-AE women, 83% and 16%, respectively, presented with PCOS according to the updated 2018 International Consortium guidelines. The sensitivity, specificity, PPV and NPV of the telephone questionnaire to predict PCOS was 88%, 77%, 84% and 81%, respectively. The sensitivity, specificity, PPV and NPV of the telephone questionnaire to predict hirsutism ( $mFG \geq 4$ ) was 81%, 80%, 81% and 80%, respectively. The sensitivity, specificity, PPV and NPV of the telephone questionnaire to predict oligo-ovulation was 98%, 87%, 84% and 98%, respectively.

**CONCLUSIONS:** A simple telephone questionnaire, based on self-assessment of body hair and menstrual status, can be used with high predictive value to identify women at risk for AE disorders, including PCOS, and to detect healthy controls. This approach could be an important tool for the undertaking of needed epidemiologic studies of AE and PCOS.

### ART LAB: OUTCOME PREDICTORS

**O-109** Tuesday, October 15, 2019 10:45 AM

**THE EFFECT OF HIGH HUMIDITY ON EMBRYO CULTURE MEDIA OXIDATION.** Carmela Albert, PhD,<sup>a</sup> Raquel Del Gallego, PhD,<sup>b</sup> Lucia Alegre, PhD,<sup>b</sup> Zaloa ZL. Larraategui, PhD,<sup>c</sup> Julian Marcos, Sr., PhD, Belén Aparicio-Ruiz, PhD,<sup>a</sup> Marcos Meseguer, PhD<sup>a</sup> <sup>a</sup>IVIRMA, Valencia, Spain; <sup>b</sup>IVIRMA Global, Valencia, Spain; <sup>c</sup>EMBRYOLOGIST, BILBAO, Spain; <sup>d</sup>IVIRMA Global, Valencia, Spain, Tel Aviv, Israel.



**OBJECTIVE:** Oil overlay has supported the successful use of a dry incubator to culture human embryos, preventing changes in the pH and temperature. However, dry conditions may affect the osmolality due to the evaporation of the culture media. The use of humid conditions avoids osmolality changes. Our aim in this study was to know how culture conditions might affect embryo culture related to an oxidative stress profile.

**DESIGN:** Retrospective multicentric study including a total of 7,544 embryos from 1,043 patients undergoing egg donation and autologous IVF treatment.

**MATERIALS AND METHODS:** Embryos were cultured in a time-lapse incubator system GeriO (Genea Biomedix, Australia). Out of its 6 patient-individual chambers, 3 of them worked under a dry atmosphere (DC) and 3 under humid conditions (HC). Retrospectively, blastocyst and good morphology blastocyst rate were evaluated.

For the oxidative stress profiling, a total of 125 spent embryo culture media from the Geri Dishes<sup>®</sup> were analyzed using the TCL (Thermochemiluminescence) Analyzer<sup>™</sup> (Carmel Diagnostics, Kiryat-Tivon, Israel). Its mechanism consists on a heat-induced oxidation of biological fluids, leading to the production of light energy counted as photons emitted per second (cps). The use of sequential vs. single-step culture medium was taken into account when HC and DC were compared. Data was analyzed with ANOVA and Chi-squared tests (SPSS software).

**RESULTS:** No statistical differences were found in terms of embryo development. We obtained a very similar blastocyst rate when the embryos were culture under HC: 71.3% vs DC: 71.0%. Likewise, high quality embryo rate (classified as A or B according to the ASEBIR criteria) was very similar 38.1% in HC vs 37.7% in DC. Regarding the oxidative stress profile, no significant differences were found between groups HC and DC in single-step medium. However, the results showed a trend towards a higher oxidative stress level in media cultured under DC: 127.8±40.6 cps vs. HC: 106.9±44.1 cps. On the other hand, sequential medium did show a significant oxidative status difference ( $p < 0.05$ ) between media collected on day 3 (75.5±20.4 cps) and media collected on day 5/6 (105.8±39.2 cps). Moreover, no significant differences were found between the oxidative status of media coming from sequential collected on day 5/6 and single-step media. These results were quite interesting as they may depict how the oxidative metabolism in the embryos increase after day 3, when the maternal to zygotic transition takes place.

**CONCLUSIONS:** In a previous study, our results strongly suggested that culture conditions with a high humidity atmosphere promoted embryo development. However, in an attempt to increase the sample size to confirm these findings, no statistically significant differences have been found. According to the oxidative status of the spent media, DC seem to affects the media oxidation. A larger sample size would be required to confirm this trend.

**O-110** Tuesday, October 15, 2019 11:00 AM

### PUTRESCINE SUPPLEMENTATION PROMOTE THE MATURATION OF OOCYTES AND IMPROVE THE QUALITY OF OOCYTES AND THE POTENTIAL OF EMBRYOS DEVELOPMENT.



Wei Wu, MD,<sup>a</sup> Lingbo Cai, PHD,<sup>a</sup> Yuting Ling, Master,<sup>a</sup> Johne Liu, PhD,<sup>b</sup> <sup>a</sup>The First Affiliated Hospital of Nanjing Medical University, Nanjing, China; <sup>b</sup>Ottawa Hospital Research Institute, Ottawa, ON, Canada.

**OBJECTIVE:** To investigate the mechanisms of promotion the maturation of oocytes and improvement the quality of eggs and the potential of embryos development through exogenous addition of putrescine in the treatment of diminished ovarian reserve(DOR).

**DESIGN:** A self-controlled paired design was used to select a total of 136 immature eggs in the ovulation-promoting process in patients undergoing in vitro fertilization.

**MATERIALS AND METHODS:** The even number of immature eggs of the same patient, were paired and randomly assigned to the experimental group or control group. Putrescine was added to the in vitro maturation (IVM) medium of immature eggs in the experimental group, and the control group was a conventional IVM group. After 24 to 48 hours culture, the first polar body of the maturation oocyte was biopsied and then the oocytes were subjected to whole genome amplification. Next Generation Sequencing technology was used to detect changes in whole genome copy number and mitochondrial copy number content of oocytes/polar bodies.

**RESULTS:** 136 GV eggs were randomly assigned to the control group (IVM group) and the experimental group (IVM+putrescine group). The maturation rate of the experimental group was 66.18%, which was higher than the 57.35% of the control group. The mitochondrial content of mature eggs in the IVM+putrescine group was 22.23% higher than that in the IVM group. The aneuploidy rate in the IVM+putrescine group was slightly higher than that in the IVM group, but the difference was not statistically significant.

**CONCLUSIONS:** Putrescine can promote the in vitro maturation rate of GV and can also increase the content of mitochondria in oocytes, thereby improving the quality of oocytes and promoting the development potential of embryos.

**SUPPORT:** The National Key Research and Development Program of China (2017YFC1001602; 2017YFC1001300)

**O-111** Tuesday, October 15, 2019 11:15 AM

### HUMAN BLASTOCYSTS DERIVED FROM MONOPRNUCLEAR ZYGOTES: A BIOLOGICAL MODEL FOR THE STUDY OF PLOIDY, EUPLOIDY, TOPOGRAPHY AND HETEROARENTAL INHERITANCE.



Noelia Grau, PhD,<sup>a</sup> Nuria Soler, MSc,<sup>b</sup> Ana González-Picazo, MSc,<sup>c</sup> Xavier Vendrell, PhD,<sup>d</sup> María José Escribá, PhD,<sup>c</sup> Pilar Gámiz, PhD,<sup>a</sup> <sup>a</sup>IVIRMA-Valencia, Valencia, Spain; <sup>b</sup>Universidad de Valencia, Valencia, Spain; <sup>c</sup>IVI Foundation, Valencia, Spain; <sup>d</sup>Sistemas Genómicos, Paterna, Spain; <sup>e</sup>IVIRMA Valencia, Valencia, Spain.

**OBJECTIVE:** To describe the ploidy and euploidy, chromosomal concordance between different regions of the trophectoderm (TE) and also between TE and inner cell mass (ICM). Besides, we aimed to identify chromosomal inheritance (paternal/maternal) of haploid, diploid and polyploid blastocysts derived from monopronuclear (MPN) zygotes. Additionally, it will be discussed the eventual "rescue" of these blastocysts for reproductive purposes.

**DESIGN:** Prospective experimental study that includes 910 ICSI cycles from 892 couples registered from April 2016 to December 2018. 1081 MPN zygotes (1.2%) were obtained. A total of 199 zygotes reached the blastocyst stage (18.5%, blastocyst rate). Seventy-six blastocysts were assigned to three experimental series, according to the genetic analysis performed (ploidy, topography and parental inheritance).

**MATERIALS AND METHODS:** The study was carried out in 3 series. Series 1: 26 blastocysts were fixed by FISH (chromosomes X, Y, and 18) to assess ploidy. Series 2: 35 blastocysts were biopsied in three samples, two from TE (TE1 and TE2) and ICM. TE1 let us to determinate ploidy by FISH (chromosomes X, Y, 18); TE2 and ICM were used for 24-chromosomes study by NGS. Series 3: 15 blastocysts were biopsied as described in Series 2. TE1: study of 24 chromosomes by NGS. TE2: study of 24 chromosomes and SNPs (single nucleotide polymorphisms) by SNP-array of 750K in a "trio" format (simultaneous study of paternal/maternal/TE2 DNAs) and bioinformatic analysis. R-package for statistical analysis. The rest of the embryo was used for ploidy determinations by FISH (chromosomes X, Y, 18).

**RESULTS:** 80.5% of MPN-derived blastocysts were diploid, 8% mosaic and 11.5% haploid ( $P < 0.01$ ). Diploid blastocysts showed a normal sex ratio (1:1); 50% diploid blastocysts were aneuploid. In relation to chromosomal topography, results showed different patterns, according to the chromosomal instability grade. Correlation between compartments (TE and ICM) was perfectly matched when both compartments were euploid or whole-chromosome aneuploid (trisomies and monosomies). Incomplete matching between compartments was observed in complex (>3 chromosomes involved), segmental or mosaic samples, which were more frequently observed in those from TE. 70% MPN-derived blastocysts showed two copies of both parental genomes. In relation to parental inheritance, 40% of blastocysts were diploid heteroparental.

**CONCLUSIONS:** The MPN experimental model confirms the chromosomal correlation between ICM and different regions of TE, in cases of euploidy or pure aneuploidy. The chromosomal instability associated to segmental aneuploidy seems to be confined equally to both TE and ICM compartments. A high percentage of MPN-derived blastocysts showed two copies of both parental and euploid genomes. These data re-open debate on their convenience for clinical reproductive use.

ACIF/2018/076

APOTI/2019/A/010

SUPPORT: IMIDCA/2018/25 (IVACE; PIDCOP-CV)

**O-112** Tuesday, October 15, 2019 11:30 AM

#### USE OF NEXT GENERATION SEQUENCING FOR THE ANALYSIS OF *IN VITRO* MATURED OOCYTES AND THEIR POLAR BODIES.

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**OBJECTIVE:** Approximately 15% of retrieved oocytes are immature, 11% at the germinal vesicle (GV) stage, 4% at metaphase I (MI). To date the success rates of *in vitro* maturation (IVM) have been low. It has been suggested that IVM oocytes, if fertilized, may lead to abnormal embryos and an increased risk of spontaneous miscarriages. Consequently, immature oocytes are usually discarded.

The aim of our study was to perform a detailed cytogenetic analysis of *in vitro* matured oocytes and compare with the chromosome constitution of oocytes which were mature (metaphase II; MII) at the time of retrieval.

**DESIGN:** Prospective non-randomized study.

**MATERIALS AND METHODS:** The oocytes examined were generated by sixteen young and healthy oocyte donors participated in the study. The average female age was 23.3 years (95%CI 21.0-25.6). A total of 43 oocytes were included (22 MII and 21 GV). Oocytes identified to be at the GV stage were cultured individually in 25  $\mu$ l of Gens<sup>®</sup> Geri<sup>®</sup> medium in a time-lapse incubator for up to 50.0 hours to achieve IVM and to determine the time needed for polar body (PB) extrusion. Biopsy of the 1<sup>st</sup> PB was performed once these oocytes matured to MII. Similarly, mature MII oocytes (n=22) underwent 1<sup>st</sup> PB biopsy. The cytogenetic constitution of IVM and mature oocyte-PB pairs was assessed using a well validated next generation sequencing (NGS) strategy for the identification of chromosome and chromatid errors arising during female meiosis.

**RESULTS:** Sixty-two percent of GV oocytes matured *in vitro* to MII after an average culture of 26.1 hours (95% CI 23.2-29.0). A total of 35 oocyte-PB pairs underwent NGS analysis. Of these, 13 originated from GVs and 22 that were at the MII stage at retrieval. The overall euploidy rate observed was very similar between the two groups, i.e. 76.9% for the oocyte-PB pairs which were retrieved at the GV stage and 78.4% for the mature MII oocyte-PB pairs. The majority of abnormalities (60%) scored were due to unbalanced chromatid predivision, with the remaining 40% arising due to whole chromosome non-disjunction. No difference in IVM culture length was observed between normal and abnormal GV oocytes that reached the MII stage (26.1 hrs. [95%CI 22.3-29.9] vs. 25.93 hrs. [95%CI 17.4-34.5]).

**CONCLUSIONS:** To our knowledge, this is the first study to describe the use of NGS for cytogenetic analysis of *in vitro* matured human oocytes. Our findings suggest that GV oocytes, matured to MII *in vitro*, segregate their chromosomes in a manner equivalent to those that mature within the follicle. The fact that aneuploidy is not increased following IVM supports the idea

that an attempt should be made to “rescue” immature oocytes, rather than discarding them. Further work is required to understand the basis of the poorer outcomes associated with IVM oocytes, but these results indicate that the cause is not cytogenetic in nature.

**O-113** Tuesday, October 15, 2019 11:45 AM

#### TIME LAPSE SELECTED ELECTIVE SINGLE EMBRYO TRANSFER IN HYALURONON ENRICHED TRANSFER MEDIUM IN PCOS IMPROVES LIVE BIRTH RATES COMPARED TO USE OF CONVENTIONAL EMBRYO TRANSFER MEDIA. A POSSIBLE ALTERNATIVE TO FREEZE-ALL CYCLES IN PCOS.



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**OBJECTIVE:** Polycystic ovarian syndrome(PCOS) is shown to impair endometrial receptivity due to disturbed receptor mediation or gene expression affecting implantation rates, miscarriage rates, and live birth rates in fresh embryo transfers(ET). Freeze-all is being used as a strategy to counter ovarian hyperstimulation syndrome(OHSS) and reduced receptivity in PCOS. The added intervention of vitrification and endometrial preparation in subsequent cycles increases cost and time associated with treatment significantly. 2014 Cochrane review of use of adherence compounds shows moderate evidence that Embryoglu<sup>e</sup> (EG) increases implantation and pregnancy rates, but no study has been designed using EG with sET in PCOS. With time lapse and sET, fresh ETs are advised, avoiding OHSS, but still, implantation rates remain low. We wanted to evaluate whether use of embryo transfer medium enriched with hyaluronan (EmbryoGlue -Vitrolife, Gothenburg, Sweden) improves outcomes like implantation rate, live birth rate and miscarriage rates in time lapse(TL) selected fresh sET in PCOS women.

**DESIGN:** Prospective randomized study.

**MATERIALS AND METHODS:** Fresh embryo transfers between January 2017 and August 2018 in PCOS patients classified by Rotterdam criteria were included. Sample size was calculated with n=152 in each arm for statistical power of the study at 80% with alpha = 0.05, beta =0.2. Time Lapse imaging as discussed by N.Desai et al for embryo selection was adopted for sET. Fertilization and embryo culture conditions were similar in both groups. Fertilization was conducted in CSCM medium (Irvine, CA,USA) and the embryos were transferred into CSCM for uninterrupted time lapse culture in Miri-TL(ESCO, Singapore). Patients were randomly allocated on day of embryo transfer into two groups: In the EmbryoGlue group (153 patients), single cleavage stage or blastocyst was transferred in EmbryoGlue medium that contains 0.5 mg/ml hyaluronan (Vitrolife, Gothenburg, Sweden). In the control group (168 patients) single cleavage stage embryo or blastocyst was transferred in medium CSCM (Irvine, CA,USA). The Chi square test was used for comparison of proportions and the Mann-Whitney U test was used to compare the differences between the groups.

**RESULTS:** The EG and control group were similar with respect to age (32.7 +/- 3.6 and 31.7 +/- 3.6 respectively), infertility duration (3.8 +/- 2.1 and 3.3 +/- 2.6 years), previous IVF cycles (1.3 +/- 1.5 and 1.1 +/- 1), oocyte number (14.4 +/- 5.2 and 13.2 +/- 5), and stage of transferred embryo (17% vs 21% cleavage stage embryos, and 83% vs 79% blastocyst stage embryos). The implantation rate in EG and control group were 39.2% vs 23.8% (p<0.005) and live birth rate were 35.9% vs 17.3% (p<0.001) respectively and miscarriage rates were 8.3% in EG vs 28% in control group (p=0.17).

**CONCLUSIONS:** The use of embryo glue for time lapse selected sET in PCOS shows significant increase in implantation and live birth rates with lower miscarriage rates compared with conventional embryo transfer medium. This strategy should also be explored to improve outcomes as an effective alternative to freeze-all cycles for PCOS patients.

References: 1. Kolibianakis, E. M. et al. Increased clinical pregnancy rates by using hyaluronan in in-vitro fertilization: a systematic review and meta-analysis. *Fertil. Steril.* 90, S349 (2008).

2. Rutkowska, A. Z. & Diamanti-Kandarakis, E. Polycystic ovary syndrome and environmental toxins. *Fertil. Steril.* 106, 948–958 (2016).

3. McDonnell, R. & Hart, R. J. Pregnancy-related outcomes for women with polycystic ovary syndrome. *Women's Heal.* 13, 89–97 (2017).

4. Quintans, C. J. et al. Human IVF Outcome in Media Containing Either Alanyl-L-Glutamine or Glycyl-L-Glutamine. *Fertil. Steril.* 84, S456 (2005).

5. Huang, J. et al. The effect of protein supplement concentration in embryo transfer medium on clinical outcome of IVF/ICSI cycles: A prospective, randomized clinical trial. *Reprod. Biomed. Online* 32, 79–84 (2016).

6. Li, L. et al. The role of heat shock protein 90B1 in patients with polycystic ovary syndrome. *PLoS One* 11, 1–16 (2016).

7. Desai, N. et al. Analysis of embryo morphokinetics, multinucleation and cleavage anomalies using continuous time-lapse monitoring in blastocyst transfer cycles. *Reprod. Biol. Endocrinol.* 12, 1–10 (2014).

8. Bontekoe, S., Blake, D., Heineman, M. J., Williams, E. C. & Johnson, N. Adherence compounds in embryo transfer media for assisted reproductive technologies. *Cochrane Database Syst. Rev.* 2014–2016 (2010). <https://doi.org/10.1002/14651858.cd007421.pub2>.

9. Bellver, J. et al. Endometrial gene expression in the window of implantation is altered in obese women especially in association with polycystic ovary syndrome. *Fertil. Steril.* 95, (2011).

10. Soares Lopes, I. M. R. et al. Endometrium in women with polycystic ovary syndrome during the window of implantation. *Rev. da Assoc. Médica Bras.* (English Ed. 57, 688–695 (2013).

11. Cakmak, H. & Taylor, H. S. Implantation failure: Molecular mechanisms and clinical treatment. *Hum. Reprod. Update* 17, 242–253 (2011).

SUPPORT: None

O-114 Tuesday, October 15, 2019 12:00 PM

### COMPARISON OF EMBRYO SPECIFIC TIME-LAPSE DISH FOR INDIVIDUAL CULTURE VERSUS AN EMBRYO SPECIFIC DISH FOR GROUP CULTURE.

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**OBJECTIVE:** There are many different types of embryo culture dishes and incubators available. Dishes for time lapse incubators are very different to traditional dishes. Testing of any new dish or incubator must be extensive in order to validate the new system. The objective of this study was first to compare outcomes following sibling oocyte splits in an established culture system (embryo-specific microdrop dishes and dry benchtop incubator) to a time-lapse incubator along with its specially designed dish. The time-lapse specific dish was then tested in the benchtop incubator using sibling oocyte splits.

**DESIGN:** Prospective randomized trial.

**MATERIALS AND METHODS:** A time-lapse incubator chambers (Geri, Serono) and a K systems G210 were utilized to culture all embryos. In phase I, all embryos were grown in groups A: in mini-GPS dishes (Life Global) in a K systems G210 incubator or B: Geri (Serono) dishes inside a dry chamber of Geri (Serono) incubator. All embryos were grown in Sage sequential media with 10% v/v complex protein under 4 mL Paraffin oil (Life Global) at 5% O<sub>2</sub>. All embryos were treated identically except for the dish and incubator. Embryos were observed and media exchanged following 24h, 72h and 120h. In Phase II, to control for the impact of the culture dish, all embryos were grown in Geri dishes and placed either in the G210 (group C) or the Geri non-humidified chamber (Group D).

**RESULTS:**

**CONCLUSIONS:** Culture in the Geri dish and the Geri time-lapse system yielded more blastocysts overall and more good quality blastocysts than in mini-GPS dishes in the K systems G210. Fertilization was increased in the Geri incubator, although not significant. No changes in cleavage embryos at day 3 was apparent. More good quality embryos were observed on day 5, 6 and 7 in the Geri incubator with Geri dish.

To determine whether the incubator or the dish were responsible for these increases, the Geri dish was used to grow all embryos in Phase II and half of the oocytes placed in the K systems G210 and half in the Geri incubator. While the n is low, more good quality embryos were observed in the Geri incubator. The Geri incubator is an effective incubator yielding good quality blastocysts. More studies are required to determine if a single step media gives the same results.

References: none

SUPPORT: none

### EARLY PREGNANCY

O-115 Tuesday, October 15, 2019 10:45 AM

### RETAINED PREGNANCY TISSUE AFTER MISCARRIAGE ASSOCIATED WITH HIGH RATE OF CHRONIC ENDOMETRITIS.

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**OBJECTIVE:** To compare the prevalence of chronic endometritis in women undergoing hysteroscopic resection of retained pregnancy tissue (RPOC) after pregnancy loss to women with unexplained recurrent pregnancy loss (RPL).

**DESIGN:** Cohort study.

**MATERIALS AND METHODS:** Institutional Review Board approval was obtained. Women undergoing hysteroscopic resection of RPOC between 6/2008 and 12/2018 were included. In addition, women with unexplained RPL undergoing endometrial sampling between 1/2016 and 12/2018 were included. Unexplained RPL was defined as two or more pregnancy losses with a TSH level under 4 mU/L, negative antiphospholipid antibodies and normal uterine anatomy. Data on pregnancy history, time since last pregnancy loss and gestational age at time of loss were collected. H&E and immunohistochemical staining for CD138 were performed on all slides. A single pathologist blinded to patient history recorded the number of plasma cells per high power field (HPF). Chronic endometritis was defined as 1 or more plasma cells/10 HPF in addition to stromal changes (spindling, edema, foci of breakdown, presence of other inflammatory cells, and pigment deposition). In order to detect a 25% difference in the rate of chronic endometritis with 80% power and alpha of 0.05, a sample size of 49 women was needed in each group.

**RESULTS:** Endometrial samples from a total of 100 women were evaluated (50 women undergoing resection of RPOC and 50 women with unexplained RPL). The mean age was similar between groups, 36.4 (SD 4.7) vs 35.2 (SD 4.1) years, P=0.18. The mean number of prior pregnancy losses was 1.9 (SD 1.0) in the RPOC group vs. 3.1 (SD 0.9) in the RPL group, P=0.0001. By H&E staining, chronic endometritis was present in 60% (30/50) of women undergoing resection of RPOC vs. 14% (7/50) of women biopsied for RPL, P<0.0001. By CD138 staining, chronic endometritis was present in 62% (31/50) of women undergoing resection of RPOC vs. 30% (15/50) of women biopsied for RPL, P=0.002. In a subgroup analysis that only included women with RPL, chronic endometritis was present in 71% (20/28) of women with both RPL and RPOC vs. 24% (12/50) of women with RPL alone, P<0.0001 (H&E). Among women with RPL without

	Group A K systems G210	Group B Geri (Non-Humidified)	Group C K systems G210	Group D Geri (Non-Humidified)
Dish type	Mini GPS	Geri	Geri	Geri
Oocyte #	104	101	35	35
% Fertilization	68.3%	80.2%	82.9%	97.1%
% Good Cleavage Rate	80.3%	84.0%	72.4%	82.4%
% Total Blasts on D5	53.5%	65.4%	41.4%	55.9%
% Blasts >= 3BB D5	23.9%	33.3%	20.7%	20.6%
% Total Blasts D6	67.6%	74.1%	62.1%	67.6%
% Blasts >= 3BB D6	45.1%	59.3%	37.9%	50.0%
% Total Blasts D7	69.0%	76.5%	62.1%	67.6%
% Blasts >= 3BB D7	52.1%	61.7%	44.8%	50.0%

suspected RPOC, an implantation site or placental site nodule was reported in three women, and all three of these women had chronic endometritis.

**CONCLUSIONS:** Following miscarriage, retained pregnancy tissue is associated with a high prevalence of chronic endometritis. A hysteroscopy to evaluate for retained pregnancy tissue may be warranted in women with RPL who are diagnosed with chronic endometritis. Further research is needed to determine if resection of retained tissue is sufficient to treat RPOC associated chronic endometritis, or if additional antibiotic treatment is necessary.

**SUPPORT:** Friends of Prentice Grant

**O-116** Tuesday, October 15, 2019 11:00 AM

**IS THERE A PREDISPOSITION TO EMBRYONIC ANEUPLOIDY IN PRE-DIABETIC PATIENTS?**

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**OBJECTIVE:** There is a correlation between glycated hemoglobin (HbA1C) values in early pregnancy (> 8%) and frequency of miscarriage and congenital malformations.<sup>1</sup> To date, no study has analyzed the risk of aneuploidy with HbA1C values in the pre-diabetic range. The purpose of this study is to examine the relationship between HbA1C and embryo aneuploidy after IVF with preimplantation genetic testing for aneuploidy (PGT-A).

**DESIGN:** Retrospective cohort study at an academic medical center.

**MATERIALS AND METHODS:** We included IVF-PGT-A cycles between 2013-17. Demographics, oocyte retrieval data, embryo development and ploidy status were reviewed. Patients with a diabetic HbA1C (> 6.5%) were excluded. Pearson correlation compared maternal HbA1C with embryo aneuploidy rate. Wilcoxon rank test compared IVF outcomes and embryo aneuploidy rates between non-diabetic (HbA1C <5.7) vs. pre-diabetic (HbA1C 5.7-6.4) patients.

**RESULTS:** A total of 1,867 blastocysts from 393 patients were analyzed with PGT-A. Three hundred twenty (81.4%) patients had normal HbA1C values and 73 (18.6%) had pre-diabetes. Three hundred thirty-five patients (85.2%) had embryos with autosomal aneuploidy. Fifty-six patients (14.2%) had sex-chromosome aneuploidies; of them, 4.8% had embryos with karyotype 45X.

Pre-diabetic HbA1C was significantly correlated with the rate of sex chromosome (but not autosomal) aneuploidy (*correlation coefficient*=0.1, *p*=0.039). Maternal age, FSH, and AMH were not correlated with sex chromosome aneuploidy rate.

There was a significant difference in sex chromosome aneuploidy rate between patients with normal vs. prediabetic HbA1C (3.2% vs. 7.2%, *p*=0.033).

**CONCLUSIONS:** 1. Sex chromosome aneuploidy rate (largely X-chromosome related) was significantly higher in patients with prediabetic vs. normal HbA1C. There was no difference in overall aneuploidy rate, likely because the majority of aneuploidies are autosomal.

2. The association of Turner's syndrome and diabetes is well known. Our findings suggest that HbA1C in the pre-diabetic range may increase the risk of 45X embryos, and supports a relationship between glycemic control and the X chromosome.

3. The American Diabetes Association cut-off between normal and pre-diabetic HbA1C (5.7) was the same value identified as conferring a significant increase in the rate of sex chromosome aneuploidy.

	Non-diabetic N=320	Pre-diabetic N=73	<i>P value</i>
Age (y)	36.7 (±4.7) <sup>a</sup>	37.6 (3.5)	0.295
FSH (mIU/L)	7.19 (3.1)	6.98 (2.8)	0.791
AMH (ng/mL)	3.60 (3.9)	4.19 (4.5)	0.484
# biopsied embryos	4.46 (3.0)	4.35 (2.8)	0.978
% aneuploid embryos	52.0% (33.5)	54.6% (34.0)	0.566
% embryos with autosomal aneuploidy	50.9% (33.9)	50.6% (34.3)	0.972
% embryos with sex-chromosome aneuploidy	3.23% (12.1)	7.09% (19.1)	0.033

<sup>a</sup>±SD

**References:** 1. Greene MF, Hare JW CJP, Benacerraf BR, Soeldner JS. First-trimester hemoglobin A1 and risk for major malformation and spontaneous abortion in diabetic pregnancy. *Teratology*. 1989;39:225–31.

**SUPPORT:** none

**O-117** Tuesday, October 15, 2019 11:15 AM

**DIRECT CORRELATION BETWEEN B-HCG LEVELS AND TROPHECTODERM MORPHOLOGY QUALITY IN SINGLE EUPLOID EMBRYO TRANSFER CYCLES.**

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**OBJECTIVE:** Embryonic trophoctoderm (TE) cells play a key role in apposition, adhesion, and invasion of the maternal endometrium during early implantation. Blastocysts are morphologically graded (expansion stage (EXP); inner cell mass (ICM); trophoctoderm (TE) cells) to better understand embryonic competence and improve selection at transfer. Data is scarce regarding the relationship of embryo TE quality and early levels of  $\beta$ -hCG, a biochemical marker of early embryo implantation and placentation. Previously, we demonstrated that embryo TE quality does not correlate with major adverse perinatal outcomes or placental weight at delivery. However, patients who had transfer of embryo(s) with a low TE grade experienced placental histological changes. (Herlihy et al. 2017) This study included patients who underwent a single, euploid frozen embryo transfer (FET) and assessed the correlation between embryo TE grade and early  $\beta$ -hCG levels.

**DESIGN:** Retrospective cohort analysis.

**MATERIALS AND METHODS:** This study included patients who underwent a single, euploid FET cycle and obtained a positive pregnancy test (serum  $\beta$ -hCG  $\geq$  5 mIU/mL) from 2015 to 2019. The  $\beta$ -hCG measurement was analyzed 9 days after FET using an electrochemiluminescence immunoassays (Immulite 2000; Siemens and/or Cobas e-601; Roche). Only cases that had a first  $\beta$ -hCG measurement on day 9 after ET were included in the analysis. Blastocyst morphology was assessed using a center-specific, modified Gardner's scoring system. ANOVA,  $\chi^2$  tests, univariate, multivariate linear regression and a mixed effects model with a random intercept model were used to evaluate serum  $\beta$ -hCG levels with regard to TE grade.

**RESULTS:** A total of 2,954 single, euploid FET cycles were included in the analysis. Cohorts were segregated by TE grade: (TE-A: n=1,076; TE-B: n=1,235; TE-C: n=643).  $\beta$ -hCG values were significantly different among cohorts (TE-A: 155.5±97; TE-B: 133.7±80; TE-C: 94.1±73, *p*<0.0001) and early pregnancy loss (EPL) was significantly higher in embryos with low TE grades: (TE-A: 14.6%, TE-B: 15.3%, TE-C: 19.2%, *p*=0.01) There was a significant correlation between TE grade and mean  $\beta$ -hCG levels ( $R^2$ : 0.06, *p*<0.001). After adjusting for age, BMI, endometrial thickness at ET, ICM grade, EXP grade, and day of embryo biopsy, the correlation between high TE grade and high  $\beta$ -hCG levels remained significant ( $R^2$ : 0.12, *P*<0.0001).

**CONCLUSIONS:** After adjusting for clinical parameters, embryonic expansion, and inner cell mass grade; our data showed euploid embryo TE grade correlates with  $\beta$ -hCG levels at first pregnancy test measurement. The ultrastructural appearance of the TE cells in euploid embryos might represent a surrogate marker of embryo's capacity to properly adhere and invade the endometrium during the early implantation process. Further studies focusing on syncytiotrophoblast and endometrial cellular and molecular interactions could help reproductive specialists to better understand the mechanisms related to early placentation physiology.

**References:** 1. Herlihy, L. Sekhon, T.G. Nazem, C.A. Hernandez-Nieto, M. Oliva, J.A. Lee, B. Sandler, T. Mukherjee, A.B. Copperman. Does morphologic grading of embryonic trophoctoderm correlate with quality of placentation and perinatal outcome? : *Fertility and Sterility*, Volume 108, Issue 3, 2017, Pages e376-e377.

**SUPPORT:** none

**O-118** Tuesday, October 15, 2019 11:30 AM

**PATERNAL CONTRIBUTIONS IN EARLY EMBRYONIC GENE EXPRESSION: ROLE IN EARLY PREGNANCY LOSS.**

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**OBJECTIVE:** The dynamic interplay of the vulnerable sperm genomic and extragenomic cargo with the early embryonic development in spontaneous and assisted conceptions has been brought to surface. The suite of sperm transcripts retained in the spermatozoa and the complex epigenetically marked sperm genome synergistically function to influence early embryonic development. Dysregulated gene expression and disrupted genomic integrity resulting in early pregnancy loss needs to be further elucidated.

**DESIGN:** A case control study.

**MATERIALS AND METHODS:** Male partners of females who experienced recurrent pregnancy loss (RPL, N=75) and recurrent implantation failures (RIF, n=75) and 75 healthy fertile controls were recruited for the study and semen samples were obtained. Gene expression analysis of the genes critical for embryonic development and DNA damage repair pathway (*FOXG1*, *SOX3*, *STAT4*, *RPS6*, *RBM9*, *RPL10A*, *RPS17*, *RPL29*, *TOMM7*, *EIF5A*, *OGG1* and *PARP1*) was analyzed by qPCR analysis after normalisation with  $\beta$ -actin and *GAPDH*. Functional assessment of semen included cardinal biomarkers of oxidative stress by reactive oxygen species (ROS), DNA damage by DNA fragmentation index (DFI) and 8-OHdG levels as well as telomere length in sperm DNA.

**RESULTS:** The relative gene expression of *FOXG1* ( $p=0.048$ ), *SOX3* ( $p=0.03$ ), *RPS6*, *RBM9* and *RPL10A* ( $p<0.001$ ) was seen to differ significantly between RPL patients and controls, while the expression *FOXG1* ( $P=0.02$ ), *RPS6*, *RBM9* and *TOMM7* ( $p<0.001$ ), *RPL10A* ( $p=0.039$ ) and *RPS17* ( $p=0.002$ ) in RIF patients as compared to controls. The levels of ROS, DFI and 8-OHdG were found to be significantly higher as compared to controls and telomere length was found to be significantly different in both RPL and RIF patients with respect to controls. The odds of occurrence of RPL and RIF was 12.41 and 12.68 times greater with ROS>29 [OR 12.41, (6.53-23.55) and 13.68 (6.52-28.71)] respectively. The odds of occurrence was 12.68 and 18.87 time greater with DFI>31 [OR 12.68 (6.28-21.22) AND 18.87 (5.43-27.67)] respectively.

**CONCLUSIONS:** The orchestration of selective paternal transcripts as well as genomic integrity and telomere length is a critical determinant of early embryonic development and embryo viability. The derangements in sperm functional characteristics and gene expression has the potential to produce adverse transgenerational fetal effects and health of future progeny. The adoption of sperm RNA expression can be established as an integral part of clinical diagnostic measures among other seminal biomarkers.

TABLE 1. Relationships between low day 5 hCG level (<5 IU/L) and transfer outcome.

Outcome	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Risk ratio (95% CI)	P-value
Implantation failure	100	89.3	70.9	100	undefined	<0.0001
Biochemical pregnancy loss	43.7	92.8	39.2	93.9	6.48 (4.32-9.74)	<0.0001
Ectopic pregnancy	75.0	89.7	3.8	99.9	25.10 (2.64-238.42)	<0.0001
Early SAB	11.8	94.3	31.1	83.0	1.83 (1.15-2.93)	0.017
Late SAB	20.0	94.3	3.1	99.2	4.04 (0.46-35.09)	0.174
All pregnancy losses	30.0	93.1	61.2	78.5	2.84 (2.10-3.85)	<0.0001

References: Dhawan V, Kumar M, Dipika D, Malhotra N, Singh N, Dadhwal V, Dada R. Paternal factors and embryonic development: Role in recurrent pregnancy loss. *Andrologia* 2018; e13171.

Burl RB, Clough S, Sandler E, Estill M, Krawetz SA. Sperm RNA elements as markers of health. *Syst Biol Reprod Med* 2018; 64(1): 25-38.

Ostermeier GC, Dix DJ, Miller D, Khatri P, Krawetz SA. Spermatozoal RNA profiles of normal fertile men. *Lancet* 2002; 360 : 772-77.

Krawetz SA. Paternal contribution: new insights and future challenges. *Nat Rev Genet* 2005; 6 : 633-42.

Jodar M, Sandler E, Moskovstev S I, Librach CL, Robert G, Swanson S, Hauser R, Diamond MP, Krawetz SA. Absence of sperm RNA elements correlates with idiopathic male infertility. *Sci Trans Med* 2015; 7(295).

**SUPPORT:** This work is supported by All India Institute of Medical Sciences (AIIMS) internal funds. The authors declare no competing interests.

**PREGNANCY VIABILITY IDENTIFIED BY EARLY SERUM HCG LEVEL MEASURED IN THE PERI-IMPLANTATION PERIOD FOLLOWING THAWED SINGLE BLASTOCYST TRANSFER.** Ankita Raman, MD,<sup>a</sup>

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**OBJECTIVE:** To assess the extent to which outcome of thawed single blastocyst transfer is predicted by serum hCG level measured 5 days post transfer.

**DESIGN:** Retrospective cohort study of vitrified-warmed single blastocyst transfers performed over a 5-year period at a private fertility center.

**MATERIALS AND METHODS:** After artificial endometrial preparation, vitrified-warmed blastocysts were transferred on the 6<sup>th</sup> day of exogenous progesterone exposure. Serum hCG levels were measured 5 and 10 days after transfer. Serum hCG level below 5 IU/L on both days defined implantation failure. Biochemical pregnancy losses were transient hCG elevations that resolved spontaneously without sonographic evidence of intrauterine pregnancy (IUP). Ectopic pregnancies included persisting pregnancies of unknown location that resolved after treatment. Early spontaneous abortions (SAB) were IUPs lost before 10 weeks gestation, while late SABs were those lost after 10 weeks gestation. Implantation failures were analyzed among all transfers. Biochemical pregnancy losses and ectopic pregnancies were analyzed among all pregnancies, early SABs were analyzed among patients with IUP, and late SABs were analyzed among patients with ongoing pregnancies at 10 weeks. Chi-square tests were used in all comparisons.  $P<0.05$  was considered statistically significant.

**RESULTS:** There were 932 vitrified-warmed single-blastocyst transfers during the study period which resulted in 192 implantation failures, 633 IUPs, 549 ongoing pregnancies and 199 pregnancy losses of all types. Sensitivity, specificity, positive predictive value, negative predictive value, and relative risk are shown in Table 1. Day 5 serum hCG level < 5 IU/ml was found to have high negative predictive value for all adverse pregnancy outcomes except late SAB.

**CONCLUSIONS:** The eventual fate of an early implanting embryo is largely determined within 5 days of transfer. Even among pregnancies with sonographically confirmed IUP, low day 5 hCG correlated with early SAB. Whether pregnancy losses following low day 5 hCG result from flawed implantation events or inherently non-viable embryos is yet to be resolved.

**SEMINAL EXOSOMES PROTEOME PROFILING REVEAL IMPAIRED CELL SIGNALING AND DEFECTS IN CHROMATIN REMODELING AS PATERNAL CONTRIBUTORS IN RECURRENT PREGNANCY LOSS PATIENTS.** Luna Samanta, PhD,<sup>a</sup> Soumya Ranjan Jena, M.Phil,<sup>b</sup>

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Environment and Public Health, Ravenshaw University, Cuttack-753003, India, Cuttack, India; <sup>c</sup>A-32, Unit-4, Kharvel Nagar, Bhubaneswar, India.

**OBJECTIVE:** Spontaneous recurrent pregnancy loss (RPL) is most often investigated from the women's perspective. However, recent evidences suggest the involvement of male factor as a plausible cause particularly in idiopathic RPL. Despite being transcriptionally and translationally quiescent, spermatozoa undergo maturation during transit through epididymal and female reproductive tract. Many proteins and regulatory RNAs associated with the exosomes (epididymosomes and prostasomes) are known selectively transfer their cargo to the sperm thereby modify sperm function. However, the proteome profile of exosomes in general and RPL in particular is largely unknown. Therefore, the main objective of the present study is to identify and understand the possible paternal factors responsible for early pregnancy loss through differential proteomic analysis of seminal exosomal proteins.

**DESIGN:** Prospective case-control study involving consented participants comprising of fertile donor (n = 21) and partners of spontaneous idiopathic recurrent pregnancy loss patients (n = 21).

**MATERIALS AND METHODS:** Seminal exosomes were isolated by ultracentrifugation and characterized by western blot, transmission electron microscopy, and nanoparticle tracking analysis followed by label free liquid chromatography mass spectrometry (LC-MS/MS) and bioinformatics pathway analysis (Ingenuity Pathway Analysis: IPA, Qiagen) and STRING protein-protein interaction (PPI) analysis.

**RESULTS:** A total of the 998 proteins were detected in the data set (Control: 939 and RPL: 935). Of the 447 differentially expressed proteins 385 underexpressed and 62 overexpressed in RPL while 63 and 59 proteins were exclusive to control and RPL, respectively. Immune response (HSA:168256 ; false discover rate p=2.67e-28), signalling proteins (HSA:376176 ; false discover rate p=3.04e-22), chromatin packaging and remodeling (GO:0031497; false discover rate p=2.78e-05), protein folding and apoptosis (HSA:109581; false discover rate p=5.93e-06) were the major pathways impaired in RPL as revealed by STRING-PPI analysis. Pathway analysis by IPA showed developmental, hereditary and immunological disorders were the top diseases while cell death and survival, cellular assembly and organization, DNA replication, recombination and repair, gene expression were the major functions that were deregulated in RPL spermatozoa. Overexpression of HNRNPC and HNRNPU in RPL may be responsible for defective chromatin organization and shortening of telomere-length while underexpression of RUVBL1 may be responsible for altered centrosome function leading to abnormal embryo development.

**CONCLUSIONS:** The result of this pilot study implies the importance of exosomes in sperm maturation and function, particularly in RPL. Further validation alongside the proteome profiling of spermatozoa may lead to identification of candidate biomarkers for determination of male factors in RPL.

**SUPPORT:** Higher Education Department, Government of Odisha, A University Grant Commission and Department of Science and Technology, Government of India

## ETHICS

O-121 Tuesday, October 15, 2019 10:45 AM

### ETHICAL AND LEGAL ANALYSIS OF ADVERTISING FOR PLANNED OOCYTE

**CRYOPRESERVATION.** Michelle J. Bayefsky, B.A., Louise Perkins King, MD, JD. Harvard Medical School, Boston, MA.



TABLE 1.

	All Sites	West Coast	East Coast	Non-mandated States	Mandated States	Non-academic	Academic
N=number of IVF websites	N=203	N=66	N=133	N=85	N=119	N=112	N=91
Direct link to the practice's clinic summary report (CSR) on <a href="http://SART.org">SART.org</a> website	103 (50.5%)	31 (46.9%)	70 (52.6%)	41 (48.2%)	62 (52.1%)	58 (51.8%)	44 (48.4%)
Supplemental success rates reported by live birth per transfer, retrieval and cycle per each age category	7 (9.5%)*	3 (12.5%)*	3 (6.1%)*	5 (16.1%)*	2 (4.7%)*	2 (6.5%)*	4 (9.5%)*
Disclaimer statement when quoting IVF success rates	106 (51.9%)	35 (53%)	69 (51.9%)	40 (47.1%)	66 (55.5%)	56 (50%)	49 (53.9%)

\*percentage reflects number of clinics that reported supplemental success rates by live birth per transfer, retrieval and cycle per each age category divided by number of clinics that reported any supplemental success rates

**OBJECTIVE:** This goal of this study is to conduct an ethical and legal analysis of advertising for planned oocyte cryopreservation (OC) targeted towards younger women.

**DESIGN:** This study involves a comparison of advertisements by commercial OC companies to ethical and legal standards for advertising.

**MATERIALS AND METHODS:** Advertisements by 8 prominent egg freezing companies were reviewed, including materials from the companies' websites, ads on social media, and comments made to the news media. Ethical standards for medical advertising were reviewed and applied, including historical codes of the American Medical Association, ACOG and ASRM policies on ethical advertising, as well as standards per the bioethics literature. Legal standards for truthful advertising were reviewed, including the Federal Trade Commission Act, the Food, Drug and Cosmetic Act and the Lanham Act. Drawing on recent studies on the safety, utility, and cost-effectiveness of planned OC for younger women, the companies' advertisements were evaluated against the legal and ethical standards described.

**RESULTS:** According to the prevailing legal and ethical standards on truth in advertising, some advertisements by leading OC companies could be considered unethical, misleading, deceptive, and/or unfair. For example, presenting anti-Mullerian hormone (AMH) as a stand-alone test of future fertility is misleading given recent studies that question the utility of AMH as a marker of fertility, particularly by itself. Furthermore, advertising by commercial egg freezing companies generally omits information regarding the low usage rates of electively cryopreserved eggs, estimates of cost-effectiveness for cryopreservation at different ages, and the risks of delaying pregnancy to an advanced maternal age.

**CONCLUSIONS:** Leading commercial egg freezing companies specifically target younger women in their advertisements and convey the message that planned OC allows women to take control of their future fertility and free themselves of biological limitations. Some of the claims made and not made in advertisements by OC companies fail to adhere to ethical and legal standards for truth in advertising. Women, including young women in their twenties, should have the option to pursue OC if they so choose, but to truly respect their autonomous decision-making, they must be presented with truthful and non-deceptive information about their options. Although patients will discuss the procedure with a reproductive endocrinologist before ultimately choosing to proceed, marketing strategies that bring patients through the door have the power to shape patients' impressions, goals and expectations. Misleading advertising places a heavy burden on reproductive endocrinologists to correct misconceptions, clarify current best evidence, and assist patients in making informed decisions. Further study is warranted to determine to what extent current advertising strategies impact young women's decision to undergo OC, and what onus is placed on clinicians to ensure balanced, knowledge-based shared decision-making with their patients.

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### IVF CLINIC WEBSITES: BUYER BEWARE THE SYSTEM IS BROKEN.

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**OBJECTIVE:** Historically, a large percentage of IVF clinics did not adhere to Society for Assisted Reproductive Technology (SART) guidelines for on-line advertising. New website guidelines, effective January 2018, are clear in expectations requiring a link to clinics' success rates on the SART site and statistical explanations alongside explicit rules about presenting supplemental data in its entirety specifying that "no partial presentation is allowed". SART emphasizes that "adherence to this advertising policy is a requirement for membership in SART". This study examined if SART member IVF centers adhere to this new SART advertising policy.

**DESIGN:** Cross-Sectional Evaluation.

**MATERIALS AND METHODS:** 203 IVF center websites were examined. Univariate analysis was used for descriptive data and Fisher's exact test was used to compare categorical data between subgroups.

**RESULTS:** Only 50.5% of clinics provided a link to the SART website and, similarly, only 51.9% provided the required disclaimer statement regarding their outcome statistics. Disturbingly, only 9.5% of websites followed SART requirements about the presentation of supplemental data. There were no significant differences between academic and non-academic centers, those in mandated vs non-mandated states, or East versus West Coast clinics in any of the above areas (table 1).

**CONCLUSIONS:** Only half of surveyed websites adhere to SART's core guidelines surrounding reporting with lower compliance percentages in other areas. Consideration for additional education could be considered and enforcement of guidelines should be enhanced.

**SUPPORT:** None

**O-123** Tuesday, October 15, 2019 11:15 AM

### **INFERTILITY IN THE DIGITAL AGE: AN OPPORTUNITY FOR REI PHYSICIANS TO COMBAT THE SPREAD OF MISINFORMATION AND FILL SUPPORT GAPS IN INFERTILITY CARE ONLINE.**

Emily A. Jacobs, MD, Ginny L. Ryan, MD, MA. University of Iowa Carver College of Medicine, Iowa City, IA.



**OBJECTIVE:** To examine infertility related content posted on Instagram, including content of posts and identity of content posters.

**DESIGN:** Retrospective content analysis.

**MATERIALS AND METHODS:** Data from Instagram were obtained on April 20, 2019. One author queried 42 popular hashtags, including both medical and lay person terminology, related to infertility diagnosis, treatment and procedures. The total number of posts from each hashtag was recorded. Each of the top ten posts (as determined by Instagram's internal algorithm) for the 42 hashtags was then analyzed to qualitatively identify the content of each post. The post category was determined by the lead author based on the content of the post and the overall message it sent to its readers. The number of likes and comments were also recorded for each post. Lastly, data on the individual who posted were also recorded by analyzing that poster's Instagram profile.

**RESULTS:** A total of 5,814,691 posts were tagged with the 42 unique hashtags queried for this study. 315 of the 420 "top posts" met inclusion criteria. Of the hashtags, #PCOS had the highest number of posts associated with it (2,000,000 posts). From the 315 included posts, 271 unique posters were identified. 239 of these posters were non-healthcare related individuals (88%) and 32 (12%) were healthcare related persons. There were 14 self-identified US physicians. All but one had verified credentials. By far, the most common type of post for non-healthcare related individuals was related to their infertility journey (60%). In contrast, the majority of posts created by healthcare-related individuals were educational (41%).

When comparing US verified physician posting versus all other posters, US physicians were more likely to post educational (33% vs 9%,  $p=0.0006$ ) and promotional posts (33% vs 1%,  $p=<0.0001$ ) and less likely to post about a personal infertility journey (5% vs 58%,  $p=<0.0001$ ). There was no significant difference in 'likes' between the two groups ( $194\pm 200$  for US physicians vs  $430\pm 787$  for all other posters,  $p=1.71$ ). There was a significant difference in the number of comments between the two groups, with fewer comments in response to US physicians than all other posters ( $12\pm 16$  vs  $36\pm 51$ ,  $p=0.015$ ).

No infertility postings by verified US physicians contained medical advice or medical questions. In contrast, 5% and 2% of postings by all other individuals gave medical advice or asked a medical question, respectively. Some of the medical advice given included taking 40mg/day of black cohosh for ovulation induction, using cannabis suppositories to shrink fibroids, and rec-

ommending supplements to increase fertility (who were often sold by the poster).

**CONCLUSIONS:** Instagram and other social media platforms have the potential to be highly influential in the infertility population. Physicians, particularly board-certified reproductive endocrinologists, should consider taking steps towards having a stronger presence online to combat the spread of misinformation that currently dominates these highly used platforms, and to help bridge gaps in access to infertility care.

**O-124** Tuesday, October 15, 2019 11:30 AM

### **ADVANCING LAWS TO PERMIT SURROGACY IN US STATES: CHALLENGES & SOLUTIONS FOR ART PROVIDERS & OTHERS.**

Robert Klitzman, MD. Columbia University, NY, NY.



**OBJECTIVE:** To understand how to advance legalization of surrogacy, through data addressing opponents' concerns

**DESIGN:** Analysis of state laws & discussions with state policymakers & others

**MATERIALS AND METHODS:** N/A

**RESULTS:** Many prospective parents face legal barriers to hiring traditional or gestational surrogates, posing critical questions of whether ART providers & others can address these obstacles & if so, how. A few US states (e.g., California) allow paid gestational surrogacy, upholding legal contracts that prohibit birth mothers from keeping the baby. Yet following the Baby M case, & largely due to fears of exploiting women as surrogates, US states range widely in whether they permit, prohibit, or limit surrogacy & how they enforce such laws. Recently, traditional surrogacy is allowed (since it is not explicitly banned) in 16 states; permitted by statute without much detail in 5; permitted by statute with restrictions in 2; permitted only if unpaid in 4; permitted but with unenforceable contracts in 9; practiced, though contracts are banned, in 2; not practiced because contracts are banned in 4; & unpredictable in 9. Gestational surrogacy is allowed by law in 3 states; allowed (since not explicitly banned) in 22; allowed by statute without much detail in 7; permitted with restrictions in 6; allowed with unenforceable contracts in 1; supported but with no law in 6; practiced, though contracts are prohibited in 5; & not practiced since contracts are prohibited in Washington, DC. States differ in how much surrogates can be paid (e.g., whether more than basic expenses); whether surrogates can change their minds & if so, in when; whether court approval & state residency are needed; & whether an intended parent must provide gametes.

Advocates have unsuccessfully tried altering laws in NY & elsewhere. Opponents tend to draw on conservative Christian arguments (and wariness of much ART) or feminist concerns that most surrogates will be poor & thus taken advantage of. Yet no data exist about these claims. Crucial questions thus arise of why women choose to be surrogates - e.g., who surrogates in fact are & how they see these issues. Anecdotally, many such women are middle class, fully grasp the risks & benefits, having given birth to their own children, & feel that the rewards are worth it. Data on gestational surrogates are thus essential - e.g., on their socioeconomic status, motivations & views of their experiences - how they perceive & experience it & whether they view it, retrospectively, favorably or regretfully - to assess whether claims of exploitation are correct. Such data, if they reveal few concerns, can prompt other states to permit surrogacy, assisting many parents. These data can also be vital in educating patients, providers & the public at large about these issues. ART providers could thus help by collecting such data. Widening use of electronic medical records can facilitate collection of some of these data. Providers could also work closely with patient groups on these goals.

**CONCLUSIONS:** ART providers & others can advance legislation of paid gestational & other surrogacy & thus aid patients through collection of key data.

**O-125** Tuesday, October 15, 2019 11:45 AM

### **EVALUATING THE SART CLINIC SUMMARY REPORTS - IS IT ONLY ABOUT THE LIVE BIRTH RATES? WHAT ABOUT THE SIGNIFICANT MORBIDITY/MORTALITY RISK FACTORS ASSOCIATED WITH MULTIPLE GESTATIONS?.**

Carrie Riestenberg, MD,<sup>a</sup> Alin Lina Akopians, MD, PhD,<sup>b</sup> Deborah E. Johnson, MA,<sup>c</sup> Zachary Haimowitz, BS,<sup>c</sup> Hal C. Danzer, MD,<sup>b</sup> Mark W. Surrey, MD,<sup>b</sup> Jason A. Barritt, PhD.<sup>c</sup> <sup>a</sup>University of California, Los Angeles, Los Angeles, CA; <sup>b</sup>Southern California



Reproductive Center, Beverly Hills, CA; <sup>c</sup>ART Reproductive Center, Beverly Hills, CA.

**OBJECTIVE:** In 1992, HR 4773, the Fertility Clinic Success Rate and Certification Act, also known as the Wyden bill, was passed mandating public reporting of fertility clinic pregnancy success rates. Currently, >90% of ART clinics in the USA report to SART. The CDC, SART and ASRM work together to publish annual reports of clinic's pregnancy outcomes. SART warns that "Accurate and complete reporting of ART success rates is complicated. Clinics may have differences in patient selection, treatment approaches, and cycle reporting practices which may inflate or lower pregnancy rates relative to another clinic. This report is best understood in consult with your physician." Furthermore, "success rates should not be used to compare treatment centers." In spite of this, patients rely on this information to decide which center they will ultimately choose for their fertility treatment. The objective of this study was to compare the ranking of live birth rate (LBR), singleton live birth rate (SLBR), and weighted 'risk score' ranking based on risk factors for morbidity/mortality associated with multiple gestation in clinics reporting  $\geq$  1000 total cycles annually.

**DESIGN:** Cross-sectional evaluation.

**MATERIALS AND METHODS:** The 2017 SART Preliminary Data report was reviewed for all reporting ART clinics. Those clinics reporting  $\geq$  1,000 total cycles annually were included in our analysis, for a total of 62 clinics. LBR, SLBR, twin and triplet rates were recorded for each of the clinics. A weighted 'risk score' was then calculated for each clinic in the following manner: twin rate x 8 + triplet rate x 21. The weighted 'risk score' assigned to twin and triplet gestations was derived from published relative risk data of prematurity and low birth weight of twin and triplet gestations compared to singletons, as these have been shown to be the principal risk factors for morbidity/mortality in multiple gestation pregnancies. All clinics were subsequently ranked into quartiles with regards to LBR and 'risk score'.

**RESULTS:** Of the 15 clinics in the top quartile with respect to LBR, only one clinic also ranked in the lowest quartile of 'risk score'. Of the remaining 14 clinics, 5 ranked in the highest 'risk score' quartile and 5 in the second highest 'risk score' quartile. Therefore, <30% of the clinics ranking in the highest quartile for LBR also ranked in the top two quartiles for safety.

**CONCLUSIONS:** Our study showed that out of the 62 highest volume ART clinics reporting to SART, two thirds of those in the top quartile for LBR are in the bottom two quartiles with respect to weighted 'risk score', with one third ranking in the highest 'risk score' quartile. Only one clinic was in the top quartile in both fields. This is a manifestation of the incentive to achieve a higher LBR, commonly acknowledged to be the most referenced statistic reported by SART, at the cost of a higher risk of multiple gestation. Though not intended to be a tool for clinic comparison and ranking, SART is clearly used in this manner. We argue that more attention should be brought to the balance of success and risk in order to optimize patient outcomes and encourage increased responsibility among ART clinics.

**SUPPORT:** None

**O-126** Tuesday, October 15, 2019 12:00 PM

#### **INSTITUTIONAL POLICIES ON POSTHUMOUS REPRODUCTION USING OOCYTES AND EMBRYOS: PRELIMINARY RESULTS FROM A CROSS-SECTIONAL STUDY.**



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**OBJECTIVE:** Posthumous assisted reproduction (PAR) raises complicated ethical and legal issues. ASRM recommends that assisted reproductive technology (ART) and fertility preservation (FP) programs develop written policies regarding cases of PAR, though little is known about adoption of such policies and how they have been implemented. Our objective was to assess the presence and content of policies toward PAR using oocytes and embryos among Society for Assisted Reproductive Technology (SART) member clinics in the U.S.

**DESIGN:** Cross-sectional questionnaire-based study.

**MATERIALS AND METHODS:** Our study consists of three phases of communication: email-, postal mail-, and phone-based survey. We report

on the first phase of anonymous email survey responses. Surveys were emailed to ASRM-member medical directors of all SART member clinics (n=332) during March and April 2019 using a modified Dillman Method; contact information was acquired from SART and ASRM membership data. The survey included 23 multiple-choice and 3 open-ended questions assessing practice characteristics (practice type, location, IVF cycle volume), presence of a clinic policy towards PAR, and the content of such policy. Descriptive data are presented as %, with Fisher's exact test used where appropriate, and thematic content analysis was applied to open-ended responses.

**RESULTS:** The first phase of the study received 39 clinic responses (12% response rate). Respondents were distributed across the U.S.; average volume of IVF cycles per year ranged from < 250 to > 1500. More than one-third (35.9%, n=14) of clinics reported participating in any cases of PAR over the past five years, and 5.1% (n=2) reported participation in more than five cases. Participation in cases of PAR was not significantly associated with practice type or IVF cycle volume (p>0.05). 57.9% (n=22) had written policies towards PAR using oocytes or embryos, while 36.8% (n=14) reported they did not have a policy. Practice type, IVF cycle volume, FP volume, and prior participation in cases of PAR were not significantly associated with the presence of a policy (p>0.05). Of those with a policy, 52.4% (n=11) reported they had used that policy, 66.7% (n=10) without a policy reported they had considered adopting one, and 60.0% (n=9) reported they had received a request for PAR services. Only 44% (n=15) of clinics specified that patients not expected to survive to use oocytes due to terminal illness were eligible for oocyte cryopreservation, while 50.0% (n=17) did not specify. Open-ended comments suggested need for case-by-case appraisal and firm consent policies regarding gamete disposition.

**CONCLUSIONS:** Our preliminary results suggest that SART programs are receiving an increasing number of requests for PAR services, but many SART programs lack PAR policies, and those with policies do not always follow ASRM recommendations. As PAR cases become more common, clinics should be equipped to manage the complexities of PAR. More data are needed as this study continues, and future research is needed to understand barriers to the creation and implementation of these increasingly needed policies.

#### **IMAGING AND REPRODUCTIVE MEDICINE**

**O-127** Tuesday, October 15, 2019 10:45 AM

#### **ORAL HYOSCINE BUTYL BROMIDE PLUS CERVICAL LIDOCAINE 5% CREAM IN REDUCING PAIN DURING HYSTEROSALPINGOGRAPHY.**



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**OBJECTIVE:** Infertility is defined as the failure of a couple to conceive during 12 months of regular unprotected intercourse. Tubal abnormalities account for 30-40% of the causes of female infertility. Hysterosalpingography (HSG) is a diagnostic procedure in the evaluation of infertile women and considered to be the traditional and the gold standard in the assessment of the patency of the fallopian tubes. The major disadvantage of HSG is pain. Our objective is to evaluate the analgesic effect of combining oral Hyoscine Butyl Bromide (HBB) with cervical lidocaine cream in alleviating pain during HSG.

**DESIGN:** Randomized double-blinded controlled trial (clinicaltrials.gov: NCT02710305).

**MATERIALS AND METHODS:** The study included reproductive-aged infertile women scheduled for HSG. Eligible women were recruited and randomized (1:1) to HBB plus lidocaine or Placebo group. All women received oral 20 mg HBB or placebo tablets 30 minutes before HSG, and then 4 ml of lidocaine 5% cream or placebo was applied to the anterior cervical lip, followed by 2 ml placed in the cervical canal using a sterile needless syringe. The study outcomes were the mean pain score reported during speculum placement, cervical tenaculum placement, injection of the dye, 5 minutes and 30 minutes post-procedure using a 10-cm Visual Analogue Scale (VAS). A 2 cm difference in VAS score between both groups was considered a clinically significant difference. Other outcomes included the number of women who asked for additional analgesics and the adverse effects of the study medications. Mann Whitney test and Fisher's exact test were used for the analysis of the outcomes. Multivariate regression analysis was

performed to determine the independent predictors of pain with the dependent variable (VAS score during the injection of the dye).

**RESULTS:** One hundred forty women were enrolled and randomized to HBB plus lidocaine arm (n=70) or placebo (n=70). Both groups were similar in age, parity, BMI, duration of infertility and the prior mode of delivery without statistically significant differences. Women in the HBB plus lidocaine group were more likely to report lower VAS scores during injection of the dye, 5 minutes and 30 minutes post procedure (median: 3 vs. 6,  $p<0.001$ ; 2.5 vs. 5,  $p<0.001$ ; 1.5 vs. 3,  $p<0.001$ , respectively). Moreover, eighteen women asked for additional analgesics in the placebo group versus seven women in the study group ( $p=0.02$ ). No difference in the rate of adverse effects. The following variables were not predictors of pain; nulliparity ( $p=0.48$ ), previous cesarean deliveries (0.28), dysmenorrhea ( $p=0.13$ ), chronic pelvic pain ( $p=0.42$ ) and prior HSG ( $P=0.45$ ).

**CONCLUSIONS:** Utility of oral HBB 30 minutes before HSG plus cervical lidocaine 5% cream significantly alleviate the induced pain during and 30 min after the HSG procedure.

**SUPPORT:** None

**O-128** Tuesday, October 15, 2019 11:00 AM

### THE DEVELOPMENT OF A SYSTEM TO AUTOMATICALLY EVALUATE THE NUMBER OF PRONUCLEI USING DEEP LEARNING TECHNOLOGY.

Yuta Kida, M.S.,<sup>a</sup> Noritaka Fukunaga, Ph.D.,<sup>a</sup> Sho Sanami, Ph.D.,<sup>b</sup> Hiroyuki Watanabe, M.S.,<sup>a</sup> Yuji Tsuzuki, M.S.,<sup>b</sup> Hiroya Kitasaka, Ph.D.,<sup>a</sup> Seiji Takeda, M.S.,<sup>b</sup> Yoshimasa Asada, M.D., Ph.D.<sup>a</sup> <sup>a</sup>Asada Ladies Clinic, Nagoya, Aichi, Japan; <sup>b</sup>Research & Development Center, Dai Nippon Printing Co., Ltd., Kita-ku, Tokyo, Japan.



**OBJECTIVE:** Embryo evaluation requires long-term experience and learning to acquire high degree of accuracy. In addition, it is difficult to maintain consistency in the quality of evaluation differences among embryologists. Therefore, we have applied an analysis using Deep learning technology (DL). DL can greatly improve learning accuracy by repeat machine learning utilizing a system called Deep Neural Network (DNN). In this study, we aimed to develop a more objective and automatic evaluation system for pronuclear (PN) evaluation using DL for time lapse images (TL) of PN embryos.

**DESIGN:** We built a stand-alone framework with DNN as the core to automatic evaluation system of the PN number in human embryos, based on TL.

**MATERIALS AND METHODS:** Part 1: TL of 0, 1 or 2 PN categories (300 of each) assessed by an experienced embryologist (total 900 embryos) were used to develop algorithms for detecting the PN number. We constructed two methods, one to output the number of PN directly to the input TL by applying general DL (M1) and a modified method (M2) which combined with DNN1 for automatically detecting the PN contours from the input TL and DNN2 for outputting the PN number judged from DNN1. The detection accuracy of the two algorithms was compared using 100 embryos for each PN categories (total 300 embryos) not used for DL. The chi-square test or Fisher's exact test were used for the significant difference test.

Part 2: TL of 0, 1, 2, 3 or multi (4 or more) PN categories (300 of each) assessed by experienced embryologist (total 1500 embryos) were used to develop algorithms for detecting the PN number. M2 was reconstructed by additional learning (M3), and used to develop a method (M4) to modify M3 by changing the number of parameter (3PN and multi PN were learned as same category). We compared the detection accuracy of the two algorithms using further batches of 100 embryos (total 500 embryos) TL, not used for DL. The chi-square test or Fisher's exact test were used for the significant difference test.

**RESULTS:** Part 1: The M1 and M2 detection rates were respectively 0 PN (69% vs 99%), 1 PN (33% vs 82%) and 2 PN (91% vs 99%). All detection rates were significantly improved in M2 compared to M1 ( $P<0.05$ ).

Part 2: The M3 and M4 detection rates were respectively 0 PN (95% vs 100%), 1 PN (68% vs 71%), 2 PN (86% vs 90%), 3 PN (33% vs 81%) and multi PN (53% vs 85%). For 0 PN, 3 PN and multi PN, the detection rate was significantly improved in M4 ( $P<0.05$ ).

**CONCLUSIONS:** In this study, the detection accuracy of 2 PN by DL showed a very high correlation with the evaluation of an embryologist. In addition, even for the detection of complex PN, improvement in detection accuracy was observed by adding improvements in learning methods. Embryo evaluation by an embryologist requires long-term experience, but the embryo evaluation accuracy by DL was able to approach that of an embryologist. This evaluation has demonstrated the possibility of DL techniques to guarantee objective gamete evaluation through automatic analysis of the image. As a next step, we are in the process of developing a system that combines automatic detection of PN by DL of TL within a continuous culture system throughout embryo development.

**O-129** Tuesday, October 15, 2019 11:15 AM

### LOW ENDOMETRIAL VOLUME IS NOT ASSOCIATED WITH DIMINISHED LIVE BIRTH FOLLOWING TRANSFER OF A SINGLE THAWED EUPLOID BLASTOCYST.

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**OBJECTIVE:** Three-dimensional ultrasound (3D US) facilitates reproducible assessment of endometrial volume (EV)<sup>1</sup>, but whether or not EV is associated with pregnancy outcomes in women undergoing in vitro fertilization (IVF) is unclear<sup>2</sup>. The objective of this study is to evaluate the association between EV and pregnancy outcomes following transfer of a single thawed euploid blastocyst.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** All patients planning to undergo a single thawed euploid blastocyst transfer between April and December 2017 at a large IVF center were eligible for inclusion. Subjects underwent endometrial preparation according to a standardized protocol. On the day prior to transfer, 3D US was performed for assessment of EV. Patients then underwent transfer of a single thawed euploid blastocyst.

EV was classified into four quartiles according to the 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> percentiles. The primary outcome was live birth. Secondary outcomes included clinical pregnancy (presence of a gestational sac on ultrasound), miscarriage (pregnancy loss after documentation of gestational sac), and ectopic pregnancy. Analysis of variance was used to compare continuous variables and chi square or Fisher's exact test was used for categorical variables. Multivariate logistic regression was performed to account for potential confounders.

**RESULTS:** A total of 638 subjects consented to participation and completed the study. There were no differences amongst EV quartiles by age at retrieval, age at transfer, or body mass index. EV was directly associated with gravidity, parity and endometrial thickness (all  $P<0.01$ ). Table 1 shows pregnancy outcomes by EV quartile. When accounting for potential confounders, there were no associations between EV and live birth [aOR 0.97 (0.90-1.05)], clinical pregnancy [aOR 1.00 (0.92-1.92)] or miscarriage [aOR 1.07 (0.95-1.21)]. There was a non-significant trend between low EV and ectopic pregnancy [aOR 1.59 (0.96-2.63),  $P=0.07$ ].

**CONCLUSIONS:** EV is not associated with clinical pregnancy, miscarriage or live birth following transfer of a single thawed euploid blastocyst. It is possible that low EV confers an increased risk for ectopic pregnancy; however this association did not reach statistical significance and warrants further investigation.

Pregnancy outcome	Quartile of EV (ml)				P-value
	Q1: < 3.7 (n=152)	Q2: 3.7-4.8 (n=180)	Q3: 4.9-6.2 (n=150)	Q4: ≥ 6.3 (n=156)	
Clinical pregnancy, n (%)	111 (73.0%)	139 (77.2%)	116 (77.3%)	117 (75.0%)	0.78
Miscarriage, n (%)	13 (8.6%)	9 (5.0%)	13 (8.7%)	17 (10.9%)	0.26
Ectopic pregnancy, n (%)	5 (3.3%)	3 (1.7%)	0 (0%)	1 (0.6%)	0.08
Live birth, n (%)	96 (63.2%)	130 (72.2%)	102 (68.0%)	99 (63.5%)	0.24

References: 1. Raine-Fenning et al. The reproducibility of endometrial volume acquisition and measurement with the VOCAL-imaging program. *Ultrasound Obstet Gynecol* 2002;19:69-75.

2. Saravelos et al. Assessment of the uterus with three-dimensional ultrasound in women undergoing ART. *HRU* 107; 23(2): 199-210.

**O-130** Tuesday, October 15, 2019 11:30 AM

**COMPARISON OF SINGLETON AND TWIN PREGNANCY OUTCOMES IN WOMEN WITH A CONGENITAL UNICORNUATE UTERUS AFTER IN VITRO FERTILIZATION-EMBRYO**



**TRANSFER.** Qingqing Wu, Bachelor's degree,<sup>a</sup> Yan Ouyang, MD./Ph.D.,<sup>b</sup> Xihong Li, MD./Ph.D.,<sup>b</sup> Pei Cai, Master.<sup>c</sup> <sup>a</sup>Institute of Reproductive & Stem Cell Engineering, Central South University, Changsha, China; <sup>b</sup>Reproductive and Genetic hospital of Citic-Xiangya, Changsha, China; <sup>c</sup>Central South University, Changsha, China.

**OBJECTIVE:** To compare the singleton and twin pregnancy outcomes in women with a congenital unicornuate uterus after in vitro fertilization-embryo transfer (IVF-ET).

**DESIGN:** A retrospective analysis.

**MATERIALS AND METHODS:** A single-center retrospective cohort study was conducted with 336 women who were diagnosed with a congenital unicornuate uterus from January 2012 to December 2017. In order to avoid selection bias, only the first pregnancy of each patient were considered. All patients were diagnosed as clinical pregnancies by early transvaginal sonography in our hospital. Ectopic pregnancy, multiple pregnancy, selective or spontaneous reduction and induced labor were excluded from this analysis.

**RESULTS:** There was no significant difference in the mean maternal age, body mass index, infertility type, infertility duration and infertility factors between the singleton-pregnancy group and the twin-pregnancy group ( $p > 0.05$ ). When compared to the twin-pregnancy group, singleton-pregnancy group had a significantly lower perinatal mortality (1.8% vs. 15.7%, OR = 0.101 (0.033-0.311),  $P < 0.001$ ) and live birth weight ( $3068 \pm 514$  vs.  $2260 \pm 476$ ,  $P < 0.001$ ), and lower rates of preterm delivery (13.0% vs. 57.6%, OR=0.110 (0.059-0.205),  $P < 0.001$ ), and low birth weight (11.7% vs. 58.1%, OR=0.096 (0.53-0.174),  $P < 0.001$ ), while a markedly higher rate of term birth (65.3% vs. 28.8%, OR=4.658 (2.517-8.619),  $P < 0.001$ ). Simultaneously, the rate of miscarriage (20.6% vs. 11.9%,  $P=0.122$ ) in the twin-pregnancy group was lower than that in the singleton-pregnancy group, and the live birth rate (76.9% vs. 76.3%,  $P=0.918$ ) of the single-pregnancy group was basically consistent with the twin pregnancy group, these difference were not statistically significant.

TABLE. The comparison between singleton-pregnancy and twin-pregnancy

Pregnancy outcomes	Singleton-pregnancy group(n=277)	Twin-pregnancy group(n=59)	P-value	OR (95%-CI)
Miscarriage, %(n)	20.6% (57/277)	11.9% (7/59)	0.122	1.925 (0.830-4.463)
Preterm delivery, %(n)	13.0% (36/277)	57.6% (34/59)	<0.001	0.110 (0.059-0.205)
Term birth, %(n)	65.3% (181/277)	28.8% (17/59)	<0.001	4.658 (2.517-8.619)
Perinatal mortality, %(n)	1.8% (4/217)	15.7% (16/102)	<0.001	0.101 (0.033-0.311)
Live birth, %(n)	76.9% (213/277)	76.3% (45/59)	0.918	1.035 (0.534-2.007)
Birth weight, (g)	$3068 \pm 514$	$2260 \pm 476$	<0.001	
Low birth weight, %(n)	11.7% (25/213)	58.1% (50/86)	<0.001	0.096 (0.53-0.174)

**CONCLUSIONS:** Singleton-pregnancy could obtain better pregnancy outcomes than twin-pregnancy in women with a unicornuate uterus anomaly after IVF-ET. Therefore, reducing the incidence of twin pregnancy in women with a unicornuate uterus is clinically necessary.

**SUPPORT:** The Science and technology project of Health and Family Planning Commission of Hunan Province (No. C20180898) and the Citic-Xiangya Research Fund (No. KYXM-201703).

**O-131** Tuesday, October 15, 2019 11:45 AM

**UTERINE SUBSEPTATIONS AN INDICATION FOR SURGICAL CORRECTION IN INFERTILITY PATIENTS: A COMPARISON OF FOUR**



**SYSTEMS.** Mary Emily Christiansen, MD,<sup>a</sup> Irene Peregrin-Alvarez, MD,<sup>a</sup> Robert Roman, MD,<sup>b</sup> Roberto Levi D'Ancona, MD,<sup>b</sup> Jennifer Gordon, MD,<sup>c</sup> Laura Detti, MD.<sup>b</sup> <sup>a</sup>The University of Tennessee

Health and Science Center, Memphis, TN; <sup>b</sup>University of Tennessee Health Science Center, Memphis, TN; <sup>c</sup>UTHSC, Memphis, TN.

**OBJECTIVE:** In this study we sought to evaluate uterine subseptations using four proposed methods: the AFS-10 mm (1988/2003), the ESHRE-ESGE (2013), the ASRM (2016), and our group's 5.9 mm cut-off length (2017), to identify the classification method which allows the most accurate diagnosis and indication for surgical incision

**DESIGN:** This was a retrospective cohort study at a University center.

**MATERIALS AND METHODS:** Patients being evaluated for infertility or recurrent pregnancy loss were included in the study if they were diagnosed with a uterine subseptation, defined as having a length  $\geq 3$  mm, as this was the minimum length measured in our population. Patients diagnosed with subseptate uteri were evaluated with 2-D and 3-D ultrasound in accordance with the four different methods. The diagnosis of uterine septum according to each method's specifications was then compared among the four groups ASF, ESHRE-ESGE, ASRM, and 5.9-mm cut-off. We compared distributions using the non-parametric Mann-Whitney U test with a p value  $< 0.05$  defining statistical significance. We used SPSS v24 for Windows (Chicago, Illinois).

**RESULTS:** 125 women had uterine subseptations and all four diagnostic systems identified septate uteri in our database. The 5.9-mm cut-off diagnosed 89 septate, and 36 normal uteri and was the most inclusive while the ASRM cut-off was the most restrictive one. Subseptation were inconsistently diagnosed by the ESHRE-ESGE classification, as some subseptations longer than 10 mm would be classified as normal uteri. Five/24 had had one previous early loss and 19/24 had suffered recurrent early pregnancy loss. The 5.9-mm system was the most sensitive, while the ASRM the least sensitive) in predicting pregnancy loss (71.2% vs. 9.5%).

**CONCLUSIONS:** The proposed 5.9-mm cut-off was the most sensitive in identifying subseptate uteri and in predicting early pregnancy loss. Conversely, the AFS-10 mm and the ASRM were too restrictive, potentially missing treatment for dangerous subseptations. When dealing with such a catastrophic outcome as a pregnancy loss, it is important to find the most sensitive system to diagnose a subseptation, and the 5.9-mm system had the highest sensitivity to diagnose a subseptation and its risk of early pregnancy loss. The current study bridges the gap undermining other studies: it correlates the diagnosis of septate uterus with obstetric outcomes and provides an objective analysis of the morphometric changes of the septate compared to the normal uterus.

**SUPPORT:** None

**O-132** Tuesday, October 15, 2019 12:00 PM

**PRE-OPERATIVE MAGNETIC RESONANCE IMAGING VERSUS ULTRASONOGRAPHY FOR PREDICTING THE OPERATIVE OUTCOMES OF ABDOMINAL OR LAPAROSCOPIC MYOMECTOMY.**



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**OBJECTIVE:** To compare the utility of pelvic magnetic resonance imaging (MRI) and pelvic ultrasonography (US) in predicting the operative outcomes of patients undergoing abdominal myomectomy (AM), laparoscopic myomectomy (LM), or robot-assisted laparoscopic myomectomy (RALM).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Women <45 years undergoing AM, LM or RALM for symptomatic leiomyomata were included. Pre-operative pelvic MRI or US was performed based on physician preference. Baseline demographics were recorded for all patients, including the number, location and dimensions of all leiomyomata on MRI or US. Total leiomyomata volumes were calculated based on recorded dimensions. Primary operative outcomes of interest were total operating time, leiomyomata weight and estimated blood loss (EBL). Spearman's correlation was used to evaluate the correlation between leiomyomata volume and operative outcomes. Receiver-operator-characteristic (ROC) curves were constructed for outcomes showing statistical significance.

**RESULTS:** A total of 117 patients were included; there was no difference in the demographics or leiomyomata characteristics of patients undergoing MRI or US in the AM (n=69), LM (n=13) or RALM (n=35) groups. The mean age and leiomyomata volume of patients undergoing LM was 36.7±7.1 years and 152.1±90.9 mL, respectively. There was a strong positive correlation between MRI leiomyomata volume and operating time (rho=0.90; P<0.001) and leiomyomata weight (rho=0.89; P=0.02). Patients in the RALM group had a mean age and leiomyomata volume of 36.9±4.1 years and 242.4±136.1 mL, respectively. A significant positive correlation between MRI leiomyomata volume and operating time (rho=0.83; P=0.03) and leiomyomata weight (rho=0.79; P=0.01) was noted in the RM group as well. These correlations were non-significant in the LM and RALM groups when using US leiomyomata volume. MRI leiomyomata volume was also predictive of LM and RALM conversion to laparotomy (area-under-the-curve=0.92). These correlations were positive but non-significant in AM group. No correlation was observed between MRI and US leiomyomata volume and EBL in all groups.

**CONCLUSIONS:** Pre-operative pelvic MRI in patients undergoing LM or RALM strongly correlates with operating time and leiomyomata weight and predicts conversion to laparotomy.

**SUPPORT:** None

### IVF OUTCOME PREDICTORS 1

**O-133** Tuesday, October 15, 2019 10:45 AM

#### GONADOTROPIN-SPECIFIC FOLLICULAR STEROIDOGENESIS IN OVARIAN STIMULATION: EVIDENCE FROM THE MENOPUR IN GNRH ANTAGONIST SINGLE EMBRYO TRANSFER - HIGH RESPONDER (MEGASET-HR) TRIAL.

Fady I. Sharara, M.D.,<sup>a</sup> Eric D. Foster, PhD,<sup>b</sup> Anshul Sinha, B.Tech,<sup>b</sup> Gaurang S. Daftary, MD, MBA,<sup>b</sup> Patrick W. Heiser, PhD.<sup>b</sup> <sup>a</sup>Virginia Center for Reproductive Medicine, Reston, VA; <sup>b</sup>Ferring Pharmaceuticals, Inc. Parsippany, NJ.

**OBJECTIVE:** To evaluate gonadotropin related differences in follicle endocrine physiology in predicted high responder women undergoing assisted reproductive technology.

**DESIGN:** Multicenter, randomized, assessor-blind, non-inferiority trial.

**MATERIALS AND METHODS:** Ovulatory women aged 21-35y, BMI 18-30 kg/m<sup>2</sup> and serum anti-Müllerian hormone (AMH) ≥ 5 ng/mL (N=620) were randomized 1:1 to a 150 IU start dose of HP-hMG or rFSH in a GnRH antagonist cycle; 75 IU dose adjustments were allowed on/after stimulation day 6. Central laboratory serum hormones were measured on

stimulation days: 1, 6, day of/after trigger. Log transformation was performed and displayed with/without modeling as a function of both the number of follicles and follicles ≥ 12 mm to account for site of steroidogenesis. Birth outcomes resulting from fresh/frozen transfers within 6 months of randomization were collected.

**RESULTS:** Demographics for the HP-hMG and rFSH arms were similar. The primary non-inferiority end-point of ongoing pregnancy was met, but a higher average number of oocytes/patient was retrieved in the rFSH (22.2) vs. the HP-hMG arms (15.1). Cumulative live birth rates were similar, but OHSS and cumulative early pregnancy loss rates were significantly higher in subjects who received rFSH. Although serum estradiol (E<sub>2</sub>) concentrations were significantly elevated on day 6 and day of trigger in the rFSH group, serum E<sub>2</sub> adjusted by follicle number was instead higher in the HP-hMG group on the day of trigger. Progesterone levels remained higher in the rFSH group independent of model. Androstenedione and testosterone levels were significantly higher regardless of adjustment, in the HP-hMG group on the day of trigger (table).

**CONCLUSIONS:** Data suggest that gonadotropin specific follicular steroidogenic responses exist. After accounting for ovarian response, HP-hMG drives higher androgen and estradiol with lower progesterone levels at the end of stimulation. Additional investigation will determine whether changes in ovarian follicle steroid output might be linked to the differences in safety parameters observed.

**SUPPORT:** Ferring Pharmaceuticals

**O-134** Tuesday, October 15, 2019 11:00 AM

#### IN VITRO FERTILIZATION WITH PERSONALIZED BLASTOCYST TRANSFER VERSUS FROZEN OR FRESH BLASTOCYST TRANSFER: A MULTICENTER, RANDOMIZED CLINICAL TRIAL.

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	DAY 6				TRIGGER DAY			
	rFSH	HP-hMG	ADJ rFSH	ADJ HP-hMG	rFSH	HP-hMG	ADJ rFSH	ADJ HP-hMG
Androstenedione pmol/L	5279 5041, 5527	4984 4778, 5200	5230 4987, 5484	5100 4861, 5350	7594 7163, 8051	9789 9258, 10351	7300 6951, 7667	10190 9692, 10713
Testosterone nmol/L	1.2 1.2, 1.3	1.2 1.2, 1.3	1.2 1.2, 1.3	1.3 1.2, 1.3	2.0 1.9, 2.1	2.4 2.3, 2.6	1.9 1.8, 2.0	2.5 2.4, 2.7
Estradiol pmol/L	2627 2407, 2867	864 768, 973	2533 2328, 2755	930 853, 1014	9570 8823, 10382	8262 7576, 9011	8310 7631, 9049	9612 8805, 10494
Progesterone nmol/L	0.5 0.4, 0.5	0.2 0.2, 0.2	0.5 0.4, 0.5	0.2 0.2, 0.2	2.3 2.1, 2.6	1.4 1.2, 1.5	2.1 1.9, 2.3	1.5 1.4, 1.7

ADJ; adjusted Value  
95% Confidence Interval

**OBJECTIVE:** To determine the effectiveness of personalized embryo transfer (pET) versus frozen embryo transfer (FET) or fresh embryo transfer (ET) in infertile patients undergoing IVF at their first appointment. In pET, embryo transfer is performed within the optimal window of implantation identified by the endometrial receptivity analysis (ERA).

**DESIGN:** Multicenter randomized clinical trial. Participants aged  $\leq 37$  years scheduled for IVF with elective blastocyst transfer at the first appointment were randomized to undergo pET, FET or ET.

**MATERIALS AND METHODS:** Setting: 16 reproductive medicine centers in Europe, America and Asia with a common reference genetic laboratory.

**Patient(s):** We recruited 569 women, and 458 were randomly assigned to pET (N=148), FET (N=154), or ET (N=156) groups.

**Intervention(s):** The ERA test was performed using hormone replacement therapy guiding embryo transfer in the pET arm. Blastocyst vitrification was performed in the pET and FET arms. Blastocyst transfer in all groups.

**Main outcome measure(s):** The primary outcome was live birth. Secondary outcomes were pregnancy and implantation rates as well as clinical miscarriage, biochemical pregnancy, and obstetric and neonatal outcomes. We performed intention-to-treat and per protocol analyses.

**RESULTS:** In the per protocol analysis, live birth rates at the first embryo transfer were 45 of 80 (56.2%) in the pET group, 39 of 92 (42.4%) in the FET group, and 43 of 94 (45.7%) in the ET group (pET versus FET relative risk [RR] 1.35, 95% confidence interval (CI) 0.97- 1.86;  $p=0.09$ ; pET versus ET [RR] 1.26, 95% (CI) 0.91-1.74;  $p=0.17$ ). Cumulative live birth rates after 12 months were 57 of 80 (71.2%) in the pET group, 51 of 92 (55.4%) in the FET group, and 46 of 94 (48.9%) in the ET group (pET versus FET [RR] 1.47, 95% (CI) 1.01-2.13;  $p=0.04$ ; pET versus ET [RR] 1.71, 95% (CI) 1.17-2.49;  $p=0.003$ ).

Pregnancy rates at the first embryo transfer in the pET, FET and ET were 72.5%, 54.3% and 58.5% respectively (RR 1.56 pET versus FET, 95% CI 1.07 to 2.29,  $p=0.01$ ; pET versus ET 1.42, 0.98-2.08,  $p=0.05$ ). Implantation rates at the first embryo transfer were 57.3%, 43.2% and 38.6%, respectively (pET versus FET RR 1.37, 95%CI 1.03-1.82,  $p=0.03$ ; pET versus ET RR 1.54, 95%CI 1.15-2.05,  $p=0.004$ ).

No differences between groups were found for clinical miscarriage, biochemical pregnancy or any other secondary outcomes. Obstetrical outcomes, type of delivery and neonatal outcomes were similar in all groups.

**CONCLUSIONS:** In this RCT, we found a statistically significant improvement in cumulative live birth rates in pET compared to FET and ET. Pregnancy and implantation rates after pET over FET and ET at first attempt as well as in cumulative rates were significantly higher. These findings indicate the potential of pET with the ERA test at the first appointment that should be confirmed in larger randomized clinical trials. ([ClinicalTrials.gov NCT 01954758](https://www.clinicaltrials.gov/NCT01954758)).

**References:** 1. Malizia B, Hacker M, Penzias A. Cumulative live-birth rates after in vitro fertilization. *New England Journal of Medicine* 2009; 360(3): 236-43.

2. Adamson G, de Mouzon J, Chambers G, et al. International Committee for Monitoring Assisted Reproductive Technology: world report on assisted reproductive technology, 2011. *Fertility and Sterility* 2018; 110(6): 1067-80.

3. Simon C, Giudice L. The Endometrial Factor: A Reproductive Precision Medicine Approach. Boca Raton. CRC Press; 2017.

4. Navot D, Veckel L, Scott R, Liu H, Droesch K, Rosenwaks Z. The window of embryo transfer and the efficiency of human conception invitro. *Fertility and Sterility* 1991; 55(1): 114-8.

5. Wilcox A, Baird D, Wenberg C. Time of implantation of the conceptus and loss of pregnancy. *New England Journal of Medicine* 1999; 340(23): 1796-9.

6. Murphy C. Uterine receptivity and the plasma membrane transformation. *Cell Research* 2004; 14(4): 259-67.

7. Ponnampalam AP, Weston GC, Trajstman AC, Susil B, Rogers PA. Molecular classification of human endometrial cycle stages by transcriptional profiling. *Mol Hum Reprod*. 2004; 10(12): 879-93.

8. Talbi S, Hamilton A, Vo K, Tulac S, Overgaard MT, Dosiou C, et al. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology*. 2006; 147(3): 1097-121.

9. Riesewijk A, Martin J, van Os R, Horcajadas JA, Polman J, Pellicer A, Simon C. Gene expression profiling of human endometrial receptivity on days LH+ 2 versus LH+ 7 by microarray technology. *Mol Hum Reprod*. 2003; 9(5): 253-64.

10. Diaz-Gimeno P, Horcajadas J, Martinez-Conejero J, et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptional signature. *Fertility and Sterility* 2011; 95(1): 50-60.

11. Diaz-Gimeno P, Ruiz-Alonso M, Blesa D, et al. The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity. *Fertility and Sterility* 2013; 99(2): 508-17.

12. Ruiz-Alonso M, Blesa D, Diaz-Gimeno P, et al. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. *Fertility and Sterility* 2013; 100(3): 818-24.

13. Ruiz-Alonso M, Galindo N, Pellicer A, Simon C. What a difference two days make: "personalized" embryo transfer (pET) paradigm: A case report and pilot study. *Human Reproduction* 2014; 29(6): 1244-7.

14. Mahajan N. Endometrial receptivity array: Clinical application. *J Hum Reprod Sci*. 2015; 8(3): 121-9.

15. Hashimoto T, Koizumi M, Doshida M, Toya M, Sagara E, Oka N, Nakajo Y, Aono N, Igarashi H, Kyono K. Efficacy of the endometrial receptivity array for repeated implantation failure in Japan: A retrospective, two-centers study. *Reprod Med Biol*. 2017; 16(3): 290-6.

16. Tan J, Kan A, Hitkari J, et al. The role of the endometrial receptivity array (ERA) in patients who have failed euploid embryo transfers. *Journal of Assisted Reproduction and Genetics* 2018; 35(4): 683-92.

17. Mol BW, Bossuyt PM, Sunkara SK, Garcia Velasco JA, Venetis C, Sakkas D, Lundin K, Simón C, Taylor HS, Wan R, Longobardi S, Cottell E, D'Hooghe T. Personalized ovarian stimulation for assisted reproductive technology: study design considerations to move from hype to added value for patients. *Fertil Steril* 2018; 109: 968-979

18. Kasius A, Smit JG, Torrance HL, Eijkemans MJ, Mol BW, Opmeer BC, Broekmans FJ. Endometrial thickness and pregnancy rates after IVF: a systematic review and meta-analysis. *Hum Reprod Update* 2014; 20: 530-41.

19. Connell MT, Szatkowski JM, Terry N, DeCherney AH, Propst AM, Hill MJ. Timing luteal support in assisted reproductive technology: a systematic review. *Fertil Steril* 2015; 103: 939-46 e3.

20. Glujovsky D, Pesce R, Fiszbajn G, Sueldo C, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. *Cochrane Database Syst Rev* 2010; 20: CD006359

21. Lensen S, Osavlyuk D, Armstrong S, et al. A randomized trial of endometrial scratching before in vitro fertilization. *New Engl J Med*. 2019; 380: 325-34.

**SUPPORT:** The study was supported by Igenomix.

**O-135** Tuesday, October 15, 2019 11:15 AM

## **ROLE OF METFORMIN IN PREVENTION OF PREMATURE LUTEINIZATION IN FRESH INTRACYTOPLASMIC SPERM INJECTION (ICSI) CYCLES: A RANDOMIZED CONTROLLED TRIAL.**

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**OBJECTIVE:** To date, an effective approach for prevention of premature luteinization (PL) is lacking. Metformin has been shown to inhibit the first step of steroidogenesis, and consequently decrease progesterone output from the granulosa cells. This study was to investigate possible roles of metformin in decreasing progesterone levels in the late follicular phase and improving pregnancy outcomes

**DESIGN:** Single-university affiliated IVF center placebo-controlled, double-blind, randomized trial (NCT 03088631).

**MATERIALS AND METHODS:** Infertile women planned for IVF with ICSI were randomized in 1:1 ratio into two groups, metformin treatment and placebo. Patients in metformin group received 1500 mg/day from the starting of contraceptive pills in the preceding cycle until ovulation triggering day. Baseline measures and ICSI characteristics were examined. Regression and correlation analysis models were utilized to evaluate the impact of metformin on PL parameters and ongoing pregnancy rates.

**RESULTS:** A total of 320 women were enrolled in the study. There was no statistical difference in demographics including age, body mass index, basal FSH, anti-mullerian hormone, duration of infertility, the indication for ICSI, and number of previous IVF cycles between the 2 groups. Women in metformin group showed more antral follicle count (AFC)



[median(IQR): 13(8) vs. 11(5),  $p < 0.005$ ]. As for cycle characteristics, both groups have comparable ovarian stimulation protocols, days of ovarian stimulation, endometrial thickness on day of triggering, peak estradiol levels, and number of mature oocytes retrieved. Metformin group received lower total gonadotrophin dose compared to the placebo group [median(IQR): 2700(1275) vs. 3300(1425),  $P < 0.001$ ]. Metformin group generated higher top quality embryo rate (TQE) [60% vs. 50%,  $p < 0.005$ ] and higher ongoing pregnancy rate [43.8% vs. 31.7%,  $p < 0.02$ ] as compared to placebo. In multiple binary logistic analysis adjusted to AFC and embryo quality, metformin group still demonstrated positive association with ongoing pregnancy [aOR 1.63 CI 1.02-2.59,  $P < 0.04$ ]. Furthermore, metformin group showed lower serum progesterone level at day of ovulation triggering, progesterone to estradiol ratio, and progesterone to mature oocyte index (calculated by dividing serum progesterone level by number of mature oocyte) [median(IQR): 0.9(0.5) vs. 1.1(0.7),  $p < 0.001$ ; 0.31(0.2) vs. 0.41(0.3),  $p < 0.002$ ; 0.08(0.07) vs. 0.11(0.1),  $p < 0.002$ , respectively]. In stepwise linear and conditional regression models, adjusted to ICSI cycle co-variables, metformin was associated with lower serum progesterone level ( $p < 0.001$ ) and more likelihood to have serum progesterone less than 1.5 [aOR: 0.4, CI 0.2-0.8,  $p < 0.009$ ].

**CONCLUSIONS:** In cases with potential PL, Metformin administration is an effective approach to improve pregnancy outcomes in ICSI cycles by preventing PL.

**References:**

- Manno M, Tomei F. Can we prevent premature luteinization in IVF cycles?. *Medical hypotheses*. 2014 Jan 1;82(1):122-3.
- Mansfield R, Galea R, Brincat M, et al. Metformin has direct effects on human ovarian steroidogenesis. *Fertility and sterility* 2003;79(4):956-62.

**SUPPORT:** This study was supported by Assiut Faculty of Medicine grant's office (27/3/2017-006).

**O-136** Tuesday, October 15, 2019 11:30 AM

**DIMINISHED OVARIAN RESERVE (DOR) IS ASSOCIATED WITH REDUCED EUPLOID RATES VIA PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) INDEPENDENT OF AGE: EVIDENCE FOR CONCOMITANT REDUCTION IN OOCYTE QUALITY WITH QUANTITY.** Eleni A. Greenwood, MD, MSc,<sup>a</sup> Charles E. McCulloch, PhD,<sup>b</sup> Marcelle I. Cedars, MD,<sup>c</sup> Mitchell P. Rosen, MD, HCLD<sup>b</sup> <sup>a</sup>University of California San Francisco, San Francisco, CA; <sup>b</sup>UCSF, San Francisco, CA; <sup>c</sup>University of California San Francisco, Department of Obstetrics and Gynecology, San Francisco, CA.



**OBJECTIVE:** Controversy surrounds whether an age-adjusted reduction in ovarian reserve quantity is accompanied by diminished oocyte quality. We sought to determine whether women with DOR (quantitatively) had lower rates of euploid blastocysts via PGT-A testing, as a proxy for oocyte quality.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** Results from all day 5 and 6 blastocyst trophoctoderm biopsies for PGT-A between 2010-2019 at a single academic were reviewed. Blastocysts graded BB (Gardner) or better are biopsied at our center. Infertility diagnoses were grouped as DOR (assigned by clinicians at initial consultation per Bologna criteria) vs non-DOR infertility. Women >42y were excluded given potential conflation with DOR in this range. Couples without infertility (for example, PGT-A for recurrent pregnancy loss, fertility preservation, or PGT-M) were also excluded. The primary outcome was euploid rate, defined as the number of euploid blastocysts divided by number of blastocysts biopsied per cycle. Generalized linear models were

used to account for the clustered nature of the data and control for age of the oocyte. Interaction analyses were performed. A secondary analysis assigned DOR on the basis of age-adjusted mature oocyte (M2) yield, comparing the lowest quartile to the remaining 3/4 by age group. Finally, we compared pregnancy outcomes after euploid single embryo transfer (SET) by DOR status.

**RESULTS:** 8,042 blastocyst PGT-A biopsies from 1,152 women over 1,675 IVF cycles were identified. 225 women (20%) had DOR as infertility diagnosis. Age was higher among DOR women (39.5y vs 37.0y). Euploid rates varied by DOR vs non-DOR (Table). Controlling for age, women with DOR had 24% reduced odds of a biopsied blastocyst being euploid vs non-DOR (Table). Impact of DOR on euploid rates did not differ by age category (interaction  $p = 0.43$ ). When assigning DOR status to women producing the lowest quartile of age-adjusted M2 yield, this relationship remained (Table). No differences were identified in rates of live birth or ongoing pregnancy between patients with and without DOR after SET of a euploid blastocyst ( $n = 944$  transfers) (56.8% vs 54.8%, respectively;  $p = 0.88$ ).

**CONCLUSIONS:** Blastocysts from women with DOR are less likely to be euploid than those from women without DOR, after adjustment for age. Given the concomitant reduction in euploid rates with quantity of oocytes observed in this study, quantitative ovarian reserve assessments (i.e. follicular machinery) may yield insight into relative ovarian aging.

**O-137** Tuesday, October 15, 2019 11:45 AM

**DAY 7 EUPLOID BLASTOCYSTS HAVE COMPROMISED DEVELOPMENTAL COMPETENCE AND HALF THE LIVE BIRTH RATE POTENTIAL OF DAY 5 EUPLOID BLASTOCYSTS.** Sue McCormick, BS, Robin Smith, BS, Laura Reed, BS, William B. Schoolcraft, MD, Mandy G. Katz-Jaffe, Ph.D. Colorado Center for Reproductive Medicine, Lone Tree, CO.



**OBJECTIVE:** To maximize the success of infertility treatment for poor prognosis patients, culture is extended until day 7 of embryo development. Day 7 represents a 48-hour delay in blastulation and questions have surfaced as to the clinical efficacy of this approach. The aim of this study was to evaluate the developmental competence and implantation potential of euploid embryos based on day of blastulation.

**DESIGN:** Retrospective analysis of a large series of euploid single embryo transfers (SET).

**MATERIALS AND METHODS:** A total of 2,947 euploid SETs performed at a single infertility clinic were identified for analysis which included patients with variable infertility diagnoses. Standard protocols for hormone replacement frozen embryo transfer were employed and the highest morphological grade, euploid blastocyst was selected for SET. Blastocyst biopsy was performed upon the identification of the inner cell mass (ICM): day 5 (D5;  $n = 1,880$ ), day 6 (D6;  $n = 986$ ) or day 7 (D7;  $n = 81$ ). Primary reproductive outcomes included implantation rate with fetal cardiac activity and live birth rate. Statistical analysis included Chi-square test for independence and ANOVA, with significance at  $P < 0.05$ .

**RESULTS:** Maternal age was significantly increased with each additional day that blastocyst development was delayed (D5 = 35.9 ± 3.7 years; D6 = 36.9 ± 3.7 years; D7 = 37.6 ± 3.5 years;  $P < 0.0001$ ). Higher aneuploidy rates were also observed for D7 blastocysts even when controlled for maternal age (D7 62.2% vs. D5/D6 45%, mean maternal age 37.5 ± 3.5 years;  $P < 0.0001$ ). No significant differences were observed between the three groups for ICM or trophoctoderm grade at the time of SET. Reproductive outcomes were

Average euploid rates per cycle, by DOR vs non-DOR; and impact of DOR diagnosis on euploid rates, adjusted for age

	Clinician-diagnosed		Lowest 1/4 M2 Yield	
	DOR	Non-DOR	DOR	Non-DOR
<35	42.8%	54.8%	45.1%	57.5%
35-37	50.3%	50.2%	43.5%	51.4%
38-40	27.5%	40.8%	34.0%	38.9%
41-42	22.4%	25.6%	28.9%	21.9%
Total	29.0%	44.9%	38.2%	43.2%
DOR*	aOR 0.76 (95% CI 0.65, 0.90)		aOR 0.80 (95% CI 0.68, 0.93)	
		$p < 0.01$		$p < 0.01$

\*Impact of DOR diagnosis on euploid rate, adjusted for age

significantly impacted by a delay in blastocyst development, with both implantation (D5 = 73.6%; D6 = 60.9%; D7 = 39.5%) and live birth rates (D5 = 68.5%; D6 = 55.2%; D7 = 37.0%) being significantly decreased ( $P < 0.0001$ ). Even when compared to a cohort of maternal age-matched counterparts (mean maternal age =  $37.6 \pm 3.6$  years), women achieved poorer live birth outcomes with the SET of a D7 euploid blastocyst (37%) compared to a D5 or D6 euploid blastocyst SET (63%;  $n = 1,314$ ;  $P < 0.0001$ ).

**CONCLUSIONS:** Aneuploidy rates and reproductive success were significantly associated with the appropriate timing of blastulation and identification of the ICM. Increased maternal age was associated with a delay in blastocyst development. However, even with maternally aged-matched counterparts, significantly compromised developmental potential was observed for D7 euploid blastocysts compared to D5 or D6 euploid blastocysts. Biochemical, metabolic and epigenetic processes that could impact embryo viability, independent of chromosome numeration, are potential contributors to the observed halving of the live birth rate for D7 euploid blastocysts. Despite poorer outcomes, these data still suggest that with appropriate patient counseling, extended culture to D7 for blastocyst biopsy is a viable clinical option for poorer prognosis patients.

**SUPPORT:** None.

**O-138** Tuesday, October 15, 2019 12:00 PM

**PROTOCOL MATTERS: A PROPENSITY GROUP ANALYSIS SHOWS THAT PROGESTERONE ELEVATIONS ON DAY OF TRIGGER DURING FRESH IVF-AFFECT LIVE BIRTH RATES DIFFERENTLY ACCORDING TO STIMULATION PROTOCOL.**

Chantal Bartels, MD,<sup>a</sup> James Grady, PhD,<sup>b</sup> Chaoran Hu, M.S.,<sup>b</sup> Grow R. Daniel, MD,<sup>a</sup> <sup>a</sup>Center for Advanced Reproductive Services, University of Connecticut, Farmington, CT; <sup>b</sup>University of Connecticut, Farmington, CT.

**OBJECTIVE:** To assess the influence of trigger day progesterone (P) levels on live birth rate (LBR) after fresh embryo transfer when using different ovarian stimulation protocols, either gonadotropin-releasing hormone (GnRH) agonist suppression or GnRH antagonist.

**DESIGN:** Retrospective propensity score matching

**MATERIALS AND METHODS:** eIVF is a multicenter database that has collected over 122,548 patient IVF cycles, 2004-2018. We use logistic regression with protocol types, namely GnRH agonist suppression and GnRH antagonist, to identify co-variables and perform propensity score matching. The two protocol cohorts were matched for age, smoking status, basal follicle stimulating hormone, number of mature oocytes, insemination type, cryopreservation of embryos, and number of embryos transferred. Each matched protocol cohort contained 6560 patient cycles. Logistic regression was used to regress the outcome live birth against protocols type and progesterone level and the (protocol / progesterone) interaction. Chi-square was used to compare categorical variables.

P level on day of trigger was divided into three groups: those with P level less than the mean ( $< 1.0$  ng/mL), between the mean and one standard deviation ( $1-1.5$  ng/mL), and greater than one standard deviation ( $> 1.5$  ng/mL).

**RESULTS:** The mean P level on day of trigger (ng/mL) was  $1.0 \pm 0.45$  and  $1.0 \pm 0.44$  for the agonist suppression and the antagonist groups respectively. The two propensity groups were similar with respect to matched prognostic factors. P level on day of trigger did not differ with different stimulation protocols. LBRs were statistically significantly higher in every P group when utilizing the agonist suppression protocol compared to the antagonist protocol ( $p < 0.001$ ) (Table 1).

TABLE 1. Live birth rate by protocol for progesterone levels

P (ng/mL) morning of trigger	Live Birth Rate (%)	
	Agonist Suppression N=6560	Antagonist N=6560
Low < 1.0	34.3 (1198/3491)	27.7 (971/3508)
Mid 1.0-1.5	35.4 (713/2013)	23.7(480/2022)
High > 1.5	36.3 (383/1056)	22.2 (229/1030)
Chi Square p-value	0.45	0.0001

**CONCLUSIONS:** Elevated serum P levels  $> 1$  ng/mL on the day of trigger is associated with a statistically reduced LBR following IVF stimulation protocols using GnRH antagonist. There is no decrease in LBR with P elevations

on day of trigger during ovarian stimulation with the GnRH agonist suppression protocol. This data suggests that protocol should be considered when recommending a freeze-all approach in the setting of elevated P levels on the day of trigger.

**SUPPORT:** None

**LGBTQ**

**O-139** Tuesday, October 15, 2019 10:45 AM

**REPRODUCTIVE FUNCTION IN A TRANSGENDER MOUSE MODEL FOLLOWING CESSATION OF TESTOSTERONE.**

Molly B. Moravek, MD, MPH,<sup>a</sup> Hadrian M. Kinnear, BA,<sup>a</sup> Vasantha Padmanabhan, MS, PhD,<sup>a</sup> Ariella Shikanov, PhD,<sup>b</sup> <sup>a</sup>University of Michigan, Ann Arbor, MI; <sup>b</sup>University of Michigan, ANN ARBOR, MI.

**OBJECTIVE:** While pregnancy is certainly possible in transgender men previously on gender-affirming testosterone (T), very little is known about overall fecundability, and thus fertility preservation is recommended prior to starting T. Studies of T-exposed ovaries at the time of gender-affirming surgery reveal aberrations in ovarian histology and follicle appearance, but functional studies have not been performed. We have established a mouse model to study the effects of gender-affirming T therapy on reproduction. The objective of the current pilot study was to examine fertility following T cessation in our mouse model, with the hypothesis that reproductive function would be fully restored.

**DESIGN:** Translational animal study.

**MATERIALS AND METHODS:** Ten 8 – 9 week old adult female C57BL/6N mice were injected with T enanthate 0.45mg and 5 control (C) mice were injected with vehicle twice weekly for 6 weeks, then all injections were stopped. Daily vaginal cytology and weekly serum hormone analysis was performed. Once cyclicity resumed, mice were divided into two groups: 1) sacrificed after 3-4 estrous cycles and ovaries harvested or 2) 14 weeks breeding 1:1 with male C57BL/6 mice. Offspring resulting from group 2 were sacrificed on day of life (DOL) 26 and organs harvested. Descriptive statistics were calculated and confidence intervals calculated via modified Wald method or using a t-distribution, as appropriate.

**RESULTS:** All T-treated mice stopped cycling after 1–2 T injections and 8/9 resumed cycling 7-15 weeks following T cessation (one mouse sacrificed early for vaginal prolapse). Control mice cycled regularly throughout. Despite resumption of cyclicity, T-treated mice sacrificed after 3-4 estrous cycles ( $n=4$ ) exhibited ovarian stromal hyperplasia and lack of corpora lutea on histologic examination. In the breeding arm, 50% of T-treated (2/4) and 50% of control mice (1/2) produced offspring. We observed a similar sex ratio (50% female in T group, 95%CI: .24, .76; 57% in control, 95% CI: .33, .79) and litter size (4.5 in T vs 4.7 in C) between groups. Mean weight on DOL 4 was 2.44g (95% CI: 1.89, 3) in T offspring vs 3.29g (95% CI: 2.93, 3.64) in control offspring, and 14.75g (95% CI: 13.68, 15.82) in T offspring and 16.33g (95% CI: 13.46, 19.2) in control mice on DOL 26. The ovaries of both T and control offspring appeared normal, both grossly and histologically.

**CONCLUSIONS:** Despite histologic aberrations noted in the ovaries of T-treated mice early after resumption of cyclicity, their fertility approximated that of controls, with no obvious aberrations noted in the offspring. These pilot data suggest that T-induced subfertility may be reversible following cessation and may not affect long-term reproductive function. Further, these data justify a larger study of fertility following T cessation, as well as further investigation into the molecular mechanisms underlying T-induced changes in ovarian architecture.

**SUPPORT:** ASRM/SREI Research Grant; University of Michigan Office of Research Grant

**O-140** Tuesday, October 15, 2019 11:00 AM

**REPRODUCTIVE LIFE PLANNING AND INTEREST IN FERTILITY PRESERVATION AMONG TRANSGENDER AND GENDER NON-BINARY INDIVIDUALS.**

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**OBJECTIVE:** Professional organizations agree that all transgender persons should be counseled on the effects of their transition on their fertility



TABLE 1.

	Sex Documented at Birth			Average Age	SD
	Female	Male	Intersex		
Referral to GHP specifically for fertility preservation	5	3	0	25.1	5.3
Those with reproductive life planning goals	20	25	2	27.1	10.1
Those who have undergone fertility preservation	1	11	0	26.4	6.8
Those who desire fertility preservation	26	20	1	27.2	10.3

as well as options for fertility preservation and reproduction prior to transition. The UCLA Gender Health Program (GHP) is a multidisciplinary medical, surgical and behavioral health team that supports transgender and gender non-binary individuals in their transition. We sought to identify characteristics of individuals who desired fertility preservation at intake to the GHP.

DESIGN: Cross-sectional study.

MATERIALS AND METHODS: When individuals establish care at the GHP, a Care Coordinator performs a telephone intake to ascertain which referrals and services they require. We obtained IRB exemption to examine this intake data for all those in the Gender Health Program from January 2018 – March 2019. The data were coded and de-identified. Descriptive statistics were then performed.

RESULTS: A total of 397 intake surveys were included in the data analysis. The average age of individuals who established care at the GHP was 29 years (SD 12.4). Forty-seven (11.8%) individuals stated they had reproductive life planning goals. Twelve (3%) had previously undergone fertility preservation, with eleven of those assigned male at birth. Forty-seven (11.8%) stated they desired fertility preservation. Of the 397, only eight (2%) endorsed presenting to the GHP for fertility preservation as their primary goal. Eleven (2.9%) stated they were interested in referral for hysterectomy with oophorectomy. None of these eleven patients who desired surgical sterilization were also interested in referral for fertility preservation (see Table 1). Neither gender identity, nor race/ethnicity was predictive of interest in fertility preservation.

CONCLUSIONS: Access to reproductive health services is desired by many transgender and gender non-binary individuals. Although forty-seven (11.8%) of individuals stated they had reproductive goals, only twelve (3%) had undergone any fertility preservation and a majority of those had sex documented as male at birth. Given the low reported rate of fertility preservation and apparent level of interest, future research should focus on barriers to receiving fertility counseling or referrals for transgender and gender non-binary individuals.

O-141 Tuesday, October 15, 2019 11:15 AM

#### MEDICAL ASPECTS OF FERTILITY PRESERVATION (FP) FOR TRANSGENDER ADOLESCENTS AND YOUNG ADULTS (TAYAS): A SYSTEMATIC REVIEW.

Shira Baram, MD,<sup>a</sup> Samantha Myers, B.A.,<sup>b</sup> Samantha Yee, Ph.D.,<sup>a</sup> Clifford Lawrence Librach, MD.<sup>a</sup> <sup>a</sup>CReATe Fertility Centre, Toronto, ON, Canada; <sup>b</sup>McMaster University, Hamilton, ON, Canada.

OBJECTIVE: Many TAYAs choose to undergo gender-affirming hormone treatment (GAHT), sex reassignment surgery (SRS) or both. While these treatment options help alleviate symptoms of gender dysphoria, there are significant fertility risks that should be considered prior to commencing the transition process. This study aimed to systematically review the current literature on risks of GAHT, FP options and outcomes specific for TAYAs, to identify gaps in the current research and future research directions.

DESIGN: Systematic review and quantitative analysis.

MATERIALS AND METHODS: We systematically searched the following electronic databases: Medline, PubMed, Embase®, and

PsychINFO to identify all studies which evaluated GAHT effects, FP options and outcomes in both male to female (MtF) and female to male (FtM) TAYAs. We included peer-reviewed papers from 2001 to March 2019. We excluded abstracts, clinical reviews, opinion pieces, editorial letters, and dissertations.

RESULTS: The search identified 745 papers. After applying exclusion and inclusion criteria, 21 were included. Among topics discussed in the selected papers are GAHT effects on hormone levels, semen parameters, testicular and ovarian morphology, spermatogenesis and oocytes as well as FP outcomes. Nine studies evaluated the effects of GAHT in MtF TAYAs. Most of the data suggest significant, yet mostly reversible, effects of GAHT on semen parameters. Interestingly, 2 of the studies described reduced parameters in TAYAs with no prior GAHT. The effects on testicular morphology and spermatogenesis vary between studies, with maturation arrest being the most common abnormality (24-100%). Normal spermatogenesis was observed in 0 to 25% of cases. Eight studies evaluated the effects of GAHT in FtM TAYAs. AMH level was found to be reduced in one study, yet unchanged in another. While some studies described Follicular distribution in the majority of specimens as polycystic pattern, others describe normal distribution. Two studies evaluated in vitro maturation of oocytes retrieved at the time of SRS. Maturation rate was 34-38%, 68% survived vitrification/thaw and 87-94% had a normal appearing spindle. The literature regarding outcomes of FP in TAYAs consists mainly of case reports and case series, suggesting the feasibility of the process. No studies regarding ovarian tissue or testicular tissue cryopreservation were identified.

CONCLUSIONS: This review brings to light the paucity of data available in the literature on the reproductive effects of GAHT and outcomes of FP in TAYAs. Current guidelines recommend that FP counselling take place before commencing GAHT, and support medical therapy initiation soon after a diagnosis is established. This paradigm, creates a dilemma for reproductive specialists as there are currently little high-quality data to rely upon when counselling TAYAs. Most available data point to some degree of effect of GAHT, on both the testis and ovaries, yet the extent and reversibility of that effect has not yet been thoroughly explored. Future research should include large scale cohort studies, throughout the entire FP process.

SUPPORT: CReATe Fertility Centre

O-142 Tuesday, October 15, 2019 11:30 AM

#### THE BURDEN OF FAMILY BUILDING AS A GAY MALE COUPLE: THE MAJORITY OF GAY MALE COUPLES SEEN AT A LARGE REPRODUCTIVE MEDICINE PRACTICE DESIRE A CHILD WITH EACH OF THEIR

GENETICS. Lisa Schuman, MSW,<sup>a</sup> Spencer S. Richlin, M.D.,<sup>b</sup> Robin Mangieri, MA,<sup>b</sup> Melissa Kelleher, MSW,<sup>c</sup> Nora Bolger, RN,<sup>d</sup> Mark Leondires, M.D.<sup>b</sup> <sup>a</sup>Reproductive Medicine Associates of Connecticut, Norwalk, NY; <sup>b</sup>Reproductive Medicine Associates of Connecticut, Norwalk, CT; <sup>c</sup>Reproductive Medicine Associates of CT, Norwalk, CT; <sup>d</sup>RMA of Connecticut, Norwalk, CT.

OBJECTIVE: In 2012, advances in reproductive endocrinology led ASRM to recommend a single embryo transfer for patients “with a good prognosis and to recipients of embryos from donated eggs”. Progress in gay rights has led to more men seeking fertility treatment to build their families over the past decade. Often same sex male couples (SSMC) desire a child from each of their genetics, which requires a donor and a surrogate, in addition to clinic fees. As a result of the high cost for IVF using an ovum donor, surrogate, surrogacy agency, and legal representation per pregnancy, these patients typically request a double embryo transfer. The aim of our study was to verify our observation that both men typically desire a child from each partner and when counseled, the majority of these men are willing to proceed with a single embryo transfer.

DESIGN: Retrospective Analysis

MATERIALS AND METHODS: Between 2017 and 2018, 46 SSMC participated in a meeting with the clinic Medical Director which included a review of risks associated with a twin gestation. These couples also received a counseling session with one of two mental health professionals who also discussed risks involved in proceeding with a double embryo transfer. These clinicians also asked, “are you interested in having children with both of your genetics?”

RESULTS: 45 (98%) couples said they desired a child from each of their genetics. One couple said they were “not sure” if they would have more than one child. Two couples had one child when they came to the clinic. Of the forty one couples who were beginning their path to parenthood, 27 (66%) decided to pursue a single embryo transfer after completing counseling.

**CONCLUSIONS:** Scientific literature addressing the importance of pursuing a single embryo transfer in regard to childhood outcome is particularly relevant when using an ovum donor (Fert. Stert. 2012; 4:838). Consideration of patient desires, including the interest to transfer two embryos for an improved chance of a successful pregnancy and concomitant costs for a second journey needs to be understood. Many clinics are only willing to transfer a single embryo created with a donor oocytes into a surrogate without further discussion. Additional consideration should be given to SSMC who face the expenses of ovum donation and surrogacy since both want to be genetic fathers. As surrogacy agencies and the public become more aware of the health risks associated with a twin gestation, it is likely fewer male couples will request a double embryo transfer. For now, fertility clinics should consider the financial difficulties inherent in two surrogacy journeys and counsel these men with sensitivity.

References: None

SUPPORT: None

**O-143** Tuesday, October 15, 2019 11:45 AM

### **PREGNANCY SUCCESS RATES FOR LESBIAN COUPLES UNDERGOING INTRAUTERINE INSEMINATION.**

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**OBJECTIVE:** To compare pregnancy rates in lesbian women undergoing donor sperm intrauterine insemination (IUI) to heterosexual women undergoing IUI using partner or donor sperm; we hypothesized that pregnancy rates would not differ significantly between the two populations.

**DESIGN:** Retrospective chart review.

**MATERIALS AND METHODS:** This study included all IUI cycles completed at Fertility centers of University of California, San Francisco from 2009-2016 and Stanford University from 2016-2017. The primary outcome of interest was clinical pregnancy rates per cycle. Student t-test and chi square test were used for statistical analysis. Significance was accepted at  $p < 0.05$ .

**RESULTS:** A total of 11,845 IUI cycles were included, 341 of which were lesbian women using donor sperm and 11,504 of which were heterosexual women with unexplained or male factor infertility using either partner or donor sperm. Baseline characteristics including maternal age, type of IUI cycle, and total motile sperm count were similar between the two groups. Lesbian women had a clinical pregnancy rate of 11% per IUI cycle similar to that of heterosexual women who had a clinical pregnancy rate of 12% ( $p=0.17$ ). Among both lesbian and heterosexual women, age was inversely correlated with clinical pregnancy rate ( $p=0.005$  and  $p=0.02$ , respectively).

**CONCLUSIONS:** Increasing numbers of lesbian women are attempting to achieve pregnancy using IUI with donor sperm. Lesbian women generally seek these treatments for procreative management and not for infertility. Nonetheless, they have an increased prevalence of smoking, obesity, sexually transmitted diseases and polycystic ovary syndrome compared to heterosexual women, which may affect their fertility and IUI success (1). Previous studies are limited with conflicting findings. More information is desperately needed and will help guide counseling, management, and treatment in this population. Despite the majority of lesbian females not having a diagnosis of infertility, in this study pregnancy rates were similar in lesbian women undergoing IUI for procreative management and heterosexual women undergoing intrauterine insemination for unexplained or male factor infertility. Pregnancy rates for both groups were comparable to nationally reported IUI success rates (2).

References: 1. Nordqvist S, Sydsjö G, Lampic C, Akerud H, Elenis E, Skoog Svanberg A. Sexual orientation of women does not affect outcome

of fertility treatment with donated sperm. *Hum Reprod.* 2014;29:704–11. <https://doi.org/10.1093/humrep/det445>.

2. Sicchieri F, Silva AB, Silva ACSRE, Navarro PAAS, Ferriani RA, Reis RMD. Prognostic factors in intrauterine insemination cycles. *JBRA Assist Reprod.* 2018;22(1):2–7. <https://doi.org/10.5935/1518-0557.20180002>.

**O-144** Tuesday, October 15, 2019 12:00 PM

### **IMPORTANT DECISION-MAKING CONSIDERATIONS FOR SAME-SEX MALE COUPLES (SSMC) AND SINGLE MEN (SM) WHEN PURSUING ASSISTED REPRODUCTIVE TECHNOLOGIES (ART).**



Shilini Hemalal, BAS, MSc Candidate,<sup>a</sup> Samantha Yee, Ph.D.,<sup>a</sup> Lori Ross, Ph.D.,<sup>b</sup> Mona Loufy, MD, MPH,<sup>c</sup> Clifford Lawrence Librach, MD.<sup>a</sup> <sup>a</sup>CReATe Fertility Centre, Toronto, ON, Canada; <sup>b</sup>Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada; <sup>c</sup>Women's college Hospital, Toronto, ON, Canada.

**OBJECTIVE:** There is an increasing trend for SSMC and SM to have children through ART. However, research on their experience accessing care is limited. Our objective was to evaluate decision-making considerations throughout the ART process which are unique to SSMC and SM who have used, or are currently using, ART.

**DESIGN:** This study was approved by the University of Toronto REB (#32847). A 58-item anonymous online questionnaire accessible through Survey Monkey was administered in order to collect quantitative data. This initial study includes only those undergoing ART in Canada.

**MATERIALS AND METHODS:** Data collection began in 08/2018 using convenience sampling techniques to recruit participants and is still ongoing. To date, 72 completed surveys have been used for this analysis.

**RESULTS:** The sample consisted of 63 partnered men and 9 SM, of which 21 had a child using ART, and 51 were actively pursuing ART at the time of filling out the survey. There were a similar number of Canadian ( $n=32$ , 44.4%) and international intended parents ( $n=39$ , 54.2%) who completed the survey. The majority ( $n=48$ , 66.7%) were in their 30s at the time of pursuing ART. The sample cohort was predominantly Caucasian ( $n=50$ ; 69.4%) and had a high socioeconomic status; 80.6% were university graduates and the median individual income before tax was \$79,500 CAD (\$59,360 USD).

With respect to the decision to pursue parenthood, the majority of participants ( $n=63$ , 87.5%) had 'a deep desire to have a child' and felt that having a child was 'a natural next step in their life' ( $n=50$ , 69.4%). Common resources for learning about ART were internet search (58.3%), social media platforms (41.7%), friends (38.9%), and attending seminars, workshops, and conferences (33.4%) focused on men pursuing parenthood. Twenty-five participants (34.7%) 'never' experienced social stigma regarding their family building plan; almost all ( $n=70$ , 97.2%) had some form of social support.

When choosing an egg donor, characteristics that were of highest importance to consider included: medical history, physical attributes, personality and temperament, ethnicity, and education. Of coupled participants, 45 (71.4%) intended to use or had used both theirs and their partners' sperm to fertilize eggs. Three quarters of participants ( $n=54$ ) used, or intended to use, preimplantation genetic testing (PGT-A) to screen their embryos. On average, participants spent 3-6 months to find a suitable surrogate. Of participants who had acquired a surrogate, the majority 'strongly agreed' with their surrogates on several value and lifestyle issues. Agencies were used in 62 cases (91.1%) for egg donor recruitment, and in 54 cases (90.0%) for surrogate recruitment.

**CONCLUSIONS:** The present study provides novel data on the unique considerations that SSMC and SM take into account when using ART in Canada to build their families. For future planned studies we will collect data from those who underwent ART in other countries and compare that data with these results.

O-145 Tuesday, October 15, 2019 10:45 AM

### THE PREVALENCE OF Y-CHROMOSOME MICRODELETIONS IN OLIGOZOOSPERMIC MEN: A SYSTEMATIC REVIEW AND META-ANALYSIS OF NORTH AMERICAN AND EUROPEAN STUDIES.

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**OBJECTIVE:** European and North American guidelines recommend Y-chromosome microdeletion (YCM) screening in azoospermic and oligozoospermic men with sperm concentrations <5 million sperm/mL; however, numerous studies have suggested that YCM are rare when sperm concentrations are >1 million sperm/mL. We systematically reviewed and meta-analyzed European and North American studies to determine the prevalence of complete YCM in oligozoospermic men with sperm concentrations of >0–1, >1–5, and >5–20 million sperm/mL and to determine whether 1 or 5 million sperm/mL is the most appropriate sperm concentration threshold for YCM screening.

**DESIGN:** Systematic Review and Meta-Analysis.

**MATERIALS AND METHODS:** We performed a systematic review of MEDLINE, EMBASE, Cochrane Library, and [ClinicalTrials.gov](http://ClinicalTrials.gov) for studies from database inception through February 2019 evaluating the prevalence of complete YCM in oligospermic men in North American and European studies, specifically assessing the sperm concentration threshold of 1 million sperm/mL. Random-effects meta-analysis was used to examine prevalence of complete YCM in oligospermic men with sperm concentrations of >0 – ≤1 million sperm/mL versus oligospermic men with sperm concentrations of >1 – 5 million sperm/mL.

**RESULTS:** Thirty-seven studies were identified during systematic review (n=12,492 oligozoospermic men). All complete YCM in oligozoospermic men were *AZFc* microdeletions. Eighteen studies contained data conducive to meta-analysis (n=10,866 men). Comparing the pooled estimated prevalence by sperm concentration, complete YCM were significantly more common in men with sperm concentrations of >0–1 million sperm/mL (5.0% [95% CI: 3.6–6.8%]) versus >1–5 million sperm/mL (0.8% [95% CI: 0.5–1.3%], *p*<0.001). YCM were similar in men with sperm concentrations >1–5 sperm/mL and >5–20 million sperm/mL (0.8% [95% CI: 0.5–1.3%] vs 0.5% [95% CI: 0.2–0.9%], *p*=0.14).

**CONCLUSIONS:** In Europe and North America, the majority of YCM occur in men with sperm concentrations ≤1 million sperm/mL, with less than 1% identified in men with >1 million sperm/mL. Male infertility guidelines for North America and Europe should reconsider the sperm concentration screening thresholds to recommend.

**SUPPORT:** None.

O-146 Tuesday, October 15, 2019 11:00 AM

### THE EFFECT OF ADVANCING PATERNAL AGE ON PREGNANCY AND NEONATAL OUTCOMES FOLLOWING A SINGLE EUPLOID FROZEN EMBRYO TRANSFER IN A DONOR OOCYTE MODEL.

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**OBJECTIVE:** Advanced maternal age is a significant determinant of oocyte quality and a risk factor for adverse obstetrical outcome. Less is known about the effect of paternal age on in vitro fertilization (IVF) and neonatal outcomes. Population-based studies have suggested that advanced paternal age may be associated with preterm birth and low birth weight.<sup>1</sup> Previously, our center demonstrated no association between paternal age and impaired fertilization, blastulation, or increased embryonic aneuploidy.<sup>2</sup> While chromosomal copy number variants are largely derived from errors in oocyte meiosis, there is evidence showing a positive correlation between paternal age and de novo germline mutation rates.<sup>3</sup> We hypothesize that a higher prevalence of de novo mutations in embryos derived from men with advancing paternal age could be associated with early pregnancy loss (EPL), lower ongoing pregnancy/live birth (OP/LB) rates, and adverse peri-

natal outcomes. Using a donor oocyte derived euploid embryo, in a frozen embryo transfer (FET) model, this study sought to elucidate the relationship between paternal age and pregnancy/perinatal outcomes.

**DESIGN:** Retrospective, cohort study.

**MATERIALS AND METHODS:** The study included patients undergoing a single euploid FET of donor oocyte-derived embryos from 2012 to 2019. Oocyte donors were ≤35 years of age. Paternal age was treated as a continuous variable. The primary outcome of the study was OP/LB rate. Secondary outcomes included clinical pregnancy (CP) rate, EPL rate, gestational age (GA) at delivery, and neonatal birth weight. Data were evaluated using multivariate linear regressions with generalized estimating equations.

**RESULTS:** A total of 303 single euploid FET cycles from 187 patients were included in this study. Paternal age ranged from 27.6 to 66.7 years (44.5 ± 6.5). There was no statistically significant association between paternal age, CP rate (OR 1.01 [95% CI 0.96–1.07], *p*=0.62), OP/LB rate (OR 0.99 [95% CI 0.94–1.05], *p*=0.75), or EPL rate (OR 1.00 [95% CI 0.96–1.07], *p*=0.96) after controlling for oocyte age, BMI, endometrial thickness at transfer, embryo morphology grade, and days required for blastulation. No association between paternal age and birth weight ( $\beta$ = 8.17, *p*=0.91) was observed after controlling for GA, fetal sex, and BMI. Paternal age was not associated with GA at delivery ( $\beta$ = -0.02, *p*= 0.83).

**CONCLUSIONS:** In a large, homogeneous cohort of single, euploid FETs derived from donor oocytes, paternal age was not associated with pregnancy or perinatal outcomes. Our results are encouraging, as they did not demonstrate a link between paternal age and preterm delivery or birth weight. While reassuring, this does not address other multifactorial diseases such as schizophrenia and autism that have been associated with advanced paternal age.<sup>1</sup> As the diagnostic capabilities of preimplantation genetic testing expand to include the detection of de novo mutations and higher resolution detection of copy number variants, future studies might investigate the impact of paternal age on the embryonic genome, pregnancy outcomes, and newborn health and development.

**References:** 1. Khandwala YS, Baker VL, Shaw GM et al. Association of paternal age with perinatal outcomes between 2007 and 2016 in the United States: population based cohort study. *BMJ* 2018; 363: k4372.

2. Chang S, Sekhon LH, Hernandez-Nieto C, et al. Is paternal age associated with embryo aneuploidy? *Fertil Steril* 2018; 109(3): e17.

3. Girard SL, Bourassa CV, Lemieux Perreault L-P, et al. Paternal Age Explains a Major Portion of De Novo Germline Mutation Rate Variability in Healthy Individuals. *PLoS One* 2016; 11(10):e0164212.

**SUPPORT:** None.

O-147 Tuesday, October 15, 2019 11:15 AM

### THE EFFECT OF TETRAHYDROCANNABINOL ON TESTOSTERONE AMONG MEN IN THE UNITED STATES: RESULTS FROM THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY.

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**OBJECTIVE:** Smoking tetrahydrocannabinol (THC) causes central suppression of gonadotropins resulting in testosterone deficiency. Emerging literature suggests that this effect may not occur, and that men using THC may actually have increased testosterone (T). Given this discrepancy, we sought to determine the association between different levels of THC usage and T levels using a nationally representative cohort.

**DESIGN:** This is a retrospective review of a cross-sectional data set, the National Health and Nutrition Examination Survey (NHANES). A survey designed by the center for disease control (CDC) to determine the health of the United States.

**MATERIALS AND METHODS:** All men ages 18–80 years who answered the substance use questionnaire and underwent laboratory testing for T were included. THC use was self-reported and categorized by number of times used monthly. Multivariate modeling, controlling for confounders identified on univariate analysis, was then used to determine the relationship between THC use and T levels.

**RESULTS:** Among the 5,146 men who met inclusion criteria, 1477 (28.7%) endorsed smoking THC at least once in their lifetime, 809 endorse smoking in the last year (15.7%), and 625 (12.1%) reported smoking the last month. Mean T level of the cohort was 430 ± 185 ng/dL. Univariate analysis revealed that men who reported smoking THC in the last year on average had a higher T (497) compared to those who did not report using THC (414 ng/

dL,  $p=0.002$ ). Multivariate analysis controlling for age, body mass index, exercise level, alcohol use, and race demonstrated an inverse U association between THC use in the past year and T (Table), ( $p<0.001$ ).

**CONCLUSIONS:** Analysis of a nationally representative cohort suggests that there is a dose-dependent effect of THC on T levels. While there is an increase in T in all THC users, increased amounts of THC usage appear to have a detrimental effect on serum testosterone levels. Future prospective work using specific doses of THC and studies elucidating the mechanism of the association is required to corroborate these findings.

TABLE. The effects of THC use on testosterone when controlling for age, body mass index, exercise level, alcohol use, and race

THC use within last year	Difference in T Level (ng/dL)
Never	—
Once a month	49.96
2-3 times a month	66.77
4-8 times a month	52.18
9-24 times a month	41.81
25-30 times a month	33.44

\* Controlling for age, body mass index, exercise, alcohol use, race, comorbidities, study years.

\*\*Linear/Quadratic trend significant.

**O-148** Tuesday, October 15, 2019 11:30 AM

**UTILIZING THE YO<sup>®</sup> HOME SPERM TEST NOVICE USERS OBTAINED ACCURATE RESULTS AS COMPARED TO TRAINED TECHNICIANS.**

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**OBJECTIVE:** Home sperm testing is emerging as an option for men to assess their fertility in a convenient and private environment. This study evaluated the ability of untrained users (NOVICE) to obtain accurate YO Home Sperm Test (YO) results compared to trained technicians (TRAINED).

**DESIGN:** Double-blind multi-center prospective study.

**MATERIALS AND METHODS:** The YO home sperm test is an FDA approved OTC device to measure Motile Sperm Concentration (MSC). Results are reported as a LOW MSC of  $<6 \times 10^6$ /mL or MODERATE/NORMAL MSC of  $\geq 6 \times 10^6$ /mL This MSC cut-off is based on the WHO 5th edition reference values for semen analysis. Statistical analysis was based on positive (PPA) and negative percent agreement (NPA) between NOVICE and TRAINED user's results. Positive results were defined as below the MSC cut-off and negative results above it, indicating absence of the condition being tested. Analysis was performed using MedCalc statistical software.

316 participants (NOVICE users) were enrolled in the study conducted at 3 sites. NOVICE users were comprised of 292 males with a mean age of 26.9 (19-61) and 24 females with a mean age of 35.0 (20-58). Ethnic breakdown was White 68%, Black 15%, Latino 11%, Asian 3% and Others 3%. Educational level was high school 23%, tech school 5%, college 58% and post graduate 14%. English, the first language in 218 cases (69%) was the second language in 98 (31%) of users. After semen collection, the NOVICE user was provided a YO kit to perform the test on either a Galaxy or iPhone up-

Site Name, Location	N	NOVICE vs. TRAINED user		
		PPA	NPA	Accuracy
Xytex Corp., Augusta, GA	82	96.3%	100.0%	98.15%
Xytex Corp., New Brunswick, NJ	136	97.1%	97.0%	97.05%
Medical Electronic Systems, Caesarea, IL	98	96.4%	100.0%	98.20%
OVERALL	316	96.7%	98.7%	97.70%
Inter-site CV		0.5%	1.7%	0.7%

loaded with the YO app. The NOVICE user conducted the test following only the instructions provided in the kit and on the YO app. Once the NOVICE user completed the test, a blinded laboratory professional (TRAINED) ran the same sample on the same Smartphone.

**RESULTS:** The NOVICE vs. TRAINED users results demonstrated a PPA of 96.7%, an NPA of 98.7% with 97.7% accuracy for all sites combined. The inter-site coefficients of variation (CV) were  $<2\%$ .

**CONCLUSIONS:**

- 316 untrained users (NOVICE) of the YO Home Sperm Test demonstrated a high level of agreement and accuracy determining Motile Sperm Concentration above and below  $6 \times 10^6$ /mL compared to trained technicians (TRAINED).
- The capability to accurately determine Motile Sperm Concentration at home may enable couples to appreciate earlier and more privately if a male factor is impacting their ability to conceive.

Reference: None.

SUPPORT: Medical Electronic Systems.

**O-149** Tuesday, October 15, 2019 11:45 AM

**UNDERUTILIZATION OF PRIMARY MEDICAL CARE AMONG MEN PRESENTING FOR FERTILITY EVALUATION.**

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**OBJECTIVE:** A growing body of evidence demonstrates an association between male infertility and significant medical comorbidities including cardiovascular disease, cancer, and even mortality. As such, it is essential that men with subfertility establish and maintain a relationship with a primary care physician (PCP). We sought to determine the proportion of young men presenting for fertility evaluation who reported an established relationship with a PCP.

**DESIGN:** Retrospective, cross-sectional study.

**MATERIALS AND METHODS:** We retrospectively examined all men presenting for initial male infertility consultation at a tertiary care center with a single reproductive urologist between 2000 and 2018. All men were asked to provide the name of their PCP at the time of initial visit. Descriptive statistics and multivariable regression were utilized to characterize the proportion of men with a PCP at the time of evaluation and associations between PCP status, patient age, and comorbidity.

**RESULTS:** Among 4,127 men presenting for initial fertility consultation, 1,324 had PCP data recorded. Of these, 480 (36.3%) did not have an established PCP at the time of evaluation. Men with a PCP were older than those without - median age 35 years (interquartile range [IQR] 23-40) versus 34 years (IQR 31-38),  $p<0.001$ . A smaller proportion of men with a PCP had elevated blood pressure (46.2% versus 56.8%,  $p=0.03$ ), however a similar proportion of men in both groups were obese (21.7% versus 24.6%,  $p=0.31$ ). Among 513 men who had a documented visit with an internal medicine physician within our tertiary care network prior to initial fertility consultation, 184 (35.9%) had not seen an internal medicine physician in over a year.

**CONCLUSIONS:** Over one-third of men presenting for fertility evaluation did not have an established PCP, and among those who did, a sizable proportion had not seen their PCP in the previous year. Given the strong link between male infertility and medical comorbidities, reproductive urologists are uniquely positioned to encourage and facilitate the critical relationship between men with subfertility and primary care physicians.

**O-150** Tuesday, October 15, 2019 12:00 PM

**SALVAGE ULTRASOUND GUIDED TARGETED CRYOABLATION OF THE PERI-SPERMATIC CORD FOR PERSISTENT CHRONIC SCROTAL CONTENT PAIN AFTER MICROSURGICAL DENERVATION OF THE SPERMATIC CORD.**

Sijo Joseph Parekattil, MD,<sup>a</sup> Jamin Brahmhatt, MD,<sup>b</sup> Nahomy Calixte, MD,<sup>b</sup> Mohamed Etafy, MD,<sup>c</sup> Richard A. Mendelson, Ph. D.<sup>d</sup> <sup>a</sup>PUR Clinic, South Lake Hospital &

University of Central Florida, Clermont, FL; <sup>b</sup>PUR CLinic, Clermont, FL; <sup>c</sup>PUR Clinic, Clermont, FL; <sup>d</sup>Keiser University, Cooper City, FL.

**OBJECTIVE:** To assess the efficacy of Ultrasound Guided Targeted Cryoablation (UTC) of the peri-spermatic cord as a salvage treatment for patients who failed microsurgical denervation of the spermatic cord (MDSC) for the treatment of chronic scrotal content pain (CSP).

**DESIGN:** Retrospective review of 279 cases (221 patients: 58 bilateral) undergoing UTC between Nov 2012 to July 2016, performed by two fellowship-trained microsurgeons.

**MATERIALS AND METHODS:** UTC was performed using a 16-gauge cryo needle (Endocare, HealthTronics, Austin, TX). Branches of the genitofemoral, ilioinguinal and inferior hypogastric nerves were cryoablated medial and lateral to the spermatic cord at the level of the external inguinal ring. Level of pain was measured preoperatively and postoperatively using the Visual Analog Scale (VAS) and Pain Index Questionnaire (PIQ-6) (QualityMetric Inc., Lincoln, RI).

**RESULTS:** Median age was 43 years, operative duration 20 minutes, and post-operative follow-up 36 months (24 to 60). Subjective VAS outcomes: 75% significant reduction in pain (11% complete resolution and 64%  $\geq 50\%$  reduction in pain). Objective PIQ-6 outcomes: 53% significant reduction at 1 month (279 cases), 55% at 3 month (279 cases), 60% at 6 month (279 cases), 63% at 1yr (279 cases), 65% at 2yrs (275 cases), 64% at 3yrs (232 cases), 59% at 4yrs (128 cases) and 64% at 5 yrs (53 cases) post-op. Complications: two wound infections, four penile pain cases (resolved in a few months).

**CONCLUSIONS:** Ultrasound Guided Targeted Cryoablation of the peri-spermatic cord is a safe potential treatment option for the salvage management of persistent CSP in patients who have failed MDSC.

**SUPPORT:** None.

## MENTAL HEALTH

**O-151** Tuesday, October 15, 2019 10:45 AM

### THE IMPACT OF KLINEFELTER SYNDROME ON QUALITY OF LIFE - A MULTICENTRE STUDY.

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**OBJECTIVE:** Klinefelter syndrome (KS) is associated with an increased risk of lower socioeconomic status and a higher risk for morbidity and mortality, which may have a significant impact on quality of life (QOL). The objective of this study is to investigate QOL in a large European cohort of men with KS and associate QOL with socioeconomic status, prevalence of somatic disease and mental illness, testosterone supplementation and age of diagnosis.

**DESIGN:** This study was part of the European dsd-LIFE study, a non-interventional, clinical, cross-sectional study.

**MATERIALS AND METHODS:** Participants were recruited in 14 clinical study centres in 6 European countries which participated in the European dsd-LIFE study. 218 men with KS were eligible for inclusion. Male normative data from the European Social Surveys (ESS) was used for comparison. Clinical data, related to quality of life, social activity and health status were collected.

**RESULTS:** The WHO physical domain score of men with KS ( $66.2 \pm 19.4$ ;  $n=206$ ) was significantly lower compared to the healthy reference population ( $76.5 \pm 16.2$ ;  $n=1324$ ;  $p<0.001$ ). The WHO psych domain score of men with KS ( $n=206$ ) was significantly lower ( $63.0 \pm 17.9$ ) compared to the healthy reference population ( $67.8 \pm 15.6$ ;  $n=1324$ ;  $p<0.05$ ). The WHO environment

domain score of men with KS ( $69.7 \pm 14.9$ ;  $n=206$ ) was comparable to the healthy reference population ( $70.52 \pm 20.7$ ;  $n=1324$ ;  $p=0.5$ ). The WHO social domain score of men with KS ( $59.1 \pm 22.1$ ;  $n=206$ ) was significantly lower compared to the healthy reference population ( $68.2 \pm 13.8$ ;  $n=1324$ ;  $p<0.001$ ). Men with KS reported less engagement in social activities compared to others of the same age (33% vs 49%,  $p<0.001$ ), and had less intimate friendships ( $p<0.001$ ). Experienced discrimination and the presence of somatic or mental health problems led to a significantly worse QOL.

**CONCLUSIONS:** Quality of life is significantly impaired in men with Klinefelter Syndrome, most likely due to discrimination and the presence of somatic and mental health problems. A multidisciplinary approach of healthcare providers might help to provide adequate counselling and treatment to improve quality of life.

**SUPPORT:** The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n°305373.

**O-152** Tuesday, October 15, 2019 11:00 AM

### INTIMATE PARTNER VIOLENCE AMONG POSTPARTUM WOMEN REPORTING PRIOR FERTILITY TREATMENT.

Jerrine Renee Morris, MD, MPH, Jennifer F. Kawwass, MD, Heather S. Hipp, MD Emory University, Atlanta, GA.

**OBJECTIVE:** To determine the prevalence of intimate partner violence (IPV) among women reporting use of fertility services compared to those who conceived spontaneously in a national sample of postpartum women.

**DESIGN:** A cross-sectional population-based study using data from the Pregnancy Risk Assessment Monitoring System (PRAMS), which included women with recent live births between 2009-2016.

**MATERIALS AND METHODS:** Women self-reported use and type of fertility treatment as well as IPV before or during their most recent pregnancy. Weighted percentages for reported IPV were calculated and compared between women with and without a prior history of fertility treatment preceding their recent pregnancy. We adjusted for maternal age, maternal race/ethnicity, maternal education, marital status, pre-pregnancy BMI, number of stressors (e.g. homelessness) experienced in the preceding 12 months prior to delivery, tobacco use in the three months prior to pregnancy, pre-pregnancy health insurance, annual household income, number of prior live births, outcome of prior pregnancy (including preterm or low birth weight), birth plurality, outcome of most recent pregnancy (including NICU admission or neonatal death), breastfeeding status of most recent neonate. Using multivariate logistic regression, the adjusted odds of IPV as a function of fertility treatment status were calculated.

**RESULTS:** Of the 37,114 women, 4,664 (12.6%) reported fertility treatment and 766 (2.1%) reported IPV. Of the women who reported use of fertility treatment, 59 (1.3%) reported IPV prior to or during their most recent pregnancy. Women who reported use of fertility treatment were less likely to endorse IPV as compared to women who did not report use of fertility treatment prior to their most recent pregnancy ( $p<0.0001$ ). After adjustment, the odds of IPV were similar among women who received fertility treatment and those who did not (adjusted odds ratio 1.10, 95% confidence interval 0.64-1.89). There was no difference in type of fertility treatment and IPV (including fertility-enhancing drugs, artificial or intrauterine insemination, assisted reproductive technology, or other medical treatment). Predictors of IPV within this population included age less than 20, greater number of reported stressors, tobacco use prior to pregnancy, and household annual income less than \$52,000. Non-Hispanic White race/ethnicity and being married were protective against IPV.

**CONCLUSIONS:** Despite the known adverse psychosocial implications of infertility, its treatment did not confer greater risk of IPV within this postpartum population. The difficulties associated with infertility, however, may have been mitigated by successful treatment but could be potentiated if unsuccessful. The preconception period, inclusive of encounters with infertility specialists, represents a unique opportunity to screen and counsel women, especially those who may be at higher risk for IPV.

**O-153** Tuesday, October 15, 2019 11:15 AM

### IMPACT OF IN-CENTER STRESS REDUCTION MODALITIES ON SART-REPORTED LIVE BIRTH RATES.

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Mineola, NY; <sup>b</sup>Northwell Health Fertility, Zucker School of Medicine at Hofstra/Northwell, Manhasset, NY.

**OBJECTIVE:** To determine whether infertility centers that offer in-center stress reduction modalities (SRM) have higher live birth rates compared to centers without such services.

**DESIGN:** Retrospective cohort study comparing LBR among a sample of SART-affiliated fertility clinics with and without in-center SRM. Information on in-center availability of massage therapy and acupuncture were collected through standardized "secret shopper" phone conversations with clinic staff and/or navigation through each center's website.

**MATERIALS AND METHODS:** The LBR from SART-affiliated fertility clinics from 6 states (NY, NJ, MA, PA, AZ, WA) were collected. Cycles utilizing gestational carriers were excluded, as were centers without finalized SART data or with unknown SRM treatments. Information regarding in-center acupuncture or massage was gathered via the centers' websites or anonymous phone conversations with center staff. The primary outcome was LBR in the primary outcome per egg retrieval cycle. LBR was weighted based on the number of cycles performed in each age group for each center. The mean LBR was compared between centers who offer in-center SRM and those who do not stratified by SART maternal age group (<35, 35-37, 38-40, 41-42, >42) using student's t-test; p<0.05 determined significance.

**RESULTS:** Ninety-four centers in 6 states (NY, NJ, MA, PA, AZ, WA) were identified using the SART website; 9 centers were excluded due to non-finalized 2016 data and 16 centers were excluded due to unavailable SRM information. Of the 69 fertility clinics included, 16 offered acupuncture and/or massage therapy in-center. LBR was significantly higher in women ages <35 (41.8% vs 37%, p-value 0.02) and 35-37 (32.8% vs 13.7%, p-value 0.04) in clinics offering SRM compared to those who do not (Table).

SART Age category	Live Birth Rate		p-value
	with SRM (n=16)	without SRM (n=55)	
<35	41.8	37.0	0.02
35 - 37	32.8	13.7	0.04
38 - 40	20.5	10.0	0.17
41 - 42	11.1	5.5	0.20
> 42	4.2	2.0	0.30

**CONCLUSIONS:** To our knowledge, this is the first study to examine the impact of stress reduction modalities including massage and acupuncture on SART-reported LBR among affiliated clinics. LBR was found to be significantly higher for women ages <35 and 35-37 among clinics that offer these complementary therapies. This suggests that incorporating alternative medical treatments, such as acupuncture and massage, may improve IVF outcomes for younger patients. It is possible that older patients have less of a benefit due to the profound relationship between age and fertility; however, expanding this analysis to include more centers may similarly suggest a benefit for a broader patient population, and help to ascertain the true utility of SRM.

**O-154** Tuesday, October 15, 2019 11:30 AM

#### HOME- OR HOSPITAL -BASED MONITORING TO TIME FROZEN EMBRYO TRANSFER IN THE NATURAL CYCLE? OUTCOMES FROM A RANDOMIZED CONTROLLED TRIAL (ANTARCTICA-2).



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**OBJECTIVE:** Frozen-thawed embryo transfer (FET) is at the heart of in vitro fertilization (IVF). Two types of FET-cycles are used: artificial cycle FET (artificial preparation of the endometrium with progesterone and estrogen) and natural cycle FET. During a natural cycle FET, women visit the hospital repeatedly and receive an ovulation trigger to time FET. The previously performed Antarctica RCT showed that natural cycle FET is more cost-effective compared to artificial cycle FET. From the woman's point of view a more natural approach using home-based monitoring of the ovulation with LH urine test to time FET may be desired. Currently, the multicentre Antarctica-2-RCT is comparing the cost-effectiveness of home-based monitoring with hospital-based monitoring. The Antarctica-2-RCT enables us to study in quality of life using patient-reported-outcome-measures (PROMs) and patient satisfaction using patient-reported-experience-measures (PREMs) in both FET-strategies.

**DESIGN:** PROMs and PREMs were assessed alongside the Antarctica-2 RCT. PROMs were assessed using the validated questionnaire EQ-5D-5L. Currently, there are no guidelines for assessing PREMs in this population. Therefore, members of the Dutch patient organisation for fertility problems (Freya) filled out an online survey and selected the following PREMs to assess: (1) anxiety to miss ovulation, (2) perceived level of partner participation, (3) level of discretion, (4) feeling of empowerment (5) satisfaction with treatment.

**MATERIALS AND METHODS:** We assessed PROMs and PREMs at three time-points after randomization: 1. prior to randomization, 2. at the time of FET and 3. before pregnancy test. A sample size of 200 participants was needed to find a difference of 0.3 with a standard deviation in both groups of 0.7 an alpha of 5%, power of 80% and drop-out rate of 10%. We performed mixed model analysis for between-group-comparison of treatment and time effects.

**RESULTS:** A total of 231 patients were randomized. Of these, 115 women were treated with home-based monitoring and 116 women were treated with hospital-based monitoring. Even though the RCT is still recruiting for the primary outcome we completed the subset of participants for the PROMs and PREMs measurements and therefore report on these results. For the PROMs we found a significant increase of anxiety/sadness symptoms over time (p=0.002) in both groups. We found no treatment effect of home-based versus hospital-based monitoring on the EQ-5D-5L. There was no interaction effect on PROMs between both groups and stage of the treatment. For the PREMs we did not find any significant treatment or time effect on any of the five PREMs.

**CONCLUSIONS:** There is no difference in PROMs and PREMs in women undergoing home-based compared to hospital-based monitoring. In both groups an increase of anxiety/sadness symptoms over time was observed. Both strategies seem equally patient friendly. Before implementation of home-based monitoring we await the results (effectiveness and costs) of the Antarctica-2- RCT.

**SUPPORT:** The Antarctica-2 RCT is supported by a grant of the Netherlands Organisation for Health Research and Development (ZonMw 843002807).

**O-155** Tuesday, October 15, 2019 11:45 AM

#### DOES UTILIZATION OF PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) ALTER DROPOUT RATES?



Denny Sakkas, PhD,<sup>a</sup> Alice D. Domar, Ph.D.,<sup>a</sup> Laura E. Dodge, ScD, MPH,<sup>b</sup> Boston IVF, Waltham, MA; <sup>b</sup>Harvard Medical School, Boston, MA.

**OBJECTIVE:** Dropout rates after an initial in vitro fertilization (IVF) cycle are ~25%. Preimplantation genetic testing for aneuploidy (PGT-A) is thought to decrease dropout rates. The aim of this study was to determine whether PGT-A influences treatment termination among IVF patients in a state with mandated insurance coverage.

**DESIGN:** Retrospective chart review.

**MATERIALS AND METHODS:** All patients whose first, fresh autologous cycle began between 1/1/2014 and 7/31/2017 were identified and stratified by whether they had used PGT-A for their first cycle. The proportion of patients terminating treatment was calculated as the number of patients who did not return to treatment divided by the number who had not had a live birth in the prior cycle. We used a chi-square test to compare the proportions of patients terminating treatment in each group. We used inverse probability weighting and adjusted Cox proportional hazard models to estimate the cumulative probability and 95% confidence interval (CI) of the first live birth in up to six IVF cycles while accounting for informative censoring and female age at the time of embryo creation.

TABLE 1. Comparing women who had PGT-A to women who did not have PGT-A in their first cycle

Cycle	All patients		PGT-A at first cycle		No PGT-A in first cycle		P*
	Cycle cohort	Did not return for treatment N/Total N (%)	Cycle cohort	Did not return for treatment N/Total N (%)	Cycle cohort	Did not return for treatment N/Total N (%)	
1	6,656	NA	273	NA	6,383	NA	
2	4,483	704/5,187 (13.6)	174	41/215 (19.1)	4,309	663/4,972 (13.3)	0.02
3	2,470	593/3,063 (19.4)	108	21/129 (16.3)	2,362	572/2,934 (19.5)	0.37
4	1,427	379/1,806 (21.0)	58	22/80 (27.5)	1,369	357/1,726 (20.7)	0.14
5	816	270/1,086 (24.9)	38	11/49 (22.5)	778	259/1,037 (25.0)	0.69
6	507	162/669 (24.2)	18	6/24 (25.0)	489	156/645 (24.2)	0.93

\*P compares PGT-A with no PGT-A.

**RESULTS:** The study included 6,656 eligible patients. Among the PGT-A group, 19.1% of those without a delivery in the first cycle did not return for a second cycle compared to 13.3% in the non-PGT-A group (P=0.02) (Table 1). The proportion not returning after subsequent cycles did not differ (all P≥0.14). The cumulative incidence of live birth after up to six IVF cycles was similar in the PGT-A (76.1%, 95% CI: 67.3–82.6%) and non-PGT-A (72.9, 95% CI: 71.2–74.5%).

**CONCLUSIONS:** Couples utilizing PGT-A were more likely than those who did not to terminate treatment after the first unsuccessful IVF cycle. In subsequent cycles, those using PGT-A were just as likely as those who did not to terminate treatment prior to achieving a live birth. Although it is accepted that PGT-A improves the likelihood of live birth per transfer, it is likely many couples did not return to care due to a lack of euploid embryos or due to the stresses of fertility treatment independent of PGT-A.

**SUPPORT:** None.

**O-156** Tuesday, October 15, 2019 12:00 PM

**HAIR CORTISOL AS A NEW BIOMARKER OF UNDERLYING CHRONIC STRESS, ANXIETY AND DEPRESSION IN INFERTILITY: A PILOT STUDY.**

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**OBJECTIVE:** To study the viability of hair cortisol levels as new biomarker of chronic stress and explore its relationship with perceived anxiety levels and depressive symptoms.

**DESIGN:** Prospective, observational, cross-sectional study.

**MATERIALS AND METHODS:** A total of 50 non-smoking women, with body mass index of 19-30 kg/m<sup>2</sup> and no previous fertility treatments, undergoing IVF were eligible for the study. Interested patients were asked to give a sample of their hair in their second consultation with the doctor and twelve weeks later. Study exclusion criteria included subjects with any recognized psychiatric or immune health condition; no drugs, alcohol consumption or high caffeine consumption. To reduce the confounding effect of risk variables, patients diagnosed with Cushing disease, asthma, on steroid medication, diabetes or other conditions known to influence cortisol levels, were excluded. The State-Trait Anxiety Inventory (STAI) and Depression Subscale (DEP) from Symptom Checklist 90-R (SCL-90-R) were used to assess anxiety and depression respectively at second appointment (T1) and twelve weeks after (T2). A score R40 on the State Anxiety scale (S-Anxiety) was used to detect clinically significant anxiety. SCL-90-R scores of DEP 2.0 were used to detect depression. Non parametric, student t-tests, Chi-Square and Shapiro-Wilk normality tests were used where appropriate and a p<0.05 was considered to be significant.

**RESULTS:** The mean age was 36.2 (0.8) prior starting treatment. No patient was receiving psychological therapy or were on psychiatric medication at the time of the treatment. Overall patients had more Trait Anxiety at T1 (mean: 29.8, p<0.001) than T2 (mean: 24.4, p<0.001), while there was a mild difference in terms of depression (mean: 0.8 vs 1.5, p<0.001) from T1 to T2. Cortisol levels increased from T1 to T2 (mean: 239.2 vs 246.9, p<0.001). On T2, 52% of women had a positive pregnancy test, and their cortisol levels were reduced from T1 to T2 (mean: 357.2 vs 151.1, p<0.001) while women who had a negative result had higher cortisol levels at T2 (mean: 106.5 vs 378.6, p<0.001). Regarding correlation only frequent physical exercise showed a significant association to lower cortisol secretion at T1 but not at T2 (0.03 vs 0.09, p<0.05). Neither

age, infertility diagnosis, anxiety levels had any significant association with cortisol.

**CONCLUSIONS:** We have shown that hair cortisol is a promising new biomarker to evaluate chronic stress in infertility patients. Cortisol secretion interacted with stress to accelerate the development of depressive symptoms, especially in those patients with a negative pregnancy test. Replication of these findings in a larger population will allow further explorations of the possible physiological mechanisms underlying stress and treatment outcomes.

**PEDIATRIC AND ADOLESCENT GYNECOLOGY**

**O-157** Tuesday, October 15, 2019 10:45 AM

**ADOLESCENTS AND ECTOPIC PREGNANCY: TRENDS IN EMERGENCY DEPARTMENT UTILIZATION BETWEEN 2006-2014.**

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**OBJECTIVE:** Ectopic pregnancies (EP), if not promptly diagnosed and treated, are associated with significant morbidity and mortality and can negatively impact future fertility in young women. A substantial portion of the work-up and management of EP occurs in the Emergency Department (ED). To better understand this condition in adolescents, we investigated trends in ED utilization for EP in girls aged 13 to 19 years old over a 9-year period.

**DESIGN:** Retrospective cross-sectional study.

**MATERIALS AND METHODS:** The Nationwide Emergency Department Sample (NEDS), Healthcare Cost and Utilization Project (HCUP), Agency for Healthcare Research and Quality, was queried for all ED visits in adolescents between 13 and 19 years old with a primary or secondary diagnosis (ICD-9-CM) of EP from 2006 to 2014. Parameters assessed included national estimated numbers of ED visits, ED charges adjusted for inflation, hospital geographic locations, patient's demographic characteristics, and methotrexate (MTX) administration (SAS 9.4 - Cary, NC).

**RESULTS:** Approximately 75% of adolescents who presented to the ED for EP between 2006 and 2014 were 18 or 19 years old. While the number of ED visits for EP in adolescents remained fairly stable between 2006 and 2010 (3,264 versus 3,180), there was a 17.0% drop in 2011 (2,707) and another 17.9% drop in 2014 (2,221). In the most recent year analyzed, 2014, the majority of ED visits for EP in adolescents were seen in metropolitan areas with a population >1M (52.4%), in the southern regions (38.4%), in patients with Medicaid insurance (57.1%) and those in the lowest quartile for household income based on zip code (35.5%). Average ED charges per visit for EP progressively increased from \$5,301 in 2006 to \$9,066 in 2014, while total ED charges for this condition remained relatively stable (\$17.2M in 2006 versus \$20.1M in 2014). Overall, admission rates decreased from 43.7% to 18.4% through the years analyzed. Admission rates were higher in 16 and 17 years old adolescents living in metropolitan areas and in the western states. Finally, the percentage of ED visits associated with MTX administration increased from 1.7% in 2006 to 6.9% in 2014.

**CONCLUSIONS:** The number of ED visits for EP in adolescents decreased substantially between 2006 and 2014, which aligns with lower teenage pregnancy rates recorded by the CDC and easier access to emergent and non-emergent contraception as made available by government initiatives

over the same time period. The drop in admission rates suggests an opportunity to shift the low-acuity cases of EP from the ED to the less expensive outpatient clinics. Additionally, adolescents who utilized the ED for EP more frequently belonged to the lowest income quartile and had Medicaid coverage which presents potential disparities in access to care and highlights a need for improved pediatric and adolescent gynecology outpatient services.

O-158 Tuesday, October 15, 2019 11:00 AM

**PARENT COMPREHENSION FOLLOWING VIDEO-BASED EDUCATION FOR PEDIATRIC FERTILITY PRESERVATION.**



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**OBJECTIVE:** Decisions about whether to pursue fertility preservation (FP) can be particularly difficult in the pediatric patient population because parents are making decisions for their children. Additionally, the therapies for prepubertal children and surgical FP are experimental. Parents often cite lack of knowledge about infertility risk and experimental nature of options as barriers in deciding whether to pursue FP. This study was designed to gauge comprehension of FP video-based educational tools and to assess parent attitudes towards the tools.

**DESIGN:** This was a prospective randomized survey-based study completed 2018-2019 at a single tertiary care children's hospital with Institutional Review Board approval.

**MATERIALS AND METHODS:** Participants were parents of pediatric patients (0-18 years old) admitted to a general surgery floor. Parents of children with a diagnosis putting them at risk for infertility or who had previously undergone FP were excluded. Participants completed pre-assessment questions, viewed two publicly available videos about FP, and completed post-assessment questions. Video A was colorful, animated, and used simple vocabulary. Video B was mostly black-and-white, more detailed, and used more complex vocabulary. Participants were randomized into two groups, each viewing the videos in a different order. Survey questions included participants' FP knowledge, comprehension, and video preference. Statistics were gathered using chi-squared analyses and Wilcoxon rank sum tests.

**RESULTS:** 45 participants completed the survey. The average age was 37.5 years old; the majority were female (76%) and had completed high school/GED or above (98%). At baseline, 64% of participants indicated that they knew nothing about options for children at risk for infertility. After watching both videos, baseline knowledge scores improved in 73% of all participants and 61% felt like they knew some or a lot about FP. There was no difference in the number of participants that improved from baseline between the two groups (p=0.946). After viewing both videos, 87% of participants correctly answered >50% of the comprehension questions with no difference after video A compared to video B (p=0.832). However, 70% of participants reported a preference for video A because it was interactive, colorful, and concise.

**CONCLUSIONS:** After utilizing FP video-based educational tools, parents experienced an increase in FP understanding, including the risk of infertility and options available for children, with preference for videos that are colorful and interactive. Our work indicates that video-based educational tools are an effective way to increase parent knowledge of FP options in the pediatric setting.

O-159 Tuesday, October 15, 2019 11:15 AM

**FACTORS ASSOCIATED WITH CHOOSING FERTILITY PRESERVATION IN A PEDIATRIC, ADOLESCENT AND YOUNG ADULT POPULATION.**



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**OBJECTIVE:** To determine patient characteristics associated with the decision to pursue fertility preservation prior to gonadotoxic therapy in a female pediatric, adolescent, and young adult patient population.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This IRB-approved study involved analysis of patient data in the Comprehensive Fertility Care and Preservation Program patient registry at Cincinnati Children's Hospital Medical Center from 10/1/2013 through 11/6/2018. All female patients who received a fertility consult from this group were included in the analysis. Demographics, clinical diagnosis, and treatment characteristics were compared between participants that selected fertility preservation versus those that declined. Continuous variables were analyzed by student's *t*-test and categorical variables were analyzed using Chi-square test. Results with *p* < 0.05 was considered statistically significant.

**RESULTS:** Of the 447 total fertility consults, 320 (71.5%) patients were eligible for fertility preservation options prior to gonadotoxic treatments, and one-third chose to pursue a fertility preservation intervention. In patients with a high-risk fertility assessment, 52.3% opted for fertility preservation. Patients receiving high risk gonadotoxic therapy and those planning bone marrow transplant (BMT) were more likely to choose fertility preservation. A higher proportion of non-English speaking patients/families declined fertility preservation than selected it (Table 1).

**CONCLUSIONS:** BMT, high fertility risk assessment, and non-English as primary language but not pubertal status or previous cancer treatment were significant factors affecting patient/family choice for fertility preservation in pediatric, adolescent and young adult setting. Based on these results, it is unclear whether a language barrier or cultural beliefs are affecting the decision making in non-English speaking participants. Further research is needed answer this and to better characterize barriers to fertility preservation in this population.

Patient Characteristic	Declined Preservation % (n=218)	Selected Preservation % (n=103)	p-value
Primary Language			<0.05
English	78.9 (172)	88.3 (91)	
Other	21.1 (46)	11.7 (28)	
Previous Treatment			=0.3
No	78.0 (170)	72.8 (75)	
Yes	22.0 (48)	27.2 (28)	
Development			=0.71
Pre-Menarchal	65.0 (141)	63.1 (65)	
Menarchal	34.5 (75)	36.9 (38)	
Unknown	0.5 (1)	0 (0)	
Risk Assessment			<0.0001
Low	39.9 (85)	2.0 (2)	
Intermediate	10.3 (22)	10.8 (11)	
High	49.8 (106)	87.2 (89)	
Care Team			<0.0001
Bone Marrow Transplant	36.2 (79)	51.5 (53)	
Neuro Oncology	10.6 (23)	3.7 (4)	
Leukemia/Lymphoma	26.2 (57)	6.8 (7)	
Solid Cancer	27.1 (59)	30.1 (31)	
Other	0 (0)	8.0 (7.8)	

O-160 Tuesday, October 15, 2019 11:30 AM

**WHO SEES FERTILITY SPECIALISTS? CANCER TREATMENTS AND SEEKING FERTILITY SERVICES IN ADOLESCENTS AND YOUNG ADULTS (AYA) WITH CANCER.**



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**OBJECTIVE:** Clinical guidelines endorse infertility risk counseling in newly diagnosed AYA cancer survivors and referral to fertility specialists in those who express interest. Limited empiric data exist on whether cancer treatment gonadotoxicity is related to patients seeking fertility services, which would suggest appropriate referrals to care. We hypothesized that AYA cancer survivors with planned gonadotoxic treatments are more likely to undergo infertility risk counseling (counseling) and fertility preservation procedures (FP procedures).

DESIGN: Cross-sectional.

**MATERIALS AND METHODS:** Female AYA survivors who were ages 18-39, were diagnosed with cancer at ages 15-35, completed primary cancer treatment, had at least one ovary were recruited from cancer registries, clinics and advocacy groups between 2015 and 2018 to the parent Reproductive Window study on ovarian function. Participants completed a web-based questionnaire on infertility risk counseling and preservation procedures prior to cancer treatment, as well as demographic, cancer, and reproductive characteristics. Cancer treatments were abstracted from primary records. Log-binomial regression models were used to test associations between gonadotoxic treatments (alkylating chemotherapy [AC], abdominopelvic radiation [RT], total body irradiation [TBI]) and fertility services (counseling and FP procedures) utilization, adjusting for confounding.

**RESULTS:** 578 survivors, mean age 33.1 (SD 4.7) years and 73.8% white, met eligibility criteria and were diagnosed with cancer at a mean age of 26.1 (SD 5.8) years. The most common cancers were breast (27.7%), thyroid (19.7%), and Hodgkin lymphoma (17.3%). Gonadotoxic treatment exposures were 49.5% to AC, 7% to  $\geq 7$  g/m<sup>2</sup> of cyclophosphamide equivalent dosing (CED), 4.3% to RT and 1.4% to TBI. Overall, 23.5% had counseling and 14.7% underwent FP procedures. In bivariable analysis, older age at diagnosis, infertility before cancer, cancer type, AC and CED, and RT were significantly associated with increased counseling. In adjusted analysis, age (aRR 1.09 [1.05-1.12]), CED  $< 7$  g/m<sup>2</sup> vs. none (aRR 1.71 [1.27-2.28]), and CED  $\geq 7$  g/m<sup>2</sup> vs. none (aRR 1.89 [1.09-3.27]) remained significantly associated with counseling. For FP procedures, older age at diagnosis, white race, AC, CED and RT were associated in bivariable analysis. In adjusted analysis, undertaking FP procedures was more likely with older age (aRR 1.09 [1.05-1.33]), white race (aRR 1.88 [1.1-3.2]), receipt of  $< 7$  g/m<sup>2</sup> CED vs. none (aRR 1.66 [1.10-2.49]), receipt of  $> 7$  g/m<sup>2</sup> CED vs. none (aRR 2.93 [1.64-5.23]), and RT (aRR 2.45 [1.36-3.30]).

**CONCLUSIONS:** A minority of AYA cancer survivors undergo fertility services before cancer treatment, indicating a continued gap in care. Survivors who received alkylating chemotherapy or abdominopelvic radiation were more likely to undergo fertility services, supporting appropriately increased use of these services in women who are at higher risk of infertility.

References: 1. Johnson, R. H., & Kroon, L. (2013). Optimizing fertility preservation practices for adolescent and young adult cancer patients. *A Journal of the National Comprehensive Cancer Network*, 11(1), 71-77.

2. Bann, C. M., Treiman, K., Squiers, L., Tzeng, J., Nutt, S., Arvey, S., ... & Rechis, R. (2015). Cancer survivors' use of fertility preservation. *A Journal of Women's Health*, 24(12), 1030-1037.

3. Shnorhavorian, M., Harlan, L. C., Smith, A. W., Keegan, T. H., Lynch, C. F., Prasad, P. K., ... & Keel, G. (2015). Fertility preservation knowledge, counseling, and actions among adolescent and young adult patients with cancer: a population-based study. *A Cancer*, 121(19), 3499-3506.

4. Letourneau, J. M., Ebbel, E. E., Katz, P. P., Katz, A., Ai, W. Z., Chien, A. J., ... & Rosen, M. P. (2012). Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. *A Cancer*, 118(6), 1710-1717.

5. Gilleland Marchak, J., Elchuri, S. V., Vangile, K., Wasilewski-Masker, K., Mertens, A. C., & Meacham, L. R. (2015). Perceptions of infertility risks among female pediatric cancer survivors following gonadotoxic therapy. *A Journal of pediatric hematology/oncology*, 37(5), 368-372.

6. Green, D. M., Kawashima, T., Stovall, M., Leisenring, W., Sklar, C. A., Mertens, A. C., ... & Robison, L. L. (2010). Fertility of male survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. *A Journal of Clinical Oncology*, 28(2), 332.

7. Letourneau, J. M., Ebbel, E. E., Katz, P. P., Katz, A., Ai, W. Z., Chien, A. J., ... & Rosen, M. P. (2012). Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. *A Cancer*, 118(6), 1710-1717.

SUPPORT: NIH HD080952-05.

O-161 Tuesday, October 15, 2019 11:45 AM

#### BEYOND PREMATURE OVARIAN INSUFFICIENCY: CHARACTERIZING REPRODUCTIVE AGING AND RISKS FOR ACCELERATED AGING IN ADOLESCENT AND YOUNG ADULT (AYA) CANCER SURVIVORS.

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**OBJECTIVE:** AYA cancer survivors undergo cancer treatments that increase their risk of premature ovarian insufficiency, but little is known about their transition to menopause and the applicability of reproductive aging staging systems, i.e. STRAW+10, to this population. We characterized AYA survivors by STRAW+10 criteria and examined the association between gonadotoxic treatment therapies and menstrual cycle patterns.

DESIGN: Retrospective cohort.

**MATERIALS AND METHODS:** Female survivors who were ages 18-39, diagnosed with cancer at ages 15-35, completed primary cancer treatment and had at least one ovary were recruited from cancer registries, clinics and advocacy groups from 2015-2018 to the parent Window Study on ovarian function. Participants collected dried blood spots (DBS), answered questionnaires on menstrual pattern, and had primary medical records abstracted. Survivors who were pregnant, breastfeeding, or on hormonal therapy were excluded. DBS were assayed for AMH and FSH (AnshLabs, Webster, TX). Intra-assay and inter-assay CV were  $< 10\%$ . STRAW+10 criteria were used to categorize participants into the following stages: reproductive (RE), menopausal transition (MT), or post-menopausal (PM). Multinomial logistic regression estimated associations between gonadotoxic treatments and STRAW stage and adjusted for confounding.

**RESULTS:** 279 survivors, mean age 33.7 (SD 4.6), were at a median of 6.9 years (IQR 5.3) post-treatment. The most common cancers were leukemia/lymphoma (35%), thyroid (21%) and breast (20%). By menstrual criteria alone, 51% were in RE stage, 42% in MT stage, and 8% in PM stage. Including biomarkers, 210/279 could be characterized by STRAW+10 (80% in RE, 12% in MT, 7% in PM). The group that did not fit STRAW+10 criteria reported menstrual patterns consistent with MT, but had AMH/FSH values consistent with RE stage; these women were younger ( $p=0.01$ ) and more proximal to cancer diagnosis ( $p=0.02$ ) compared to the MT group.

In this cohort, 48% received alkylating chemotherapy, 3% received pelvic radiation (RT), 1% received total body irradiation (TBI), and 8% had cancer recurrence. Adjusted for current age, cyclophosphamide equivalent dose of  $< 7$  grams/m<sup>2</sup> and  $\geq 7$  grams/m<sup>2</sup> were significantly associated with higher risk of being in MT stage (RR=5.3, 95% CI 2.0-13.7 and RR=8.6, 95% CI 2.1-35.6, respectively) and PM stage (RR=16.2, 95% CI 1.6-160.5 and RR=96.9, 95% CI 9.9-952.9, respectively) compared to no CED exposure. Cancer recurrence was significantly associated with higher risk of PM stage (RR=18.2, 95% CI 5.5-60.3). Age was not associated. RT ( $p=0.001$ ) and TBI ( $p=0.04$ ) were both associated with advanced reproductive aging stages, but small numbers precluded inclusion in multivariable models.

**CONCLUSIONS:** Known to be gonadotoxic, alkylating chemotherapy increased the risk of being in more advanced STRAW stages, providing novel evidence to support classifying reproductive aging in AYA survivors using STRAW+10 criteria. Menstrual pattern alone misclassifies AYA survivors with regard to their stage of reproductive aging, supporting the use of AMH and FSH in this population.

SUPPORT: NIH HD080952-05.

O-162 Tuesday, October 15, 2019 12:00 PM

#### COMPENSATORY OVARIAN HYPERTROPHY AFTER UNILATERAL OOPHORECTOMY: EVALUATION OF OVARIAN VOLUMES IN THE PEDIATRIC AND ADOLESCENT POPULATION.

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**OBJECTIVE:** Although unilateral oophorectomy is performed in the pediatric and adolescent population for indications including adnexal mass concerning for malignancy, adnexal torsion and infection, it is infrequent and the true prevalence is unknown. Limited data exists on the morphologic and physiologic effects on the remaining ovary after oophorectomy, especially in the pediatric population. Studies have shown compensatory hypertrophy of the remaining single ovary in animal studies and similar findings have been noted in the testis in human boys. Our aim is to evaluate ovarian volumes on ultrasound following unilateral oophorectomy to determine if compensatory ovarian hypertrophy occurs in the remaining contralateral ovary which may lead to important changes in clinical practice.

**DESIGN:** A retrospective chart review was performed at a large academic children's hospital after institutional review board approval on all female patients  $< 21$  years of age who underwent oophorectomy based on CPT, ICD-9 and ICD-10 codes over a 20-year collection period.

**MATERIALS AND METHODS:** The charts of 328 female patients who met the inclusion criteria were reviewed and 96 were included in the analysis. Patients were excluded if oophorectomy was not performed, they required subsequent contralateral oophorectomy, lacked follow-up ultrasound following

oophorectomy, or had sonographic abnormalities (e.g. ovarian cyst) on post-oophorectomy ultrasound. Data collected included: age, race, comorbidities, age of menarche, surgeon specialty, ultrasound findings and bilateral ovarian volumes prior to surgery, indication for surgery, surgical pathology, and ultrasound findings and ovarian volume of remaining ovary following surgery. Descriptive analysis of ovarian volume of the remaining ovary following oophorectomy calculated on ultrasound were compared to known age-matched standard volumes at the time of post-operative ultrasound.

**RESULTS:** The average age of patients at time of oophorectomy was 10.8 years (2 days – 18 years). Twenty-four (25%) were < 10 years of age and 72 (75%) were > 10 years. Average time from surgery to post-operative ultrasound was 12.1 months (0 – 129 months). Average ovarian volume age < 10 years was 2.5 ml and > 10 years was 13.7 ml. Sixty (63%) of patients had post-operative volumes greater than age-matched standards and 29 (30.2%) had smaller volumes. Of those with increased volume, average was 15.3 ml (< 10 years) and 15 ml (> 10 years). Sixty (62.5%) patients had volumes more than 10% larger than the age matched standards, and 50 (52.1%) patients had volumes more than 50% larger. Of the those with increased post-operative ovarian volume (n=60), 83.3% had volumes > 50% larger than age-matched standards.

**CONCLUSIONS:** Ovarian enlargement occurs in the contralateral ovary following unilateral oophorectomy in the pediatric and adolescent population which supports the concept of compensatory ovarian hypertrophy that has been previously demonstrated in non-human models. This knowledge is important to the future clinical management of young females who have undergone unilateral oophorectomy.

References: Arai H (1920) On the cause of the hypertrophy of the surviving ovary after semispaying (albino rat) and on the number of ova in it. *Dev Dyn* 28:59–79.

Cohen HL, Tice HM, Mandel FS (1990) Ovarian volumes measured by US: bigger than we think. *Radiology* 177:189–192.

Cohen HL, Shapiro MA, Mandel FS, Shapiro ML (1993) Normal ovaries in neonates and infants: a sonographic study of 77 patients 1 day to 24 months old. *AJR Am J Roentgenol* 160:583–586.

Dailey RA, Peters JB, First NL et al (1969) Effect of unilateral ovariectomy in the Yorkshire and Poland China prepuberal gilt. *J Anim Sci* 28:775–779.

Hartman CG (1925) Observations on the functional compensatory hypertrophy of the opossum ovary. *Am J Anat* 35:1–24.

Koff SA (1991) Does compensatory testicular enlargement predict monorchism? *J Urol* 146:632–633.

Orsini LF, Salardi S, Pilu G, et al. Pelvic organs in premenarcheal girls: real-time ultrasonography. *Radiology* 1984; 153:113–116.

SUPPORT: None.

## PRACTICE MANAGEMENT

O-163 Tuesday, October 15, 2019 10:45 AM



### CURRENT STATUS OF REPRODUCTIVE LABORATORY PROFESSION: WORKLOAD, WELLNESS, EARNINGS AND JOB SATISFACTION.

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**OBJECTIVE:** To investigate the current workplace status among reproductive laboratory professionals in the U.S., including trends in earnings and comparison to benchmarks, work environment, job satisfaction, and wellness.

**DESIGN:** Retrospective analysis of multiple years of Society of Reproductive Biologists and Technologists (SRBT) Salary and Job Satisfaction Surveys with comparable publications and benchmarks.

**MATERIALS AND METHODS:** SRBT biennial survey data 2001–2018 were analyzed to determine longitudinal trends of salary among reproductive lab personnel. Variables including work environment, benefits, salary of various job titles, clinic setting, gender, job satisfaction and burnout, as well as off-site consulting, were analyzed. Key compensation numbers were compared with national earnings data from U.S. Bureau of Labor Statistics and similar clinical lab and biotechnology sector wage surveys.

**RESULTS:** Total of 1,737 responses were analyzed. Overall, survey responses showed satisfaction with their current jobs and optimism on job market projections. However, the majority of survey responses also indicated significant stress (89% answered medium to extremely high level of stress), burnout (60.6%) and overtime work (72.8%). Most common benefits received were health and dental insurance, paid time-off, retirement plan, and support for conference attendance and certification. In 2018, the average annual clinical workload processed by each hands-on personnel included 108 fresh oocyte retrievals, 88 FETs, 79 biopsies for PGT, and 167 andrology tasks. Throughout the past two decades, nominal compensation (non-inflation adjusted) of reproductive lab professionals steadily increased throughout most of the survey period, with numbers higher than the national average for college/advanced degree workers. Such earnings were higher than most clinical lab specialties as well, with exception in a couple biotechnology sectors. Director earnings increases trended higher to advanced degree workers nationwide. Non-director categories showed a more significant salary growth than nationwide college/advanced degree workers and lab directors. Data from recent years revealed a wider distribution in salary range, which may reflect the volatility due to short supply of senior embryologists. Recent data also demonstrated an increasing portion of bonus in the compensation structure, which may indicate a broader utilization of bonus/incentives across all clinical settings, and possibly a contributing factor to the wider range of compensation among lab personnel. Gender-related difference in compensation remains significant despite an overall smaller gap than nationwide college/advanced degree workers.

**CONCLUSIONS:** The salary trend of reproductive lab profession show a steady increase throughout the period, a good indication compared to national labor wage and related clinical lab wage benchmarks. However, work-related stress, burnout, overtime duties, and gender pay gap remain issues to be resolved. Potential factors and impacts on these trends warrant further investigation.

O-164 Tuesday, October 15, 2019 11:00 AM

### DOES THE OPERATOR PERFORMING THE EMBRYO TRANSFER SIGNIFICANTLY INFLUENCE THE CYCLE OUTCOME?

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**OBJECTIVE:** Although embryo transfer (ET) is recognized to be an operator dependent technique, it is still unclear whether there are factors that can influence a correlation between success and operator. This study sought to analyze whether Ongoing Pregnancy Rate (OPR) is associated to the operator and whether there is a learning curve to become proficient.

**DESIGN:** Retrospective comparative analysis including all the fresh ET performed between 1996 and 2016 at a University-affiliated Center. Only embryo transfers performed by the surgeon on duty on that day were included. For operators with previous experience, the number of previous procedures was their entering threshold.

**MATERIALS AND METHODS:** A logistic regression model with a random intercept for the surgeon was specified, accounting for the heterogeneity among surgeons. To investigate the role of experience on OPR, a two-step procedure was implemented: a logistic regression for every surgeon to estimate a linear term expressing the relationship between experience and OPR and then the estimated slopes were compared through meta-analysis techniques.

**RESULTS:** We included in the analysis 19,829 fresh ET performed by 32 operators. The random effects logistic model included: woman age, FSH, number of oocytes retrieved, fertilization rate, year of the procedure, number and stage of transferred embryos. The likelihood-ratio test for the heterogeneity among operators was highly significant (p-value = 0.0066). From the worst to the best operator the difference between intercepts varied from a coefficient of -0.205374 to a coefficient of 0.1458145: this result can constitute a very big burden. Performing a random effects meta-analysis on these slopes, we found that the overall estimate was near zero, with a total pooled effect = 0.000 (-0.001 - 0.001). No evidence arose of an increase in OPR according to the operator's experience. The I<sup>2</sup> of the heterogeneity among slopes was 43.7%. From our data, some operators perform worse than the

mean and do not improve with additional transfers. This observation can be explained because ET is generally performed by a single operator who learn on his own, with little opportunity of comparison.

**CONCLUSIONS:** This study shows that the operator factor can affect OPR but there is no significant increase in the outcome with experience. In future a very useful method could be the digital simulator, which could help operators to ameliorate without practicing on real patients.

**SUPPORT:** None.

**O-165** Tuesday, October 15, 2019 11:15 AM

**ONLINE PATIENT REVIEWS ARE INFLUENCED BY TYPE OF PHYSICIAN-BASED INFERTILITY PRACTICE.**



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**OBJECTIVE:** In the field of reproductive endocrinology and infertility (REI), physician online presence plays a large marketing role with success rates and procedures reported on clinic/hospital websites<sup>1</sup> and patient assessments on physician rating sites. Compared to other specialties, REI clinics place a strong emphasis on optimizing patient-centered care in order to enhance their experience, increase treatment compliance and patient wellbeing while minimizing anxiety and depression during their often extended treatment cycles<sup>2</sup>. The objective of this study is to determine if patient online ratings are influenced by infertility insurance coverage. We hypothesize that patient reviews of physicians will be more positive in areas where health insurance mandates fertility coverage given that financial burden on patients is often cited as a major stressor of their experience.

**DESIGN:** Retrospective Cohort Study.

**MATERIALS AND METHODS:** Online physician ratings submitted between 2016-2019 from popular websites (Vitals, RateMD, Healthgrades) were recorded for REI specialists in the U.S. registered through SART and CDC. Overall rankings of physicians were compared based on infertility insurance coverage, clinic location, and type of clinical practice (university/hospital vs. private practice). Infertility insurance coverage was determined as covered if state health insurance mandates any type of coverage for fertility treatment and not covered if the state does not mandate fertility coverage.

**RESULTS:** Data was collected from 1,097 REI specialists. An average rating of 4.09 out of 5 was found for physicians in states with mandated insurance coverage and an average rating of 4.08 out of 5 was found for those without insurance coverage (p = 0.762). The average rating for physicians based within a university/hospital practice was 3.96 compared to 4.13 for physicians in a private practice setting (p = 0.011). Among regions in the U.S., the South scored significantly higher mean average rating (p<0.01) than the Northeast and Midwest region. There was no significant difference (p>0.05) between West and South region (see Table).

**CONCLUSIONS:** A statistically significant higher rating was found for physicians in private practice compared to those affiliated with a university/hospital. No difference was found between the average rating in states with mandated insurance coverage for infertility treatment compared to states without insurance coverage. Furthermore, the South region had significantly higher mean average ratings compared to other regions in U.S except the west.

Region	N	Mean Average Rating +/- standard deviation
Northeast	327	3.99 +/- 0.930*
West	241	4.14 +/- 0.95* <sup>^</sup>
South	354	4.22 +/- 0.85* <sup>^</sup>
Midwest	175	3.91 +/- 1.01*

\*p = 0.01 – 0.049; <sup>^</sup>p = 0.765

References: 1. Wilkinson J, Vail A, Roberts SA. Direct-to-consumer advertising of success rates for medically assisted reproduction: a review of national clinic websites. *BMJ Open*. 2017;7(1):e012218.

2. Gameiro S, Canavaro MC, Boivin J. Patient centred care in infertility health care: Direct and indirect associations with wellbeing during treatment. *Patient Education and Counseling*. 2013;93(3):646-654.

**O-166** Tuesday, October 15, 2019 11:30 AM

**FREQUENCY AND CLAIMS BASIS FOR LAWSUITS OVER LOST, DISCARDED AND DAMAGED FROZEN EMBRYOS OVER A 10 YEAR PERIOD.**



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**OBJECTIVE:** Cryopreservation technology has opened options to preserve fertility and maximize family building options. These opportunities create liability risks for providers not directly related to clinical practice and quality controls but also for maintenance of laboratory equipment and environment. Insights into how best to deliver care and assure optimal outcomes may be gained from a first-ever review of an increasing body of recent case law brought over embryos that have been lost, damaged discarded, misimplanted or contaminated. Our objectives are to review claims, basis of claims and frequency of lawsuits over lost frozen or damaged frozen embryos.

**DESIGN:** Retrospective review of case law in state and federal courts over a 10 year period.

**MATERIALS AND METHODS:** Case law was researched from January 1, 2009 to April 22, 2019. Bloomberg, Westlaw and Lexis Nexis databases were searched to provide coverage of state court dockets regarding allegations and basis of claims made. Bloomberg Law included all federal court dockets. Cross-referenced terms included embryo, fertilized oocyte, frozen or cryopreserved embryo, discarded, lost and damaged embryo/s and implanted embryos. Data extracted included claims arising in federal and state courts.

**RESULTS:** A total of 131 cases were identified: 121 and 10 lawsuits in the state and federal court dockets respectively. 87 cases involved the recent cases in California and Ohio in 2018-19. Allegations for these relate to freezer storage tank failure. In the remaining 44 cases, the majority (37) were brought across a broad range of allegations including: personal injury; breach of contract or warranty; product liability; professional negligence; unfair business practices and miscellaneous tort. A minority of cases (7) were brought for medical malpractice. The locations of these 44 cases included New York, Delaware, Illinois, Arizona and North Dakota.

**CONCLUSIONS:** The frequency of suits for damaged, lost or destroyed embryos is low with the exception of the recent events in California and Ohio. The basis of the claims is seldom for medical malpractice. These findings suggest that insurance coverage directed to claims outside of medical malpractice may be warranted given the expanding inventories of frozen oocytes, embryos and sperm and varying basis for claims.

**SUPPORT:** None.

**O-167** Tuesday, October 15, 2019 11:45 AM

**IMPROVED MONITORING OF HUMAN EMBRYO CULTURE CONDITIONS USING A DEEP LEARNING-DERIVED KEY PERFORMANCE INDICATOR (KPI).**



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**OBJECTIVE:** The clinical outcome of an in-vitro fertilization (IVF) cycle is perhaps the best indicator of system efficiency with ongoing pregnancy rates providing the most robust marker of embryo quality. Several early developmental stage markers are widely used to monitor culture conditions, however, their association with clinical outcomes is unclear. The objective of this study was to determine whether the use of an artificial intelligence (AI) algorithm, trained to predict in-vitro human embryo developmental fate, can be effectively used as a key performance indicator (KPI) for monitoring the performance of the embryo culture system.

**DESIGN:** Retrospective cohort study using a pre-developed deep neural network<sup>1</sup>. The deep neural network (AI) analyzed embryos images acquired at 70 hours post insemination and provided a score (KPI score) taking into account all embryos within a given group.

Key Performance Indicator	Medium A (n=151)	Medium B (n=137)	Medium C (n=124)	Medium D (n=137)	Medium E (n=167)	Medium F (n=160)	R <sup>2</sup>
Day 2: % 4-Cell	35.8%	41.6%	34.7%	38.0%	36.5%	42.5%	0.1144
Day 3: % 8-Cell	27.8%	26.3%	16.1%	27.0%	30.5%	31.9%	0.1144
Day 3: % 6-10 Cell	46.4%	38.7%	32.3%	38.0%	40.7%	44.4%	0.0415
Day 3: % ≥ 7-Cell	64.9%	70.1%	66.1%	66.4%	77.8%	74.4%	0.0557
Day 3: % AI Generated KPI	33.8%	30.2%	41.5%	38.9%	38.6%	37.8%	0.9063
% Ongoing Pregnancy Rate	50.0%	42.9%	58.3%	58.8%	58.8%	57.9%	

**MATERIALS AND METHODS:** A total of 876 embryos were cultured in 6 different lots of media (Medium A-F; CSC-Complete, Irvine Scientific) and under identical conditions at 37°C, 5% O<sub>2</sub> and 6.5% CO<sub>2</sub> with oil overlay (OvOil, Vitrolife). The percentage of 2 pronucleus (2PN) zygotes at the 4-cell stage on Day 2, 8-cell, 6 to 10-cell, ≥ 7-cells and those predicted to develop into high quality blastocyst stages using an AI-based generated KPI on Day 3 of embryo development was compared with ongoing pregnancy rates using a regression analysis. The low threshold value for ongoing pregnancy rates in the Massachusetts General Hospital (MGH) fertility clinic is set at 50%.

**RESULTS:** The AI-based generated KPI for predicting high quality blastocyst formation had the highest association with ongoing pregnancy rates (R<sup>2</sup>=0.9063). This was the only cleavage stage KPI examined that was able to detect changes in our embryo culture environment that resulted in the pregnancy rates dropping below the threshold of 50%.

**CONCLUSIONS:** The most important aspect of quality assurance data analysis is the identification of KPIs that will provide meaningful insight into laboratory functioning. This study demonstrated the power of using AI predictions in monitoring the performance of the embryo culture environment.

Reference: I. P. Thirumalaraju, J. Y. Hsu, C. L. Bormann, M. Kanakasabapathy, I. Souter, I. Dimitriadis, K. A. Dickinson, R. Pooniwala, R. Gupta, V. Yogesh and H. Shafiee, *Fertility and sterility*, 2019, **111**, e29.

**SUPPORT:** This work was partially supported by the Brigham Precision Medicine Developmental Award (Brigham Precision Medicine Program, Brigham and Women's Hospital) and R01AI118502, R01AI138800, and R21HD092828 (National Institute of Health).

**O-168** Tuesday, October 15, 2019 12:00 PM

#### **AUTOMATED QUALITY ASSESSMENT OF INDIVIDUAL EMBRYOLOGISTS PERFORMING ICSI USING DEEP LEARNING-ENABLED FERTILIZATION AND EMBRYO GRADING TECHNOLOGY.**



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**OBJECTIVE:** Data analysis is a crucial part of an effective in-vitro fertilization (IVF) quality assessment (QA) program. Routine review of identified key performance indicators (KPIs) are important to ensure proper laboratory functioning and, perhaps more importantly, to identify potential problems to permit timely corrections. Fertilization assessment is the primary outcome used to measure embryology staff proficiency with Intracytoplasmic sperm injection (ICSI). However, tracking the developmental fate of ICSI derived embryos may provide a more complete picture of how well this procedure is being performed. Current quality assessments require manual examination and recording of fertilization status and embryo developmental scores. These processes are labor-intensive and highly subjective in nature. Convolutional neural networks (CNNs) have been used to assess fertilization and blastocyst development with accuracies rivaling highly trained embryologists. The objective of this study was to compare quality assessments of staff performing ICSI using standard manual grading measurements and fully automated AI-trained image measurements.

**DESIGN:** Retrospective cohort study using a deep convolutional neural network<sup>1</sup>. The deep neural network (AI) was trained to evaluate embryos at day 1 and day 5 post insemination.

**MATERIALS AND METHODS:** In our study, performances of 7 individual embryologists were calculated through manually analyzing the developmental outcome rates with the outcome rates measured automatically by an

AI system through morphological analyses. The AI system developed to evaluate fertilization and blastocyst development was utilized for this work. We compared the rates of fertilization, blastocyst development, and high-quality blastocyst (HQB) development in a total of 947 embryos that were divided between the 7 embryologists. To evaluate the difference between the two analysis methods, we performed a Wilcoxon matched-pairs signed rank test and a coefficient of variation (%CV) analysis.

**RESULTS:** The Wilcoxon tests revealed that the two approaches performed with negligible differences (P>0.05) for all three rate estimations (Fertilization, Blastocysts, and HQB). The medians of difference for estimations of fertilization, blastocysts, and HQB were -1.3% (P>0.31), 1.8% (P>0.09), and -3.6% (P>0.18), respectively. The %CV estimations also showed that the difference between manual and AI-generated estimations for each embryologist in all three rates was low. The median of %CV between the two approaches in measuring the rates of fertilization, blastocysts, and HQB were 1.9%, 3.4%, and 10.9%, respectively.

**CONCLUSIONS:** This study is the first to describe the use of artificial intelligence to monitor individual embryologists performing ICSI in a clinical setting. The extremely low coefficient of variation between the manual and AI-based QA assessment methods demonstrate the high accuracy of the automated AI system. This study demonstrates an alternative method for monitoring KPIs in the IVF laboratory without the need for manual assistance.

Reference: I. I. Dimitriadis, C. L. Bormann, P. Thirumalaraju, M. Kanakasabapathy, R. Gupta, R. Pooniwala, I. Souter, J. Y. Hsu, S. T. Rice, P. Bhowmick and H. Shafiee, *Fertility and sterility*, 2019, **111**, e21.

**SUPPORT:** This work was partially supported by the Brigham Precision Medicine Developmental Award (Brigham Precision Medicine Program, Brigham and Women's Hospital) and R01AI118502, R01AI138800, and R21HD092828 (National Institute of Health).

#### **PROCEDURES AND TECHNIQUES**

**O-169** Tuesday, October 15, 2019 10:45 AM

#### **EMBRYO TRANSFER MANEUVERS AND MANIPULATIONS – THE EFFECT ON IN VITRO FERTILIZATION (IVF) OUTCOMES.**



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**OBJECTIVE:** To determine the effect of maneuvers performed on the embryo transfer (ET) catheter during ET on in vitro fertilization (IVF) outcomes.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This study included all women undergoing IVF/ICSI with a subsequent Day 3 or Day 5 ET at a single academic hospital IVF practice from 1/2013 to 1/2018. The first ET during the study period was included from each patient. A 'trial followed by transfer' method was routinely employed and all ETs were performed under abdominal ultrasound guidance. Each ET was systematically scored on ease by the transferring physician (easy, some difficulty, extreme difficulty) and any additional maneuver or instrumentation that was needed to perform the ET, such as bending the outer sheath of the catheter, extending the outer sheath over the inner catheter, and retaining the external sheath (conversion into an 'afterload' method).

The primary outcome was live birth rate. Secondary outcomes included clinical pregnancy rate (CPR), implantation rate (IR), ectopic, biochemical and miscarriage rate.

Direction of the uterus, catheter used, infertility diagnosis, BMI, age, donor egg, fresh versus frozen embryo, use of a gestational carrier, preimplantation genetic testing, endometrial thickness, presence of blood and mucus on the transfer catheter, distance from the fundus, and physician performing the ET

(fellow or attending) were all tested as potential confounders in univariate analyses. Log-binomial models and Poisson regression models, adjusted for catheter used, presence of mucus, donor egg, and fresh versus frozen, were used to estimate the adjusted relative risks (ARR) with a 95% confidence interval. A stratified analysis, based on ease of ET, was performed.

**RESULTS:** A total of 3,995 ETs were included (76% fresh, 24% frozen). Overall CPR was 46% and LBR was 36%. Twenty-six percent of ETs were performed with the afterload technique with a retained external sheath. A bend was placed in 37% of ETs, and the outer sheath was extended in 49% of transfers. The univariate analyses showed that a bent or extended sheath conferred no difference in outcomes, whereas the afterload technique conferred a lower live birth rate and clinical pregnancy rate ( $p=0.001$ ). Amongst transfers that were easy or performed with some difficulty, the live birth rate with and without a retained sheath was 32% and 38%, respectively. Within the stratified model based on transfer ease, after adjustment for confounding, there was no significant difference in outcomes when ET catheter maneuvers were performed.

**CONCLUSIONS:** Amongst ETs performed with ease, some difficulty, and extreme difficulty, there was no significant impact of bending the sheath, extending the sheath, or retaining the external sheath on cycle outcomes. However, there was a trend toward worse clinical pregnancy and live birth rates when the external sheath was retained, even amongst easy transfers. Consideration should be made in light of this finding for centers that use 'afterload' as their primary ET technique.

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#### MINIMALLY INVASIVE UTERINE ASPIRATION 24 HOURS AHEAD OF EMBRYO TRANSFER CHARACTERIZES THE COMPROMISED RIF UTERINE MICROENVIRONMENT AND IS PREDICTIVE OF REPRODUCTIVE OUTCOME.

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**OBJECTIVE:** Repeat implantation failure (RIF) is particularly challenging to treat in ART, resulting in limited success even when adequate preparation of the endometrium is established and a transfer is performed with a high grade euploid blastocyst. The objective of this study was to utilize a multidisciplinary approach to decipher the complexity of RIF through investigations of the maternal molecular components ahead of an embryo transfer.

**DESIGN:** Research study.

**MATERIALS AND METHODS:** Patients were recruited with IRB consent 24 hours prior to a programmed frozen embryo transfer (FET) with a euploid blastocyst. Uterine secretions were collected by gentle aspiration (~2-5ul) under ultrasound guidance and grouped according to reproductive outcomes: Failed euploid FET (RIF patients,  $\geq 3$  prior IVF failures) and Positive live birth FET (maternally age-matched patients; mean  $36.6 \pm 3.8$  years). Total and small RNA ( $n = 22$ ) was isolated for sequencing on the NovaSeq 6000 (Illumina). Reads were aligned to hg38 using GSNAP and analyzed with edgeR (FDR cutoff of 5%;  $P < 0.01$ ). Metabolite analysis ( $n = 20$ ) was performed by UHPLS-MS (Thermo) using MassMatrix and Maven (Princeton Univ). Proteomic analysis ( $n = 6$ ) involved FASP digestion and LC-MS/MS, with protein identifications generated by Mascot ( $v 2.6$ ) and Scaffold ( $v 4.8.9$ ) ( $\alpha$  of 0.05; fold change  $> 1.5$  or  $< 0.5$ ).

**RESULTS:** A unique uterine microenvironment was observed for RIF patients and negative implantation outcomes 24 hours prior to an embryo transfer ( $P < 0.05$ ). An interplay of several biological processes were evident in RIF failed aspirates with a focused interest on 13 significantly reduced transcripts, 7 significantly increased maternal miRNAs, 12 significantly decreased amino acids and 16 proteins of significantly altered abundance ( $P < 0.05$ ). Specific examples included decreased expression of PLA2G4D ( $P < 0.0001$ ) which regulates the eicosanoid pathway, thereby impacting downstream synthesis of prostaglandins like PGE2. Decreased expression of TET1 ( $P < 0.0001$ ), an epigenetic regulator required for DNA methylation. Increased expression of miR-17, a known negative regulator of VEGFA, required for successful implantation ( $P < 0.01$ ). Decreased quantities of arginine, essential for blastocyst activation and trophectoderm motility ( $P < 0.05$ ). Lastly, an increased abundance of SERPING1, a protein associated with inflammation, which regulates complement activation ( $P < 0.05$ ).

**CONCLUSIONS:** Analysis of uterine secretions 24 hours prior to FET, allowed for an in-depth molecular characterization of the compromised RIF uterine microenvironment and was predictive of reproductive outcome. The negative influence on key miRNAs and gene transcription levels, in addition

to altered amino acid and protein concentrations, were all identified as critical contributors to poor RIF outcomes. Ongoing investigations into the relationships of these molecular networks will lead to the possibility of more effective clinical interventions for this difficult patient population.

**SUPPORT:** None.

O-171 Tuesday, October 15, 2019 11:15 AM



#### EFFECTS OF INTRAOVARIAN INJECTION OF AUTOLOGOUS PLATELET RICH PLASMA ON OVARIAN RESERVE AND IVF OUTCOMES IN WOMEN WITH PREMATURE OVARIAN INSUFFICIENCY.

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**OBJECTIVE:** Premature ovarian insufficiency (POI) affects 1% of reproductive age women. There are currently no effective treatment options that allow women with POI to conceive with their own eggs. Autologous platelets have been used as a source of proteins for healing and tissue regeneration for more than two decades, and platelet-rich plasma (PRP) is reported to promote the development of isolated human primordial and primary follicles to the preantral stage. In this study, we aimed to investigate the effects of intraovarian injection of autologous PRP on ovarian reserve and IVF outcomes in patients with POI.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Women (age range 20-48) diagnosed with POI based on ESHRE criteria (amenorrhoea or oligomenorrhoea for at least four months and increased follicle-stimulating hormone (FSH)  $> 25$  IU/l measured twice with a four-week interval) were recruited for the study. Antral follicle counts (AFC), anti-mullerian hormone (AMH), and FSH levels were determined at baseline. PRP was prepared from peripheral blood using routine techniques. PRP injection was performed transvaginally, under ultrasound guidance, into at least one ovary using a 35 cm 17 G single lumen needle. Cyclic estrogen (4 mg estradiol daily on days 1-25) and progesterone (200 mg progesterone daily on days 16-25) were used to induce vaginal bleeding after PRP treatment. On the 2-4th days of induced menses after the procedure, AFC, AMH, and FSH levels were re-assessed. Patients with at least one antral follicle were started on ovarian stimulation for IVF-ICSI and embryo banking at cleavage stage. Markers of ovarian reserve (AFC, FSH, AMH), and IVF laboratory outcome parameters (number of MII oocytes, 2PN embryos, cleavage stage embryos) were followed.

**RESULTS:** At the time of this submission, a total of 70 patients (mean age  $\pm$  SD:  $40.8 \pm 4.8$ ) with the diagnosis of POI were included in the study. PRP treatment resulted in improved AFC ( $2.6 \pm 1.3$  vs  $0.9 \pm 0.8$ ;  $p < 0.01$ ), increased serum AMH ( $0.19 \pm 0.11$  vs  $0.11 \pm 0.08$ ;  $p = 0.01$ ), and lower serum FSH ( $32.6 \pm 9.6$  vs  $37.4 \pm 11.2$ ;  $p = 0.06$ ) levels. Total number of MII oocytes, 2PN and cleavage embryos obtained were  $2.38 \pm 1.58$ ,  $2.00 \pm 1.47$ , and  $1.94 \pm 1.08$ , respectively. In 24 patients (34.2%), no changes were observed in terms of AFC or other laboratory parameters after the PRP procedure, therefore IVF-ICSI was not attempted. Another 24 patients (34.2%) failed to respond to stimulation or had fertilization failure. In 21 patients (30%), at least one cleavage stage embryo was obtained and embryo banking was performed. Importantly, spontaneous pregnancy occurred in six patients (7.1%, mean age  $\pm$  SD:  $39.5 \pm 5.8$ ) one or two cycles after the PRP procedure. At the time of this report, one of these pregnancies had resulted in missed abortion while the others were ongoing.

**CONCLUSIONS:** In women with POI, intraovarian injection of autologous PRP might be considered as an alternative experimental treatment option. Future studies with larger sample size and randomized prospective study design will be necessary to determine whether this intervention truly results in improved clinical outcomes.

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#### ENDOMETRIAL RECEPTIVITY ANALYSIS DOES NOT INCREASE LIVE BIRTH RATES IN FIRST FROZEN EMBRYO TRANSFER WITH A EUPLOID BLASTOCYST.

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Center, Austin, TX; <sup>b</sup>Ovation Fertility, Austin, TX; <sup>c</sup>School of Public Health, University of Texas Health Science Center at Houston, Houston, TX.

**OBJECTIVE:** Previous studies suggest an endometrial receptivity analysis (ERA) may improve implantation and live birth rates for patients with previous frozen embryo transfer (FET) failure. The purpose of this study is to evaluate whether performing an ERA prior to first time FET with a euploid blastocyst improves the live birth rate.

**DESIGN:** This is a single institution retrospective cohort study.

**MATERIALS AND METHODS:** A retrospective review was performed including all patients in 2017 who underwent first time FET with a euploid blastocyst(s) (n=220). All patients in this study underwent PGT-A by Nex-Gen sequencing. The embryos were then vitrified. Patients were stratified by ERA status (n=46) vs. no ERA (n=174) prior to undergoing FET. The primary outcome was to measure the live birth rate. Secondly, we measured the implantation rate and the clinical pregnancy rate. A two-sample t-test was used to compare continuous outcomes between groups, and Chi-square testing was used to compare proportions between the two groups.

**RESULTS:** The implantation rate for patients that underwent ERA vs. no ERA prior to FET was 64.6% vs. 60.5% (p=0.71). The clinical pregnancy rate for ERA vs. no ERA prior to FET was 56.5% vs. 52.8% (0.56). The live birth rate for ERA vs. no ERA prior to FET was 52.2% vs. 51.1% (p=0.9). The single embryo transfer rate was 96% for the ERA group vs. 98% for the non-ERA group.

**CONCLUSIONS:** Performing an ERA prior to first time FET with a euploid blastocyst did not increase the live birth rate compared to patients who did not have an ERA before their first FET. The differences in implantation and clinical pregnancy rates between the two groups were also not statistically significant. Our findings warrant an adequately powered randomized controlled trial to determine the efficacy of ERA prior to the transfer of a euploid blastocyst.

O-173 Tuesday, October 15, 2019 11:45 AM



#### SAFETY OF OIL-BASED CONTRAST MEDIUM FOR HYSTEROSALPINGOGRAPHY: A SYSTEMATIC REVIEW.

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**OBJECTIVE:** A hysterosalpingography (HSG) with oil-based contrast increases pregnancy rates in women with subfertility. However, there have been some concerns regarding complications, most importantly the risk of intravasation of the contrast resulting in oil-embolisms, pelvic infections and thyroid dysfunction. Here, we present a clear overview on the frequency of the reported complications.

**DESIGN:** A systematic review and meta-analysis.

**MATERIALS AND METHODS:** We searched electronic databases up to March 2018 as well as textbooks (published before 1960) and reference lists to identify eligible studies. There were no language or publication date restrictions. We performed a systematic review and meta-analysis of relevant RCTs, cohort studies and case reports/series. We looked at women and their offspring.

**RESULTS:** We included 120 studies, published between 1928 and 2017, of which 76 case reports/series. The 44 cohort studies reported on 20,438 HSG's. Intravasation occurred in 1.9% (389/20,438), no treatment was needed in the majority of cases. Embolisation occurred in 0.1% (24/20,438). A total of four deaths have been reported in the cohort studies; three caused by peritonitis (last report in 1950) and one caused by an oil-embolism (1955), which occurred in a 45-year old woman who received an HSG for another indication than subfertility. Among the cohort studies published since 1970, 22 studies reported on 7027 HSG's, intravasation occurred in 1.5% (102/7027) and embolisation in 0.2% (13/7027), without fatal complications.

The 76 case reports/series, published since 1928, reported on a total of 204 intravasations and 27 embolisations, with locations in the lungs, cerebrum and retina. There have been seven deaths described in the case reports/series; one caused by an anaphylactic shock and one caused by an oil-embolism in a woman of 60 years who received an HSG for a different indication than subfertility. The other five deaths were caused by infection after the HSG and/or a subsequent laparotomy. The latest fatal infection occurred in 1950.

**CONCLUSIONS:** HSG with oil-based contrast for tubal testing is a safe procedure, when performed in a modern setting with antibiotic prophylaxis when indicated.

**SUPPORT:** Guerbet, France.

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**NATIONAL TRENDS IN EMBRYO TRANSFER TRAINING.** Dana B. McQueen, M.D., M.A.S., Jared C. Robins, MD, Eve C. Feinberg, M.D. Northwestern University, Chicago, IL.



**OBJECTIVE:** To evaluate national trends in embryo transfer training for Reproductive Endocrinology and Infertility Fellows.

**DESIGN:** Cross Sectional study.

**MATERIALS AND METHODS:** Institutional Review Board approval was obtained. Reproductive Endocrinology and Infertility (REI) Fellowship program directors and fellows were surveyed to assess their experience with live embryo transfers performed by fellows and potential barriers to fellowship training in live embryo transfer.

**RESULTS:** Anonymous surveys were sent to 51 REI fellowship program directors and 142 fellows. Responders included 25 program directors and 47 fellows (10 first-year, 14 second-year and 23 third-year fellows). 35% practiced in the Midwest, 35% in the North East/Mid Atlantic, 18% in the South West/South East and 18% in the West/Northwest. Among all 72 responders, 19% (14/72) reported that no live embryo transfers were performed by fellows in their program, 16% (4/25) of program directors and 21% (10/47) of fellows. 70% (7/10) of first year fellows, 43% (6/14) of second year fellows and 44% (10/23) of third year fellows had performed < 10 live embryo transfers at the time of survey. The median number of live embryo transfers performed during fellowship was 20 (range 0-370, mean 65.1, SD 95). On a scale of 1-10, the program directors' reported level of comfort with fellows performing live embryo transfer was 8.1 in the Midwest, 8.5 in the North East/Mid Atlantic, 6.9 in the South West/South East and 5.9 in the West/Northwest. Barriers to live embryo transfers performed by fellows included: attending physician acceptance (50%, 36/72), perceived patient acceptance (44%, 32/72), physician-patient relationship (42%, 30/72), history of difficult transfer (25% 18/72), perceived fellow skill (21%, 15/72), concerns regarding competition with private practice (18%, 13/72), and lack of simulator training (8%, 6/72). There was no agreement regarding the number of live embryo transfers that should be performed prior to graduation from fellowship, with program directors reporting a range of 0 to 100 (median 25, mode 25) and fellows reporting a range of 0 to 250 (median 30, mode 50).

**CONCLUSIONS:** There are significant differences between fellowship programs regarding the availability of live embryo transfer training, with nearly half of third year fellows reporting < 10 live embryo transfers. Data suggests that embryo transfers performed by fellows have similar live birth rates to embryo transfers performed by attending physicians. However, perceptions among fellowship program directors regarding physician and patient acceptance likely influence experience during fellowship training. Efforts should be made to address these barriers and set minimum standards for number of transfers performed during fellowship.

#### REPRODUCTIVE BIOLOGY: ANIMAL AND EXPERIMENTAL STUDIES

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**HIGH-FAT DIET CAUSES DYSREGULATION OF OVARIAN ENDOTHELIN-2 EXPRESSION ACROSS THE ESTROUS CYCLE.** Natalie M. Hohos, PhD, Emily M. Elliott, BS, Malgorzata E. Skaznik-Wikiel, MD UNIVERSITY OF COLORADO - ANSCHUTZ MEDICAL CAMPUS, Aurora, CO.



**OBJECTIVE:** We have previously shown that high-fat diet (HFD) feeding in female mice leads to abnormal estrous cyclicity, subfertility, and aberrant ovarian expression of genes important in ovulatory function, regardless of obese phenotype<sup>1,2</sup>. We found that a gene critical to normal ovulation, *endothelin-2* (*Edn2*), is significantly downregulated in animals exposed to HFD. *Edn2*'s ovarian expression increases sharply right before ovulation. However, it is unknown how *Edn2* is expressed in the ovary across the estrous cycle and how that expression is impacted by HFD. We aimed to evaluate ovarian *Edn2*

Estrous Stage	<i>Edn2</i> Ovarian Expression		<i>Ece</i> Ovarian Expression	
	HFD Compared to Chow Controls (fold change)	p-value	HFD Compared to Chow Controls (fold change)	p-value
Diestrus	6.2	0.013	6.6	<0.0001
Proestrus	10.9	0.039	4.2	0.0002
Estrus	-6.25	0.26	7.9	<0.0001
Metestrus	-4.76	0.21	6.1	0.0002

expression throughout the estrous cycle in HFD exposed mice and compare it with chow fed controls.

DESIGN: Prospective laboratory animal study.

MATERIALS AND METHODS: 5-week-old C57Bl/6J mice were fed a standard chow or 60% HFD for 10 weeks. Estrous cyclicity was evaluated daily for the last two weeks of feeding and ovaries were collected in each of the four estrous cycle stages (N = 9/group/stage). T-test and chi-square tests were used for statistical analysis, as appropriate.

RESULTS: After 10 weeks of diet, HFD mice weighed more than chow controls (28.8 ± 0.7g, 21.1 ± 0.2g p < 0.0001). HFD mice also had a higher prevalence of abnormal estrous cycles compared to chow controls (58.3% and 21.6% p = 0.0018). In chow controls, *Edn2* was expressed as expected with basal levels during diestrus and proestrus, increased 11.6-fold during estrus, and decreased back to basal levels during metestrus. In the HFD mice, *Edn2* was dysregulated across the entire estrous cycle (table 1), and when *Edn2* expression was examined across all cycle stages in HFD mice, there was no characteristic peak of *Edn2* expression in estrus with the lowest levels of *Edn2* observed. Endothelin converting enzyme (*Ece*, cleaves *Edn2* pre-peptide to active form) transcript expression levels were found to be uniformly upregulated in the HFD exposed mice across all stages of the estrous cycle (table 1).

CONCLUSIONS: Our data suggest that *Edn2* and its post-translational regulation is dysregulated across the estrous cycle in HFD-fed mice. Work is currently underway to examine ovarian protein *Edn2* levels across the estrous cycle to confirm our gene expression data. Future research should investigate mechanisms behind dysregulated *Edn2* expression with HFD feeding. Collectively, this work will allow us to better understand how HFD leads to ovulatory dysfunction and to develop strategies targeting HFD-induced ovulation defects.

Reference: 1) Skaznik-Wikiel et al. Biol Reprod. 2016; 94(5):180. 2) Hosos et al. Mol Cell Endocrinol. 2018; 470:199.

SUPPORT: Colorado NORC Pilot Grant (P30DK048520) to M.ES-W.

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#### REGULATION OF EMBRYONIC DEVELOPMENT BY PLATELET-ACTIVATING FACTOR IS MOST LIKELY VIA THE INTRINSIC APOPTOSIS PATHWAY.

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OBJECTIVE: Platelet-activating factor (PAF) is a potent signaling phospholipid produced by preimplantation embryos and is required for development and implantation. The mechanistic process(es) by which PAF regulates embryo development has not been fully elucidated. In other physiological systems, PAF modulates apoptotic activity, by both inhibition and activation. Apoptosis is initiated via two different cellular pathways: extrinsic and intrinsic. Each pathway leads to programmed cell death but is initiated by different signals (e.g. aneuploidy; DNA damage) and utilizes caspase-8 and caspase-3 activities in different steps. The sea urchin is a time-honored model for investigational studies in developmental biology and is beneficial for understanding similar events for human embryos. Therefore, this study utilized the sea urchin to investigate the effect of PAF on early embryo development including apoptosis activity.

DESIGN: Prospective, randomized controlled experimental laboratory animal study utilizing the sea urchin (*Lytechinus variegatus*) model.

MATERIALS AND METHODS: Two-cell stage sea urchin embryos were cultured (20 embryos/replicate; 6 replicates per treatment group) in synthetic sea water (50µL) and in the presence or absence (control) of 10<sup>-7</sup>M PAF, and

10<sup>-7</sup>M lyso-PAF (biologically inactive form of PAF). Following a 24-hour culture period at 22°C, embryo development was recorded, and apoptotic activity was assessed by monitoring cleavage of 30µM DEVD-AMC and 50µM IETD-AMC peptide substrates of caspase-3 and caspase-8 respectively. Cleavage of the peptide substrates by sea urchin embryonic extracts were determined by detecting absolute fluorescence (absolute fluorescence units; AFU) over time (once/minute; 30 minutes).

RESULTS: The PAF group (57.2%) had significantly (P<0.01) more gastrula stage embryos than the control (11.1%) or lyso-PAF (5.0%) groups. There was a significant difference (P<0.05) in caspase-3 enzyme activity between sea urchin embryos cultured in PAF (17.872 AFU/minute) versus controls (8.764 AFU/minute) and versus lyso-PAF (31.787 AFU/minute). Therefore, PAF treated sea urchin embryos extracts cleaved the caspase-3 specific peptide substrate differently than either control or lyso-PAF treated sea urchin embryos. No significant differences were found between PAF (32.909 AFU/minute), lyso-PAF (32.622 AFU/minute) or control (24.236 AFU/minute) groups regarding caspase-8 enzyme activity. Therefore, exposure of sea urchin embryos to PAF did not yield any effect on caspase-8 activity towards the peptide substrate.

CONCLUSIONS: Exogenous PAF induced advanced stages of development in sea urchin embryos. Sea urchin embryos exposed to PAF affected caspase-3 but not caspase-8 protease activities suggesting a greater involvement of the intrinsic pathway for initiating apoptosis. Thus, PAF's impact on enhanced embryo development may result from intracellular cues to modulate apoptotic activities via the intrinsic pathway. Additional studies will further elucidate the mechanism by which PAF regulates apoptosis during early embryonic development.

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#### OOCYTE SPECIFIC TRANSCRIPTION REGULATORS, NOBOX AND FIGLA, ARE IDENTIFIED AS KEY CONTRIBUTORS TO THE DECLINE IN FECUNDITY ASSOCIATED WITH OVARIAN AGING.

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OBJECTIVE: Advanced maternal age (AMA; ≥35 years) is associated with a decline in fecundity that is largely attributed to loss of oocyte number and quality. The aim of this study was to explore at a molecular level the relationship between aging, ovarian environment and oocyte quality.

DESIGN: Longitudinal research study.

MATERIALS AND METHODS: Young outbred CD1 female mice (3-4 months old; Young) and naturally aged outbred CD1 female mice (10-12 months old; Aged) were super-ovulated and oocytes collected (n = 10 group) for quantitative immunofluorescence of endoplasmic reticulum (ER) stress indicators, pIRE1 and ATF6. Total RNA was isolated from unstimulated ovaries (n = 6 per group), sequence libraries were prepared using the TruSEQ Total RNA library kit (Illumina) and sequenced on the Illumina NovaSeq 6000. Differentially expressed genes (DEGs) were generated using edgeR, with an FDR cutoff of 5% (q value <0.01) and Student's t-test significance at p<0.001, followed by Ingenuity Pathway Analysis (Qiagen).

RESULTS: Oocyte numbers significantly declined with natural aging (mean: Aged = 4.6, Young = 12.9; p<0.0001). Total RNA sequencing revealed 281 significant DEGs in Aged versus Young ovaries (120 increased and 161 decreased; p<0.0001). Unsupervised hierarchical clustering of the 281 DEGs cleanly separated the ovaries according to female age. Enriched pathway analysis revealed signaling pathways including Citrulline-Nitric Oxide Cycle, VEGF Family Ligand Receptor Interactions, and HIF1α signaling. Nitric oxide is a common signaling molecule in these pathways, and has been shown to maintain diplotene arrest in pre-ovulatory oocytes. Aged ovaries displayed a significant decrease in nitric oxide gene expression

( $p < 0.0001$ ) that could lead to premature meiotic resumption. Interestingly, a significant increase in the proportion of immature oocytes was also observed with natural aging (mean: Aged = 79.1%, Young = 24.0%;  $p < 0.0001$ ). Oocyte specific upstream transcription factors, NOBOX and FIGLA, were identified as significantly inhibited in Aged ovaries ( $p < 0.0001$ ). Vital oocyte genes targeted by these regulators include Gdf9 (folliculogenesis), H1foo (germinal vesicle maturation), Rfpl4 (oogenesis) and Zp3 (zona pellucida glycoprotein), all significantly decreased in Aged ovaries ( $P < 0.0001$ ). Additionally, FIGLA plays a role promoting ubiquitin-mediated proteolysis via genes Fbxw21 and Nlrp4f, both significantly decreased in Aged ovaries ( $p < 0.0001$ ). Inhibition of protein degradation results in cellular stress identified by increased intensity of staining and nuclear localization of ER stress indicators, ATF6 ( $p < 0.01$ ) and pIRE1 ( $p < 0.05$ ), in Aged oocytes.

**CONCLUSIONS:** This study revealed that at a molecular level the widespread, damaging impacts of natural aging on ovarian function, including significant transcriptomic and protein expression changes that directly contribute to the decline in oocyte quality and overall fecundity. Ongoing studies are focused on manipulating identified genomic targets with the potential to slow down ovarian aging.

**SUPPORT:** None.

**O-178** Tuesday, October 15, 2019 11:30 AM

#### **SAFE AND EFFICIENT DETECTION OF EGG MATURITY WITHOUT CUMULUS CELL REMOVAL BY NON-INVASIVE TOMOGRAPHY.**

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<sup>a</sup>Colorado Center for Reproductive Medicine, Lone Tree, CO; <sup>b</sup>Animated Dynamics, Inc., Indianapolis, IN; <sup>c</sup>CCRM NY, New York, NY.



**OBJECTIVE:** Currently, eggs must be denuded to assess maturity. However, maintenance of the cumulus cell investment is critical to support oocyte quality during maturation. A novel tomography device using near infra-red light has been developed to detect the first polar body without removing cumulus cells. Our objective was to determine the effect of tomographic imaging for polar body detection on mouse oocyte developmental competence and subsequent embryo quality.

**DESIGN:** Prospective research study. The experimental design consisted of three treatment groups; control eggs (C, standard IVF/IVC protocol;  $n=135$ ), imaged eggs (I;  $n=45$ ), and eggs that were treated identically to I but not exposed to tomography (NI;  $n=63$ ). Two replicates were performed.

**MATERIALS AND METHODS:** In vivo matured mouse (outbred CF1,  $n=8$  females) eggs were collected following ovarian stimulation. A subset of oocytes was randomly selected and immediately placed into fertilization medium containing sperm (C). The remaining eggs were placed into two imaging dishes, consisting of 50  $\mu$ L drops of MOPS buffered medium under oil. Both dishes were moved to a heated stage on the imaging system. Eggs in one dish were imaged (I; 8-10 eggs/microdrops); oocytes in the second dish (NI) served as an environmental control. Presence or absence of the polar body was recorded for each egg. After imaging, both I and NI groups were placed into fertilization medium containing sperm. After IVF, 2PN zygotes were placed into sequential culture medium.

**RESULTS:** It required 93 seconds on average to image each oocyte and determine if it contained a polar body (range, 40-150 sec/oocyte). Tomography evaluation revealed that 78% of the eggs were mature. After IVF, C had fewer ( $p < 0.01$ ) 2PN zygotes than either I or NI (60%, 87%, 76%, respectively). The percentage of mature eggs (78%) was slightly underestimated compared to the percentage of successfully fertilized zygotes (87%). There were no differences in blastocyst development or hatching between treatments (C, 64% and 57%; I, 67% and 69%; NI, 75% and 67%, respectively). There were no differences in ICM or total cell number between treatments, although NI tended ( $p=0.08$ ) to have fewer TE cells than C (NI,  $83.6 \pm 5.3$ ; C,  $100.8 \pm 6.1$ ). The percentage of ICM cells was increased ( $p < 0.05$ ) in I ( $12.3 \pm 1.1\%$ ) compared to C ( $8.9 \pm 0.7\%$ ). The expression of 8/9 genes related to blastocyst viability (BMP15, DNMT3A, FOXO3A, GLUT1, GRP78, NANOG, PASG, PLAC8) did not differ between treatments, although expression of ATF4 was decreased ( $p < 0.05$ ) in I and NI compared to C.

**CONCLUSIONS:** Assessment of cumulus enclosed mouse eggs to determine maturity using near infra-red tomography does not have any negative effects on fertilization, blastocyst development or embryo quality. This data suggests that tomography could be used to safely make clinical decisions about the most appropriate fate of each retrieved egg prior to cumulus removal, thereby improving quality of the oocyte cohort.

**SUPPORT:** Supported by Colorado Center for Reproductive Medicine, and Animated Dynamics, Inc.

**O-179** Tuesday, October 15, 2019 11:45 AM

#### **THE PLASMINOGEN ACTIVATOR SYSTEM IN THE PRIMATE ENDOMETRIUM DURING THE OVARIAN CYCLE AND MENSTRUATION.**

Reem Sabouni, MD,<sup>a</sup> Esra Demirel, MD,<sup>b</sup> David F. Archer, MD.<sup>c</sup> <sup>a</sup>EVMS/ Jones Institute for Reproductive Medicine, Norfolk, VA; <sup>b</sup>North Shore University Hospital & Long Island Jewish Medical Center, Manhasset, NY; <sup>c</sup>Eastern Virginia Medical School, Norfolk, VA.



**OBJECTIVE:** The endometrium undergoes dynamic morphologic changes reflecting hormonal fluctuations. The plasminogen activator system (PAS) is an enzymatic cascade involved in hemostasis and matrix turnover that is activated by tissue plasminogen activator (tPA) and inhibited by plasminogen activator inhibitors-1 (PAI 1). Evidence supports PAS's role in remodeling the endometrium in human endometrial cancers, yet little is known on the dynamic alterations of these enzymes within a controlled ovarian cycle. This study seeks to characterize the expression of PAI 1 and tPA in the primate endometrium during an artificial cycle.

**DESIGN:** Animal in vivo experiment.

**MATERIALS AND METHODS:** Endometrial biopsy samples were obtained from 4 adult cycling female rhesus macaques monkeys during an artificial cycle controlled with estrogen and progesterin implants at 3 separate time points: menstrual, proliferative and secretory. The tissue sections were stained via immunohistochemistry (IHC) with specific PAI 1 and tPA antibodies. Controls using immunofluorescence and IHC were captured with standardized settings with 4 representative images of stroma, gland and vasculature from each tissue. Four separate areas of stroma, gland or vasculature were analyzed from different parts of each slide. Values were expressed as integrated optical density and analyzed using Image-J software. Statistics were performed on means  $\pm$  standard deviation of  $n=4$ /group and subjected to ANOVA with Tukey's multiple comparison test at  $p < 0.05$ .

**RESULTS:** Stromal PAI 1 was highest in the secretory phase ( $184.7 \pm 9.1$ ), then proliferative ( $151 \pm 5.6$ ) and menstrual phase ( $88.4 \pm 18.6$ ) with a statistically significant difference between secretory phase compared to the proliferative and menstrual phases ( $p < 0.0001$ ). Glandular PAI 1 was highest in the secretory phase ( $99.6 \pm 36.9$ ), followed by proliferative ( $48 \pm 9.8$ ) and menstrual phase ( $44.1 \pm 3.1$ ). Vascular PAI 1 was highest in the secretory phase ( $127.7 \pm 23$ ), followed by proliferative ( $89 \pm 16.3$ ) and menstrual phase ( $61.9 \pm 4.7$ ). Statistically significant differences were seen between secretory compared to proliferative and menstrual phases for the gland ( $p < 0.1$ ) and vasculature ( $p < 0.001$ ).

Stromal tPA was highest in the secretory phase ( $181 \pm 17.6$ ), then proliferative ( $169.6 \pm 10.7$ ) and menstrual phase ( $145 \pm 33$ ). Glandular tPA was highest in the secretory phase ( $123 \pm 20.7$ ), then menstrual ( $113 \pm 29$ ) and proliferative phases ( $104 \pm 11.7$ ). Vascular tPA was highest in the secretory phase ( $119.9 \pm 26.6$ ), followed by menstrual ( $109 \pm 29$ ) and proliferative phase ( $94.5 \pm 6.6$ ). The differences between the menstrual phases for tPA were not statistically significant.

**CONCLUSIONS:** PAI 1 was noted to be significantly expressed in the secretory phase in the stroma, gland and vasculature supporting its possible role in the endometrial decidualization. High PAI 1 levels in the secretory stroma may reflect protease activity with the onset of menses. Comparing expression of PAI 1 and tPA demonstrates that PAI 1 appears to have a more dynamic expression within the monkey endometrium suggesting a larger role in endometrial remodeling.

**O-180** Tuesday, October 15, 2019 12:00 PM

#### **THE ROLE OF GLYPHOSATE IN INFERTILITY: THE MECHANISTIC LINK.**

Charalampos Chatzicharalampous, MD, PhD, Zeina A. Yahfoufi, BS, David Bai, BS, Awoniyi Olumide Awonuga, MD, Husam Abu-Soud, PhD Wayne State University, Detroit, MI.



**OBJECTIVE:** In light of the recent Roundup lawsuit, glyphosate has been widely accepted as a significant environmental toxin that may affect humans in various ways including cancer and infertility. Exposure to even low doses of glyphosate-based herbicides during pregnancy has been found to impair fertility, cause intrauterine growth restriction and induce fetal malformations. In this study

we sought to determine the underlying mechanism by which glyphosate negatively impacts oocyte quality, fertilization rates as well as embryo development.

**DESIGN:** Experimental case-control study of mouse oocytes and pronuclear embryos, exposed *in vitro* to increasing glyphosate concentrations and followed through day 5 of development. We utilized multiple assays including reactive oxygen species (ROS) generation and zinc depletion to examine the possible underlying detrimental mechanisms.

**MATERIALS AND METHODS:** Metaphase II mouse oocytes (n=200) were retrieved from 8-10 week female mice and a subset (n=100) were fertilized using IVF. The oocytes as well as the fertilized mouse embryos were then exposed to increasing concentrations of glyphosate (0-200  $\mu$ M) for 2h - 4h as per protocol. The oocytes were divided into four groups that were treated as follows: Group A: ROS detection assay, Group B: Zinquin ethyl ester assay, Group C: fixed, stained and scored based on the spindle structure (microtubule morphology -MT and chromosomal alignment - CH) as indicators of the oocyte's capacity to sustain exposure. All groups were compared to Group 4: untreated controls.

Exposed embryos were incubated for up to 120 hours post fertilization and evaluated for full and hatching blast rate conversion. They were photographed and graded daily based on their appearance and development using published embryo grading protocols. A subset of the treated embryos (n = 10 for each concentration) were treated, in a similar fashion as the oocytes, in order to evaluate for ROS overproduction and zinc depletion. Confocal microscopy was used to assess the embryos. Statistical analysis was performed using t-test, ANOVA and chi-square. A p-value < 0.05 was considered statistically significant.

**RESULTS:** Oocytes treated with increasing glyphosate concentrations > 50  $\mu$ M were found to have poor scores for MT and CH and that difference was statistically significant as compared to controls (p< 0.05). Embryos followed to 96 hours post fertilization (early blastocyst) and 120 hours (full and hatching blastocyst) after glyphosate exposure (0-200  $\mu$ M) were assessed and those exposed to glyphosate concentrations > 100  $\mu$ M showed significantly increased arrest rates and poor morphology scores compared to controls. ROS overproduction as well as zinc depletion was evident in embryos treated with high glyphosate concentrations. These observations were statistically significant compared to untreated controls (p<0.05).

**CONCLUSIONS:** This work suggests the possible underlying mechanisms by which glyphosate negatively affects reproductive health in the mouse model. Possible fertility implications in humans will require further research.

**SUPPORT:** None.

## ART LAB: TECHNOLOGY

O-181 Wednesday, October 16, 2019 10:45 AM

### SHORTER TELOMERE LENGTH OF WHITE BLOOD CELLS IS ASSOCIATED WITH HIGHER RATES OF ANEUPLOIDY IN WOMEN UNDERGOING IN VITRO FERTILIZATION.

Brent M. Hanson, MD,<sup>a</sup> Xin Tao, Ph.D,<sup>b</sup> Yiping Zhan, Ph.D,<sup>c</sup> Julia G. Kim, MD, MPH,<sup>a</sup> Emily K. Osman, MD,<sup>a</sup> Ashley W. Tiegs, MD,<sup>a</sup> Shelby A. Neal, MD,<sup>a</sup> Richard Thomas Scott, Jr., MD,<sup>a</sup> Emre Selhi, M.D.<sup>a</sup> <sup>a</sup>IVI-RMA New Jersey, Basking Ridge, NJ; <sup>b</sup>The Foundation for Embryonic Competence, Basking Ridge, NJ; <sup>c</sup>Foundation for Embryonic Competence, Basking Ridge, NJ.



**OBJECTIVE:** Telomeres are tandem repeats of the sequence TTAGGG located at the ends of chromosomes. Telomere shortening is a key mechanism of cell senescence and aging. Telomere shortening has been associated with decreased oocyte quality through disruption of chromosome alignment and spindle structure. In this study, we sought to evaluate whether the telomere length of cumulus cells (CC) or white blood cells (WBC) in an infertile population is associated with reproductive aging.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Women undergoing IVF between July 2017 and December 2018 were recruited for the study under Institutional Review Board approval. Blood and CC were collected at the time of oocyte retrieval. Genomic DNA was isolated and stored at -80°C. Telomere DNA length was measured by quantitative real-time PCR and normalized to AluYa5 sequence as an endogenous control for each sample. Linear regression was applied to determine if telomere length (TL) of WBC or CC was associated with patient age, number of oocytes retrieved, number of mature (M2) oocytes retrieved, blastulation rate, aneuploidy rate, serum anti-mulle-

rian hormone (AMH), and serum estrogen (E2) level on day of hCG or GnRH $\alpha$  administration.

**RESULTS:** TL data was available for WBC samples from 156 individuals and for CC samples from 142 patients. Data was available for both tissue types in 139 patients (age range 25.7 to 45.0 years, mean 35.0  $\pm$  4.0 years). As expected, WBC telomere length declined with increasing age (p=0.006). In contrast, CC TL was equivalent in patients of all ages and failed to show anticipated age-related shortening. As such, CC TL was unrelated to any index of ovarian performance including number of oocytes retrieved (p=0.95), M2 oocytes retrieved (p=0.81), blastulation rate (p=0.98), aneuploidy rate (p=0.30), AMH level (p=0.32), or mid-cycle E2 level (p=0.77). Consistent with these data, CC TL was not associated with patient age (p=0.99). While WBC TL declined with increasing maternal age, it was a poor predictor of quantitative ovarian performance [total oocytes retrieved (p=0.47), number of M2 oocytes retrieved (p=0.30), AMH level (p=0.13), E2 level (p=0.36), and blastulation rate (p=0.48)]. However, TL of WBC samples was associated with embryonic competence as evidenced by aneuploidy rate (p=0.02), with shorter TL associated with higher aneuploidy.

**CONCLUSIONS:** The TL of CC was not associated with patient age or any index of ovarian or embryonic performance. Declining WBC TL was associated with increasing maternal age and increasing rates of embryonic aneuploidy. Further studies are necessary to determine if changes in peripheral somatic cell TL are truly prognostic for aneuploidy rate within a given maternal age group or if this finding is simply reflective of a simultaneous change which occurs with age.

**Reference:** None.

**SUPPORT:** None.

O-182 Wednesday, October 16, 2019 11:00 AM

### NON-INVASIVE OOCYTE SELECTION INCREASES CLINICAL PREGNANCY RATE: A PROSPECTIVE STUDY OF 108 PATIENTS.

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**OBJECTIVE:** To compare clinical pregnancy outcome by non-invasive cumulus testing using gene expression in combination with embryo morphology versus morphology alone in eSET patients.

**DESIGN:** A prospective blinded interventional clinical trial.

**MATERIALS AND METHODS:** Planned fresh transfers of day-3 ICSI eSET. Patients were stimulated with GnRH antagonist and HP-HMG. Oocytes underwent single denudation after pick-up. The cumulus cells were analysed with QRT-PCR for three predictive genes CAMK1D, EFNB2 and SASH1 (Corona Test) and two control genes. The analysis resulted in a single score for each oocyte. The score was used to select and transfer a single day 3 embryo with excellent or good morphology. The control group was matched (blinded for outcome) under the same conditions as the intervention group (same age, same number of embryos, same stimulation protocol).

The primary outcome was clinical pregnancy (fetal heartbeat confirmed by endovaginal ultrasound at 7 weeks), with stratification for age and number of excellent/good quality embryos (GQE). Secondary outcome included cumulative pregnancies from frozen embryo transfers. Outcomes were compared among treatment arms using one-tailed chi-square test.

**RESULTS:** A total of 108 patients underwent the Corona Test and were matched with 108 control patients nearest in time to the treated cases. There was an 80% increase in clinical pregnancy rate on a day 3 eSET (61% Corona Test group vs 34% control group). The cumulative pregnancy rate in the Corona Test group was 79% and 50% in the control group. In our center, outcome for the same patient population with day-5 blastocyst transfer is 50% with eSET and 71% cumulatively.

**CONCLUSIONS:** Using Corona Test as a non-invasive test to select a day 3 embryo could almost double the clinical pregnancy rates on day 3 and shows an increase compared to a day 5 blastocyst transfer. These data indicate that morphology selection supported by non-invasive cumulus testing can drastically increase pregnancy rates.

	Corona Test group	Control group	Chi-square (p-value)
Overall clinical pregnancy (n=108) (%)	61	34	<0,0001
Overall cum. clinical pregnancy (n=108) (%)	79	50	<0,0001
Clin. preg. Age <35 (n=65) (%)	62	35	0,0014
Clin. preg. Age [35-38] (n=43) (%)	60	33	0,0047

SUPPORT: This study was funded by IWT/VLAIO and Vrije Universiteit Brussel IOFPOC26.

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### mtDNA CONTENT IS NOT ASSOCIATED WITH EMBRYONIC REPRODUCTIVE COMPETENCE.



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**OBJECTIVE:** Cytoplasmic maturation, fertilization, embryogenesis and placentation all rely on sufficient energy production and therefore mitochondrial biogenesis. Reactive oxygen species which are potentially harmful to the developing embryo are a side-product of the mitochondrial oxidative phosphorylation integral to energy production. This study's objective was the assessment of predictive value of mitochondrial DNA (mtDNA) copy number in the context of reproductive potential in the human blastocyst.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** mtDNA content in 700 euploid human embryos following single embryo transfer were used to assess this question, determining if the implantation outcome is associated with mtDNA copy number. Additionally, 78 euploid paired sibling cycles (two consecutive transfer cycles performed on the same patient, using euploid embryos from the same cohort, where the first cycle failed to result in implantation and the second cycle resulted in sustained implantation) were included to assess whether or not within a given cohort the mtDNA content was predictive. Relative mtDNA copy number was determined using targeted amplification followed by quantitative real-time PCR (qPCR) for 2 mitochondrial loci (16S and MajArc) relative to a multicopy nuclear genome locus (AluYb8). A logistic regression model was used to determine whether mtDNA content was associated with the odds of achieving pregnancy. Covariates were maternal age and day of biopsy. A ROC curve was created to determine if there were threshold values above which there was a meaningful change in clinical outcomes. Finally, a paired analysis was done to determine if the pregnancies which occurred in the second transfer cycle after a failed first euploid transfer were more likely to have lower mitochondrial concentrations.

**RESULTS:** The range of maternal age was 21.8-45.3, and the sustained implantation rate at 9th gestational week was 65.3%. mtDNA copy number was not associated with sustained implantation rates ( $p=0.74$ ), and there was no threshold value above or below which ongoing implantation was more or less likely. There was also no correlation between mtDNA copy number and maternal age ( $p=0.45$ ). In addition, in women who underwent a second single embryo transfer following a failed transfer ( $n=39$ ), there was no association between relative mtDNA levels of sibling embryos in the 2<sup>nd</sup> transfer relative to the 1<sup>st</sup> transfer and ensuing implantation and delivery rates ( $p=0.67$ ).

**CONCLUSIONS:** Neither the 700 euploid single embryo transfers nor the set of 78 paired transfers suggest that mtDNA copy number analysis is a predictive biomarker of euploid human embryo reproductive competence. In addition, no relationship between blastocyst mtDNA copy number and female age was identified. These data do not support the clinical utilization of mtDNA copy number in clinical decision making when selecting which embryo to transfer.

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### IS THERE ANY ROOM TO IMPROVE EMBRYO SELECTION? ARTIFICIAL INTELLIGENCE TECHNOLOGY APPLIED FOR LIVE BIRTH PREDICTION ON BLASTOCYSTS.



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Marco Toschi, MSc,<sup>d</sup> Raquel Del Gallego, PhD,<sup>c</sup> Jose Celso Rocha, PhD.<sup>e</sup> <sup>a</sup>IVIRMA Global, Valencia, Spain, Tel Aviv, Israel; <sup>b</sup>Imperial College London, London, United Kingdom; <sup>c</sup>IVIRMA Global, Valencia, Spain; <sup>d</sup>IV-IRMA, Rome, Italy; <sup>e</sup>State University of São Paulo Júlio de Mesquita Filho, Assis, Brazil.

**OBJECTIVE:** To apply Artificial Intelligence (AI) technology on time-lapse (TLM) embryo images and morphokinetic parameters to predict live birth.

**DESIGN:** The morphokinetic parameters ( $n=131$ , ICSI only), with known live birth data from single blastocyst transfers, and 131 TLM images of embryos at 111.5 hours post ICSI were used to train (70%), validate (15%), and blindly test (15%) for prediction of live birth by an AI feature-extraction system. Inclusion criteria involved recipients from our oocyte donation program with single blastocyst transfer and non-PGT.

**MATERIALS AND METHODS:** Absolute and interim cleavage time points (t2 to t8) were used, along with 33 independent numerical variables extracted from standardized TLM images as an input data. The artificial neural network (ANN) architecture associated with the genetic algorithm was used to produce a predictable output of live birth. The efficacy of prediction of live birth was quantified and assessed using ROC curves, AUC and confusion matrices (True Positive -TP, True Negative -TN, False Positive -FP, and False Negative-FN).

**RESULTS:** Overall accuracy of prediction of live birth by AI using morphokinetic data was 96.2% (126/131; TP= 37, TN= 69, FP= 1, FN= 4, AUC= 0.946). In the training dataset, the accuracy was 95.5% (86/91, AUC 0.96), and in the blind test dataset, accuracy was 100% (20/20, AUC=0.961). The overall accuracy of prediction of live birth by AI using image analysis was 90.1% (100/111, TP=39, TN= 61, FP= 7, FN= 4, AUC= 0.91). In the training dataset, the accuracy was 89% (81/91, AUC 0.887), and in the blind test dataset, accuracy was 95% (19/20, AUC=0.67-0.94). The combination of morphology and morphokinetics, the AUC for positive were similar (0.96) but for negative live birth were less predictive (0.65).

**CONCLUSIONS:** This is the first time that AI is used to evaluate human embryo quality using morphokinetic and morphological assessment in a data set of single embryo transfers from an oocyte donation program with known live birth. Our data suggests that AI can be used to enhance the efficacy of embryo selection performed by the standard morphology or the existing algorithms of morphokinetics. Applying AI in conjunction with morphokinetic or image analysis has the potential for being the platform of embryo selection, with similar predictive abilities when treated independently although its combination may not improve the performance of AI.

SUPPORT: Agencia Valenciana de Innovació, Generalitat Valenciana.

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### A DEEP LEARNING FRAMEWORK OUTPERFORMS EMBRYOLOGISTS IN SELECTING DAY 5 EUPLOID BLASTOCYSTS WITH THE HIGHEST IMPLANTATION POTENTIAL.



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**OBJECTIVE:** To evaluate the performance of an artificial intelligence-based approach, using a deep convolutional neural network (CNN) combined with a genetic algorithm (GA), in selecting top quality day 5 euploid blastocysts compared to those selected by highly trained embryologists.

**DESIGN:** Historical Prospective Double-Blinded Multi-Center Cohort Study.

**MATERIALS AND METHODS:** Using a dataset of 3,469 embryos, the deep CNN model was trained and tested to primarily classify images of embryos captured at 113 hours post insemination (hpi). A non-overlapping set of 97 euploid embryo images with known implantation outcomes was then used to compare the embryo predicting accuracy of 15 highly trained embryologists from multiple centers in the US to that of the CNN. Only euploid embryos that had undergone preimplantation genetic testing for aneuploidies (PGT-A) were included to remove the bias introduced by chromosomal abnormalities.

**RESULTS:** The CNN performed with an accuracy of 75.3% while the embryologists performed with an average accuracy of 67.4% (min-max: 64.5%-70.2%) in differentiating euploid embryos based on their implantation outcome. The CNN performed with a sensitivity and specificity of 84.2% (CI: 72.1% to 92.5%) and 62.5% (CI: 45.8% to 77.3%), respectively. The positive predictive value (PPV) and negative predictive value (NPV) of the network were 76.2% (63.8% to 86.0%) and 73.5% (55.6% to 87.1%), respectively. A one sample t-test revealed that the CNN significantly outperformed embryologists in predicting embryo implantation of euploid embryos using a static image obtained at a single time-point (113 hpi) ( $P < 0.0001$ ).

**CONCLUSIONS:** The trained artificial intelligence framework outperformed trained embryologists in identifying PGT-A euploid embryos destined to implant. A large randomized controlled trial is warranted to confirm that the developed CNN can improve in-vitro fertilization outcomes by prospectively selecting embryos with higher implantation potential than those selected with the current methods.

**SUPPORT:** This work was partially supported by the Brigham Precision Medicine Developmental Award (Brigham Precision Medicine Program, Brigham and Women's Hospital) and 1R01AI118502, R01AI138800, and R21HD092828 (National Institute of Health).

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#### THE ASSOCIATION BETWEEN RAPIDLY DIVIDING EMBRYOS AND EMBRYONIC EUPLOIDY DETECTED VIA NEXT GENERATION SEQUENCING (NGS).

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**OBJECTIVE:** Previous research has suggested that rapid embryo development may be a strong predictor of outcomes [1]. Rapid cell division of the early embryo was thought to be "chaotic," and cleavage stage embryos with > 8 cells thought to have poor developmental potential. However, others have found that early cleavage embryos have higher implantation rates [2]. Studies evaluating the relationship between cleavage development and embryonic aneuploidy [3] are limited by use of older technologies. Thus, our goal was to assess whether rapid cell division of an early embryo is correlated with copy number variation and embryonic competence.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The study included patients at a single academic center who underwent in vitro fertilization and had at least one embryo that reached cleavage stage from 2016 to 2019. Day 3 embryos were divided into 3 groups: slow (< 6 cells), intermediate (6-8 cells), and fast (>8 cells). Our primary outcome was euploidy as diagnosed by trophectoderm (TE) biopsy for preimplantation genetic testing for aneuploidy (PGT-A). All tested embryos were evaluated using NGS. Secondary outcomes included number of blastocysts, biopsied blastocysts, ongoing pregnancy/live births (OP/LB), and clinical losses (CL). Data were analyzed using students ANOVA, chi square tests, and a multivariate logistic regression, with  $p < 0.05$  considered significant.

**RESULTS:** A total of 40,916 Day 3 embryos from 3,565 patients were assessed in the study. In our unadjusted analysis, there were significant differences between slow ( $n=5,651$ ), intermediate ( $n=23,907$ ), and fast ( $n=11,358$ ) Day 3 embryos that developed to blastocysts (30.30%; 77.50%; 80.08%,  $p < 0.0001$ ) and that were biopsied (9.68%; 46.89%; 52.22%,  $p < 0.001$ ). Euploidy was similar among groups (47.71%; 49.07%; 50.76,  $p = 0.07$ ). A sub-analysis of intermediate vs fast embryos showed a higher rate of euploidy in the fast group ( $p=0.04$ ). After adjusting for confounders, and using the intermediate group as a control, fast Day 3 embryos were significantly associated with increased odds of reaching blastocyst stage (OR 1.13, CI 1.06-1.20,  $p = 0.0001$ ) and having blastocysts that were eligible for TE biopsy (OR 1.18, CI 1.12-1.24,  $p < 0.0001$ ). After controlling for confounders, we found no association between fast growing Day 3 embryos and odds of euploidy (OR 1.04, CI 0.97-1.11,  $p = 0.23$ ). There was no association

between fast growing Day 3 embryos and odds of OP/LB (OR 0.97, CI 0.82-1.14,  $p = 0.69$ ) or CL (OR 1.02, CI 0.77-1.35,  $p = 0.91$ ).

**CONCLUSIONS:** Rapidly dividing cleavage embryos perform as well as, if not better than, intermediate or slow growing cleavage embryos. Prior studies of rapidly dividing embryos may have witnessed embryo/endometrial dyssynchrony and not necessarily implantation failure related to embryonic competence. Our study demonstrated that rapidly dividing embryos have high rates of euploidy and clinical potential. Morphokinetic measurements combined with genomic and non-genomic markers provide the ideal support to optimize embryo selection and improve patient outcomes.

**References:** 1. Bos-Mikich, A., A.L. Mattos, and A.N. Ferrari, *Early cleavage of human embryos: an effective method for predicting successful IVF/ICSI outcome*. Hum Reprod, 2001. **16**(12): p. 2658-61.

2. Lee, M.J., et al., *Cleavage speed and implantation potential of early-cleavage embryos in IVF or ICSI cycles*. J Assist Reprod Genet, 2012. **29**(8): p. 745-50.

3. Luna, M., et al., *Human blastocyst morphological quality is significantly improved in embryos classified as fast on day 3 (>or=10 cells), bringing into question current embryological dogma*. Fertil Steril, 2008. **89**(2): p. 358-63.

**SUPPORT:** None.

## ENDOMETRIOSIS

**O-187** Wednesday, October 16, 2019 10:45 AM

#### CORRELATION BETWEEN FOLLICULAR LEVELS OF INTERLEUKIN 6 (IL-6) AND ANTI-MULLERIAN HORMONE (AMH) AND ICSI OUTCOME IN WOMEN WITH PROVEN ENDOMETRIOSIS.

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**OBJECTIVE:** Investigating a potential correlation between follicular AMH and IL-6 in women with endometriosis and thus a potential influence of the inflammatory process in endometriosis on ICSI outcome.

**DESIGN:** A matched case-control study was conducted in the Reproductive Medicine center at Aziza Othmana Hospital in Tunis. The study population included a total of seventy-five patients; twenty-five patients with proven endometriosis and fifty patients diagnosed with other causes of infertility, each undergoing an ICSI cycle between March and August 2018.

**MATERIALS AND METHODS:** All patients followed a controlled ovarian stimulation protocol for an ICSI cycle. The follicular fluid was collected from 75 patients at the time of oocyte retrieval, and then stored at -80°C until assay. AMH and IL6 concentrations in follicular fluid were determined by electrochemiluminescence immunoassay. Comparisons of data between the two groups were performed with t student test and with chi 2 test. Correlations were assessed with the Pearson correlation test.

**RESULTS:** Two groups were formed; an endometriosis group and a control group. Both groups were comparable regarding clinical parameters and those of the ovarian stimulation. As for the biological parameters measured in the follicular fluid, IL-6 levels showed a statistically significant increase in the "endometriosis" group compared to the "control" one (162.32 vs 19.93;  $p=0.02$ ). The follicular AMH levels were comparable between the two groups (2.22 vs 2.71;  $p=0.41$ ). No correlation was shown between the follicular levels of IL6 and AMH ( $r = 0.01$ ,  $p = 0.3$ ). The comparison of ICSI outcomes between the "endometriosis" group and the "control" group showed that the fertilization rate (69.90% vs 62.98% ;  $p > 0.05$ ), the Top embryo rate (41.71% vs 37.64%;  $p > 0.05$ ) and the pregnancy / transfer rate (38.09% vs 34%;  $p > 0.05$ ) were comparable between the two groups. The miscarriage rate was higher in women with endometriosis (37.5% vs 18.75%;  $p > 0.05$ ).

**CONCLUSIONS:** The higher miscarriage rate in women with endometriosis suggests that the endometrial receptivity is the target of the deleterious effect of the inflammatory process caused by endometriosis rather than by the ovarian function or the oocyte quality. Further investigations are needed to confirm such a theory.

**SUPPORT:** This study was funded by the research department of Aziza Othmana Hospital in Tunis.

### PLATELETS DRIVE FIBROGENESIS THROUGH INDUCTION OF ENDOTHELIAL-MESEMCHYMAL TRANSITION (ENDOMT) IN ENDOMETRIOSIS.

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**OBJECTIVE:** One conundrum in endometriosis arising from a recent study is that, while endometriotic epithelial and stromal cells supposedly co-exist, the two cellular components seem to take independent developmental trajectories. This is due to the finding that, while cancer-associated somatic mutations were found to be enriched in the epithelial component, the stroma does not carry much. Given that endometriotic lesions are fundamentally wounds undergoing repeated tissue injury and repair that ultimately progress to fibrosis, we hypothesized that the stromal component of endometriotic lesions may recruit other cells and turn them into mesenchymal cells. One possible candidate cell is endothelial cell. Essentially, endothelial cells in lesions transdifferentiate into mesenchymal cells, likely induced by transforming growth factor  $\beta 1$  (TGF- $\beta 1$ ) released by activated platelets, contributing further to lesional fibrosis. This study was undertaken to test this hypothesis.

**DESIGN:** Laboratory study using human tissues, in vitro experimentation using an human umbilical vein endothelial cell line HUVEC.

**MATERIALS AND METHODS:** Immunofluorescent analysis of 30 each ovarian endometrioma (OE) and deep endometriosis (DE) tissue samples, using antibodies against CD31 and fibroblast-specific protein 1 (FSP-1), was performed. Immunohistochemistry analysis of CD31, FSP-1 and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) was also performed. Masson trichrome staining was used to evaluate the extent of lesional fibrosis. In vitro experiments, we evaluated morphological changes, gene and protein expression levels, migratory and invasive propensity, cellular contractility, and collagen production for HUVEC co-cultured with vehicle, activated platelets or thrombin only. We used A83-01, a TGF- $\beta 1$  inhibitor, to neutralize TGF- $\beta 1$ .

**RESULTS:** Endometriotic lesions clearly exhibited signs consistent with EndoMT, especially in OE lesions. Activated platelets, through the induction of TGF- $\beta 1$  signaling pathway, induced EndoMT in HUVECs, resulting in increased migratory and invasive propensity, cellular contractility, and collagen production. Prolonged exposure of HUVECs to activated platelets induced increased expression of  $\alpha$ -SMA, desmin and F-actin suggesting further transdifferentiation into smooth muscle-like cells. Neutralization of TGF- $\beta 1$  abolished these changes. OE lesions had significantly higher staining levels of CD31, but lower  $\alpha$ -SMA and FSP-1 staining, concomitant with lower lesional fibromuscular content than that of DE lesions. The staining levels of CD31 correlated negatively with the staining levels of  $\alpha$ -SMA, as well as the extent of lesional fibrosis.

**CONCLUSIONS:** EndoMT contributes to fibrogenesis in endometriosis. Because of EndoMT, the endometriotic stroma is constantly replenished by endothelial cells and other cells. These cells generally have much lower mutation rates than that of the endometriotic epithelium. Thus, we provide an explanation for the above mentioned conundrum of apparent independent developmental trajectories taken by endometriotic epithelium and stroma.

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### SOMATIC CANCER DRIVER MUTATIONS IN ENDOMETRIOSIS LESIONS CONTRIBUTE TO SECONDARY CANCER RISK.

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**OBJECTIVE:** To determine whether cancer driver mutations contribute to the development and progression of endometriosis and endometriosis associated cancers.

**DESIGN:** Endometriosis lesions might arise as an autotransplant, as a hamartoma, through metaplasia, or as a neoplasm. Some endometriosis lesions are progressive, invasive, and possibly metastatic, and cancers sometimes arise in endometriosis lesions. Recent studies have shown that somatic mutations accumulate during the clonal evolution of individual endometriosis lesions. We conducted whole exome sequencing to investigate the presence

of known cancer driver mutations in endometriosis lesions and to correlate these mutations with long term outcomes.

**MATERIALS AND METHODS:** 276 women (age 12 to 95) operated on at OHSU between 2003 and 2014 with a confirmed histologic diagnosis of endometriosis were considered. Exome sequencing was performed on DNA extracted from formalin-fixed paraffin-embedded tissue samples exhibiting endometriosis histology to varying degrees. Within a 5 to 16 year follow-up interval, 55/276 (20%) of these women had a subsequent diagnosis of cancer at OHSU.

Whole exome sequencing (WES) was performed using Ion Proton Instrument with the AmpliSeq Exome Capture Kit. All missense, truncating (stopgain, stoploss, splicing and frameshifts), and synonymous variants listed in the IntOGen database were considered (20,302 TIER1 cancer driver mutations). Tier 1 cancer driver genes have epidemiologic, mutational and functional evidence to support their role in oncogenic transformation.

**RESULTS:** 113 Tier 1 cancer driver mutations (4 splicing, 15 stopgain and 94 missense) were seen in tissue from 66 women. 24% of the 276 surgical samples show at least one cancer driver mutation; 7.3% carried at least 2 cancer driver mutations, a single sample was observed to have 9 cancer driver mutations, and one sample had multiple deletions (runs of homozygosity) including a hemizygous driver mutation. The TP53 gene had the highest rate of cancer driver mutations with 5 mutations detected. 14.7% of the women without a detected driver mutation had a diagnosed cancer during the follow-up interval while 24 of the 66 (36%) women with endometriosis lesions harboring a cancer driver mutation developed a cancer during the follow-up interval [(p=0.0003) odds ratio=3.3 (95% confidence limits 1.8-6.2)]. The majority of the cancers developing in these 18 women were cancers known to be associated with endometriosis. Of note, the mean age of endometriosis diagnosis for the 18 women with a somatic driver mutation who developed cancer was 53.2, and the age at diagnosis was 36.3 for those with no cancer to date (Wilcox p=0.00001).

**CONCLUSIONS:** Somatic cancer driver mutations are common in endometriosis lesions. When a cancer driver mutation is present in an endometriosis lesion, the risk of a secondary cancer appears to be elevated.

**SUPPORT:** Juneau Biosciences, LLC.

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### CYTOSKELETAL AND EXTRA CELLULAR MATRIX GENES ARE KEY CONTRIBUTORS IN THE PATHOGENESIS OF ENDOMETRIOSIS.

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**OBJECTIVE:** Our recent exome sequencing study of 2,668 Caucasian women with surgically diagnosed endometriosis identified 1,113 low-frequency exome variants in 925 genes that showed association with endometriosis (MAF<0.01, p<0.05). To elucidate the pathogenic process underlying endometriosis, we here seek to determine if these genes share any biologic pathways.

**DESIGN:** In the present study we used gene ontology (GO) enrichment analysis to determine if the genes identified in our rare exome variant study show structural or molecular enrichment.

**MATERIALS AND METHODS:** Gene symbols for the 925 genes were imported into WebGestalt (<http://www.webgestalt.org>) and analyzed for enrichment. Significantly enriched terms were identified using a hypergeometric test with Benjamini-Hochberg (BH) correction, and False Discovery Rate (FDR) significance of p<0.05. STRING (<https://string-db.org>) was used to assess enrichment of Protein-Protein interactions (PPI).

**RESULTS:** 914 of the 925 implicated genes had GO annotations in WebGestalt. The most significantly enriched cellular components are shown in Table 1. PPI was also significantly enriched with 3388 observed interactions compared with 3009 expected interactions (p=6.32E-12).

**CONCLUSIONS:** The results show that the genetically associated variants are enriched for proteins in the membrane, cytoskeleton, and extra cellular matrix, and that they show significantly elevated PPI. We have previously proposed that common endometriosis variants identified by GWAS implicate cytoskeletal regulation in the pathogenesis of endometriosis, and that epithelial-to-mesenchymal transition (EMT) govern mesothelial barrier homeostasis and integrity during wound healing (Albertsen et al., *Reprod. Sci.*, 2017, 24(6):803-811). Here we show further support for this hypothesis by showing that low-frequency exome variants cluster in the same cellular compartments and pathways. Endometriosis is recognized as an estrogen-dependent

TABLE 1.

Gene Set	Description	Gene Set Size	Expected Count	Observed Count	Fold Enrichment	P Value	FDR
GO:0005887	Integral plasma membrane	1596	65.6	126	1.92	3.58E-13	2.75E-10
GO:0044430	Cytoskeletal part	1620	66.6	127	1.91	4.68E-13	2.75E-10
GO:0009986	Cell surface	782	32.1	67	2.08	9.24E-09	1.36E-06
GO:0098590	Plasma membrane region	1175	48.3	86	1.78	1.08E-07	0
GO:0031012	Extracellular matrix	496	20.4	43	2.11	3.39E-06	0.0002
GO:0030054	Cell junction	1268	52.1	83	1.59	0	0.0006

inflammatory disease, but genetic evidence suggest that the initiation of endometriosis is linked to the structural features of the cell. This non-hormonal pathogenic model suggest that it may be possible to *prevent* endometriosis by inhibiting EMT and by stabilizing mesothelial barrier integrity.

SUPPORT: Juneau Biosciences, LLC.

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### ENDOMETRIOSIS DOES NOT IMPACT LIVE BIRTH RATES IN FROZEN EMBRYO TRANSFER (FET) OF EUPLOID BLASTOCYSTS.

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**OBJECTIVE:** One explanation proposed for subfertility among women with endometriosis is impaired endometrial receptivity. We sought to test this hypothesis by comparing pregnancy and live birth (LB) outcomes in women with endometriosis versus two control groups without suspected endometrial factor: (1) non-infertile patients who underwent ART in order to test embryos for a single gene disorder and (2) couples with isolated male factor infertility.

**DESIGN:** Retrospective Cohort.

**MATERIALS AND METHODS:** FETs of PGT-A normal blastocysts performed from January 2016 through March 2018 were included in the analysis. Patients with endometriosis were compared to those with male factor infertility and non-infertile patients using PGT-M for a single gene disorder. Endometriosis was confirmed surgically in 90% of the endometriosis cohort. Patients with multiple infertility diagnoses and those using gestational carriers or donor oocyte were excluded from the analysis. All blastocysts vitrified and warmed for transfer were grade BB or better. Comparisons were made with multigroup chi-square and  $P < 0.05$  was considered statistically significant.

**RESULTS:** 472 euploid FET cycles were available for analysis. 59 transfers occurred in patients with endometriosis, 362 transfers in patients with male factor infertility, and 51 transfers in non-infertile patients. There was no difference in patient age in each treatment group and age was not associated with live birth in euploid embryo transfers. An equal number of embryos were transferred in each group. Patients with endometriosis had similar rates of clinical pregnancy (CP) and spontaneous abortion (SAB) when compared to male factor and non-infertile patients. There was no difference in LB in patients with endometriosis (63%) compared to patients with male factor

	Endometriosis (n = 59)	Male Factor (n = 362)	PGT-M (n = 51)	P value
No. Embryos Transferred	1.05	1.09	1.06	0.97
Positive hCG	80%	74%	80%	0.44
CP	73%	65%	63%	0.43
SAB	10%	14%	10%	0.63
LB	63%	53%	51%	0.34

(51%) and non-infertile patients (53%). While the study was only powered to detect a 20% decrease in LB, the 63% LB rate in endometriosis patients did not suggest a negative effect.

**CONCLUSIONS:** Whether endometriosis primarily affects IVF outcomes via oocyte quality or the endometrium is debated. By controlling for embryo quality using euploid FET cycles, we found no difference in pregnancy outcomes in patients with endometriosis compared to those with male factor and non-infertile patients.

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### NON-INVASIVE DIAGNOSIS OF ENDOMETRIOSIS: USING MACHINE LEARNING INSTEAD OF THE OPERATING ROOM.

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**OBJECTIVE:** Endometriosis affects an estimated 1 in 10 women during their reproductive years, and up to 30% to 50% of women with endometriosis may experience infertility. Classically, endometriosis is a surgical diagnosis, and excision or ablation of endometriosis is known to be technically challenging with little added benefit for patients undergoing in vitro fertilization (IVF). However, the presence of an endometriosis diagnosis may impact clinical recommendations during fertility treatment. A previous study developed classifiers for prediction of endometriosis in a cycle-phase specific manner by using margin tree classification within one dataset. Our aim was to build on this research by utilizing machine learning to predict and independently validate the presence or absence of endometriosis, regardless of cycle phase and other uterine pathology, through endometrial biopsy (EMB) samples.

**DESIGN:** Retrospective cohort analysis of publicly available genomic data.

**MATERIALS AND METHODS:** We trained Random Forest classifiers on ten gene-expression based modules, derived from spectral decomposition of the discovery dataset (n = 148) to predict the presence of endometriosis. These classifiers were validated in an independent gene expression dataset (n = 37) of eutopic EMB samples obtained from patients with and without endometriosis.

**RESULTS:** We identified a 280-gene predictor of endometriosis using Random Forests that was found to predict the presence of endometriosis, regardless of the endometrial phase and other pathology, with an accuracy of 84% (area under ROC = 0.84; p-value: 6.14e-05), with a negative predictive value of 86% and a positive predictive value of 81%. We reduced model over-fitting by performing 10-fold cross-validation of our discovery data.

**CONCLUSIONS:** Using machine learning, we developed a new genomic signature with the ability to accurately predict the presence of endometriosis from an EMB sample regardless of cycle phase or other pathology. Ongoing work is interrogating the findings in the IVF population, and the role played by DNA methylation in regulating expression of key genes and pathways in our predictive model. In a move towards personalized, noninvasive medicine, the EMB diagnosis of endometriosis could provide meaningful clinical information without subjecting patients to the risks and expense of surgery.

References: 1. Tamarisks JS et al. A Molecular classification of endometriosis and disease stage using high-dimensional genomic data. *Endocrinology*. 2014 Dec;155(12):4986-99. <https://doi.org/10.1210/en.2014-1490>.

2. A Bulletti C, Coccia ME, Battistoni S, Borini A. Endometriosis and infertility. *J Assist Reprod Genet*. 2010;27(8):441-447. <https://doi.org/10.1007/s10815-010-9436-1>.

SUPPORT: None.

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**METABOLIC SYNDROME (MetS): FECUNDABILITY AND ADVERSE PREGNANCY OUTCOMES IN UNEXPLAINED INFERTILITY.**



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**OBJECTIVE:** To determine the association of MetS with fecundability and pregnancy complications after ovarian stimulation–intrauterine insemination (OS-IUI) for unexplained infertility.

**DESIGN:** Secondary analysis of a randomized clinical trial (RCT) investigating clinical pregnancy, live birth, and multiple pregnancy rates with OS-IUI for couples with unexplained infertility.

**MATERIALS AND METHODS:** This secondary analysis included all 900 couples undergoing OS-IUI treatment as part of The Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation (AMIGOS) clinical trial. Briefly, this trial enrolled women at 12 sites, age 18–40 with at least one patent fallopian tube and regular menses who underwent OS-IUI with letrozole, clomiphene citrate (CC) or gonadotropins for up to four treatment cycles. Male partners were required to have a semen analysis with at least 5 million total motile sperm in the ejaculate. Chi-Square/Fisher exact, Student's t, and logistic regression were utilized as appropriate. A p-value of < 0.05 was considered statistically significant. MetS was defined by the International Diabetes Federation criteria. Overweight/obese = HiBMI (>25 kg/M<sup>2</sup>), Very high BMI (VHBMI) >35kg/ M<sup>2</sup>. Pregnancy complications included pre-eclampsia (Pr-E), gestational diabetes (GDM), preterm delivery, placental abruption, large for gestational age, IUGR and postpartum infections.

**RESULTS:** Prevalence of Hi BMI was 51.09%, VHBMI 10.4%, and MetS 17.6%. BMI or MetS was not associated with clinical pregnancy or live birth rates. Pregnancy complications occurred in 40.18% overall (CC 30.0%, letrozole 41.4% and gonadotropins 46.9%). For CC and letrozole, the odds for any pregnancy complication with MetS were 2.72 (1.27, 5.82). With MetS, 22.7% had Pr-E and 27.3% had GDM vs. 5.2% and 8.3% without MetS. When given gonadotropins, MetS was not associated with complications, however multiple pregnancies were more common (33% of triplet pregnancies had Pr-E). For those with VHBMI, the odds of a complication were 4.30 (1.17, 15.79), and 65% had MetS. The overall odds for a complication with MetS present were 3.10 (1.44, 6.67) adjusting for HiBMI and multiple pregnancies.

**CONCLUSIONS:** MetS did not influence fecundability. However, it is significantly associated with pregnancy complications beyond the risk conferred by obesity alone. MetS portends pregnancy complications, as does VHBMI, with OS-IUI for patients with unexplained infertility.

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**THE AROMATASE INHIBITOR, LETROZOLE: A NOVEL TREATMENT FOR ECTOPIC PREGNANCY.**



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**OBJECTIVE:** Study the use of the aromatase inhibitor, letrozole, for the treatment of ectopic pregnancy compared to methotrexate.

**DESIGN:** Non-randomized prospective cohort study.

**MATERIALS AND METHODS:** A series of 42 consecutive patients with undisturbed ectopic pregnancy were counseled regarding the treatment op-

tions including surgical treatment (control group), medical treatment with the methotrexate (study group 1) or letrozole (study group 2). Each group included 14 patients. Primary outcome was complete resolution of ectopic pregnancy determined by serum hCG levels below laboratory immunoassay detection. Secondary outcomes included changes in the biochemical parameter of ovarian reserve, Anti-Mullerian Hormone (AMH), as well as hematological and hormonal changes associated with the two medical treatments compared to surgical treatment.

**RESULTS:** Complete resolution of ectopic pregnancy occurred in equal number of patients, 12 out of 14 (86%) in each of the two study groups. The two patients who failed methotrexat treatment had to undergo surgery after becoming hemostatically unstable, while in the letrozole group, one patient had to go to surgery when she became hemostatically unstable, while in the second patient, a decision to do surgery was due to failure of hCG level to decline four days after letrozole treatment. Treatment with methotrexate was associated with higher levels of liver enzymes, and lower levels of platelets (the differences in both parameters were statistically significant). The decline in hCG levels was faster in the letrozole group, when compared to the methotrexate group. Three months after treatment, AHM levels were lower in the methotrexate group when compared to the letrozole and the surgery group. However, the decline in the hCG and AMH levels were not statistically significant.

**CONCLUSIONS:** Up to our knowledge, this is the first report in the literature on the success of letrozole in medical treatment of ectopic pregnancy. In the absence of estrogen priming, progesterone may not exert its physiological functions due to a negative effect on progesterone receptors. We hypothesized that by inhibiting the estrogen synthetase (the aromatase enzyme), the progesterone would not exert its physiological function in maintaining pregnancy, including ectopic pregnancy. The small sample size and non-randomized design of our study would limit our conclusion about letrozole success in treating ectopic pregnancy. However, the promising high resolution rate and better safety profile that letrozole has over a chemotherapeutic agent like methotrexate, should encourage studying the letrozole as a promising medical treatment for ectopic pregnancy through more definitive randomized clinical trials, that are adequately powered. Furthermore, letrozole may also be a safer alternative instead of surgical approach in managing early pregnancy loss, and pregnancy termination when medically indicated and ethically appropriate. In our study, a long follow up is intended to compare ovarian reserve in the two study groups and the surgery control group.

**References:** *Mitwally*, A et al. April 1, 2014, A United States Patent 8,685,950.

Use of aromatase inhibitors for treatment of ectopic pregnancy.

Document Identifier, US 20080025991 A1.

Prior Publication Date, Jan 31, 2008.

Family ID: 36148881 Appl. No.: 11/664,768 Filed: October 4, 2005 PCT. Filed: October 04, 2005 PCT No.: PCT/US2005/035864 371(c)(1),(2),(4) Date: April 04, 2007 PCT Pub. No.: WO2006/041941 PCT Pub. Date: April 20, 2006.

**SUPPORT:** None.

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**REPRODUCTIVE OUTCOMES FOLLOWING A RUPTURED ECTOPIC PREGNANCY.**



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**OBJECTIVE:** Ectopic pregnancies account for 2% of all pregnancies in the United States. Subsequent pregnancy outcomes following ruptured versus non-ruptured ectopic pregnancy have been poorly reported in the literature. Non-peer reviewed websites have reported that ruptured ectopic pregnancies are damaging for future fertility; however, only one single study has reported no difference. As rupture of an ectopic pregnancy could lead to hemoperitoneum, inflammation, and scar formation, we hypothesized that ruptured ectopic pregnancies will decrease future fertility. Therefore, the primary objective of this study is to determine if fewer subsequent intrauterine pregnancies occur following surgical excision of a ruptured tubal ectopic compared to surgical excision of a non-ruptured ectopic pregnancy.

**DESIGN:** Retrospective cohort study at a University-affiliated hospital.

**MATERIALS AND METHODS:** All patients undergoing salpingectomy for a tubal ectopic pregnancy from 1/1991-12/2016 were considered. Patients were excluded if: it was not possible to determine ruptured versus non-ruptured status; if the patient had documented contraceptive use or no sexual

activity within 12 months of the procedure; or if the patient had insufficient follow-up, defined as less than 2 visits within 12 months of the procedure. All data was statistically analyzed using Fisher exact tests.

**RESULTS:** A total of 1,171 tubal ectopic pregnancies were identified, 77 of which met inclusion criteria. Ruptured ectopic pregnancies did not result in a significant decrease in subsequent intrauterine pregnancy rate nor a significant increase in future ectopic pregnancy rate during the 12-month follow-up period. 10 out of 27 (37%) patients with ruptured ectopic pregnancy had an intrauterine gestation within 12 months, while 17 out of 50 (34%) patients with a non-ruptured ectopic achieved an intrauterine pregnancy within 12 months ( $p=0.8070$ ). 4 out of 27 (15%) cases with a ruptured ectopic and 7 out of 50 (14%) cases with a non-ruptured ectopic had a subsequent ectopic pregnancy within 12 months ( $p>0.99$ ).

**CONCLUSIONS:** Ruptured ectopic pregnancy did not adversely affect the intrauterine pregnancy rate nor recurrent ectopic pregnancy rate 12 months following the rupture, compared to non-ruptured ectopic pregnancies.

References: 1. Ectopic pregnancy—United States, 1990-1992. Centers for Disease Control and Prevention (CDC). *MMWR Morb Mortal Wkly Rep.* 1995;44:46-8.

2. Creanga AA, Syverson C, Seed K, Callaghan WM. Pregnancy-related mortality in the United States, 2011-2013. *Obstet Gynecol* 2017;130:366-73.

3. Mol F, van Mello NM, Strandell A, et al. Salpingotomy versus salpingectomy in women with tubal pregnancy (ESEP study): an open-label, multicentre, randomised controlled trial. *Lancet* 2014;383:1483-1489.

4. Desroque D, Capmas P, Legendre G, Bouyer J, Fernandez H. Fertility after ectopic pregnancy. *Journal de Gynecologie Obstetrique et Biologie de la Reproduction* 2010;39:395-400.

5. Job-Spira N, Fernandez H, Bouyer J, Pouly JL, Germain E, Coste J. Ruptured tubal ectopic pregnancy: risk factors and reproductive outcome: results of a population-based study in France. *Am J Obstet Gynecol* 1999;180:938-944.

6. Bernoux A, Job-Spira N, Germain E, Coste J, Bouyer J. Fertility outcome after ectopic pregnancy and use of an intrauterine device at the time of the index ectopic pregnancy. *Human Reproduction* 2000;15(5):1173-1177.

7. Juneau C, Bates GW. Reproductive outcomes after medical and surgical management of ectopic pregnancy. *Clin Obstet Gynecol.* 2012;55(2):455-60.

8. Kostrzewa M, Zyla M, Kolasa-Zwierzchowska D, et al. Salpingotomy vs salpingectomy—a comparison of women's fertility after surgical treatment of tubal ectopic pregnancy during a 24-month follow-up study. *Ginekol Pol* 2013;84(12):1030-5.

9. de Bennetot M, Rabischong B, Aublet-Cuvelier B, Belard F, Fernandez H, Bouyer H, Canis M, Pouly JL. Fertility after tubal ectopic pregnancy: results of a population-based study. *Fertility and Sterility* 2012;98:1271-1276.

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**PROGESTIN THERAPY FOR WOMEN WITH COMPLEX ATYPICAL HYPERPLASIA: LEVONORGESTREL INTRAUTERINE DEVICE VERSUS SYSTEMIC THERAPY.**

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**OBJECTIVE:** For women with complex hyperplasia with atypia (CAH) who do not undergo hysterectomy, either for fertility preservation or due to poor surgical candidacy, the effectiveness of the levonorgestrel-releasing intrauterine device (LNG-IUD) compared to systemic therapy has not been well studied. We sought to examine differences in treatment response between the LNG-IUD and systemic therapy in women with CAH.

**DESIGN:** A retrospective observational study at a tertiary care center between 2003-2018.

**MATERIALS AND METHODS:** Time dependent analyses of complete response (CR) and progression to cancer were performed for LNG-IUD vs. systemic therapy. A propensity score inverse probability of treatment weighting (IPTW) model was used to create a weighted cohort that differed based on treatment type but was similar with respect to other characteristics. An interaction-term analysis was performed to examine the impact of body habitus on treatment response, and an interrupted time-series analysis was employed to assess changes in treatment patterns over time.

**RESULTS:** Among 245 women with CAH, 69 (28.2%) had the LNG-IUD and 176 (71.8%) received systemic therapy. Mean age and body mass index

were 36.9 years and 40.0 kg/m<sup>2</sup>, respectively. In the patient level analysis (Table 1), women who received the LNG-IUD were three times more likely to have CR and had a 75% lower likelihood of progression to cancer compared to those who received systemic therapy (both,  $P<0.001$ ). In particular, women with class III obesity derived significant benefit from the LNG-IUD vs. systemic therapy (CR rates, 70.3% vs. 40.6%, HR 4.34, 95%CI 2.75-6.86,  $P<0.001$ ) compared to those with class I-II obesity (95.3% vs. 53.5%, HR 1.85, 95%CI 1.16-2.97,  $P=0.010$ ). In the cohort level analysis, LNG-IUD use significantly increased after 2007 (6.3% to 82.7%, 13.2-fold increase,  $P<0.001$ ), and this increase was associated with an increasing number of women who achieved CR (32.9% to 81.4%, 2.5-fold increase,  $P=0.005$ ).

Outcome	Progestin route	1-year rate	Adjusted-HR (95%CI)	P-value
Complete response	Systemic	46.7%	1	
	LNG-IUD	80.7%	3.14 (2.35-4.21)	<0.001
Overall response	Systemic	57.0%	1	
	LNG-IUD	80.9%	2.07 (1.61-2.66)	<0.001
Cancer	Systemic	15.7%	1	
	LNG-IUD	4.1%	0.25 (0.12-0.52)	<0.001

**CONCLUSIONS:** Our study suggests that the LNG-IUD may be more effective than systemic therapy with oral progestins for women with CAH who opt for non-surgical treatment, particularly in morbidly obese women.

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**PRECONCEPTION A1c AND TIME TO PREGNANCY, PREGNANCY LOSS, AND LIVE BIRTH.**

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**OBJECTIVE:** Reproductive aged women are increasingly at risk of comorbid conditions resulting from obesity and sedentary lifestyles. Past research indicates that increasing A1c in healthy populations is positively associated with markers of inflammation and the development of diabetes in the future. It is unknown if increasing A1c in healthy women during the preconception period impacts reproductive success. Our goal was to examine the relationship of preconception A1c and time-to-pregnancy (TTP), pregnancy loss, and live birth.

**DESIGN:** Prospective cohort from the Effects of Aspirin in Gestation and Reproduction trial included 1,228 healthy women ages 18-40 years with a history of one or two pregnancy losses attempting spontaneous conception, and no known diagnosis of infertility, diabetes, or PCOS.

**MATERIALS AND METHODS:** A1c was measured using high performance liquid chromatography (Tosoh Bioscience) at the baseline visit prior to pregnancy. Pregnancy was detected with hCG and ultrasound. Fecundability odds ratio (FOR) and 95% confidence intervals (CI) were estimated using discrete Cox proportional hazards regression models, accounting for left truncation and right censoring. Weighted log-binomial regression models were used to estimate relative risk (RR) and 95% CIs for live birth and pregnancy loss. Models were adjusted for age, BMI, race, income, and treatment arm.

**RESULTS:** Preconception A1c results were available for 1,194 participants. The lower 10<sup>th</sup> percentile consisted of A1c values of 3.8-4.6% (n=121), the middle group A1c of 4.7-5.5% (n= 975), and upper 90<sup>th</sup> percentile A1c was 5.5-7.5% (n= 98). Increasing preconception A1c was associated with longer TTP (FOR 0.74; 95% CI 0.57, 0.96) in unadjusted models, however, there was no association in adjusted models after accounting for BMI and other markers of obesity and insulin resistance (FOR 0.92; 95% CI 0.69, 1.22). Preconception A1c was not associated with differences in live birth (RR 1.03; 95% CI 0.84, 1.25) or pregnancy loss (RR 0.74; 95% CI 0.49, 1.12).

**CONCLUSIONS:** Among healthy women, we observed no association of A1c with live birth rate or pregnancy loss. The association of A1c and TTP appeared to be influenced by BMI, a strong risk factor for both diabetes and

infertility. It is expected that increased adiposity contributes to alterations in glucose metabolism, inflammation, and lowered reproductive efficiency. Our data supports current guidelines that reserve preconception A1c assessment in patients with risk factors for diabetes.

**O-198** Wednesday, October 16, 2019 12:00 PM

### **A LONGITUDINAL ASSESSMENT OF OVARIAN RESERVE AFTER MYOMECTOMY.**

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**OBJECTIVE:** Myomectomy is the preferred treatment option for symptomatic fibroids in women desiring fertility-sparing treatment. However, the effect of myomectomy on ovarian reserve is largely unknown. There is evidence to show that other treatments for fibroids including uterine artery embolization and hysterectomy may diminish ovarian reserve. Additionally, the use of a tourniquet transiently decreases blood supply to the ovaries, which may impact ovarian reserve. This study sought to determine whether open and minimally invasive myomectomy are associated with immediate and/or long-term changes in serum anti-Mullerian hormone (AMH).

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Patients undergoing minimally invasive (robot-assisted or laparoscopic) or open abdominal myomectomy by one primary surgeon from May 2018 through March 2019 were included. A Penrose drain tourniquet was used for all open myomectomies. Vasopressin was injected into the myoma subserosa for all minimally invasive myomectomies (MIS). Baseline data collected included age, BMI, and race. Surgical data collected included surgical approach, additional procedures, estimated blood loss (EBL), length of procedure, and weight of fibroids removed. Serum AMH was collected prior to the procedure. Follow-up serum AMH levels were measured at 2 weeks, 3 months, and 6 months after the procedure. To achieve 80% power to detect a 15% difference in mean AMH level, with  $p < 0.05$ , a minimum of 43 subjects needed to be recruited. Paired t-tests were used to detect the mean difference between baseline AMH and 2 week, 3 month, and 6 month AMH respectively. Univariate linear regression was used to detect the effect of surgical approach and covariates on the percent difference in AMH from baseline to each follow-up time point. All follow-up visits will be completed by September 2019, therefore a preliminary analysis was conducted for the purpose of this abstract.

**RESULTS:** A total of 56 patients were included in the study. 32 had open myomectomies and 24 had minimally invasive myomectomies. A significant decrease in serum AMH was found between baseline and 2 weeks post-operatively ( $n=42$ ) ( $b=0.26 \pm 0.75$  (95% CI 0.03-0.49)  $p=0.029$ ). This transient effect was no longer significant after 3 months ( $n=20$ ) and 6 months ( $n=14$ ). Linear regression showed a significantly greater decrease at 2 weeks post-operatively in the open compared to MIS group ( $b=-0.56$ ,  $p=0.039$ ). No significant differences in AMH were seen between open and MIS groups at 3 and 6 months. Surgical factors such as EBL, length of surgery, and fibroid weight were not significantly associated with post-operative changes in serum AMH level.

**CONCLUSIONS:** AMH levels appear to undergo a transient decline in the immediate post-operative period after myomectomy, with a more pronounced effect with an open compared to MIS approach. The use of a tourniquet might cause a more significant decrease in AMH in the immediate post-operative period, but does not appear to have a sustained effect. Patients can be reassured that undergoing a myomectomy does not have a long-term impact on ovarian reserve, regardless, of the approach.

Reference: None.

SUPPORT: None.

## **FERTILITY PRESERVATION**

**O-199** Wednesday, October 16, 2019 10:45 AM

### **HOW OPEN IS THE WINDOW OF OVARIAN FUNCTION AFTER CANCER TREATMENT?**

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**OBJECTIVE:** The remaining window of ovarian function after cancer treatment is clinically important, yet largely unknown. This study estimated the trajectory of AMH over two decades following cancer treatment in female survivors of adolescent and young adult cancers (AYA survivors). We hypothesized that AMH levels would initially rise, then plateau and finally fall over time after cancer treatment, and trajectories would vary by treatment gonadotoxicity.

**DESIGN:** Cross-sequential.

**MATERIALS AND METHODS:** Female AYA survivors who were ages 18-39, were diagnosed with cancer at ages 15-35, completed primary cancer treatment and had at least one ovary were recruited from cancer registries, clinics and advocacy groups between 2015 and 2018. Followed for 18 months, participants collected dried blood spots (DBS) and answered questionnaires every 6 months. DBS were assayed for AMH levels (LOD 0.03 ng/mL, inter- and intra-assay CV < 10%) using the picoAMH assay (Ansh-Labs, Webster, TX. Cancer treatment data were abstracted from primary records. Functional Principal Component Analysis (FPCA) modeled AMH trajectory over time since treatment. Principal components were compared by gonadotoxicity (low, moderate, high) and age at diagnosis (<25, 25 to <35, ≥35) groups.

**RESULTS:** 763 survivors, mean age 33.3 (SD 4.7), contributed 1968 AMH levels at a median of 6.5 years post-treatment (IQR 2.0-9.1). The most common cancers were breast (27%), lymphoma (25%) and thyroid (18%). By treatment gonadotoxicity, 30% were low, 62% were moderate, and 8% were high. For the overall trajectory, post-treatment AMH levels began low, then rose to plateau at 2.5 years, and maintained until levels began to fall at 10 years. FPCA showed that trajectories differed significantly by gonadotoxicity group ( $p < 0.001$ ) and age at cancer diagnosis ( $p < 0.05$ ). The low group displayed rising levels until 2.7 years post-treatment, then maintained at similar levels until 10 years post-treatment before levels began to fall. The moderate group trajectory was similar, but the magnitude of peak AMH recovery was approximately two-thirds of the low group. In contrast, the high group displayed a quick recovery (plateau by 1.5 years) and no appreciable time interval during which AMH was maintained before a steep fall of levels. Younger age at diagnosis was associated with higher levels of AMH at plateau, but similar maintenance intervals prior to fall of levels, compared with older age at diagnosis. The predicted trajectories showed overlapped among groups.

**CONCLUSIONS:** Using the hybrid longitudinal and cross-sectional design, with the FPCA approach, we show novel data on the trajectory of AMH beyond the first 5 years after cancer treatment. The AMH trajectories suggest that for low and moderate toxicity groups, the duration during which AMH stays plateaued appears long, in contrast to a narrow window in the high toxicity group. These trajectories aid in counseling AYA survivors on their family building plans.

SUPPORT: NIH HD080952-05.

**O-200** Wednesday, October 16, 2019 11:00 AM

### **IN SEARCH OF THE CRYSTAL BALL - HOW MANY EGGS TO A LIVE BIRTH? A 2-STEP PREDICTION MODEL FOR EGG FREEZING COUNSELING BASED ON INDIVIDUAL PATIENT AND CENTER DATA.**

Serena H. Chen, M.D.,<sup>a</sup> Yajing Angela Xie, PhD,<sup>b</sup> Natalie A. Cekleniak, MD,<sup>a</sup> Debbra A. Keegan, MD,<sup>a</sup> Mylene WM. Yao, MD,<sup>b</sup> <sup>a</sup>Division of Reproductive Medicine, IRMS at St Barnabas, Livingston, NJ; <sup>b</sup>Univfy Inc., Los Altos, CA.



**OBJECTIVE:** We aim to develop a two-step egg freezing counseling tool that provides personalized expected live birth (LB) rates before oocyte retrieval (Pre-OR) and adjusts the expectations after oocyte retrieval (Post-OR), when the oocyte yield is known.

**DESIGN:** We applied machine learning (ML) to a retrospective IVF-LB outcomes data set. Due to limited LB outcomes from egg freezing itself, this large, diverse IVF patient population served as proxy for women considering egg-freezing to preserve fertility potential.

**MATERIALS AND METHODS:** We applied the boosted tree method and cross-validation to train and test Pre- and Post-OR models in predicting LB outcomes. The dataset comprises linked IVF-ET data from 1,166 IVF cycles started at our center in 2015 for women under 42. Both Pre- and Post-OR

models use clinical predictors such as age, BMI, AMH, day 3 FSH, any clinical infertility diagnosis, reproductive history, and semen analysis, but only the Post-OR model uses the oocyte yield. Models with optimal discrimination (AUC) and prediction accuracy relative to an age-control model were selected. This approach does not rely on assumptions about per-oocyte live birth rates or per-embryo aneuploidy rates. However, it does assume 1) clinical predictors have the same relative impact on LB rates in IVF patients and women without infertility diagnosis and 2) the freeze-thaw survival rates of oocytes and blastocysts are similar.

**RESULTS:** Model Evaluation: The AUC for the Pre-OR, Post-OR and age-control models were 67%, 73%, and 57%, respectively. Compared to age-control, AUC improved by 17% (Pre-OR) and 28% (Post-OR). Prediction accuracy, measured by the posterior log of odds ratio compared to age-control (i.e. "how many times more accurate compared to age-control") is improved by 25-folds (Pre-OR) and 67-folds (Post-OR) using natural log scale. Based on the Pre-OR model, 84% of our IVF patients have a personalized LB rate over 32% from transfer(s) of embryo(s) generated from one IVF-COH cycle. Relevance for Egg Freezing Counseling - Example 1: Based on the Pre-OR model, a 30 year old woman (BMI 26, AMH 3.5 ng/mL, no infertility) has 69-70% (95% CI) LBR per egg-freezing cycle (per cycle here on) which would be adjusted if oocyte yield is less than expected. For example, if her oocyte yield were 5-9 oocytes (less than the expected 10-15), her expected LBR per cycle would decrease to 48-53% (95% CI). Example 2: Based on the Pre-OR model, a 36 year old woman (BMI 28, AMH 2.5 ng/mL, no infertility) has 52-53% (95% CI) LBR per cycle. However, if her oocyte yield were > 15 oocytes (higher than expected), her expected LBR per cycle would increase to 60% (95% CI).

**CONCLUSIONS:** We have developed a two-step egg freezing counseling tool that sets expectations about LB outcomes before and after knowing the actual oocyte yield while personalizing LB expectations to each woman's reproductive health profile and maximizing transparency with ML-based models validated against our center's IVF outcomes. User experience testing is required to optimize how to best convey LB expectations provided by the models.

**SUPPORT:** Each organization funded its own research efforts.

**O-201** Wednesday, October 16, 2019 11:15 AM

**CHEMOTHERAPY CAUSES PRIMORDIAL FOLLICLE DEATH IN THE HUMAN OVARY VIA MULTIPLE APOPTOTIC PATHWAYS AND NOT BY "BURN OUT".** Shiny Titus, Ph.D., Kutluk H. Oktay, M.D., Ph.D., Yale University School of Medicine, New Haven, CT.



**OBJECTIVE:** It has been proposed that gonadotoxic chemotherapy results in the "burn out" of primordial follicle reserve by activating PI3K/PTEN/Akt signaling pathway. Others have challenged this concept and put forward DNA damage and apoptosis as the main mechanism of follicle loss. We conducted this study to answer this controversy and conclusively determine the mechanism of chemotherapy-induced damage to ovarian reserve in women.

**DESIGN:** Ovarian cortical pieces from organ donors aged  $\leq 32$  years were xenografted subcutaneously to SCID mice ( $n=12$  mice/ tissue from 4 donors each). After 10 days, the mice were given an injection of cyclophosphamide (75mg/kg) or the vehicle. The tissues were recovered 12 hours later.

**MATERIALS AND METHODS:** The recovered xenografts were assessed for apoptosis by anti-caspase-3 (AC3) and DNA double strand breaks by  $\gamma$ -H2AX immunostaining as well as follicle growth initiation rate (FGIR) by primary/primordial follicle ratios. Single primordial follicle oocytes were laser captured for RNA sequencing and single cell quantitative real time PCR (qRT-PCR) to determine the signaling pathways activated in response to chemotherapy exposure. Immunofluorescence microscopy and image analysis were used to assess phosphorylation of PI3K/PTEN/Akt pathway and Bad-Bcl2 co-localization.

**RESULTS:** There was no difference in the primordial follicle growth initiation rate (FGIR) between the vehicle and the cyclophosphamide-treated xenografts ( $p=0.35$ ). However, cyclophosphamide caused a significant increase in the percentage of apoptotic ( $65.02 \pm 3.55\%$  vs.  $28.27 \pm 5.77\%$ ;  $p=0.001$ ) and DNA-damaged ( $82 \pm 2.7\%$  vs.  $23.82 \pm 8.66\%$ ;  $p=0.0001$ ) primordial follicles. Ingenuity Pathway Analysis (IPA) of the RNA sequencing data from laser captured primordial follicles showed significant decrease in the expression of PECAM ( $p=0.00009$ ), IKKBE ( $p=0.0001$ ), and ANGPT1 ( $p=0.003$ ) after cyclophosphamide treatment. These are positive regulators of the Akt pathway and hence results indicate that cyclophosphamide suppresses rather than activating Akt. In addition, IPA predicted that suppression of these pathways favor apoptosis in single primordial follicles. By qRT-PCR

analyses, there was no change in the expression of Akt ( $p=0.9$ ). Further, cyclophosphamide treatment did change the expression of the phospho-Akt ( $P=0.14$ ), phospho-Foxo3a ( $p=0.17$ ), or phospho-rpS6 (0.48) compared to the vehicle. By qRT-PCR, chemotherapy increased the expression of the anti-apoptotic Bcl2 ( $p=0.0003$ ) accompanied by enhanced colocalization of the pro-apoptotic BAD-Bcl2 complex ( $p=0.006$ ) in the primordial follicles, confirming that cyclophosphamide induces follicle death via apoptosis.

**CONCLUSIONS:** This single cell transcriptomic and immunohistochemical analysis of human primordial and primary follicles prove that gonadotoxic chemotherapy agents do not cause follicle activation; they rather create a pro-apoptotic state resulting in massive loss of ovarian reserve. Future research on pharmacological fertility preservation should target preventing DNA damage and apoptosis rather than follicle activation.

**SUPPORT:** This work was supported by RO1 HD061259 from NICHD.

**O-202** Wednesday, October 16, 2019 11:30 AM

**METFORMIN: A NOVEL OPTION OF FERTILITY PRESERVATION DURING CYCLOPHOSPHAMIDE-CONTAINING CHEMOTHERAPY.** Chu-Chun Huang, MD,<sup>a</sup> Mei-Jou Chen, MD, PhD,<sup>b</sup> Shee-Uan Chen, MD, PhD,<sup>a</sup>



Hong-Neng Ho, MD, PhD,<sup>c</sup> Yu-Shih Yang, MD, PhD.<sup>d</sup> <sup>a</sup>Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei, Taiwan; <sup>b</sup>National Taiwan University Livia Shangyu Wan Scholar, Taipei, Taiwan; <sup>c</sup>Taipei Medical University, Taipei, Taiwan; <sup>d</sup>Department of Obstetrics and Gynecology, Fu Jen Catholic University Hospital, New Taipei, Taiwan.

**OBJECTIVE:** Cyclophosphamide (CP) could cause premature primordial follicle activation and depletion, and finally premature ovarian failure, through the imbalanced activation of mTOR signaling pathway. Whether metformin, a widely prescribed anti-diabetes agent with mTOR inhibitory effect, could preserve fertility during CP treatment is still unknown.

**DESIGN:** A murine study.

**MATERIALS AND METHODS:** The female C57BL/6 mice aged 6-8 weeks were randomized into five groups ( $n=8$  per group), including the control group without any medical treatment, the CP-alone group (75mg/kg, i.p. weekly), the treatment groups which CP was co-administrated with either oral metformin (50mg/kg/day) or two specific mTOR inhibitors (sirolimus 0.67mg/kg/day or everolimus 0.167mg/kg/day). After four weeks of treatment, five mice per group were sacrificed to collect the ovarian tissue and serum, and three mice per group were mated with male breeders 8 weeks after the end of treatment. The data were analyzed by one-way analysis of variance, the chi-square test or Fishers exact test where appropriate. A P value of  $< 0.05$  was considered statistically significant.

**RESULTS:** The number of primordial follicle, tertiary follicle, and corpus luteum significantly decreased in the CP-alone group compared with the control group. The deleterious effects of CP were significantly rescued when oral metformin was given that the follicular counts were significantly higher in the CP + metformin group than CP-alone group (number of primordial follicle:  $16.7 \pm 6.3$  v.s.  $9.6 \pm 4.7$ ,  $p=0.004$ ; tertiary follicle:  $5.4 \pm 1.1$  v.s.  $2.6 \pm 1.8$ ,  $p=0.002$ ; corpus luteum:  $8.2 \pm 1.5$  v.s.  $5.6 \pm 1.3$ ,  $p=0.029$ ). The other two specific mTOR inhibitors, sirolimus and everolimus, also exhibited similar protective effects on the ovarian follicular counts against CP damage. The serum level of anti-mullerian hormone, a reliable objective marker of ovarian reserve, was significantly decreased in the CP-alone group and increased in CP + metformin group (Control v.s. CP-alone v.s. CP + metformin:  $5.8 \pm 0.3$  v.s.  $2.1 \pm 1.0$  v.s.  $4.6 \pm 1.2$  ng/ml,  $p<0.0001$ ). The number of the offspring was also significantly decreased in the CP-alone group and increased in the CP + metformin group (Control v.s. CP-alone v.s. CP + metformin:  $6.7 \pm 1.2$  v.s.  $1.0 \pm 1.0$  v.s.  $4.0 \pm 2.0$ ,  $p=0.004$ ). The IHC stain showed that the expression of phospho-mTOR protein and TUNEL protein within mice ovaries were increased when treated with CP and were significantly decreased when co-treated with metformin.

**CONCLUSIONS:** This is the first research showing that metformin could preserve ovarian function and fertility in mice treated with CP. The underlying mechanism might be related to both the mTOR inhibitory and anti-apoptotic effects of metformin. It could be a safe, effective and also economic fertility preserving agent during CP-containing chemotherapy thus showing promising potential in future clinical research and application.

**SUPPORT:** This study was supported by grants MOST 105-2314-B-002-109-MY3 (H.N. Ho), MOST 102-2321-B002-093-MY3 and 105-2628-B002-043-MY4 (M.J. Chen), and MOST 105-2628-B-002-031-MY3 (C.C. Huang) from the Ministry of Science and Technology of Taiwan and the National Taiwan University Hospital (107-004058, 108-004336).

**WORLD WIDE UPDATE: RESULTS WITH CRYOPRESERVED OVARIAN TISSUE TRANSPLANT.** Sherman Silber, MD,<sup>a</sup> Yuting Fan, M.D.,<sup>b</sup> Sierra Goldsmith, B.S.<sup>a</sup> <sup>a</sup>Infertility Center of St. Louis, Chesterfield, MO; <sup>b</sup>University of Michigan, Ann Arbor, MI.



**OBJECTIVE:** There have been many scattered case reports, with a confusing literature therefore, on results with ovarian cryopreservation for cancer patients. There have been only three other series reported, with differing and changing techniques. Our objective was to report a consistent series, and to mine from the literature the number of babies, and whether the procedure (after 22 years) should still be labeled as “experimental”.

**DESIGN:** Patients who have had ovary freezing, and came back years later to re-implant their frozen tissue, were studied monthly post-op for many years for ovarian function, pregnancy, and live birth. In addition, the scattered world literature was scanned to determine the total number of live births.

**MATERIALS AND METHODS:** 115 patients, age 2 to 35 years, had frozen ovary cortex stored at our center since 1997. 15 of them up till now have had the tissue thawed and re-implanted. Three were leukemia, one was multiple sclerosis, two were premature ovarian failure, and the rest were solid tissue cancers. All were menopausal for at least 3 years. The technique for re-implantation was the same in all cases. After thaw of cortical tissue, three to five slices were quilted into one piece with 9-0 nylon interrupted sutures. The dead cortex was removed in entirety, and the quilted slices were sutured to the underlying medulla with 9-0 nylon interrupted sutures after hemostasis was achieved with micro-bipolar forceps and irrigation with pulsatile heparinized media to avoid adhesions. All transplants were orthotopic so that the patients could be allowed to conceive spontaneously. Patients were followed monthly for hormones, return of menses, and pregnancy, and delivery. In addition, the literature was reviewed to try to tabulate the number of live births to date in the world.

**RESULTS:** Of the fifteen patients who had their frozen tissue re-implanted, none underwent IVF, all pregnancies were spontaneous from intercourse. 15 healthy babies were delivered to 10 of the 15 women (66%). Two women had 4 babies from the thawed, transplanted tissue. Two of the three with leukemia had a total of 4 healthy babies. In the literature, we counted a total of 170 babies in addition to our 15, making a total of 185. Live baby pregnancy rate in the literature ranged from a low of 31% to our 66%, in the only four reported series, including ourselves, thus far. There have been no cases of transmission of cancer.

**CONCLUSIONS:** Ovarian cryopreservation for cancer patients should no longer be labeled “experimental”.

O-204 Wednesday, October 16, 2019 12:00 PM

**ARE GENDER DYSPHORIA PATIENTS COUNSELED ON FERTILITY PRESERVATION PRIOR TO INITIATING HORMONAL THERAPY?** Ross G. Everett, MD MPH, Bryce A. Toburen, BA, Kaylee M. Luck, BS, Johnathan Doolittle, MD, Jay I. Sandlow, MD. Medical College of Wisconsin, Milwaukee, WI.



**OBJECTIVE:** National guidelines recommend counseling patients with gender dysphoria on the impacts of hormone or surgical therapy on their fertility prior to beginning either intervention. In this study, we aim to identify the compliance to these guidelines at our institution.

**DESIGN:** Retrospective, single institution chart review.

**MATERIALS AND METHODS:** Utilizing ICD codes, we identified patients with a diagnosis of gender dysphoria [GD] treated at our institution between 2008-2018. Various parameters regarding medication regimen, surgical intervention, fertility counseling, and fertility preservation were obtained through retrospective review. Patient demographics and interventions were compared. All data was analyzed in a standard statistical fashion utilizing Stata software.

**RESULTS:** Upon review, 269 patients met inclusion criteria. Of these, 114 (42.4%) had a chromosomal sex of female and 155 (57.6%) were chromosomal males. Race was divided as 75.5% White, 16.7% Black and 7.8% Other. The average age was 30.9 (S.D.±13.7). Regarding management of GD, 171 (63.6%) had been managed by Endocrinology, 118 (43.9%) by Gynecology, and 25 (9.3%) had seen Urology. 74 patients (27.5%) ultimately pursued some surgical intervention. 97 patients (36.1%) were on hormonal therapy for GD prior to evaluation at our institution and were excluded from subsequent analysis. Another 26 patients did not have record of pursu-

ing hormonal therapy to date. Of the remaining 146 patients, 96 (65.8%) had documented counseling regarding fertility. On chi-square tests, age was the only demographic found to be significantly different between those counseled on fertility and those not. Additionally, on multinomial logistic regression, individuals ≤30 years were significantly more likely to be counseled regarding fertility than those 31-50 years (RR 2.45, p=0.049) and those 51+ years (RR 5.47, p=0.013). Factors such as race, chromosomal sex, and managing specialty were not found to be predictive. Among those patients ≤30 years of age, 71 of 98 (72.5%) were counseled regarding fertility preservation.

**CONCLUSIONS:** Compliance with national guidelines to counsel GD patients on fertility preservation is best among younger patients, most notably those less than or equal to 30 years of age. This appears consistent among patients of both chromosomal sexes and across different managing specialties. Further research is needed regarding other risk factors for poor counseling as well as to predict those patients who will be interesting in fertility preservation.

**References:** 1. Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an endocrine society clinical practice guideline. *Endocr Pract.* 2017; 23(12): 1934-2403.

2. The World Professional Association for Transgender Health, “Standards of Care for the Health of Transsexual, Transgender, and Gender Non-conforming People. 7<sup>th</sup> Version.” 2011, <http://www.wpath.org>.

**SUPPORT:** I have no financial support to disclose.

## FIBROIDS

O-205 Wednesday, October 16, 2019 10:45 AM

**EFFECT OF FIBROID LOCATION AND SIZE ON EFFICACY OF ELAGOLIX: RESULTS FROM PHASE 3 CLINICAL TRIALS.** Ayman Al-Hendy, MD PhD,<sup>a</sup> James Simon, MD,<sup>b</sup> Sandra M. Hurtado, MD,<sup>c</sup> Linda D. Bradley, MD,<sup>d</sup> Charlotte D. Owens, MD,<sup>e</sup> Ran Liu, PhD,<sup>e</sup> Kurt T. Barnhart, MD, MSCE,<sup>f</sup> Veronica Gillispie, MD,<sup>g</sup> <sup>a</sup>University of Illinois College of Medicine, Chicago, IL; <sup>b</sup>IntimMedicine Specialists, Washington DC; <sup>c</sup>The Woman’s Hospital of Texas Clinical Research Center, Houston, TX; <sup>d</sup>Cleveland Clinic, Cleveland, OH; <sup>e</sup>AbbVie, Inc., North Chicago, IL; <sup>f</sup>University of Pennsylvania, Philadelphia, PA; <sup>g</sup>Ochsner Baptist Hospital, New Orleans, LA.



**OBJECTIVE:** Evaluate influence of uterine fibroid (UF) size/location on the efficacy of elagolix with add-back therapy in women with heavy menstrual bleeding (HMB) associated with UF.

**DESIGN:** Data was pooled from two 6-month, randomized, double-blind, placebo-controlled phase 3 studies, Elaris UF-1 and UF-2. Premenopausal women (18-51 years) were included if they had HMB [ $>80$ mL menstrual blood loss (MBL)/cycle; alkaline hematin methodology] and ultrasound-confirmed UF diagnosis. Women were randomized 1:1:2 to placebo, elagolix 300mg twice daily (BID), or elagolix 300mg BID with 1 mg estradiol/0.5mg norethindrone acetate (E2/NETA) once daily.

**MATERIALS AND METHODS:** This subgroup analysis evaluated the influence of UF location and size on the efficacy of elagolix+E2/NETA. Uterine volume, and size and location of UF were assessed by ultrasound. Subgroups were defined by baseline (BL) FIGO categories which were grouped FIGO 0-3, FIGO 4, or FIGO 5-8, median BL primary fibroid volume and median BL uterine volume. The primary endpoint was the proportion of women with  $<80$ mL MBL during the final month and  $\geq 50\%$  MBL reduction from BL to the final month. Adverse events (AEs) were monitored.

**RESULTS:** Overall 72.2% (95% CI, 67.65, 76.73) who received elagolix+E2/NETA were responders for the primary endpoint. When stratified by FIGO classification, the results were similar for all subgroups: FIGO 0-3, 77.7% (95% CI, 65.10, 90.22), FIGO 4, 68.1% (95% CI, 60.98, 75.18), and FIGO 5-8, 74.0% (95% CI, 67.21, 80.85). Similar results were seen in women with a primary fibroid volume of either  $<$  or  $\geq 36.2$  cm<sup>3</sup> (median) and uterine volume of either  $<$  or  $\geq 356.5$  cm<sup>3</sup> (median). Overall AEs for elagolix+E2/NETA included hot flushes (20.0%), nausea (9.4%), headache (9.4%), night sweats (8.6%), and fatigue (6.1%).

**CONCLUSIONS:** The effect of elagolix in reducing HMB associated with UF was not impacted by uterine volume, or UF location and size.

**SUPPORT:** This work was funded by AbbVie Inc. AbbVie participated in the study design, research, data collection, analysis and interpretation of data, writing, reviewing, and approving the publication.

### SUBLINGUAL MISOPROSTOL 400 VS. 200 MCG FOR REDUCING BLOOD LOSS DURING ABDOMINAL MYOMECTOMY: A RANDOMIZED DOUBLE-BLINDED CLINICAL TRIAL.



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**OBJECTIVE:** Uterine leiomyomas are the most frequent benign gynecologic pelvic tumors in women, which originate from smooth muscle cells of the uterus. Intra-operative bleeding and the increased need for blood transfusion and iron therapy are major challenges with abdominal myomectomy. Our objective is to compare the effectiveness of preoperative sublingual misoprostol 200 vs. 400 mcg for reducing blood loss during and after abdominal myomectomy.

**DESIGN:** A randomized, double-blind, clinical trial (ClinicalTrials.gov: NCT02709564).

**MATERIALS AND METHODS:** Patients with documented uterine fibroids on pelvic imaging and scheduled for abdominal myomectomy were invited to participate in our study. We included women aged (18-50 years) with five or less symptomatic subserous or intramural fibroids, Preoperative hemoglobin level is >8 g/dl, and uterine size is less than 24 weeks gestation. The eligible women were randomized (1:1) to either (group A) received two tablets of sublingual misoprostol 400 mcg at 3 hours and 1 hour before the surgery and (group B) received one tablet of sublingual misoprostol 200 mcg and one placebo tablet at the previously mentioned schedule. The primary outcome was the difference in the mean amount of intraoperative blood loss during myomectomy. The secondary outcomes included the change of hemoglobin (HB) before and 24 hours after surgery, duration of surgery, post-operative blood transfusion and the side effects of the drug. Mann Whitney test and Fisher's exact test were used for the analysis of the outcomes.

**RESULTS:** Eighty women were enrolled and randomized (n=40 in each arm). No difference between both groups regarding age, parity, BMI, type, number, size of fibroids and the total uterine size. Estimated blood loss was significantly lower in the misoprostol 400 mcg group (373.3±55.6 vs. 560.0±105.2 ml, p<0.001). Moreover, the reduction in HB level was significantly lower in the misoprostol 400 mcg group (0.8±0.18 vs. 1.7±0.38 g/dL, p<0.001). The operative duration was significantly shorter in the misoprostol 400 mcg group (91.3±5.7 vs. 111.2±6.3 minutes, p<0.001). Seven cases required a blood transfusion in the misoprostol 200 mcg group versus two cases in the other group (p=0.03). No difference between both groups reading the side effects of misoprostol.

**CONCLUSIONS:** Sublingual misoprostol 400 mcg is an effective and safe method for reduction of blood loss and need for blood transfusion during abdominal myomectomy.

**SUPPORT:** None.

O-207 Wednesday, October 16, 2019 11:15 AM

### URINARY PARABEN CONCENTRATIONS AND INCIDENCE OF UTERINE LEIOMYOMATA: A PROSPECTIVE ULTRASOUND STUDY.



Lauren A. Wise, Sc.D.,<sup>a</sup> Olivia R. Orta, Ph.D.,<sup>a</sup> Traci N. Bethea, Ph.D., M.P.A.,<sup>b</sup> Amelia K. Wesselink, Ph.D.,<sup>a</sup> Jennifer Weuve, Sc.D.,<sup>a</sup> Michael McClean, Sc.D.,<sup>a</sup> Paige L. Williams, Sc.D.,<sup>c</sup> Russ Hauser, MD, MPH, Sc.D.,<sup>c</sup> Donna D. Baird, Ph.D.<sup>d</sup> <sup>a</sup>Boston University School of Public Health, Boston, MA; <sup>b</sup>Boston University School of Medicine, Boston, MA; <sup>c</sup>Harvard T.H. Chan School of Public Health, Boston, MA; <sup>d</sup>National Institute of Environmental Health Sciences, Durham, NC.

**OBJECTIVE:** Parabens are a group of alkyl esters of p-hydroxybenzoic acid that are widely used as preservatives in personal care products, cosmetics, pharmaceuticals, and food processing. While parabens have shown weak estrogenic effects in animal models, epidemiologic studies of their health effects are limited. There have been no studies of the association between paraben exposure and UL incidence. We evaluated the association between urinary concentrations of parabens and incidence of uterine leiomyomata (UL).

**DESIGN:** Case-cohort analysis nested within the Study of Environment, Lifestyle and Fibroids (SELF), a prospective cohort study of 1,693 Black women aged 23-35 from Detroit, MI.

**MATERIALS AND METHODS:** After enrollment in 2010-2012, women were followed with telephone-assisted interviews, self-administered questionnaires, and in-person office visits that involved standardized ultrasound examinations and first-morning urine sample collection at baseline, 20 months, 40 months, and 60 months of follow-up. The CDC measured urinary concentrations of ethylparaben (EP), methylparaben (MP) and propylparaben (PP) using liquid chromatography with tandem mass spectrometry. UL were ascertained using ultrasound. Among the UL-free subcohort randomly-selected at baseline and all incident UL cases (N=765 women, including 298 cases), we estimated rate ratios (RR) and 95% confidence intervals (CI) using age-stratified Cox regression adjusted for age at menarche, smoking, alcohol, BMI, education, reproductive history, and current hormonal contraceptive use.

**RESULTS:** We found detectable concentrations of EP, MP, and PP in the baseline urine of 79%, 100%, and 100% of participants, respectively, with median concentrations of 2.34, 115, and 16.6 µg/g creatinine. Individual paraben concentrations were weakly to moderately correlated across time points, with Spearman correlations (r) ranging from as low as 0.23 for PP (baseline vs. 40 months) to as high as 0.43 for MP (20 vs. 40 months). MP and PP concentrations were highly correlated with each other (r: 0.76-0.80), but other pairs of parabens were not. RRs comparing highest vs. lowest quartiles of cumulatively-averaged (i.e., baseline, 20 and 40 month samples) creatinine-adjusted concentrations of EP, MP, and PP were 0.90 (CI: 0.65-1.24), 0.72 (CI: 0.51-1.02), and 0.66 (CI: 0.46-0.93), respectively, but no dose-response associations were observed. For the total molar sum of all parabens, RRs comparing quartiles 2, 3, and 4 (highest) vs. 1 (lowest) of cumulatively-averaged creatinine-adjusted concentrations were 0.85 (CI: 0.61-1.17), 0.87 (CI: 0.63-1.21), and 0.65 (CI: 0.46-0.93), respectively. Models using baseline urinary concentrations produced comparable findings. Results were similar across strata of BMI and parity, but were attenuated among current users of hormonal contraception.

**CONCLUSIONS:** Among reproductive-aged Black women, urinary concentrations of selected parabens were weakly inversely associated with UL incidence.

**SUPPORT:** Support: Extramural (R01-ES024749) and intramural funding from NIH, NIEHS and the American Recovery and Reinvestment Act.

O-208 Wednesday, October 16, 2019 11:30 AM

### VITAMIN D LONG-TERM TREATMENT DECREASES HUMAN UTERINE LEIOMYOMA SIZE THROUGH SPECIFIC MOLECULAR MECHANISMS IN A XENOGRAFT ANIMAL MODEL.



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**OBJECTIVE:** Uterine leiomyomas (LM) are benign estrogen-dependent tumors, composed of smooth muscle cells interspersed in an abundant extracellular matrix (ECM). In women, an increased risk of LM is associated with Vitamin D (VitD) deficiency. Our group have demonstrated *in vitro* the anti-proliferative action of VitD on human LM primary cells, elucidating the potential role of VitD as a therapy to shrink LM. In this study, we aim to corroborate *in vivo* the VitD effect at both short and long terms in a xenograft mouse model through specific mechanisms: cell proliferation, ECM degradation and apoptosis.

**DESIGN:** Preclinical study of human uterine LM treatment with VitD in an animal model.

**MATERIALS AND METHODS:** Human LM fragments (4mm<sup>2</sup>) were implanted intraperitoneal in ovariectomized NOD-SCID mice (hormonally supplemented). One week after, we established 3 groups: control, VitD 0.5 µg/kg/day and VitD 1 µg/kg/day. Treatments were delivered by micro-osmotic pumps for 21 days in the short-term (10 mice/group) and for 60 days in the long-term (6 mice/group) treatments. Human LM implants were monitored *in vivo* and sizes were measured by PET/CT using <sup>18</sup>F-FDG. At the end of the treatments, LM were collected and measured macroscopically, proliferation was analyzed by immunohistochemistry (Ki67), apoptosis by Western Blot (WB) (CASPASE 3) and TUNEL assay; and ECM formation by WB (COLLAGEN I and PAI-1) and qRT-PCR (TGFβ3, MMP2, MMP9 and TIMP1).

**RESULTS:** In VitD short-term treatment, *in vivo* monitoring did not show significant differences in LM size. Regarding macroscopic LM size, control group showed a growing trend, while both VitD-treated groups maintained LM size. In addition, any VitD treatment decreased cell proliferation, while only VitD 1µg/kg/day increased apoptotic cells and reduced COLLAGEN I expression (not statistically significant). In the long-term treatment, *in vivo*

monitoring showed a statistically significant reduction of  $^{18}\text{F}$ -FDG uptake in both VitD-treated groups ( $p < 0.05$ ), indicating a reduction in LM size. Likewise, macroscopic LM size diminished significantly in VitD  $1 \mu\text{g}/\text{kg}/\text{day}$  dose group ( $p = 0.025$ ). Besides, the high dose of VitD significantly decreased cell proliferation in LM ( $p = 0.025$ ). Further, CASPASE 3 expression was induced by VitD in a dose-dependent manner and apoptotic cells increased in VitD  $1 \mu\text{g}/\text{kg}/\text{day}$  group ( $p = 0.009$ ). Regarding ECM, VitD treatment decreased COLLAGEN I expression at both doses ( $p < 0.02$ ) and at the highest dose we observed decrease of PAI-1, TGF $\beta$ 3, TIMP1 and increase of MMP2 and MMP9 expression ( $p < 0.05$ ).

**CONCLUSIONS:** VitD short-term treatment is only capable to maintain human uterine LM size, while at long term VitD significantly reduces LM size by cell proliferation inhibition and ECM degradation and apoptosis increase, without side effects. Our data strongly suggest that long-term treatment with VitD could be considered as an effective adjuvant treatment for uterine LM in women.

AC & HF contributed equally.

SUPPORT: P115/00312; P117/01039; P118/00323; CD15/00057; ACIF/2016/444.

**O-209** Wednesday, October 16, 2019 11:45 AM

### MULTI-OMIC ANALYSIS OF UTERINE LEIOMYOMAS IN AFRICAN AMERICANS AND CAUCASIANS: INSIGHTS INTO HEALTH DISPARITY.



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**OBJECTIVE:** Uterine leiomyomas (ULMs) are the most common tumor of the female genital tract. Prevalence of ULMs are higher in African-American (AA), who also experience greater severity of symptoms and different responses to treatment than Caucasian (CA) women.

**DESIGN:** We performed whole genome sequencing (WGS) and in-depth global proteomic analyses of ULMs from Black and White women to evaluate for a biologic basis underlying the health disparities seen in this disease.

**MATERIALS AND METHODS:** Flash-frozen ULMs from AA ( $n = 23$ ) and CA ( $n = 25$ ) patients and matched myometrium ( $n = 20$  total cases) were obtained from a single institution. Tissues were processed for genomic DNA to support WGS analysis (Illumina HiSeq X) or by pressure cycle-assisted lysis and tryptic digestion to support global proteomic analysis using liquid chromatography-tandem mass spectrometry (Thermo Fusion Lumos) using a multiplexed, quantitative proteomics/tandem mass tag-10 labeling strategy. Differential expression and functional inference analyses was performed using commercial and in-house bioinformatic pipelines.

**RESULTS:** Standard estimates of ancestral admixture from WGS data revealed that 95% of self-described AA women were of African descent and 87% of self-described CA women were of European descent, with remaining clustering as admixed American ancestry. Preliminary somatic mutation analyses of a patient subset ( $n = 14$ ) revealed  $n = 5$  AA and  $n = 4$  CA cases ( $> 64\%$ ) exhibited recurrent mutations or deletions in a hotspot window with the *MED12* gene. Proteomic analyses resulted in the quantification of 3,257 total proteins and revealed 401 significantly altered (LIMMA  $P < .05$ ) between AA vs. CA ULMs. Protein alterations suggested marked remodeling of extracellular matrix within AA versus CA ULMs and included diverse collagen (COL3A1, COL5A2 and COL4A1) as well as matrix metalloproteinases (MMP2/10) isoforms. Functional analyses revealed activation of the glycoprotein VI signaling as well as pathways supporting muscle formation and intercellular signaling, but inhibition of JAK-STAT signaling and pathways supporting fibrinogenesis and proliferation of connective tissue cells in AA versus CA ULMs. Comparative analyses of protein alterations with historic differential gene expression analyses comparing 52 ULMs from AA ( $n = 8$ ) and CA ( $n = 3$ ) women (Davis BJ et al, 2013) revealed  $> 41\%$  (168) of proteins altered between AA vs. CA ULMs were also significantly altered at the transcript level and exhibited high correlative abundance trends (Spearman Rho = 0.559,  $P < 0.001$ ).

**CONCLUSIONS:** Quantitative analyses revealed distinct proteomic changes among White and Black ULMs, providing insight into the pathogenesis of disparities seen in this common disease. These findings may also clarify novel therapeutic strategies that support individualized treatment.

**O-210** Wednesday, October 16, 2019 12:00 PM

### PREOPERATIVE TREATMENT WITH LEUPROLIDE ACETATE AND ULIPRISTAL ACETATE BEFORE OUTPATIENT HYSTEROSCOPIC MYOMECTOMY: PROSPECTIVE COMPARATIVE STUDY.



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**OBJECTIVE:** Outpatient hysteroscopic myomectomy can be usually performed in case of single submucosal myoma with largest diameter up to 2 cm. The volume of the myoma has a critical role in outpatient myomectomy because larger myomas require a longer resection time and, thus, these procedures may be less tolerated by patients. One of the major advantages of preoperative therapy is to decrease the volume of uterine myomas. This prospective study compared outpatient hysteroscopic myomectomy performed by using the Versapoint system in patients who received 3-month preoperative treatment with leuprolide acetate (LA), ulipristal acetate (UPA) or who did not receive any preoperative hormonal therapy.

**DESIGN:** Single center prospective non-randomized study.

**MATERIALS AND METHODS:** This study included patients of reproductive age requiring outpatient resection of single FIGO type 0-1 myoma with largest diameter  $< 2$  cm. Exclusion criteria were: previous surgical treatment of uterine myomas, previous administration of hormonal therapies for uterine myomas, additional endometrial conditions requiring hysteroscopic treatment (such as uterine polyps), additional surgical procedures performed by other approaches (such as laparoscopy), suspicion of malignancy. Study patients underwent either preoperative treatment with UPA (5 mg/day; group UPA) or LA (11.25 mg/ml, group LA) for 3 months or immediate surgery (without preoperative hormonal therapy, group S). The choice of receiving preoperative treatment was based on patients' preference. Hysteroscopic myomectomy was performed by using the Versapoint system. The primary objective of the study was to compare the rate of complete resections in the three study groups. The secondary objective of the study was to compare the operative results between the study groups. The tertiary objective of the study was to assess the characteristics of the myomas and the endometrium in patients treated with UPA and LA. Data were analyzed according to intention to treat.

**RESULTS:** 138 patients were included in the study. The percentage decrease in the volume of uterine myomas was higher in patients receiving LA than in those treated with UPA ( $p = 0.015$ ). Before surgery, myoma volume was lower in group LA and in group UPA than in group S ( $p = 0.026$  and  $0.043$ , respectively). The percentage of complete resection was significantly higher in group LA (83.0%; 39/47) than in group UPA (60.5%; 23/38;  $p = 0.020$ ) and in group S (62.2%; 33/53;  $p = 0.021$ ). The volume of fluid infused was significantly lower in group LA than in group S ( $p < 0.005$ ). There was no significant difference in the volume of fluid absorbed between the three study groups ( $p = 0.341$ ). Concerning the characteristics of the endometrium, completely atrophic endometrium was significantly more frequent in the LA group compared with the other study groups. The texture of the myoma was rubbery or soft more frequently in the UPA group than in the other groups.

**CONCLUSIONS:** Compared with UPA or no treatment, LA improves the rates of complete resection in patients undergoing outpatient hysteroscopic myomectomy.

### IVF OUTCOME PREDICTORS 2

**O-211** Wednesday, October 16, 2019 10:45 AM

### IMPACT OF BODY WEIGHT ON OVARIAN RESPONSE AFTER INDIVIDUALIZED OR FIXED FOLLICLE-STIMULATING HORMONE DOSING IN WOMEN UNDERGOING OVARIAN STIMULATION FOR IN VITRO FERTILIZATION.



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Glasgow, Glasgow, United Kingdom; <sup>b</sup>University of Modena and Reggio Emilia and Clinica Eugin Modena, Modena, Italy; <sup>c</sup>IVI Madrid, Rey Juan Carlos University, Madrid, Spain; <sup>d</sup>Ferring Pharmaceuticals, Copenhagen, Denmark.

**OBJECTIVE:** To explore the influence of body weight on ovarian response and related clinical outcomes after individualized follicle-stimulating hormone (FSH) dosing versus fixed starting dosing of 150 IU FSH in women undergoing in vitro fertilization (IVF).

**DESIGN:** Randomized, assessor-blind, controlled trial; 1326 women undergoing their first ovarian stimulation cycle were randomized 1:1 to follitropin delta or follitropin alfa. In the follitropin delta group, women with anti-Müllerian hormone (AMH) <15 pmol/L received fixed daily doses of 12 µg and women with AMH ≥ 15 pmol/L received individualized doses, based on AMH level and body weight. Women randomized to follitropin alfa received a fixed starting dose of 150 IU, regardless of their AMH and body weight.

**MATERIALS AND METHODS:** Oocytes retrieved 36±2 hours after triggering of final follicular maturation were inseminated by IVF or intracytoplasmic sperm injection. Good-quality blastocysts (≥ 3 BB) was based on Gardner-Schoolcraft classification (1999), blastocyst transfer was performed on day 5, and ongoing pregnancy was confirmed by ultrasound at 10-11 weeks after transfer. Data were evaluated descriptively by calculating the mean number of oocytes, good-quality blastocysts, and the ongoing pregnancy rate for nested subgroups of women based on increasing body weight.

**RESULTS:** Exposure to serum FSH showed an inverse relationship with body weight for both follitropin delta and follitropin alfa. In women with AMH <15 pmol/L, the ovarian response in terms of number of oocytes did not show any body weight dependence. In women with AMH ≥ 15 pmol/L treated with follitropin alfa, the number of oocytes decreased from an overall mean of 13 oocytes to a mean of 10 oocytes with increasing body weight. In the individualized follitropin delta group, the number of oocytes was not affected by body weight. Accordingly, the number of good-quality blastocysts decreased with increasing body weight in women with AMH ≥ 15 pmol/L in the follitropin alfa group, from an overall mean of 2.3 to a mean of 2.0, whereas body weight did not affect the number of good-quality blastocysts in the individualized follitropin delta group. The ongoing pregnancy rate after fresh transfer tended to decrease with increasing body weight in the follitropin alfa group but not in the individualized follitropin delta group.

**CONCLUSIONS:** With fixed starting doses of FSH, ovarian response declined with increasing body weight, whereas ovarian response remained stable after individualized FSH dosing.

Reference: **Trial registration number:** NCT01956110.

**SUPPORT:** The study was funded by Ferring Pharmaceuticals, Copenhagen, Denmark.

**O-212** Wednesday, October 16, 2019 11:00 AM

**PREDICTORS OF EARLY PREGNANCY LOSS FOR 7,261 INFERTILE PATIENTS AFTER IN VITRO FERTILIZATION.**

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**OBJECTIVE:** Due to the patients undergo in vitro fertilization (IVF) have a high rate of early pregnancy loss (EPL), our study aims to investigate the predictors of EPL for those patients in order to facilitate early treatment for the high-risk patients.

**DESIGN:** Prospective study.

**MATERIALS AND METHODS:** All participants underwent IVF treatment at our reproductive center between January and December 2017. During this period, 7,286 women were identified with a singleton pregnancy by the first routine TVS at day 27-29 after IVF. The gestational sac diameter (GSD), crown-rump length (CRL), embryonic heart rate (EHR) and yolk sac diameter (YSD) were measured and recorded. Meanwhile, the clinical characteristics were also collected. The first trimester pregnancy outcome of these women was noted at 12 weeks of gestation. Twenty-five cases were lost at follow-up. There were 966 cases of spontaneous miscarriage ≤ 12 weeks of gestation, which were assigned as EPL and 6,295 women with an ongoing pregnancy for >12 weeks of gestation. Multinomial logistic regression analysis was used to identify the probability predictive factors of EPL.

**RESULTS:** Compared with the ongoing pregnancy group, the maternal age (MA), duration of infertility and transfer cycle were significantly higher, and the day14 human chorionic gonadotrophin and the endometrium thickness on transfer day were significantly lower in the EPL group (P < 0.001).

In addition, the GSD (18.5±3.6 vs. 13.2±4.8 mm), CRL (3.5±0.9 vs. 1.2±1.6 mm) and YSD (3.6±0.4 vs. 2.6±1.5 mm) were significantly greater in the ongoing pregnancy group than the EPL group (p < 0.01). There was a higher likelihood of cardiac activity being present in the ongoing pregnancy group (99.2% vs. 39.0%, P < 0.001). However, the presence of intrauterine hematomas (16.0% vs. 18.8%, p = 0.026) and a gestation approximate to the uterine serosa (GATUS, 1.6% vs. 3.1%, p = 0.001) were detected more often in the EPL group.

Finally, GATUS, MA, GSD, CRL, EHR and YSD were identified by multinomial logistic regression model after stepwise screening. The probability of EPL was:  $\exp(z)/(1 + \exp(z))$ , where  $z = 7.339 + (0.723 \times MA) + (0.650 \times GATUS) - (0.664 \times GSD) - (1.272 \times CRL) - (1.719 \times EHR) - (0.632 \times YSD)$ .

**CONCLUSIONS:** The MA, GATUS and GSD, CRL, EHR, YSD on day 27-29 were the probability factors of EPL after IVF. The multinomial logistic model could be used to calculate the probability of EPL and thus, proper early treatment could be applied to the high-risk patients.

TABLE. The predictive factors for the EPL

Parameters	β	OR (95% CI)	P
MA	0.723	2.061 (1.721-2.467)	<0.001
GATUS	0.650	1.916 (1.050-3.497)	0.034
GSD	0.664	0.515 (0.382-0.694)	<0.001
CRL	1.272	0.280 (0.194-0.405)	<0.001
EHR	1.719	0.179 (0.144-0.223)	<0.001
YSD	0.632	0.532 (0.410-0.690)	<0.001
Constant	7.339		

**SUPPORT:** The Science and Technology Project of the Health and Family Planning Commission of Hunan Province (No. C20180289) and the Citic-Xiangya Research Fund (No. KYXM-201703).

**O-213** Wednesday, October 16, 2019 11:15 AM

**THE IMPACT OF AGE BEYOND PLOIDY: OUTCOME DATA FROM 9,101 EUPLOID SINGLE EMBRYO TRANSFERS.**

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**OBJECTIVE:** Rate of embryonic aneuploidy increases significantly with increasing female age and is the primary cause of lower pregnancy and live birth rates observed in older reproductive age women. This study evaluates single euploid embryo transfers to eliminate the impact of aneuploidy on reproductive efficiency. It then seeks to determine if an age-related decline in reproductive efficiency persists indicating that other factors may contribute to impaired outcomes in aging women.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** A total of 9,101 single embryo transfers that had undergone pre-implantation testing for aneuploidy (PGT-A) and cryopreservation were included. These were divided into five groups according to the age of the woman at the time of oocyte retrieval: <35 years old (n=4,585 embryos transferred), 35-37 years old (n=2,272), 38-40 years old (n=1,665), 41-42 years old (n=330), and >42 years old (n=249). Biochemical (positive serum β-hCG 10 days after transfer), clinical (visualized gestational sac), and live birth rates were calculated for each group as percentage of embryos

Age	% of embryos transferred		
	Implantation	Clinical preg.	Live birth
<35	81 %	68 %	59 %
35-37	78 %	65 %	56 %
38-40	78 %	63 %	54 %
41-42	73 %	58 %	51 %
>42	73 %	57 %	51 %
p value	< 0.0001	<0.0001	0.0144

transferred, and then compared using a Chi-square for trend. Similarly, the clinical pregnancy rate was also analyzed for trend as a percentage of biochemical pregnancies, and the live birth rate as a percentage of clinical pregnancies, in order to detect at what stage increasing age has the greatest impact.

**RESULTS:** Implantation rates as a percentage of embryo transfers negatively correlated with oocyte age, with the percentage of embryos transferred ranging from 73.1% in the oldest group to 81.5% in the youngest ( $p < 0.0001$ ). This difference was consistent throughout clinical pregnancy rates (57.4% - 67.5%;  $p < 0.0001$ ), and live birth rates (50.5% - 58.5%;  $p = 0.01$ ). Interestingly, the proportion of clinical pregnancies which were lost did not change with age, strongly suggesting that factors contributing to decline in reproductive potential with age have their impact prior to the establishment of a clinical pregnancy.

**CONCLUSIONS:** Age-related diminution in reproductive efficiency is largely overcome by selection of euploid embryos for transfer. However, an age-related decrease in implantation, clinical pregnancy, and live birth rates persists indicating that aneuploidy is not the only factor contributing to reproductive senescence. The additional factors, which remain to be defined, seem to impact the reproductive process prior to implantation as the overall probability of progressing to delivery after implanting was not impacted by age.

**O-214** Wednesday, October 16, 2019 11:30 AM

### **THREE-DIMENSIONAL ULTRASOUND DIAGNOSIS OF ADENOMYOSIS IS NOT ASSOCIATED WITH DIMINISHED LIVE BIRTH FOLLOWING SINGLE THAWED EUPLOID BLASTOCYST TRANSFER: A PROSPECTIVE COHORT STUDY.**



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**OBJECTIVE:** To evaluate the impact of adenomyosis, diagnosed using three-dimensional ultrasound (3D US), on pregnancy outcomes following single thawed euploid blastocyst transfer.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Patients planning to undergo a single thawed euploid blastocyst transfer between April and December 2017 at a large IVF center were eligible for inclusion. Exclusion criteria were body mass index  $\geq 40$  kg/m<sup>2</sup>, uterine anomalies, history of myomectomy, use of a gestational carrier, and communicating hydrosalpinx. Consenting patients underwent endometrial preparation according to a standardized protocol. On the day prior to embryo transfer, 3D US was performed and images were stored for subsequent evaluation. Subjects then underwent transfer of a single thawed euploid blastocyst and pregnancy outcomes were accrued.

All 3D US images were de-identified and independently reviewed by five reproductive endocrinologists for the presence of seven sonographic features of adenomyosis: globular uterine configuration, asymmetry of the myometrial walls, heterogeneous echotexture, irregular junctional zone, myometrial cysts, linear striations, and presence of an adenomyoma. Adenomyosis was considered to be present if at least three out of five reviewers noted a minimum of one sonographic feature. Inter- and intra-rater agreement was evaluated using Fleiss' kappa. Clinical and cycle characteristics of subjects with and without adenomyosis were compared using Student's *t*-test and chi-square test. The primary outcome of interest was live birth rate. Secondary outcomes included clinical pregnancy rate and miscarriage rate. Logistic regression was performed to account for potential confounders.

**RESULTS:** The prevalence of adenomyosis in the study population was 15.3% (99/648). The inter- and intra-rater agreement amongst five independent reproductive endocrinology and infertility specialists who conducted 3D US assessment of adenomyosis were fair ( $\kappa = 0.23$ ) and moderate ( $\kappa = 0.58$ ), respectively. Subjects with adenomyosis were older (37.1 versus 35.9 years,  $P = 0.02$ ) and more likely to have undergone a GnRH agonist downregulation protocol when compared to subjects without adenomyosis (16.2% vs. 5.1%,  $P = 0.02$ ). Clinical pregnancy (80.0% vs. 75.0%) and live birth rates (69.5% vs. 66.5%) were similar between groups. When adjusting for potential confounders, there was no difference in clinical pregnancy [aOR 1.47 (0.85-2.56)], miscarriage [aOR 1.3 (0.62-2.72)] or live birth [aOR 1.28 (0.78-2.08)] in subjects with adenomyosis and those without adenomyosis. No individual sonographic marker of adenomyosis was found to be predictive of pregnancy outcomes.

**CONCLUSIONS:** Adenomyosis diagnosed on 3D US is not associated with pregnancy outcomes following transfer of a single thawed euploid blas-

tocyst. These findings suggest that routine screening for adenomyosis in an unselected infertile patient population undergoing frozen embryo transfer is not warranted.

**O-215** Wednesday, October 16, 2019 11:45 AM

### **ART OUTCOMES AMONG PRE-PREGNANCY CANCER SURVIVORS: LINKAGE OF MASSACHUSETTS SART CORS AND CANCER REGISTRY.**



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**OBJECTIVE:** To investigate fertility treatment outcomes among childhood, adolescent, and young-adult cancer survivors.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** ART cycles in Massachusetts to women  $\geq 18$  years old from 2004-2013 from the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) ( $n = 90,928$  cycles) were linked to the Massachusetts Cancer Registry. Main outcomes of interest were treatment patterns, number of oocytes retrieved, number of oocytes fertilized with or without ICSI, number of embryos transferred, implantation rates, and clinical intrauterine gestation (CIG). We used generalized estimating equations to take into account multiple pregnancies per woman with a log link and a Poisson distribution to estimate relative risks (RR) and 95% confidence intervals (CI) *a priori* adjusted for maternal age and cycle year. To investigate mechanism of association, we stratified by autologous/donor gametes and compared autologous embryo quality between women with and without cancer history.

**RESULTS:** Among women who utilized ART, 587 (1,273 ART cycles) were childhood and early-life cancer survivors. In crude models, women cancer survivors undergoing ART were more likely to use donor gametes (RR:1.27 (1.01-1.61)) compared to women with no history of cancer, although this attenuated after adjustment for age and cycle year (RR:1.04 (0.82-1.30)). We saw no difference in number of oocytes retrieved (RR: 1.02 (0.96-1.09)) or proportion of oocytes fertilized (RR:0.97 (0.94-1.01)) between autologous cycles with and without a history of cancer, however cancer survivors had higher total FSH administered (3735.9 IU/mL; RR:1.14 (1.09-1.19)) compared to cycles with no history of cancer (3362.2 IU/mL). Among cycle starts, cycles to women with a history of cancer were less likely to result in CIG (RR: 0.71 (0.64-0.78)) compared to cycles without a history of cancer; this relationship was strongest among autologous cycles (RR:0.64 (0.57-0.72)) but absent from donor cycles (RR:1.06 (0.91-1.23)). When restricted to cycles with embryos transferred, there was no difference in CIG between cycles with and without a history of cancer (RR:0.98 (0.90-1.08)). Among autologous single embryo transfers, no significant difference was seen in the proportion of good quality embryos transferred at the cleavage (RR: 1.13 (0.91-1.42)) or blastocyst (RR:1.20 (0.98-1.47)) stage in cancer survivors compared to women with no history of cancer.

**CONCLUSIONS:** Women cancer survivors may require more FSH and potentially different ART protocols compared to women with no history of cancer. Our analyses further suggest that cancer may influence ovarian stimulation response, given that autologous cycles to cancer survivors were less likely to result in CIG among cycle starts but not among embryo transfers. Future studies should investigate stimulation protocols to maximize successful implantation and CIG among women starting ART cycles who have a history of cancer.

**SUPPORT:** NIH R01HD067270.

**O-216** Wednesday, October 16, 2019 12:00 PM

### **ENDOMETRIAL COMPACTION (DECREASED THICKNESS) IN RESPONSE TO PROGESTERONE RESULTS IN HIGHER ONGOING PREGNANCY RATE.**



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**OBJECTIVE:** For a pregnancy to occur, implantation of an embryo into a receptive endometrium is crucial. There are few methods to reliably assess the receptivity of the endometrium during an in-vitro fertilization (IVF) cycle. Some methods are invasive such as endometrial biopsy for histologic dating or for the Endometrial Receptivity Array (ERA) and cannot be done in the cycle of interest. Other non invasive methods that can be performed in the treatment cycle include ultrasound (US) for endometrial pattern & thickness or for sub-endometrial waves. We have previously shown a significant increase in ongoing pregnancy if the endometrium became thinner (compacted) during the progesterone phase in hormonally replaced (HRT) frozen embryo transfer (FET) cycles with untested embryos. The objective of the present study was to evaluate whether endometrial compaction was also associated with improved ongoing pregnancy rates in fresh IVF cycles and in FET cycles involving euploid embryos after preimplantation genetic testing for aneuploidies (PGT-A).

**DESIGN:** A retrospective observational cohort study.

**MATERIALS AND METHODS:** We retrospectively evaluated cycles from 3 cohorts: 271 HRT cycles with untested single blastocyst FET, 250 HRT single FET cycles of euploid embryos, after PGT-A, and 370 cycles of single fresh embryo transfers after controlled ovarian hyperstimulation. We evaluated recorded digital US images of the endometrium using imaging software and measured endometrial thickness. We calculated the change in endometrial thickness from the end of the estrogen stage/trigger day to the day of embryo transfer. We divided the patients into two groups: 1) cycles with a compaction rate of 10% or greater; 2) cycles with no change or an increase in thickness. The primary outcome was ongoing pregnancy defined as visualization of fetal cardiac activity at 12 weeks gestation or later.

**RESULTS:** Similar to our previous findings in HRT cycles with untested single blastocyst transfers, we found a significantly higher ongoing pregnancy rate in the euploid embryo cohort and in the fresh embryo transfer cohort with a 10% or greater compaction of the endometrial lining thickness.

Ongoing pregnancy:

	# of cycles	Compacted	Not Compacted	p Value
Frozen Embryo Transfer	271	43/83 51.8%	45/188 23.9%	<0.001
Euploid embryo FET	250	47/99 47.5%	49/151 32.5%	0.017
Fresh Transfers	370	52/130 40.0%	61/240 25.4%	0.004

**CONCLUSIONS:** Compaction of the endometrial lining results in a better ongoing pregnancy rates in FET cycles with euploid embryos and in fresh embryo transfers. Our results suggest that an US measurement in the estrogen phase and again in the progesterone phase demonstrating endometrial compaction may be a new non-invasive determinant of endometrial receptivity in IVF cycles.

## MALE FACTOR

O-217 Wednesday, October 16, 2019 10:45 AM



### A MICROFLUIDIC SPERM-SORTING DEVICE REDUCES THE PROPORTION OF SPERM WITH DOUBLE STRAND DNA FRAGMENTATION.

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**OBJECTIVE:** To determine whether the use of the microfluidic sperm sorting device Fertile Chip diminishes the proportion of sperm with double strand DNA fragmentation (dsSDF) compared to *swim up*.

**DESIGN:** This is a matched cohort study of samples from nine patients. All were diagnosed with 60% or more dsSDF in their spermatozoa, as assessed by a neutral COMET. The study was approved by the local IRB. The number of patients included was calculated to detect a difference of 20% in dsSDF between study groups, with an alpha risk of 0.05 and a beta risk of 0.05.

**MATERIALS AND METHODS:** One semen sample of each participant was collected for the study. After a basic sperm analysis, a part was frozen and a part was split into two further aliquots; one aliquot was processed using Fertile Chip and then frozen, and the other was processed using *swim up* and then also frozen. The three frozen aliquots were analysed by neutral COMET assay for the detection of dsSDF.

**RESULTS:** The nine patients included in the study had a mean age of 38.9 years (range 34 – 53) and their mean BMI was 26.78 kg/m<sup>2</sup> (range: 20.9 – 32.84). Five of them had a history of miscarriage (range: 1-7). Their basic semen characteristics were: the mean volume was 2.88 ml (range: 1-4); the mean concentration was 94.13 M/ml (range 5.98 - 321.4) and the mean percentage of motile sperm (a+b forms) was 39.77% (range 20.9 - 59.8). Processing semen samples using *swim up* did not change the percentage of spermatozoa with dsSDF (64.8% in the raw samples and 65.1% post *swim up*). On the other hand, microfluidic sorting of the fresh semen sample using Fertile Chip lowered the percentage of dsSDF to 34.9%; a reduction of 45.2% (p<0.001).

**CONCLUSIONS:** The selection of spermatozoa using Fertile Chip diminishes significantly the percentage of spermatozoa with dsSDF, either compared to the fresh ejaculate or after *swim up*. Fertile Chip can be used in patients with a high proportion of spermatozoa carrying dsSDF and undergoing ICSI; although this study did not evaluate reproductive results, it is reasonable to expect an improvement of clinical variables in this kind of patients.

**SUPPORT:** None.

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### A STEP TOWARDS THE AUTOMATION OF INTRACYTOPLASMIC SPERM INJECTION (ICSI): REAL TIME CONFIRMATION OF OOCYTE PENETRATION BY ELECTRICAL RESISTANCE MEASUREMENT.



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**OBJECTIVE:** Automated (robotic) intracytoplasmic sperm injection (ICSI) requires confirmation of plasmatic membrane penetration. Visual assessment using image processing algorithms have been developed but remain unreliable. We hypothesized that an increase in electrical resistance upon oocyte plasmatic membrane piercing during ICSI can serve as an objective tool to confirm oocyte penetration.

**DESIGN:** Experimental study.

**MATERIALS AND METHODS:** Oocyte membrane piercing with the ICSI pipette was performed by advancing the pipette towards mature (metaphase II) oocytes collected from 6 to 12-week-old mice and immature (germinal vesicle stage and metaphase I) oocytes donated by women who underwent oocyte retrieval. Electrical resistance at the ICSI pipette tip was measured using a conventional electrophysiological setup that includes an electrical resistance meter and two electrical wires located in the lumens of the holding and ICSI pipettes. Our mouse experiments included four groups: a study group, two negative and one positive penetration control groups. In the study group, egg penetration was determined visually by 3 investigators through 2D light microscopy. The first negative penetration control group consisted of oocytes with fragmented membrane (non-viable). In the second negative control group, the ICSI pipette tip was advanced to the perivitelline space and the absence of oocyte penetration was confirmed by applying fluid pressure to demonstrate oocyte compression and zona pellucida expansion. In the positive penetration control group, the plasmatic membrane was ruptured after the application of pressure through the pipette tip (confirming that the tip was inside the egg and not in the perivitelline space). Our human oocyte experiments included two groups: morphologically normal (viable) and fragmented (non-viable) oocytes, as determined visually by two investigators through 2D light microscopy. In all experiments, median resistance changes and their ranges were calculated.

**RESULTS:** In mouse oocytes, significant electrical resistance (**R in mΩ**) increases were detected in all positive penetration control group cases (n=11): ΔR = 8.2 (3.0 – 106.0), P < 0.001. In these cases, rupturing the

membrane, by positive pressure, led to an immediate resistance drop to around the extracellular resistance values. In the two negative penetration control groups (n=19), no significant resistance changes were detected. In the study group (n=45), resistance increase was detected after visual observation of egg penetration:  $\Delta R = 6.5$  (0.1 – 191.7),  $P < 0.001$ . In human oocytes, a marked increase in resistance was observed in all visually normal (viable) oocytes (n=28):  $\Delta R = 2.2$  (0.9 - 6.7),  $P < 0.001$ . In the fragmented/non-viable oocytes (n=6), no significant change in resistance was detected.

**CONCLUSIONS:** An electrical resistance increase can serve as a reliable tool to confirm oocyte penetration, independent of optical visualization. Following further validation and safety assessment, this technology can potentially be integrated into manual or robotic ICSI systems.

References: 1.Permans S, Grant E, Walker GM, Yoder JA. A review of automated microinjection systems for single cells in the embryogenesis stage. *IEEE/ASME Transactions on Mechatronics*. 2016 Oct;21(5):2391-404.

2.Saadat M, Hajiyavand A, Singh Bedi AP. Oocyte Positional Recognition for Automatic Manipulation in ICSI. *Micromachines*. 2018 Sep;9(9):429.

3.Zhang Y, Chen X, Gueydan C, Han J. Plasma membrane changes during programmed cell deaths. *Cell research*. 2018 Jan;28(1):9.

4.Narahashi T. Principles of electrophysiology: An overview. *Current protocols in toxicology*. 2003 Aug 1;17(1):11-0.

O-219 Wednesday, October 16, 2019 11:15 AM

### **SIMPLE VITRIFICATION OF A SMALL NUMBER OF TESTICULAR SPERMATOZOA USING RAPID-I CARRIERS IN NON-OBSTRUCTIVE AZOOSPERMIA.** Yoza Nagao, MS, Keiko Tanaka, MS, Hitomi Otsubo, BS, Shigetoshi Mizumoto, Ph.D., Takeshi Kuramoto, M.D., Ph.D., Masao Murakami, PhD. Kuramoto Women's Clinic, Fukuoka, Japan.



**OBJECTIVE:** Testicular sperm extraction (TESE) combined with ICSI has made biological fatherhood possible for many men with non-obstructive azoospermia (NOA), the most severe form of male infertility. For the men with a limited number of testicular spermatozoa, efficient sperm storage is crucial to avoid complications related to repeated TESEs in cases of failed ICSI cycles. However, reports on ideal carriers and techniques for cryopreserving a small number of spermatozoa that can be used universally are still limited. In this study, the clinical efficacy of our method for vitrifying a small number of spermatozoa using Rapid-i carriers (ESHRE 2011) was evaluated for men with NOA and a small number of testicular spermatozoa.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Vitrification of a small number of spermatozoa was performed for 14 men with NOA following conventional (3 men) or micro-dissection (11 men) TESE from February 2012 to January 2019. Vitrification: 1–10 spermatozoa were aspirated into an ICSI pipette and added to 1.5  $\mu$ L of cryoprotective solution (K-SISC, Cook Medical) on a Rapid-i carrier strip (Vitrolife), which was placed in LN<sub>2</sub> vapor (2 min), inserted into a pre-cooled RapidStraw (Vitrolife), ultrasonically sealed, and plunged into LN<sub>2</sub>. Warming: the cryopreserved strip was warmed in mineral oil and placed in a 2- $\mu$ L droplet of K-SISC. Spermatozoa were recovered using an ICSI pipette and placed in a 2- $\mu$ L droplet of modified HTF medium. After ICSI using warmed spermatozoa, 2PN oocytes and good-quality embryos were vitrified by day 6. Data for vitrified ET performed until the end of March 2019 were analyzed.

**RESULTS:** In total, 409 spermatozoa (78% motile) were vitrified. The average number of vitrified spermatozoa per patient was  $29.2 \pm 3.4$ . During ICSI (22 cycles), 219 spermatozoa were warmed; the sperm recovery rate was 87%. The average number of warmed spermatozoa per patient was  $11.9 \pm 1.8$ . The motile sperm rate per recovered spermatozoa was 49%. The fertilization rate was 31%. Of the warmed ET cycles (17 cycles), clinical pregnancy rate per ET, live delivery rate per ET (not including 2 ongoing pregnancies), and miscarriage rate per pregnancy were 41%, 18%, and 29%, respectively.

**CONCLUSIONS:** In this study, we report 87% sperm recovery rate and the first successful use of warmed spermatozoa for ICSI-ET with our modification of the Rapid-i protocol in men with NOA and a small number of spermatozoa following TESE. Our approach shortens the laborious and time-consuming search for warmed individual spermatozoa from hours to minutes. Thus, in addition to avoiding repeated TESEs, this method may provide benefits in men with a small number of spermatozoa, such as improving ICSI outcomes and avoiding the risk of finding no sperm on the day of oocyte retrieval. Although NOA is relatively rare in the overall population, follow-up studies with a larger cohort are warranted to validate the efficacy of the approach.

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### **COMPARISON OF CRYOPROTECTANT FREE VITRIFICATION OF HUMAN SPERMATOZOA IN A NEW SEMEN SIMULANT WITH RAPID FREEZE OF SPERMATOZOA IN GLYCEROL.** Soundarya Janani Senthil Kumar, MBBS, M.Sc.<sup>a</sup> N. Sanjeeva Reddy, MD (Obstetrics and Gynaecology), DGO,<sup>b</sup> Manjula Daniel G, PhD,<sup>c</sup> Sindhuja Namboori Srinivasan, MBBS, M.Sc Clinical Embryology, PhD Research Scholar.<sup>d</sup>



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**OBJECTIVE:** Seminal fluid contains materials with cryoprotectant properties. However, ROS and free radicals generated by the spermatozoa and seminal leukocytes within the semen may have a detrimental role in the protection of spermatozoa during cryopreservation. The purpose of this study was to formulate a new medium similar to the seminal fluid and to compare the effects of vitrification of sperms in the newly prepared semen simulant medium with rapid freezing of sperms in glycerol and to survey the effect of these two methods on the sperm quality parameters and DNA fragmentation after thawing/warming.

**DESIGN:** This prospective study was done in 100 normozoospermic semen samples from male partners who attended our infertility clinic from February 2018 to February 2019.

**MATERIALS AND METHODS:** A semen simulant embodying the salient physical and chemical properties of human semen was prepared in-house. It had all energy sources including fructose, glucose, pyruvate and lactate along with antioxidants such as ascorbic acid and urate that are naturally present in the seminal fluid. Consenting men attending the infertility clinic provided their semen samples. The sperm quality parameters were analysed according to WHO 2010 and the DNA fragmentation Index (DFI) was analysed by sperm chromatin dispersion test. Each sample was divided into two aliquots, one half was vitrified in the semen simulant and the other half was frozen by rapid freeze in Glycerol. Post-thaw samples were subjected to all the tests performed in the fresh semen sample and the results were compared. Statistical analysis was done with IBM.SPSS software. Repeated measures of ANOVA was used for multiple comparison. The probability value 0.05 was considered as significant level.

**RESULTS:** There was a significant increase in the total motility and vitality in the samples vitrified with the semen simulant after warming when compared with the rapid freeze group (mean  $\pm$  SD Total motility:  $31.85 \pm 8.52$  vs  $29.53 \pm 8.15$  p-0.001; Vitality: 45.84% vs. 43.65% p-0.034 respectively). The percentage of normal morphology in the samples frozen by rapid freeze with glycerol was significantly lower in comparison with those vitrified in the simulant ( $1.33 \pm 0.33$  % vs.  $4.57 \pm 2.05$  % p- 0.0005). There was a significant increase in DFI in the samples frozen by rapid freeze when compared to those frozen-warmed by vitrification with the semen simulant ( $32.7 \pm 6.68$  % vs.  $26.10 \pm 5.45$  % p-0.0005).

**CONCLUSIONS:** The total motility, viability and the sperm chromatin integrity was comparatively better in the sperms vitrified in the semen simulant. The current work assumes that the main cause of damage in the rapid freezing group was osmotic shock, because it requires cryoprotective agent (CPA). On the other hand, CPA was not used in vitrification and the speed of cooling is high, avoiding extracellular ice formation. In conclusion, vitrification in the semen simulant medium has great potential for human sperm cryopreservation and does not require CPA. Due to the cost effectiveness and inhouse preparation, vitrification in the semen simulant could be an effective alternative for commercial media.

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### **SURGICAL SPERM EXTRACTION VS. SEMEN CENTRIFUGATION: METHOD OF SPERMATOZOA RECOVERY DOES NOT CORRELATE WITH EUPLOIDY RATES IN PATIENTS WITH CRYPTOZOOSPERMIA.** Carlos Hernandez-Nieto, MD,<sup>a</sup> Joseph A. Lee, BA,<sup>a</sup> Martha Luna-Rojas, MD,<sup>a</sup> Tamar Alkon, MD,<sup>a</sup> Christine Britton-Jones, PhD, HCLD,<sup>a</sup> Natan Bar-Chama, MD,<sup>b</sup> Alan B. Copperman, MD,<sup>b</sup> Benjamin Sandler, MD.<sup>b</sup>



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**OBJECTIVE:** Cryptozoospermia is defined as spermatozoa not identified in the ejaculate, but observed in pellet following centrifugation (World Health Organization). Fertility specialists differ in opinion whether there might be benefits to surgically retrieving sperm in these patients. Previous studies have described a correlation between testicular extracted sperm and spermatid aneuploidy in patients with non-obstructive azoospermia (1). However, there are currently no peer reviewed publications associating rates of embryonic ploidy with Cryptozoospermia. The aim of this study is to evaluate the rate of embryonic ploidy in blastocysts derived from testicular versus ejaculated sperm in cryptozoospermic patients.

**DESIGN:** Retrospective cohort analysis.

**MATERIALS AND METHODS:** The study included couples who suffer from Cryptozoospermia and underwent an autologous IVF cycle(s) with pre-implantation genetic testing (PGT-A) from 2014 to 2019. Only cases where oocyte insemination was conducted with intra-cytoplasmic sperm injection (ICSI) were evaluated. Cohorts were separated based on the source of sperm (Ejaculated vs. Testicular (TESE)). Demographic and clinical embryology parameters were compared. Student's t-test, Wilcoxon' rank test, chi-square test, and multivariate logistic regression models were used for data analysis.

**RESULTS:** Of the 87 IVF/PGT-A cases on cryptozoospermia patients (n=573 blastocysts) included, 74 cases (n= 474 blastocysts) utilized ejaculated sperm while 13 cases (n= 99 blastocysts) utilized testicular sperm. No significant differences were found in demographic and stimulation parameters among cohorts. No differences between the ejaculated and testicular cohorts were found in fertilization (63.2%; 61.1%, p=0.32); blastulation (64.5%; 66.6%, p=0.69); and rate of embryo euploidy (49.7%; 52.1%, p=0.76) respectively. No differences were found in rate of cycle cancellation due to unavailable embryos for TE biopsy (18.9% vs 7.6%, p=0.32). After adjusting for female and male's age, BMI, AMH, and number of biopsied embryos, there were no association with utilizing surgical extracted sperm and lower odds of embryo euploidy (OR 0.69, CI95% 0.11-4.3, p=0.69).

**CONCLUSIONS:** Normal chromosomal composition is a primary driver of embryonic competence and reproductive success in patients undergoing ART. In our review of the literature, this is the first study analyzing the euploidy rate on a large cohort of embryos in patients with Cryptozoospermia. Our data demonstrate that the odds of the resulting embryo being euploid is not associated with the source of sperm recovery. Regardless of the method of collection, a number of researchers have raised concerns about genetic and epigenetic risks of utilizing sperm cells prone to increased DNA integrity damage or exposed to different environmental factors (i.e. free oxygen radicals). Our study findings show that there is no genomic advantage to surgical sperm retrieval in cryptozoospermic patients. These data can be used to counsel patients who suffer from cryptozoospermia about the potential chromosomal composition of their embryos.

Reference: 1. Weng SP, Surrey MW, Danzer HC, Hill DL, Chen PC, Wu TC. Chromosome abnormalities in embryos derived from microsurgical epididymal sperm aspiration and testicular sperm extraction. *Taiwan J Obstet Gynecol.* 2014 Jun;53(2):202-5.

**SUPPORT:** None.

O-222 Wednesday, October 16, 2019 12:00 PM

### TRANSIT THROUGH THE MALE GENITAL TRACT INCREASES SPERM CHROMATIN FRAGMENTATION.

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**OBJECTIVE:** To map the level of sperm chromatin fragmentation (SCF) at different areas of the male genital tract.

**DESIGN:** Male partners from consenting couples had their ejaculated specimens screened for SCF with a commercially available kit. ICSI clinical outcomes were compared between those with normal and abnormal levels of SCF.

Men with abnormal SCF had the option to undergo spermatozoa retrieval from the vas deferens, epididymis, and testes to be concurrently screened for SCF.

Clinical outcomes were compared between the ejaculated and surgically retrieved (SR) spermatozoa. Encouraged by these results, men with ICSI fail-

ure and high SCF in their ejaculate agreed to undergo surgical spermatozoa retrieval and ICSI at our center, and the results were compared to their historical ejaculated cycles.

**MATERIALS AND METHODS:** SCF levels were assessed by terminal deoxynucleotidyl dUTP nick-end labeling (TUNEL). A threshold of <15% was considered normal, with at least 500 spermatozoa assessed per patient. ICSI was performed in the standard fashion, and the outcome was recorded.

**RESULTS:** A total of 200 couples underwent 439 ICSI cycles utilizing spermatozoa with normal SCF. When these outcomes were compared with those from 122 couples who underwent 278 ICSI cycles utilizing spermatozoa with abnormal SCF (9.3±3% vs. 22.6±9%;  $P < 0.01$ ), the results demonstrated that high SCF hindered implantation rates ( $P < 0.05$ ).

Topographical mapping through the male genital tract showed that SCF was 20.4±9.6% in the vas deferens, 15.8±7.7% in the epididymis, and 11.4±5.7% in the testis, which was much lower compared to the ejaculated control (32.9±20%;  $P < 0.05$ ).

Couples (n=25) who underwent ICSI with SR spermatozoa had lower SCF ( $P < 0.0001$ ) and higher implantation, clinical pregnancy (CP), and delivery rates ( $P < 0.05$ ). Epididymal spermatozoa performance was superior to both ejaculated and testicular sperm for implantation, CP, and delivery rates ( $P < 0.01$ ).

Finally, couples (n=45) with a history at other medical institutions of ICSI failure with ejaculated spermatozoa were treated solely with SR spermatozoa at our center. When compared to the historical cycles, SR spermatozoa had lower fertilization rates ( $P < 0.05$ ) but enhanced implantation (19.1%), CP (40.0%), and delivery rates (34.3%;  $P < 0.01$ ), with epididymal spermatozoa performing even better ( $P < 0.001$ ).

**CONCLUSIONS:** For the first time, we have demonstrated that SCF increases progressively through the testicle, to the epididymis, the vas deferens, and is highest in the ejaculate. Reproductive physicians can guide their patients toward the use of SR spermatozoa, which can enhance the success of reproductive treatments.

## MALE REPRODUCTION AND UROLOGY: RESEARCH

O-223 Wednesday, October 16, 2019 10:45 AM

### A NOVEL MOUSE MODEL TO INVESTIGATE PLACEMENT, PROCESSING AND REMOVAL OF SPERM PROTAMINES.

Samantha B. Schon, MD, MTR,<sup>a</sup> Lindsay Moritz, BS,<sup>b</sup> Sue Hammoud, Ph.D.<sup>a</sup> <sup>a</sup>University of Michigan, Ann Arbor, MI; <sup>b</sup>University of Michigan, Cellular and Molecular Biology Graduate Program, Ann Arbor, MI.



**OBJECTIVE:** Protamines, consisting of protamine 1 (P1) and protamine 2 (P2) are essential for packaging paternal DNA into the sperm nucleus. Proper histone-to-protamine exchange is critical for normal fertility with aberrations in this process associated with infertility, altered semen parameters, decreased fertilization rates in couples undergoing IVF and even decreased pregnancy rates. Despite their critical importance, our understanding of the mechanism by which protamines are processed, placed or removed from DNA remains poorly understood due to the unavailability and/or unreliability of commercially available antibodies. To circumvent these limitations, the objective of our study was to generate a novel mouse model with the endogenous P2 loci epitope-tagged and to identify novel interacting proteins to gain insight into P2 placement, processing and removal.

**DESIGN:** Laboratory experiments utilizing transgenic murine testes and sperm.

**MATERIALS AND METHODS:** Epitope-tagged P2 mice were generated via CRISPR/Cas9. Incorporation of two tags was validated with western blot and immunofluorescence (IF). Phenotypic and fertility assessments were performed using testes and epididymal weights, sperm counts, motility assessment and breeding trials. Immunoprecipitation followed by mass spectrometry (IP-MS) from whole testes using both transgenic (P2<sup>Tg/+</sup>) and wild-type control mice was performed for identification of interacting proteins. Newly identified proteins were validated via reciprocal IP-MS, western blot and IF.

**RESULTS:** We demonstrate successful incorporation of both of the two tags in sperm. P2<sup>Tg/+</sup> and P2<sup>Tg/Tg</sup> mice are fertile with normal litter size and fertility parameters. IP-MS revealed over 500 interacting proteins, a

number of which have been validated and are known to have enzymatic or chaperone/chromatin remodeling roles in other cell types.

**CONCLUSIONS:** We have successfully generated an epitope-tagged protamine 2 transgenic mouse. Through IP-MS we have further identified a number of candidate interacting proteins. Future studies will focus on continued validation of these proteins and investigation of their specific functions. This work is critical to elucidating the currently unknown mechanism by which protamines are placed, processed and removed in both sperm the early embryo.

**SUPPORT:** 5K12HD065257-07 (SBS) and 1DP2HD091949-01 (SSH).

**O-224** Wednesday, October 16, 2019 11:00 AM

**POLYMORPHISMS IN THE HUMAN *PRDM9* GENE MAY LEAD TO MEIOTIC ARREST AND AZOOSPERMIA.**

M. Blake Evans, DO, Sherry Ralls, BA, Mohamed Mahgoub Mohamed, MD, PhD, Alan H. DeCherney, MD, Todd Macfarlan, PhD NIH-NICHD, Bethesda, MD.



**OBJECTIVE:** *PRDM9* is responsible for directing the location of programmed double strand breaks and subsequent crossover events between homologous chromosomes during meiosis in human gametes. *Prdm9* is also essential for meiosis and fertility in mice, with *PRDM9* knockout males displaying complete azoospermia. *PRDM9* contains a rapidly evolving DNA binding zinc finger array that is coded by a ~84 nucleotide repeating unit mini-satellite sequence. The human A-type allele, which accounts for ~90% of the alleles in the human population, contains 13 repeating units. We sought to develop a strategy to effectively genotype this mini-satellite with PacBio sequencing and to determine whether *PRDM9* variation, including mini-satellite length polymorphisms, is associated with infertility in the human male.

**DESIGN:** Observational study.

**MATERIALS AND METHODS:** Using a normospermic human control, a two-step polymerase chain reaction (PCR) protocol was established to successfully amplify the *PRDM9* zinc finger array mini-satellite and sequence it using both Sanger and PacBio next generation sequencing, which has the advantage of circular consensus sequencing and long reads. We next amplified *PRDM9* from the genomic DNA of 48 azoospermic men and 5 controls. The samples were visually analyzed with gel electrophoresis, and potential mutant alleles that had an atypical band appearance were compared to the wild type.

**RESULTS:** PacBio sequencing results from the normospermic control were found to be an identical match to the known human A allele, confirming our ability to effectively genotype the mini-satellite array in a single PacBio run. Gel electrophoresis of PCR amplified *PRDM9* alleles from azoospermic men identified 6 potential mutant variants of distinct mini-satellite lengths differing from the A-allele.

**CONCLUSIONS:** We have developed a protocol to effectively genotype the human *PRDM9* zinc finger array mini-satellite to evaluate a potential etiology of azoospermia in the infertile human male. We found 6 potential *PRDM9* alleles differing from the known A-allele in a small (n=48) azoospermia cohort. Current/ongoing research includes applying our PacBio sequencing protocol to genotype all 48 azoospermic men in comparison to a control group and evaluate if there is an association between *PRDM9* mini-satellite repeat length, polymorphisms, or de novo mutations and azoospermia.

**O-225** Wednesday, October 16, 2019 11:15 AM

**A RANDOMIZED CONTROLLED ANIMAL TRIAL: EFFICACY OF A 4K3D VIDEO MICROSCOPE VERSUS AN OPTICAL OPERATING MICROSCOPE FOR UROLOGIC MICROSURGERY.**

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**OBJECTIVE:** Operating microscopes continue to use classical optics to provide magnification whereas video microscopy utilizes an image sensor and video monitors. Early video microscopes lacked the technology to pro-

duce a comparable experience to optic-based systems. Modern 4K3D video surgical systems can theoretically outperform traditional optics in flexibility, working depth and user ergonomics. We conducted a randomized controlled trial in rats comparing the efficacy and safety of a 4K3D video microscope to a traditional operating microscope.

**DESIGN:** Randomized controlled animal trial.

**MATERIALS AND METHODS:** The FDA approved ORBEye surgical platform (Olympus/Sony), which provides 4K3D video images via light-weight eyewear, was compared to a traditional operating microscope (S3, Zeiss). Male Wistar rats weighing 250 - 375 gm were used. Each rat underwent vasovasostomy (VV) and vasopididymostomy (VE) with a single-armed needle microsurgical technique, with laterality deemed by coin toss. 16 animals were randomized just prior to incision to either operating microscope. An additional eight rats were used as shams, and another 2 were used for initial training on the ORBEye. All animals were euthanized 6 weeks post-op. Operating time per anastomosis, patency, and sperm granuloma formation were compared for each arm. Prism v7 (GraphPad Software) was used for statistical analyses.

**RESULTS:** 23 rats survived to week-6 post-op. VV patency rates were 57.1 and 62.5% (p = 0.9) for the S3 and ORBEye, respectively. VE patency rates were 87.5 and 75% (p = 0.9) for the S3 and ORBEye, respectively. There was no statistical difference in granuloma formation for either VV (p = 0.9) or VE (p = 0.6). Granuloma size did not differ significantly for VV (S3 7mm vs. ORBEye 10mm, p = 0.3) or for VE (2mm vs. 7mm, p = 0.2). Anastomosis time was not different between the two microscopes (VV: S3 31min vs. ORBEye 36.5min, p = 0.2; VE: 29min vs. 33min, p = 0.1).

**CONCLUSIONS:** Using a well-established microsurgery training model, performance of the ORBEye did not differ from a traditional operating microscope in terms of patency, granuloma formation, or operative time. Based on this data, the ORBEye appears to be noninferior for urologic microsurgery procedures. The system can be applied to microsurgery in any specialty. Further study is warranted to substantiate these results and to assess for meaningful differences in working depth and user ergonomics.

**SUPPORT:** The ORBEye was provided on loan from the Olympus Corporation of America.

**O-226** Wednesday, October 16, 2019 11:30 AM

**MALE PARTNER AGE AND THE RISK OF ISCHEMIC PLACENTAL DISEASE IN AUTOLOGOUS IVF PREGNANCIES.**

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**OBJECTIVE:** Advanced maternal age has been extensively studied and is a known risk factor for adverse pregnancy outcomes including ischemic placental disease (IPD), defined as the obstetrical diagnosis of preeclampsia, small for gestational age (SGA), or placental abruption. Advancing paternal age has been associated with decreased fecundity, early pregnancy loss, and adverse outcomes for the offspring; however, little is known about the impact of advanced paternal age on IPD. Our objective was to evaluate the association between male partner age and the risk of IPD among pregnancies 20 weeks of gestation or greater.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** We identified all deliveries from January 1, 2004 to December 31, 2013 at a tertiary hospital that resulted from autologous in vitro fertilization (IVF) cycles. Cycles using donor sperm were excluded. Male partner age at the time of oocyte retrieval was categorized as <30, 30-<35, 35-<40, and 40 years or older. Couples whose male partner was 30-<35 was used as the reference group. IPD was defined as preeclampsia, placental abruption, SGA, or intrauterine fetal demise due to placental insufficiency. We identified pregnancies complicated by preeclampsia or placental abruption using ICD-9 codes and medical record review. We defined SGA as <10<sup>th</sup> percentile using gestational age and sex-adjusted U.S. growth curves. All IUFDs were reviewed in the medical record to determine the cause, if known. We used log-binomial regression and generalized estimating equations with an independent correlation matrix to estimate risk ratios (RR) and 95% confidence intervals (CI), accounting for multiple

pregnancies per woman. All models were adjusted for maternal age, paternal age, year of delivery, cycle number, and nulliparity.

**RESULTS:** We identified 1,133 deliveries from 1,023 couples. The overall incidence of IPD was 26.4%. The risk of IPD was similar across categories of male age (range: 23.0-29.4%). When compared to couples with a male partner 30-<35 years of age, the risk of IPD was 0.72 (95% CI 0.43-1.2) in the male age <30 group, 0.89 (95% CI 0.65-1.2) in the male age 35-<40 group, and 1.1 (95% CI 0.60-1.9) in the male age 40+ group. When evaluating subgroups of IPD, compared to couples whose male partner was 30-<35, deliveries from couples whose male partner was <30 had a lower risk of SGA (RR 0.28, 95% CI 0.10-0.76). The risk of the other individual components of IPD was similar in all of the male partner age categories.

**CONCLUSIONS:** There is no association between male partner age and the risk of IPD; however, the risk of SGA is lower in the youngest male age category. Larger studies are needed to confirm these findings.

O-227 Wednesday, October 16, 2019 11:45 AM

### EVALUATION OF SPERM PROTEOME IN CANCER PATIENTS PRIOR TO TREATMENT.

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**OBJECTIVE:** Cancer has an adverse effect on sperm health. Conventional semen analysis does not explain the fertility status of cancer patients. Currently, proteomics is being used as a powerful tool to identify the fertility associated molecular pathways affected in spermatozoa. The objective of this study was to evaluate the sperm proteome of cancer patients compared with healthy fertile men and infertile men.

**DESIGN:** Cryopreserved semen samples of cancer patients before starting cancer therapy were used in the current study. Type of cancer patients included were: Testicular cancer (n=28), Hodgkin's disease (n=20), Lymphoma (n=8) and Leukemia (n=5). Pooled samples from the cancer patients were used for proteomic analysis. The proteome of cancer group, was compared with fertile men (n=7) and infertile men (n=9).

**MATERIALS AND METHODS:** Proteomic profiling of sperm (cancer patients, fertile men, and infertile men) was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Proteins and peptides were identified using search programs MASCOT and SEQUEST. Sperm proteins of cancer patient group were compared separately with fertile and infertile men groups. Differentially expressed proteins (DEPs) obtained from two different analysis were subjected to comparison analysis using ingenuity pathway analysis (IPA) software.

**RESULTS:** The functional bioinformatic analysis revealed that proteins associated with mitochondrial dysfunction, oxidative phosphorylation, and sirtuin signaling pathways are dysregulated in cancer patients in comparison to fertile and infertile men. Furthermore, comparison analysis of two sets of DEPs predicted deactivation of oxidative phosphorylation and TCA cycle (Table 1).

**CONCLUSIONS:** Current proteomic findings indicate that the cellular pathways associated with oxidative phosphorylation and TCA cycle are affected in spermatozoa of cancer patients. Further in-depth investigation and validation of specific proteins associated with both the pathways in cancer patients are warranted.

TABLE 1. Deactivated pathways in spermatozoa of cancer patients

Pathways	Z score*	
	Cancer vs. Fertile	Cancer vs. Infertile
Oxidative Phosphorylation	-3.46	-4.00
TCA cycle II	-2.45	-2.45
Fatty acid $\beta$ -oxidation I	-2.00	-2.24
Glycolysis I	-2.24	-2.00

\*Considered significant when Z score is >2 or <-2.

Reference: None.  
SUPPORT: None.

O-228 Wednesday, October 16, 2019 12:00 PM

### NOVEL EX VIVO CULTURE OF NEONATAL MOUSE TESTICULAR ORGANOID MAINTAINED IN A HANGING DROPLET WITH RETINOIC ACID.

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**OBJECTIVE:** To propose a method to sustain germ cell characteristics of neonatal germinal epithelial cells in the form of self-organized organoids and initiate meiotic differentiation under the exposure of retinoic acid (RA).

**DESIGN:** We tested the feasibility of utilizing an ex vivo three-dimensional culture system maintained in a hanging droplet (HD) to sustain and induce maturation of murine neonatal testicular cells.

**MATERIALS AND METHODS:** After trypsinization of 5-day-old newborn mouse testicular tissue, isolated cells were cultured in medium designed for spermatogonial stem cells (SSCs) composed of DMEM/F12 with GDNF, FGF2, 2-mercaptoethanol, L-glutamine, and B27 supplement in a gelatin-treated well for 3 days. Cell culture was then trypsinized and washed in SSC medium. The resulting cell pellet was resuspended in SSC medium void (control) or with 1  $\mu$ M RA (HDRA). Cell suspensions were then adjusted to 40,000 cells/ml, and approximately 1,000 testicular cells were placed in each 25- $\mu$ l HD. Cell characterization was performed every 3 days by germ cell stage-specific markers on an H&E-stained background.

**RESULTS:** After culturing neonatal testicular cells in HDs, initial aggregation was observed 48 hours after HD culture. The earliest complete self-formation of spheroidal organoids was observed at day 3 for both control and HDRA. In the control group, continuing and consistent expression of OCT4 (>70%) and nuclear DAZL (>75%) throughout the experiment until day 21 determined that the SSCs retain stemness. In HDRA, a downregulated expression of OCT4 was recorded as early as day 3 in approximately 50% of the cells. A shift from nuclear to perinuclear positivity of DAZL in 16% of the cells in the HDRA group at day 21 confirmed differentiation into spermatocytes. Cytoplasmic VASA expression in the HDRA group confirmed meiotic/post-meiotic differentiation of the germ cells. Positive vimentin staining in 25% of the cells indicated the presence of nurturing pre-Sertoli cells in both groups.

**CONCLUSIONS:** The attempt to maintain germ cell characteristics of neonatal testicular cells in the form of self-organized organoids appears to be an effective strategy for studying ex vivo spermatogenesis in the long-term. With the essential supplement of RA, germinal epithelial maturation was achieved. Once the ability to induce late-stage gametogenesis is confirmed, this technique may benefit cancer survivors who underwent gonadotoxic therapy in prepubertal age with irreversible damage of the germinal epithelium.

### NURSING

O-229 Wednesday, October 16, 2019 10:45 AM

### TRIGGER PREPARATION ONLINE VIDEOS IMPROVE WORKPLACE EFFICIENCY & NURSING SATISFACTION.

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**OBJECTIVE:** To test the hypothesis that implementation of tailored ovulation trigger injection instruction videos would (1) reduce average nursing time spent counseling a patient for trigger by 20%, (2) reduce overall calls made to the after-hours physician regarding trigger instructions, (3) increase average nursing and patient satisfaction scores.

**DESIGN:** Prospective Intervention Trial.

**MATERIALS AND METHODS:** Nine total videos were created to instruct patients on how to mix and inject specified doses of Human

### THE EFFECTIVENESS OF EDUCATION USING VIDEOS WITH SMART PHONES AND A BOOKLET ON FERTILITY WOMEN - FOCUSED ON THE PROCESS OF IN VITRO FERTILIZATION.



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**OBJECTIVE:** The purpose of this study was to develop a 'Q&A related to in vitro fertilization' for the first-time trial women and to produce a booklet with the contents of the existing video education 'In Vitro fertilization' and compare the effectiveness of education through knowledge and educational satisfaction surveys and to find a more effective educational plan for women with in vitro fertilization.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** From September 10th, 2016 to October 25th, 2016, a total of 131 women who participated in the first trial of in vitro fertilization in the CHA Fertility Center Seoul Station, and 9 participants were eliminated those who they did not see the educational data to the end or did not respond to the questionnaire sincerely. The selection of educational materials was selected by the participants. The selection result was selected by a total of 122 participants; 45 pamphlets, 35 videos, and 42 videos and pamphlets. The survey tool, Knowledge Measurement Problem Questionnaire, consisted of 17 items by the validity of the contents of the expert group and the difficulty of the content of the general population. It modified and supplemented education satisfaction items in web-based virtual classrooms developed by Jeong In-seong and Lim Jung-hoon (1999). The reliability of the tool was  $\alpha = 0.895$  for Cronbach. The participants were asked about the level of knowledge and satisfaction of education on the 7th - 8th day of menstruation, which is the next hospital visit. Data analysis was performed using SPSS WIN 21.0. The general characteristics of the participants were asked by descriptive statistics and frequency analysis. ANOVA and crossover analysis were used for homogeneity. ANOVA, Scheffe, regression analysis and correlation analysis were used respectively. Percentage of correct answer which can be an important parameter of the paper was calculated as a percentage using the right answer and the number of samples \*100.

**RESULTS:** As a result of this study, the total score of the knowledge level items was the highest with correct answer rate of 86.83%, and followed by the booklet group with 85.49% and the video group was 82.02%. The results of the ANOVA showed that there was no significant difference in the level of knowledge among the three groups: 13.91 2.369 in the video group, 14.64 1.540 in the booklet group, and 14.74 2.165 in the booklet + video group. It was confirmed that the score of booklet + video group was high in the order of booklet group 4.14 0.534, video group 4.31 0.581, booklet + video group 4.41 0.538.

**CONCLUSIONS:** Based on the results of this study, it is shown that if the education needs of the participants who are in vitro are analyzed, and if systematic and standardized educational materials are produced accordingly and brochures and videos are appropriately provided, it is possible to increase education satisfaction. The purpose of this study has its meaning in conducting the study about the content and method of education for infertility women and the content and method of education provided to the participants of in vitro baby are specifically suggested.

**References:** Covington SN. The role of the mental health professional in reproductive medicine. *Fertil Steril* 1995;64:895-7.

CP Hawkes(2013): Smartphone technology enhances newborn intubation knowledge and performance amongst paediatric trainees February 2013, Pages 223–226.

Gromik Nicolas A(2015): The Effect of Smartphone Video Camera as a Tool to Create Digital Stories for English Learning Purposes 64-79.

Jenkinson D., Davison J., Jones S.,Hawtin P.(1988). Comparison of effects of a self management booklet and audiocassette for patients.

JH Lee, HK Jung, G Lee, HY Kim(2013). Effect of behavioral intervention using smartphone application for preoperative anxiety in pediatric patients 2013 Dec;65(6):508-518. English with asthma. *British Medical*.

Thomas McNeal(2006) Anytime, anywhere: Using mobile phones for learning. *Journal of the Research Center for Educational Technology (RCET)* Vol. 2, No. 2, Fall 2006.

Chorionic Gonadotropin (hCG): 1500 units (u), 2500u, 3300u, 5000u, and 10,000u from a 10,000u vial of hCG; 1500u, 3300u, 5000u, and 10,000u from a 5000u vial of hCG. Nurses logged total time spent counseling patients on trigger instructions over a 2-week period in June 2018, prior to video implementation. Videos were emailed to all patients on the day of planned trigger starting mid-November 2018. Nurses logged time spent counseling per patient 1 month following video implementation over a 2-week period. Nursing satisfaction surveys were sent following video initiation and compared to pre-video scores. Patient surveys were sent retrospectively to gauge adequacy of information received, satisfaction, confidence, and need to page on-call physician prior to and following video implementation. Means were compared using a paired t-test for each of the measured outcomes on the patient surveys.

**RESULTS:** Time spent counseling patients by staff was on average 29 minutes prior to video implementation and decreased by 40% to 17.5 minutes following initiation of videos. 5 nurses completed the satisfaction survey with improved average scores from 20% pre-video to 84% post-video. 148 patients were sent a survey 1 month before and after video implementation with a response rate of 38.5% pre-video and 25% post-video. Overall trends revealed that patients completing a trigger injection for the first time reported improved scores on information received, satisfaction, and confidence, though none of these values approached significance. Patients undergoing repeat trigger injection reported significantly lower satisfaction scores following video implementation (9.44/10 to 8.38/10,  $p=0.028$ ). There was a reduction in overall calls made to the on-call physician with 10.5% calling prior to the video and 8.1% post-video.

**CONCLUSIONS:** Trigger videos resulted in a 40% reduction in nursing time spent counseling patients and fewer calls to the on-call physician. This improved efficiency was associated with improved nursing satisfaction and stable patient satisfaction and confidence. Practices seeking efficiency gains should consider utilization of video-based instruction.

O-230 Wednesday, October 16, 2019 11:00 AM

### ELECTIVE EGG FREEZING AND MALE SUPPORT: A QUALITATIVE STUDY OF MEN'S HIDDEN ROLES IN WOMEN'S FERTILITY PRESERVATION.



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**OBJECTIVE:** Do men participate in women's fertility preservation decisions and procedures? Emerging evidence suggests that lack of a male partner is the primary reason why women are pursuing elective egg freezing (EEF). However, this qualitative study asked women whether men played any supportive roles in their fertility preservation decisions and procedures.

**DESIGN:** In this binational, qualitative study, 150 women (114 in the United States, 36 in Israel) who had completed at least one cycle of EEF were interviewed by two senior medical anthropologists, one in each country, during the period from June 2014 to August 2016.

**MATERIALS AND METHODS:** Study participants were recruited through 4 American IVF clinics (2 academic, 2 private) and 3 in Israel (1 academic, 2 private). In-depth, semi-structured, open-ended interviews were audio-recorded, transcribed, and entered into a qualitative data analysis program (Dedoose) for thematic analysis, along with detailed interview summaries.

**RESULTS:** Although 85% of women identified the lack of a male partner as their main reason for pursuing EEF, nearly two-thirds (63%) relied on some form of male support during their EEF decision making processes and procedures. Five categories of men, in order of support, included: 1) fathers (or other male father figures), 2) male partners (past or present), 3) male friends, 4) brothers, and 5) male judges (who supported EEF in divorce settlements). More than a dozen different forms of assistance were offered by men in four major categories (instrumental, financial, physical, and psychological).

**CONCLUSIONS:** Five different categories of men played supportive roles in women's EEF, offering 12 forms of instrumental, financial, physical, and psychological assistance. Although one-third of women went through EEF alone or with only female support, this study reveals the "hidden" roles men play in supporting female family members, friends, and partners.

**SUPPORT:** US National Science Foundation, Cultural Anthropology Program, BCS-1356136.

**EVALUATION OF ANXIETY IN FREEZE-ALL**

**PATIENTS.** Nagihan Dinçer, Bsc,<sup>a</sup> Aysel Salgın, Bsc,<sup>a</sup> Necati Findikli, Ph.D.,<sup>b</sup> Fazilet Kubra Boynukalin, M.D., MSc,<sup>c</sup> Mustafa Bahçeci, M.D., Ph.D.<sup>b</sup> <sup>a</sup>BAHÇEÇİ FULYA IVF CENTER, İSTANBUL, Turkey; <sup>b</sup>Bahçeci Health Group-Fulya IVF Centre, Istanbul, Turkey; <sup>c</sup>Bahçeci Health Group-Fulya IVF Centre, İSTANBUL, Turkey.



**OBJECTIVE:** To investigate the anxiety scores and depression scales of infertile women that applied to Bahçeci Fulya IVF Center for elective frozen blast embryo transfer between February 26, 2019, and April 18, 2019.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** This study is a prospective cohort study which included 178 patients. Face to face interview in the patient's room 1 hour ago the embryo transfer was performed and Beck Depression Inventory (BDI) with 21 items and Anxiety and Depression Scale (HADS) with 14 items were fulfilled. The age of the patients, number of previous failed trials, total follicle stimulating hormone (FSH) and human menopausal gonadotropin (HMG) doses during ovulation induction period, number of oocytes obtained after oocyte pick up, number of m2, number of frozen embryos, number of embryos remaining were recorded from the patients file. The data obtained after BDI and HADS were evaluated with SPSS 15.0 program. All these parameters were analyzed in multiple regression analysis.

**RESULTS:** BDI and HADS score was found to be correlated ( $\rho:0.57$   $p<0.001$ ). In the multivariate analysis no factor such as women age, number of previous failed trials, FSH and HMG doses during ovulation, number of oocytes retrieved, number of m2, number of frozen embryos, number of embryos remaining were found to have an effect the BDI and HADS. Also, the pregnancy rates were not affected according to the BDI and HADS scores stratified.

**CONCLUSIONS:** Anxiety scales does not affected by the patient's ovulation induction and embryological parameters.

O-233 Wednesday, October 16, 2019 11:45 AM

**CLINICAL EXPERIENCE OF NURSING TEAM IN PRE-CONCEPTION GENETIC COUNSELING AT A LARGE, DIVERSE INFERTILITY PRACTICE.**

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**OBJECTIVE:** While ethnicity based-carrier screening was once a customary component of preconception genetic testing, expanded carrier screening (ECS) is being increasingly utilized to identify recessive or X-linked mutations. The role of the nursing team has evolved to include provision of genetic counseling for patients facing new information to process and potential use in treatment. This study assesses nursing involvement and patient decision making in infertile couples accessing ECS.

**DESIGN:** Retrospective.

**MATERIALS AND METHODS:** The study included patients who underwent ECS (panels with >200 diseases) from May 2017 – March 2018. Patients were identified as either non-carriers, carriers whose partner tested negative for the mutation, carrier couples, or female carriers (X-linked pathogenic variant). We evaluated the mutation prevalence and the decision-making process regarding use of preimplantation genetic testing for monogenic/single gene defects (PGT-M).

**RESULTS:** A total of 2439 patients (980 couples) underwent ECS. 1575 (64.6%) patients were found to carry  $\geq 1$  mutation. The most prevalent being 8.1% Alpha Thalassemia (n=198), 5.8% Biotinidase Deficiency (n=142), 5.7% GJB2-related Non-Syndromic Hearing Loss (n=139), 4.1% Cystic Fibrosis (n=100), 3.8% Familial Mediterranean Fever (n=93). 864 patients (35.4%) tested negative for all mutations.

Of 980 participating couples, 31 (3.1%) were identified as being carrier couples. 39 of the 1527 females tested (2.5%) were carriers for X-linked con-

ditions. The 39 X-linked females and 31 carrier couples underwent formal genetic counseling to assist with ART treatment decision-making. 30 proceeded with PGT-M while 30 declined PGT-M.

Of the carrier couples who decided to access PGT-M technology, the most prevalent conditions included Cystic Fibrosis (n=5), Beta-Globin Related Hemoglobinopathies (n=4), GJB2-related Non-Syndromic Hearing Loss (n=3), Familial Mediterranean Fever (n=3), and Gaucher Disease (n=2). Of the females who pursued PGT-M for X-linked conditions, 5 were Fragile X Pre-mutation carriers and 1 a Fragile X Intermediate carrier.

Of the 30 individuals/couples who declined PGT-M, 19 were Fragile X Intermediate carriers, 7 were Fragile X Pre-mutation carriers, 2 were GJB2-related Non-Syndromic Hearing Loss carrier couples, 1 Familial Mediterranean Fever carrier couple, and 1 Beta-Globin Related Hemoglobinopathies (City of Hope variant) carrier couple. Patients positive for intermediate Fragile X were most likely to waive PGT-M.

**CONCLUSIONS:** Expanded genetic carrier counseling and screening have become integral parts of preconception counseling. Given decreasing costs of sequencing and increasing awareness or the discordance between self-reported ethnicity and ancestral inheritance markers, pan-ethnic is now widely utilized. The modern infertility nursing team is increasingly being called upon to provide pre- and post-test counseling to infertility patients. By educating patients about contemporary reproductive options, patients will obtain a greater sense of autonomy across their family building journey.

Reference: None.

SUPPORT: None.

O-234 Wednesday, October 16, 2019 12:00 PM

**WHAT IS THE IDEAL NUMBER OF VIALS OF DONOR SPERM TO PURCHASE FOR PATIENTS UNDERGOING DONOR SPERM INTRAUTERINE INSEMINATION (DIUI)?**

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**OBJECTIVE:** Gamete donation has provided patients who would not otherwise have the ability to conceive the opportunity to have a healthy child via screened selected eggs and sperm. Donor sperm is a limited resource, and scarce literature exists to inform patients regarding the optimal number of vials to purchase to maximize the chances of conceiving while minimizing cost. The objective of this study is assess the number of donor sperm vials needed to achieve ongoing pregnancy (OP) for patients who are undergoing donor sperm intrauterine insemination (DIUI).

**DESIGN:** Retrospective, cohort study.

**MATERIALS AND METHODS:** The study included patients at a single academic center who underwent a natural cycle or medicated ovulation induction cycle (with clomiphene citrate or letrozole) with DIUI from 2010-2019. Exclusion criteria included gonadotropin use or imaging showing tubal pathology, uterine myomas, or polyps >0.5 cm. r-hCG was administered when  $\geq 1$  18mm follicle was visualized. DIUI was performed 36 hours later. The primary outcome was OP. A Kaplan-Meier curve was created for each SART age group to determine the cumulative probability of OP from each DIUI cycle. A second curve stratified by anti-Mullerian hormone (AMH) levels: (low <0.7 ng/mL, normal 0.7-8.4 ng/mL, and high >8.4 ng/mL).<sup>1</sup> Patients were censored when they dropped out or progressed to IVF.

**RESULTS:** A total of 913 patients were included in the study (Groups A: 257, B: 199, C: 168, D: 142, E: 147). The cumulative percent of patients that achieved OP in each cycle is shown in Table 1.

**CONCLUSIONS:** Until now, there has not been a personalized algorithm to predict how many vials of donor sperm should be purchased prior to attempting DIUI. Using Kaplan-Meier curves stratified by age and AMH, we developed a starting point from which clinicians can further tailor their recommendations to incorporate patient characteristics and preferences for family size. The cumulative OP rate per cycle can also be used to counsel patients about when to transition their treatment strategy to one that includes assisted

reproductive technologies. Future studies might include a financial analysis that includes time and cost as variables of both low-tech and high treatments, so that we can best inform our patients.

TABLE 1. Cumulative OP rate per cycle (%)

Cycle	SART Age Group					AMH Group		
	A	B	C	D	E	Low	Normal	High
1	18.3	14.6	11.9	11.2	2.0	3.3	7.7	15.4
2	32.5	23.0	19.1	16.0	4.0	3.3	14.9	27.9
3	42.5	28.1	28.2	20.5	4.0	12.8	21.2	<b>36.9</b>
4	50.0	32.8	31.7	25.6	<b>6.4</b>	12.8	27.4	36.9
5	53.6	44.6	38.9	37.0	6.4	12.8	36.2	
6	61.1	50.1	42.3	<b>46.7</b>		<b>30.2</b>	44.5	
7	64.4	<b>54.7</b>	42.3	46.7		30.2	44.5	
8	69.5	54.7	42.3				49.8	
9	<b>76.3</b>		<b>55.1</b>				<b>57.0</b>	
10	76.3		55.1				57.0	

Reference: 1. Steiner AZ, Pritchard MS, Stanczyk FZ, et al. Association between biomarkers of ovarian reserve and infertility among older women of reproductive age. *JAMA* 2017; 318(14):1367-1376.

SUPPORT: None.

### PROFESSIONAL DEVELOPMENT

O-235 Wednesday, October 16, 2019 10:45 AM

#### IMPROVING THE REI FELLOWSHIP INTERVIEW

**EXPERIENCE: A SURVEY.** Erika P. New, MD, MPH,<sup>a</sup> Papri Sarkar, MD,<sup>b</sup> Ruben J. Alvero, MD,<sup>c</sup> Anthony N. Imudia, MD.<sup>d</sup> <sup>a</sup>University of South Florida, Brandon, FL; <sup>b</sup>USF, Tampa, FL; <sup>c</sup>Stanford University, Palo Alto, CA; <sup>d</sup>University of South Florida, Tampa, FL.



**OBJECTIVE:** The interview process for residents applying to Fellowship in Reproductive Endocrinology and Infertility (REI) is a highly competitive process with many challenges for applicants such as conflicting interview dates, the expense of traveling, and missing days from work. The goal of this study is to collect data on the current REI fellowship interview process so that it may be improved in the future.

**DESIGN:** An anonymous survey was sent to individuals who have gone through the REI fellowship interview process. In addition, fellowship program directors and coordinators were contacted by e-mail to gain information on typical interview dates for each program.

**MATERIALS AND METHODS:** The survey designed for applicants was distributed over social media and the REI fellow e-mail list-serv. Some survey questions included:

- How many days of work or vacation did you take off for Fellowship interviews?
- Did you ever miss an opportunity to interview at a program you were interested in? If so, what was the reason?
- How often did you have to travel to the same city more than once for an interview?
- How much money did you spend on average per interview?
- What recommendations do you have for how the interview process could be improved?

The fellowship program information was obtained by contacting each program using the publicly available contact information on the Accreditation Council for Graduate Medical Education (ACGME) website.

**RESULTS:** There were 44 survey respondents. Of those, 38.6% participated in the 2018 REI interview season, 29.5% in 2017 and 31.8% participated more than 2 years ago. The mean number of interviews attended was 12.6 (range of 1-22). On average 13.4 (0-30) days off work or vacation were used to interview. 67.4% of respondents

missed an opportunity to interview at a program they were interested in, with most common reasons: the interview date was the same day as another interview, could not attend due to geographic location, and cost was too great. 72% traveled to the same city more than once for an interview. The average cost per interview was \$478 (range \$200-\$1,000) and average cost per interview season was \$5,660 (range \$900-\$15,000). Fellowship program data was available from 43 of 48 programs contacted. The 2018 interview season spanned from June 4-August 30. The most popular interview date was Monday, August 27 (5 interviews). The number of dates that had conflicting interviews scheduled were 26. Most programs offered 2 interview dates (46.5%), 30% offered 3 interview dates, 16% offered 1 date, and 6.9% offered 4 dates.

**CONCLUSIONS:** This data supports the need to coordinate the REI fellowship recruitment process between programs to reduce conflicting interview dates and mitigate cost. Recommendations from respondents include having programs notify applicants of interview offers at the same time, geographically aligning interviews by region, including a virtual component to interviews such as video interviews or interviewing at a central location, and helping applicants with costs such as hotels or flights. Encouraging collaboration between fellowship programs would increase applicant satisfaction.

References: 1. Franasiak et al. *Fertility Research and Practice* (2017) 3:18. DOI [10.1186/s40738-017-0045-x](https://doi.org/10.1186/s40738-017-0045-x).

2. ERAS and Reproductive Endocrinology and infertility Working Together. A Welcome from the ERAS Senior Director. May 2016. [www.aamc.org/eras](http://www.aamc.org/eras).

3. National Resident Matching Program, Results and Data: Specialties Matching Service 2018 Appointment Year. National Resident Matching Program, Washington, DC. 2018.

4. Watson SL, Hollis RH, Oladeji L, Xu S, Porterfield JR, Ponce BA. The burden of the fellowship interview process on general surgery residents and programs. *J Surg Educ*.2017 Jan - Feb;74(1):167-172. <https://doi.org/10.1016/j.jsurg.2016.06.008>. Epub 2016 Jul 11.

5. Gressel GM, Van Arsdale A, Dioun SM, Goldberg GL. The gynecologic oncology fellowship interview process: challenges and potential areas for improvement. *Gynecol Oncol Rep*.2017 Apr 7;20:115-120. <https://doi.org/10.1016/j.gore.2017.04.003>. eCollection 2017 May.

6. Frishman G, Bell CL, Botros S, Brost BC, Robinson RD, Steinauer J, Wright JD, Adams KE. Applying to subspecialty fellowship: clarifying the confusion and conflicts! *Am J Obstet Gynecol*.2016 Feb;214(2):243-246. <https://doi.org/10.1016/j.ajog.2015.10.936>. Epub 2015 Nov 12.

SUPPORT: None.

O-236 Wednesday, October 16, 2019 11:00 AM

#### THE STATE OF WOMEN IN ACADEMIC REPRODUCTIVE ENDOCRINOLOGY PROGRAMS.

Jessica Selter, MD, Emily Spurlin, MD, Paula C. Brady, MD Columbia University Medical Center, New York, NY.



**OBJECTIVE:** To identify gender differences in leadership and academic rank within academic reproductive endocrinology programs with fellowships in the United States.

**DESIGN:** Cross-sectional study.

**MATERIALS AND METHODS:** Official institutional websites of the 2017-2018 American Board of Obstetrics and Gynecology (ABOG)-accredited reproductive endocrinology fellowship programs were reviewed, and gender representation at each leadership position and academic rank (Division and Fellowship Director; Full, Associate, and Assistant Professor) was recorded. Three programs did not consistently report academic rank, so only leadership positions were recorded. Associate Fellowship Directors were rarely reported and therefore excluded. Only medical doctors (MD, DO, MBBS) who completed postgraduate training in OB/GYN were included. Private practice physicians affiliated with universities were included only if present on academic department websites with academic titles. Univariate comparisons were performed using Chi-squared tests, with significance at  $p \leq 0.05$ .

**RESULTS:** Among 49 ABOG-accredited reproductive endocrinology programs, 263 faculty were identified, 129 (49%) male and 134 (51%)

	Male (n=129) (%)	Female (n=134) (%)	P-Value
Leadership			
Division Director (n=49)	34 (69.4%)	15 (30.6%)	0.006
Fellowship Director (n=49)	32 (65.3%)	17 (34.7%)	0.03
Academic rank			
Full Professor (n=101)	71 (70.3%)	30 (29.7%)	<0.001
Associate Professor (n=60)	31 (51.7%)	29 (48.3%)	0.79
Assistant Professor (n=102)	27 (26.5%)	75 (73.5%)	<0.001

female. Division directors were 69.4% male and 30.6% female ( $p=0.006$ ). Similarly, fellowship directors were 65.3% male and 34.7% female ( $p=0.03$ ). Full professors ( $n=101$ ) were more frequently male (70.3% vs. 29.7%  $p<0.001$ ). There was no difference in gender among associate professors ( $n=60$ , 51.7% male vs. 48.3% female,  $p=0.79$ ), while significantly more assistant professors were female than male ( $n=102$ , 73.5% vs. 26.5%,  $p<0.001$ ).

**CONCLUSIONS:** While a majority of residents in obstetrics and gynecology and half of reproductive endocrinology academic faculty are female, women are still underrepresented among leadership positions and full professors in academic reproductive endocrinology programs. More women than men are currently assistant professors, suggesting the possibility of a more equal distribution of leadership and tenure in the future.

**O-237** Wednesday, October 16, 2019 11:15 AM

#### EVALUATION OF OFFICE HYSTEROSCOPY SIMULATION AS PART OF AN OB/GYN RESIDENCY TRAINING CURRICULUM.

Jessica Selter, MD, Sally F. Vitez, MD, Eric J. Forman, MD, Samuel Zev Williams, M.D., Ph.D. Columbia University Medical Center, New York, NY.



**OBJECTIVE:** To evaluate the impact of an office hysteroscopy simulation on Ob/Gyn resident comfort with the procedure and performance metrics.

**DESIGN:** One-group pretest-posttest design study.

**MATERIALS AND METHODS:** Ob/Gyn residents at a single institution were recruited to voluntarily attend an office hysteroscopy 2-hour training session. Residents underwent testing on the Endosee procedure. They then went through a series of Endosee simulation exercises with increasing level of difficulty, and then were re-tested on the original Endosee procedure. Residents were evaluated via both objective measures by a pre/post multi-metric scoring system and subjective measures with pre- and post-surveys. The multi-metric scoring system included an overall score that incorporated procedure time, patient comfort, visualization, and precision. Univariable analysis with paired student's t-test, wilcoxon-signed rank test, and fisher exact tests were performed as appropriate.

**RESULTS:** A total of 17 residents completed the office hysteroscopy simulation program. The average age was 28.8 with 87% females, and an even distribution of years of training. The distribution of intended subspecialty training included 11.7% into reproductive endocrinology, 29.4% into gynecologic oncology, 11.7% into minimally invasive gynecologic surgery, and 23.5% into generalist/unknown. All residents reported little to no experience with office hysteroscopy prior to the training experience and 88% felt extremely uncomfortable with performing the office hysteroscopy procedure. Following training, there was a significant increase in subjective comfort (76% vs. 11.4%,  $p<.01$ ) with a majority of residents reporting slight/moderate comfort with performing the procedure. Following training, all residents agreed that Endosee simulation was a good preparation for office hysteroscopy. Furthermore, there was a significant increase in residents who agreed that office hysteroscopy simulation should be integrated into Ob/Gyn curriculum (68% vs. 94%,  $p=.04$ ). After training, residents had an improved overall score (218 vs. 242,  $p<.01$ ), decreased procedure

time (116sec vs. 73sec,  $p<.01$ ), shorter cumulative path length (24.4 vs. 17.8cm,  $p=.01$ ) and a trend towards improved navigation percentage (61% vs. 70%,  $p=.06$ ).

**CONCLUSIONS:** This study demonstrates that office hysteroscopy training using a simulator improves both subjective resident comfort and objective performance. Despite the small sample size, the overall enthusiasm regarding office hysteroscopy simulation suggests the need for a larger study group and a possible role for integrating office hysteroscopy into resident Ob/Gyn curriculum.

**SUPPORT:** CooperSurgical provided surgical simulators for the study.

**O-238** Wednesday, October 16, 2019 11:30 AM

**FERTILITY KNOWLEDGE IN OBSTETRICS AND GYNECOLOGY RESIDENTS.** Leah Roberts, MD,<sup>a</sup> Rashmi Kudesia, MD,<sup>b</sup> Shaliz Dolan, MD,<sup>a</sup> Marisa Rose, MD,<sup>a</sup> Temple University Hospital, Philadelphia, PA; <sup>b</sup>CCRM Fertility Houston, Houston, TX.



**OBJECTIVE:** Reproductive Endocrinology and Infertility is taught in every obstetrics and gynecology (OB-GYN) residency program in the country, however, resident knowledge in this area has never been assessed by a validated instrument. The goal of this study was to evaluate fertility knowledge among current OB-GYN residents using a recently published validated instrument, the Fertility and Infertility Treatment Knowledge Score (FIT-KS).

**DESIGN:** Survey.

**MATERIALS AND METHODS:** OB-GYN residents in the United States were recruited through an email to all residency coordinators nationwide. They were asked to voluntarily respond to a short questionnaire including demographic information and the FIT-KS instrument, through an online survey platform.

**RESULTS:** One hundred and seventy-seven residents responded to the survey (approximately a 4% response rate). The sample was 91% female, with 69% between the ages of 26 and 30. They represented an equal distribution between all four levels of training with 40, 47, 39 and 40 respondents of each year of training. Mean score was 21.2 (73%). Several knowledge gaps were noted. In terms of understanding natural conception, 27% of respondents believed having less than nine periods a year could be normal, and 27% also responded that a male partner's age did not impact fertility. Only 56% knew that sperm would survive for three to five days in the female reproductive tract. For risk factors, the fertility effects of moderate alcohol consumption and sexual lubricants were most commonly mischaracterized. Residents could define the common assisted reproductive technologies; however, grossly overestimate their success rates per cycle. No statistically significant differences were noted across the level of training. The majority of all residents (95%) stated that they do discuss fertility with their patients, however 18% stated they do not feel comfortable answering their patient's questions. Residents who did not feel comfortable answering their patient's questions mean score was 20.9 (72%), not significantly different from those residents who did feel comfortable providing fertility counseling 21.3 (73%).

**CONCLUSIONS:** Substantial gaps exist in fertility knowledge among OB-GYN residents, with understanding of male fertility and success

rates of assisted reproductive technologies being particularly limited. Knowledge of fertility does not change throughout residency training, demonstrating consistent gaps in fertility knowledge. Knowledge during PGY1 year is consistent with mean scores found in prior research in Internal Medicine residents (65%), as well as a cohort of female medical students and obstetrics and gynecology residents and fellows (64.9%) [1,2].

References: 1. Kudesia R., et al. Low fertility awareness in United States reproductive-aged women and medical trainees: creation & validation of the fertility and infertility treatment knowledge survey (FIT-KS). *Fertility and Sterility*, 2017.108(4):p711-717.

2. Roberts L., et al. A didactic intervention to improve fertility knowledge among resident physicians. *Fertility and Sterility*, 2018. 110(4): p e239.

SUPPORT: None.

O-239 Wednesday, October 16, 2019 11:45 AM

#### COLLABORATIVE AND MULTIDISCIPLINARY APPROACH TO THE REI FELLOWSHIP APPLICATION.

Randi H. Goldman, M.D., Christine Mullin, M.D., Esther Lopez, M.P.A., Jeanette Tomasino, Ph.D., Martina Borovica, M.B.A., Avner Hershlag, M.D., Northwell Health Fertility, Zucker School of Medicine at Hofstra/Northwell, Manhasset, NY.



**OBJECTIVE:** To describe the development and implementation of a Reproductive Endocrinology and Infertility (REI) fellowship program and the process of attaining initial accreditation, with a focus on the multidisciplinary collaborative effort of the OB/GYN department, GME committee, and affiliated programs.

**DESIGN:** Descriptive study at an academic medical center.

**MATERIALS AND METHODS:** This is a descriptive study completed at an academic institution that evaluates the critical aspects of establishing an REI fellowship program. We specifically explored how to utilize the broad network of interdisciplinary opportunities already established at our institution to provide a collaborative, inclusive learning environment for fellow trainees.

**RESULTS:** REI sub-specialists are expected to master the medical knowledge and surgical procedures involved with all aspects of reproductive health, including care for infertile women and couples, disorders that threaten fertility such as cancer and systemic disease, pediatric and adolescent gynecologic care, and fertility preservation. When designing our fellowship program, we built upon the multidisciplinary strengths of our institution by consulting with other departments and divisions. Minimally Invasive Gynecologic Surgery, Male Infertility, Endocrinology, Pediatric Endocrinology, and Genetics – disciplines that encompass the broad aspects of our field – have partnered to educate our fellows and actively participated in developing the curriculum. The Feinstein Institute for Medical Research provides methodological, step-wise classes on study design and research, and a thorough biostatistics course for fellow learners. This research-oriented environment is committed to giving fellows the tools to conduct well-designed and meaningful basic, translational, and clinical studies. The process of building our program highlighted the collaborative nature of existing programs, and establishing an REI fellowship became a natural extension for our department. The multidisciplinary nature of the fellowship reflects the ever-expanding horizons of our specialty, equipping our fellows with strong exposure to all aspects of reproductive health both in and out of the division. Our new program was granted two fellows each year.

**CONCLUSIONS:** This descriptive study emphasizes the need for a collaborative effort in the accreditation of a new fellowship program. Utilizing existing institutional resources and designing a fellowship program with input from all teams that will comprise the fellows'

education fosters an environment for individual and group learning, opportunities for sharing clinical information between divisions, as well as collaborative research that will support the training of future leaders and advancement of the field. We hope that other programs that are considering establishing a training program will use this model, taking advantage of existing opportunities at their institutions.

O-240 Wednesday, October 16, 2019 12:00 PM

#### REI EXPOSURE IN RESIDENCY: HOW MUCH IS ENOUGH?

Jason A. Schneider, M.D.,<sup>a</sup> Tomer Singer, MD,<sup>b</sup> Avner Hershlag, M.D.,<sup>b</sup> Randi H. Goldman, M.D.<sup>b</sup>



<sup>a</sup>Zucker School of Medicine at Hofstra/Northwell at Lenox Hill Hospital, New York, NY; <sup>b</sup>Northwell Health Fertility, Zucker School of Medicine at Hofstra/Northwell, Manhasset, NY.

**OBJECTIVE:** Exposure to Reproductive Endocrinology and Infertility (REI) is critical for Obstetrics and Gynecology (OB/GYN) resident learners to ensure adequate fund-of-knowledge and for career planning. The purpose of this study is to assess residents' exposure to clinical and research opportunities in REI and identify how experience and comfort with REI vary by demographic characteristics.

**DESIGN:** Cross-sectional electronic survey study.

**MATERIALS AND METHODS:** A web-based questionnaire was completed anonymously by 100 U.S.-based OB/GYN residents, with questions regarding the structure of their residency programs and REI rotations, access to REI faculty, patients, and research, career plans, and demographics. Outcome measures were whether residents felt their REI experience was "too little," "just right," or "too heavy," and whether they anticipated feeling comfortable completing a basic infertility workup at the completion of training. Fisher's exact tests determined differences in outcomes;  $p < 0.05$  determined significance.

**RESULTS:** Most residents (89%) reported having a dedicated REI rotation and 1/3 of trainees had rotations over multiple years; 24% occurred during the PGY-1 year, 43% during PGY-2, 40% during PGY-3, and 25% during PGY-4. Approximately 1/4 of respondents have an REI fellowship at their institution and 3/4 have the opportunity to participate in REI-related research. Among those who described their REI experience as "too light" (N=53), 66% anticipated feeling comfortable completing an infertility workup at the end of residency, while 93% of residents who felt their REI exposure was "just right" (N=46) anticipated feeling comfortable, a statistically significant difference ( $p=0.001$ ). Residents training in the Northeast felt least prepared to perform a workup vs. the rest of the U.S. (69% vs. 86%,  $p=0.048$ ). Significantly fewer fellowship-aspiring residents felt their REI exposure was "just right" (32%) vs. residents who were undecided or planning to be general OB/GYNs (55%) ( $p=0.038$ ). Residents planning to pursue REI fellowship were least likely to feel their REI exposure was "just right" (18%, vs. 82% "too light"). Only 1 resident felt that their REI exposure was "too heavy." Residents whose REI curricula included ASRM teaching modules were significantly more likely to feel their REI exposure was "just right" (62% vs. 38%,  $p=0.035$ ). No differences were seen based on rotation length, university- vs. community-based programs, having a sub-specialty trained program director, or presence of an institutional REI fellowship.

**CONCLUSIONS:** Although 53% of residents feel REI exposure during residency is "too light," most (79%) trainees expect to feel comfortable completing a basic infertility workup upon graduation. ASRM teaching modules may be a simple intervention to standardize REI curricula and help residents feel more content with their REI exposure during training. Importantly, adequate exposure to REI may help inform career choices of trainees.

O-241 Wednesday, October 16, 2019 10:45 AM

**DIFFERENTIATION OF EMBRYONIC STEM CELLS INTO MALE GERM LINE CELLS IN A BIOREACTOR USING DECELLULARIZED SEMINIFEROUS TUBULE MATRIX IMMERSED IN A CONDITIONED MEDIUM.**



Philip Xie, B.S., Aysha Trout, B.A., Zev Rosenwaks, M.D., Gianpiero D. Palermo, M.D., Ph.D., The Ronald O. Perleman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY.

**OBJECTIVE:** To propose a method to sustain mouse embryonic stem cells (mESCs) utilizing a decellularized seminiferous tubule matrix (DSTMs) as a biomechanical scaffold, and induce differentiation into male germ line cells in conditioned medium.

**DESIGN:** We tested the potential of a bioreactor concept-based culture system supported by a biological scaffold to allow de novo generation of meiotic male germ line cells from mESCs.

**MATERIALS AND METHODS:** Male mESCs were cultured in epiblast cell-like cell (EpiLC) medium containing activin A, bFGF, and KSR for 3 days to allow differentiation into EpiLCs. Subsequent exposure to primordial germ cell-like cell (PGCLC) medium subsidized with BMP4, BMP8b, SCF, LIF, and EGF in hanging droplet (HD) allowed the formation of embryoid bodies (EBs) rich in PGCLCs. Isolated cells from 80 EBs were utilized in the bioreactor, in directed contact with DSTMs, and loaded with DMEM in a gelatin-treated culture well equipped with a 0.4-µm pore size mesh inlet. 14-week-old adult male B6D2F1 mice were sacrificed for DSTMs and conditioned medium. DSTMs were prepared by immersion in 1% sodium dodecyl sulfate for 24 hours. Eighty 3-mm sections of DSTM, longitudinally sliced open and flattened, were placed below the mesh; interstitial cells were isolated from the respective contralateral testes by differential plating and loaded above the mesh. Cell characteristics were analyzed by germ cell stage-specific markers on an H&E-stained background.

**RESULTS:** After culturing mESCs in EpiLC medium, the continuing expression of OCT4 (>90%) and the decreased positivity of Nanog (45%) indicated progression to EpiLCs. EBs rich in PGCLCs expressed positive surface SSEA-1 after six days of culture in HD with PGCLC medium. Isolated cells of PGCLCs were derived from the digestion of EBs and layered on the DSTMs. The earliest attachment of PGCLCs onto DSTM occurred on day 3, and complete recellularization was observed at approximately day 10. Following complete recellularization, about half of all isolated cells obtained from the enzymatic digestion of recellularized tubule displayed decreased expression of OCT4, while 5% displayed nuclear DAZL positivity at day 10. In 1% of the cells, perinuclear DAZL confirmed spermatocyte differentiation at day 21. At around day 16, cytoplasmic VASA positivity in 5% of the cells suggested meiotic/post-meiotic germ line differentiation.

**CONCLUSIONS:** The timeline of our bioreactor system was comparable with in vivo spermatogenesis in the mouse, occurring in the course of 21 days. Once the ability of a 3D biocompatible scaffold to induce late-stage gametogenesis is confirmed, it will be possible to study spermatogenesis in vitro. Neogametogenesis from genotyped stem cells performed in a scaled-down microfluidic device may help to treat men afflicted by Sertoli cell-only syndrome.

O-242 Wednesday, October 16, 2019 11:00 AM

**EFFICIENT GENERATION OF GRANULOSA CELL LIKE CELLS FROM HUMAN ENDOMETRIUM DERIVED IPS CELLS AS A SOURCE FOR AUTOLOGOUS ESTRADIOL PRODUCTION.**



Joo Hyun Park, MD, PhD, Heeyon Kim, M.D., Hyun Kyung Kim, MS, SiHyun Cho, M.D., Ph.D., Yonsei University College of Medicine, Gangnam Severance Hospital, Seoul, Korea, Republic of (South).

**OBJECTIVE:** To derive granulosa cell like cells from induced pluripotent stem cells (iPSCs) which are driven from discarded endometrial stromal cells as a novel source of autologous estradiol production.

**DESIGN:** iPSCs were driven using human endometrial cells obtained from five benign hysterectomies and stepwise granulosa cells were differentiated to successfully induce estradiol production.

**MATERIALS AND METHODS:** After pathologic confirmation, human endometrial cells free from pathologic findings were obtained from five hysterectomy specimens of benign indications. Using episomal vectors for Sox2,

Oct4, cMyc and Klf4, 3 cell lines were driven per donor. Embryoid bodies (EBs) were first formed from these patient endometrium derived iPSCs and to induce primitive streak-mesendoderm and intermediate plate mesoderm lineage. Consequently estradiol(E2) producing granulosa like cells were differentiated. Human embryonic stem cell(ESC) line H9 was used as control. To induce mesodermal lineage differentiation, produced EBs were supplemented with BMP4 (10 ng/ml), WNT3a (6 ng/ml), Activin A (6 ng/ml) and bFGF (5 ng/ml) for 6 days. After confirming mesodermal commitment, differentiation was further directed using BMP4 (10 ng/ml), Follistatin (25 ng/ml) and bFGF (5 ng/ml) for an additional 6 days. During the differentiation process markers indicative of granulosa cell differentiation(AMH, FOXL2, FSHR, AMHR2, LHR, CYP19A1) was performed via real-time PCR and FACS analysis. After a differentiation period of 12 days, these cells were seeded at a density of 4x10<sup>5</sup> per one 6-well plate and after adding androstenedione for an additional period of time, E2 assay was performed using ELISA.

**RESULTS:** After a differentiation process of 6 days, FACS analysis of brachyruyry expression for H9 was 30% and 21.7% (SD +/- 3.5%) for human iPSCs. This primitive streak-mesendoderm marker, brachyruyry showed marked expression at day 6 of differentiation and decreases upto day 12 of differentiation. Donor and cell line variabilities with regards to efficiency and time requirements were observed.

The aforementioned AMH, FOXL2, FSHR, AMHR2, LHR and CYP19A1 expression were increased after estradiol producing granulosa cell differentiation in both iPSCs and H9. Real-time PCR analysis showed relative AMH expression of 10.8 (SE +/- 0.11) in the iPSCs and 2.4 (SE +/- 0.1) in the H9 driven granulosa cell like cells compared with the undifferentiated state. According to FACS analysis, AMHR2 expression at differentiation day 12 was as follows; 53.2% for iPSCs and 93.6% for H9. FSHR at day 12 for iPSCs was 32.4% versus 30.2% for H9, CYP19A1 expression for H9 differentiation at day 6 was 38.5% and 83.9% at day 12. However, CYP19A1 expression for iPSCs was not observed at day 6 but a 100% expression at differentiation day 12.

Control estradiol concentration in the basal media was 9.38 pg/ml(SE +/-4.4), 2119.7 pg/ml(SE +/- 211.9) for iPSCs 1364.3 pg/ml(SE +/- 107.9) for H9.

**CONCLUSIONS:** Granulosa cell like cells expressing the appropriate markers and functional for estradiol production could be successfully derived from human endometrium derived iPSCs

**SUPPORT:** None.

O-243 Wednesday, October 16, 2019 11:15 AM

**TARGETING ACTIVATED PRO-INFLAMMATORY PATHWAY IN PRIMED MYOMETRIAL STEM CELLS WITH VITAMIN D3 AND PARICALCITOL.**



Hoda Elhossiny Elkafas, MSc,<sup>a</sup> Osama A. Badary, PhD,<sup>b</sup> Engy Elmorsy, PhD,<sup>c</sup> Rehab Kamel, PhD,<sup>c</sup> Ayman Al-Hendy, MD PhD,<sup>a</sup> Qiwei Yang, PhD.<sup>d</sup> <sup>a</sup>University of Illinois at Chicago College of Medicine, Chicago, IL; <sup>b</sup>National Organization for Drug Control and Research (NODCAR), Giza, Egypt; <sup>c</sup>pharmacology and toxicology department faculty of pharmacy Helwan University, cairo, Egypt; <sup>d</sup>University of Illinois at Chicago, Chicago, IL.

**OBJECTIVE:** Uterine fibroids (UFs) are a benign monoclonal neoplasm of the myometrium and recognized as the most prevalent gynecologic tumor among reproductive age women. Previous studies showed that early life exposure to xenoestrogens such as diethylstilbestrol (DES) increased the frequency of UF development. However, the underlying mechanism is largely unknown. This study is to determine the pro-inflammatory mediators which contributes to the activated inflammatory pathways in myometrial stem cells (MMSCs) in response to developmental exposures to DES, and characterize the role of Vitamin D3 and its analog in reversing the DES exposure-induced activated inflammatory pathway.

**DESIGN:** Laboratory research studies using the Eker rat fibroid model (*Tsc2*-mutant Eker (*Tsc2*<sup>EK/+</sup>): MMSCs).

**MATERIALS AND METHODS:** Female newborn Eker rats were treated S.C. with vehicle (VEH) or 10 µg/kg of DES, a tool compound of environmental EDCs, on postnatal days (PND) 10-12, a key period of uterine development. MMSCs were isolated from adult (5 months) myometrium tissue (N=5 for each group) using dual Stro-1 and CD44 surface markers. Whole genome RNA-sequencing was performed to identify pro-inflammatory markers in DES- and VEH-MMSCs. The protein expression levels of a panel pro-inflammatory genes were measured using a cytokines antibody array. RNA expression was determined by qRT-PCR. Student T and ANOVA test were used for statistical analysis.

**RESULTS:** Ingenuity Pathway Analysis of RNA-seq data demonstrated that inflammatory pathway was activated in response to DES exposure in MMSCs.

RNA-seq demonstrated that several key inflammatory mediators including TNF- $\alpha$ , IL1a, IL1b, IL17, IL-12, CINC-1, ICAM-1, IL1ra, CXCL5, and TIMP-1 were upregulated in DES-MMSCs as compared to VEH-MMSCs. The RNA expression of IL-10 which is an anti-proinflammatory mediator was down regulated in DES-MMSCs. qRT-PCR analysis confirmed the alteration of RNA expression of these inflammatory mediator genes ( $P < 0.05$ ). Cytokines antibody array analysis exhibited an increased expression of CINC-1, ICAM-1, IL1ra, CXCL5, TIMP-1, and VEGF in DES-MMSCs vs VEH-MMSCs. q-PCR analysis demonstrated that treatment with Vitamin D3 and its analogue Paricalcitol reversed the effect of DES exposure by down-regulating those pro-inflammatory cytokines ( $P < 0.01$ ). Cytokines antibody array further demonstrated that vitamin D3 and Paricalcitol reversed the DES-induced upregulation of pro-inflammatory mediators including CINC-1, ICAM-1, IL1ra, CXCL5, TIMP-1, and VEGF in DES-MMSCs.

**CONCLUSIONS:** Our data strongly demonstrate that developmental exposure to DES increases the risk of adult onset of UFs by creating an inflammatory milieu in the myometrium. Vitamin D3 and Paricalcitol treatment are capable of reversing the effect of DES exposure-induced activation of pro-inflammatory pathway in MMSCs, suggesting that vitamin D3 and its analogue as a treatment option could be useful to decrease the incidence of UFs.

**SUPPORT:** NIH RO1 ES028615 and U54 MD007602.

**O-244** Wednesday, October 16, 2019 11:30 AM

#### **BONE MARROW MESENCHYMAL STEM CELLS ARE MOBILIZED TO THE CIRCULATION DURING PREGNANCY AND ARE A SOURCE OF DECIDUAL STROMAL CELLS.**

Reshef Tal, MD, PhD,<sup>a</sup> Pallavi Pallavi, MD,<sup>a</sup> Yuan-Yuan Fang, MD,<sup>a</sup> Shruti Chinchankar, MBBS,<sup>a</sup> Hugh S. Taylor, M.D.<sup>b</sup> <sup>a</sup>Yale School of Medicine, New Haven, CT; <sup>b</sup>Yale University School of Medicine, New Haven, CT.



**OBJECTIVE:** Bone marrow-derived progenitor cells contribute to various nonhematopoietic cell populations in the endometrium including decidual stromal cells (DSCs). However, the exact BM lineage of BM-derived DSCs is unknown. Our objective was to explore the dynamics of circulating bone marrow mesenchymal stem cells (BM-MSCs) during pregnancy and investigate whether BM-MSCs may be the origin of BM-derived DSCs.

**DESIGN:** Animal Study.

**MATERIALS AND METHODS:** BM cells from whole body GFP or GFP under regulation of Hoxa11 promoter (Hoxa11-GFP) transgenic mice were used for BM transplantation (BMT) into wild-type C57BL/6J syngeneic females following established 5-FU based submyeloablation regimen (~45% chimerism). Hoxa11 expression in the BM is restricted to MSCs and is not expressed in hematopoietic cells, allowing in-vivo tracking of BM-MSCs. Following 1-month recovery, mice were mated with proven males. Multicolor flow cytometry was performed on peripheral blood, BM and uterus/decidua of non-pregnant, and pregnant mice on E5.5 and E9.5, timing of peak BMDCs recruitment to the uterus. Live cells were gated on Sca1+ and Lin- to identify stem cell populations and further divided into MSCs (Sca1+/CD45-/Lin-) and hematopoietic stem cells (HSCs) (Sca1+/CD45+/Lin-). Extracted BM cells were cultured in MSC expansion media, and characterized by flow cytometry, trilineage MSC differentiation, and decidualization assays.

**RESULTS:** Flow cytometry analysis showed that circulating MSCs were increased in pregnant mice from 0.002% in nonpregnant to 0.007% on E5.5, and further increased to 0.014% on E9.5 (~7-fold compared to nonpregnant,  $p < 0.01$ ,  $n = 5$ /group). In contrast, circulating HSCs remain unchanged from nonpregnant through E9.5 (0.10% vs. 0.11%), as did BM MSCs and HSCs. In the Hoxa11-GFP BMT model, BM-derived Hoxa11+GFP+ cells were detected in the E9.5 pregnant decidua (0.25±0.4% of total cells) and were CD45- by flow cytometry analysis, indicating that BM-MSCs are a likely origin of BM-derived DSCs. In-vitro, we cultured BM cells from GFP+ BMT recipients and analyzed P-2 cells for MSC characteristics. BM GFP+ cells were plastic-adherent and grew in MSC expansion media. Flow cytometry demonstrated that 9.5% of GFP+ cells were triple-positive for MSC markers Sca-1<sup>+</sup>CD29<sup>+</sup>CD44<sup>+</sup> and CD45<sup>-</sup>. Cultured BM GFP+ cells had trilineage differentiation ability to adipogenic, osteogenic and chondrogenic lineages, confirming their MSC characteristics. Moreover, cultured BM cells showed characteristic decidual morphological changes in response to decidualization stimuli, most prominent in the combined cAMP+17-MPA treatment group. In addition, expression of decidual prolactin prl8a2 mRNA was upregulated 10-fold on culture day 8 and 14 in the BM cells subjected to decidualization treatment vs. control ( $p < 0.01$ ).

**CONCLUSIONS:** These data show that BM-MSCs are increasingly mobilized to the circulation during murine pregnancy, are able to undergo decidualization in-vitro, and are a source of Hoxa11+ nonhematopoietic decidual cells in pregnancy. These suggest that BM-MSCs play an important previously unrecognized role in pregnancy.

**Reference:** Rux DR, Song JY, Swinehart IT, Pineault KM, Schlientz AJ, Trulik KG, et al. Regionally Restricted Hox Function in Adult Bone Marrow Multipotent Mesenchymal Stem/Stromal Cells. *Dev Cell.* 2016;39(6):653-66.

**SUPPORT:** 5K12HD047018-14 (to H.S.T and R.T.) American Society for Reproductive Medicine (to R.T.) Robert E. Leet and Clara Guthrie Patterson Fellowship award (to R.T.)

**O-245** Wednesday, October 16, 2019 11:45 AM

#### **USE OF CRISPR/dCas9 SYSTEM FOR INDUCTION OF MESENCHYMAL STEM CELLS.**

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**OBJECTIVE:** The objective of this study is to use CRISPR/dCas9 gene editing technology in order to increase gene activation for induction of differentiation of mesenchymal stem cells obtained from human umbilical cord matrix.

**DESIGN:** Human umbilical cord mesenchymal stem cell line was used for differentiation via chemical induction or CRISPR/dCas9 genome activation system. Osteogenic, adipogenic, and neurogenic differentiations were established. The experiments were designed as three biological replicates and three technical replicates for each biological replicate. The efficiency of differentiation capacity was evaluated by qPCR, Western blotting and super resolution microscopy.

**MATERIALS AND METHODS:** Human umbilical cord mesenchymal stem cells (MSCs) (Cell line: PCS500010/ATCC) was first differentiated to osteocytes, adipocytes and neurons via use of chemicals. Dexamethasone, glycerol-3-phosphate, ascorbic acid in DMEM with 10% FBS were used for osteogenic induction. Dexamethasone, insulin, indomethacin, isobutyl xanthine in DMEM with 10% FBS were used for adipogenic induction. Valproic acid, potassium Chloride, butylated hydroxyanisole in DMEM with 10% FBS were used for neurogenic induction. The inductions lasted for 21-28 days. An other group of MSCs were induced via use of dCas9 by viral transduction. In order to induce differentiation related transcriptional and/or activator factors were activated via use of dCas9-SAM (Synergistic Activation Mediator) system. Guide RNAs (gRNAs) for PPAR-gamma, RUNX2 and SOX were designed to target the area of 0 to -200 basepair according to TSS (transcription starting site). For 48 hours gRNAs were transduced to MSCs via lentivirus that holds the plasmids and gRNAs. At the end of induction period (28 days after chemical and 2 days after CRISPR/dCas9 induction) MSCs were assessed for morphological and biochemical changes. Osteopontin and alizarin red were used for osteogenic; oil red o and adiponectin were used for adipogenic and MAP2, NeuN, beta-III-tubulin were used for evaluation of neurogenic induction. qPCR, IF, and WB were used.

**RESULTS:** The induction of MSCs via CRISPR/dCas9 showed significantly efficient results in terms of both phenotypical and biochemical changes, by 35% for adipogenic induction, 45% for osteogenic induction, and 25% for neurogenic induction ( $p < 0.05$ ) as depicted by qPCR analysis. Superresolution microscopy evidently elaborated the changed morphology of cells with positive stainings for osteopontin in osteogenic cells, lipid granules in adipogenic cells. The neurogenic cells showed long dendrite-like extensions that reach out to each other. These cells were positive for NeuN, beta-III-tubulin and MAP2.

**CONCLUSIONS:** CRISPR/dCas9-SAM activation system was significantly more efficient for differentiation of human umbilical cord MSCs into different lineages. The differentiation was more rapid, did not need constant use of induction medium and did not reverse by time.

**O-246** Wednesday, October 16, 2019 12:00 PM

#### **POST-MEIOTIC MALE GERM CELL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS BY EXPOSURE TO CONDITIONED MEDIUM.**

Aysha Trout, B.A., Philip Xie, B.S., Alessandra Parrella, M.Sc., Zev Rosenwaks, M.D., Gianpiero D. Palermo, M.D., Ph.D., The Ronald O. Perleman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY.



**OBJECTIVE:** To determine whether the exposure of mouse embryonic stem cells (mESCs) to medium conditioned by adult peritubular or neonatal germinal epithelial cells efficiently increases differentiation into primordial germ cell-like cells (PGCLCs) and induces meiosis.

**DESIGN:** We tested a novel culture system utilizing a mesh interphase system to initiate meiotic differentiation of mESCs under media conditioned by adult peritubular cells consisting mainly of Leydig cells, or by neonatal germinal epithelial cells containing pre-Sertoli cells, which are adapted to peracrine signaling. mESCs without exposure of these cells served as a control.

**MATERIALS AND METHODS:** Over a 6-month period, mESCs were differentiated into epiblast-like cells (EpiLCs) and PGCLCs. Peritubular cells derived from adult mouse testes were isolated by differential plating; germline cells from neonatal mice were isolated by trypsinization of testes and placed on the mesh. After 7 passages,  $1.2 \times 10^6$  mESCs were plated on each well with MEFs and cultured in the mesh interphase system subsidized with either adult or neonatal cells, or voided at 37°C and 5% CO<sub>2</sub>. Germ cell stage-specific biomarkers (Nanog, Oct4, DAZL, and VASA) were assessed at regular intervals of culture.

**RESULTS:** At day 3 of differentiating mESCs in mesh interphase-conditioned medium containing Activin A, bFGF, and KSR, decreased positivity of Nanog (45%) indicated the successful differentiation of mESCs into EpiLCs. Continued expression of Oct4 (>90%) was detected in the cells at day 3, suggesting the retention of stemness. Cytoplasmic DAZL positivity at day 5 demonstrated early meiotic differentiation into spermatocyte lineage. On day 8, approximately 30% expressed VASA positivity, indicating further progression into meiosis. Cells cultured in media conditioned by adult interstitial cells had greater expression of DAZL and VASA than those cultured with neonatal interstitial cells. The optimal condition was determined to be a 1:5 ratio of adult cells to mESCs. DAZL and VASA expression were negative in the control group, suggesting the important role of the medium conditioned by testicular cells.

**CONCLUSIONS:** These results indicate that our novel culture system can promote differentiation of mESCs into PGCLCs and further meiotic differentiation. Initiation of neospermatogenesis using mESCs can be optimized in the presence of factors secreted from Leydig cells derived from adult mouse testes. Reproducing spermatogenesis in vitro may provide valuable information on overcoming male infertility due to spermatogenic arrest or germ cell aplasia.

## REPRODUCTIVE ENDOCRINOLOGY

**O-247** Wednesday, October 16, 2019 10:45 AM

### VARYING LEVELS OF SERUM ESTRADIOL DO NOT ALTER THE TIMING OF THE EARLY ENDOMETRIAL SECRETORY TRANSFORMATION.

Emily K. Osman, MD,<sup>a</sup> Tianren Wang, M.D., Ph.D.,<sup>b</sup> Min Yang, Ph.D.,<sup>b</sup> Yiping Zhan, Ph.D.,<sup>b</sup> Caroline R. Juneau, MD,<sup>c</sup> Scott J. Morin, MD,<sup>d</sup> Emre Seli, M.D.,<sup>a</sup> Richard Thomas Scott, Jr., MD,<sup>a</sup> Jason M. Franasiak, MD,<sup>a</sup> <sup>a</sup>IVI-RMA New Jersey, Basking Ridge, NJ; <sup>b</sup>Foundation for Embryonic Competence, Basking Ridge, NJ; <sup>c</sup>Audubon Fertility, New Orleans, LA; <sup>d</sup>IVI-RMA Northern California, San Francisco, CA.



**OBJECTIVE:** Endometrial receptivity is induced by the systematic exposure of estradiol (E2) followed by progesterone (P4). There has been concern that the exaggerated E2 levels seen in stimulated cycles may attenuate the impact of P4 rise and initiation of secretory transformation, ultimately altering the window of receptivity. This study aimed to determine if supra-physiologic E2 levels in the ranges attained during normal and high response superovulation cycles can modify the onset of secretory transformation.

**DESIGN:** Prospective, randomized, paired.

**MATERIALS AND METHODS:** A total of 30 biopsies were collected from 10 volunteers that were enrolled and randomized to the order in which they completed 3 different uterine stimulation cycles: physiologic (approximately 180 pg/mL), moderately supra-physiologic (600-800 pg/mL) or supra-physiologic (2000 pg/mL) ranges in order to approximate natural, controlled ovarian stimulation, and in vitro fertilization (IVF) cycles. E2 valerate, selected for constant steady state levels that more accurately simulate conditions during the proliferative phase, was administered in physician adjusted doses for 12 days. Intramuscular P4 in oil 10 mg/day, a dose known to mimic the P4 rise seen prior to the onset of secretory transformation, was administered and after two completed days of P4 exposure, an endometrial biopsy was performed. DNA was isolated from the specimens and bisulfite sequencing was performed to construct a methylation array. Differential methylation analysis was conducted based on differences in M-values of individuals across treatment groups for each probe as well as carrying out *t*-tests. RNA was isolated for RNAseq analysis and gene expression values were compared using Cufflinks 2.1.1. Genes were deemed differentially expressed if they demonstrated an FDR (adjusted *p*-value) of less than 0.05. All

analyses were performed in a pairwise fashion to compare among the three stimulation cycles within individual patients and secondarily to compare all patients in each of the cycles.

**RESULTS:** The mean peak E2 level in the physiologic group was 275 pg/mL, moderate group was 909.7 pg/mL and 2043.4 pg/mL in the supra-physiologic group. Principal component analysis of 834,913 CpG sites was performed on M-values of individual patients within the low, moderate and supra-physiologic conditions in a paired approach. There were no differences in genome-wide methylation within individual patients across E2 groups. A paired analysis revealed that gene expression profiles did not differ within the same individual at each of the three E2 levels. No significant alterations in gene expression were identified between the low, moderate and supra-physiologic groups in an interpatient analysis.

**CONCLUSIONS:** Highly supra-physiologic E2 levels do not alter the ability of physiologic levels of P4 to induce secretory transformation. These data suggest that the diminution in implantation seen in stimulated cycles results from embryonic-endometrial dysynchrony following early P4 elevations or slowly blastulating embryos, which may be independent of the magnitude of the E2 rise.

**O-248** Wednesday, October 16, 2019 11:00 AM

### DISRUPTION OF MITOCHONDRIAL DYNAMICS IN OOCYTES RESULTS IN INFERTILITY AND DIMINISHED OVARIAN RESERVE.

Man Zhang, M.D., Ph.D.,<sup>a</sup> Muhammed Burak Bener, B.S.,<sup>a</sup> Zongliang Jiang, Ph.D.,<sup>b</sup> Tianren Wang, M.D., Ph.D.,<sup>c</sup> Ecem Esencan, M.D.,<sup>a</sup> Richard Scott, III, B.S.,<sup>d</sup> Emre Seli, M.D.,<sup>a</sup> <sup>a</sup>Yale School of Medicine, New Haven, CT; <sup>b</sup>Louisiana State University, Baton Rouge, LA; <sup>c</sup>Foundation for Embryonic Competence, Basking Ridge, NJ; <sup>d</sup>Foundation of Embryo Competence, Basking Ridge, NJ.



**OBJECTIVE:** Mitochondrial fusion and fission (collectively referred to as mitochondrial dynamics) allow mitochondria to adapt to changes in their metabolic milieu and to respond to environmental stressors. Mitofusin-1 (MFN1) regulates mitochondrial dynamics by promoting mitochondrial fusion. The aim of the current study was to determine the role of MFN1 in female reproductive competence using a mouse model with oocyte-specific deletion of *Mfn1*.

**DESIGN:** Experimental study.

**MATERIALS AND METHODS:** *Mfn1*<sup>fllox/fllox</sup> mice were crossbred with *Zp3-Cre* mice to produce mice with oocyte-specific *Mfn1* deletion (*Mfn1*<sup>-/-</sup>). Fertility was assessed by mating 2-month-old *Mfn1*<sup>-/-</sup> and wild type (WT) female mice (n=7 per group) with WT fertile males for 12 weeks. Follicle development was assessed by staining serial ovarian sections with hematoxylin and eosin. Ability to generate oocytes (germinal vesicle [GV] and metaphase II [MII]) was assessed after injection with PMSG (5IU) or PMSG and hCG (5IU). RNA sequencing analysis was performed using pooled *Mfn1*<sup>-/-</sup> and WT secondary follicle-enclosed oocytes (n=3 mice per group). Protein and mRNA expression were assessed using immunofluorescence and qRT-PCR, respectively.

**RESULTS:** *Mfn1*<sup>-/-</sup> female mice were infertile and did not produce any pups. *Mfn1*<sup>-/-</sup> mice (8-weeks-old) ovaries had similar number of primordial, primary, and secondary follicles compared to WT, but no antral follicles. They also did not produce mature (MII) oocytes (p < 0.001), and generated a significantly lower number of immature oocytes (17±3.6 vs 40±3.0, p < 0.01). When changes in follicular pool was assessed across mouse reproductive lifespan, *Mfn1*<sup>-/-</sup> mice were found to have significantly lower number of primordial and primary follicles compared to WT at 6 months, and depletion of follicles of all stages at 12 months (p < 0.01 for all comparisons). RNA-seq analysis revealed a total of 982 genes that were differentially regulated in *Mfn1*<sup>-/-</sup> oocytes with a number of affected pathways including cell death (apoptosis) signaling and ceramide biosynthesis (p < 0.01). As suggested by RNAseq analysis, caspase 6 (mediator of apoptosis) and ceramide levels were elevated in *Mfn1*<sup>-/-</sup> secondary follicle-enclosed oocytes compared to WT (p < 0.01). Because elevated intracellular ceramide may induce apoptosis, we tested whether decreasing ceramide levels in *Mfn1*<sup>-/-</sup> mice would rescue reproductive phenotype. Indeed, treatment with ceramide synthesis inhibitor myriocin (1.5 mg/kg daily injection for 21 consecutive days) rescued follicular defects in *Mfn1*<sup>-/-</sup> mice and resulted in development of antral follicles.

**CONCLUSIONS:** Absence of MFN1 in oocytes results in infertility and diminished ovarian follicular reserve. Importantly, arrested follicular development that ensues in the absence of MFN1 can be rescued by myriocin, which blocks the accumulation of pro-apoptotic ceramide. Whether these mechanisms can be exploited to improve human reproductive efficiency will need to be further investigated.

### CELL TYPE SPECIFIC EFFECTS OF HYPERLIPIDEMIA AND HYPERINSULINEMIA, CHARACTERISTIC OF REPROMETABOLIC SYNDROME, ON PITUITARY FUNCTION.

Rosemary McDonald, BS,<sup>a</sup> Katherine Kuhn, MS,<sup>b</sup> Andrew P. Bradford, PhD,<sup>b</sup> Nanette Santoro, M.D.<sup>b</sup> <sup>a</sup>University of Colorado Anschutz Medical Campus, Aurora, CO; <sup>b</sup>University of Colorado School of Medicine, Aurora, CO.



**OBJECTIVE:** Obesity has a profound impact on reproductive function, reducing fertility and increasing the risk of pregnancy complications and birth defects. Obesity in women is associated with hyperlipidemia, hyperinsulinemia, and decreased basal and GnRH-stimulated FSH and LH secretion from gonadotrope cells in the pituitary. We have termed this phenotype 'Reprometabolic Syndrome'. We have previously shown that acute infusions of lipid/insulin into healthy, normal weight, cycling women recapitulates this reprometabolic phenotype of obesity. However, the underlying mechanisms are not understood. We sought to confirm that the decreased FSH and LH were not attributable to differential hemodilution, and investigated if the effects of lipid/insulin infusions were confined to gonadotropes or impacted other anterior pituitary cell types.

**DESIGN:** 8 normal weight, regularly cycling women underwent 6-hour visits with either a saline and heparin (control) infusion, or a hyperinsulinemic-euglycemic clamp with heparin and Liposyn (Abbott laboratories). GnRH stimulation was applied at 240 minutes.

**MATERIALS AND METHODS:** Frequent blood sampling (q10 min) was conducted at each visit, which occurred in random order, between days 2-5 in sequential menstrual cycles. Anterior pituitary hormones TSH and prolactin (PRL), thyroid hormones (free T4, total T3) and cortisol were measured in serum samples. TSH was measured in pooled samples q30 min. PRL, fT4, total T3, and cortisol samples were pooled to measure approximately 0, 30, 160, and 360 min time points. Lastly, creatinine was measured hourly in pooled samples.

**RESULTS:** In contrast to the decrease in gonadotropins, TSH increased in the lipid/insulin-treated samples, with the most dramatic percent change after 160 minutes (28.2% increase), significantly different from TSH levels in the saline infusions ( $p < 0.0005$ ), which slightly decreased (-11.4%). Thyroid hormones (fT4 and total T3), PRL, cortisol, and serum creatinine did not differ between saline or lipid/insulin infusion conditions.

**CONCLUSIONS:** Lack of change in serum creatinine showed there was no hemodilution due to variable infusion volumes. fT4 and total T3 were unchanged, suggesting that the increase in TSH was a thyrotrope cell response to lipid/insulin and not a result of altered thyroid function. Cortisol, an inhibitor of TSH production, was unaffected by infusion condition. Levels of the lactotroph hormone PRL were not impacted by lipid/insulin, confirming that effects on the pituitary are both complex and cell type specific. Our results imply that the impact of obesity on the hypothalamic-pituitary-gonadal axis is not simply suppression, and extends beyond reproductive functions. Further research is needed to elucidate mechanisms underlying the selective modulation of pituitary trophic hormones in response to changes in diet and metabolism.

O-250 Wednesday, October 16, 2019 11:30 AM

### CHRONIC EXPOSURE TO AMH MAY ACCELERATE GROWTH OF FOLLICLES VIA DOWNREGULATION OF AMHR II.

Limor Man, M.D., M.Med.Sc.,<sup>a</sup> Eleni Kallinos, B.S.,<sup>b</sup> Daylon James, PhD,<sup>c</sup> Zev Rosenwaks, M.D.<sup>c</sup> <sup>a</sup>Assistant Professor of research, NYC, NY; <sup>b</sup>NYC, NY; <sup>c</sup>The Ronald O. Perleman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY.



**OBJECTIVE:** Anti-Müllerian hormone (AMH) has been suggested to exert a repressive input on activation and growth during folliculogenesis. We have previously shown that direct paracrine delivery of AMH via engineered endothelial cells (ECs) reduces premature follicular mobilization and growth in short-term human ovarian tissue xenografts (Man et al., 2017). Others, using continuous administration of AMH via an osmotic pump or intraperitoneal injection of lentivirally encoded AMH in a murine model, have shown similar results (Kano et al., 2017). Here, we investigated the influence of AMH-producing ECs on follicle growth in long-term grafts.

**DESIGN:** Xenograft of human ovarian tissue into NOD scidgamma (NSG) mice with co-transplantation of control ECs or ECs that constitutively secrete human AMH.

**MATERIALS AND METHODS:** We used lentiviral vectors encoding both a fluorescent reporter and human AMH to modify ECs. We obtained a large volume of ovarian tissue from a 26-year-old organ donor to serve

as a source of patient-matched material for study. We co-transplanted cortical fragments from this donor with AMH-expressing or control ECs to establish xenografts in oophorectomized NSG mice. We recovered xenografts at 2 (CTRL, n=7; AMH, n=7), 8 (CTRL, n=6; AMH, n=8), and 14 (CTRL, n=7; AMH, n=8) weeks. We assessed the ratio of primordial to growing follicles in each treatment in histologic sections using H&E staining and light microscopy. Antral follicles at 14 weeks were stained for AMH type II receptor (AMHR II), and the level of expression was quantified by measuring the ratio of area of AMHR II-positive staining to the total nuclear area using a confocal microscope.

**RESULTS:** In contrast to short-term grafts (2 weeks), in which AMH-ECs promoted quiescence (primordial:growing follicles ratio: CTRL 0.32 vs. AMH-ECs 1.39,  $P < 0.01$ ), the AMH group in long-term grafts revealed a shift in the follicular pool toward accelerated growth (primordial:growing follicle ratio: 8 weeks - CTRL 0.55 vs. AMH ECs 0.19,  $P = 0.06$ ; and 14 weeks - CTRL 0.29 vs. AMH 0.12,  $P = 0.05$ ). Notably, at 14 weeks, antral follicles from the AMH group exhibited 3-fold larger diameter than antral follicles in the CTRL group (CTRL median diameter 0.5 mm vs. AMH-ECs 1.5 mm,  $P = 0.01$ ). Lastly, measurement of AMHR II protein level revealed a median ratio of 22.4 in the CTRL compared to 1.15 in the AMH group ( $P = 0.001$ ).

**CONCLUSIONS:** As autotransplantation of ovarian tissue becomes more widely practiced, resolving the mechanisms mediating follicular activation and growth is increasingly relevant. Our unexpected finding, given previous results indicating a suppressive input of AMH, suggests that a chronic AMH stimulus of the ovary may initially suppress activation but ultimately induces a rebound effect, in part, via negative feedback and downregulation of AMHR II.

Reference: Kano M, Sosulski AE, Zhang L, Saatcioglu HD, Wang D, Nagyky N, Sabatini ME, Gao G, Donahoe PK, Pépin D. AMH/MIS as a contraceptive that protects the ovarian reserve during chemotherapy. *Proc Natl Acad Sci U S A* 2017;114: E1688-E1697.

Man L, Park L, Bodine R, Ginsberg M, Zaninovic N, Man OA, Schattman G, Rosenwaks Z, James D. Engineered endothelium provides angiogenic and paracrine stimulus to grafted human ovarian tissue. *Sci Rep* 2017;7: 8203.

**SUPPORT:** Internal funding, CRMI.

O-251 Wednesday, October 16, 2019 11:45 AM

### ANDROGEN HORMONES AND SEXUAL FUNCTION AMONG CANCER SURVIVORS: SHORT AND LONG-TERM TREATMENT EFFECTS.

Leigh A. Humphries, MD,<sup>a</sup> Katherine E. Cameron, MD, MBE,<sup>b</sup> Mary D. Sammel, ScD,<sup>c</sup> Clarisa R. Gracia, MD, MSCE<sup>b</sup> <sup>a</sup>Hospital of the University of Pennsylvania, Philadelphia, PA; <sup>b</sup>University of Pennsylvania, Philadelphia, PA; <sup>c</sup>Department of Biostatistics, Epidemiology and Informatics, University of Pennsylvania, Philadelphia, PA.



**OBJECTIVE:** Sexual function is a critical component of quality of life for many cancer survivors. Given the importance of androgen production in sexual health, we sought to characterize circulating androgen levels before and after cancer therapy as well as sexual function.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Reproductive age female patients (15-39 years) with a cancer diagnosis and controls completed sexual health questionnaires and tests of serum androgens (free testosterone, DHEAS) and ovarian reserve (FSH, AMH, AFC). This study included a) women with a new cancer diagnosis, assessed pre-treatment and every 3 months post-treatment to examine short-term effects (N=117), and b) cancer survivors  $\geq 1$  year from the end of therapy with no evidence of disease to examine long-term effects (N=120). These participants were compared to similar-aged controls (N=100) and also late-reproductive aged controls (N=63) using linear regression adjusted for age and BMI.

**RESULTS:** In adjusted models, women with a new cancer diagnosis (median age 27) had significantly lower testosterone and DHEAS levels than similar-aged controls, even prior to the start of therapy (median testosterone 0.35 ng/mL vs 0.49 ng/mL,  $p < 0.01$ ; DHEAS 0.79  $\mu$ g/mL vs 1.3  $\mu$ g/mL,  $p < 0.01$ ); with no difference in AMH levels pre-treatment. After cancer therapy, testosterone levels were further suppressed, with a median within-person decrease of 62% ( $p < 0.01$ ) at 2 months and 39% ( $p < 0.01$ ) at 6 months after treatment end. By 12 months after treatment, testosterone levels among cancer patients exhibited some recovery, at a rate of 6% per month ( $p < 0.01$ ).

Reported sexual dysfunction before and after treatment was prevalent regardless of testosterone or DHEAS levels, with 41% of sexually active participants reporting decreased libido at their pre-treatment visit and 42% at 6 months post-treatment.

In long-term survivors, women (median age 24) remote from therapy (median 8.2 years) had significantly lower testosterone and DHEAS levels compared to similar-aged controls ( $p=0.01$  and  $p<0.01$ , respectively), though higher than late-reproductive age controls (median age 47), ( $p=0.02$  for testosterone,  $p<0.01$  for DHEAS). However, sexual dysfunction did not differ from controls (20% in survivors, 16% in similar-aged controls, 17% in late reproductive-aged controls,  $p=0.83$ ), and symptom prevalence was not associated with androgen levels.

**CONCLUSIONS:** Prior to chemotherapy, women with a new cancer diagnosis have lower androgen levels than controls, possibly due to pre-existing suppression of the hypothalamic pituitary ovarian axis. Androgen levels drop further after therapy. While there is some recovery, long-term levels remain lower than controls. Sexual dysfunction is prevalent immediately post therapy; yet, in long-term survivors, androgen levels do not correlate with self-reported sexual dysfunction, and thus androgens may not play a major role in sexual functioning in this population. More studies investigating sexual function and quality of life in cancer survivors, particularly immediately post-therapy, may help guide counseling for these women.

O-252 Wednesday, October 16, 2019 12:00 PM

**ETHNIC DISCORDANCE IN SERUM MÜLLERIAN HORMONE (AMH) IN HEALTHY WOMEN; POPULATION STUDY FROM CHINA AND EUROPE.** Scott M. Nelson, MD, PhD,<sup>a</sup> Gemma L. Clayton, PhD,<sup>b</sup> Abigail Fraser, PhD,<sup>c</sup> Sun Aijun, PhD,<sup>d</sup> <sup>a</sup>University of Glasgow, Glasgow, United Kingdom; <sup>b</sup>University of Bristol, Bristol; <sup>c</sup>University of Bristol, Bristol, United Kingdom; <sup>d</sup>Peking Union Medical College Hospital, Beijing, China.



**OBJECTIVE:** Chinese women are known to have an earlier age at natural menopause than their European counterparts, whether they also have a lower functional ovarian reserve is unknown. This study was designed to assess whether there are ethnic differences in Anti-Müllerian Hormone (AMH) in women of reproductive age.

**DESIGN:** Non-select cohort of women in the Netherlands and China.

**MATERIALS AND METHODS:** Women with regular menstrual cycles, not on hormonal contraception or with any medical history of note, were recruited to provide a day 2-5 early follicular sample in China and Europe. AMH was determined using the Roche Elecsys assay. AMH decline was modelled with a linear, quadratic and quadratic with interaction on age equations to assess the impact of ethnicity.

**RESULTS:** 1348 subjects met the inclusion criteria and participated in the study; 887 European and 461 Chinese women. Despite the Chinese population being slightly younger  $34.07 \pm 8.38$  years than their European counterparts  $34.75 \pm 8.87$  years, their median AMH was lower 1.87 (IQR 0.28, 3.64) as compared to 2.11 (IQR 0.73, 3.96), with evidence of increasing discordance from age 25 years. In all regression models of the AMH age-related decline, there was evidence of a difference between Chinese and European women. On average AMH was 51% (geometric mean ratio=0.49 (95% CI 0.44 to 0.55)) lower in the Chinese population compared to the European population.

**CONCLUSIONS:** There were independent effects of age and ethnicity on serum AMH concentrations, with Chinese women having a substantially lower AMH in adult life than their European counterparts from age 25 onwards.

**SUPPORT:** None.

## REPRODUCTIVE IMMUNOLOGY

O-253 Wednesday, October 16, 2019 10:45 AM

**INTERFERON GAMMA-INDUCED PROTEIN 10 (IP-10) IS SIGNIFICANTLY LOWER AT EARLY IMPLANTATION IN TWIN VERSUS SINGLETON PREGNANCIES.**

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**OBJECTIVE:** To determine if pro-inflammatory cytokines in maternal serum differ between twin and singleton gestations in the implantation phase.

**DESIGN:** A prospective longitudinal cohort of women.

**MATERIALS AND METHODS:** Women with an initial positive  $\beta$ -hcg serum blood draw after a double or single embryo transfer following in vitro

fertilization (IVF) were eligible to participate. Patients were selected for analysis based on healthy term singleton ( $n=21$ ) or healthy term dichorionic diamniotic (di/di) twin ( $n=6$ ) delivery. Cytokines tumor necrosis factor alpha (TNF $\alpha$ ) and interferon gamma-induced protein 10 (IP-10) were analyzed in serial serum samples ( $n=94$ ) from day 9-15 after blastocyst embryo transfer using the SimplePlex immunoassay platform. Samples were compared throughout the sample period using t-tests and Cohen's D.

**RESULTS:** TNF $\alpha$  and IP-10 were detected in all sera samples. From day 9-15, IP-10 was significantly lower in di/di twin gestations than in singleton gestations (day 9-11,  $84.5 \pm 28.5$  v  $129.1 \pm 67$  pg/mL,  $p=0.01$ ; day 12-13,  $93.8 \pm 29.2$  v  $120.8 \pm 40.7$  pg/mL,  $p=0.05$ ; day 14-15,  $102.7 \pm 19.9$  v  $145.6 \pm 63.7$  pg/mL,  $p=0.01$ ). Looking at the overall trend, Cohen's D was -0.59, indicating that IP-10 was significantly lower in days 9-15 in twin pregnancies (95% confidence interval -1.15 to -0.04). During this same time frame, TNF $\alpha$  showed no significant difference (day 9-11,  $5.3 \pm 1.7$  v  $6.2 \pm 1.5$  pg/mL; day 12-13,  $5.2 \pm 1.4$  v  $5.8 \pm 1.4$  pg/mL; day 14-15,  $5.4 \pm 1.2$  v  $6.4 \pm 2.2$  pg/mL). Cohen's D for TNF $\alpha$  also was not significantly different (-0.29, 95% confidence interval -0.83 to 0.26).

**CONCLUSIONS:** This is the first report describing IP-10 in serum in the early implantation phase, and the first report comparing pro-inflammatory cytokines between patients with singletons and di/di twins. We demonstrate that serial IP-10 concentrations are significantly lower throughout the early implantation phase in di/di twin pregnancies when compared to normal singleton pregnancies, while TNF $\alpha$  concentrations are not. This strengthens the inhibitory roles ascribed to IP-10 regarding angiogenesis in pregnancy, as increased angiogenesis would be expected in a healthy di/di twin pregnancy.

**SUPPORT:** Prelude Fertility - Scientific Advisory Board Grant.

O-254 Wednesday, October 16, 2019 11:00 AM

**SUPEROVULATION ALTERS THE HUMAN UTERINE NATURAL KILLER CELL REPERTOIRE DURING THE WINDOW OF IMPLANTATION.**

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**OBJECTIVE:** Adverse perinatal outcomes associated with fresh IVF, including pre-eclampsia and growth restriction, have been at least partially attributed to abnormal placentation secondary to the maternal hormonal environment following superovulation. Trophoblast invasion and uterine vascular remodeling is regulated in part by maternal immune cells, with multiple adverse pregnancy outcomes associated with disturbances in the immune cell populations. A recent study defined three subtypes of natural killer (NK) cells (NK1, NK2, NK3) in the endometrium that may play a role in human placentation. The aim of this study was to evaluate the effect of superovulation on human endometrial immune cell distribution during the window of implantation.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Endometrial biopsies and peripheral blood samples were collected from 25 subjects: 16 samples obtained in natural cycles 8 days after a positive ovulation predictor kit and 9 samples obtained 7 days after egg retrieval in gonadotropin stimulated IVF cycles. All participants had regular menstrual cycles, no known history of endometriosis or autoimmune disorders and were free of hormonal and chronic anti-inflammatory treatment prior to their biopsies. Immune cell populations were analyzed using flow cytometry. Serum estradiol (E<sub>2</sub>) and progesterone (P<sub>4</sub>) levels were measured by chemiluminescent competitive immunoassay. Student t-test or Mann-Whitney U-test was used to evaluate between-group differences.

**RESULTS:** Baseline characteristics were comparable for both groups. As expected, serum E<sub>2</sub> levels on the day of biopsy were significantly higher in patients following gonadotropin stimulation. No differences in serum P<sub>4</sub> levels were noted. Characterization of the total leukocyte population (CD45+ cells) revealed a statistically significant reduction in CD56<sup>bright</sup> endometrial NK cells in stimulated cycles compared to natural cycles ( $24.54 \pm 3.62$  vs.  $37.23 \pm 2.63$  % of total CD45+ cells,  $p=0.009$ ). When NK cell subtypes were analyzed, there was a significant increase in endometrial NK1 subpopulation as a proportion of all NK cells ( $19.14 \pm 3.04$  vs.  $9.48 \pm 2.28$  % of total CD56<sup>bright</sup> cells,  $p=0.008$ ) and a decrease of endometrial NK3 subpopulation ( $28.8 \pm 3.3$  vs.  $49.29 \pm 3.88$  % of total CD56<sup>bright</sup> cells,  $p=0.043$ ) in the stimulated cycles compared to natural cycles. The fraction of NK2 cells as a proportion of all NK cells was unchanged. No changes were seen in the percentage of endometrial CD14<sup>bright</sup> monocytes/macrophages. There were no differences in the immune cell populations in peripheral blood on the day of biopsy.

**CONCLUSIONS:** These findings demonstrate that superovulation affects the distribution of NK cells in the endometrium during the window of implantation. Uterine NK cell, specifically NK3 cells, based on marker expression and cytokine production, appear to function in regulating trophoblast invasion during early implantation. These data provide a potential mechanism by which alterations in the maternal hormonal environment may lead to an increased risk of disorders of placentation and adverse perinatal outcomes.

**O-255** Wednesday, October 16, 2019 11:15 AM

**UNRAVELLING THE IMMUNOGENETICS OF PREGNANCY: PARENTAL HLA-C ALLOTYPES ARE PREDICTIVE OF PREGNANCY LOSS AFTER SINGLE EUPLOID EMBRYO TRANSFERS.** Diego Marin, M.S.,<sup>a</sup>



Xin Tao, Ph.D.,<sup>b</sup> Li Sun, Ph.D.,<sup>b</sup> Richard Thomas Scott, Jr., MD.<sup>a</sup> <sup>a</sup>IVI-RMA New Jersey, Basking Ridge, NJ; <sup>b</sup>Foundation for Embryonic Competence, Basking Ridge, NJ.

**OBJECTIVE:** Uterine natural killer cells (uNK) orchestrate correct placentation through cellular responses mediated by their killer cell immunoglobulin-like receptors (KIR) and the HLA-C ligands (C1 or C2) presented by trophoblast cells. It has been reported that when patients have KIR AA genotypes (inhibitory) and their embryos are homozygous for the HLA-C2 allele, the risk of miscarriage increases significantly compared to other combinations. Since it is not always feasible to know the embryonic HLA-C ligands before a transfer, this study aimed to evaluate if parental HLA-C genotypes are predictive of clinical outcomes in a context of euploid single embryo transfer (SET).

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** Patients undergoing a euploid SET with own eggs were included in the study. Only the first SET per couple and SETs that resulted in a positive B-hCG were included in the analysis since the effect of KIR-HLA interactions occurs after implantation. Maternal KIR and parental HLA-C genotyping was performed using quantitative PCR. The variable "nC2" was created to score each couple based on the number of their C2 alleles. Next, the number of maternal C2s were subtracted from the paternal ones (PC2-MC2) so as to assess if a higher HLA-C2 load from either parent is associated with outcomes. Primary endpoints were ongoing pregnancy and clinical loss rates. Logistic regressions and the Fisher's exact test were computed when appropriate.

**RESULTS:** 790 euploid SETs were included in the analysis. Mean maternal age was  $35.96 \pm 3.74$  years. The overall frequency of maternal KIR AA and Bx genotypes was 28.7% and 71.3% respectively. For parental HLA-C allotypes, the frequencies were: C1C1 37.4%, C1C2 46.6% and C2C2 16%. Overall ongoing pregnancy rate was 77.59% (95% CI 74.52-80.45) and remained statistically unchanged irrespective of parental HLA-C or maternal KIR. However, clinical pregnancy loss was positively dependent of parental nC2 in KIR AA patients only ( $\beta=0.73$ ,  $p=0.0027$ ), reaching 33.33% when both parents were homozygous C2C2. Regarding each parent's C2 load, a higher paternal C2 load was significantly associated with lower risk of pregnancy loss in Bx patients only ( $\beta=-0.41$ ,  $p=0.0074$ ), which also shows that a higher paternal C1 load is associated with a higher risk of clinical loss in this group. In fact, when there were more C1 alleles from father than mother per couple (PC1>MC1) the risk of clinical loss was significantly increased in the Bx population (OR: 2.37, 95% CI: 1.33 – 4.18).

**CONCLUSIONS:** The risk of miscarriage increased significantly in relation to parental C2 allotypes in KIR AA patients. Notably, when the parental origin of HLA-C allotypes was investigated, a higher paternal C1 load increased the risk of clinical loss in patients with activating KIR (Bx), a finding in alignment with the theory of immunological memory of uNK cells with maternal HLA-C allotypes. This data also suggests that parental KIR-HLA-C genotyping could be useful for counselling patients undergoing euploid SET in cases where HLA-C-based embryo selection is not feasible.

**O-256** Wednesday, October 16, 2019 11:30 AM

**EFFICIENCY OF IMMUNOMODULATION OF ENDOMETRIUM WITH MIXED PATERNAL AND MATERNAL PERIPHERAL MONONUCLEAR CELLS IN REPEATED IMPLANTATION FAILURES.** Hanen Elloumi, Dr,



Khaled Mahmoud, Dr Sonia Mnallah, Dr, Mariem ben Khelifa, Phd, Fathi Zhioua, Dr, Khaled Terras, Dr, Mohamed Khrouf, Dr clinique la rose, centre FERTILLIA, Tunis, Tunisia.

**OBJECTIVE:** To date, implantation is the rate-limiting step for the success of IVF. The process of implantation is a complicated process that requires the

orchestration of a series of events involving both the embryo and the endometrium. Recently, accumulating evidence has suggested that local immune cells at the implantation site have actively contributed to embryo implantation. Some studies suggested the role of endometrium immuno-modulation with maternal activated peripheral mononuclear cells (PBMCs) in implantation success. However, the effect of intra uterine insemination of mixed paternal and maternal activated PBMCs before embryo transfer in RIF cases has not been studied enough. In this direction, the aim of our work is to examine the influence of the type and the number of intrauterine peripheral blood mononuclear cells application on embryo implantation rates for infertile patients.

**DESIGN:** Prospective study conducted between February 2018 and February 2019. Forty one couples with RIF were included. The patients were categorized into two groups with regard to their treatment type, autologous PBMC: group A (n=18) and co-cultured maternal and paternal PBMC: group B (n=26). Subgroups were defined according to the number of PBMC inseminated: < 2 millions (Group A1 (n=8) and group B1 (n=10); and  $\geq 2$  millions (Group A2 (n=11) and group B2 (n=13)).

**MATERIALS AND METHODS:** Mononuclear cells were isolated from patient's peripheral blood by density gradient centrifugation using commercially available lymphocyte preparation and then cultured for 3 days and transferred into the endometrium cavity prior to embryo transfer. All patients were selected on the following inclusion criteria: failure to achieve a pregnancy following a minimum of three IVF/ICSI cycles in which more than 5 high-grade embryos were transferred, age  $\leq 40$  years old, primary infertility and absence of uterine pathology.

**RESULTS:** Baseline clinical parameters and number of embryos transferred were found to be comparable in all groups. Our study demonstrates that activated PBMC promote clinical pregnancy rates (CPR) (39%). The CPR were significantly higher when at least 2 millions of co-cultured maternal and paternal PBMC were inseminated, group B2, (62% in comparison respectively to group A1, A2 and B1 (38%; 37%; 10%); ( $p<0.05$ ). The implantation rate was also significantly higher in group B2 (35.3% in comparison respectively to group A1, A2 and B1 ((19%; 22%; 8.3%); ( $p<0.05$ ).

**CONCLUSIONS:** In conclusion, we provide for the first time the effect of the adjuvant of paternal activated PBMC to immune modulate endometrium. Intra Uterine insemination of paternal cultured-activated PBMC 48h prior embryo transfer can provide biological signals specifically paternal antigen and cytokines that can exert a considerable influence on female reproductive tract physiology by inducing pro inflammatory cytokines, chemokines and interleukines profiles changes to mediate maternal immune tolerance of the embryo at implantation. The precise mechanism of PBMCs action still unclear and both in vitro and in vivo experiments are needed in order to clarify the mechanism.

**O-257** Wednesday, October 16, 2019 11:45 AM

**UTERINE NK CELL ACTIVITY IN RECURRENT IMPLANTATION FAILURE- ROLE OF INTRALIPIDS.** Krishna Deepika, MS, FRM,<sup>a</sup>



Arveen Vohra, MS, FRM.<sup>b</sup> <sup>a</sup>Consultant in Rep Med, Bangalore, India; <sup>b</sup>Senior Consultant, Milann Fertility Centre, Bangalore, India.

**OBJECTIVE:** To evaluate Uterine NK cell activity among women with Recurrent Implantation Failure (RIF) and to derive a range of uterine NK cell activity so as to identify the subset of RIF population who would benefit from intralipid infusion therapy.

**DESIGN:** Prospective study cohort consisted of RIF patients who underwent uterine NK cell analysis and depending on NK cell activity, intralipid therapy was administered (n=120). The retrospective control group comprised of RIF patients who did not undergo uterine NK cell analysis nor receive intralipid therapy (n=40).

**MATERIALS AND METHODS:** Inclusion criteria: Woman who had RIF (defined as failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years) who had undergone antagonist protocol IVF cycles with frozen embryos.

**Exclusion criteria:** Fresh embryo transfer, Thrombophilias, uterine cavity abnormalities, fibroid uterus, adenomyosis and those with contraindications to intralipids such as lipid nephrosis, impaired renal function, pathological hyperlipidemia and acute pancreatitis.

Prospective cohort (n=120) underwent endometrial biopsy for uterine NK cell activity on Day 21 of previous cycle after obtaining informed written consent. The Navios-CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 is a standardised instrument reagent system from Beckman Coulter for evaluation of percentage T cells, B cells and NK cells from whole blood / tissue samples. Absolute counts were calculated from the total

lymphocyte counts obtained from a Beckman Coulter LH500 analyzer. A level greater than 30% of total endometrial lymphocyte population was adjudged the criteria for administering intravenous intralipid infusion of 20% as bolus dose, when optimal endometrium (thickness  $\geq$  8mm and Applebaum Zone 3-4 vascularity) was obtained on transvaginal sonography.

Sample size calculation was based on to achieve 80% power to detect a difference of proportion of 0.2 of clinical pregnancy rate between the groups. The test statistic used was the two-sided Z test with pooled variance. The significance level of the test was targeted at 0.05.

**RESULTS:** Receiver operator curve analysis shows that at an elevated uterine NK cell level of 30-38% (at a sensitivity of 60.7% and specificity of 52.2%), a minimal benefit was discernible with Intralipids in achieving a positive clinical pregnancy (positive predictive value 49.2%, negative predictive value 63%). The area under the curve depicting predictive value of uterine NK cell level among patients receiving intralipids for predicting clinical pregnancy is only 53%, which is very low – hence the benefit was deemed to be minimal. Above 38% uterine NK cell activity, no benefit was observed with intralipid therapy. Clinical pregnancy rate among those who received intralipid was comparable to those who did not (43.2% vs 42.2%).

**CONCLUSIONS:** Uterine NK cell activity of 30-38% in RIF patients, minimal benefit of intralipid therapy was discernible. However, there seems to be no significant increase in the pregnancy outcome with Intralipids.

**SUPPORT:** NIL.

**O-258** Wednesday, October 16, 2019 12:00 PM

### **EXPRESSION AND FUNCTION OF THE PD-1 IMMUNE CHECKPOINT IN THE HUMAN OVARY AND FALLOPIAN TUBE.**

Joshua Johnson, PhD,<sup>a</sup>

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**OBJECTIVE:** The Programmed Cell Death Protein-1 (PD-1/PDCD-1/CD279) signaling pathway has powerful immunomodulatory action in the context of disease, including cancer.<sup>1-3</sup> PD-1 receptor activation by its ligands suppresses immune responses, and cancer cells avoid surveillance via PD-1 and/or ligand expression. Conversely, blocking PD-1 signaling can improve cancer immune surveillance and in some cases, treatment outcomes and patient survival. A soluble variant of PD-1 (sPD-1) has been shown to modulate immune response(s), including T cell activation. Because immune and inflammatory pathways impact ovarian function, we asked whether the PD-1 immune checkpoint functions in the human ovary or fallopian tube.

**DESIGN:** Subjects were i) pre- and postmenopausal women whose ovaries were removed electively or for benign conditions excluding endometriosis; ii) women whose fallopian tubes were removed and evaluated for evidence of transformation; and iii) women undergoing IVF for clinical indications. IVF patients underwent controlled ovarian hyperstimulation per standard clinical protocols. FF from the lead follicle was collected undiluted at the time of retrieval. All specimen collection was approved by the local IRB.

**MATERIALS AND METHODS:** Deidentified normal patient ovary and oviduct tissue (n>10 per group), and, follicular fluid (FF) collected during clinical oocyte retrievals (n=60 unique samples) were screened for PD-1 pathway expression using immunohistochemical (IHC) staining and ELISA for FF soluble proteins, respectively. Bioactivity of soluble pathway factors was assessed by adding FF to activated human T cells, with and without blocking antibody controls, and interferon gamma (IFN $\gamma$ ) was measured as a surrogate for T cell activation.

**RESULTS:** PD-1, PD-L1 and PD-L2 are expressed by ovary and fallopian tube resident immune cells (T cells and macrophages), and also by non-immune cells, including granulosa cells and oocytes. PD-L1 is expressed by normal and transformed (e.g., p53 mutant) cells of the tubal epithelium. Most FF samples contained sPD-1 (30/36 assayed), and all contained soluble ligands sPD-L1 and sPD-L2 (50/50). Soluble receptor and ligands were present in FF at bioactive levels that can control the degree of T cell activation. Compared to PBS negative control samples, FF addition was found most often to significantly enhance T cell IFN $\gamma$  production. In samples that contained the highest levels of sPD-1, T cell IFN $\gamma$  production was instead lower than control levels. IFN $\gamma$  was negligible in the media of non-activated T

cells. Additional controls included alternative first antibodies and no-first-antibody controls for IHC, and blocking antibody controls for T cell activation assays; all of these minimize but do not eliminate the role of chance.

**CONCLUSIONS:** We herein present data on novel PD-1 signaling in non-immune cells of the ovary that suggest that the pathway may be involved in physiological ovarian functions including the enforcement of follicle, oocyte and embryo immune privilege. These data are immediately relevant to the ontogeny of ovarian cancer and the tubal origins of the disease.<sup>3</sup>

**References:** 1. Okazaki, T., et al. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. *Proc. Natl. Acad. Sci. U.S.A.* 98, 13866–13871 (2001).

2. Okazaki, T. & Honjo, T. PD-1 and PD-1 ligands: from discovery to clinical application. *Int. Immunol.* 19, 813–824 (2007).

3. Karyampudi, L. et al. PD-1 Blunts the Function of Ovarian Tumor-Infiltrating Dendritic Cells by Inactivating NF $\kappa$ B. *Cancer Res.* 76, 239–250 (2016).

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### **PARENTAL AGING**

**O-259** Wednesday, October 16, 2019 10:45 AM

### **INFLUENCE OF MATERNAL AGE AND OVARIAN RESERVE ON THE DECISION TO CONTINUE OR TO CANCEL IVF CYCLES IN PATIENTS WITH ONE OR TWO LARGE FOLLICLES.**

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Alina Mariana Mahfoudh, M.A.,<sup>b</sup> Jacques Balayla, M.D.,<sup>c</sup> Naama Steiner, M.D.,<sup>d</sup> Alexander Volodarsky-Perel, M.D.,<sup>c</sup> Sara Henderson, M.Sc.,<sup>c</sup> Atif Zeadna, M.D.,<sup>c</sup> Weon-Young Son, Ph.D.,<sup>f</sup> Mali Salmon-Divon, Ph.D.,<sup>g</sup> Michael H. Dahan, MD<sup>a</sup> McGill University, Montreal, QC, Canada; <sup>b</sup>Montreal, QC, Canada; <sup>c</sup>Affiliation not provided; <sup>d</sup>McGill University Health Centre, Montreal, QC, Canada; <sup>e</sup>MUHC Reproductive Centre, Montreal, QC, Canada; <sup>f</sup>Division of Reproductive Endocrinology and Infertility, McGill University Health Care Centre, Montreal, QC, Canada; <sup>g</sup>Senior Lecturer, Ariel, Israel.



**OBJECTIVE:** To determine factors that influence IVF stimulation that result in one or two mature follicles

**DESIGN:** A retrospective cohort study evaluating patients who underwent 465 IVF cycles at the McGill University Health Center (MUHC) between 2011 and 2014.

**MATERIALS AND METHODS:** 465 patients that developed 1-2 follicles  $\geq$  14mm on hCG administration day were included. We divided the cycles into three groups based on the female age:  $\leq$  34 years old, 35-39 years old,  $\geq$  40 years old. Sub-analysis based on antral follicle count and female age was conducted. Statistical analysis was performed with multivariate logistic regression. Power analysis demonstrated a 99.7% power to detect a difference of 25% with alpha 5% in a sample of 365.

**RESULTS:** Of the 465 cycles included in the study, 365 cycles (78.8%) ended in embryo transfer. The live birth rate was 5.2% per cycle. The pregnancy rates and LBR per cycle respectively were: 35.5% and 15.6%, for the  $\leq$  34-year-olds; 14.7% and 6.4%, for the 35-39-year-olds and 8.4% and 2.7%, for the  $\geq$  40-year-olds (P value <0.0001 and 0.0001, respectively). Evaluating odds of pregnancy and live birth (LB) as a function of age and antral follicular count (AFC) with multivariate logistic regression controlling for confounding effects, revealed that a one-year increase in age reduces the likelihood of pregnancy and LB by 11% (p=0.002 and 0.026 respectively), and one unit increase in AFC count lead to a 9% increase in the odds of both outcomes (p=0.005 for pregnancy and 0.017 for LB). Having one follicle vs. two follicles only affect the odds of pregnancy (p=0.02) but not that of LB. Outcomes were low in all cases in women 35 years old or above. However in women under the age of 35 years, with AFC  $\geq$  11, live birth rates were 56%, while if AFC was  $\leq$  10, live birth rates were 5.5%.

**CONCLUSIONS:** This data suggests a paradigm shift in reasoning from age being the predictor of outcomes in women with a low response at IVF to both age and ovarian reserve needing to be taken into consideration. Patients aged less than 35-year-olds with  $\leq$  two mature follicles have excellent success rates if the AFC was greater than 10. Young women with AFC of 10 or less or women 35 years of age or older with an emphasis on those at least 40 years old, should be advised of the low chance of success and a sizable percentage of not having an embryo to transfer when one or two mature

follicles have developed with IVF. This result while controlling for the effect of age and ovarian reserve elucidates that ovarian reserve plays a role in both quality and quantity.

SUPPORT: None.

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### SUBDERMAL HORMONE IMPLANT AS TREATMENT FOR THE IMPROVEMENT OF MENOPAUSAL SYMPTOMS IN A PRIVATE FERTILITY CENTER.

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**OBJECTIVE:** To evaluate the effect of testosterone subdermal implants on improvement of menopausal symptoms.

**DESIGN:** A prospective, observational, inferential analytical study.

**MATERIALS AND METHODS:** A total of 96 consecutive patients who attended the menopause clinic from August 2017 to December 2018 were prospectively enrolled. Symptoms evaluation was performed with the Menopause Rating Scale (MRS), a specific scoring scale of menopausal symptoms. It is composed of 11 points or items of symptoms that are grouped into three subscales or dimensions: 1) Somatic-vegetative. 2) Psychological. 3) Urogenital. Higher MRS scores are associated with increased deterioration in the quality of life.

Subcutaneous testosterone (2 mg/kg) implants were applied for symptoms relief in all patients and MRS scores assessed before and after. The application of testosterone was carried out in the office under local anesthesia, in the subcutaneous tissue of the gluteal or abdominal regions. The time of effect of the implant was 6 months and no patients required implant removal. The follow-up questionnaire was performed 12 weeks after initial placement. Paired Student t tests were performed to compare variables.

**RESULTS:** The mean age of the 96 patients was 51 years  $\pm$  6.51 and the onset of menopausal symptoms was less than 5 years in all. The mean MRS score (31.93  $\pm$  7.46) had a significant decline (12.37  $\pm$  7.4, P <0.001) once the implants were applied. Patients experienced the most improvements in Hot flashes (P <0.001), Cardiac palpitations (P <0.001), Sleep disorders (P <0.001), Mood (P <0.001), Irritability (P <0.001), Tiredness (P <0.001), Vaginal Dryness (P <0.001).

**CONCLUSIONS:** Testosterone hormone subdermal implants represent a valid alternative to hormone replacement therapy, effectively improving major menopausal symptoms. Further studies and follow-ups are required to evaluate continuous efficacy, tolerability and safety of testosterone implants.

O-261 Wednesday, October 16, 2019 11:15 AM

### CIRCULATING ESTRADIOL (E2) LEVELS IN POST-MENOPAUSAL USERS AND NON-USERS OF VAGINAL ESTROGEN.

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**OBJECTIVE:** The U.S. Food and Drug Administration (FDA) applies the same "black box" warning to vaginal estrogens as for systemic estrogens, implying they carry similar cardiovascular, thromboembolic, and breast cancer risks, yet the systemic impact of topical vaginal estrogen use is largely unknown. Using data from the Study of Women Across the Nation (SWAN), we examined serum E2 levels in postmenopausal women using vaginal estrogen products (V-E2) compared to non-estrogen users (NoE2), with the hypothesis that serum E2 levels would be equivalent among the two groups.

**DESIGN:** Prospective, observational study of 3302 women at baseline who were followed through the menopausal transition and beyond (SWAN) and queried annually about hormone use.

**MATERIALS AND METHODS:** Serum E2 was measured using a direct, ultrasensitive chemiluminescent immunoassay (England, Clin Chem 2002, 48: 1584; detection limit 6pg/mL). We compared postmenopausal V-E2 and NoE2 users before and after adjusting for smoking, body mass index (BMI), race/ethnicity, and study site. We also compared serum E2 levels between recent (<2-4 weeks), remote ( $\geq$  4 weeks), and never users. Serum E2

values were calculated for all menopause stages for reference. Observations with concurrent systemic estrogens or androgens were excluded from analysis. Data were analyzed using linear mixed models following log transformation of E2 levels; results were backtransformed for presentation.

**RESULTS:** There were 215 postmenopausal observations (from 131 women) containing V-E2 use and 10232 postmenopausal observations (from 2409 women) with NoE2 use. White women and women with a normal BMI were more prevalent among V-E2 than NoE2 users (68 vs 45% and 57 vs 32%, respectively). Prior to covariate adjustment, serum E2 did not differ between V-E2 and NoE2 users (18.55 (95%CI:16.92, 20.33) vs 18.01(95% CI:17.72, 18.31) pg/mL, p=0.53). Serum E2 was not related to smoking (p=.26), but higher E2 was associated with higher BMI (p<.0001) and African American race (p<.0001). After covariate adjustment, mean serum E2 was slightly higher in V-E2 users compared to NoE2 users (20.16 (95% CI:18.43, 22.05) vs 17.93 (95%CI: 17.66, 18.2) pg/mL, p=0.01). Adjusted mean serum E2 levels were 20.62 pg/mL in recent V-E2 users, 17.23 pg/mL in remote users, and 17.93 pg/mL in never users; the difference between never users and recent users was statistically significant (p=0.007). Mean (95% CI) serum E2 (pg/mL) was 50.72 (48.69, 52.83) in premenopause, 48.69 (47.73, 49.67) in early perimenopause, 28.13 (27.10, 29.21) in late perimenopause, 17.87 (17.55, 18.21) in natural postmenopause, and 17.81 (16.46, 19.28) in surgical postmenopause.

**CONCLUSIONS:** Serum E2 levels are greater in V-E2 users compared to NoE2 users, and in recent V-E2 users compared to never users, but are overall lower than pre- and early perimenopause levels. Women with higher BMI appear less likely to use V-E2, and have slightly higher circulating E2, which may indicate they are less symptomatic. The clinical significance of these small differences in E2 is not known, and may be further clarified by the use of mass spectrometry-based methods.

**SUPPORT:** Supported by R25HD075737 (to NFS) and SWAN. The Study of Women's Health Across the Nation (SWAN) has grant support from the National Institutes of Health (NIH), DHHS, through the National Institute on Aging (NIA), the National Institute of Nursing Research (NINR) and the NIH Office of Research on Women's Health (ORWH) (Grants U01NR004061; U01AG012505, U01AG012535, U01AG012531, U01AG012539, U01AG012546, U01AG012553, U01AG012554, U01AG012495). The content of this abstract is solely the responsibility of the authors and does not necessarily represent the official views of the NIA, NINR, ORWH or the NIH.

O-262 Wednesday, October 16, 2019 11:30 AM

### DO ORAL OVULATION INDUCTION AGENTS OFFER BENEFITS IN WOMEN 38 YEARS AND OLDER?

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**OBJECTIVE:** Gonadotropins generally have higher pregnancy and multiples rates when compared to oral ovulation inducing medications. This study was performed to compare outcomes in women with age factor infertility, who traditionally were felt not to be good candidates for oral medications with intrauterine insemination (IUI).

**DESIGN:** This retrospective cohort study was performed to compare the success of ovulation induction using oral agents versus gonadotropins in women  $\geq$  38 years old, compared to a control group of younger women.

**MATERIALS AND METHODS:** First to third stimulated IUI cycles in a single academic fertility center between January 2011 and March 2018. Primary outcome was pregnancy rate (defined by a Beta HCG > 10 mIU/ml) per cycle. A total of 5405 IUI cycles were included. Of these, 3816 IUIs were for women < 38 years at the time of insemination, 1537 (40.3%) cycles with oral agents (Letrozole or Clomiphene citrate), and 2279 (59.7%) cycles were Gonadotropin-stimulation. 747 IUIs cycles were for women 38-39 years old, 254 (34%) cycles with oral agents and 493 (65%) cycles with Gonadotropins. The last group included 842 IUIs for women 40-43 years old, 202 (24%) cycles with oral agents and 640 (76%) cycles with Gonadotropins. Fisher exact test was performed. Power analysis demonstrated a 98% power to detect a 10% difference with alpha-error 5% and 1589 cycles in subjects at least 38 years of age.

**RESULTS:** Among women < 38 years, the pregnancy rate did not differ between IUIs using oral agents (N=166/1537, 10.8%) compared to IUIs with Gonadotropins (N=267/2279, 11.7%) (p=0.40). Among women

38-39 years old, the pregnancy rate also did not differ between IUIs using oral agents (N=28/254, 11.02%) compared to IUIs with Gonadotropins (N=52/493, 10.55%) (p=0.90). Surprisingly, in women 40-43 years, the pregnancy rate was significantly higher in the oral agents (N=26/202, 12.87%) compared to IUIs with Gonadotropins (N=39/640, 6.09%) (p=0.0036).

**CONCLUSIONS:** Likely an undetected bias resulted in lower gonadotropin pregnancy rates among women 40-43 years of age. However, the use of oral agents for ovarian stimulation with IUI in women older than 40 years of age is an effective treatment strategy given pregnancy rates above 10% per cycle.

Women Age (years old)	Pregnancy rate Oral agents	Pregnancy rate Gonadotropins	p-value
<38	166/1537 10.8%	267/2279 11.7%	0.4052
38-40	28/254 11.02%	52/493 10.55%	0.9007
40-43	26/202 12.87%	39/640 6.09%	<b>0.0036</b>

**O-263** Wednesday, October 16, 2019 11:45 AM

**A 5-YEAR ANALYSIS OF DEMOGRAPHICS, CYCLE CHARACTERISTICS AND REPRODUCTIVE OUTCOMES OF 907 EGG FREEZING CYCLES IN PATIENTS WITH DIMINISHED OVARIAN RESERVE AND AGE-RELATED FERTILITY DECLINE.**



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**OBJECTIVE:** Since the adoption of new regulations on ART procedures in 2014, non-medical oocyte cryopreservation has been legalized in Turkey for childless women with diminished ovarian reserve (DOR). As older women face age related fertility decline regardless of their ovarian reserve, they also benefit from egg freezing under this regulation. Since there are no studies to date addressing the cycle characteristics and reproductive outcomes of egg freezing cycles in patients with DOR, we aim to evaluate cycle characteristics and reproductive outcomes of women with DOR and those with normal ovarian reserve facing age-related fertility decline.

**DESIGN:** Retrospective data analysis.

**MATERIALS AND METHODS:** Electronic databases or charts of patients who underwent egg freezing in 3 IVF centers in Istanbul between 2014 and 2019 were retrospectively reviewed. Egg freezing cycles of patients with DOR (DOR group) or patients who were  $\geq 38$  years with normal/high ovarian reserve according to their age (NR-Aged group) were included into the study.

**RESULTS:** This study reviewed 907 egg freezing cycles of 586 patients. Sixteen percent of women were  $<35$  years old, whereas 66% were over the age of 38. 517 patients with DOR underwent 825 egg freezing cycles and 69 NR-Aged patients underwent 82 egg freezing cycles. In the DOR group, 76 cycles (9%) were cancelled due to inadequate follicular development, premature ovulation, no oocyte or no mature oocytes collected. The mean age and AMH of the DOR group at the time of freezing were  $37.4 \pm 5.2$  years and  $0.6 \pm 0.4$  ng/dl, respectively. Mean number of frozen MII oocytes per cycle was  $3.4 \pm 2.3$ . The average number of egg freezing cycles per patient was  $1.6 \pm 1.1$  resulting in a total frozen MII oocyte number of  $5.3 \pm 3.7$  per patient. In the NR-Aged group the mean AMH level was  $2.2 \pm 1.4$  and the mean number of frozen MII oocytes was  $11.8 \pm 4.1$ .

A total of 20 patients returned to use their frozen oocytes. None of the three patients returned in the NR-Aged group who had more than 12 frozen oocytes got pregnant. Of the 17 patients in the DOR group, 6 patients did not have embryo transfers and only 3 patients had live births (17.6%), of the two from thawed oocytes (11.7%). However, the third patient got pregnant in the following fresh cycle after thawed oocytes could not be fertilized. In order to increase the number of oocytes, 7 thawing cycles were combined with fresh cycles.

**CONCLUSIONS:** To our knowledge, this is the first reported analysis of egg freezing cycles of patients with DOR. Young women with DOR is the most important group who will benefit from preventive egg freezing. Since the cycle cancellation rate is high and reproductive outcomes of these patients are very low, these patients should be counseled accordingly about the risks and expectations, and advised to have higher number of oocytes

frozen. In accordance with previously published reports, despite high number of frozen oocytes, the chances of getting pregnant for older patients are extremely low. Follow-up and future reproductive outcomes of patients with DOR who undergo egg freezing is very important as these results will be helpful in counseling this patient population.

**SUPPORT:** None.

**O-264** Wednesday, October 16, 2019 12:00 PM

**THE IMPACT OF PATERNAL AGE ON REPRODUCTIVE OUTCOMES IN THE SETTING OF A EUPLOID SINGLE EMBRYO TRANSFER ACHIEVED WITH SURGICALLY EXTRACTED SPERM.**



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**OBJECTIVE:** While clinical outcomes from in vitro fertilization (IVF) cycles performed using surgically extracted sperm and ejaculated sperm have been reported to be similar, men requiring surgical sperm extraction represent a unique patient population (1,2). Additionally, the relationship between paternal age and assisted reproductive technology (ART) outcomes is a controversial topic which is often confounded by factors arising from the female partner. This study sought to determine whether increasing paternal age is associated with adverse outcomes in the setting of a single embryo transfer (SET) of a euploid embryo created with surgically extracted sperm. Preimplantation genetic testing for aneuploidy (PGT-A) was utilized to minimize the potential effects of aneuploidy.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This study was performed at a large fertility practice. Couples were included if they underwent a first cycle of IVF between 2012 and 2019 with surgically extracted sperm and then underwent intracytoplasmic sperm injection (ICSI) and PGT-A followed by SET of a euploid embryo. Wilcoxon rank sum test, Chi-square analysis, Fisher's exact test, logistic regression models, and linear regression models were utilized to assess the relationship between paternal age and rates of implantation, delivery, biochemical loss, and clinical loss. An analysis of the relationship between paternal age and fertilization rate, blastulation rate, and euploid rate was also performed.

**RESULTS:** 207 couples met inclusion criteria. Mean male partner age was  $37.2 \pm 7.5$  years, with 69 male patients age 40 or older. Mean female partner age was  $33.8 \pm 4.7$  years. Among couples undergoing SET of a PGT-A tested embryo with surgical sperm, implantation rate was 84.5%, delivery rate was 58.8%, biochemical loss rate was 12.0%, and clinical loss rate was 6.5%. Adjusting for female age, there was no statistically significant association between male partner age and implantation rate (p=0.54), delivery rate (p=0.34), biochemical loss rate (p=0.07), or clinical loss rate (p=0.92).

In a sub-group analysis of men age 40 or above compared to men younger than 40, there was no significant association observed between paternal age and fertilization rate (p=0.11), blastulation rate (p=0.67), or euploid rate (p=0.43) while adjusting for female age.

**CONCLUSIONS:** When a couple undergoes SET of a euploid embryo using sperm obtained via surgical extraction techniques, increasing paternal age does not appear to affect pregnancy outcomes (implantation rate, delivery rate, biochemical loss rate, and clinical loss rate). Furthermore, no relationship was demonstrated between paternal age and the embryologic outcomes of fertilization rate, blastulation rate, and euploid rate. While multiple factors undoubtedly contribute to the overall health and development of an early pregnancy, the role of paternal age is unlikely to be significant if surgically extracted sperm is utilized.

**References:** 1. CC Tsai, FJ Huang, LJ Wang, YJ Lin, FT Kung, CH Hsieh, KC Lan. Clinical outcomes and development of children born after intracytoplasmic sperm injection (ICSI) using extracted testicular sperm or ejaculated extreme severe oligo-astheno-teratozoospermia sperm: a comparative study. *Fertil Steril* 2011; 96(3):567-71.

2. JF Kawwass, J Chang, SL Boulet, A Nangia, A Mehta, DM Kissin. Surgically acquired sperm use for assisted reproductive technology: trends and perinatal outcomes, USA, 2004-2015. *J Assist Reprod Genet* 2018; 35(7):1229-1237.

**SUPPORT:** None.

P-1 Tuesday, October 15, 2019 6:30 AM

**DOES AN INSURANCE MANDATE TO COVER INFERTILITY TREATMENT INCREASE ACCESS TO IN VITRO FERTILIZATION?**

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**OBJECTIVE:** Financial constraints limit many patients from being able to access infertility care, especially assisted reproductive technologies (ART) such as *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI). We sought to determine the impact of state insurance mandates for infertility coverage on the utilization of IVF within each state.

**DESIGN:** Retrospective analysis of publicly available data.

**MATERIALS AND METHODS:** All IVF centers in the United States in 2017, their zip code, and number of cycles performed were extracted from CDC data. Using US census data, the median salaries for zip code and state were extracted. The number of IVF centers and number of IVF cycles between states with and without infertility coverage insurance mandates were compared. The association between geographic region, income and the number of IVF cycles was evaluated. IVF centers in mandate and non-mandate states were sequentially sorted by the median household income of the zip code they are located in and grouped into successive increments of \$10,000 of median household income. Total number of cycles per successive \$10,000 income bracket were compared in mandate and non-mandate states. Paired and unpaired Student's T-tests were performed for continuous variables.

**RESULTS:** Fifteen states mandate some degree of infertility coverage. States with insurance mandates for infertility coverage had a greater number of yearly IVF cycles per 100,000 residents compared to states without infertility coverage mandates (104 cycles vs 57 cycles per 100,000 p=0.029). However, there was no difference between the number of IVF centers per person between states with and without infertility coverage mandates (0.16 vs 0.13 per 100,000 residents, p=0.058). On average, IVF centers were located in zip codes with greater median incomes than their respective states (\$73,325.17 vs \$62,607.53, p<.0001). This relationship held true for both states with infertility insurance mandates (\$79,894.00 vs \$66,820.87, p<.0001) and without infertility insurance mandates (\$65,813.11 vs \$ 57,759.77, p<.0001). There was no significant difference between number of cycles in mandate and non-mandate states at IVF centers located in median household income brackets below \$80,000. In centers located in median household income brackets greater than \$80,000, the total number of cycles performed was significantly greater in mandate states vs. non-mandate states (p=0.0461).

**CONCLUSIONS:** States with insurance mandates for infertility coverage have a greater number of IVF cycles per 100,000 residents, but surprisingly do not have a greater number of IVF centers carrying out these cycles. On the whole, IVF centers are located in relatively wealthier zip codes in both mandate and non-mandate states. Only at IVF centers in zip codes with median household income greater than \$80,000 do mandate states have a greater number of IVF cycles than non-mandate states. This suggests that state insurance mandates for infertility coverage may not be making IVF more accessible for all but may be selectively benefitting wealthier geographic regions.

P-2 Tuesday, October 15, 2019 6:30 AM

**DEFINING INFERTILITY: HOW THE LANGUAGE USED TO DESCRIBE INFERTILITY SHAPES PUBLIC PERCEPTION AND POLICY.**

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**OBJECTIVE:** To investigate if the label associated with infertility has a causal impact on public perceptions of infertility and infertility treatment.

**DESIGN:** Cross-sectional study.

**MATERIALS AND METHODS:** Participants aged 18 or older were recruited using an online survey platform. Quotas were set so gender, age, and race/ethnicity reflected national rates.

Participants read a description of infertility that randomly included the label of infertility as a 1) condition, 2) disease, or 3) disability. Participants were then asked about their thoughts on different policies related to infertility including insurance coverage and public assistance programs for treatment, sex education, and public awareness campaigns. At the end of the survey, participants were asked which label they thought best defined infertility. Demographic data collected included age, gender, marital status, political party, religiosity, schooling, income, and history of infertility.

Comparisons were made between both the assigned label and the preferred participant label with the policy outcomes using ANOVA for continuous and chi-square for categorical variables.

**RESULTS:** Of the 1221 participants, a majority (78%) preferred labeling infertility as a "condition" compared to "disability" (12%) or "disease" (10%). Participants who preferred the label condition were older and less likely to have a previous infertility diagnosis (p<.001) and those reporting higher religiosity were more likely to prefer disease (p=.028). There were no other demographic differences. Participants who preferred labeling infertility as a disability or disease were more likely to support infertility policies such as insurance coverage for treatments and fertility preservation, public awareness campaigns, coverage during sex education, and public assistance programs for treatment (Table).

	All	Condition	Disability	Disease	p-value
Infertility Insurance Coverage	58*	56	63	68	<.001
Fertility Preservation Insurance Coverage	60	59	66	66	<.001
Infertility Public Awareness Campaigns	71	70	76	73	.023
Infertility Discussion in Sex Education	68	67	73	71	.010
Public Assistance Programs for Infertility Treatment	71	69	74	76	<.001

\*Scale 0-100 with 100 indicating complete support.

The assigned infertility label had little effect on the public perception measures, however, participants were more likely to prefer labeling infertility as a disease or disability if they had been exposed to those labels (p<.001).

**CONCLUSIONS:** Although there was an overall preference for labeling infertility as a condition, participants who thought of infertility as a disease or disability were more likely to support public policies supporting infertility treatment coverage and increasing infertility awareness.

P-3 Tuesday, October 15, 2019 6:30 AM

**PATIENT ACCESS AND UNTAPPED POTENTIAL: CAN NEW DATA DRIVE PROGRESS?**

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**OBJECTIVE:** Determine gaps and factors that cause a discrepancy between the potential for, and utilization of, infertility care in the U.S.

**DESIGN:** Multivariate models to determine potential number of patients for, and actual numbers receiving, treatment in all Nielsen determined U.S. Designated Market Areas (DMAs).

**MATERIALS AND METHODS:** The number of women receiving treatment by a reproductive endocrinologist was calculated using fresh cycle data reported by the CDC, combined with an estimate of IUI-only patients based on state insurance mandate. A multivariate prediction model was created using >20 variables including insurance mandate, age, gender and psycho-demographics in 115 DMAs to predict numbers of patients receiving treatment. Variable coefficients related to treatment potential (defined as need and predisposed) were separated from those impacting

utilization (e.g. doctors, locations and distance) to construct a sub-model to identify numbers of potential patients. Accuracy was determined using R<sup>2</sup> coefficient. Analysis was used to obtain results for all 211 DMAs by zip code.

**RESULTS:** The multivariate model predicted the number of patients being treated with a R<sup>2</sup> > .93 (p<.001) among 115 DMAs with fertility clinics. Of the 330,200 women determined to have potential for treatment (need and predisposed), only 137,000 are estimated to be receiving it (41% utilization) with a gap of 193,200 who would receive treatment if it were more accessible. Although insurance requirements and affluence were significant factors, other variables such as psycho-demographics were even more so. The number of patients with potential for treatment vs. utilization varied significantly among and within DMAs. Of all DMAs, 47 had high incidence (>mean 23%) of potential patients; of these 30 had high utilization levels (>mean 41%) whereas 17 had low utilization levels (<mean 41%), resulting in a mean of 3,202 underserved patients per DMA. If just the 17 low-use DMAs increased use to match the high-utilization DMAs, patient access would increase by 11% nationally. Similar access opportunities exist in every DMA.

**CONCLUSIONS:** Intuition rather than objectivity have often guided efforts to improve patient access by factoring variables such as wealth and proximity to medical centers. New data identifies and quantifies populations who need and are predisposed to treatment by incorporating provider and patient based variables that impact treatment utilization. This data can be used to expand geographic access to care and optimize patient outreach.

Potential (Predisposed)	Low	High	
# DMAs	19	30	<b>High Util</b>
Potential as % of Need	18%	33%	
High % Util	52%	52%	
Underserved per DMA	462	2,466	
# DMAs	145	17	<b>Low Util</b>
Potential as % of Need	14%	25%	
Low % Util	24%	34%	
Underserved per DMA	388	3,202	

P-4 Tuesday, October 15, 2019 6:30 AM

**GENDER AND FERTILITY STATUS AFFECT PERCEPTIONS OF INFERTILITY AND SUPPORT FOR ACCESS TO CARE: A CROSS SECTIONAL STUDY.**

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**OBJECTIVE:** To determine if gender and fertility status impact perceptions of infertility and support for treatment access.

**DESIGN:** Cross sectional study.

**MATERIALS AND METHODS:** Subjects aged 18 years and over were recruited to complete an online survey, and quotas were used to reflect gender, age, and race/ethnicity national rates. Exclusion criteria included transgender, non-binary, those who identified themselves as “other,” as well as any missing gender data. Respondents were surveyed about their attitudes towards infertility treatment-related health insurance coverage, fertility preservation, public awareness campaigns, sex education, public assistance programs, sex selection, embryo disposition and other related beliefs.

**RESULTS:** Of the 1221 subjects recruited, 1157 were included based on our criteria. A total of 54 out of 564 (9.6%) females reported infertility, and 49 of 593 (8.6%) males reported infertility. Overall, females with infertility were more likely to support public assistance programs for infertility treatments (p=.001; see Table 1), especially for persons unable to afford treatment or will undergo cancer treatment that may cause infertility. They were also more supportive of infertility public awareness campaigns (p=.015). They were least likely to support the use of IVF to choose the sex of their desired child (p=.027). Males with infertility

were most likely to support the use of IVF both in people with and without infertility to choose the sex of their child (p=.027 and p<.001, respectively) and as well as government regulation to limit the number of embryos transferred to avoid twins (p<.001) and triplets or greater (p=.034). They were also more likely to have negative infertility beliefs (p<.001) and agree that if someone is unable to have children, they were “not meant to have children”, “it is the will of God,” they “did something to become infertile,” and “they just need to relax and they will get pregnant.”

**CONCLUSIONS:** Males and females with infertility were more likely to support greater access to care with coverage of treatment through insurance and public assistance programs. However, males with infertility were most likely to have negative beliefs regarding infertility.

TABLE 1. Perceptions of infertility by gender and fertility status

Infertility status	Female		Male		P value
	No	Yes	No	Yes	
Support for infertility insurance coverage	57.6	64.2	54.8	67.7	.091
Support for fertility preservation insurance coverage	59.5	64.2	59.1	69.1	.977
Support for infertility public awareness campaigns	73.5	81.3	70.8	73.4	<b>.015</b>
Support for public assistance programs for infertility treatments	71.8	78.0	67.9	71.0	<b>.001</b>
Negative fertility beliefs	39.8	41.4	46.5	59.7	<b>.000</b>

P-5 Tuesday, October 15, 2019 6:30 AM

**VARIATION IN AVAILABILITY OF FERTILITY CARE TREATMENTS ACROSS US REGIONS.**

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**OBJECTIVE:** Across the US, the number of patients being cared for in fertility centers has grown considerably over the last years. However, the differential access to infertility care in the regions of the country remains to be understood. Taking into consideration population growth, this study describes and quantifies the evolution and regional variation of the utilization of assisted reproduction (AR) treatment in the US over five years.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** We used the publicly available Society for Assisted Reproductive Technology (SART) dataset to measure utilization of AR services in the US between 2011 and 2015. Clinics were grouped into states and into the 4 US census regions. The number of clinics per million (Clinics/1M) and cycles per thousand (Cycles/1k) per region was standardized using the US Census data on the number of females of reproductive age (20-44 years). Trends were assessed using Mann-Kendall test and statistical significance set at P<0.05.

**RESULTS:** There were 958,231 cycles performed in the US during the study period. On average, there were 8.7 fertility clinics per million females, ranging from an average of 7.6 per million in the South to 10.5 per million in the Northeast. Contrary to the absolute number of cycles, which increased in all regions, the number of cycles per thousand females only increased significantly in the West and Midwest, remaining stable in the Northeast and South (Table). The results for states can be visualized at <http://bit.ly/asrm19var>.

**CONCLUSIONS:** The number of cycles in the US increased significantly over the last five years, but the population-standardized rate remained stable in two of the US regions. In association with the unchanging number of clinics, this suggests that in some regions the expansion of fertility services is driven by the female population growth and not by an increasing fraction of this population seeking infertility care. Further research is needed to evaluate the reasons for these variations and their impact on outcomes. These results should be considered when evaluating policies aimed at

	2011	2012	2013	2014	2015	p-value * = <0.05
<b>Midwest</b>						
Clinics/1M	8.22	8.12	8.28	8.17	8.75	
Cycles/1k (N)	2.24 (26,622)	2.39 (29,807)	2.54 (31,458)	2.73 (33,423)	3.07 (38,457)	* (*)
<b>Northeast</b>						
Clinics/1M	10.76	10.75	10.84	10.30	10.32	
Cycles/1k (N)	4.90 (49,886)	4.97 (57,673)	4.71 (59,873)	4.80 (64,038)	5.35 (67,500)	(*)
<b>South</b>						
Clinics/1M	7.55	7.58	7.82	7.71	7.61	
Cycles/1k (N)	2.51 (42,213)	2.61 (45,984)	2.60 (51,476)	2.78 (57,812)	3.05 (64,879)	(*)
<b>West</b>						
Clinics/1M	9.00	9.22	9.29	9.06	9.07	
Cycles/1k (N)	2.33 (32,961)	2.59 (42,536)	2.78 (47,707)	3.11 (53,058)	3.65 (60,868)	* (*)

expanding access to fertility care and the allocation of resources by new fertility centers.

SUPPORT: None.

P-6 Tuesday, October 15, 2019 6:30 AM

**NATIONWIDE SURVEY OF ACCESS TO CARE INITIATIVES IN REI PRACTICES ASSOCIATED WITH OB/GYN RESIDENCY PROGRAMS.** Tia Jackson-Bey, MD MPH,<sup>a</sup> Holly Mehr, MD MSEd,<sup>b</sup> Jacqueline Ho, MD MS,<sup>c</sup> Lusine Aghajanova, MD PhD,<sup>d</sup> Molly M. Quinn, MD,<sup>b</sup> Jacquelyn Rose Hoffman, BA,<sup>c</sup> Christopher N. Herndon, MD.<sup>f</sup> <sup>a</sup>University of Illinois at Chicago, College of Medicine, Chicago, IL; <sup>b</sup>University of California, Los Angeles, Los Angeles, CA; <sup>c</sup>University of Southern California, Los Angeles, CA; <sup>d</sup>Stanford University School of Medicine, Stanford, CA; <sup>e</sup>University of Arizona College of Medicine - Tucson, Tucson, AZ; <sup>f</sup>University of Washington, Seattle, WA.



**OBJECTIVE:** To survey practice patterns designed to increase access to infertility care among REI practices associated OB/GYN residency programs in the United States.

**DESIGN:** Cross-sectional survey.

**MATERIALS AND METHODS:** A total of 281 ACGME certified OB/GYN residency programs were identified. Contact information was found for 270 programs, which were contacted via email and asked to have their REI division director or REI resident rotation director complete an anonymous online survey. The survey included 28 questions on demographics of the residency program, associated REI practice, and presence of initiatives at the institution to expand access to infertility care. Responses were analyzed with logistic regression analysis using STATA software, with significance as  $p < 0.05$ .

**RESULTS:** A total of 80 responses were received for a 30% response rate. Of these, 41% (n=33) of REI practices associated with OB/GYN residency programs identified as academic, 24% (n=19) private practice, 26% (n=21) hybrid academic/private, 4% (n=3) military practices and 5% (n=4) other. Responses were received in all US geographic regions with 22% (n=17) located in the Northeast, 33% (n=26) South, 29% (n=23) Midwest, and 16% (n=13) West. In regards to practice size, 20% (n=16) of practices had 1-2 REI providers, 40% (n=32) had 3-5 providers, and 31% (n=25) had 6 or more providers. Eighty eight percent (n=70) of practices offered IVF and of those 78% (n=55) reported utilizing an onsite embryology lab. Thirty eight percent (n=30) of practices reported having an REI fellowship. Of clinical initiatives to expand access to infertility care to lower income patients, respondents reported offering discounted infertility services (38%, n=30), utilization of a low-cost IVF program (28%, n=22), and utilization of a resident and/or fellow staffed clinic to provide infertility care (39%, n=31). The most commonly discounted infertility services included IVF (73%, n=22), clinical consultation (70%, n=21), and IUI (53%, n=16). The provision of discounted prices for infertility services was correlated with increasing practice size (OR 2.29, 95% CI 1.23, 4.24,  $p=0.01$ ) and number of ART cycles performed annually (OR 3.65, 95% CI 1.48, 9.02,  $p=0.05$ ). Academic REI practices (OR 3.6, 95% CI 0.98, 13.25,  $p=0.05$ ) trended to be more likely to have a low-cost IVF program although sample size was low. The lower costs were achieved through use of mild stimulation (50%, n=11), less lab draws and/or ultrasound during cycle monitoring (32%, n=7), institutional based discounts or write-offs (41%, n=9),

and pharmaceutical company based medication discount programs (36%, n=8). Of practices with a low-cost IVF program, 40.9% (n=9) were developed within the past five years.

**CONCLUSIONS:** To our knowledge, this study of REI practices associated with OB/GYN residency programs is among the first to broadly survey clinical access to infertility care initiatives across the United States. Our findings demonstrate utilization of diverse approaches to expand access to care. Larger practices and academic REI programs were more likely to have clinical initiatives to increase access to care.

## CANCER

P-7 Tuesday, October 15, 2019 6:30 AM

### ONCOLOGIC OOCYTE CRYOPRESERVATION FOR FERTILITY PRESERVATION: NATIONAL TRENDS AND COMPARISON OF CYCLE CHARACTERISTICS BETWEEN WOMEN WITH AND WITHOUT CANCER.

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**OBJECTIVE:** To compare trends, cycle characteristics, and outcomes between women freezing oocytes for fertility preservation due to cancer diagnosis versus elective social reasons. We also compared cancer-related oocyte cryopreservation (OC) outcomes to those for other medical or fertility-related diagnoses.

**DESIGN:** Retrospective cohort study using national surveillance data reported to the Society for Assisted Reproductive Technology Clinical Outcomes Reporting System from 2012-2016 in the United States.

**MATERIALS AND METHODS:** Cycles were divided into 4 distinct groups: 1. cancer only, 2. elective only, 3. medically-indicated, 4. infertility-indicated. Trends in absolute number and proportion of cycles within each group were calculated. Multiple imputation was used to characterize cycles with a missing indication (32.3%), race/ethnicity (47.2%), and body mass index (22.0%). Cycle and outcome characteristics were compared between the 4 groups. We used multivariable log-binomial models to estimate pooled adjusted risk ratios (aRRs) and 95% confidence intervals (CIs) for associations between reason for cryopreservation and hyperstimulation, gonadotropin dose, and cycle cancellation. Poisson regression models were used to estimate oocyte yield. Models controlled for age, body mass index, stimulation protocol, geographic region, gonadotropin dose, and race/ethnicity.

**RESULTS:** Between 2012-2016, 29,631 autologous OC cycles were reported to SARTCORS. The total number of cycles performed for a cancer-related indication increased from 2,925 to 8,828 cycles from 2012 to 2016 and comprised a similar proportion (range 5.6-6.1% annually) of all OC cycles performed in the United States. Compared to purely elective OC cycles, cycles completed for a cancer diagnosis were more likely to be performed among women under 35 years old, with a higher BMI, living in the South, and were more likely to use an antagonist protocol. Compared to purely elective OC, gonadotropin dose (aRR 0.89, [CI] 0.80-0.99), cycle cancellation (aRR 0.90, 95% CI 0.70-1.14), and hyperstimulation (aRR 1.46, 95% CI 0.77-2.29) were not clinically different for cancer-related cycles. Average oocyte yield (approximately 16) and percent maturity (approximately 80%) were comparable in both groups. Oocyte

cryopreservation performed for medical indications was associated with higher gonadotropin dose (aRR 1.22, 95% CI 1.12-1.33) and higher likelihood of cancellation (aRR 1.68, 95% CI 1.46-1.92) compared to elective OC.

**CONCLUSIONS:** The number of OC cycles among women with a cancer diagnosis has increased over the past 5 years; however the percentage OC cycles for cancer has remained stable. While patient demographic characteristics were different among those freezing eggs for fertility preservation due to cancer, the cycle outcomes were comparable to elective OC after controlling for potential confounding. Women freezing eggs for oncologic reasons can be reassured that their cycle outcomes are comparable to those freezing eggs electively. The outcomes of the subsequent egg thaw, fertilization, and transfer cycles remain unknown.

**SUPPORT:** N/A.

**P-8** Tuesday, October 15, 2019 6:30 AM

### **RISK FACTORS FOR ATYPICAL HYPERPLASIA AND ENDOMETRIAL CANCER IN THE INFERTILITY POPULATION: A CASE-CONTROL STUDY.**

Jenna Lipson Kahn, M.D.,<sup>a</sup> Lindsey Buckingham, M.D.,<sup>b</sup> Nathanael C. Koelper, MPH,<sup>c</sup> Mary D. Sammel, ScD,<sup>d</sup> Divya Kelath Shah, MD, MME<sup>c</sup> Pennsylvania Hospital, University of Pennsylvania Health System, Philadelphia, PA; <sup>b</sup>Affiliation not provided; <sup>c</sup>University of Pennsylvania Health System, Philadelphia, PA; <sup>d</sup>University of Pennsylvania Health System, Philadelphia, CA; <sup>e</sup>University of Pennsylvania, Philadelphia, PA GA.

**OBJECTIVE:** The reported incidence of atypical endometrial hyperplasia (AH) and endometrial cancer (EC) in American women under the age of 50 years old is <0.01%. The incidence of AH and EC diagnosed on routine infertility evaluation in a diverse American population is unknown. The study objectives were to estimate the incidence and identify independent risk factors for AH/EC in infertile women.

**DESIGN:** Case-control study.

**MATERIALS AND METHODS:** Data were abstracted from the electronic medical record on all female patients ages 18-50 years seeking initial evaluation at an academic infertility center from 1/1/2009 to 12/1/2018. Patients with a prior diagnosis of breast, ovarian, or colon cancer, or known genetic pre-disposition to cancer were excluded, leaving 11,569 infertile women contributing information. For the case-control study, cases were defined as patients who were diagnosed with AH or EC as part of an infertility workup (n=22). Controls without AH or EC were randomly selected from other women whose infertility was attributed to any female or unexplained factor and underwent an infertility evaluation during the same year in a 10:1 ratio (n=220). A logistic regression was used to estimate odds of AH or EC accounting for covariates such as age, race, BMI, and presence of ovulatory dysfunction. A forward variable selection method was used to arrive at a multivariable model of independent risk factors and to examine potential confounders.

**RESULTS:** Among 11,569 female patients undergoing routine infertility workup between 2009-2018, 22 cases of AH or EC were identified (incidence 2 per 1000 women, 95% CI 1.2-2.9 per 1000). Of these, 12 were diagnosed with AH and 10 were diagnosed with EC. Twenty-five percent of controls had a BMI  $\geq 30$  kg/m<sup>2</sup> as compared to 75% of cases (p<0.001). Adjusting for age, race, and ovulatory dysfunction, women with BMI  $\geq 30$  kg/m<sup>2</sup> had 5.9 times the odds of developing AH or EC (AOR 5.9, 95% CI 2.0-17.2). Odds of AH or EC were 3.4 times higher in women with ovulatory dysfunction after adjusting for age, race, and BMI (AOR 3.4, 95% CI 1.1-10.1). Age and race were not independently associated with odds of AH or EC after controlling for BMI.

**CONCLUSIONS:** The incidence of AH and EC identified during an infertility workup is approximately 10 times higher than that reported in a general population of women of comparable age. Obesity is the strongest independent risk factor for development of AH and EC in the infertile population.

References: 1. A Henley, S.J. et al., 2018. Uterine Cancer Incidence and Mortality - United States, 1999-2016. *MMWR. Morbidity and mortality weekly report*, 67(48), pp.1333-1338.

2. A Fujiwara, H. et al., 2009. Frequency and characteristics of endometrial carcinoma and atypical hyperplasia detected on routine infertility investigations in young women: a report of six cases. *Human reproduction (Oxford, England)*, 24(5), pp.1045-1050.

3. A Tohma, Y.A., Haberal, A., & Gogsen, O., 2018. Prevalence of endometrial cancer or atypical hyperplasia diagnosed incidentally in infertility clinic.

**SUPPORT:** None.

**P-9** Tuesday, October 15, 2019 6:30 AM

### **UTILIZATION OF GONADOTROPIN-RELEASING HORMONE AGONISTS FOR PRESERVATION OF OVARIAN FUNCTION IN WOMEN WITH BREAST CANCER RECEIVING CHEMOTHERAPY.**

Sally F. Vitez, MD, Ling Chen, MD MPH, Paula C. Brady, MD, Jason D. Wright, MD, Columbia University Medical Center, New York, NY.

**OBJECTIVE:** To determine the use and predictors of GnRH (gonadotropin-releasing hormone) agonists for ovarian conservation in young, reproductive age women with newly diagnosed breast cancer undergoing chemotherapy.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The MarketScan database was used to identify women 15-45 years of age with newly diagnosed breast cancer from 2008-2017. All patients underwent cancer directed surgery (lumpectomy, mastectomy, biopsy or lymph node evaluation) and received cytotoxic chemotherapy within three months before or after surgery. Patients were considered to have received GnRH agonist therapy if they had one claim for a GnRH agonist in the same period. All women with history of oophorectomy before or during the study period were excluded from the study. Trends and predictors of GnRH agonist use were described and compared using Cochran-Armitage trend test and Chi-square tests.

**RESULTS:** We identified a total of 13,634 women with breast cancer who underwent treatment with chemotherapy. The median age was 39 years with 755 women <30 (5.5%) years of age and 12,879 >30 (94.5%) years old. GnRH agonists were administered to 112 (0.8%, 95% CI 0.7-1.0%) women. The rate of GnRH agonist use was higher in women age 15-30 years compared to women age 30-45 (2.8% vs 0.7%) (P < 0.001). During the study period, the utilization of GnRH agonists increased from 0.3% in 2008 to 1.3% in 2017 (P<0.001). Use of GnRH agonists was higher in the Northeast (2.0%) compared to the north central (0.7%), southern (0.6%) and western (0.5%) U.S. (P<0.001).

**CONCLUSIONS:** The utilization of GnRH agonists among reproductive age women with breast cancer undergoing chemotherapy is extremely low.

**P-10** Tuesday, October 15, 2019 6:30 AM

### **EVIDENCE THAT ALKYLATING CHEMOTHERAPY IS NOT RELATED TO CLINICAL INFERTILITY IN ADOLESCENT AND YOUNG ADULT (AYA) CANCER SURVIVORS.**

Kelsey Pinson, MD,<sup>a</sup> Christina Lam, MD,<sup>a</sup> Alexa CO Medica, MD,<sup>b</sup> Brian W. Whitcomb, PhD,<sup>c</sup> Ksenya Shliakhtsitsava, MD,<sup>d</sup> H. Irene Su, M.D., M.S.C.E.<sup>e</sup> <sup>a</sup>University of California, San Diego, La Jolla, CA; <sup>b</sup>UCSD resident, San Diego, CA; <sup>c</sup>University of Massachusetts, Amherst, Amherst, MA; <sup>d</sup>UT Southwestern, Dallas, TX; <sup>e</sup>University of California San Diego, La Jolla, CA.

**OBJECTIVE:** Gonadotoxic cancer treatments adversely impact ovarian reserve, but it is unknown if they are related to clinical infertility in AYA cancer survivors. We tested the hypothesis that alkylating chemotherapy (AC) and abdomino-pelvic radiation (RT) increase risks of infertility after cancer.

**DESIGN:** retrospective cohort.

**MATERIALS AND METHODS:** Female AYA survivors who were ages 21-40 at enrollment, 15-35 at cancer diagnosis, and completed primary cancer treatment, were recruited to the parent Reproductive Window Study on ovarian function. Survivors completed an enrollment questionnaire which included demographic, cancer, and reproductive characteristics, including infertility, pregnancy attempts, and pregnancies. Primary medical records were abstracted for cancer treatments. This analysis included the first episode of pregnancy attempt after cancer (N=200). Primary exposures were AC and RT. The primary outcome was clinical infertility (no pregnancy after 12 months of trying). Chi-square, Fischer's Exact, and log binomial regression were used to estimate associations between patient characteristics and infertility.

**RESULTS:** Mean age at time of cancer diagnosis was 26 +/- 5.5 years, 72% were Caucasian, and 17% were Hispanic. The most common cancers were lymphoma (27%), breast (24%), and thyroid (22%). Mean age at pregnancy attempt was 30 (15% age <25 yr, 35% 25-30 yr, 37% 30-35, 14% >35yr). 47% of participants received AC while only 3% received RT. In the first pregnancy attempt, 156 survivors (78%) achieved a pregnancy, 78% by 6 months, 88% by 12 months, and 12% after 12 months. RT was significantly associated with infertility RR 3.75 (95% CI 2.4-5.9). However, alkylating chemotherapy exposure (RR 1.03, CI 0.6-1.7, p=0.88) and cyclophosphamide dose by tertile (tertile 2 vs 1, RR 1.5 (95% CI 0.2-9.9); tertile 3

vs 1, RR 0.6 (95% CI 0.1-5.3)) were not associated with infertility. The composite cyclophosphamide equivalent dose (CED) showed no dose dependent association with infertility (< 4 g/m<sup>2</sup> vs. none, RR 0.86 (95% CI 0.5-1.7); 4-8 g/m<sup>2</sup> vs. none, RR 0.56 (95% CI 0.15-2.1), > 8g/m<sup>2</sup> vs. none, RR 1.1 (0.4-2.9)). The interaction between age at pregnancy attempt and CED was also not significant (p=0.71). No associations were found between race, BMI, age at cancer diagnosis, or age at pregnancy attempt and risk of infertility.

**CONCLUSIONS:** Abdominopelvic radiation was associated with a 3-fold higher rate of infertility. Exposure to alkylating chemotherapy, cyclophosphamide, and CED were not associated with infertility in AYA survivors. While these agents are known to decrease ovarian reserve, our novel time to pregnancy data suggests no association with infertility, which is consistent with childhood cancer survivor data that did not observe lower rates of ever pregnancy with alkylating chemotherapy exposure. Although this sample size is relatively small, there is no signal suggesting an association between alkylating chemotherapy and infertility. These data suggest no worse prognosis for becoming pregnant after cancer even with high dose alkylating exposure.

**SUPPORT:** NIH HD080952-05.

**P-11** Tuesday, October 15, 2019 6:30 AM

### **REPRODUCTIVE POTENTIAL OF VITRIFIED OOCYTES AND EMBRYOS PRODUCED FROM IN VITRO MATURATION CYCLES OF CANCER PATIENTS FOR FERTILITY PRESERVATION.**

Weon-Young Son, Ph.D., Helene Creux, M.D., Sara Henderson, M.Sc., Shaoguang Jin, Ph.D., Jln-Tae Chung, M.Sc., William Buckett, M.D. Division of Reproductive Endocrinology and Infertility, McGill University Health Care Centre, Montreal, QC, Canada.



**OBJECTIVE:** Few clinical options for fertility preservation (PF) are available to women with cancer. Although vitrification of oocytes/embryos obtained from IVF cycles has been used successfully in the PF program, controlled ovarian stimulation (COH) is contraindicated for patients with certain forms of cancer. In addition, many cancer patients have limited time to do COH before therapy. In these cases, immature oocyte collection followed by in vitro maturation (IVM) can be an alternative. This vitrification technique has also been applied to cryopreserve oocytes/embryos obtained from IVM program, but data about embryological and clinical outcomes is limited. The aim of this study was to evaluate post-thawing outcomes of immature oocytes collected by transvaginal aspiration in a fertility preservation program for women with cancer.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** IVM treatment for cancer patients was performed whatever the phase of the menstrual cycle. IVM oocyte retrieval was performed 38 h after an administration of 10,000 IU hCG. Immature oocytes obtained were cultured in vitro until 48 hours. The matured oocytes were cryopreserved using vitrification method either mature stage or cleavage stage after fertilization with partner sperm. We conducted study of cancer patients treated in a university based IVF center for 16 years (2003-2018). We reviewed the records of 213 cancer patients who underwent IVM cycles (n=237) for PF for cancer. All embryos and oocytes that were vitrified and warmed were included in the study. Post-warming embryological and clinical outcomes were evaluated.

**RESULTS:** Most frequent cancer for FP in our IVM program was breast cancer (67.6%) followed by hematological cancer (17.8%). The median time lapsed before returning to attempt pregnancy was 6.0 [4-13] years for IVM oocyte and 5.5 [4-13] years for IVM embryo cryopreservation. Thirty-two stored IVM embryos from eight patients (12 cycles) were warmed (mean = 2.7 (range:1-4)). Survival rate per embryo was 92.4 % and 24 embryos (mean=2.0) were transferred. Three patients became clinically pregnant (25.0% per cycle), resulting in the normal delivery of a healthy baby, one ongoing for 34 weeks and one miscarriage. Live birth/ongoing pregnancy rate per patient was 25.0% (2/8). In the IVM oocyte cryopreservation, 77 oocytes were warmed from 8 patients (9 cycles), survival rate per oocyte was 71.6 %, 56.8 % normal fertilization and cleavage rate per embryo was 68.1%. Two cycles of embryo transfers were canceled due to no cleavage. Out of 7 ET cycles, there were 2 biochemical pregnancies, but no clinical pregnancy.

**CONCLUSIONS:** IVM embryos can be stored with a reasonable result for cancer patients. However, cryopreservation of oocytes collected from IVM program seems to have poor reproductive potential. Therefore, more studies are urgently required to improve IVM- and vitrification method to successfully preserve oocytes collected from cancer patients.

**P-12** Tuesday, October 15, 2019 6:30 AM

### **FEASIBILITY OF FERTILITY PRESERVATION IN PRE-PUBERTAL MALES SCHEDULED TO RECEIVE CHEMOTHERAPY.**

Helen Levey Bernie, DO MPH, Elizabeth Schofield, MPH, Nicole Benfante, BS, John P. Mulhall, MD MSc FECSM FACS Memorial Sloan Kettering Cancer Center, New York, NY.



**OBJECTIVE:** Overall incidence rates of childhood cancer vary between 50-200/million children across the world. Childhood cancer survival rates are high, as are the late effects of gonadotoxic agents, such as infertility, in cancer survivors. While cryopreservation of sperm in adults is widely used, cryopreservation of testicular tissue in pre-pubertal boys has only recently been applied as a means to help preserve future fertility in pre-pubertal boys with cancer. The objective of this study is to present our experience of pre-pubertal pre-chemo fertility preservation.

**DESIGN:** We reviewed all pre-pubertal patients who underwent pre-chemo fertility preservation at a large cancer center for feasibility and safety.

**MATERIALS AND METHODS:** All pre-pubertal pre-chemotherapy patients who underwent fertility preservation using testicular sperm extraction (TESE) were analyzed. Parents were informed that: the procedure was experimental, given that it is unknown if the tissue will be of use in the future; the procedure had to be performed under an anesthetic for another procedure (infusion port, bone marrow aspirates); the procedure would not be covered by insurance. A unilateral window technique was utilized as most patients were to undergo chemotherapy within 48 hours of TESE. Safety data including rates of infection, hematoma development or delay in chemotherapy initiation were recorded.

**RESULTS:** A total of 22 pre-pubertal males had a mean age of 7.613 years constituted the study population. Mean FSH level was 6.9±6.3 (range 0.3-15.4) iU/ml. Most (71%) were Tanner stage 1, mean testicular volume 3.6±1.9. The most common childhood cancers in this cohort were sarcomas (55%), immune deficiencies (23%), and leukemias (14%). The procedure took an average of 22 . The tissue was sent to the sperm bank where it was cryopreserved. Viability testing on the first 10 specimens revealed healthy testicular tissue. No wound infections or scrotal hematomas occurred postoperatively. All patients were able to commence chemotherapy on schedule, usually within 24 hours or less of the fertility procedure.

**CONCLUSIONS:** Fertility preservation in pre-pubertal pre-chemotherapy patients is a safe and feasible operation.

**SUPPORT:** None.

**P-13** Tuesday, October 15, 2019 6:30 AM

### **FERTILITY PRESERVATION DISCUSSION IN CANCER PATIENTS IS UNDERUTILIZED AND VARIES BASED ON AGE.**

Peter N. Dietrich, MD, G Luke Machen, MD, Pranav Dadhich, MD, Johnathan Doolittle, MD, Jay I. Sandlow, MD Medical College of Wisconsin, Milwaukee, WI.



**OBJECTIVE:** The American Society of Clinical Oncology recommends that all patients with a cancer diagnosis be counseled on the impact of their disease and treatment on fertility. Despite this, oncofertility is often omitted in pretreatment discussion and planning. The reason for lack of adequate counselling and referrals for fertility preservation is unclear. This study seeks to evaluate the prevalence of cryopreservation discussions.

**DESIGN:** A retrospective review was performed on 3133 male patients aged 18-60 years with a cancer diagnosis at a single institution. Patient's charts were queried for "vasectomy", "semen", "sperm", "fertility" and "preservation".

**MATERIALS AND METHODS:** Patients were excluded if they had a vasectomy or sperm banking prior to their cancer diagnosis, as well as if they had no treatment for their cancer diagnosis. Data was collected for cryopreservation discussion, discussion prior to treatment, discussion before chemotherapy, and if cryopreservation of sperm was performed. Age, race, cancer location, primary treatment, and chemotherapy status was also recorded.

**RESULTS:** A total of 2504 patients were included for analysis. Mean age was 49 years. There was documentation of counseling on cryopreservation in 353 (14.1%)—280 (79.3%) before primary treatment and 298 (84.4%) before chemotherapy. 126 (5.0%) patients underwent cryopreservation. A logistic regression indicated a significant effect of age, race, site of cancer, primary treatment, and chemotherapy treatment on whether cryopreservation was discussed (chi<sup>2</sup> <0.001, pseudo R<sup>2</sup>=0.29). Chemotherapy at any time of treatment (OR 6.99, p<0.001) was significantly associated with cryopreservation counseling. Testicular and prostate cancer patients were significantly more

likely to be offered cryopreservation ( $p < 0.001$  and  $p = 0.005$ , respectively). Patients aged 30-39, 40-49, and 50-60 were significantly less likely to receive counseling when compared to patients aged 18-29 while controlling for other variables (OR 0.41, 0.12 and 0.05 respectively,  $p < 0.001$  for all 3 groups).

**CONCLUSIONS:** Reproductive side effects are not as commonly discussed as other systemic side effects when a patient receives a cancer diagnosis or when they start treatment. Our study indicates that cryopreservation is vastly underdiscussed. Younger patients, those undergoing chemotherapy during their treatment period, and a diagnosis of testicular and prostate cancer were more likely to receive cryopreservation counseling. As assisted reproductive techniques have become more successful and readily available, it is important to include options and counseling for all patients.

**P-14** Tuesday, October 15, 2019 6:30 AM

**FERTILITY-SPARING TREATMENT (FST) AND ASSISTED REPRODUCTIVE TECHNOLOGY (ART) IN PATIENTS WITH ENDOMETRIAL CARCINOMA (EMCA) AND ENDOMETRIAL INTRAEPITHELIAL NEOPLASIA (EIN); PREGNANCY OUTCOMES AFTER EMBRYO TRANSFER (ET).** Hilary Friedlander, MD,<sup>a</sup> Jennifer K. Blakemore, MD,<sup>b</sup> David H. McCulloh, Ph.D.,<sup>c</sup> Mary Elizabeth Fino, MD.<sup>d</sup> <sup>a</sup>NYU School of Medicine, New York, NY; <sup>b</sup>NYU Langone School of Medicine, New York, NY; <sup>c</sup>NYU Langone Health, New York, NY; <sup>d</sup>NYU Langone Fertility Center, New York, NY.



**OBJECTIVE:** Non-surgical management for patients desiring future fertility with EMCA and its precursor, EIN, has the goal of clearance of affected tissue and reversion to normal endometrial function (1). Only approximately 15% of these patients will have a livebirth (LB) without the need for ART (2). Despite this low number, little information exists on the pregnancy outcomes for patients who will go on to utilize ART. We investigated the pregnancy outcomes for patients who underwent ET after FST.

**DESIGN:** Retrospective cohort study of all patients who underwent ET after FST for EMCA or EIN at a single center between 1/2003 and 12/2018.

**MATERIALS AND METHODS:** An analysis of all patients and ET outcomes after FST was performed. Patients who utilized ART but did not yet return for ET were excluded. Descriptive data are presented as mean  $\pm$  SD. Observed ET outcomes were sub-grouped into 1) LB + ongoing pregnancy (OP) and 2) spontaneous abortion (SAB) + not pregnant (NP). Observed outcomes were compared to expected outcomes matched for age and type of transfer [fresh or frozen, number of embryos transferred, and with or without pre-implantation genetic testing (PGT) at our center] with a Wilcoxon Signed-Rank Test,  $p < 0.05$  considered significant.

**RESULTS:** 14 patients, 3 with EMCA and 11 with EIN, met criteria for inclusion for a combined total of 40 ETs. The mean age at initiation of ART following FST was  $35.14 \pm 4.77$  (range 28 to 44) and includes two patients, aged 40 and 44, who ultimately used donor eggs. The average BMI at diagnosis was  $26.51 \pm 6.17$ . FSTs prior to ET included megestrol acetate ( $n=7$ ), oral progesterone ( $n=5$ ), levonorgestrel intrauterine device ( $n=1$ ), and polypectomy ( $n=1$ ). The average time from diagnosis to first ET was 1.62 years  $\pm$  1.46. The average number of ETs per patient was  $2.86 \pm 2.03$ , with a range of 1 to 9. Of 40 ETs, 10 transfers were fresh ETs with an average of  $2.70 \pm 1.06$  embryos transferred per cycle. Three ETs were untested donor eggs, each with a single embryo transferred per cycle. Thirteen were frozen untested ETs, with an average of  $1.77 \pm 0.81$  embryos transferred per cycle. Six patients elected to use PGT [Array Comparative Genomic Hybridization (aCGH) and Next Generation Sequencing (NGS)] for a total of 14 frozen euploid ETs, with an average of  $1.69 \pm 0.97$  embryos transferred per cycle. Outcomes for all ETs included 7 LB, 1 OP, 8 SAB, and 24 NP. An analysis of observed outcomes by sub-group, compared to the expected from matched controls (age, ET type and number, and PGT as described above) showed that patients with EMCA/EIN after FST had a significantly lower LB/OP rate than expected,  $Z = -5.04$ ,  $df = 39$ ,  $p < 0.01$ . A sub-group analysis of the 14 euploid ETs (7 single by NGS, 4 single by aCGH, 3 double by aCGH) resulted in a LB/OP rate of 21.4% compared to an expected rate of 62.8% ( $Z = -3.32$ ,  $df = 13$ ,  $p < 0.001$ ).

**CONCLUSIONS:** Patients who have undergone FST for EMCA/EIN have significantly poorer outcomes than expected after ET. Further evaluation of the impact of the diagnosis, treatment and repeated cavity instrumentation for EMCA/EIN is necessary to create an individualized and optimized approach for this unique patient population.

References: 1. ACOG Committee Opinion #631. Reaffirmed 2017. Endometrial Intraepithelial Neoplasia.

2. Gallos ID, Yap J, Rajkhowa M, et al. Regression, relapse, and live birth rates with fertility-sparing therapy for endometrial cancer and atypical complex endometrial hyperplasia: a systematic review and metaanalysis. *Am J Obstet Gynecol* 2012;207:266.e1-12.

SUPPORT: None.

**P-15** Tuesday, October 15, 2019 6:30 AM

**OVARIAN STIMULATION IN CANCER PATIENTS: RANDOM VERSUS CONVENTIONAL START.** Andrea Natalia Coscia, MD,<sup>a</sup> Mariana Miguens, M.D.,<sup>a</sup> Mariana Cecilia Calvo, MD,<sup>a</sup> Rocío Belén Anria, M.D.,<sup>a</sup> Milfra Espinal, MD,<sup>b</sup> Elayne Margarita Vasquez, MD,<sup>a</sup> Sergio D. Papier, Sr., M.D.<sup>a</sup> <sup>a</sup>CEGYR, Ciudad Autonoma de Buenos Aires, Argentina; <sup>b</sup>Cegyr, Buenos Aires, Argentina.



**OBJECTIVE:** To determine if random start ovarian stimulation in cancer patients provides similar results compared to conventional stimulation starting in follicular phase.

**DESIGN:** Retrospective data analysis at a single center (CEGYR).

**MATERIALS AND METHODS:** All patients undergoing oocyte cryopreservation for fertility preservation due to recent cancer diagnosis were reserved from 2012 to 2018.

Patients were grouped according to random start or conventional start of the ovarian stimulation. Conventional start was defined as scheduled in early follicular phase initiation of gonadotrophins; random start was initiated at any other moment of the menstrual cycle.

The analyzed variables were: number of oocytes, number of matured oocytes (metaphase II), and cycle length.

**RESULTS:** 71 cycles met inclusion criteria.

Oocytes were collected of 23 (33%) patients on the random start group and 48 (67%) from the conventional one.

Mean age was 33.8 years old in the conventional and 33.25 years old in the random start groups. ( $p: 0.65$  IC95%, 2.04-3.23).

The mean number of oocytes collected were similar 11.9 (conventional) versus 10.4 (random) ( $p: 0.47$  IC95%, 2.65-5.66) and mean number of mature oocytes vitrified was also similar (metaphase II): 9.30 (conventional) vs 7.6 (random) ( $p: 0.34$  IC95%, 1.81-5.13).

The cycle duration was different, being the conventional shorter (9.7 days) than the random group (11.3 days) ( $p: 0.0019$  IC95%, 0.61-2.58).

**CONCLUSIONS:** Random start stimulation cycles for cancer patients has comparable results and allows patients to start gonadotrophin stimulation irrespective of menstrual cycle phase, with no impairment of oocyte yield and only a small increase of cycle duration.

Random start is a good opportunity for patients who are run out of time and face a fertility threatening medical condition.

**P-16** Tuesday, October 15, 2019 6:30 AM

**ADDED BENEFIT OF IMMATURE OOCYTE MATURATION FOR FERTILITY PRESERVATION IN WOMEN WITH MALIGNANCY.** Yoni Cohen, M.D., Ph.D.,<sup>a</sup> Samer Tannus, M.D.,<sup>b</sup> Alexander Volodarsky-Perel, M.D.,<sup>b</sup> Weon-Young Son, Ph.D.,<sup>b</sup> Togas Tulandi, M.D.,<sup>b</sup> William Buckett, M.D.<sup>b</sup>



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**OBJECTIVE:** To assess the added value of maturing immature oocytes collected during fertility preservation treatments in women with malignancy.

**DESIGN:** A retrospective case control study conducted at a tertiary academic IVF unit.

**MATERIALS AND METHODS:** Patients: 327 cancer patients undergoing fertility preservation treatment from 2009 to 2017. We compared oocyte maturation rates and cycle parameters from 3 types of fertility preservation treatments: 1. Stimulated IVF cycle ( $n=143$ ), 2. Non-stimulated IVM cycle ( $n=158$ ), 3. Follicle aspiration and oocyte collection from ovarian tissue prepared for ovarian tissue cryopreservation followed by in vitro maturation of the immature oocytes ( $n=48$ ). The primary outcome measure was the maturation rate and the number of mature oocytes. The secondary outcomes were oocyte fertilization and embryo development rates.

**RESULTS:** The mean maturation rate in IVF cycles was 38% and in the non-stimulated IVM cycles was 55%. In women who chose to cryopreserve their embryos, similar fertilization and embryo cleavage rates were found in oocytes that matured after stimulated IVF cycles compared to non-stimulated

IVM cycles. Gonadotropin releasing hormone agonist triggering, treatment with aromatase inhibitor or oral contraceptives use before the cycle, did not affect the maturation rate.

**CONCLUSIONS:** Although the maturation rate of immature oocytes collected in IVF cycles is low, it is still a viable source of oocytes that can be used to improve the efficacy of fertility preservation treatments by increasing the number of mature oocytes available for freezing or fertilization.

**P-17** Tuesday, October 15, 2019 6:30 AM

### ACCESS TO FERTILITY-SPARING TREATMENT OF ENDOMETRIAL INTRAEPITHELIAL NEOPLASIA: REPRODUCTIVE OUTCOMES AND PATIENT SATISFACTION.

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**OBJECTIVE:** Endometrial intraepithelial neoplasia (EIN) is the precursor to type 1 endometrioid adenocarcinoma, which is caused by unopposed estrogenic proliferation of the endometrium. Hysterectomy is curative in up to 98% of cases, but women desiring fertility preservation now have access to hormonal therapy as a treatment option. Our objective was to characterize patient satisfaction and subsequent reproductive outcomes among patients with EIN or grade 1 endometrial adenocarcinoma who elected fertility-sparing treatment.

**DESIGN:** retrospective cohort, survey.

**MATERIALS AND METHODS:** We performed a retrospective medical record review for all patients seen in consultation for EIN or grade 1 endometrial adenocarcinoma from a single gynecologic oncology practice at a tertiary care hospital from 2007 through 2016. The abstracted data included patient characteristics, fertility treatment, and reproductive outcomes. We also invited patients to complete a survey, either online or via telephone, to understand patient experiences with fertility-sparing management of EIN and type 1 endometrioid adenocarcinoma. Reported data are from a combination of the medical record review and the survey.

**RESULTS:** There were 64 eligible patients, and 41 (64%) completed the survey. Among the 64 patients, the majority (77%) had EIN. Initial treatment included a progestin-containing intrauterine device for 61%, an oral progestin for 33%, and both for 1%; initial treatment was unknown for 5%. Complete regression was documented in 69% of patients, and 26% underwent hysterectomy. Roughly half of patients (56%) had a documented infertility consultation, and 36% pursued treatment with ovulation induction or stimulation, intrauterine insemination and/or in vitro fertilization. Of the 24 patients who we know attempted pregnancy after fertility-sparing treatment, 11 (46%) had a total of 16 pregnancies and 8 patients had at least one live birth. Among the survey respondents, 87% agreed or strongly agreed that they were pleased with the advice they received about treatment options, and 82% felt they had a choice about their treatment. Most respondents (65%) felt their initial treatment was helpful in treating their cancer. Nearly all respondents (91%) agreed or strongly agreed they would make the same decision to try hormonal treatment.

**CONCLUSIONS:** The majority of patients with EIN or grade 1 endometrial adenocarcinoma who elected fertility-sparing treatment subsequently pursued consultation with an infertility specialist and many underwent infertility treatment. Among those who we know attempted pregnancy, one third had a live birth. Overall, patients were very satisfied with their fertility-sparing treatment, and nearly all expressed that they would make a similar choice again. In future studies, we aim to further elucidate the unique aspects of fertility treatment in patients with EIN who elect fertility-sparing treatment.

**P-18** Tuesday, October 15, 2019 6:30 AM

### LETROZOLE AND FERTILITY PRESERVATION IN PATIENTS WITH BREAST CANCER.

Marouen Braham, Sr., Associate professor,<sup>a</sup> Sarah Amari, Medical Degree,<sup>b</sup> Khadija Feriel Kacem Berjeb, Associate professor,<sup>c</sup> Haihem Khalil, Sr., Doctorate in Medicine,<sup>a</sup> Manel Hamdoun, Medical Degree,<sup>d</sup> Mounir Ben Meftah, Sr., Medical Degree,<sup>a</sup> Habiba Essoussi, Medical Degree,<sup>c</sup> Olfa Bahri, Sr., Professor,<sup>d</sup> Anis Fadhlouli, Associate Professor,<sup>a</sup> Fethi Zhioua, Pr.<sup>a</sup> <sup>a</sup>Aziza Othmana University hospital, Tunis, Tunisia; <sup>b</sup>Gynecology, Obstetric and Reproductive Medicine Department, Aziza Othmana University Hospital, Tunis, Tunisia; <sup>c</sup>Reproductive Medicine Laboratory, Aziza Othmana University Hospital, Tunis, Tunisia; <sup>d</sup>Biochemistry Department, Aziza Othmana University hospital., Tunis, Tunisia.



**OBJECTIVE:** Ovarian stimulation with exogenous gonadotropins leads to a significant rise in circulating estrogen levels which could aggravate the spread of breast cancer. The use of anti aromatase agents such as letrozole could prevent this elevation. But is it safe and effective in fertility preservation?

**DESIGN:** We conducted a prospective comparative study.

**MATERIALS AND METHODS:** A total of 171 patients were referred to our FP consult. Only 143 patients underwent fertility preservation (oocyte/embryo vitrification) and 75 amongst them had breast cancer.

A FP consultation is provided by both a gynecologist and a biologist of the department. A complete physical examination is performed. An informed consent is signed before starting the procedure. The evaluation of the ovarian reserve is done by Antral Follicle Count (AFC) ultrasound and AMH dosage.

The stimulation is conducted according to a random-start antagonist protocol using GnRH agonist triggering. For patients with breast cancer, the adjunction of letrozole 5mg/day was started the first day of ovarian stimulation and continued 7 days after oocyte pick up, while closely monitoring estrogen levels during COS.

We compared the number of mature oocytes obtained between patients with breast cancer (Groupe 1) and patients diagnosed with other types of cancer (group 2).

**RESULTS:** The average age of our patients (years) in group 1 was 30.3 +/- 3.7 and 26.9 +/- 6.7 in group 2; with no significant statistical difference (p=0.73). The evaluation of ovarian reserve using AFC (12.3 +/- 6.2 vs 13.9 +/- 6.4; p = 0.7) and serum AMH levels (2.43 +/- 2.3 ng/ml vs 2.8 +/- 2.45 ng/ml, p = 0.5) showed similar results in both groups.

The duration of the ovarian stimulation was not significantly different between both two groups: 10.2 +/- 2.3 days vs 11.8 +/- 3.1 days; p = 0.5).

Estradiol level on the day of ovulation triggering was 479 +/- 323 pg/ml in the breast cancer group versus 1701 +/- 682 pg/ml in the other group (p = 0.02).

The number of CCOs obtained in the breast cancer group was 10.76 +/- 8.39 compared with 9.11 +/- 6.81 in the group 2 and the difference was not significant (p=1.83).

The mean number of mature metaphase II oocytes collected in the breast cancer group was 7.38 +/- 6.11 oocytes versus 6.09 +/- 4.72 oocytes in group 2. The difference was not statistically significant either (p=1.33).

**CONCLUSIONS:** Breast cancer is one of the most frequent malignancies in women worldwide and the demand for fertility preservation is on the rise. Letrozole would provide much ease and safety during emergency controlled ovarian stimulation, without negatively impacting its outcome.

## CRYOPRESERVATION

**P-19** Tuesday, October 15, 2019 6:30 AM

### OOCYTE YIELD WITH IMMEDIATE SUBSEQUENT OOCYTE CRYOPRESERVATION (OC) CYCLES COMPARED TO INTERVAL CYCLES.

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**OBJECTIVE:** Women undergoing OC cycles often desire multiple cycles to increase oocyte yield. Traditionally, an interval of 1 menstrual cycle (MC) or more was advised. However, data supporting this recommendation are limited. The aim of this study is to evaluate the difference in oocyte yield between 1st and 2nd retrievals in women undergoing immediate subsequent cycle compared to those with an interval of 1 or more MCs, and to evaluate whether interval length is associated with 2<sup>nd</sup> cycle oocyte yield.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All women undergoing 2nd OC cycles at Extend Fertility Medical Practice from 4/2016-12/2018 were included in the study. Demographic and cycle data were abstracted from the electronic medical record. Difference in days and MII cryopreserved oocytes between retrievals were calculated and categorized. Subjects were also categorized by whether they yielded more, equal, or fewer oocytes in 2nd cycle compared to 1st cycle. Comparisons between groups were made utilizing Mann-Whitney-U, Kruskal-Wallis or X<sup>2</sup>, where appropriate. A multinomial logistic regression was performed to assess the association between retrieval interval and 2nd cycle oocyte yield, while controlling for age and AMH.

**RESULTS:** 399 subjects with 2nd retrievals were included in the study. For the cohort, mean age was 36.5±3.0 years, median AMH was

1.51±1.32 ng/mL, median 1st cycle MII cryopreserved oocyte yield was 6.0±5.0, and median 2nd cycle MII cryopreserved oocyte yield was 8.0±7.0. Difference in median oocyte yield was +2.0±6.0 (range; -12 to +52). Median interval days between retrievals was 72±60 (range; 15-739).

88 (22.1%) of subjects completed their 2nd retrieval within 1 MC of their initial retrieval. No significant difference between those whose interval was <1 MC compared to ≥1 MC was noted in age (p=0.58), AMH (p=0.22), or 1st cycle yield (p=0.09). No significant difference was noted in oocyte yield with interval of <1 MC vs 1 MC (p=0.92) or with interval of <1 vs ≥1 MC (p=0.19).

244 (61.5%) subjects yielded more, 38 (9.6%) yielded equal, and 115 (29.0%) yielded fewer oocytes in their 2nd retrieval compared to 1st. Median interval days was significantly higher in those with fewer (81±94) vs same (55.5±71) vs more (60±64) oocytes in the 2nd retrieval (p=0.04). Subjects with intervals <3 MCs were significantly more likely to yield equal or more oocytes in 2<sup>nd</sup> vs 1<sup>st</sup> cycle (RR 1.21; 95% CI 1.02-1.45). In the multinomial regression model, shorter interval was significantly associated with having more vs fewer oocytes, even when controlling for age and AMH (p=0.03).

**CONCLUSIONS:** Our data suggest that there is no evidence to support waiting 1 MC before undergoing a subsequent retrieval in women undergoing OC. Furthermore, intervals of ≥3 MC may be associated with decreased yield. While this study may be limited by its sample size, it represents the largest to date evaluating oocyte yield in subsequent cycles in either IVF or OC cycles.

**P-20** Tuesday, October 15, 2019 6:30 AM

### INITIAL VALIDATION OF AN AUTOMATED CRYOSTORAGE AND INVENTORY MANAGEMENT SYSTEM.

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<sup>a</sup>TMRW Life Sciences, New York, NY; <sup>b</sup>TMRW Life Sciences, Inc., New York, NY.



**OBJECTIVE:** To validate an automated and robotic liquid nitrogen vapor-based storage tank for cryopreserved embryos and gametes.

**DESIGN:** Experimental study.

**MATERIALS AND METHODS:** Cryopreserved mouse embryos (2PN-stage) in straws (Embryotech, Haverhill, MA) were distributed into platform-specific containers and either robotically uploaded into the automated tank (Group A) or transferred to a liquid nitrogen-filled dewar (Group B). Five days of storage ensued after which the embryos were robotically or manually retrieved from the tank or dewar, respectively, for warming. In each group, post-warming survival was evaluated and embryos were randomly allocated to culture in groups of 10 for incubation at 37°C in an atmosphere of 5%CO<sub>2</sub>/5%O<sub>2</sub> for 96 hours. At the end of the incubation period, blastocyst formation rate (#blastocysts/#2PN) was assessed. One-way ANOVA was applied for statistical analysis.

**RESULTS:** In both Groups A and B, post-warming survival of 2PN was 100%. Blastocyst formation rates were 93.2±7.9% (S.D.) (97/104) in Group A and 91.3±8.5% (95/104) in Group B. One-way ANOVA indicated no statistical difference (p=0.575).

**CONCLUSIONS:** This initial validation study suggests that application of a robotic, liquid nitrogen vapor-based tank for storage of cryopreserved mammalian embryos is feasible. Although studies utilizing human embryos and gametes remain to be done, this proof of concept study provides grounds for further development and testing of the system for application to Assisted Reproductive Technology. A number of key features of the system promises increased security, stability and efficiency compared to conventional methods of cryostorage that have been in use for four decades. These features include sample management through robotic handling, digital inventory control as well as continuous telemetric reporting of multiple tank parameters such as temperature, liquid nitrogen (LN2) level and LN2 consumption rate with predictive analytics that can help avoid system failure.

**P-21** Tuesday, October 15, 2019 6:30 AM

### CRYOPRESERVATION OF BOTH MALE AND FEMALE GAMETES LEADS TO REDUCED EMBRYO DEVELOPMENT AND IMPLANTATION POTENTIAL.

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**OBJECTIVE:** Egg-sharing is an effective way to speed up the waiting time for recipients and to provide treatment for infertile patients who need help funding their own treatment. The impact of egg and sperm cryopreservation on the outcomes of recipients' cycles is uncertain. The objective of this study was to investigate the influence of oocyte and sperm cryopreservation on donated eggs in terms of laboratorial and clinical outcomes of intracytoplasmic sperm injection (ICSI) cycles.

**DESIGN:** Historical cohort study.

**MATERIALS AND METHODS:** Data analyzed in this study were obtained via chart review of 115 oocyte donor ICSI cycles (age range 21-34 years), and 122 oocyte recipients (age range 31-48) undergoing 152 oocyte recipient ICSI cycles, participating in an egg-sharing donation program, from 2016 to 2018, in a private university-affiliated IVF center. The sample size calculation suggested that 148 cycles would be enough to demonstrate a 20% effect with 80% power and 5% significance level considering as primary outcome implantation rate. Cycles were split into four groups according to the origin of oocytes and semen, as follows: Fresh O/S Group, recipients in which fresh oocytes were injected with fresh sperm (n=19); Fresh O / Cryo S Group, recipients in which fresh oocytes were injected with cryopreserved sperm (n=14); Cryo O / Fresh S Group, recipients in which cryopreserved oocytes were injected with fresh sperm (n=85); and Cryo O/S Group, recipients in which cryopreserved oocytes were injected with cryopreserved sperm (n=34). The impact of oocyte and semen cryopreservation on recipients' ICSI outcomes was investigated by using General Mixed Models fit by restricted maximum likelihood, followed by Bonferroni post hoc test for the comparison of means amongst the four groups. The model was generated using covariates as fixed effects and egg-donors and egg-recipients as random effects, with unstructured covariance structure, adjusted for potential confounders.

**RESULTS:** Normal cleavage speed rate on day 3 was significantly lower in Cryo O/S Group (55.5%) compared to all other groups (Fresh O/S Group: 76.4%, Fresh O / Cryo S Group: 75.7%, and Cryo O / Fresh S Group: 62.4%, p: 0.046). Blastocyst development rate was also significantly lower in Cryo O/S Group (24.0%) compared to all other groups (Fresh O/S Group: 53.0%, Fresh O / Cryo S Group: 41.1%, and Cryo O / Fresh S Group: 33.0%, p: 0.020). A statistically significant gradual decline was observed in implantation rate (Fresh O/S Group: 36.7%, Fresh O / Cryo S Group: 32.9%, and Cryo O / Fresh S Group: 29.5%, Cryo O/S Group: 14.5%, p: 0.047). The rates of fertilization, high quality embryos on days 2 and 3, normal cleavage speed on day 2, high-quality blastocyst, clinical pregnancy, and miscarriage were similar between the groups.

**CONCLUSIONS:** In an egg-sharing donation program, embryo developmental competence and implantation potential are reduced when vitrified oocytes are injected with frozen sperm.

Reference: NA.

SUPPORT: None.

**P-22** Tuesday, October 15, 2019 6:30 AM

### AUTOMATED VITRIFICATION FOR EMBRYO CRYOPRESERVATION: PRELIMINARY COMPARATIVE RESULTS AND FIRST LIVE BIRTH IN EUROPE.

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**OBJECTIVE:** Automated vitrification has been made available recently, but its clinical efficiency has not been properly addressed in relation to manual vitrification. Therefore, the objective of this ongoing study is to compare clinical outcomes following automated embryo vitrification with those achieved after manual vitrification.

**DESIGN:** In June 2018 we began a study in which, so far, we have vitrified 92 cleavage stage embryos and 161 blastocysts using the automated vitrification system (Gavi®-Genea Biomedx), and 203 cleavage stage embryos and 255 blastocysts using the universally accepted/gold-standard system for manual vitrification (Kitazato Vitrification - Cryotop® Kit). This study is still in progress.

**MATERIALS AND METHODS:** Participants are couples undergoing embryo transfer following vitrification from June 2018 in our fertility centre. Embryos were cryopreserved using Gavi® according to manufacturer's

instructions or with Cryotop®. Embryo quality, number of transferred embryos and patient age have been balanced in both groups. Embryos were thawed according to the manufacturer's instructions and transferred in double (cleavage stage embryos) or single transfers (blastocysts). The clinical end-point for the comparative analysis is clinical pregnancy rate. Clinical pregnancy has been diagnosed by ultrasound examination 7 weeks after transfer, following a positive  $\beta$ -hCG test.

**RESULTS:** So far, we thawed 30 cleavage stage embryos vitrified with Gavi®, which were utilised in 15 double embryo transfers. During the same period, 66 cleavage stage embryos vitrified with Cryotop® were also double transferred (33 DET). So far, clinical pregnancy rates after automated and manual vitrification of cleavage stage embryos are 26,7% (4/15) and 33,3% (11/33), respectively. In parallel, we thawed 36 blastocysts after automated vitrification and 77 blastocysts after manual vitrification, all of them utilised in single transfers, except for 1 blastocyst from the manual vitrification group which was classified as degenerated after thawing. So far, clinical pregnancy rates after automated and manual vitrification of blastocysts are 44,4% (16/36) and 32,9% (25/76), respectively. No miscarriages have been observed so far after automated vitrification of cleavage stage embryos (0/4), whereas the abortion rate following manual vitrification of cleavage stage embryos is 27,3 % (3/11) so far. For cryopreserved blastocysts, abortion rates are 12,0% (3/25) and 12,5% (2/16) with manual and automated vitrification, respectively, so far. These transfers resulted in the first twenty pregnancies following automated embryo vitrification in Europe. At the time this abstract was written, one live birth, the first in Europe, had already occurred.

**CONCLUSIONS:** These data suggest that automated vitrification is technically efficient and may benefit the consistency of clinical outcomes following vitrification, as well as the logistics of the fertility centres.

**P-23** Tuesday, October 15, 2019 6:30 AM

**PROLONGED SEMEN CRYOPRESERVATION DECREASES MOTILE CONCENTRATION.**

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**OBJECTIVE:** There is a paucity of data regarding the viability of cryopreserved sperm. Good laboratory practice involving cryopreservation include QA/QC, equipment maintenance and stable temperatures of -196°C. These ensure tissues remain viable for later use. However, cryopreservation has been shown to alter the structure and function of stored spermatozoa. This study aims to determine if sperm viability is affected by long-term storage.

**DESIGN:** Before-After Study.

**MATERIALS AND METHODS:** Patients sperm samples that were abandoned over the years of 1995-2014 (average 14.5 years) were thawed and analyzed before discard. Samples were considered abandoned if patients couldn't be contacted within the past 5 years to continue storage. Prior to initial freeze, semen analyses were performed. Semen samples were stored in a 1:1 mixture of test yolk buffer, aliquoted into a 1.5 ml cryovial and suspended in nitrogen vapor for 30 minutes prior to being plunged into liquid nitrogen. Vials were thawed by placing cryovials into 37°C heat blocks for 20 minutes. Post-thaw survival of sperm was determined by calculating sperm concentration, motility and progression. Pre and post analyses were analyzed using regression analyses with a cluster analysis to account for pairwise comparisons.

**RESULTS:** 131 patient semen samples were grouped into four categories listed in Table 1. Age, initial sperm concentration and motility were not

TABLE 1. Summary of difference between initial and post-thaw motile concentrations

Years Stored	n	Pt Age	Initial Motile Concentration (M/ml)	Post-Thaw Motile Concentration (M/ml)	$\Delta$
<10	30	38.8±4.9	37.6±33.5	19.6±29.8	0.26±0.26
10-14	36	37.7±7.5	28.2±29.3	16.6±22	0.28±0.18
15-19	36	38±7.7	68.8±36	10.1±21.9	0.18±0.21
>20	29	34.7±8.7	45±58.2	11.7±24.1	0.09±0.13*

\*p<0.05

different between groups. Overall there is a significant decline in sperm motility with years of storage as vials stored for 20 years and longer show a 70% decrease compared to 10 years and less (p=0.0012). The patient age or initial sperm concentration at the time of freeze has no impact on sperm survival.

**CONCLUSIONS:** Despite appropriate measures to maintain specimen in storage, it appears that prolonged storage in liquid nitrogen may impact sperm survival. This result warrants further study as viability of tissues may be affected over time and reduce the success of fertility outcomes.

**P-24** Tuesday, October 15, 2019 6:30 AM

**EMBRYOS FROZEN WITHIN A SHORT TIME OF REACHING THE EXPANDED BLASTOCYSTS FROM THE EARLY BLASTOCYSTS HAVE HIGH VIABILITY: TIME-LAPSE INVESTIGATION OF 5177 BLASTOCYSTS.**



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**OBJECTIVE:** Morphological grading of blastocysts is in widespread use; however, morphokinetic grading by time-lapse monitoring is much less commonly applied. As PGS is not permitted in Japan, a method for estimating the potential viability of embryos using morphokinetic evaluation is required. It is known that Day5 blastocysts have higher viability than Day6 blastocysts. However, it is less understood whether an interval between early blastocysts and expanded blastocysts affects the embryo fertility.

**DESIGN:** The data was obtained in a retrospective study of 4097 cycles (mean patient age 38.2 years old) in the period 2013–2017.

**MATERIALS AND METHODS:** In total, 7283 embryos derived from IVF or ICSI were monitored using a time-lapse system (EmbryoScope, Vitrolife, Denmark) and the time from reaching the blastocysts to freezing the expanded blastocysts was recorded. The blastocysts selected for freezing had an ICM and inner diameter of more than 160  $\mu$ m. 5177 blastocysts were subsequently thawed for transfer. The survival rate at thawing, the pregnancy rate after single embryo transfer, and the live birth rate were determined. These three metrics were compared among embryos classified by the time between reaching the blastocysts and freezing the expanded blastocysts.

**RESULTS:** Three groups of thawed embryos were compared: 0–20 hours (3083), 21–40 hours (2011), and 41 hours or more (83) from blastocoel formation to expanded blastocysts frozen-thawed. Survival rates at thawing were 97.6% (3010/3083), 95.0% (1911/2011), and 92.8% (77/83), respectively, in these groups; the rate was significantly higher in the 0–20 hour group compared to the other 2 groups. Pregnancy rates of 58.8% (1767/3004), 42.9% (819/1907), and 14.3% (11/77) were obtained; the rate in the 0–20 hour group was significantly higher than the other 2 groups. Live birth rates were 68.6% (1212/1767), 62.6% (513/819), and 54.5% (6/11); the 0–20 hour group showed a significant difference to the 21–40 hour group.

**CONCLUSIONS:** As the viability of the thawed embryos with a short interval between reaching blastocysts and freezing expanded blastocysts was higher than in embryos with longer intervals, we suggest that patients with multiple blastocysts should be preferentially transplanted with those frozen a short time of developing expanded blastocysts.

Reference: None.

SUPPORT: None.

**THE TWO-STEP ASYNCHRONOUS VITRIFIED-THAWED BLASTOCYST EMBRYO TRANSFER STRATEGY. THE IMPACT ON MULTIPLE PREGNANCY RATE.** Viktor Veselovskyy, MD Nadiya Clinic, Kyiv, Ukraine.



**OBJECTIVE:** To compare clinical and multiple pregnancy rate among women who underwent two-step asynchronous blastocyst embryo transfer (TSABET) versus DET in the frozen embryo transfer (FET) cycle with patients who had at least two vitrified blastocysts.

**DESIGN:** Retrospective single-center cohort study.

**MATERIALS AND METHODS:** All patients (534 consecutive IVF/ICSI cycles) at NADIYA Clinic from 6/30/2015-12/31/2018 who met such criteria as age <38, good quality day 5-6 blastocysts, had at least 2 remaining cryopreserved blastocysts and subsequently underwent FET, were included in this study. Exclusion criteria were preimplantation genetic testing (PGT) cycles and donor oocyte cycles.

Primary outcomes were clinical pregnancy rate per transfer and multiple pregnancy rate. Secondary outcomes were miscarriage rate, ectopic pregnancy rate.

All women received estradiol for the preparation of the endometrium. The administration of progesterone (50 mg in oil, daily) was initiated when endometrium thickness exceeded 8 mm. In the DET group (433 cycles), on day 6 (P+6) or 7 (P+7) after the initiation (P+1) of progesterone treatment, two blastocysts were transferred. In the TSABET (101 cycles), on day P+6 and day P+9 the blastocysts were transferred twice.

The results between the DET and the TSABET cycles were compared (see Table below).

Chi-squared tests and t-tests were used to compare demographic, cycle characteristics and outcome data between groups.

**RESULTS:** Demographic characteristics were similar between DET and TSABET patients. TSABET patients had a significantly higher clinical pregnancy rate than DET patients (70,3% vs 57,0%). However, DET patients had a significantly higher multiple pregnancy rate (44,1% vs 9,9%). There was no difference in ectopic pregnancy and abortion rate seen between groups.

	DET (n=433)	TSABET (n=101)	P
Age, mean±σ	31,6±3,5 (19-37)	31,7±3,1 (23-37)	0,975
Clinical pregnancy	247 (57,0%)	71 (70,3%)	0,015
Multiple pregnancy	109 (44,1%)	7 (9,9%)	<0,001
Ectopic pregnancy	2 (0,8%)	1 (1,4%)	0,646
Miscarriages <20 w	48 (19,4%)	12(16,9%)	0,631

**CONCLUSIONS:** To our knowledge, this is the first study of using two-step vitrified-thawed blastocyst transfer strategy with 72h interval between transfers.

In the group of good prognosis patients TSABET strategy resulted in a higher clinical pregnancy rate than DET, but also was associated with a much lower multiple pregnancy rate. When deciding on the two embryos to transfer in this group, TSABET strategy should be preferable.

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**IMPACT OF EQUILIBRATION DURATION DURING OOCYTE VITRIFICATION PROTOCOL: PRELIMINARY RESULTS OF A PROSPECTIVE OBSERVATIONAL STUDY.** Charlene Herbemont, Pharm.D,<sup>a</sup>



Isabelle Cedrin-Durnerin, M.D.,<sup>b</sup> Michael Grynberg, M.D., Ph.D.,<sup>b</sup> Christophe Sifer, M.D.<sup>b</sup> Jean Verdier Hospital, Bondy, France; <sup>b</sup>Jean Verdier Hospital, bondy, France.

**OBJECTIVE:** Oocyte cryopreservation is a valuable technique in the field of fertility preservation (FP) as well as oocyte donation programs. Numerous studies have already analyzed outcomes following oocyte vitrification. Regarding technical aspects, open versus (vs) closed carriers have mainly been investigated. However, vitrification protocols commercially available do describe various durations of the equilibration step (from 6 to 10 minutes (min)). To date, a potential impact of this variability on the outcomes after warming has never been investigated.

**DESIGN:** This prospective observational study has been in progress since 2014, including all oocyte cryopreservation cycles. Vitrification/warming (n=64) were performed using commercialized media (Kitazato, Japan). Dur-

ing equilibration, according to the manufacturer's procedure, 9 oocytes maximum were deposited by 3 in 3 drops of equilibration solution (ES). After 6min, the vitrification step was initiated for the first 3 oocytes (duration 1min), and the following oocytes straw-by-straw were vitrified immediately thereafter, respectively after 7 and 8min of equilibration.

**MATERIALS AND METHODS:** Oocyte vitrification/warming required patients' written consent. To date, 64 couples underwent the whole procedure of warming and ICSI. The allocation of oocytes per straw depended on the total number of mature oocytes, explaining the variable number of oocytes in groups 6/7/8min. Survival, fertilization, embryo quality and suitability for transfer/freezing were assessed per oocyte and compared according to the duration of equilibration.

**RESULTS:** Indications for cryopreservation were: oocyte accumulation (46%); oocyte donation (33%); FP prior to cancer treatment (3%), for endometriosis (10%) poor ovarian reserve (1%); absence of sperm on the day of ICSI (7%). Overall, 388 oocytes were warmed, and 329 of them survived (survival rate (SR)=84.8%). The analysis according to the equilibration duration showed a slight difference in terms of SR: 82.5% (188/228) in group 6min vs 85.6% (101/118) in group 7min vs 95.2% (40/42) in group 8min (global p value=0.06). Interestingly, SR after 8min of equilibration was significantly or close to significantly higher than after respectively 6min (p=0.02) and 7min (p=0.07). After ICSI, fertilization rate (68.1 vs 74.5 vs 60.6%, p=0.29), Day 2 top (28.9 vs 37.1 vs 19.0%, p=0.23) and good quality embryo rates (43.0 vs 47.1 vs 33.3%, p=0.52) and rates of embryos suitable for transfer/freezing (70.3 vs 67.1 vs 52.4%, p=0.28) were statistically similar whatever the duration of the equilibration phase.

**CONCLUSIONS:** The equilibration duration during oocyte vitrification protocol might influence SR after warming but might not impact further embryo development of the surviving oocytes. If these data were confirmed, larger investigations should be performed on the different vitrification media commercially available. Then, manufacturers' recommendations on vitrification protocol should be amended accordingly. Furthermore, clinical outcomes should be analyzed.

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**COMPARATIVE STUDY OF FERTILITY PARAMETERS IN VITRIFIED HUMAN SPERM IN THE PRESENCE AND ABSENCE OF EMBRYORP®: A NOVEL ANTIOXIDANT.** César Rodrigo Coria, College degree,<sup>a</sup>



Paulina Torres, Master Degree,<sup>a</sup> Lina Villar, Sr., MD,<sup>b</sup> Israel Maldonado, MD,<sup>b</sup> Israel Jiménez, Sr., MD,<sup>a</sup> Claudia Lydia Treviño, PhD.<sup>a</sup> <sup>a</sup>Instituto de Biotecnología, Cuernavaca, MR, Mexico; <sup>b</sup>Clínica de Reproducción Asistida en la Ciudad de México, Ciudad de México, DF, Mexico.

**OBJECTIVE:** Find out if presence of the antioxidant EmbryoORP® in vitrification medium is a critical element that may reduce cryodamage in native sperm samples.

**DESIGN:** This study included 20 nomozoospermic sperm samples from healthy donors between 23 and 40 years old, that were used to evaluate a novel antioxidant: EmbryoORP® on functional and structural sperm quality parameters in a standard vitrification protocol.

**MATERIALS AND METHODS:** All samples were vitrified with the Easy-Sperm kit Sperm and divided into two aliquots: vitrified (V) and vitrified + EmbryoORP® (V-E). Concentration, motility and pH were assessed with a novel instrument LensHooke<sup>X1PRO</sup>®. Oxidation-reduction potential (ORP) were evaluate with the MiOXSYS® system. Acrosomal reaction, mitochondrial membrane potential (MMP) and vitality were also assessed by flow cytometry and compared between both groups.

**RESULTS:** Previously we measured the ORP in the seminal fluid of 50 infertile patients with an average of 30.53±17.88mV which is significantly lower to the estimate of 280mv in the vitrification medium. 10ul of Embryo-ORP® per milliliter diminish the ORP to 94.9mv in the vitrification medium and to 92.7mv in devitrification medium, values that are closely to physiological parameters of the cell. We found out no statistical difference in the progressive motility (p =0.2068), non-progressive (p = 0.3225) vitality (p=0.1610), morphology (p=0.8881), preservation of the acrosome (p=0.3031) and MMP (0.2743) post cryopreservation in both groups. The presence of the antioxidant lowers the pH of the medium to near 7 while the vitrification medium remains if physiological values (p <0.0001). The concentration the V-E group was lower after the freezing protocol compared to the V group (p = 0.0145). The ORP was lower in vitrified cells supplemented with the antioxidant (p < 0.0001).

**CONCLUSIONS:** We concluded from these results that the use of Embryo-ORP® is not the best option from sperm cryopreservation

**IS THE INCREASE IN EGG FREEZING CYCLES RELATED TO INCREASED NUMBERS OF SINGLE WOMEN IN THE UNITED STATES?**

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**OBJECTIVE:** In 2017, the United States Census reported 110.6 million unmarried persons over the age of 18, 53.2% of which were women. At the same time, the number of women choosing to freeze their eggs has increased over the last decade. The objective of this study was to determine whether there is an association between the rise in egg freezing and the number of single women in the United States.

**DESIGN:** Retrospective Cohort.

**MATERIALS AND METHODS:** Data on oocyte banking for fertility preservation from the SART database from 2014-2017 was analyzed. In addition, data from the United States Census Bureau on marital status of single women was obtained for the same years. The total number of single women reported was compared with the number of oocyte banking cycles. The Pearson correlation test was used to investigate associations between the number of egg freezing cycles and single women. Significance was defined as  $p < .05$ .

**RESULTS:** Between 2014-2017, a total of 33,324 egg freezing cycles were recorded from the SART database. Over this time frame, the number of cycles has increased by 79% while the total number of single women reported has increased 5% (Table 1). We found a high correlation between the increasing number of egg freezing cycles in the USA and the increasing numbers of single women nationwide, However, it did not reach statistical significance (coeff=.87,  $p = .13$ ).

Year	Number of egg freezing cycles (n)	Single (n)	Married (n)	Total(n)	Percentage single (%)
2014	6090	37,311,000	66,732,000	129,871,000	28.73
2015	7591	37,394,000	67,217,000	131,395,000	28.46
2016	8707	38,995,000	67,450,000	132,662,000	29.39
2017	10,936	39,087,000	68,082,000	133,403,000	29.30

**CONCLUSIONS:** 1. In just 4 years, egg freezing has seen an exponential rise of 79%.

2. During the same period, the number of single women in the USA increased by 5%.

3. These two observations are highly correlated, yet did not reach statistical significance. We suggest that an increase in the numbers of years of egg freezing cycles reported could reach significance.

4. Likely, the sharp rise in egg freezing is also related to improvements in egg freezing technique and results, increased awareness of reproductive ageing, the impact of social media and advertising and more. These factors cannot be enumerated and, might have diluted impact of the increase rate of single women over the same time period.

5. Last but not least: the relationship between egg freezing statistics and the number of single women may have already shifted from uni-directional to bi-directional. How many women delay marriage because egg freezing is readily available and increasingly more reliable?.

**THE IMPACT OF THE SHORT-TERM HUMAN SPERM STORAGE IN THE CRYOPROTECTANT-FREE MEDIUM ON SPERM MOTILITY AND VITALITY.**

Nabil Sayme, Dr. med.,<sup>a</sup> Marija Kljajic, Master



of Biology Science,<sup>b</sup> Thomas Krebs, Biology,<sup>a</sup> Dieter Maas, Prof. Dr. med.<sup>a</sup>  
<sup>a</sup>Team Kinderwunsch Hannover, Hannover, Germany; <sup>b</sup>Saarland University Medical Center, Homburg, Germany.

**OBJECTIVE:** Slow freezing is currently the most commonly used technique for sperm cryopreservation since the vitrification of spermatozoa is still a rather unexplored methodology. Storage sperm at +4 C is a relatively new technique and in the aim of reach better recovery rates, many studies confirmed that freezing/store sperm without cryoprotectants gives better results. The purpose of the study was to investigate does it the sperm storage in the cryoprotectant-free medium a good alternative for short-time preservation (up to 4 weeks) compare to the conventional slow freezing, as well as the impact of the short-term human sperm storage on sperm motility and vitality.

**DESIGN:** The study included 20 sperm samples collected between February 2018- April 2018. Out of 20 samples, 10 were normozoospermic, 5 were teratozoospermic and 5 were asthenozoospermic. Native samples with volume higher than 3 ml before preparation was divided equally in the aim to reach the same sperm concentration. After gradient preparation, the volume of 200µl sample was treated with the same volume of cryoprotectant (GM501 Sperm store, Gynemed, Germany ) or sperm preserve medium (Sperm Preserve, Gynemed, Germany).

**MATERIALS AND METHODS:** After slow freezing two straws of each sample were preserved into liquid nitrogen for a period of one and four weeks. Samples treated with Sperm Preserve were divided as well into two straws and stored in fridge on 4 C degrees for the same period. After this period samples were thawed with Sperm Active (GM501 Sperm Active, Gynemed, Germany) and motility and vitality after these two freezing procedures were compared. For statistical analysis, a One-Way ANOVA was used.

**RESULTS:** After one week of slow freezing and storage in liquid nitrogen, 24.9±11.22% of spermatozoa regained their motility compare to samples which were stored on 4 C where recovery rate was 32.75±11.02%. One-Way ANOVA confirmed that there is a significant difference between these two groups (  $p = 0.31$  ). The sperm motility rate after four weeks was slightly lower 20.4±6.87% in the slow freezing sample group, compare to 28.05±9.81% after storage at 4 C but still, further statistical analysis confirmed a significant difference between these two groups ( $p = 0.005$ ). Asthenozoospermic samples stored at 4 C had better motility recovery rate after one week 28.8±11.6% vs 13.6±2.96% ( $p = 0.02$ ) than after four weeks 24.2±14% vs 12.8 ±4.7% where the difference between these two groups was not statistically significant as well as neither between teratozoospermic samples. Vitality was one of the characteristics which we analyzed as well and the difference was significant especially after one week ( $p = 0.0001$ ) where survival rate after slow freezing was 40.5±11.80% compared to the storage sample where that number was 54.25±13.20 %. After four weeks as well, a higher percentage of sperm survive in the storage group 39.75±7.88% compare to the 30.7±6.78% of slow frozen samples ( $p = 0.001$ ).

**CONCLUSIONS:** The cryoprotectant-free sperm storage protocol tested in this study renders considerably better recovery rates (motility and vitality) of the sperm compared to slow freezing.

**SUPPORT:** Gynemed.

## DONOR GAMETES

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**CHANGES IN U.S. UTILIZATION OF DONOR EGG IVF CYCLES AT DIFFERENT FEMALE AGES BETWEEN 2005-2016.** Norbert Gleicher, MD, Sarah K. Darmon, PhD, David F. Albertini, PhD, David H. Barad, MD, MS. Center for Human Reproduction, New York, NY.



**OBJECTIVE:** To investigate as part of a larger study of changing U.S. practice patterns in IVF, how the utilization of third-party donor eggs has changed between 2005-2016.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** IVF outcome data are generated annually under Congressional mandate by the Center for Disease Control and Prevention (CDC), including almost all (in 2016, 463/502) of the nation's IVF centers, and are publicly reported with approximately two-and-a-half years delay. Most recent available data are, therefore, in the 2016 Annual Assisted Reproduction Summary Report from the CDC. We here report on CDC outcome reports longitudinally between years 2005-2016, with years 2005, 2010, 2015 and 2016 serving as index years.

**RESULTS:** With advancing female age, third-party donor egg cycles universally increased in all years as a subgroup of all IVF cycles until around 2010, when they peaked, representing 37% of all cycles. By 2015 they were only 34% and by 2016 only 33% of all cycles at ages 43-44; at ages above 44 years, they in 2010 represented as much as 73% of all cycles but by 2015 only 71% and by 2016 only 65%. We also observed a dramatic switch from use of fresh to frozen donor eggs, also starting in 2010, gaining ground much quicker especially above age 42: Above age 42 in 2005, 36% of donor cycles utilized frozen eggs, by 2010 38% of 43-44 year-olds and 45% of women above age 44 utilized frozen oocytes; by 2015 those numbers had further risen to 65% of 43-44 year-olds and 70% of women above age 44 years.

**CONCLUSIONS:** These data reveal a welcome decline in third party donor egg cycles after 2010, suggesting that more IVF centers are offering older patients the chance of pregnancy and delivery with use of own eggs. They, however, also raise concern about the rapid switch from use of fresh to frozen donor oocytes since 2010, likely caused by growth in commercial frozen egg banks, since frozen donor eggs produce ca.10% lower live birth rates than fresh eggs.<sup>1</sup> These developments, therefore, may adversely affect live birth rates with third-party donors.

**References:** <sup>1</sup> Kushnir VA, Darmon SK, Barad DH, Gleicher N. New national outcome data on fresh versus cryopreserved donor oocytes. *J Ovarian Res* 2018;11:2

**SUPPORT:** Intramural funds from The Center for Human Reproduction and grants from The Foundation for Reproductive Medicine.

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**DONOR OOCYTE PREGNANCIES AND FETAL FRACTION: MANAGING PATIENT EXPECTATIONS AND PROVIDING ACCURATE INFORMATION.** Melissa K. Maisenbacher, MS, Georgina Goldring, MS, Wendy DiNonno, MS, Allison Ryan, PhD Natera, San Carlos, CA.



**OBJECTIVE:** Determine if differences in fetal fraction (FF) are observed in donor oocyte pregnancies compared to the general population.

**DESIGN:** Retrospective analysis

**MATERIALS AND METHODS:** Noninvasive prenatal testing (NIPT) samples from singleton pregnancies were analyzed at a single reference lab. NIPT was performed using a SNP-based method with FF measured as previously described.<sup>1</sup> FF from 1611 donor oocytes was analyzed and compared to a large set of reference cases matched for maternal weight (MW) and gestational age (GA). A z-score was calculated for each donor oocyte compared to its reference data. If no impact to FF from the use of donor or IVF, the average z-score is expected to be zero.

Statistical analysis was performed using a z-test to establish if this was the case.

**RESULTS:** For donor cases the average z-score was -0.4. A z-test determined this deviation from normal to be significant ( $p < 0.00001$ ), showing that donor cases have lower FF than their corresponding reference data. The average MW was 154.3 lbs. (range 79.2-370.4 lbs.), average GA was 12.9 weeks (range 9-33 weeks) and average FF was 8.4%.

**CONCLUSIONS:** The adoption of NIPT over other screening and diagnostic methods continues to grow, especially among women using donor oocyte/IVF. This population's preference for NIPT may stem from increased anxiety, higher false positive rates with traditional serum screening and avoidance of diagnostic procedures carrying miscarriage risk. Therefore, understanding the differences in FF in this population is critical.<sup>2-5</sup>

Previous studies have reported lower FF in patients undergoing IVF and in donor oocyte populations.<sup>6,7</sup> Lower FF has also been associated with increased MW, early GA, certain maternal health conditions, and abnormal fetal results (T18/T13/triploidy).<sup>6,8,9</sup> Our results reveal statistically significant lower FF in donor oocyte pregnancies compared to matched reference data.

It is unknown why FF is lower in donor oocyte/IVF pregnancies. Hormone treatment and higher rates of abnormal cord insertion among IVF pregnancies and a high degree of antigenic dissimilarity among donor oocyte pregnancies suggest that the IVF process may impair implantation.<sup>2,5-7,10,11</sup> However, lower fetal fractions are also associated with increased risk for chromosome abnormalities. Thus, choosing a NIPT lab with high FF accuracy will reduce the risk of false negatives for this vulnerable population.

Identifying factors that affect FF can optimize NIPT algorithms for various populations and be useful during genetic counseling. Women choosing NIPT after donor oocyte/IVF cycles should be informed of risks for lower FF and higher test failure rates which are associated with increased risks of adverse perinatal outcomes and obstetric complications.<sup>2,6</sup>

Future analysis should determine the effect on FF of donor oocyte versus non-donor IVF cases, of various IVF techniques (ICSI, fresh vs. frozen embryo) used to achieve pregnancy and of different etiologies of infertility.

**References:** 1. Zimmerman et al., Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. *Prenatal Diagnosis* 2012; 32: 1-9.A

2. Amor D.J. et al., Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for Down syndrome. *Human Reproduction*. 2009; 24(6):1330-1338.

3. Kilmelman D. et al., Do patients who achieve pregnancy using IF-PGS do the recommended genetic diagnostic testing in pregnancy? *J Assist Reprod Genet*. 2018; 35:1881-1885.

4. Darwich J et al., Anxiety and Psychological Stress Before Prenatal Screening in First-Time Mothers Who Conceived Through IVF/ICSI or Spontaneously. *Women & Health*. 2014; 54:5: 474-485

5. Takyi et al., Prenatal screening for chromosomal abnormalities in IVF patients that opted for preimplantation genetic screening/diagnosis (PGS/D): a need for revised algorithms in the era of personalized medicine. *J Assist Reprod Genet*. 2017;34(6):723-724.

6. Lee TJ et al., Cell-free DNA testing in singleton IVF conceptions. *Human Reprod*. 2018; 33(4):572-578.

7. van der Hoorn ML et al., Clinical and immunologic aspects of egg donation pregnancies: a systematic review. *Hum Reprod Update*. 2010;16(6):704-12

8. Zhou Y et al., Effects of Maternal and Fetal Characteristics on Cell-Free Fetal DNA Fraction in Maternal Plasma. *Reproductive Sciences*. 2015; 22(11): 1429-1435.

9. Revello R. et al., Screening for trisomies by cell-free DNA testing of maternal blood: consequences of a failed result. *Ultrasound Obstet Gynecol*. 2016 Jun;47(6):698-704.

10. Yanaihara A. et al., Difference in the size of the placenta and umbilical cord between women with natural pregnancy and those with IVF pregnancy. *J Assist Reprod Genet*. 2018 Mar;35(3):431-434.A

11. Ebbing C. et al., Prevalence, risk factors and outcomes of velamentous and marginal cord insertions: a population-based study of 634,741 pregnancies. *PLoS One*. 2013 Jul 30;8(7):e70380.

**SUPPORT:** Natera, Inc.

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**FRESH OOCYTE CYCLES YIELD IMPROVED EMBRYO QUALITY AND SURPLUS EMBRYO CRYOPRESERVATION RATES COMPARED TO FROZEN OOCYTE CYCLES IN AN EGG-SHARING DONATION**



**PROGRAMME.** Amanda Souza Setti, MSc,<sup>a</sup> Daniela Paes de Almeida Ferreira Braga, PhD,<sup>a</sup> Matheus de Castro Azevedo, BSc,<sup>b</sup> Assumpto Iaconelli, Jr., MD,<sup>a</sup> Edson Borges, Jr., PhD<sup>a</sup> <sup>a</sup>Fertility Medical Group / Sapientiae Institute, Sao Paulo, Brazil; <sup>b</sup>Fertility Medical Group, Sao Paulo, Brazil.

**OBJECTIVE:** An egg-sharing programme provides a good opportunity for recipients and donors to achieve motherhood. At present, there are no evidences to ensure that the cryopreservation of shared eggs is not detrimental to recipients' treatment outcomes. The objective of this study was to investigate the influence of cryopreservation on donated eggs in terms of laboratory and clinical outcomes of intracytoplasmic sperm injection (ICSI) cycles.

**DESIGN:** Historical cohort study.

**MATERIALS AND METHODS:** Data analyzed in this study were obtained via chart review of 267 oocyte donor ICSI cycles (age range 19-34 years), and 320 oocyte recipients (age range 26-48) undergoing 307 vitrified and 119 fresh oocyte recipient ICSI cycles, participating in an egg-sharing donation program, from 2015 to 2018, in a private university-affiliated IVF center. The sample size calculation suggested that 199 cycles would be enough to demonstrate a 20% effect with 80% power and 5% significance level considering as primary outcome blastocyst development rate. The impact of oocyte cryopreservation on recipients' ICSI outcomes was investigated using General Mixed Models fit by restricted maximum likelihood, followed by Bonferroni post hoc test for the comparison of means between fresh and warm oocyte donation groups. The model was generated using covariates as fixed effects and egg-donors and egg-recipients as random effects, with unstructured covariance structure, adjusted for oocyte dysmorphisms and other potential confounders.

**RESULTS:** The fertilization rate (80.7% vs. 75.8%,  $p = 0.034$ ), high quality embryos rate on days 2 (70.3% vs. 57.8%,  $p = 0.047$ ) and 3 (50.2% vs. 34.6%,  $p = 0.003$ ), normal cleavage speed rate on days 2 (90.6% vs. 77.2%,  $p = 0.027$ ) and 3 (83.6% vs. 61.6%,  $p = 0.001$ ), and blastocyst development rate (47.1% vs. 19.8%,  $p < 0.001$ ) were significantly higher on fresh oocyte donation cycles compared to warmed oocyte donation cycles. There were no statistically significant differences between fresh and warmed oocyte donation cycles in terms of high-quality blastocyst rate (71.2% vs. 62.0%,  $p = 0.328$ ), implantation rate (35.7% vs. 25.7%,  $p = 0.182$ ), clinical pregnancy rate (54.1% vs. 42.9%,  $p = 0.313$ ), and miscarriage rate (12% vs. 15.9%,  $p = 0.745$ ). The surplus embryos cryopreservation rate was significantly higher on fresh cycles compared to warmed cycles (65.4% vs. 24.1%,  $p = 0.015$ ).

**CONCLUSIONS:** In an egg-sharing donation program, fertilization and embryo developmental competence are reduced when vitrified oocytes are used for ICSI compared to fresh oocytes. Despite no statistical significant differences were observed in terms of pregnancy outcomes, cycles using fresh oocytes had higher rates of surplus embryo cryopreservation, which is interesting for those patients with a negative pregnancy outcome, allowing them to resort to warmed embryo transfer instead of a new cycle of oocyte donation. Efforts must be made so donor-recipient matching makes it possible to receive fresh eggs.

**References:** NA.

**SUPPORT:** None.

**P-33 Tuesday, October 15, 2019 6:30 AM**

**DONOR DIALOGUE: A CROSS-SECTIONAL ASSESSMENT OF LONG-TERM MEDICAL AND PSYCHOLOGICAL HEALTH STATUS AFTER ELECTIVE OOCYTE DONATION.**



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**OBJECTIVE:** There is an inverse relationship between the use of elective oocyte donation and the understanding of long-term potential impact. We sought to assess the long-term medical and psychological health status of all elective oocyte donors (anonymous, directed, agency) at a single institution.

**DESIGN:** Anonymous quantitative and qualitative survey.

**MATERIALS AND METHODS:** An anonymous survey was emailed to all donors with a working email who donated between 2008 – 2019 (n=161).

**RESULTS:** 36 donors completed the survey (response rate 22.4%). The majority identified as Caucasian (77.1%). Most identified as not religious (33.3%), atheist (19.4%) or spiritual (16.7%). 44.4% reported they are currently single and 33.3% as currently married. 41.6% had at least a Bachelors degree, 38.9% a Masters and 16.7% a Doctorate. 48.6% reported half altruistic/half financial motivations, 14.3% reported pure altruism and another 14.3% purely financial. Most (54.3%) were between 25-30 years old at time of first donation. 40.0% donated between 2-5 years ago and another 34.3% 5-10 years ago. 40.0% of respondents donated once, 17.1% twice, 17.1% three times, and 25.7% 4 or more times. Most reported no

post-op complications (34.3%) or minor symptoms only (51.4%). 30.6% reported at least 1 pregnancy but 57.1% hadn't tried or are not interested. Of donors reporting pregnancies, none required Assisted Reproductive Technology for conception but 13.3% reported >2 losses. Of donors with living biological children, 75% reported their children had no medical problems. 1 directed donor reported her niece has Schaff-Yang syndrome. 80% reported no update in their medical history; 2 reported new allergies, 1 epilepsy, 1 anemia, 1 collagenous colitis, 1 fibrocystic breasts, and 1 reported a keratoacanthoma removal. 1 respondent each reported a diagnosis of ovulatory dysfunction, blocked fallopian tubes, unexplained fertility and fibroids respectively. Over half of donors reported being treated for or having ever experienced symptoms of depression or anxiety. Birth control was the most reported new medication. 63.0% reported no update in their family history. 2 reported new cancers in grandmothers (breast, cervical) and 3 reported a family death (depression, colon cancer, old age). 31.3% reported they knew children were born from their oocytes and all wrote positive comments about the knowledge of livebirth. 80.6% reported that they would make the same choice to donate and 58.1% reported they would recommend donation. 62.5% would still have donated under open donation or ID disclosure models. 81.3% were interested in maintaining contact for future updates.

**CONCLUSIONS:** Most donors did not have major medical history updates. The majority felt positively about donation but also reported a high rate of depression/anxiety, which could be related. Donors felt positively about open disclosure and maintaining contact with recipients. Continued long-term follow up will help provide better counseling about the medical risks/benefits of donation. Moving toward an open disclosure may expand the psychological benefits for both donor and recipient.

**References:** None

**SUPPORT:** None

**P-34 Tuesday, October 15, 2019 6:30 AM**

**IS PATERNAL AGE PLAYING A MAJOR ROLE IN OOCYTE DONATION PROGRAM?**



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**OBJECTIVE:** To determine the impact of paternal age on clinical pregnancy in oocyte donation program.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** The present study evaluated our oocyte donation program from January 2015 to December 2018. In total, 152 donors donated their oocytes to perform 475 IVF cycles. The paternal age was classified in group 1: younger than 40 years (N=229); group 2: 40 to 50 years (N=183) and group 3: older than 50 years (N=63). The main result was clinical pregnancy. A linear Poisson log regression models was used.

	Clinical Pregnancy n = 290 (%)	No pregnancy n = 185 (%)	p-value
Paternal Age			
< 40	150 (65.5)	79 (34.5)	0.082
40 - 50	108 (50.0)	75 (41.0)	
> 50	32 (50.8)	31 (49.2)	
Mean of oocytes assigned per cycle	11.0 ± 2.6	10.9 ± 3.1	0.616**
Fertilization rate	0.7 ± 0.2	0.7 ± 0.2	0.246**
Blastulation rate	0.6 [0.4 - 0.8]	0.6 [0.3 - 0.7]	<b>0.005***</b>
Embryo quality (SART criteria)			
Good	0.5 [0.3 - 0.6]	0.4 [0.1 - 0.6]	<b>0.056***</b>
Fair	0.4 [0.3 - 0.6]	0.4 [0.2 - 0.6]	0.529
Poor	0 [0 - 0.3]	0 [0 - 0.3]	0.220
Donor oocyte recipient age	41.6 ± 4.6	42.2 ± 5.2	0.204
Insemination technique			
FIV	71 (63.4)	41 (36.6)	0.561
ICSI	219 (60.3)	144 (39.7)	

**RESULTS:** Young women who donated oocyte to our program give oocytes to 3 partners as an average. Pregnancy was not associated to paternal age (see table 1). Additionally, number of oocytes, fertilization rate, donor oocyte recipient age and insemination technique (ICSI or FIV) were not related to clinical pregnancy. Interestingly, blastulation rate and the embryo quality were the only parameters associated to pregnancy.

**CONCLUSIONS:** The strength of the present study was that the same donors give oocytes to different partners. Our results suggest that paternal age does not influence the clinical pregnancy. Although, prospective studies are needed by using sibling oocytes.

**SUPPORT:** None

### EMBRYO CULTURE

**P-35** Tuesday, October 15, 2019 6:30 AM

#### PROSPECTIVE RANDOMIZED MULTICENTER STUDY ON CULTURE OF SIBLING HUMAN OOCYTES IN A SEQUENTIAL MEDIA SYSTEM WITH AND WITHOUT ANTIOXIDANTS : THE EFFECT OF FEMALE AGE.



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**OBJECTIVE:** To investigate the combined effect of three antioxidants Acetyl-L-Carnitine (ALC), N-Acetyl-L-Cysteine (NAC) and  $\alpha$ -Lipoic Acid (ALA) in a sequential culture media system on human embryo development and clinical outcome in relation to maternal age

**DESIGN:** Prospective randomized sibling oocyte multicenter study

**MATERIALS AND METHODS:** This study included couples intending to undergo IVF or ICSI, with female age  $\leq 40$  years old and at least eight cumulus-oocyte-complexes after retrieval. Cycles involving PGT, split IVF/ICSI and surgically retrieved sperm were excluded.

Human oocytes were randomly distributed to Vitrolife G-Series with or without a combination of three antioxidants (10  $\mu$ M ALC /10  $\mu$ M NAC /5  $\mu$ M ALA (A3)). IVF/ICSI and embryo culture were conducted in 5% oxygen. Embryo quality on day 3 and day 5/6 and clinical outcome were assessed in relation to maternal age (< 35 versus >35). Good embryo quality on day 3 was defined as 8 to 10-cells with even cells and low fragmentation; good quality blastocysts as equal or greater than 3BB. Clinical outcome was assessed in either fresh or vitrified-warmed embryo transfer cycles. The study was registered with [clinicaltrials.gov](http://clinicaltrials.gov) (NCT02999958).

**RESULTS:** A total of 133 patients participated in the study. The mean female age was 33.8  $\pm$  3.1 years. 1783 oocytes were collected of which 890 were allocated to G-Series media with A3 and 893 to standard G-Series media. When analyzing for age groups in G-Series with A3 compared to standard G-Series, the following results were obtained:

- Good quality Day 3 embryo development was significantly higher in the younger age group in G-Series with A3 (<35: 50.2 % vs 38.2 %, P < 0.05;  $\geq$  35: 48.6 % vs 41.1 %, n.s.)
- The overall blastocyst rate on Day 5 + 6 was higher in both age groups in G-Series with A3 (<35: 61.3 % vs 56.6 %;  $\geq$  35: 66.2 % vs 60.7 %) but not significant.
- The GQB rate on Day 5 + 6 was higher in both age groups in G-Series with A3 (<35: 32.8 % vs 28.2 %;  $\geq$  35: 27.8 % vs 25.9 %).

- More blastocyst were used for cryopreservation and transfer on Day 5 +6 in G-Series with A3 in both age groups (<35: 41.2 % vs 37.2 %;  $\geq$  35: 43.5 % vs 38.8 %).

We noted no difference between G-Series with A3 vs G-Series in the younger age group for implantation per fetal sac, per fetal heart and for the ongoing pregnancy rate (< 35: 50.6 % vs 55.3 %, 48.2 % vs 52.6 % and 48.1 % vs 52.6 %, respectively). A significant difference (P < 0.05) was found for the same parameters in the older age group for G-Series with A3 ( $\geq$  35: 57.5 % vs 23.5 %, 50.0 % vs 26.5 % and 50.0 % vs 25.8 %, respectively).

**CONCLUSIONS:** In general the presence of antioxidants during IVF and embryo culture imparts significant benefits on day 3 embryo quality and a trend to better day 5 embryo quality and utilization rate. Implantation rates and ongoing pregnancy rates are significantly higher in media with A3 in patients with advanced maternal age but not in younger patients, but cumulative pregnancies could increase as more embryos were cryopreserved. Supplementation of antioxidants to culture media may improve the viability of human embryos in ART, plausibly through the reduction of oxidative stress, and improve clinical outcomes in certain age groups.

**SUPPORT:** Vitrolife sponsored part of the media for the study.

**P-36** Tuesday, October 15, 2019 6:30 AM

#### MODELING OF AIRBORNE EMBRYOTOXIC VOLATILE ORGANIC COMPOUNDS (VOCs) IN THE IVF CULTURE ENVIRONMENT – THEIR CONCOMITANT CYTOTOXIC CONCENTRATION WITHIN THE GROWTH MEDIA AND EMBRYO.



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**OBJECTIVE:** VOCs are a common component of laboratory ambient air. VOCs are unique in their polarity, molecular weight and structure and play a critical role in preimplantation toxicology and epigenetic processes. This study sought to define the mechanisms of cytotoxicity associated with VOCs found in the IVF culture environment. The concomitant concentrations of VOCs common to IVF laboratories were modeled with Henry's Law (HL) from the gaseous to aqueous phase, and the final resulting concentration within the embryo was modeled with octanol water partitioning coefficients (OWPC).

**DESIGN:** HL was used to model VOC mass transfer from the air to the water/media phase. This model uses the air-water partitioning coefficient and the definition that the ratio between the liquid and air phase concentration is defined and unique for each organic compound. The OWPC was used for each compound to correlate the mass transfer from the water/media phase to the embryo using the ratio between the organic phase and water phase concentration.

**MATERIALS AND METHODS:** Evaluation of over 40 IVF laboratories identified the mean total VOC (TVOC) levels and 6 most common VOCs. HL and OWPC calculations determined the concomitant VOC concentrations in the culture media, embryo in culture, and time required to reach equilibrium for each compound. Research has shown that TVOC concentrations greater than or equal to 500 ppb in the media is embryotoxic and exerts a statistically significant impact on blastocyst conversion rates. Air phase VOC concentrations were compared to known embryotoxic VOC levels in cell culture media to determine if typical VOC levels in IVF laboratories are embryotoxic.

**RESULTS:** The concentration of each VOC within the embryo ( $C_{\text{embryo}}$ ) was modeled based on airborne VOC levels measured. This modeled  $C_{\text{embryo}}$

Compound	Air (ppb) Common to the IVF Laboratory	Time for Airborne VOC to Reach Equilibrium in Media (min)	Embryotoxic $C_{\text{embryo}}$ (mg VOC/kg of embryos)	Modeled $C_{\text{embryo}}$ (mg VOC/kg of embryos) Based on Tested IVF Laboratories Air
Acetone	425	24.1	285	421.8 (toxic)
Acrolein	85	0.2	489	47.1
Formaldehyde	225	2,162.0	1,132	48,992 (toxic)
Isopropanol	510	90.7	15	119.2 (toxic)
Styrene	170	4.1	445,715	5,989.9
Toluene	225	4.2	244,963	1,521.4

was compared to the embryotoxic level when embryos were cultured in an aqueous environment of 500 ppb VOCs. Levels of acetone, formaldehyde and isopropanol measured in IVF laboratories resulted in cytotoxic cellular levels.

**CONCLUSIONS:** Airborne VOCs are driven to reach equilibrium and can be magnified in concentration as they partition from the air to the cell culture media, and ultimately, into the embryo. Once cellular, the VOCs exert a negative influence on blastocyst conversion, implantation, and clinical pregnancy rates. This study related the measured concentration of airborne VOCs to the modeled concentration within the embryo. This novel study further defines the mechanisms of cytotoxicity of VOCs by defining their partition from the gaseous to aqueous phase, and most importantly, to the cellular phase. This data furthers our understanding of the role of VOCs in epigenetic variation and cytotoxicity.

**P-37** Tuesday, October 15, 2019 6:30 AM

**RANDOMIZED STUDY OF EMBRYO COMPETENCE AND DEVELOPMENTAL POTENTIAL IN GLOBAL BLASTOCYST MEDIUM AND G-TL MEDIUM, DESIGNED FOR TIME LAPSE.**

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**OBJECTIVE:** Continuous uninterrupted culture of embryos to the blastocyst stage requires that the medium be able to support growth to day 6 without refreshment. Global Blastocyst (GB) medium initially formulated for conventional culture with medium exchange on day 3 has been used successfully in time-lapse (TL) chambers. Our study objective was to compare zygote performance in G-TL medium, designed for continuous culture to Global Blastocyst medium.

**DESIGN:** Randomization of sibling zygotes between culture media and retrospective analysis of embryo morphokinetic and outcome data

**MATERIALS AND METHODS:** A total of 7331 zygotes from consecutive non-PGS patients undergoing IVF from 2016 thru December 2018 were cultured in the Embryoscope TL chamber. G-TL (with human serum albumen-HSA) and GB medium with 10% added HSA protein supplement with globulins (SPS) were placed in the Embryoscope slide (6 wells per medium). Sibling zygotes were randomly distributed amongst wells and cultured at 37 °C with 6% CO<sub>2</sub> /6% O<sub>2</sub>. Time lapse videos were annotated daily for cell divisions and dysmorphisms. The following kinetic markers were assessed: tSyn (syngamy), t2 (time to 2c), t3, t4, t5, t8, tM (fully compacted morula), tSB (start of blastulation), tBL (time of blastulation), tEBL (time of expanded blastocyst). Blastocyst grade (BG) based on maturity (4=hatched, 3=expanded, 2=full blastocyst, 1=early) and ICM /TE quality (3=good, 2=fair and 1=poor were scored using ESHRE criteria. Overall embryo utilization (transferred or frozen) and percent good quality embryos (GQE) for cryopreservation was calculated for each medium. Embryonic competence based on implantation (sac, fetal heart-FHT) in fresh and frozen single embryo transfer (SET) cycles was assessed. Differences between treatment groups were analyzed using ANOVA and the chi-square test. P values of <0.05 were considered statistically significant.

**RESULTS:** With G-TL ,80% of blastocysts were BG3/4 as compared to 77% of GB blastocysts (p=0.03) but ICM/TE scores did not differ. Multinucleation was higher in GB vs G-TL (44 % vs 40%,respectively; p=0.009).

**CONCLUSIONS:** This large study with randomization of sibling zygotes allowed a more robust comparison between culture media. Both media performed well. No difference was detected in clinical outcomes in either fresh or frozen SET cycles.

	GB	GT-L	p Value
Cultured zygotes (n)	3859	3472	
Blastocysts (%)	2592 (67%)	2218 (64%)	0.002
Expanded blastocysts (%)	1990 (52%)	1722 (50%)	NS
Embryo utilization (%)	2273 (59%)	1889 (54%)	0.001
GQE- Frozen(%)	1851 (48%)	1599 (46%)	NS
Transfers			
Fresh SET IR-Sac (%)	82/142 (58%)	51/82 (62%)	NS
Fresh SET IR-FTHT (%)	80/142 (56%)	49/82 (60%)	NS
Frozen SET IR-Sac (%)	81/124 (65%)	76/126 (60%)	NS
Frozen SET IR-FTHT (%)	77/124 (62%)	69/126 (54%)	NS

**References:** Desai N, Ploskonka S, Goodman L,Austin C,Goldberg J and Falcone T. Analysis of embryo morphokinetics, multinucleation and cleavage anomalies using continuous time-lapse monitoring in blastocyst transfer cycles. *Reprod Biol Endocrinology* 12:54 (20 June 2014).Å

Desai N, Goldberg J, Austin C, and Falcone T. Are cleavage anomalies, multinucleation, or specific cell cycle kinetics observed with time-lapse imaging predictive of embryo developmental capacity or ploidy? *Fertil Steril* 2018 109 (4)Å 665–674

**SUPPORT:** None

**P-38** Tuesday, October 15, 2019 6:30 AM

**3D GYRATORY ROCKER MAY ACCELERATE EMBRYONIC ZOI EXPRESSION AND FURTHER EMBRYONIC DEVELOPMENT IN MOUSE EMBRYOS.**



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**OBJECTIVE:** Before implantation, zygote travels the oviduct and uterus along fluid flow by beating of the cilia and muscle contraction. This condition makes fluid flow, shear stress, rolling effect of embryo and external stimuli to support embryo movement and development. Oviductal condition lead to movement of embryos with punctuate fluid velocity. To mimic these physical/mechanical environment, new static culture system is developed includes a well of the well system, GPS culture dish, sub-micro drop culture. And dynamic micro-vibration culture system.

**DESIGN:** MII eggs from inbred and outbred mouse were fertilized and cultured in vitro with or without the 3D gyrotory rocker in the traditional incubator.

**MATERIALS AND METHODS:** At 14hr post hCG injection, MII-cumulus mass were collected from both oviducts. Pooling the MII eggs from several mouse with 0.1% hyaluronidase and fertilized with epididymis sperm from fertile male mouse. 8hr post IVF, zygote with 2PN were collected and cultured in KSOM on the 3D gyrotory rocker or regular shelves. 3D gyrotory rocker is controlled 70 rpm in speed, and 12 degree in angle of tilt continually. We investigated three different medium size to make shear stress. After 3 day culture, morula from both culture condition were subjected real time-PCR with embryonic ZO-1 alpha form. After 5 day culture, blastocyst and hatching rate were observed and counted ICM/TE cells. ICM/TE counts were performed by immunostaining with anti-Oct 4 and DAPI staining. P value were calculated by t-test with Graphpad Prism.

**RESULTS:** In both inbred or out bred mouse, blastocyst formation and hatching rate are higher percentage in 3D gyrotory rocker than regular platform culture significantly in same incubator (P < 0.05). Hatching process was faster in 3D gyrotory than the regular platform. There is no differences between the RPM or angle fit of 3D gyrotory rocker. Also there is no differences between medium drop sizes from 0.01 to 0.6ml on 3D gyrotory rocker. ICM/TE count in day 5 blastocysts were not different. From real time-PCR, ZO-1 alpha form highly expressed in 3D gyrotory than the regular platform. According to these results, mouse embryo culture on 3D gyrotory rocker accomplished higher blastocyst and further hatched embryos.

**CONCLUSIONS:** The shear stress by 3D gyrotory rocker may induced mechanical stimuli and auto/paracrine effectors and improve the embryo development. This research was supported by NRF-017R1D1A1B03028155.

**SUPPORT:** This research was supported by NRF-017R1D1A1B03028155.

**P-39** Tuesday, October 15, 2019 6:30 AM

**GROUP EMBRYO CULTURE STRATEGIES AFFECT THE OXIDATIVE STATUS OF THE SPENT CULTURE MEDIA AND EMBRYO DEVELOPMENT**



**RESULTS.** Lorena Bori, PhD,<sup>a</sup> Raquel Del Gallego, PhD,<sup>a</sup> Lucia Alegre, PhD,<sup>a</sup> Silvia Azaña, MSc,<sup>a</sup> Tamara Viloria, PhD,<sup>a</sup> Marcos Meseguer, PhD<sup>b</sup> <sup>a</sup>IVIRMA Global, Valencia, Spain; <sup>b</sup>IVIRMA Global, Valencia, Spain, Tel Aviv, Israel.

**OBJECTIVE:** To describe the impact of the group embryo culture over the oxidative profile of the medium and over the fertilization and the blastocyst rates in two types of culture dishes from two time-lapse incubators.

**DESIGN:** A retrospective analysis, including 299 IVF cycles from May 2017 to December 2018, was conducted. Culture media from 413 groups

of embryos (15  $\mu$ l/group) monitored with EmbryoScope Plus® (ES+) and Geri Plus® were analysed by the Thermochemiluminescence (TCL) Analyzer™ (Carmel Diagnostics, Israel).

**MATERIALS AND METHODS:** A total of 299 spent embryo culture media from ES+ and 114 from Geri were analyzed. Sequential medium was used in 227 embryo groups and single-step medium in 186. The TCL Analyzer™ consists on the heat-induced oxidation of biological fluids, leading to the production of light energy counted as photons emitted per second (cps). The oxidative parameters were obtained after 55 sec. (H1), 155 sec. (H2) and 255 sec. (H3). A smoothing algorithm (sm) was used to normalize data. Data were analyzed with ANOVA and Chi-squared tests (SPSS software).

**RESULTS:** Higher fertilization rates were found as the number of oocytes increased in the same group. However, blastocyst rate and the number of good quality blastocysts decreased when the number of embryos per group increased:  $73.6 \pm 30.3\%$  for  $\leq 6$  embryos,  $69.0 \pm 23.7\%$  for 7-8 embryos,  $67.4 \pm 23.1\%$  for 9-12 embryos and  $64.9 \pm 23.2\%$  for  $\geq 13$  embryos. The comparison between two time-lapse incubators with this kind of embryo culture showed significantly ( $p < .05$ ) higher fertilization rates for Geri ( $78.9 \pm 17.3\%$  for Geri vs.  $73.7 \pm 20.6\%$  for ES+) and higher blastocyst rates for ES+ ( $70.6 \pm 26.7\%$  for ES+ vs.  $65.7 \pm 22.6\%$  for Geri). According to our data, sequential culture medium worked significantly better ( $p < .05$ ) than single-step medium in terms of blastocyst rate ( $72.13 \pm 24.5\%$  for sequential medium vs.  $65.57 \pm 26.6\%$  for single-step medium). In addition, oxidative stress level of the medium the fifth day post ICSI was significantly higher as more oocytes were successfully fertilized (Table).

Table: Mean and standard deviation of the TCL parameters according to the number of successfully fertilized oocytes.

Oocytes fertilized	N (Groups)	H1sm (cps)	H2sm (cps)	H3sm (cps)
$\leq 4$	117	$88.7 \pm 32.8$	$91.5 \pm 34.9$	$98.3 \pm 39.4$
5-6	108	$83.6 \pm 35.9$	$85.8 \pm 37.5$	$92.9 \pm 41.5$
7-8	93	$103.3 \pm 49.2$	$108.0 \pm 54.5$	$117.14 \pm 63.8$
$\geq 9$	95	$105.1 \pm 32.3$	$109.9 \pm 32.6$	$121.3 \pm 37.1$
P Values		$<0.001$	$<0.001$	$<0.001$

**CONCLUSIONS:** Group embryo culture strategies affect embryo development results: as the number of oocytes cultured per group increased fertilization rates were improved, but not blastocyst rates, which were higher when medium was replaced. Moreover, media's oxidative stress level was higher when more fertilized oocytes were cultured per group.

**P-40** Tuesday, October 15, 2019 6:30 AM

**THE EFFICACY OF THE NEW EMBRYO CULTURE MEDIUM WHICH DESIGNED FROM THE COMPONENTS OF HUMAN TUBAL FLUID; PROSPECTIVE RANDOMIZED TRIAL.**

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**OBJECTIVE:** Sequential and single step media are the most widely used for embryo cultures in IVF. There is no medium which designed from human oviductal fluid. Almost all media that available to use at present are derived and arranged from previous somatic cell culture media. In 2017, the medium (HiGROW OVIT; Fuso Pharma, Japan) composed of amino acid concentrations by the data of human oviductal fluid became available to use in Japan, which contained different concentrations of amino acids from previous media. In this study, we examine the clinical availability of the new media in terms of utilization of embryos and giving birth to healthy baby in IVF.

**DESIGN:** prospective randomized trial.

**MATERIALS AND METHODS:** Human oviductal fluid was aspirated laparoscopically from 28 women aged 26–39 years with no major intrapelvic abnormality to formulate new embryo culture medium. Liquid chromatography with tandem quadruple mass spectrometry and ion chromatography were used to analyze 31 components of the oviductal fluid sample. We conducted an RCT to evaluate the medium using 3,418 embryos obtained from 674 cycles of patients who underwent IVF or intracytoplasmic sperm injection (ICSI) between September 2017 and March 2019. Before fertilization,

the oocytes were divided into two groups: cultures using the new medium composed of human oviductal amino acid (OVIT); and cultures using current medium (Medium A). The embryo grade during culture period to the blastocyst stage and clinical outcome after embryo transfer were compared between the OVIT group and the Medium A group.

**RESULTS:** Between the two groups, patient characteristics were not significantly different. The number of embryos in the OVIT group on day 3 which showed "8-cell 2-grade" by Veeck's criterion, was larger (18.6% (318/1,709)) than in the Medium A group (14.1% (241/1,709));  $P < 0.01$ . The OVIT group showed significantly higher rates of blastocyst development (62.4% (1,024/1,641)) than the Medium A group (58.5% (961/1,643));  $P < 0.05$ . The number of embryos which were elected for ET or cryopreserved was larger in the OVIT group (44.4% (759/1,709)) compared with the Medium A group (37.2% (636/1,709));  $P < 0.01$ . Furthermore, in the OVIT group had higher implantation rates (28.1% (124/442)) after ET than that in the Medium A group (25.5% (94/368)); the difference was not statistically different. Birth weight of the OVIT group was  $3,179.2 \pm 473.3$  grams ( $n=39$ ) and the Medium A group was  $3,087.1 \pm 409.1$  grams ( $n=28$ ). The ratio of male to female was 20/19 in the OVIT group ( $n=39$ ), and 10/16 in the Medium A group ( $n=26$ ). One trismus nascentium child was confirmed in the OVIT group and one congenital ear fistula child was confirmed in the Medium A group.

**CONCLUSIONS:** Medium composed of human oviductal amino acids enhances embryonic ability more than the current single step medium, and it may make a contribution to clinical success in IVF treatment.

References: none

**SUPPORT:** none

**P-41** Tuesday, October 15, 2019 6:30 AM

**A NOVEL ZONA-FREE CULTURE SYSTEM FOR SEVERE CYTOPLASMIC FRAGMENTATION CASES: A PILOT STUDY USING 3PN EMBRYOS AND TIME-LAPSE CINEMATOGRAPHY.**

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**OBJECTIVE:** A study in 2017 observed perivitelline threads in more than 50% of cleavage-stage human embryos using time-lapse imaging, and the rate of cytoplasmic fragmentation (at the first cleavage) was significantly decreased in embryos without perivitelline threads ( $P < 0.001$ ). While it is proposed that perivitelline threads play an important role in crosslinking the cumulus cells and oocyte during maturation, the mechanism underlying such a role remains unclear. It is also unknown whether the threads still function in mature MII oocytes. Therefore, in this study, zona pellucida of abnormally-fertilized oocytes which were donated by patients was removed at pronuclear stage. Those zona-free oocytes were observed in time-lapse culturing system in order to examine developmental morphology.

**DESIGN:** Prospective study.

**MATERIALS AND METHODS:** This study used 57 abnormally fertilized (3PN) embryos (cIVF:  $n = 51$ , ICSI:  $n = 6$ ) donated by assisted reproduction technology patients in our clinic with informed consent since 2017. After confirming the three pronuclei, we removed the ZP from each 3PN embryo using a laser, and the resultant zona-free embryos were cultured and observed in an incubator equipped with a time-lapse imaging system. For ZP removal, 3PN embryos were placed in drops of 0.125M sucrose-containing HEPES media that had been covered with mineral oil and warmed to 37°C. Despite a small reduction in ooplasm size, half of the ZP was removed by laser (Saturn 5; Origio, Lykos; Hamilton Thorne). Subsequently, the ooplasm was completely separated from their ZPs by pipetting, and these zona-free 3PN embryos were cultured continuously for 5 days with time-lapse imaging.

**RESULTS:** Of 58 zona-free embryos in total, 54 (94.7%) were cleaved, and there was no significant decrease in cleavage rate compared to 2PN embryos (98%) used routinely in our clinic. Furthermore, 28 of the 54 embryos (51.9%) developed to the morula stage after third cleavage, and 18 embryos (33.3%) formed a blastocoel and became blastocysts. Thus, removing the ZP before cleavage did not adversely affect the embryo development. In terms of the amount of fragmentation, based on the modified Veeck's criteria, 36 of 54 zona-free 3PN embryos (66.7%) showed less than 20% of the volume in fragments compared to the total volume of cytoplasm at the first cleavage (Grade 1 and 2), 14 (25.9%) showed 20-40% fragments (Grade 3), and only 4 (7.4%) showed  $> 40\%$  fragments (Grade 4). These results suggested that the rate of fragmentation was decreased by ZP removal before the first cleavage.

**CONCLUSIONS:** This study revealed that the ZP is not always necessary for normal development after the pronuclear stage because the zona-free

embryos studied herein developed normally, maintained their cell adhesion well, and showed a decreased rate of fragmentation. This innovative culture system might provide the major breakthrough needed for patients who have difficulty obtaining good-quality embryos.

**SUPPORT:** None

**P-42** Tuesday, October 15, 2019 6:30 AM

### TYPE OF CULTURE MEDIUM IS ASSOCIATED WITH PREIMPLANTATION EMBRYO DEVELOPMENT.



**DEVELOPMENT.** Linette van Duijn, MD,<sup>a</sup> Melek Rousian, MD, PhD,<sup>a</sup> Eva S. van Marion, MD,<sup>b</sup> Joop S. E. Laven, MD, PhD,<sup>b</sup> Régine P. M. Steegers-Theunissen, MD, PhD,<sup>a</sup> Esther B. Baart, PhD,<sup>c</sup> <sup>a</sup>Erasmus University Medical Centre, Rotterdam, Netherlands; <sup>b</sup>Erasmus University Medical Center, Rotterdam, Netherlands; <sup>c</sup>Erasmus MC University Medical Center, Rotterdam, Netherlands.

**OBJECTIVE:** Previous research has demonstrated several influences of the periconception maternal environment on health later in life. The culture medium used in IVF/ICSI treatment, however, can be considered as an artificial environment of the preimplantation embryo. Since the introduction of the EmbryoScope™ time-lapse incubator preimplantation embryo development can be closely observed. The aim of this study is to investigate the influence of two commercially available culture media on the developmental kinetics of the pre-implantation embryo, and IVF/ICSI treatment outcome.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Data were obtained between 2012-2017 of 545 women undergoing their first IVF/ICSI treatment at the Erasmus MC. In this period Vitrolife G1 (n=255) and Sage 1-Step (n=290) culture media were used subsequently. Embryos were cultured until day 3 of development in the EmbryoScope™ time-lapse incubator and morphokinetic parameters of all transferred and frozen embryos were retrospectively annotated and not used for embryo selection. Treatment and patient characteristics were retrieved from medical records. Crude and adjusted associations between culture media and morphokinetic parameters were investigated using linear mixed models. Differences in treatment outcome were assessed by logistic regression.

**RESULTS:** Embryos cultured in Sage 1-Step medium show faster development over all developmental stages (from fading of pronuclei to 8-cell stage) compared to Vitrolife G1. For example, embryos cultured in Sage 1-Step reach the 2-cell stage 2.08 (95%CI 1.57-2.60) and 8-cell stage 3.61 (95%CI 1.78-5.44) hours faster, respectively. After adjustment for female age, fertilisation method, type of ovarian stimulation, lowered oxygen culture and overall embryonic improvement over time, embryos cultured in Sage 1-Step reach the 2-cell stage 3.07 (95%CI 1.18-5.62) and 8-cell stage 9.89 (95%CI 2.80-16.99) hours faster. After adjustment for female age, fertilisation method and type of ovarian stimulation, embryos cultured in Vitrolife G1 demonstrated similar odds for positive β-hCG-test, fetal heartbeat and liveborn, when compared with embryos cultured in Sage 1-Step medium.

**CONCLUSIONS:** When compared to embryos cultured in Vitrolife G1, embryos cultured in Sage 1-Step culture medium are associated with faster development, however ongoing pregnancy rate is not significantly different. Our statistical approach enables analysis of the whole cohort of usable embryos per patient for an association between the type of culture medium and developmental kinetics. As embryo kinetics are likely to reflect embryo metabolism, the type of culture medium may impact embryo metabolism but not implantation potential. Further prospectively collected data is needed to unravel the relation between pre-implantation embryo kinetics and post-implantation development.

**P-43** Tuesday, October 15, 2019 6:30 AM

### BLASTULATION AND GOOD BLASTULATION BETWEEN DAYS 5 AND 6 OF EXTENDED EMBRYO CULTURE.



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**OBJECTIVE:** To identify the blastulation rate (BR) of pre-blastocysts between days 5 (D5) and 6 (D6) of embryo culture and its determinants.

**DESIGN:** Retrospective cohort

**MATERIALS AND METHODS:** We analyzed *in vitro* fertilization (IVF) cycles occurring between 2016 and 2018 at a single academic center. Due to embryo culture being performed in a grouped fashion, only fresh IVF cycles where the total embryo numbers in culture were equal on D5 (after embryo transfer [ET] or cryopreservation) and D6 were included. Blastulation was defined as the presence of a new blastocyst on D6 (number of D6 blastocysts minus number of D5 blastocysts, divided by the number of embryos). Good blastulation was defined as the presence of a good/excellent grade expanded or further developed blastocyst on D6 (number of good/excellent grade expanded or further developed blastocysts on D6 divided by the number of embryos). Frozen ET and donor cycles were excluded.

Patient and cycle characteristics were extracted from our IVF database and are listed in Table 1. Generalized estimating equations with logistic regression were used in iterative fashion to calculate adjusted odds ratios (OR) with 95% confidence intervals (CI) for selected parameters.

**RESULTS:** We analyzed 1037 cycles from 859 patients, characteristics of which are listed in Table 1. BR between D5 and D6 was 24.57% (1639/6670). Good BR was 23.18% (1546/6670). Determinants of blastulation were; number of pre-blastocysts on D5 (OR: 1.70, 95% CI: 1.51-1.92), number of good/excellent blastocysts on D5 (OR: 1.15, 95% CI: 1.08-1.23), and fresh ET on D5 (OR: 1.52, 95% CI: 1.07-2.16). Determinants of good blastulation included; number of embryos (OR: 1.17, 95% CI: 1.09-1.26) and existence of good/excellent blastocysts on D5 (OR: 17.25, 95% CI: 10.91-27.26) and age (OR: 0.95, 95% CI: 0.90-1.00).

**CONCLUSIONS:** The BR between D5 and D6 is important for patients considering fresh ET with a pre-blastocyst on D5. The good BR would guide about their odds of having an embryo for PGT and/or cryopreservation on D6. Number of pre-blastocysts, grading of their sibling blastocysts and fresh ET on D5 positively predict new blastocysts on D6. Older patients with few cultured embryos and no good/excellent blastocyst on D5 are unlikely to have an advanced stage good/excellent blastocyst on D6.

TABLE 1. Patient and cycle characteristics.

Variable	Mean ± SD or n (%)*
Cultured Embryo Cohort	9.80 ± 6.15
Age	33.54 ± 4.56
BMI	28.02 ± 6.82
White Race	914 (90)
Gravidity	1.03 ± 1.40
Parity	0.40 ± 0.68
AFC	24.32 ± 12.86
Male Factor Diagnosis	351 (34)
History of Smoking	180 (17)
Current Smoker	27 (3)
Sum of Metaphase 1 & 2 Oocytes	13.93 ± 8.21
ICSI use	600 (58)
Pre-Blastocysts on D5	3.78 ± 3.51
Good/Excellent Blastocysts on D5	2.59 ± 2.99
Fresh ET on D5	771 (75)

\*SD: standard deviation

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### EMBRYO CULTURE IN TIME-LAPSE SYSTEM PROVIDES BETTER RATES OF BLASTOCYST FORMATION, DECREASES EMBRYO DEVELOPMENT ARREST RATE COMPARED TO TRADITIONAL TRIPLE-GAS CULTURE SYSTEM.



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**OBJECTIVE:** Evaluate rates of blastocyst formation and embryo development arrest between uninterrupted (time-lapse) and triple-gas (90% N<sub>2</sub>, 5% CO<sub>2</sub>, 5% O<sub>2</sub>) systems.

**DESIGN:** This is a cohort study analyzing laboratory data between January 2018 and March 2019 at Huntington Medicina Reprodutiva Clinic in São Paulo, SP, Brazil.

**MATERIALS AND METHODS:** A total of 1.276 cycles of FIV were evaluated, 681 cycles formed the traditional triple-gas system group (90% N<sub>2</sub> / 5% CO<sub>2</sub> / 5% O<sub>2</sub>), mean age 37.56±3.59 years old and embryos were externally evaluated on days 1, 3, 5, 6 and 7, if applicable. In the time-lapse group (EmbryoScope®), 595 cycles, mean age 37.54±3.47 years old, were cultured uninterrupted. Blastocyst formation rate (no. blastocysts/no. 2PN), blastocyst mean number formed per cycle, and cycle cancellation rate due to embryo development arrest were compared.

**RESULTS:** A total of 9.482 mature oocytes followed in vitro fertilization, 4.936 in the triple-gas group and 4.546 in the time-lapse group, being fertilized 3.565 (72.23%) and 3.423 (75.30%) oocytes, respectively. From those, 1.791 blastocysts were formed in the traditional incubator group and 1.942 in the time-lapse group (50.2% versus 56.7%, p=0.001, chi-square test). Blastocyst mean number formed in the time-lapse group were generally higher than the control group (3.4±2.8 versus 2.7±2.8 p<0.0001, t-test). When maternal age was considered in analysis, ages between 35 and 42 years old showed gains in blastocysts mean number formed in time-lapse group: a) 34 years or less, no difference (4.1±3.1 versus 3.9±3.3, p = 0.34); b) 35 to 37 years, higher in time-lapse group (3.8±3.2 versus 3.0±2.7, p = 0.02); c) 38 to 40 years, higher in time-lapse group (3.2±2.6 versus 2.4±2.5; p = 0.0003); d) 41 to 42 years, higher in time-lapse group (2.6±2.2 versus 1.8±2.5; p = 0.001) and e) 43 years or older, no difference (1.7±1.4 versus 1.4±1.4, p> 0.05). Embryo developmental arrest rate was also lower in the time-lapse group (11% versus 15%, respectively; p=0.05, chi-square test).

**CONCLUSIONS:** The uninterrupted culture available at the time-lapse system produced better blastocyst formation rates, especially between 35 and 42 years old, lower embryo arrest rate and mainly with the ability to produce approximately 01 (one) extra blastocyst per cycle, which could increase the cumulative pregnancy rates. Considering younger (<34 yo) or older women (> 43 yo) no benefits were shown from time-lapse to traditional culture systems, possibly due to a better or worst oocyte quality. Our results indicate that uninterrupted systems are important to enhance blastocyst formation and may play a fundamental aspect for cumulative pregnancy rate producing an extra embryo.

## EMBRYO PHYSIOLOGY

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### INHIBITION OF LINE-1 TRANSPOSITION BLOCKS TELOMERE ELONGATION AND DOWNREGULATES TOTIPOTENCY GENES DURING MOUSE EMBRYO DEVELOPMENT.

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**OBJECTIVE:** During preimplantation embryo development, the telomere aging clock resets and 2-cell genes establish totipotency. Like most long-lived cells, oocytes have short telomeres, but telomeres elongate markedly during early embryo development, even in the absence of telomerase (1). Recent studies report that the retrotransposon, LINE-1, is activated after fertilization, when telomeres elongate. Retrotransposons protect chromosome ends in many species, so we hypothesized that inhibiting retrotransposition of LINE-1 during mouse early embryos would disrupt telomere reprogramming in mouse preimplantation embryos. We also evaluated the effect of LINE-1 on the 2-cell, totipotency genes.

**DESIGN:** Laboratory experiment

**MATERIALS AND METHODS:** Sixty thawed mouse zygotes (B6C3F1 X B6D2F1) (Embryotech Laboratories) were grouped (15 zygotes/group) and cultured in Global medium containing 0.4% BSA with or without 1µM of the reverse transcriptase inhibitor, AZT, for up to 15 (mid 2-cell stage) or 24 hours (late 2-cell stage). DNA and mRNA were isolated from embryos. Gene expression was measured by RT-qPCR, and LINE-1 copy number and telomere length were measured by qPCR. Data (Mean±SEM) was analyzed with GraphPad Prism 8 software by one-way ANOVA Tukey's multiple comparisons test.

**RESULTS:** As expected, AZT inhibited LINE-1 copy number in late 2-cell embryos compared to controls (1.000±0.113 vs 1.942±0.089, P<0.0001). Telomeres were longer in late 2-cell (1.563±0.107) than mid 2-cell embryos (1.128±0.059) (P=0.001), consistent with prior observations of progressive

telomere elongation during early development(1). AZT blocked telomere elongation between mid- and late-2 cell stages stage (1.152±0.039 vs 1.000±0.107, P=0.1994). Telomere length of late 2-cell embryos exposed to AZT was shorter than untreated controls (1.000±0.107 vs 1.563±0.107, P=0.0002). Transcripts of LINE-1, as well as the 2-cell genes, DUX and Zscan4, were highly expressed in mid- 2-cell compared to late 2-cell embryos (P<0.0001), regardless of inhibition of LINE-1. This suggests that 2-cell genes are activated at synthesis phase of the 2-cell stage. Moreover, AZT decreased expression of DUX, Zscan4d and LINE-1 in mid- 2-cell embryos (0.312±0.021, 0.727±0.055 and 1.266±0.066 respectively) compared to control embryos (1.913±0.197, 1.912±0.202 and 1.913±0.216 respectively, P<0.001).

**CONCLUSIONS:** The retrotransposon, LINE-1, regulates telomere lengthening during preimplantation embryo development- inhibition of LINE-1 synthesis by AZT blocks telomere elongation. AZT also inhibits the 2-cell genes, DUX and Zscan4d, suggesting that LINE-1 is essential not only for telomere reprogramming but also for the establishment of totipotency during early development.

**References:** 1. Liu L, Bailey SM, Okuka M, Muñoz P, Li C, Zhou L, Wu C, Czerwicz E, Sandler L, Seyfang A, Blasco MA, Keefe DL. Telomere lengthening early in development. Nat Cell Biol. 2007 Dec; 9(12):1436-41.

**P-46** Tuesday, October 15, 2019 6:30 AM

### HYPERGLYCOSYLATED HUMAN CHORIONIC GONADOTROPIN IN BLASTOCYST CULTURE MEDIUM OF MALE AND FEMALE HUMAN EMBRYOS.

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**OBJECTIVE:** The hyperglycosylated human chorionic gonadotropin (hCG-H) is considered a good marker of early trophoblast invasion. However, little is known about its production and secretion during the first five days of embryo development. The aim of this study is to compare the levels of hCG-H in culture media from male and female human embryos.

**DESIGN:** Observational study.

**MATERIALS AND METHODS:** Single-step culture media samples from 78 good quality embryos, derived from good prognosis patients undergoing intracytoplasmic sperm injection (ICSI), were collected on the fifth day of embryo cultivation. All embryos were tested by next-generation sequencing (NGS) technique and only the balanced ones were used for analysis. hCG-H levels in the culture media were evaluated by ELISA kit (Cusabio Biotech, CBS-E15803h) according to the manufacturer's instructions. The absorption was measured on a microplate reader (Beckman Coulter DTX 880 Multimode detector) at 450 nm. Statistical analysis was performed using SPSS v.21 (IBM Corp., Armonk, NY, USA). Descriptive parameters and patients' characteristics were reported as mean ± SD. P<0.05 was considered statistically significant.

**RESULTS:** The NGS analysis revealed that 37% of the embryos (n=29) were balanced, 48% (n=14) of them were female (XX) and 52% (n=15) were male (XY). The presence of hCG-H was confirmed in all embryo culture media samples. When comparing culture media samples from male versus female embryos hCG-H levels were not significantly different (0.09 ± 0.01 mIU/ml vs. 0.10 ± 0.03 mIU/ml; p=0.37, respectively). However, the variety in hCG-H concentration was significantly lower in the samples from male embryos compared to female ones (Levene's Test for Equality of Variances, p = 0.004).

**CONCLUSIONS:** Our results suggest that the chromosomal sex of human embryos could have an effect on the secretion of hCG-H. The female embryos produce more variable quantities of hCG-H compared to the male ones.

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### STUDY ON THE RELATIONSHIP BETWEEN PRONUCLEAR PROXIMITY IN ZYGOTE AND MULTINUCLEATION OF EARLY CLEAVAGE EMBRYOS IN HUMAN USING TIME-LAPSE

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**OBJECTIVE:** It has recently been reported that two separate bipolar spindles aligned their poles before anaphase keeping the parental genomes apart during the first cleavage in mammalian zygote including mouse and human. In mouse, the failure of spindle alignment by increasing the distance between the two pronuclei, which led to a larger gap between the spindles, gives rise to multinucleated two-cell embryos phenocopying frequently observed errors in IVF clinics. The purpose of our study is to examine the relationship between pronuclear (PN) proximity in zygote and multinucleation (MN) of early cleavage embryos in human using time-lapse system (TLS).

**DESIGN:** Data were retrospectively collected from TLS performed from May 2018 to February 2019 in the Fertility center of CHA Gangnam Medical Center. We analyzed 220 zygotes from 64 patients.

**MATERIALS AND METHODS:** We assessed the PN proximity, distance, MN, and development of early cleavage embryos using EmbryoScope™ (Vitrolife, Gothenburg, Sweden). Drawing tool was used to measure the distance from a PN center to another PN center in the same focal plane before PN fade. Zygotes were divided into two groups according to the proximity (group Gap [G; n=17] and group Juxtaposition [J; n=203]). The number of MN in 2-cell and 4-cell embryo was checked then the embryos were divided into three groups (No MN, MN, and N/A; not available due to abnormal division). Embryo development was checked up to day 3. The quantitative variables were expressed as mean ± SD and statistically analyzed with Student *t* test. *p* < 0.05 was considered to be statistically significant.

**RESULTS:** The incidence of gap between PN was considerably low (G; 7.7% vs. J; 92.3%). The average of PN distance was significantly different between two groups (G;  $31.2 \pm 5.1 \mu\text{m}$  vs. J;  $21 \pm 4.9 \mu\text{m}$ , \* *p* < 0.05). The rate of No MN (23.5% [2C] and 52.9% [4C]) was highly decreased but the rate of MN (41.2% [2C] and 5.9% [4C]) and N/A (35.3% [2C] and 41.2% [4C]) was increased in the Gap group compared with Juxtaposition group (49.8%/72.9% [2C/4C], 38.4%/5.4% [2C/4C], and 11.8%/21.7% [2C/4C]). Also the rate of 3D good quality embryo is slightly decreased in Gap group (G; 82.4% vs. J; 89.7%).

**CONCLUSIONS:** This study suggests that the PN proximity is also one of the applicable explanations for the MN in early cleavage embryos of human due to the failure of zygotic spindle alignment as other mammalian embryos such as mouse. As the presence of MN in human embryos, especially during the first and second mitotic divisions, is generally considered to be abnormal, our study on the relationship between PN proximity and MN combined with TLS could be used as a noninvasive technique to enhance selection of competent embryos likely to have the greatest potential of development. This may be of particular benefit to patients desiring elective single embryo transfer without PGS screening.

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#### **OXIDATIVE STRESS IN HUMAN TESTICULAR TISSUE BEFORE AND AFTER CRYOPRESERVATION: A COMPARATIVE STUDY.**

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**OBJECTIVE:** To compare oxidative stress in human testicular tissue in both cases of obstructive(OA) and non- obstructive or functional azoospermia (NOA) before and after cryopreservation.

**DESIGN:** Comparative study.

**MATERIALS AND METHODS:** Azoospermic patients with OA and NOA were subject to surgical sperm retrieval with needle aspiration using a 14 G cannula. Cryopreservation was done in cryovials immersed in liquid nitrogen (-196°C). Assay of Catalase activity (CAT) and Malondialdehyde level (MDA) using colorimetric methods in fresh testicular samples and after cryopreservation of samples with positive sperm retrieval. In addition, assessing the number of retrieved sperms and Johnson spermatogenic score were done in fresh testicular samples.

**RESULTS:** The study included 21 OA (group A), 16 positive NOA (group B with positive sperm retrieval) and 21 negative NOA (group C with negative sperm retrieval). Mean CAT activity in positive and negative NOA groups ( $151.90 \pm 122.32$  U/gm protein) ( $146.00 \pm 121.7$  U/gm protein respectively), were significantly higher than OA group ( $65.67 \pm 72.99$  U/gm protein) (*P*=0.017, *P*=0.018 respectively). MDA level was also significantly higher in positive and negative NOA ( $31.50 \pm 15.81$  nmol/gm) ( $40.38 \pm 14.42$  nmol/gm) groups than OA group ( $21.33 \pm 9.61$  nmol/gm) (*P*=0.043, *P*=0.000) respectively. CAT activity and MDA level correlated

negatively with mean number of retrieved sperms (in groups with positive sperm retrieval A&B) (*r*= - 0.261, *P*= 0.048, *r*= -0.402, *P*=0.002) respectively. After thaw there was significant increase in CAT activity in OA only ( $213.67 \pm 160.36$  v  $65.67 \pm 72.99$  U/gm protein) (*P*= 0.000), while there was no significant difference in MDA level in both OA and positive NOA. However, after thawing mean MDA level was still significantly higher in NOA than OA ( $26.94 \pm 11.21$  v  $24.19 \pm 15.97$  nmol/gm) (*P*= 0.049).

**CONCLUSIONS:** Men with NOA seem to have increased basal testicular oxidative stress compared to those with OA as indicated by increased CAT activity and MDA level in fresh testicular samples. These markers of oxidative stress correlated negatively with spermatogenic activity. Furthermore, OA seem to resist oxidative injury induced by cryopreservation by enhancing CAT activity more efficiently than NOA.

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#### **SELF-CORRECTION OF ANEUPLOIDY IN HUMAN BLASTOCYSTS AND SELF-ORGANIZING GASTRULOIDS.**

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**OBJECTIVE:** Finding of aneuploid cells in trophectoderm biopsies at blastocyst stage is currently considered cause for disposal of embryos. Degree of tolerated aneuploidy and whether embryos self-correct downstream have, however, become one of the most controversial issues in reproductive medicine. Objective of this study was, therefore, investigation of degree of self-correction of aneuploidy in human blastocyst-stage embryos and in an *in vitro* model of early human gastrulation, - gastruloids.

**DESIGN:** We tracked aneuploidy in pre-implantation human embryos using single-cell RNA-seq data and conducted prospective *in vitro* studies on the impact of aneuploidy on human gastrulation.

**MATERIALS AND METHODS:** We induced aneuploidy in human embryonic stem cells by treatment with reversine, an inhibitor of MPS1, crucial for the spindle assembly checkpoint and the error correction pathway during cell division. Aneuploid and euploid cells were mixed to generate chimeric human gastruloids and their developmental outcomes were measured using a highly quantitative micropattern platform. To provide *in vivo* relevance, we used a computational approach to track aneuploidy in pre-implantation human embryos using single-cell RNA-seq data.

**RESULTS:** Aneuploid colonies did not affect maintenance of pluripotency, albeit displaying increased TP53. Chimeric euploid-aneuploid differentiated gastruloids showed differential cell death. This was particularly acute in the ectodermal (SOX2+) and mesendodermal (BRA+, SOX17+) lineages, without affecting extra-embryonic (GATA3+CDX2+) tissue. Using bioinformatics, we showed the presence of wide-spread but selective chromosomal instability in human blastocysts. The gene expression signature of aneuploid cells was closely associated to that of euploid cells and, consistent with the above noted gastruloid studies. Originally high levels of aneuploidy (up to 50%) gradually corrected themselves with time.

**CONCLUSIONS:** Similarly to the mouse,<sup>1</sup> aneuploidy is tolerated in humans in extra-embryonic tissue but not in cells contributing to the embryo proper. Altogether, these results strongly suggest that presence of aneuploidy in human trophectoderm is not an indicator of embryo quality to be used for embryo selection in human IVF.

References: <sup>1</sup>Bolton H, Graham SJ, Van der Aa N, Kumar P, Theunis K, Fernandez Gallardo E, Voet T, Zernicka-Goetz M., Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential. *Nat Commun.* 2016; 7:11165 (2016)

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**P-50** Tuesday, October 15, 2019 6:30 AM

#### **EMBRYO RESPIRATION TO EVALUATE THE EMBRYO QUALITY AND VIABILITY, AND ITS CLINICAL OUTCOME.**

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**OBJECTIVE:** Earlier prediction of the quality and viability of *in vitro* developing embryo is very important. The measurement of embryo oxygen consumption, that is embryo respiration may be one of the objective methods to know the embryo quality and viability. Oxygen consumption is an ideal indicator of overall metabolic activity because ATP is generated

predominantly by oxidative phosphorylation. Cell respiratory assay system (CRAS) is a less invasive technique in which the tip of a microelectrode monitors the local distribution of oxygen near embryo. It is reported that the bovine embryos with higher oxygen consumption were better candidates to further development into good quality embryo and yielded higher pregnancy rate after embryo transfer. We have also reported that cleaved stage human embryo's morphological analysis and oxygen consumption become good predictor for development into good quality blastocyst using surplus embryos. The objective of this study is to clarify whether embryo respiration reflect quality and viability of the embryo or not in clinical use.

**DESIGN:** A prospective study.

**MATERIALS AND METHODS:** Two hundred twenty-seven normal fertilized embryo and thirty-six frozen-thawed blastocyst for frozen-thawed embryo transfer (FET) enrolled in this study. All study subjects had signed informed consents prior to entering the study, in accordance to local IRB protocol. Embryo respiration and morphology were evaluated every day for fertilized embryo and good quality blastocysts were frozen for future FET. For frozen-thawed blastocyst, embryo respiration was evaluated just before FET.

**RESULTS:** Embryo respiration of embryo finally developed into good quality blastocyst was significantly higher than that did not on day 4 ( $8.2 \pm 2.2$  vs.  $6.1 \pm 2.0 \times 10^{15}/\text{mol s}^{-1}$ ,  $p < 0.01$ ) and day 5 ( $9.4 \pm 1.3$  vs.  $5.8 \pm 2.3 \times 10^{15}/\text{mol s}^{-1}$ ,  $p < 0.01$ ). Moreover, embryo respiration on day 2 of embryo that arrested to develop on day 3 was significantly lower than that did not ( $6.9 \pm 1.3$  vs.  $5.9 \pm 1.7 \times 10^{15}/\text{mol s}^{-1}$ ,  $p < 0.05$ ). Besides, embryo respiration on day 2 of embryo that arrested to develop on day 4 was also significantly lower than that did not ( $7.1 \pm 1.4$  vs.  $6.1 \pm 1.4 \times 10^{15}/\text{mol s}^{-1}$ ,  $p < 0.01$ ). For frozen-thawed transfer cycle, embryo respiration just before transfer was significantly higher in pregnant group compared with failed group ( $12.2 \pm 2.5$  vs.  $9.9 \pm 2.3 \times 10^{15}/\text{mol s}^{-1}$ ,  $p < 0.05$ ) in spite of morphologically grade was not different between two groups.

**CONCLUSIONS:** Embryo respiration measured on day 4 or day 5 may be a predictor to know the good quality blastocyst development. Embryo respiration on day 2 may be a predictor for not to arrest the development of embryo. Moreover, measurement of embryo respiration just before transfer is very useful to evaluate the embryo quality to implant. So, embryo respiration may become good option to know the embryo quality and viability.

**References:** Hiroyuki Abe. A Non-invasive and Sensitive Method for Measuring Cellular Respiration with a Scanning Electrochemical Microscopy to Evaluate Embryo Quality. *J Mamm Ova Res* 2007;24:70-8.

A. Fukui, A. R. Takeyama, Y. Wakimoto, Y. Ukita, H. Shibahara. Embryo oxygen consumption to evaluate the embryo quality. *Fertil Steril* 110 (4), Suppl, September 2018, e354

**SUPPORT:** None

**P-51** Tuesday, October 15, 2019 6:30 AM

#### REPLICATION STRESS LIMITS DEVELOPMENTAL POTENTIAL OF HUMAN PREIMPLANTATION EMBRYOS.

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**OBJECTIVE:** Human preimplantation embryos show abnormal nucleation and DNA damage, compromising cell cycle progression and developmental potential. The molecular mechanisms and timing of these abnormalities are unknown. We identified endogenous DNA damage and repair mechanisms in human embryos and developed a murine model to study developmental consequences of replication stress in preimplantation embryos.

**DESIGN:** Prospective laboratory study of human and mouse embryos

**MATERIALS AND METHODS:** Endogenous DNA damage and repair pathways were evaluated in humans using donated oocytes ( $n=25$ ) and embryos ( $n=20$ ). Immunofluorescent staining detected DNA damage ( $\gamma$ H2AX, RPA), repair (RPAS33, RPA S4/S8, Rad51, 53BP1), and micronucleation.

Consequences of genomic instability were studied in a murine model using aphidicolin, a DNA polymerase inhibitor that increases endogenous chromosome fragility<sup>1</sup>. Mouse zygotes ( $n=500$ ) were briefly exposed to aphidicolin in the first cell cycle, then evaluated at 1-cell, 2-cell, 4-8-cell and blastocyst stage. Immunofluorescent staining detected DNA damage and repair. Cleavage progression, blastulation and embryo quality were assessed versus controls.

Human and mouse karyotypes were determined with NGS.

**RESULTS:** Human preimplantation embryos show endogenous DNA damage, demonstrated by  $\gamma$ H2AX, RPA and abnormal nucleation. Cleavage embryos had significantly greater foci and micronucleation vs blastocysts ( $\gamma$ H2AX cleavage mean 2.3 vs blastocyst 1.0,  $p < 0.0001$ ; RPA cleavage mean 1.7 vs 0.3,  $p < 0.0001$ ; abnormal nucleation cleavage mean 15.9% vs blastocyst 4.2%,  $p < 0.0001$ ). DNA damage foci coincided with RPAS33, indicating RPA phosphorylation by G2 checkpoint kinase ATR, Rad51, indicating repair by homologous recombination, and 53BP1, indicating unrepaired DNA is passed to daughter cells.

Aphidicolin-induced replication delay resulted in DNA damage ( $\gamma$ H2AX and RPA), and RPAS33, indicating an ATR-dependent G2 checkpoint. Additional DNA repair mechanisms included Rad51 and 53BP1, similar to human embryos. Though some unreplicated DNA is tolerated in mitosis and compatible with euploidy, aphidicolin-induced under replication in the first cell cycle precipitated instability in later cell cycles, leading to decreased blastulation (45% after 8h aphidicolin vs 91.8% control,  $p < 0.0001$ ), and poor quality embryos as evidenced by significantly fewer total cells and inner cell mass with significantly greater DNA damage and micronucleation with increasing duration of aphidicolin exposure compared to controls.

**CONCLUSIONS:** DNA damage responses to incomplete replication in G2 (ATR and Rad51), and the G1 response to unreplicated DNA (53BP1) mirror endogenous repair activity in human preimplantation embryos. Developmental consequences of replication stress likely persist beyond the preimplantation stage and may contribute to failed implantation or miscarriage. The murine model of genomic instability enables further study of these processes and the development of targeted therapeutics.

**References:** 1 Glover, T. W., Berger, C., Coyle, J. & Echo, B. DNA polymerase alpha inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. *Hum Genet* 1984 (67): 136-142.

**SUPPORT:** New York Stem Cell Foundation (NYSCF), New York State Stem Cell Science Program (NYSTEM).

**P-52** Tuesday, October 15, 2019 6:30 AM

#### THE EFFECT OF ZONA PELLUCIDA THICKNESS VARIATION ON FERTILIZATION AND SUBSEQUENT EMBRYONIC DEVELOPMENT: A LARGE SINGLE CENTER COHORT STUDY.

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**OBJECTIVE:** During in vitro fertilization (IVF), variations in zona pellucida (ZP) thickness are frequently observed in retrieved oocytes. It is possible that these variations in ZP appearance are caused by the alteration of patterning glycoprotein matrix, which may be associated with oocyte cytoplasmic competence for embryonic development. In the present study, a large cohort of 1,664 oocytes was evaluated to understand the relation between ZP thickness and embryonic outcomes.

**DESIGN:** This was a retrospective, single-center, cohort study.

**MATERIALS AND METHODS:** A retrospective study on 1,665 oocytes from 978 cycles (827 patients, mean age:  $40.7 \pm 0.1$  years) was conducted from August 2018 to January 2019 in a single center. All patients underwent clomiphene citrate-only minimal stimulation IVF cycles. Maturation status of oocytes was confirmed by the appearance of meiotic spindles and oocytes were inseminated by intracytoplasmic sperm injection (ICSI). The ZP thickness was measured relative to a line drawn along the major axis of the oocyte; two ZP thickness measurements were taken at opposite sides of the line and values were averaged. Spearman's correlation coefficient was used to evaluate the relationship between age of the female and ZP thickness. Multivariable logistic regression analysis, which included all significant confounding factors, yielding adjusted odds ratios (ORs) and 95% confidence intervals (CIs), was used to evaluate the correlation of ZP thickness to embryonic outcomes. Values were considered statistically significant when  $p$ -values were  $< 0.05$ .

**RESULTS:** The mean ZP thickness was  $19.0 \pm 0.1 \mu\text{m}$ . A significant negative correlation was observed between ZP thickness and age of the female (Spearman's correlation coefficient,  $r = -0.0656$ ,  $p = 0.0078$ ). Fertilization, cleavage, blastocyst formation, and blastocyst utilization rates in this cohort were 86.6% (1,439/1,664), 85.4% (1,419/1,664), 56.0% (930/1,664), and 42.7% (709/1,664), respectively. Multivariable logistic regression analysis revealed that there were no statistically significant associations between ZP thickness and fertilization (adjusted OR: 1.011, 95% CI: 0.960-1.065,  $p = 0.6725$ ), cleavage (adjusted OR: 0.991, 95% CI: 0.942-1.042,  $p = 0.7303$ ), blastocyst formation (adjusted OR: 0.974, 95% CI: 0.940-

1.011,  $p=0.1637$ ), and blastocyst utilization (adjusted OR: 0.003, 95% CI: 0.957-1.031,  $p=0.7168$ ).

**CONCLUSIONS:** The properties of ZP have been considered to reflect the history of oocyte cytoplasmic maturation. Our results demonstrate that ZP thickness has no relation with embryonic outcomes, suggesting that variations in ZP thickness do not influence oocyte cytoplasmic competence for embryonic development.

**SUPPORT:** none

**P-53** Tuesday, October 15, 2019 6:30 AM

#### **IDENTIFICATION OF NUCLEOLAR CHANNEL SYSTEMS (NCSS) IN ENDOMETRIAL SECRETIONS AT THE TIME OF FROZEN EMBRYO TRANSFER IN ARTIFICIAL CYCLES WITH SUCCESSFUL**

**IMPLANTATION.** Rachel S. Gerber, MD,<sup>a</sup> Erkan Buyuk, MD,<sup>a</sup> Harry Lieman, MD,<sup>a</sup> U. Thomas Meier, Ph.D.<sup>b</sup> <sup>a</sup>Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY; <sup>b</sup>Dept. of Anatomy & Structural Biology, Albert Einstein College of Medicine, Bronx, NY.



**OBJECTIVE:** NCSs are histological markers of the window of implantation in natural and controlled ovarian hyperstimulation cycles. Thus far, NCSs have not been detected in frozen embryo transfer artificial (FET-A) cycles. This study aims to detect NCSs in endometrial aspirations obtained immediately prior to embryo transfer during blastocyst FET-A cycles without affecting implantation.

**DESIGN:** Prospective study at a single university-affiliated site

**MATERIALS AND METHODS:** Patients undergoing FET-A using estradiol and progesterone for endometrial preparation are consented for a lower uterine segment aspiration using an open tip embryo transfer catheter during a mock embryo transfer performed immediately prior to the actual embryo transfer. The aspirated endometrial secretions containing endometrial cells are then analyzed for the presence of NCSs using indirect immunofluorescence. Based on a prior study, positive NCS status was defined as the presence of NCSs in at least 3 endometrial epithelial cells (EECs). Pregnancy outcomes are monitored to ensure that there is no effect of the aspiration on implantation rates.

**RESULTS:** Uterine secretions were obtained from 5 patients immediately prior to embryo transfer. The average age of women was  $37.2 \pm 4.2$  years. NCSs were detected in exfoliated EECs of uterine secretions in 4 of 5 (80%) samples and could not be unequivocally identified in 1 of 5 (20%), which was designated as indeterminate. Implantation as evidenced by a positive BHCG was seen in 5 of 5 (100%) of the patients who underwent aspiration with a clinical pregnancy rate of 40% and an ongoing pregnancy rate of 20%.

**CONCLUSIONS:** This is the first report of NCS detection in FET-A cycles in the absence of follicular development and ovulation. NCS status can be determined in exfoliated EECs of uterine secretions obtained at the time of embryo transfer while maintaining implantation. Our study provides proof of principle to determine endometrial receptivity through individualized point of care testing of NCS status during frozen embryo transfer in artificial cycles.

**SUPPORT:** This study was supported by a grant from the NIH (HD094293 to U.T.M.).

**P-54** Tuesday, October 15, 2019 6:30 AM

#### **SINGLE-CELL MITOCHONDRIAL STAINING OF HUMAN BLASTOCYSTS.**

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**OBJECTIVE:** Physicians are looking beyond chromosomal copy number to understand why some euploid embryos fail to implant. Studies investigating the contribution of mitochondrial (mt) DNA levels on implantation have shown some association between mt and embryonic competence, but, these studies are limited, as DNA count has been estimated based on limited sampling of trophectoderm (TE). As mt are the primary energy source of embryonic cells, characterization of mt activity rather than DNA copy number might offer insight into embryonic competence. This study aimed to characterize mt activity on the single cell level in human blastocysts as a marker of embryonic quality.

**DESIGN:** Prospective cohort

**MATERIALS AND METHODS:** Previously vitrified human blastocysts that had undergone TE biopsy and next-generation sequencing from 2017-2018 were included. Embryos were thawed and immersed in trypsin to dissociate cells. Cells were washed with blastocyst culture and suspended in RPMI (with 10% FBS). Cells were incubated at room temperature with Cytopainter mitochondrial stain and loaded onto the Beacon single cell-handling platform into tracked pens. Cells were imaged in the Texas Red channel. Analysis was performed using Fiji and Prism 8.

**RESULTS:** A total of 515 cells were analyzed from seven embryos in four patients. Two embryos biopsied for PGT-A on day 6 of development from a 35 yo woman were analyzed: embryo A (grade 4AB) was euploid and embryo B (grade 4BA) was aneuploid. Three euploid embryos from a 33 yo patient, each biopsied on a different day of development, were also included: embryo C (Grade 4BB) was biopsied on day 5, embryo D (grade 6BB) was biopsied on day 6, and Embryo E (grade 6BB) was biopsied on day 7. Two euploid embryos biopsied on day 5 with grades of 4BB were included: embryo F from a 33 year old (yo) patient and embryo G from a 40 yo patient.

For embryo A, 72 cells panned and 66 retained staining (91%). Of the 91 cells that panned for embryo B, 78 (86%) stained. Comparing embryo A to B, a higher level of staining was observed with embryo A ( $P<0.0001$ ). Of the 68 cells that panned for embryo C, 49 showed staining (72%). Embryo D had 58 cells panned, of which 52 cells stained with Cytopainter (90%). Embryo E had 18 cells panned with 10 cells retaining staining (56%). When comparing embryos C, D, and E, there was a significantly higher level of mitochondrial staining in the earlier stage embryos ( $p=0.0013$ ). A total of 77 cells from embryo F panned and 36 showed staining (47%). Embryo G had 131 cells panned and 68 (52%) stained. Of the stained cells, embryo F was observed to have higher intensity staining than embryo G ( $p=0.6$ ).

**CONCLUSIONS:** This study is one of the first to demonstrate single cell mt activity from a human embryo. We found that cells from euploid, faster growing embryos derived from younger patients possess higher mt content, perhaps representing an increase in embryonic competence. Mt activity might therefore be a useful clinical marker of embryonic quality. Future study will focus on correlating mt activity and gene expression profiles on the single cell level to gain insight into genomic markers and drivers of embryonic development and competence.

**References:** None

**SUPPORT:** None

**P-55** Tuesday, October 15, 2019 6:30 AM

#### **COMPARATIVE ANALYSIS OF DNA METHYLATION IN EUPLOID AND ANEUPLOID HUMAN EMBRYOS USING REDUCED REPRESENTATION BISULFITE SEQUENCING.**

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**OBJECTIVE:** DNA methylation (DNAm) is a fundamental epigenetic control mechanism that occurs by the addition of methyl (CH<sub>3</sub>) groups to cytosine residues and guides differentiation during embryonic development. As its accuracy and efficiency is essential for embryo viability, DNAm has the potential to be a marker for embryonic reproductive competence. Reduced representation bisulfite sequencing (RRBS) is a high-throughput technique that combines restriction digestion and bisulfite sequencing to enrich for highly methylated regions of the genome, allowing analysis of genome-wide methylation profiles on a single nucleotide level. In this study, we used RRBS to characterize differences in DNAm profiles of euploid and aneuploid human blastocysts.

**DESIGN:** Experimental study

**MATERIALS AND METHODS:** Two trophectoderm (TE) biopsies from each of the 10 previously diagnosed euploid and 10 aneuploid embryos were analyzed RRBS. The libraries were prepared with cell lysis, MspI digestion, end repair/dA-T tailing, adapter ligation, bisulfite conversion, and amplification, then sequenced using Illumina HiSeq 2500 with paired-end 150 bp reads. The sequencing reads were trimmed to remove the adapter read through and filtered to eliminate the reads without the MspI recognition sites, then aligned to the reference using Bismark software. Unconverted reads were filtered. The methylome profiles at the GC-rich regions were compared in euploid and aneuploid human embryos.

**RESULTS:** The overall whole genome CpG coverage of the TE biopsies was 5-10%, which was the expected as RRBS detects 1-2 million CpG sites

of whole genome (28.7 million CpG sites). The average DNAm level was 15%. Aneuploid embryos showed significantly lower DNAm levels compared to euploid embryos ( $p < 0.0001$ ). Increased patient age was correlated with elevated DNAm levels in blastocysts ( $p = 0.04$ ). Blastocysts cryopreserved on day 6 had significantly lower DNAm compared to those that were cryopreserved on day 5 ( $p = 0.001$ ). Whole chromosomal aneuploidy predicted by calculating the fraction of read count from each chromosome showed 100% consistency with previous diagnosis. The chromosomes involved in monosomy embryos (-4, -13, -16, and -18) showed reduced methylation rates compared to the other chromosomes.

**CONCLUSIONS:** DNAm levels detected by RRBS in trophoctoderm biopsies from human blastocysts is associated with ploidy status, maternal age, and embryo growth characteristics. This novel tool could provide a foundation for the development of epigenetic biomarkers of reproductive competence.

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### **MATERNAL AGE AND BLASTOCYST QUALITY DO NOT INFLUENCE THE EMBRYO PRODUCTION OF HYPERGLYCOSYLATED HUMAN CHORIONIC GONADOTROPIN.**

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**OBJECTIVE:** The purpose of the study was to evaluate the associations between the human embryo quality, maternal age and the amount of human chorionic gonadotropin (hCG-H) in the secretome of in-vitro cultured embryos.

**DESIGN:** Observational study.

**MATERIALS AND METHODS:** Individual embryos from 49 women were cultured to the blastocyst stage in 25  $\mu$ L of single-step culture medium in the EmbryoScope. Media samples ( $n = 54$ ) were collected on day 5 from wells containing good, fair or poor quality blastocysts, respectively. Media from wells without an embryo were also collected as controls. The quality of the embryos was assessed morphologically. Measurement of hCG-H concentration in culture media is performed with ELISA kit (Cusabio Biotech, CBS-E15803h) according to the manufacturer's instructions. The absorption is measured on a microplate reader (Beckman Coulter DTX 880 Multimode detector) at 450 nm. SPSS v.21 is used for the statistical analysis (IBM Corp., Armonk, NY, USA).  $P < 0.05$  indicates the statistical significance between the compared groups.

**RESULTS:** The mean age of the patients included in the study was 38.08  $\pm$  4.28 years. The number of observed Day 5 good, fair and poor quality blastocysts was 26, 22 and 6, respectively. The presence of hCG-H was confirmed in all embryo culture media samples but was absent in the controls. Comparison between poor, good and excellent quality embryos was made by Mann-Whitney U test as parameters were not normally distributed. The measured mean hCG-H levels were not significantly different between poor, fair and good quality embryos (0.095 mIU/ml vs. 0.095 mIU/ml vs. 0.08 mIU/ml;  $p = 0.91$ , respectively). In addition, there was a lack of significant correlation between women's age and the level of hCG-H, produced by embryos ( $R = 0.17$ ,  $p = 0.23$ ).

**CONCLUSIONS:** Our results suggest that the embryo's secretion of hCG-H is not influenced by maternal age and morphological quality of human blastocysts.

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### **EVALUATING THE ABILITY OF AN OOCYTE TO REPAIR FRAGMENTED SPERM CHROMATIN.**

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**OBJECTIVE:** To evaluate the ability of oocyte DNA repair mechanisms to detect and fix deficiencies in sperm chromatin integrity and support embryo implantation.

**DESIGN:** From 2006-2017, ejaculates from 127 men were assessed for sperm chromatin fragmentation (SCF). Intracytoplasmic sperm injection

(ICSI) clinical outcomes were divided into groups according to the SCF level of the male partner and the proportion of mature oocytes obtained at retrieval. Proportional oocyte nuclear maturity was considered an indirect marker for presumed cytoplasmic readiness of the oocyte, suggesting its ability to repair the male genome during deprotonation.

**MATERIALS AND METHODS:** Samples from consenting couples were screened for SCF levels by terminal deoxynucleotidyl dUTP nick-end labeling (TUNEL) utilizing a commercially available kit. A minimum of 500 spermatozoa were assessed per patient, and an SCF of 15% or below was considered normal. From the retrieved cohort, the proportion of metaphase-II oocytes at the time of ICSI was recorded and used for this assessment. ICSI was performed in the standard fashion. Female partners were limited to  $\leq 35$  years of age to control for eventual confounding female factors.

**RESULTS:** A total of 127 couples underwent 191 ICSI cycles. Of them, 84 couples in which the male partner had a normal SCF level ( $9.8 \pm 3\%$ ) underwent 125 ICSI cycles; conversely, 43 couples in which the male partner had an abnormal SCF level ( $24.1 \pm 11\%$ ;  $P < 0.0001$ ) underwent 67 ICSI cycles.

When the proportion of mature oocytes was over 80% at the time of retrieval, there was no difference in the ICSI clinical outcome between couples with normal and abnormal SCF levels, indicating that a mature ooplasm overcomes compromised genomic integrity. However, when the proportional oocyte maturity dropped below 80%, ICSI cycles carried out with spermatozoa with a normal SCF level ( $n = 62$ ) retained an implantation rate of 25.0%, whereas the embryos generated from spermatozoa with an abnormal level of SCF ( $n = 27$ ) showed an impaired ability (8.3%) to implant ( $P < 0.05$ ). The latter implies that the ooplasm of the MII oocyte was unable to properly repair male genomic deficiencies.

**CONCLUSIONS:** These findings support the notion that an appropriate nuclear and cytoplasmic maturity is needed to unravel the male genome and repair eventual nicks and breaks within the chromatin. The implied ooplasmic dysmaturity, occurring with a suboptimal MII cohort, may not efficiently overcome compromised sperm chromatin integrity.

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### **THE MITOCHONDRIAL DNA QUANTIFICATION IN CUMULUS CELLS AND IMPLANTATION POTENTIAL OF EMBRYOS.**

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**OBJECTIVE:** Recent studies have suggested that age-related decreased competence of oocytes may be due to low quantity of mitochondrial DNA (mtDNA) copy number. Moreover, quantification of mtDNA in the oocyte-cumulus-cell complexes (OCCCs) may serve as a predictor of blastocysts viability. The purpose of this study to investigate relative levels of mtDNA in the OCCCs in association with female age, ovarian reserve, embryo morphology, ploidy and blastocyst implantation rate.

**DESIGN:** Prospective clinical study performed on 470 OCCCs retrieved from 72 advanced reproductive age patients undergoing ART treatment with intracytoplasmic sperm injection (ICSI) and preimplantation genetic testing for aneuploidy. Inclusion criteria: age 35-45 years; BMI: 18 - 29 kg/m<sup>2</sup>; FSH  $\leq 15$  IU/ml; normal female/male karyotype; non-smokers. Exclusion criteria: genital endometriosis III-IV; severe extragenital pathology; polycystic ovary syndrome; chronic endometritis; <sup>\*</sup> 96% of sperm with abnormal morphology according to WHO criteria. The morphological assessment of embryos was carried out according to the Gardner classification. Out of the 130 obtained blastocysts 56 embryos were diagnosed as aneuploid, and 74 as euploid. Presently, 51 frozen euploid embryos were transferred (FET). All transferred euploid blastocysts ( $n = 51$ ) divided into 2 groups: 1 group ( $n = 21$ ) - implanted embryos, 2 group ( $n = 30$ ) - non-implanted.

**MATERIALS AND METHODS:** Cumulus cells (CCs) were removed from OCCCs using fine needles. Collected CCs were placed into the Eppendorf tube and frozen at  $-80^{\circ}$  C for subsequent DNA analysis. MtDNA was assessed by using a quantitative real-time polymerase chain reaction technique. DNA from the trophectoderm samples were amplified and subjected to aneuploidy analysis using array comparative genomic hybridization. In statistic analysis was used Pearson's correlation coefficients and Fisher's exact test;  $p < 0.05$  was considered significant.

**RESULTS:** The median age was 37.8 years old (range, 35-45), the mean level of anti-Mullerian hormone (AMH) was  $2.66 \pm 1.09$ , and the mean body

mass index was 22.3±1.5. A positive correlation of the relative level of mtDNA in the CCs with the patients' age (p = 0.008) and AMH levels (p = 0.003) was revealed. There was no statistically significant correlation between mtDNA copy number and embryo morphology on day 5 (p=0.7). There was a tendency to increase mtDNA copy number in group 1 vs. group 2, 390 and 299, respectively (p >0.05). In this study we didn't find relationship between median mtDNA content of CCs and embryos ploidy (356 vs. 325, in euploid (n=74) and aneuploidy (n=56) blastocyst, respectively, p >0.05).

**CONCLUSIONS:** mtDNA content in CCs correlated with female age and AMH level. However, the determination of mtDNA copy number in CCs don't predict embryos implantation potential.

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### DYNAMIC AND VIABILITY OF HUMAN DAY-6 TROPHOCTODERM CELLS DURING 141 DAYS OF CELL CULTURE.

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**OBJECTIVE:** To determine the dynamic and viability of derived human trophoctoderm cells (TE) in vitro cultured for 141 days.

**DESIGN:** Research ongoing study.

**MATERIALS AND METHODS:** Study trophoctoderm cells were obtained from day-6 blastocysts determined to be non-viable after undergoing in vitro fertilization. This study was conducted in accordance with an IRB. Biopsied mass of approximately 25 TE cells was placed in three individual time-lapse wells with 25 µl of RPMI 1640 with 20% HSA in a time-lapse incubator (Embryoscope). This culture media was supplemented with human fibroblast growth factor-4. A controlled environment of 5% O<sub>2</sub>, 6% CO<sub>2</sub>, 37°C, and a 7.2 pH were used to provide cell culture conditions. Culture media was changed every 24 hours. TE cells were settled at the bottom of three wells in a central depression with a diameter of 200 µm. TE cells were video monitoring in the 10-minute cycle time with seven focal planes for 141 days. TE cells from the same well were counted (using a cell counter) three times to obtain the average number. A sample of 5 µl aliquot of cells was taken for each well and analyzed by Veriseq (high-resolution Next Generation Sequencing) to confirm the viability and the chromosomal analysis of the TE cells. Genetic testing was performed by Reprogenetics Recombine Genesis Genetics (Cooper Genomics). The remaining cells were discarded.

**RESULTS:** Dynamic reproduction of TE cells was observed in all biopsied samples. TE cells responded with aggressive expansion and growth during the first 30 days of culture and then remained in a plateau growth pattern during the rest of the cell culture time. The number of cells was an estimated average, but it could be inaccurate due to the size of the cells and the focal plane of the video picture. The viability of the cells was determined by the genetic information outcome, color, and integrity of the cells.

TE Cells derived and in vitro cultured for 141 days

Biopsied Specimen	Initial Number of TE Cells (Average)	Number of TE Cells at 30 Days of Cell Culture (Average of 3 Counts)	Number of TE Cells at 141 Days of Cell Culture (Average of 3 Counts)	Genetic Screening at 141 days of Cell Culture (Next Generation Sequencing)
1	25	1200	5000	Aneuploid
2	25	2300	6000	Euploid
3	25	3500	8000	Euploid

**CONCLUSIONS:** Deriving TE cells from human day-6 blastocysts is possible. This outcome opens the opportunity to explore new trophoctoderm cell culture conditions. Increment in trophoctoderm cells might offer new alternatives to improve our knowledge of this type of cells on IVF patients with a poor number of trophoctoderm cells on day-6 embryos.

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### IS LOW MITOCHONDRIAL DNA (MTDNA) CONTENT AFTER FERTILIZATION FAILURE DUE TO OOCYTE AGING IN CULTURE?.

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PhD,<sup>c</sup> Carmen Vidal, M.D., Ph.D.,<sup>b</sup> Ma José de los Santos, PhD,<sup>b</sup> <sup>a</sup>IVI foundation, Instituto de Investigación Sanitaria la Fe, Valencia, Spain; <sup>b</sup>IVIRMA Valencia, Valencia, Spain; <sup>c</sup>IVIRMA ROMA, Roma, Italy.

**OBJECTIVE:** Low mitochondrial DNA (mtDNA) content in oocytes has been correlated with oocyte fertilization failures. However, all the studies with failed-fertilized oocytes have been performed in in-vitro aged oocytes. As the results have relevant clinical implications on understanding oocyte competence, the aim of the study was to evaluate the effect of in vitro aging in mtDNA content in failed-fertilized oocytes.

**DESIGN:** A prospective cohort study was performed with 101 samples consisting on 36 "fresh" non-inseminated MII donated oocytes, 31 in-vitro aged failed-fertilized oocytes, 17 "fresh" failed-fertilized oocytes from patients and another 17 from donors.

**MATERIALS AND METHODS:** Samples were collected in PCR tubes the same day of follicle aspiration in the case of donated MII oocytes, after 19-22 hours post-ICSI for "fresh" failed-fertilized oocytes and after 5-6 days of culture in the case of in vitro aged failed-fertilized oocytes. Q-PCR was performed with SurePlex DNA Amplification System (Illumina) using specific primers for the ATP8 gene to assess the total mtDNA copy number. Data was analyzed by ANOVA test with Scheffé multiple comparison.

**RESULTS:** Significant higher mtDNA content was found in "fresh" non-inseminated MII oocytes comparing with "fresh" and failed-fertilized ones (P<.05) in both patients and donors. Besides, there were no significant differences in terms of mtDNA content between "fresh" and in-vitro aged failed-fertilized oocytes (P>.05).

	N	P25	Median	P75
MI I donated oocytes	36	5.51	7.59	9.43
Fresh failed-fertilized oocytes (patients)	17	4.89	5.42	6.01
Fresh failed-fertilized oocytes (donors)	17	4.89	5.28	6.14
Total fresh failed-fertilized oocytes	34	4.91	5.34	5.99
In-vitro aged failed-fertilized oocytes	31	3.77	4.39	5.10

**CONCLUSIONS:** As it appears in literature, we have observed a significant decrease in mtDNA content associated with failed-fertilized oocytes compared to "fresh" non-inseminated MII oocytes. Such decrease occurs regardless of the in vitro aging. In addition, we observed that the decrease of mtDNA content in failed-fertilized oocytes is independent of the maternal age. Furthermore, it seems that the decrease of mtDNA content observed in failed-fertilized oocytes compared to non-fertilized oocytes is due to the fail of fertilization itself and not because of the mtDNA degradation in culture, since when we compare fresh failed-fertilized oocytes and failed-fertilized oocytes that were sampled after 5 or 6 days after ICSI, we do not observe significant differences. Therefore, we can conclude that the fail of fertilization is related to oocytes with an unusually low mtDNA content and this finding supports the importance of the mtDNA content in oocytes as a biomarker for embryo viability.

**SUPPORT:** This work was funded by a grant from the Generalitat Valenciana (Spain).

**P-61** Tuesday, October 15, 2019 6:30 AM

### DO MALE EMBRYOS GROW FASTER THAN FEMALE EMBRYOS?.

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**OBJECTIVE:** Preimplantation genetic testing for aneuploidy (PGT-A) gives us insight into the chromosomal makeup of an embryo. It has been

suggested that male embryos grow faster than female embryos and as a result embryologists are more likely to select a male embryo for transfer. The objective of this study is to assess whether an embryo's sex and/or chromosomal normalcy may be related to their rate of development.

**DESIGN:** A retrospective study of PGT-A results obtained between 2016 and 2018.

**MATERIALS AND METHODS:** Information was derived from PGT-A results of 151 patients (691 embryos; 21 uncounted due to being beyond parameters or missing information). We determined the ratios of day-5 XY to XX, labeling this as group A, day-6 XY to XX as group B, day-5 euploid (N) to aneuploid (AN) as group C, and day-6 N to AN as group D. These ratios were then tested to determine whether embryo growth exhibited any significant patterns.

**RESULTS:**

A chi-square test for independence was performed on the ratios of four groups: A, B, C, and D. The former three groups' ratios yielded no significant differences, with group B and C possessing ratios very close to an even split 50/50, which is consistent with the average prediction of embryo development. However, the latter group yielded a significant difference with 92:128 euploid to aneuploid ratio ( $p = 0.015$ ).

group A	total Day 5 XY	242	Expected Value	225
	total Day 5 XX	208	Expected Value	225
Chi-Square Test	P-Value:	0.11		
Group B	Total Day 6 XY	108	Expected Value	110
	Total Day 6 XX	112	Expected Value	110
Chi-Square Test	p-Value:	0.79		

**CONCLUSIONS:** Our results do not support the notion that male embryos grow faster than female embryos. There was a significant difference in the rate of aneuploidy seen when comparing day 6 blastocysts to those that reached the blastocyst stage on day 5.

References: n/a  
**SUPPORT:** n/a

**P-62** Tuesday, October 15, 2019 6:30 AM



**TROPHECTODERM CELL DEVELOPMENT FROM DAY-6 HUMAN BLASTOCYSTS. IS IT POSSIBLE TO REPRODUCE THEM IN VITRO?.**

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**OBJECTIVE:** To create trophectoderm (TE) cells in vitro from discarded day-6 blastocysts.

**DESIGN:** Research ongoing study

**MATERIALS AND METHODS:** Trophectoderm cells from non-viable, discarded, day-6 blastocysts were selected for this study. Specimens were derived from unused cells obtained from in vitro fertilization patients who had consented to have these discarded cells used for this IRB-approved research study. Three biopsied cell masses were cultured in RPMI 1640 medium supplemented with 20% HSA. TE cells were cultured with human fibroblast growth factor-4 in a time-lapse incubator (Embryoscope®). TE cells were video monitored every ten minutes with seven different focal planes for 500 hours of cell culture. Derived TE cells from the same specimen were divided into two groups according to the size of the cell in  $\mu\text{m}$ . TE cells were counted (using a cell counter) three times to obtain the group number.

Development of trophectoderm cells in vitro

Time of Cell Culture	Specimen 1		Specimen 2		Specimen 3	
	TE Cells $\geq 10 \mu\text{m}$ (n)	TE Cells $\leq 10 \mu\text{m}$ (n)	TE Cells $\geq 10 \mu\text{m}$ (n)	TE Cells $\leq 10 \mu\text{m}$ (n)	TE Cells $\geq 10 \mu\text{m}$ (n)	TE Cells $\leq 10 \mu\text{m}$ (n)
0 hours	25	0	25	0	25	0
500 hours	200	1000	300	2000	250	2500

**RESULTS:** Trophectoderm cell derivation started after initial cell culture. Proliferated TE cells resulted in smaller cells of approximately 2-5  $\mu\text{m}$ . Some of these smaller cells grew and reached the same size as the original TE cell source. The number of cells was an estimate number, but it could be inaccurate due to the size of the cells and the focal plane of the video picture.

**CONCLUSIONS:** Developing trophectoderm cells in vitro is possible. Although some of the derived cells resembled the same origin, the growth pattern of most cells was prolonged. Moreover, most of the resulted TE cells were smaller than 5  $\mu\text{m}$ . These smaller TE cells might need different culture protocols and growth factors to express similar characteristics as the original TE cells from day-6 blastocysts.

**P-63** Tuesday, October 15, 2019 6:30 AM

**CAN GROWTH HORMONE REALLY IMPACT ANEUPLOIDY RATES?** Ruchi Kaushik Amin, MD,<sup>a</sup> Lauren Grimm, MA,<sup>b</sup> Elisabeth Rosen, BS, MA,<sup>b</sup> Angeline Beltsos, MD,<sup>b</sup> Roohi Jeelani, MD,<sup>b</sup> <sup>a</sup>Wayne State University, Detroit, MI; <sup>b</sup>Vios Fertility Institute, Chicago, IL.



**OBJECTIVE:** To specifically look at the effect of growth hormone (GH) on preimplantation genetic testing for aneuploidy (PGT-A).

**DESIGN:** Retrospective chart review at a private infertility center.

**MATERIALS AND METHODS:** All patients enrolled in poor-responder in-vitro fertilization (IVF) protocols from 2016-2018 were included in this study. Cycles, in which GH was administered, were analyzed for PGT-A results, when available, and compared to the results of other poor responder IVF protocols without the use of GH. A two sample t-test was used to analyze the data using Stata 14.0 (StataCorp LLC., College Station, TX, USA).

**RESULTS:** A total of 171 cases that utilized PGT-A were identified. The euploidy rate in the GH group was 28.4% compared to 23.3% in the age-matched control group. Although not statistically significant, this study shows that addition of GH in patients with poor oocyte quality may lead to a successful pregnancy and lower chances of miscarriage ( $p=0.256$ ).

**CONCLUSIONS:** Historically, GH has been implemented for the improvement of oocyte quality. Previous literature has shown that the addition of GH improves clinical pregnancy rates and decreases miscarriage rates, which is consistent with the fact that PGT-A also decreases miscarriage rate. Although, not statistically significant, our results demonstrate that GH not only improves oocyte quality, but helps improve euploidy rates in patients with diminished ovarian reserve. We stipulate that the mechanism of action of GH is to decrease aneuploidy, thereby improving clinical pregnancy rates.

**ENDOMETRIAL PHYSIOLOGY**

**P-64** Tuesday, October 15, 2019 6:30 AM

**THE INFLUENCE OF HYPOXIA ON ANGIOGENESIS AND METABOLISM IN HUMAN ENDOMETRIAL STROMAL CELLS.** Hidetaka Okada, MD, Takeharu Kido, MD. Kansai Medical University, Hirakata, Japan.



**OBJECTIVE:** Hypoxia is a physiological event that occurs in the endometrial tissues during the premenstrual period and implantation. Hypoxia-inducible factor-1 (HIF-1) is the master regulator of the cellular response to hypoxia. HIF-1 $\alpha$  activation by the hypoxic microenvironment is involved in angiogenesis and metabolism. The aim of this study is to investigate the effects of hypoxic stress on the regulation of HIF-1 $\alpha$  and vascular endothelial growth factor (VEGF), and on the changes in the metabolic pathway in the endometrium.

**DESIGN:** Prospective in vitro studies using human primary endometrial stromal cell cultures.

**MATERIALS AND METHODS:** Human endometrial tissues were obtained from 21 patients aged 32–46 years undergoing hysterectomy for benign reasons with regular menstrual cycles. The human endometrial stromal cells (ESCs) were purified by the standard enzyme digestion method. ESCs were cultured under hypoxic (2% O<sub>2</sub>) or normoxic (20% O<sub>2</sub>) conditions with echinomycin, a small-molecule inhibitor of HIF-1 $\alpha$  activity. The mRNA levels and production of VEGF were assessed by real-time PCR and ELISA, respectively. The HIF-1 $\alpha$  protein levels were measured using western blot analysis. Metabolome analysis was measured by capillary electrophoresis electrospray ionization time-of-flight mass spectrometry and capillary electrophoresis-triple quadrupole mass spectrometry. Differences in the measured parameters across the different groups were statistically assessed using ANOVA followed by Dunnett's test and a level of P < 0.05 was considered statistically significant.

**RESULTS:** Real-time PCR analysis demonstrated that hypoxia caused a significant increase in the levels of VEGF mRNA expression (P<0.01). Hypoxia caused a significant increase of VEGF production after 6 h of culture compared with normoxia (P<0.01), and this effect continued to increase until the end of the study at 48 h. Hypoxic stress significantly induced the expression of HIF-1 $\alpha$  protein (P<0.05), and its highest expression was observed at 6 h. Echinomycin inhibited hypoxia-induced VEGF production without affecting the HIF-1 $\alpha$  protein level. These results suggest that hypoxia acts to increase VEGF via HIF-1 $\alpha$ -dependent manner. A total of 116 metabolites were analyzed. Hypoxia significantly increased glucose 6-phosphate and fructose 6-phosphate in the glycolytic pathway (P<0.05). However, while hypoxia suppressed cis-aconitic acid, isocitric acid, and citric acid in the tricarboxylic acid cycle, the decrease only reached the borderline of significance (P = 0.08).

**CONCLUSIONS:** These results indicate a potential mechanism for the action of hypoxic conditions that could influence angiogenesis and metabolism in the human endometrium.

**P-65** Tuesday, October 15, 2019 6:30 AM

**DOES THE ENDOMETRIUM HAVE A ROLE IN SELECTING EMBRYO GENDER?** Javier Herreros, Sr, MSc, Hector Huete Ferriz, MSc, Mireia Florensa, MSc, Marga Esbert, PhD IVI RMA Barcelona, Barcelona, Spain.



**OBJECTIVE:** It is still unknown if the endometrium can select the embryo depending on the gender. To date, it has been thought that the gender of the newborn children depends on the spermatozoa which fertilized the oocyte was carrier of Y or X chromosome. Nevertheless it is possible that the endometrium could have an important role on this event. The aim of our study is to prove if the endometrial receptivity could change depending on the gender of the transferred embryo.

**DESIGN:** Retrospective study

**MATERIALS AND METHODS:** This retrospective study includes 2237 IVF cycles performed in our center between January 2004 and May 2018. Patients were divided into 2 different branches:

Branch 1: study the gender percentage of the newborn children in couples who have undergone DET (double embryo transfer) in one cycle, obtaining all the possible combinations: male-male, female-female and male-female.

Branch 2: compare if there is any tendency towards the embryo's implantation depending on whether the replaced embryo has the same sex as the pre-

vious children or not in couples with two or more newborn children resulting from cycles at IVI Barcelona.

We have analyzed our data with a Chi-squared test.

**RESULTS:** Depending on the embryo gender of the newborn children, we have classified the different combinations in 10 groups.

♀+♂=1 ♀+♀=3 ♀+♀+♂=5 ♀+♀+♀+♂=7 ♂+♂+♂=9  
♂+♂=2 ♂+♂+♀=4 ♀+♀+♂+♂=6 ♀+♀+♀=8 ♂+♂+♂+♀=10

In the first branch (n=1763) there are no difference among the frequency of the three groups: 46.5% (1), 25.7% (2) and 27.6% (3). Analyzing the combinations 2 and 3 (n= 979) the probability of having two females is slightly higher than having two males, 52.19% vs 47.8%, but without statistical significant difference.

As for the second branch (n=474), we have divided the cycles into three different groups depending on the number of newborn children. Group A (n=384) with two newborn children, group B (n=84) with three newborn children and the group C (n=6) with four newborn children. We have observed that the results agrees with the natural proportion in all three groups. As an example, in the group A the percentages of combination 1 is 54%, combination 2 is 25% and combination 3 is 21%.

**CONCLUSIONS:** Our findings suggest that having a first newborn child has no influence on the gender of the following newborn children irrespective of the number of embryos replaced or the number of cycles performed. Therefore, according to our results, the endometrium does not play a role in selecting the embryo gender.

## EUPLOID EMBRYO PREDICTORS

**P-66** Tuesday, October 15, 2019 6:30 AM

**THE ASSOCIATION OF AGING MARKERS IN LUTEINIZED GRANULOSA CELLS AND EMBRYO ANEUPLOIDY RATE IN PREIMPLANTATION GENETIC TEST FOR ANEUPLOIDY CYCLES.** Tsung-Hsien Lee,

MD, PhD,<sup>a</sup> En-Hui Cheng, PhD,<sup>b</sup> Maw-Sheng Lee, MD, PhD.<sup>c</sup> <sup>a</sup>Chung Shan Medical University Hospital, Taichung, Taiwan; <sup>b</sup>Lee Women's Hospital, Taichung, Taiwan; <sup>c</sup>Chung Shan Medical University, Taichung, Taiwan.



**OBJECTIVE:** The ovarian aging is associated with poor quality oocytes, especially increasing aneuploidy rate. In addition to chronological age, several biomarkers could represent the aging status of individual person, such as telomere length and mitochondrial copy number in somatic cells. Nonetheless, the correlation between these aging biomarkers and embryo aneuploidy rate in ART cycles is not clear.

**DESIGN:** This prospective cohort study was performed for the patients for preimplantation genetic test for aneuploidy (PGT-A) programs in a single reproductive center in Taiwan.

**MATERIALS AND METHODS:** The telomere length and mitochondrial copy number in leukocytes and luteinized granulosa cells were measured as aging biomarkers. The association among these aging biomarkers was explored. The correlation between these aging biomarkers and embryo aneuploidy rate was investigated with Spearman correlation test and linear regression model.

**RESULTS:** A total of 110 PGT-A cycles was recruited for this study. The telomere length and the mitochondria copy number are intimately correlated

TABLE 1. Correlation between biomarkers of aging (Spearman correlation test)

Spearman correlation coefficient	Leukocyte telomere length	Granulosa cell telomere length	Leukocyte mitochondrial copy number	Granulosa cell mitochondrial copy number
Age	-0.093 P=0.334	-0.186 P=0.051	-0.069 P=0.472	-0.019 P=0.846
AMH	-0.015 P=0.875	0.385 P<0.001	0.006 P=0.954	0.261 P=0.006
Leukocyte telomere length	1.000	0.008 P= 0.931	0.477 P<0.001	0.095 P=0.321
Granulosa cell telomere length	0.008 P= 0.931	1.000	-0.075 P=0.437	0.361 P<0.001
Leukocyte mitochondrial copy number	0.477 P<0.001	-0.075 P=0.437	1.000	-0.020 P=0.839
Granulosa cell mitochondrial copy number	0.095 P=0.321	0.361 P<0.001	-0.020 P=0.839	1.000

**THE IMPACT OF LEAD FOLLICLE SIZE AND DURATION OF STIMULATION ON THE PROBABILITY OF EUPLOID EMBRYOS.** Denis Schapira Wajman, MD,<sup>a</sup>

David L. Keefe, M.D.,<sup>b</sup> David H. McCulloh, Ph.D.,<sup>c</sup> James A. Grifo, MD, PhD,<sup>c</sup> Cheongeun Oh, PhD<sup>a</sup> <sup>a</sup>NYU Langone, New York, NY; <sup>b</sup>New York University School of Medicine, Department of Obstetrics and Gynecology, New York, NY; <sup>c</sup>NYU Langone Fertility Center, New York, NY.



**OBJECTIVE:** Clinical guidelines on the optimal duration of controlled ovarian stimulation and ideal follicle size were developed for fresh embryo transfer cycles. Whether these apply to freeze all cycles remain unclear. We evaluated the impact of lead follicle size and duration of stimulation on the probability of euploid embryos in women undergoing IVF/PGT-A

**DESIGN:** Cross-sectional study

**MATERIALS AND METHODS:** Data from 721 patients undergoing at least two cycles of COS for IVF with preimplantation genetic testing for aneuploidy (PGT-A) via Next Generation Sequencing (NGS) (1859 cycles). Mixed-effect logistic regression, which can account for correlations among repeated outcomes within sample patients, was used to evaluate the association between independent variables and probability of achieving euploid embryos. We first conducted a mixed-effect logistic regression in a univariate manner. All variables then were evaluated in a multivariate model to control for confounding effects. Significant variables to  $p < 0.05$  were retained in the final model.  $p$ -values  $< 0.05$  were considered significant. Statistical analyses were performed using “nlme” and “lme4” package from R project. Results are reported as odds ratios (OR) with 95% confidence intervals (CI).

**RESULTS:** Increasing sum (1.034 [1.022 1.046]/ $p < 0.001$ ) and mean diameter (1.129 [1.046 1.219]  $p = 0.002$ ) of the 5 largest follicles increased the probability of forming euploid embryos. Increasing days of stimulation showed a non-significant trend toward lower chance of forming euploid embryos (0.976 [0.923 1.031]/ $p = 0.382$ ) (Table 1).

**CONCLUSIONS:** Allowing the lead follicles to exceed 18mm increases the total number of euploid embryos formed per cycle, presumably by enabling retrieval of additional mature oocytes. Evidence of a detrimental effect of excessive follicle size was not evident in our study, though the number of cycles with follicles exceeding 24 mm was limited. The non-significant trend toward decreased euploid embryos following prolonged stimulation may reflect the effects of poor responders.

TABLE 1.

Variables	Unadjusted OR [95% CI]/p-value	Adjusted OR [95% CI]/p-value
<b>Age (years)</b>		
<35	Reference	
[35, 38)	0.586 [0.399 0.861]/0.007	0.665 [0.447 0.989]/ 0.044
[38, 41)	0.382 [0.265 0.550]/ <0.001	0.371 [0.254 0.541]/ <0.001
[41, 43)	0.158 [0.107 0.233]/ <0.001	0.172 [0.114 0.258]/ <0.001
43+	0.051 [0.030 0.087]/ <0.001	0.067 [0.039 0.117]/ <0.001
<b>Follicle size</b>		1.003 [0.991 1.015]/ 0.609
Maximum		
Top 5 average	1.002 [0.996 1.007]/0.584	
Top 5 sd	1.129 [1.046 1.219]/0.002	
Top 5 sum	1.034 [1.022 1.046]/<0.001	
<b>Stimulation days</b>		
<10	Reference	
[10, 12)	1.050 [0.785 1.405 ]/0.741	
12+	0.876 [0.635 1.207 ]/0.417	
<b>Mature Eggs Retrieved</b>	1.150 [1.124 1.178]/ <0.001	0.976 [0.907 1.051]/ 0.526

with each other within leukocytes or granulosa cells, but not correlated between leukocytes and granulosa cells. In addition, serum anti-Mullerian hormone (AMH) is closely correlated with telomere length and mitochondrial copy number in granulosa cells, but not those in leukocytes. Linear regression model revealed that chronological age is the sole aging biomarker associated with aneuploidy rate of embryo in ART cycles.

**CONCLUSIONS:** Although the serum AMH, telomere length of granulosa cells, mitochondrial copy number in granulosa cells are closely correlated with each other, the chronological age is the main factor to affect aneuploidy rate of embryos in PGT-A cycles. The results suggest that the main source of aneuploidy is oocyte meiosis, especially if the oocyte stayed at diplotene stage of meiosis I for a long period of time.

**SUPPORT:** The study was supported by a grant from Ministry of Science and Technology for Maw-Sheng Lee (MOST 106-3114-B-040-001-).

**BLASTOCYST PLOIDY IS NOT RELATED TO THE NUMBER OF EMBRYOS GENERATED NOR TO THE TYPE OF OVARIAN**



**STIMULATION.** Sandro C. Esteves, M.D., Ph.D.,<sup>a</sup> Peter Humaidan, M.D., Ph.D.,<sup>b</sup> José F. Carvalho, Ph.D.,<sup>c</sup> Danilo Cimadomo, PhD,<sup>d</sup> Alberto Vaiarelli, MD.,<sup>d</sup> Hakan Yarali, M.D.,<sup>e</sup> Irem Y. Ozbek, PhD.,<sup>c</sup> Thor Haahr, MD.,<sup>b</sup> Alessandro Conforti, MD.,<sup>f</sup> Carlo Alvisi, MD, PhD,<sup>f</sup> Filippo Maria Ubaldi, M.D., Ph.D.<sup>d</sup> <sup>a</sup>Medical Director, Campinas, Brazil; <sup>b</sup>The Fertility Clinic, Skive Regional Hospital, Skive, Denmark; <sup>c</sup>Statistika Consulting, Campinas, Brazil; <sup>d</sup>GENERA, Center for Reproductive Medicine, Rome, Italy; <sup>e</sup>Anatolia IVF, Ankara, Turkey; <sup>f</sup>Department of Neuroscience, University of Naples Federico II, Naples, Italy.

**OBJECTIVE:** More than half of human embryos are aneuploid which is the main reason for the decreased live birth rates among advanced maternal age (AMA) patients undergoing Assisted Reproductive Technology (ART). However, concerns were raised regarding a putative detrimental effect of ovarian stimulation (OS) regimens on embryo ploidy status. We aimed to investigate whether euploidy is related neither to the number of blastocysts generated nor to the intensity of ovarian stimulation

**DESIGN:** Multicenter retrospective analysis of 3,108 trophectoderm biopsies from 1,109 infertile couples undergoing ICSI and preimplantation genetic testing for aneuploidy (PGT-A) between 2016 and 2017.

**MATERIALS AND METHODS:** Ovarian stimulation regimens included conventional OS using GnRH antagonist co-treatment (n=1,011 patients) and minimal OS (n=98 patients). PGT-A was indicated due to AMA, severe male factor, recurrent miscarriage, repeated implantation failure, and due to concerns about their embryonic ploidy status. Biopsied trophectoderm cells were analyzed by next-generation sequencing analysis (NGS) or real-time quantitative polymerase chain reaction (qPCR). Logistic regression was applied to the dataset. The dependent variable was blastocyst genetic status (euploid/aneuploid) whereas the independent variables were female age, the number of blastocysts biopsied, and type of OS (conventional versus minimal). Mosaic blastocysts detected by NGS (5.7% of biopsied blastocysts) were excluded from the analysis. Computations were performed using JMP 13 ([www.jmp.com](http://www.jmp.com)).

**RESULTS:** The mean female age was 39.0 years (95% confidence interval [CI]: 34.8-43.0 years), and the mean number of blastocysts available for PGT-A per patient was 3.0 (95% CI: 1.0-5.0). Overall, the percentage of euploid embryos in our cohort was 42.0% whereas the mean number of euploid blastocysts per patient was 0.89 (95% CI: 0.0-3.0). The number of euploid blastocysts per woman followed a negative binomial distribution. The fitted model selected only female age as a predictor (Estimate: -0.18; 95% CI: -0.21; -0.15,  $p < 0.001$ ), whereas the number of blastocysts available for biopsy (Estimate: 0.03; 95% CI: -0.00; 0.06) and type of OS (Estimate: 0.06; 95% CI: -0.59; 0.73) were not significant. The logistic model generated the probability, ‘p’, as an output (where ‘p’ is the probability that a biopsied blastocyst would be euploid) as a function of blastocyst cohort size and type of OS, adjusted by female age. There was a significant ( $p < 0.0001$ ) decrease in the probability of a blastocyst being euploid with every year of female age. However, this probability was not significantly associated with the blastocyst cohort size and type of OS. Introduction of the clinic into the regression model essentially did not change the estimates.

**CONCLUSIONS:** The probability of a blastocyst being euploid decreases with female age but is not affected by blastocyst cohort size and type of OS. This information might aid clinicians counseling patients undergoing ART about their chances of producing euploid blastocysts according to the treatment regimen and the age of the female.

**SUPPORT:** None

**EXOGENOUS GONADOTROPIN USE NOT ASSOCIATED WITH INCREASE IN ANEUPLOIDY OF IN VIVO RECOVERED BLASTOCYSTS.**



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**OBJECTIVE:** Does exogenous gonadotropin stimulation increase the risk of aneuploidy for in vivo blastocysts?

**DESIGN:** We performed 134 stimulated uterine lavage cycles to evaluate the safety and efficacy of a new lavage system (Previvo Genetics, Inc., San Carlos, CA). Patients gave their written informed consent. All lavages were performed in Punta Mita, Mexico from August 2017 to June 2018. Subjects were followed for 30-days post-lavage to monitor for complications. Following completion of this series, retrospective data analysis was performed.

**MATERIALS AND METHODS:** Subjects were pretreated with oral contraceptives and stimulated with gonadotropins. After ovulation was triggered, an intrauterine insemination (IUI) was performed 36 hours after trigger. Uterine lavage occurred 4-6 days after the insemination. Subjects had an endometrial biopsy and given GnRH antagonist after lavage to cause lysis of the corpora lutea. Recovered embryos underwent trophectoderm biopsy, vitrified and stored in liquid nitrogen. Biopsies were analyzed using Next Generation Sequencing (NGS).

**RESULTS:** In 134 uterine lavage cycles, 46 (34%) resulted in recovery of one blastocyst. Mean age and BMI of the subgroup were 26 years and 24.2 kg/m<sup>2</sup>, respectively. Subjects were stimulated for an average of 9.4 days with a mean total gonadotropin dosage of 1789 IU, mean total hMG dose of 888 IU. At the time of trigger, subjects had a mean maximum E2 of 2613 pg/mL (range 394-6377 pg/mL) and 9.6 follicles 16mm.

A total of 96 blastocysts were recovered and biopsied. After the initial biopsy, 37% (33/89) were euploid, 63% (56/89) aneuploid, and 7 blastocysts had no determination. Due to the high rate of aneuploidy, a second biopsy was performed in 64% (61/96) of the blastocysts (10 euploid, 48 aneuploid, 3 no determination). The second biopsy result was used to determine the euploid status of

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**OBJECTIVE:** To determine the effect of adjuvant human growth hormone (hGH) during IVF/PGT-A cycles on euploid embryo rate in women with and without poor ovarian response (POR).

**DESIGN:** Retrospective non-randomized cross over study

**MATERIALS AND METHODS:** The study was carried out at a single clinic site from 2014-2018. Inclusion criteria: women who underwent one cycle of IVF-PGT-A and at least one consecutive IVF-PGT-A cycle with hGH within a 1-year period. hGH (1.45 mg) was started on the first day of ovarian stimulation and continued until trigger. Patients were stratified as POR by Bologna criteria. Women without POR were offered hGH if prior cycles had a suboptimal response or suboptimal blastocyst development as predicted by age/antral follicle count/AMH. Using a two-tailed paired t-test, sample size of 34 was sufficient to detect an effect size of 0.5 or greater at 0.8 power. Paired t-test analysis was performed with GraphPad Prism 8.0 to detect statistical significance of p = 0.05.

**RESULTS:** 51 patients underwent 79 cycles during the study period and met inclusion criteria. Table 1 shows cycle outcomes for POR women compared to non-POR. Interestingly, in POR patients there was a small but statistical increase in number of biopsied blastocysts but no difference in number of euploid embryos/euploid rate. In women classified as non-POR, there were significant improvements in all cycle parameters. When the data was stratified according to age, euploid rate was increased in hGH cycles in both <37 years (8.75% vs. 53.9%, p =0.001) and >38 years (12.7% vs. 26.7%, p = 0.03). However, when stratified by AMH, women with AMH <1 did not show a significant increase in euploid rate (5.26% vs. 11.8%, p=0.4), whereas women AMH>1 did show a significant benefit (14.5% vs. 40.0%, p=0.0003).

**CONCLUSIONS:** Prior studies have focused on use of hGH in poor responder women during IVF, but little is known about the utility in non-POR. This study shows that hGH significantly increases the number of euploid embryos/euploid rate in women who are not poor responders but had suboptimal response or poor embryo development. Interestingly, this study shows that in POR patients, all cycle outcomes are significantly improved by hGH except euploid rate. In conclusion, our study shows the use of hGH should be considered in non-POR women to improve IVF cycle outcomes.

Table 1 IVF-PGT-A cycle outcomes in women with and without adjunct use of hGH: Mean (SD)

	Non-POR (n=60)			POR (n=19)		
	No hGH	hGH	p value	No hGH	hGH	p value
Mature oocytes (MII)	7.7 (4.85)	10 (6.6)	0.0009	3.15 (1.38)	5.5 (2.9)	0.001
Biopsied Blastocysts	1.28 (1.29)	2.83 (2.56)	<0.0001	0.26 (0.45)	0.94 (1.22)	0.03
Euploid Blastocysts	0.23 (0.5)	1.51 (2.4)	<0.0001	0.05 (0.22)	0.26 (0.56)	0.1
% Euploid	14.5 (31.8)	40.0 (42.0)	0.0003	5.26 (22.9)	11.8 (27.4)	0.4

the blastocysts for this analysis resulting in a euploid rate of 53% (49/92), 47% (43/92) aneuploid, and 4 no determinations. In 8.2% (11/134) of the cycles, positive hCG was present 13 days after IUI. The hCG levels in all cycles resolved spontaneously, or after curettage with or without methotrexate.

A logistical regression was performed to determine whether there was any correlation between covariates (days of stimulation, mean total gonadotropins, mean total hMG, mean maximum estradiol and follicles 16mm at trigger) and euploid status. No significant associations were found between any of the variables and euploid status.

**CONCLUSIONS:** This study reinforces existing IVF data that imply gonadotropin stimulation is not associated with higher rates of aneuploidy but now performed in an in vivo data set. These findings support the continued use of stimulation in the process of uterine lavage. However, the sample size is small, and the lavage system is not fully optimized to recover all embryos.

**SUPPORT:** Previvo Genetics, Inc.

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**ADJUVANT GROWTH HORMONE USE IMPROVES EUPLOID RATE IN WOMEN UNDERGOING IVF/PGT-A WHO ARE NOT POOR RESPONDERS.**



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**EMBRYO EUPLOIDY RATES DIFFER IN SAME-SEX MALE COUPLES UTILIZING A SINGLE OVUM DONOR.**



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**OBJECTIVE:** It is common practice for same-sex male couples to each want to provide a semen sample for insemination at the time of IVF using a single ovum donor. This practice provides each individual an opportunity to have a genetically related embryo for future family planning. The objective of this study was to determine if IVF euploidy rates differs among embryos generated using 2 different normospermic males and a single ovum donor.

**DESIGN:** A retrospective data analysis from a single large private fertility center.

**MATERIALS AND METHODS:** Same-sex male couples each collected and provided the IVF laboratory separate semen samples. Semen was processed identically for both samples by gradient separation and washing prior to ICSI. Oocytes were cultured for an hour following donor oocyte retrieval, prior to denudation and oocyte maturity assessment. Mature oocytes were divided equally and placed into labeled culture dishes separately, in the

same incubator for embryogenesis. Fertilization was assessed on Day 1 and resulting embryos were cultured until Days 5, 6 and 7 for blastocyst trophectoderm biopsy. Biopsied samples were PGT-A analyzed by Next Generation Sequencing. The total number of euploid embryos were compared between the two males in each couple. The male partner with the higher number of euploid embryos was denoted as Male A; and the other male with an equal or fewer number of euploid embryos was denoted Male B. The data was analyzed using Chi-square analysis with a significance set at  $p < 0.05$ .

**RESULTS:** The data from eight same-sex male couples was analyzed, with a total of 187 mature oocytes inseminated; 94 and 93 oocytes for Males A and B, respectively. There was a total of 35 euploid embryos (57.4% of those biopsied), 22 for Male A and 13 for Male B, demonstrating a statistically significant difference ( $p < 0.05$ ) in euploidy rates among the groups analyzed.

**CONCLUSIONS:** This study demonstrates that same-sex male couples desiring to generate embryos equally using the same oocyte donor at the time of a single IVF cycle, do not yield an equivalent number of euploid embryos even when both males are normospermic and under the same exact culture conditions. This study indicates that there is likely an underlying sperm factor that impacts euploidy rates even in fertile normospermic males. More research is needed to elucidate the cause of this observation in order to help clinicians in counseling same-sex male couples.

**P-72** Tuesday, October 15, 2019 6:30 AM

### **ANEUPLOIDY RATE IN BRCA CARRIERS IS SIMILAR TO AGE-MATCHED INFERTILE WOMEN.**

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**OBJECTIVE:** *BRCA 1* and *BRCA 2* are tumor suppressor genes involved in DNA mismatched repair. Studies have shown that ovarian aging is accelerated in women with *BRCA* mutations secondary to diminished ovarian reserve and accumulation of DNA damage in the oocytes of primordial follicles. [i] It is unclear whether this DNA damage seen in primordial follicles translates to a higher aneuploidy rate. This study sought out to compare aneuploidy rates between *BRCA 1* and *BRCA 2* mutation carriers undergoing in vitro fertilization (IVF) and preimplantation genetic testing for monogenic condition (PGT-M) and for aneuploidy (PGT-A) with age-matched control women with infertility undergoing IVF/PGT-A.

**DESIGN:** Retrospective analysis of an anonymous database at a commercial genetics laboratory.

**MATERIALS AND METHODS:** This study included *BRCA 1/2* mutation carriers undergoing IVF with PGT-M for *BRCA 1/2* mutations and PGT-A from 2018-2019. Infertile, non-carriers undergoing IVF with PGT-A during the same period were included as controls. All embryos were biopsied at the blastocyst stage. PGT-A for both groups was performed by Next Generation Sequencing (NGS). The primary outcome of this study was to compare the aneuploidy rates between *BRCA* carriers and age-matched controls. For both cases and controls the aneuploidy rates were stratified into four age categories: <35, 35-37, 38-40 years old. Chi square test was used to compare the aneuploidy rates between *BRCA* carriers and non-carriers in the different age groups.

**RESULTS:** A total of 73 *BRCA 1/2* mutation carriers were included in this study. There were 584 embryos tested in the carrier group with a mean number of blastocyst embryos obtained per cycle of 8 (range 4-11). Of the embryos tested, 268 (46%) did not have a pathogenic *BRCA* mutation. A total of 24,850 embryos were tested in the control group. No information regarding incidence of diminished ovarian reserve was available for either group. There was no statistical difference between carriers and non-carriers in all age categories: <35-year-old 42% vs 49% ( $p = 0.117$ ), 35-37 years-old 47% vs 54% ( $p = 0.16$ ), 38-40 years-old 51% vs 63% ( $p = 0.063$ ) respectively.

**CONCLUSIONS:** Aneuploidy rates in *BRCA* carriers were similar to infertile controls of the same age. As *BRCA* carriers become older, the increase in aneuploidy rate is similar to that observed in the general infertile population. *BRCA* carrier status does not seem to affect aneuploidy rates however further studies including non-infertile controls may provide further information on the effect of *BRCA* carrier status and aneuploidy risk.

References: [i] Impairment of *BRCA1*-related DNA double-strand break repair leads to ovarian aging in mice and humans. *Sci Transl Med.* 2013 Feb 13;5(172):172ra21. 2. *BRCA* Mutations, DNA Repair Deficiency, and Ovarian Aging. *Biol Reprod.* 2015;93(3):67

**P-73** Tuesday, October 15, 2019 6:30 AM

### **SUBOPTIMAL STIMULATION IS PREDICTIVE OF INCREASED ANEUPLOIDY AND REDUCED PREGNANCIES PER CYCLE START.**

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**OBJECTIVE:** To correlate a measurable stimulation outcome parameter to its effect on blastocyst (BL) development, aneuploidy and pregnancy outcomes, and determine if oocyte cohort maturity effects the implantation potential of a euploid BL?

**DESIGN:** Oocyte quality is difficult to independently quantify, while the percent of metaphase II (i.e., mature) oocytes within a cohort of cumulus oocyte complexes (COC) retrieved can be used to assess overall stimulation effectiveness. A 5-year (2014-2018) retrospective analysis was conducted on 1264 autologous cycles using PGT-A and 796 vitrified-warmed euploid BL transfers. Cycle cohort maturity was subdivided into patients with  $\geq 70\%$  mature eggs (Group 1) or  $< 70\%$  (Group 2).

**MATERIALS AND METHODS:** Single physician clinic stimulated 1046 patients for 1264 cycles. Oocyte maturity was evaluated, and all fertilized zygotes were grown to BL for biopsy/PGT-A and vitrification all cycles. Only first transfer attempts were compared in the per cycle analysis. Stimulation protocols were predominantly antagonist based. Oocytes were retrieved 35.5h post-hCG trigger, COC denuded 2-3h post-retrieval before ICSI and the % mature oocytes calculated. Comparisons using t-test and chi-square were performed for cycles failing to produce a BL, cycles resulting normal embryos, aneuploidy and implantation.

**RESULTS:** Average patient age was 37 and 38 years old for Groups 1 and 2, respectively ( $p < 0.01$ ), with a mean maturation rate of 88% and 57%. Oocyte maturity  $\geq 70\%$  (Group 1) occurred in 1038 cycles, while 226 cycles (18%) were identified as having suboptimal cohort maturity (Group 2). Group 2 had a reduced ( $p < 0.01$ ) mean BL yield of 2.4 blastocysts per cycle compared to 5.2 per Group 1 cycle. Yet, % BL production per cycle was not significant. Group 2 BL had a higher ( $p < 0.01$ ) aneuploidy rate (63 vs 56%) and more cycles failed to yield a normal embryo (52 vs 33%). Implantation of euploid BL derived from either group was not statistically different averaging 77%. While our overall live birth rate, independent of age, exceed 65% per euploid ET, the overall clinical pregnancy rate per cycle start was lower for Group 2 (35%) than Group 1 (52%).

**CONCLUSIONS:** It is well understood that multiple factors influence stimulation and oocyte maturity. Nonetheless, after a half decade of data collection, this study has identified a measurable outcome, oocyte cohort maturity, which predicts an increased risk of aneuploidy, a decreased euploid cycle outcome and embryos with a reduced implantation potential. Cohort maturity is influenced by several factors, including age, AMH, FSH, stimulation protocol, and endocrine/ovarian conditions. When those factors produce suboptimal maturity, cycles are adversely affected, likely due to incomplete cytoplasmic maturation of fertilized zygotes. A further understanding of the genetic regulation/omics, oocyte in-vivo genetics and basic cell receptor biology is needed to better identify why suboptimal cycle maturity negatively effects the developmental potential of the pending BL as assessed by PGT-A.

**SUPPORT:** None

### **FERTILITY PRESERVATION**

**P-74** Tuesday, October 15, 2019 6:30 AM

### **ESTIMATES OF INFERTILITY IN AN ERA OF INCREASING STI RATES, 2002-2015.**

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**OBJECTIVE:** Has the decline in infertility remained uniform across subgroups? How do factors like PID and status of STI care affect infertility?

**DESIGN:** Pelvic inflammatory disease (PID) has declined in an era of increasing sexually transmitted infection (STI) rates. Meanwhile, access to sexual and reproductive health (SRH) services remains tenuous for young and low-income women. This study aims to estimate the changes in infertility from 2002 to 2015 and explain the impact of PID and receipt of SRH services on fertility in the United States.

**MATERIALS AND METHODS:** Periodic data from the 2002, 2010, 2013, and 2015 cycles of the National Survey for Family Growth (NSFG) were

used for this analysis. The NSFG is comprised of samples of the household-level population of women aged 15 to 44 years in the United States, collected by the National Center for Health Statistics. We used the measure of infertility as constructed by the NSFG, which defines married or cohabiting women as “infertile” if she has not conceived over a period of greater than a year of unprotected intercourse with a male spouse or cohabiting partner. We first examined the rates of infertility across subgroups of married or cohabiting women. We then performed bivariate and multivariate logistic regression models using the pooled sample (N=14,208) to determine the effect of individual-level characteristics, including age, parity, PID treatment, education, income, race or ethnicity, and receipt of SRH services, on the odds of 12-month infertility among married or cohabiting women.

**RESULTS:** The decline in infertility among married and cohabiting women from 7.0% in 2002 to 5.8% in 2010 is significant; the increases to 6.3% and 7.0% in 2013 and 2015 respectively, however, are not. This trend was present across nearly all subgroups. The multivariate model showed that women who were nulliparous, had fewer years of education, or were not receiving SRH services were more likely to be infertile.

**CONCLUSIONS:** This study confirms that parity and education level continue to impact infertility. Further, the results demonstrate that access to SRH services plays an important role in infertility. In contrast to previous studies, infertility in the United States is no longer on the decline, and age, race, and ethnicity did not have significant impacts on infertility. Given the rise of STIs and the persistent lack of access to SRH services, particularly among already vulnerable groups, the connection between access to care and infertility is ripe for further investigation.

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#### **FERTILITY PRESERVATION FOR SOCIAL REASONS IN A POPULATION OF OLDER WOMEN. ¿MYTH OR REALITY?**

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**OBJECTIVE:** Oocyte cryopreservation for social reasons in older women is increasingly being performed in Argentina. Reproductive results derived from such approach remains controversial. The aim of this study was to evaluate the reproductive performance of our oocyte vitrification program in this older population that chose fertility preservation for social reasons.

**DESIGN:** Retrospective descriptive study.

**MATERIALS AND METHODS:** The results of our Anticipated Gamete Exhaustion (AGE banking) program during the 2008-2017 year-period are presented. A total of 490 women were included in our study. Of these 80.8% were  $\geq$  36 years old (396/490); 265 were between 36-39 and 131  $\geq$  40 years old at the time of vitrification.

**RESULTS:** The average age of the patients at the time of oocyte vitrification was  $37.6 \pm 3.5$  years old, and the number of vitrified MII oocytes was  $6.2 \pm 4.9$ . Only 32 women (6.5%) used their oocytes stored to date. In this group, the average age at cryopreservation was  $39.1 \pm 2.9$  years and the average storage time was  $2.8 \pm 1.8$  years. The average age at the time of thawing was  $41.9 \pm 3.4$  years old. The average number of vitrified oocytes was  $5.2 \pm 3.1$  with a survival rate of  $96.5 \pm 9.0\%$ . The average number of injected oocytes was  $5.0 \pm 3.0$  and  $3.5 \pm 2.2$  achieved fertilization. Fertilization rate was  $70.7 \pm 27.8\%$ . The average number of cleaved embryos was  $3.2 \pm 2.0$  and the average number of day-3 embryos that were transferred was  $2.2 \pm 1.0$ . Seventy-five percent of women who thawed their vitrified oocytes used sperm samples belonging to their male partners (24/32) while 25% (8/32) used donor sperm at the time of the procedure. A total of 36 ICSI procedures were performed in 32 women and 34 embryo transfers were done; two of the patients had no embryos for transfer. A total of 79 embryos were transferred. Clinical pregnancy rate was 29.4% (10/34), implantation rate was 12.6% (10/79), abortion rate 20% (2/10) and live birth rate was 23.5% (8/34). The live birth/cryopreserved oocyte rate was 5.1% (8/158). Of the 8 births recorded, 3 corresponded to women who vitrified their oocytes at 40 years of age, 2 at 41 years, 1 at 37 years, 1 at 38 years and 1 at 42 years.

**CONCLUSIONS:** According to our results, it is a myth that cryopreservation of any number of oocytes at any age in patients who choose to postpone their motherhood for social reasons ensures future biological motherhood. For that matter, it is a reality that a clear advice, based on the number of oocytes retrieved and the age of the patient at the time of cryopreservation, should be given regarding the real possibilities of becoming a biological

mother when later using the cryopreserved material. Nonetheless, the ready availability of this reproductive strategy in this older age group offers, perhaps, the only chance of having their own genetic children in the future.

**P-76** Tuesday, October 15, 2019 6:30 AM

#### **THE QUANTIFICATION OF RESIDUAL CRYOPROTECTANTS IN THE THAWED OVARIAN TISSUE FOR OVARIAN TISSUE TRANSPLANTATION.**

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**OBJECTIVE:** Ovarian tissue cryopreservation for cancer patients has been gradually increasing in numbers. Though the standard method for ovarian tissue cryopreservation is slow freezing, the simple and feasible vitrification method has been gaining popularity, especially after the reports of live births using vitrification. Since vitrification uses high concentration of cryoprotectant, the safety concerning the residual cryoprotectant in thawed tissues should be verified.

**DESIGN:** This study quantified the residual cryoprotectant in thawed ovarian tissue and demonstrated the minimal culturing time needed before transplantation.

**MATERIALS AND METHODS:** Bovine ovaries were used to make ovarian tissue pieces (10x10x1mm) for slow freezing (DMSO 1.5M and PrOH1.5M) and vitrification (EG 35%). One week later, the frozen ovarian tissues were thawed/warmed in media for either 60 minutes or 120 minutes. Then the amount of residual cryoprotectant in the thawed ovarian tissue were measured by gas chromatography.

**RESULTS:** Before thawing, DMSO and PrOH concentration were  $8.77 \pm 0.19\%$  and  $7.77 \pm 0.75\%$  in slow frozen ovarian tissues, respectively, and EG concentration was  $27.9 \pm 1.63\%$  in vitrified ovarian tissues. Immediately after thawing, DMSO and PrOH concentration dropped to  $0.71 \pm 0.18\%$  and  $0.66 \pm 0.08\%$ , respectively; however, EG concentration remained relatively high ( $3.17 \pm 0.13\%$ ). After 60 minutes media culturing, DMSO, PrOH and EG concentrations were measured at  $0.0072 \pm 0.0027\%$ ,  $0.025 \pm 0.012\%$  and  $0.038 \pm 0.011\%$ , respectively. When doubling the media culturing time to 120 minutes DMSO, PrOH and EG concentration to minimal at  $0.00078 \pm 0.00046\%$ ,  $0.0038 \pm 0.0016\%$  and  $0.0093 \pm 0.0069\%$ , respectively.

**CONCLUSIONS:** The ovarian tissues, cryopreserved either by slow freezing or vitrification, needs to be thawed/warmed for at least 120 minutes in media to completely remove the cryoprotectants from the thawed ovaries. The concentration of cryoprotectants is removed by free diffusion. This research demonstrated the safety of thawed ovarian tissue for transplantation.

**References:** Yusuke Nakamura, Ryuichiro Obata, Noriyuki Okuyama, Nobuya Aono, Tomoko Hashimoto, Koichi Kyono. Residual ethylene glycol and dimethyl sulphoxide concentration in ovarian tissue during warming/thawing steps following cryopreservation. *Reprod Biomed Online*. 2017 Sep;35(3):311-313.

**SUPPORT:** Grant-in-Aid for Scientific Research(B), Nao Suzuki.

**P-77** Tuesday, October 15, 2019 6:30 AM

#### **EMPLOYER-BASED INSURANCE COVERAGE DRAMATICALLY INCREASES UTILIZATION OF PLANNED OOCYTE CRYOPRESERVATION**

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**OBJECTIVE:** Planned oocyte cryopreservation (OC) is gaining recognition in the public and medical communities as a viable option for fertility preservation. However, cost is a significant barrier to planned OC utilization for many women, and most insurance plans do not include this benefit. Beginning in 2018, our tertiary care academic medical center initiated coverage for planned OC. The purpose of this study is to determine the impact on planned OC utilization at our center in the year immediately prior to and the year of insurance coverage commencement for employees.

**DESIGN:** Retrospective Cohort Study

**MATERIALS AND METHODS:** Planned OC cycles from 2017 and 2018 were analyzed. Patient demographics and cycle outcomes were compared

between cycles occurring in 2017 vs. 2018 according to insurance coverage and insurance type, maternal age, number of oocytes retrieved, and number of oocytes frozen. Only a patient's first cycle was included in the final analyses. Two-tailed Fisher's exact tests were performed;  $p < 0.05$  determined significance.

**RESULTS:** Between January 2017 and December 2018, 123 unique patients presented to our fertility clinic and underwent planned oocyte cryopreservation. Patient age ranged from 23 to 43 years and the mean age did not significantly differ between 2017 and 2018 (34.9 vs. 35.2, respectively). There was a 12% overall increase in planned OC utilization from 2017 (N=58) to 2018 (N=65). Significantly more patients had any insurance coverage in 2018 vs. 2017 (71.9% vs. 40.4%,  $p=0.001$ ), a 78% increase. From 2017 to 2018, the number of patients undergoing planned OC who were employees with hospital-based insurance coverage increased by a factor of 9 (5% to 41.5%,  $p < 0.001$ ). In contrast, the number of self-pay patients significantly decreased from 2017 to 2018 ( $p=0.001$ ). No significant differences were found regarding cycle outcomes, including number of oocytes cryopreserved.

Variable	2017	2018	P-value
% with any insurance coverage	71.9	40.4	0.001
% employees	5	41.5	<0.001
Age (y)	35.0	35.2	NS
AMH (ng/mL)	2.94	3.13	NS
Number oocytes retrieved	14.6	16.2	NS
Number oocytes cryopreserved	11.6	12.5	NS

**CONCLUSIONS:** A greater proportion of women seeking planned OC at our facility had insurance coverage for treatment in 2018 vs. 2017. Employer-based insurance coverage for planned OC yielded a significant increase in planned OC utilization by hospital employees. This data underscores the impact insurance coverage has on planned OC utilization rates in just one year. As awareness of coverage increases and other employers begin to expand benefits, we expect planned OC utilization rates to continue to rise.

**P-78** Tuesday, October 15, 2019 6:30 AM

**OOCYTE VITRIFICATION FOR ANTICIPATED GAMETE EXHAUSTION (AGE-BANKING) - A SYSTEMATIC REVIEW AND META-ANALYSIS OF SOCIAL TRENDS AND EFFICACY.** Shira Baram, MD,<sup>a</sup>



Noga Fuchs Weizman, MD,<sup>a</sup> Janice Montbriand, Ph.D,<sup>b</sup> Clifford Lawrence Librach, MD.<sup>a</sup> <sup>a</sup>CreAtE Fertility Centre, Toronto, ON, Canada; <sup>b</sup>Department of Obstetrical Anesthesia, Sunnybrook Health Sciences Centre, Toronto, ON, Canada.

**OBJECTIVE:** To explore current trends in attitudes and knowledge of oocyte vitrification freezing (VF) for fertility preservation, as well as to provide an update on the efficacy of the process.

**DESIGN:** A systematic review and meta-analysis.

**MATERIALS AND METHODS:** We conducted a systematic search using PubMed/MEDLINE, EMBASE, the Cochrane Database and PsychINFO, using appropriate controlled vocabulary, to identify all relevant studies published from Jan 2007 to Nov 2018. The review protocol followed PRISMA guidelines in PECO format, and was registered with PROSPERO (#CRD42019128268). The protocol was comprised of two parts; the first addressed attitudes and knowledge regarding AGE-banking, the second focused on evaluating AGE-banking efficacy and outcomes while comparing these metrics with efficacy and outcomes in vitrified donor oocytes and in cases of supernumerary oocytes vitrified following infertility treatments. Only original articles published in peer-reviewed journals written in English were included.

**RESULTS:** The literature search yielded 8038 articles of which 58 were included in the meta-analysis: 20 in the section exploring attitudes towards AGE-banking, and 38 in the section exploring its efficacy. Most respondents were aware of AGE-banking, mainly from online sources, and believed the ideal timing for AGE-banking is before women turn 35y/o. Only 40% of respondents answered correctly, when asked about the procedure and its associated risks and anticipated success rates. While two-thirds endorse AGE-banking for others, only a third would consider it for themselves. The

main factors affecting the decision whether or not to perform AGE-banking in declining order were; 1. being wary of potential health implications, 2. perceived low success rate of the procedure, 3. financial considerations, and 4. time commitment. The results of AGE-banked vitrified oocytes, were favorable and approached those obtained utilizing donor oocytes, with a post-thaw survival of 84%, fertilization rate of 74%, cleavage rate of 89%, implantation rate of 41%, clinical pregnancy rate of 50%, and live birth rate of 32%. Results obtained by utilizing supernumerary vitrified oocytes lagged behind. Currently there is no available data regarding blastulation rate and embryo quality for embryos derived from AGE-banked oocytes.

**CONCLUSIONS:** AGE-banking provides a reasonable and adequate method for preserving fertility, yet gaps in attitudes and knowledge, as well as affordability, result in under-utilization. This review points to a general lack of awareness regarding the process, its efficacy, and the ideal time to pursue AGE banking. Future research should include large scale cohort studies to further evaluate changes in attitudes and knowledge. There is also a need for establishing international registries for AGE-banking that would provide information on the efficacy of the process as well as on related health implications.

**SUPPORT:** Create Fertility Centre

**P-79** Tuesday, October 15, 2019 6:30 AM

**FERTILITY PRESERVATION: FROZEN IN TIME?** Stephanie R. Baum, MD,<sup>a</sup> Randi H. Goldman, M.D.,<sup>b</sup> Tomer Singer, MD,<sup>c</sup> Christine Mullin, M.D.<sup>d</sup> <sup>a</sup>Lenox Hill Hospital - Northwell Health, New York, NY; <sup>b</sup>Northwell Health Fertility, Manhasset, NY; <sup>c</sup>NY; <sup>d</sup>Northwell Health Fertility, Zucker School of Medicine at Hofstra/Northwell, Manhasset, NY.



**OBJECTIVE:** There has been an increase in the number of patients opting to undergo oocyte and embryo banking since removal of the "experimental" egg freezing label. However, to date, relatively few women have returned to use frozen gametes. The purpose of this study is to examine the relationship between number of oocyte and embryo banking cycles and use of previously banked oocytes and embryos.

**DESIGN:** Retrospective study of select SART-affiliated clinics

**MATERIALS AND METHODS:** A total of 179,982 cycles from 69 SART-affiliated clinics from four states (Georgia, Illinois, Massachusetts, and New York) were identified; 10 clinics were excluded due to missing SART data. Information on number of oocyte and embryo banking cycles as well as number of cycles from previously frozen oocytes and embryos was collected from the years 2015-2017. The ratio between number of new oocyte and embryo banking cycles to cycles utilizing previously banked oocytes and embryos was calculated.

**RESULTS:** From 2015 to 2017, there was an increase in the total number of cycles from 54,540 to 65,138, representing an increase of approximately 19%. The number of embryo banking cycles was largely unchanged in this time frame, ranging from 918 to 1,007, approximately 1.5-1.7% of the total number of cycles. The number of oocyte banking cycles increased linearly from 2,563 to 3,185, representing an increase of 19.5%. In 2015, 2016, and 2017, the number of embryo banking cycles converted from fertility preservation was 25%, 18%, and 20% of the total number of embryo banking cycles, respectively. The number of oocyte banking cycles converted from fertility preservation was only about 1-1.5% of the number of oocyte banking cycles.

**CONCLUSIONS:** Fertility preservation continues to be a major focus of reproductive health and utilization of planned oocyte cryopreservation is increasing. In this select population, the number of embryo banking cycles converted from fertility preservation cycles is approximately 20-25% of the number of embryo banking cycles, but the number of oocyte banking cycles converted from fertility preservation remains a small percentage of the number of oocyte banking cycles. To date, relatively few women have returned to use their frozen gametes. We expect this percentage to rise.

References: None

**SUPPORT:** None

**P-80** Tuesday, October 15, 2019 6:30 AM

**PROVIDING PATIENTS WITH SUBSIDIZED FERTILITY PRESERVATION SERVICES IMPROVES ACCESS TO CARE.** Lauren T. Bouchard, MD,<sup>a</sup> Kristin Van Heertum, MD,<sup>b</sup> Amelia Baffa, MSN, RN, PMHNP-BC,<sup>c</sup> Kathryn D. Coyne, MD,<sup>d</sup> Daniel L. Kuhr, MD,<sup>e</sup> James Goldfarb, MD, MBA,<sup>f</sup> Rachel S. Weinerman, MD.<sup>b</sup> <sup>a</sup>University



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**OBJECTIVE:** Fertility preservation counseling is recommended for all patients undergoing fertility impacting cancer treatments. Although multiple options exist, patients face significant barriers to fertility preservation including knowledge deficits, access issues, possible treatment delays, psychological stress and high cost. Our fertility division has partnered with a non-profit foundation to provide fully funded fertility preservation to patients undergoing cancer treatment. The goal of this project is to explore referral patterns and follow up for fertility preservation when all options are provided with no cost to the patient.

**DESIGN:** Retrospective chart review

**MATERIALS AND METHODS:** All female patients ages 15-39, with cancer or precancerous diagnoses, who were referred for outpatient fertility preservation between 2010-2018. All procedure and monitoring costs were covered by the foundation for patients who did not have insurance coverage for fertility preservation and the met the income criteria. Data on demographics, cancer treatment and fertility preservation were collected from medical records. Outcomes included age, insurance status, fertility preservation treatment offered, and acceptance and follow-through of fertility preservation services.

**RESULTS:** 122 women met the inclusion criteria with a mean age of 28 at the initial visit. 90.2% (n=110) of patients presented for the scheduled appointment. Of those scheduled, 26.7% had public insurance, 66.7% had private insurance, 5.0% were uninsured and 1.6% had no insurance data available for review. 95.5% (n=105) of patients were offered oocyte or embryo cryopreservation. 66% (n= 69) of those patients who were offered fertility preservation followed through with cryopreservation. 40% of uninsured patients and 56% of those with public insurance underwent oocyte/embryo cryopreservation.

**CONCLUSIONS:** Fertility preservation counseling is essential in the care of adolescent and reproductive-aged patients with cancer. However, fertility preservation can be expensive and is often not covered by insurance, which limits many patients' abilities to proceed with oocyte or embryo cryopreservation. The majority of patients in this study not only presented for their appointments but also followed through with treatment when offered in the setting of a cost-covering grant. This type of non-profit foundation could serve as a model for others practicing in states without broad insurance coverage for fertility preservation.

**P-81** Tuesday, October 15, 2019 6:30 AM

**SPERM CRYOPRESERVATION FOR FERTILITY PRESERVATION IN MEN WITH NON-MALIGNANT DISEASES.** Tepei Takeshima, M.D., Shinnosuke Kuroda, M.D., Yasushi Yumura, Ph.D. Yokohama City University, Medical Center, Yokohama, Japan.



**OBJECTIVE:** Advanced cancer treatments have improved the prognosis of cancer survivors. Simultaneously cancer treatments such as chemotherapy have been known to cause harmful effect on fertile capacity. Therefore, the peri-treatment fertility preservation in AYA patients is of crucial importance and recommended in the guideline of ASCO. However, as for non-malignant disease, there have been few reports on peri-treatment fertility preservation ever. Gonadotoxic agents such as alkylating agents are often used for non-malignant diseases. Of course, pretreatment fertility preservation for such cases are thought to be essential.

**DESIGN:** In this study, we retrospectively investigated the cases sperm cryopreservation was attempted for non-malignant diseases.

**MATERIALS AND METHODS:** This study retrospectively assessed the medical records of patients with non-malignant diseases who attempted sperm cryopreservation at the Reproduction Center of Yokohama City University Medical Center between January 2012 and September 2017. The following information was extracted from the medical records: age, type of disease, timing of consultation (the status prior to or immediately after treatment), treatment to be performed or ongoing treatment, pre-freeze semen parameters, success or failure of cryopreservation, and maintenance status of cryopreservation. Moreover, we compared semen characteristics and feasibility of cryopreservation with cases of malignancies observed during same period in our facility.

All patients provided written informed consent prior to their participation. The study design was approved by the institutional review board of our facility.

**RESULTS:** A total of 217 patients were referred and attempted sperm cryopreservation in Yokohama City University Medical Center from January 2012 to September 2017. Of those, 12 patients (5.5 %) were in status pre-treatment of non-malignant diseases at the time of consultation.

The median age was 29.5 years (range: 18–51 years). Breakdown of original diseases was aplastic anemia (3), interstitial pneumonia (2), eosinophilic granulomatosis with polyangiitis (2), and others (5: collagen disease etc). Breakdown of therapeutic regimen was cyclophosphamide with hematopoietic stem cell transplantation (9), cyclosporine (1), and methotrexate (1). Mean sperm concentration was significantly higher than that of patients with malignancies ( $58.26 \pm 42.53$  vs.  $27.13 \pm 29.08$  million/ml,  $P < 0.001$ ). And in all cases, sperm cryopreservation was successfully carried out. Of the 5 cases referred from our own institution, 3 were still in maintenance, and in 2 cases, samples were discarded on their request. On the other hand, of 7 cases referred from other institutions, 5 patients have not visited our hospital.

**CONCLUSIONS:** For patients with non-malignant diseases, pretreatment sperm cryopreservation should be carried out before gonadotoxic treatment. In addition, establishing a network that encourages patients to visit us for maintenance of cryopreservation is thought to be essential because patients from other facilities did not visit for maintenance at a higher rate.

**P-82** Tuesday, October 15, 2019 6:30 AM

**EQUAL OPPORTUNITY FOR ALL? AN ANALYSIS OF RACE AND ETHNICITY IN FERTILITY PRESERVATION (FP) IN A MAJOR AMERICAN CITY.** Paxton E. Voigt, BA,<sup>a</sup> Jennifer K. Blakemore, MD,<sup>b</sup>

David H. McCulloh, Ph.D.,<sup>c</sup> Mary Elizabeth Fino, MD.<sup>d</sup> <sup>a</sup>NYU School of Medicine, New York, NY; <sup>b</sup>NYU Langone School of Medicine, New York, NY; <sup>c</sup>NYU Langone Health, New York, NY; <sup>d</sup>NYU Langone Fertility Center, New York, NY.



**OBJECTIVE:** It has been suggested that socio-demographic factors may affect access to FP opportunities<sup>1</sup>. In one of America's most racially diverse cities, we sought to compare the racial make-up of patients with cancer (Ca) who completed FP against the overall racial diversity (including Hispanic origin) identified in the incidence of Ca in women of reproductive age in our city.

**DESIGN:** A retrospective cohort study and cross-sectional comparison of all medical embryo banking (Em) and egg freezing (Eg) cycles from 1/2017-12/2018 at our center.

**MATERIALS AND METHODS:** All patients who completed at least one medical Em or Eg cycle were reviewed. Race was self-reported at time of consultation. A calculation of the expected incidence of Ca by race in women of reproductive age in our city was determined using the most recent Ca incidence data by race<sup>2</sup> and available city census data by race, age and gender<sup>3</sup>. Statistical analysis included chi square goodness of fit and test for independence where appropriate, with  $p < 0.05$  considered statistically significant.

**RESULTS:** 107 patients who completed medical FP were included. Overall, 55 (51.4%) identified as White, 3 (2.8%) as Black, 13 (12.2%) as Asian, 6 (5.6%) as Hispanic, 3 (2.8%) as other and 27 (25.2%) did not report. 40.2% of patients were diagnosed with Breast Ca, 15.0% Gynecologic Ca, 15.0% Hematologic Ca, 5.6% Neurologic Ca, 4.7% GI Ca, 4.7% Sarcoma, 3.7% Endocrine Ca, 2.8% other Ca and 7.5% tested BRCA+ with scheduled BSO. There was no significant difference in racial distribution by Ca type ( $p=0.255$ ). A subgroup analysis excluding the BRCA+ patients and those races not reported by the census<sup>3</sup> ( $n=69$ ) was then performed to compare the racial distribution of patients who completed medical FP at our center with the racial distribution of women of reproductive age who were diagnosed with Ca in our city. Based on the calculated frequency of race within the incidence of Ca in women of reproductive age (42% White, 21% Black, 8.9% Asian, 2.8% Hispanic), an expected number of FP cases for each race was calculated and compared. Results show that there is a statistically significant difference between observed (O) and expected (E) cases of FP by race at our center; White 470/29E, Black 30/15E, Asian 130/6E and Hispanic 60/19E ( $X^2$  36.9, df 3,  $p < 0.001$ ). This FP subgroup was further analyzed by FP type [Em ( $n=31$ , 44.9%) vs Eg ( $n=38$ , 55.1%)]. A statistically significant difference in racial distribution by FP type was observed; White 66.0% Eg vs 34.0% Em, Black 33.3% Eg vs 66.7% Em, Asian 46.2% Eg vs 53.8% Em and Hispanic 0% Eg vs 100% Em ( $X^2$  10.60, df 3,  $p < 0.014$ ).

**CONCLUSIONS:** There is a difference in the observed versus expected racial distribution of patients completing medical FP at our clinic, as well as a difference in the racial distribution between procedure types (Eg vs Em). Black and Hispanic patients were underrepresented in FP and White patients had a higher incidence of Eg, while non-White patients had a higher incidence of Em. Further studies are needed to determine if these differences generalize beyond our clinic and to identify modifiable factors that can improve equal opportunity to all patients.

**References:** 1. Letourneau JM, Smith JF, Ebbel EE, Craig A, Katz PP, Cedars MI & Rosen MP. Racial, Socioeconomic, and demographic disparities in access to fertility preservation in young women diagnosed with cancer.

2. National Cancer Institute. (2019, April 9). State Cancer Profiles. Retrieved April 9, 2019, from <https://statecancerprofiles.cancer.gov/quick-profiles/index.php?state=newyork>

3. United States Census Bureau. (2018, July 1). Population estimates. Retrieved April 9, 2019, from <https://www.census.gov/quickfacts/fact/table/newyorkcitynewyork/PST045217>

**SUPPORT:** None

**P-83** Tuesday, October 15, 2019 6:30 AM

**WHAT IS IMPORTANT TO WOMEN CONSIDERING FERTILITY PRESERVATION BEFORE CANCER TREATMENT? COMPARING DECISION-MAKING VALUES WITH AND WITHOUT USING THE PATHWAYS PATIENT DECISION AID WEBSITE.**



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<sup>a</sup>Baylor College of Medicine, Houston, TX; <sup>b</sup>The University of Texas MD Anderson Cancer Center, Houston, TX.

**OBJECTIVE:** Deciding whether to undergo fertility preservation treatment prior to initiating cancer treatment is a complex personal decision. Patient decision aids have been proposed to help women navigate these decisions by providing up-to-date, balanced information and helping women clarify how they value key factors in their decision. The objective of this study was to compare female cancer patients' decision-making values (i.e., the importance of 10 key factors) and treatment preferences with and without the use of the *Pathways* patient decision aid website.

**DESIGN:** Randomized Controlled Trial

**MATERIALS AND METHODS:** *Pathways – a fertility preservation patient decision aid website for women with cancer* explains the risk of cancer-related infertility and describes fertility preservation treatments (tailored by cancer type) and other family-building options. It also provides structured decision-making activities to help women personalize the information and prepare to discuss the options with their providers. Thirty newly-diagnosed reproductive-age women were randomized to view *Pathways* or standard educational brochures, then rate how important 10 patient-identified key factors were in their decision (“Not Sure”, or from 0 = Not Important to 10 = Very Important) and to indicate their treatment preferences. At 2 months, women indicated whether they had completed a fertility preservation treatment.

**RESULTS:** Among the 10 factors, women rated *Avoiding regret about my decision, Starting my cancer treatment as soon as possible, and Being able to genetically screen my future child for cancer* as most important in their decision-making (9.4, 9.2, and 7.5 out of 10). As expected, decision-making values were highly individual and no systematic differences were observed between groups. Women who viewed the patient decision aid were more

confident in their treatment preferences (9.3 versus 8.2 out of 10). All of the women in the control group indicated they were *Not Sure* or would *Wait and See*, while half of the women who viewed *Pathways* chose egg or embryo freezing. However, only 11 women were able to complete the study and only one woman had chosen fertility preservation at 2 months.

**CONCLUSIONS:** Interacting with an interactive patient decision aid may help women become more clear and confident in their fertility preservation decisions. It may also help providers assess patients' values and preferences. However, addressing dissemination challenges may be key in providing timely care for all women.

**P-84** Tuesday, October 15, 2019 6:30 AM

**ELECTIVE OOCYTE CRYOPRESERVATION COUNSELING TOOL BASED ON NEXT GENERATION SEQUENCING RESULTS.** Mariana Miguens, M.D.,<sup>a</sup> Andrea Natalia Coscia, MD,<sup>a</sup> Daniela Lorenzi, B.Sc.,<sup>b</sup> Melina Elena Bilinski, B.Sc.,<sup>b</sup> Mariana Cecilia Calvo, MD,<sup>a</sup> Rocío Belén Anria, M.D.,<sup>a</sup> Milfra Espinal, MD,<sup>a</sup> Sergio D. Papier, Sr., M.D.<sup>a</sup> <sup>a</sup>CEGYR, Ciudad Autonoma de Buenos Aires, Argentina; <sup>b</sup>NOVA-GEN, Ciudad Autonoma de Buenos Aires, Argentina.



**OBJECTIVE:** The aim of this study was to determine the appropriate age for counseling and referral in fertility preservation, based on the number of mature oocytes (MII) needed to achieve an euploid embryo. We consider that age is the main variable that determines the quantity and quality of oocytes as well as the average and ploidy of embryos obtained.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** 150 patients who performed preimplantation genetic testing of aneuploidies (PGT-A) cycles from 2016 to 2018 at a private IVF practice were included for this study. The molecular analysis was performed by Next-generation sequencing (Veriseq-PGS, Illumina).

The age range was between 27 and 46 years. Patients were arbitrarily divided into four groups: <35, 35-37, 38-40 and >40 years.

The variables analyzed per cycle were: the number of metaphase II oocytes (MII), the average of euploid blastocyst, the number of MII required to obtain an euploid blastocyst. Statistical analysis was performed by ANOVA

**RESULTS:**

**CONCLUSIONS:** Oocyte cryopreservation by social reasons represents a legitimate exercise of women reproductive autonomy. One of its main advantages is that oocytes can be stored for a long time without this implying a quality detriment. There is a worldwide tendency to postpone motherhood. As a woman ages, the chance of having an aneuploid embryo increases. PGT-A is an alternative to perform in advanced maternal age.

Based on our results, we can infer that oocyte recovery is lower after the age of 35, affecting mainly patients older than 37 years old. Aneuploidy increases after 38 years old. The most relevant data for counseling is the number of MII oocytes required to obtain an euploid embryo. Also, we can conclude that up to 35 years old, patients would have enough oocytes to have at least two euploid embryos in a single cycle. Between 35-37 years old patients would only achieve one euploid embryo per cycle. After 38 years, between two to four cycles would be needed to have an euploid embryo.

Women age has a great impact on her reproductive capacity. We recommend assessment on fertility preservation between 30-34 years as the first approach and a second assessment between 35-37 years old.

Our conclusions emerge from an indirect analysis of the embryonic ploidy and there is still a need for comprehensive studies to develop an accurate clinical counseling tool.

Age group	Number of cycles	MII average / cycle	Euploid blastocysts average / cycle	MII required to achieve at least one euploid blastocyst
<35	18	13.28 (Min:5 Max: 24)	1.89	7.02 (CI95%: 4.08-9.03) *
35-37	33	9.42 (Min:2 Max: 26)	1.15	8.19 (CI95%: 6.59-10.07)*
38-40	55	8.15 (Min:3 Max: 22)	0.53	15.38 (CI95%: 14.16-16.75)*
>40	44	8.18 (Min:1 Max: 24)	0.29	28.2 (CI95%: 26.83-29.6)*

(\*p<0.05, min: minimum, max: maximum)

## INVITRO OOCYTE MATURATION

P-85 Tuesday, October 15, 2019 6:30 AM

### ENHANCING REPRODUCTIVE OPPORTUNITIES: THE BIOLOGIC POTENTIAL OF VITRIFIED *IN-VITRO* MATURED OOCYTES.

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**OBJECTIVE:** Oocyte *in-vitro* maturation (IVM) is a technique aimed to maximize reproductive potential for patients undergoing fertility preservation. Patients who suffer from compromised ovarian reserve, poor oocyte quality, or low number of MII oocytes at retrieval may benefit from employing IVM prior to cryopreservation. Over the past decade, optimization of oocyte maturation and cryopreservation techniques has enhanced cellular survival rates. More recent studies have suggested clinical utility of vitrified/thawed IVM oocytes (1), but data remains limited within the literature regarding reproductive potential. This study aims to assess the reproductive potential and genomic composition of blastocysts derived from vitrified/thawed IVM oocytes.

**DESIGN:** Retrospective cohort analysis.

**MATERIALS AND METHODS:** The study included all patients who underwent an elective oocyte vitrification cycle(s) with subsequent thawing and fertilization from 2010 and 2019. After oocyte retrieval, immature oocytes (Metaphase I and Germinal Vesicle stages) were cultured and were first assessed for maturity after 6 hours: Early-IVM (E-IVM). A second assessment was performed after 24 hours in culture: Late-IVM (L-IVM) as described by Escrich L et al. Matured oocytes were vitrified/thawed, underwent ICSI, and cultured sequentially to blastocyst stage. Cohorts were segregated into 2 groups: E-IVM and L-IVM oocytes. Fertilization, blastulation, and euploid rates were compared among cohorts.  $\chi^2$ , T-test, and logistic regression analyses were performed, significance was considered at ( $p < 0.05$ ).

**RESULTS:** 292 IVM oocytes obtained from 105 patients were thawed over the course of the study. 203 oocytes were E-IVM, while 89 oocytes were L-IVM. No differences were found in survival rates (81.2%, 80.8%,  $p=0.94$ ), fertilization rate (53.9%, 56.9%,  $p=0.67$ ), percentage of zygotes reaching cleavage stage (87.6%, 90.2%,  $p=0.93$ ), and blastulation rate (49.4%, 40.2%,  $p=0.24$ ). Utilizable blasts number was similar among groups (E-IVM: 38.6%, L-IVM: 37.9%,  $p=0.95$ ), though a difference was found in the percentage of good quality blastocysts among groups: (70.5%, 9%,  $p=0.0004$ ). Biopsied blasts per group (34%, 27.5%,  $p=0.56$ ) and euploidy rates (25%, 37.5%,  $p=0.53$ ) were similar among cohorts. After adjusting for age, BMI, AMH, and total number of eggs retrieved per cycle, no association was found between the time to maturation and the odds of aneuploidy (OR 0.6, CI95% 0.05-7.85,  $p=0.74$ ) or the odds of developing a good quality embryo (OR 0.18, CI95% 0.02-1.4,  $p=0.11$ ).

**CONCLUSIONS:** Formerly, the culture of embryos derived from cryopreserved IVM oocytes was perceived as having low survival rates, suboptimal developmental potential, and limited clinical utility. Our study demonstrated that IVM oocytes can be successfully cultured to the blastocyst stage and detected as chromosomally balanced. By employing IVM, we can optimize the reproductive potential per oocyte retrieval. Moreover, implementation of IVM in ART centers may increase the total number of transferable euploid blastocysts and enhance patients' ability to build a healthy family.

**REFERENCES:**

- Khalili MA, et al. Vitrification of human immature oocytes before and after *in vitro* maturation: a review. *J Assist Reprod Genet.* 2017 Nov;34(11):1413-1426.1111 <https://doi.org/10.1007/s10815-017-1005-4>.
- Escrich L, et al. Do immature and mature sibling oocytes recovered from stimulated cycles have the same reproductive potential? *Reprod Biomed Online.* 2018 Dec;37(6):667-676.

**SUPPORT:** None.

P-86 Tuesday, October 15, 2019 6:30 AM

### SUPPLEMENTING *IN-VITRO* MATURATION MEDIA WITH HUMAN FOLLICULAR FLUID IMPROVES BOTH THE MATURATION RATE OF MOUSE IMMATURE OOCYTES AND THE SUBSEQUENT EMBRYONIC DEVELOPMENT.

Takashi Horikawa, MD,<sup>a</sup> Yasuyuki Araki,



Ph.D.,<sup>b</sup> Koji Nakagawa, MD, PhD,<sup>a</sup> Keiji Kuroda, MD, PhD,<sup>a</sup> Satoru Takamizawa, MD, PhD,<sup>a</sup> Yuichi Sato, MD., PhD.<sup>c</sup> Rikikazu Sugiyama, MD, PhD<sup>a</sup> <sup>a</sup>Sugiyama Clinic Shinjuku, Tokyo, Japan; <sup>b</sup>The Institute for Advanced Reproductive Medical Technology, Maebashi, Gunma, Japan; <sup>c</sup>Takasaki ART clinic, Takasaki, Japan.

**OBJECTIVE:** Patients of an advanced maternal age who have sometimes shown unsynchronized follicle growth often receive ovarian stimulation. In those cases, immature oocytes are collected from smaller follicles. Even immature oocytes are valuable for those patients, but it is necessary for these immature oocytes to be allowed to effectively mature *in vitro*. The aim of the present study was to evaluate whether supplementing culture media with human follicular fluid (HFF) could improve the maturation and subsequent embryonic development of immature mouse oocytes.

**DESIGN:** This was an experimental study. Mouse germinal vesicle (GV) oocytes derived from 8-10 week-old female B6D2F1 mice were divided into three groups according to their concentration of HFF supplementation: The first group used 100% HFF without culture media (**all-FF group**), the second group used culture media with 50% HFF (v/v) (**half-FF group**), and the third group used only culture media (**non-FF group**).

**MATERIALS AND METHODS:** After obtaining informed consent, HFF obtained from the first puncture of a follicle during oocyte retrieval without blood contamination was used in this study. The culture media for *in-vitro* maturation (IVM) involved conventional media (Universal IVF media<sup>®</sup>) for human IVF treatment in addition to recombinant FSH (0.075 IU/ml) and hCG (0.1 IU/ml). The maturation rates of GV oocytes and the blastocyst formation rates were evaluated among the three groups. Maturation was defined as confirmation of MII stage chromosomes via staining Hoechst solution under fluorescence microscopy. The mature oocytes after IVM were inseminated and fertilized oocytes were additionally extended to the blastocyst stage (up to 124 hours).

**RESULTS:** The maturation rate following IVM in the half-FF group was 100%, which was significantly higher than that of the non-FF group (67.0%,  $p < 0.05$ ), but was similar to the all-FF group (92.0%). The fertilization rates of the all-FF, half-FF and non-FF groups were 78.1, 56.2 and 21.9%, respectively, which showed a significant increase in accordance with the inclusion of HFF. The blastocyst formation rates of the all-FF and half-FF groups were 61.1 and 63.3%, respectively, whereas that in the non-FF group (16.7%) was significantly lower ( $p < 0.05$ ).

**CONCLUSIONS:** Supplementing the culture media with HFF during IVM significantly improves the maturation rate of immature mouse oocytes, and the mature oocytes derived from IVM media with HFF possessed higher developmental potential for blastocyst formation. Supplementing IVM culture media with HFF could be useful option for the IVM of human immature oocytes.

P-87 Tuesday, October 15, 2019 6:30 AM

### IN VITRO OOCYTE MATURATION (IVM) IS A SUCCESSFUL ART OPTION FOR PCOS

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**OBJECTIVE:** *In vitro* maturation (IVM), while successful in domestic and laboratory species, has not been widely adopted in human ART. This is due in part to low oocyte maturation *in vitro*, as well as the widespread success of stimulation protocols that include administration of hCG to induce ovulation followed by retrieval of mature oocytes. However, some patients are susceptible to ovarian hyper-stimulation (OHSS) using this approach. Retrieval of immature oocytes after minimal ovarian stimulation without an ovulation trigger would alleviate this concern. The objective of this IRB approved clinical trial is to evaluate the efficacy of a newly developed IVM system as a clinical treatment for infertile patients.

**DESIGN:** Prospective cohort study

**MATERIALS AND METHODS:** Beginning on D2 until D5 of the cycle, patients received 150 IU Menopure at 2 PM. Patients had an ultrasound and blood drawn prior to oocyte retrieval on D7, 42-46 hr after the last injection. Oocytes were retrieved into medium containing meiotic inhibitors (Pre-IVM) and cultured in this medium for 20-24 hr, at which time oocytes were washed and moved into IVM medium for 27-30 hr. Following IVM, cumulus cells were removed and eggs assessed for maturity. Mature (MII) oocytes underwent ICSI; immature oocytes were returned to IVM medium for an additional 18 hr, when any mature oocytes underwent ICSI. Zygotes (2PN) were cultured in sequential culture medium and good quality blastocysts vitrified on days 5, 6, and 7. All blastocysts were biopsied for PGT-A.

**RESULTS:** To date (January-April, 2019), 8 patients have participated in the clinical study. Four patients were diagnosed with PCOS, two patients with PCO and recurrent pregnancy loss, one patient with fibroids and unexplained infertility, and one patient with DOR and poor embryo quality. Average patient characteristics include: Age, 31.5 y; AMH 7.0 ng/mL; D3 FSH 6.3 mIU/mL; AFC, 45.1; and BMI 28.5. On the day of retrieval, average E2 was 248 pg/mL, and the average size of the largest follicle was 9.1 mm. In total, 234 immature oocytes were retrieved (average 29.3 oocytes per patient, range 5-55), of which 27 were atretic (11.5%). After pre-IVM and IVM, 114 (55.1%) oocytes matured; an additional 41 oocytes matured the following day for a total maturation percentage of 74.9% (155/207). After ICSI, 81/155 (52.3%) of eggs fertilized normally. Following culture, 6 good quality blastocysts (7.4%) were produced on D5, and 16 (19.8%) overall. Five of the eight patients (62.5%) produced at least 1 good quality blastocyst; all of these 5 were PCO/PCOS patients. Ten of the 16 blastocysts produced were euploid (62.5%). To date, 2 patients have undergone FET; one has an ongoing pregnancy.

**CONCLUSIONS:** IVM is successful in a clinical setting, and is logistically feasible in the typical IVF laboratory work flow. This approach alleviates concerns of hyper-stimulation, and drastically reduces medication costs and injections. Thus, IVM is a realistic alternative ART approach for PCOS patients.

**SUPPORT:** None.

**P-88** Tuesday, October 15, 2019 6:30 AM

**THE TIMING OF THE RELEASE OF THE FIRST POLAR BODY PREDICTS THE CLEAVAGE RATE AFTER PARTHENOGENETIC ACTIVATION FOR HUMAN OOCYTES OBTAINED BY IN VITRO MATURATION.**



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**OBJECTIVE:** Human parthenogenetic blastocysts (HPB) are important materials for making parthenogenetic human embryonic stem cells (pHESC) and so on. Though, the efficient method for making HPB considering the progress of time-lapse imaging (TLI) during in vitro maturation (IVM) has not been established. The objectives were to clarify the behavior of oocytes obtained by IVM during parthenogenetic activation (PA) and to characterize the features of TLI of cleaved embryos by focusing on the timing of the first polar body (fPB) releases during IVM.

**DESIGN:** Basic research study.

**MATERIALS AND METHODS:** 55 immature oocytes were collected from resected ovaries of the 5 patients with endometrioid adenocarcinoma. These oocytes were assigned to either early or late groups based on the results of 24 hour IVM. Oocytes that released fPB within 24 hours were defined as the early group, and underwent PA. On the other hand, oocytes that did not release fPB after 24 hour IVM were subjected to an additional 24 hours of IVM, and the oocytes that subsequently released the fPB were defined as the late group and underwent PA. PA was carried out using calcium ionophore and 6-dimethylaminopurine, and subsequently oocytes were cultured in single step medium with observation by TLI. Based on the data from TLI of PA, the duration of pronucleus (PN) formation and oocytes cleavage rate were analyzed. Oct4 and Cdx2 of cleavage embryos were detected by immunofluorescence staining.

**RESULTS:** Oocytes were collected from five patients with a mean age of 36.0 ± 4.0 years, and IVM was performed on 50 oocytes. 15 oocytes were assigned to the early group and 13 to the late group. The rate of the oocytes which released fPB was 56.0%. The duration of PN formation was significantly longer in the early group (60.2 ± 13.0 hours) than in the late group (23.9 ± 8.4 hours) (p=0.045). The overall cleavage rate was 39.3%, and the results was summarized in table 1. The rate of cleavage to 8 or more cells were significantly higher in the late group than in the early group. The overall rate of cleavage to morula was 7.1%. Revers cleavage was observed in 54.5% (early group 80.0%, late group 15.4%) of all cleaved embryos, and these did not all cleaved to morula. Oct4 was detected in the HPB in late group.

	Early group (n=15)	Late group (n=13)	p value
≥ 2cell	33.3% (n=5)	46.2% (n=6)	p=0.488
≥ 8cell	0%	30.8% (n=4)	p=0.020
≥ morula	0%	15.4% (n=2)	p=0.206

**CONCLUSIONS:** The timing of release of fPB within IVM may related to the results of PA. This finding is an important point in creating an efficient pHESC, which we have clarified for the first time. It is probable that the time difference between nuclear maturation and cytoplasmic maturation is

related, though the impact of the duration of PN formation is unknown at this time, and should be investigated in the future.

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**SUPPORT:** This study was supported by Â Kanzawa Medical Research Foundation.

**P-89** Tuesday, October 15, 2019 6:30 AM

**EMBRYOLOGIST FRIENDLY PROGRAMMED IVM WITH DELAYED BLASTOCYST**



**TRANSFER.** Bruce I. Rose, MD, PhD, Kevin Nguyen, MS, Samuel Brown, MD. Brown Fertility LLC, Jacksonville, FL.

**OBJECTIVE:** To design an approach to IVM (in vitro maturation) which can be easily integrated into a busy IVF laboratory and which results in oocytes with a high maturation rate, a good blastocyst production rate, and a reasonable pregnancy rate.

**DESIGN:** Our IVM protocol uses a programmed approach to enable scheduling cases and requires only laboratory techniques already used by the embryologist. Retrieval is designed to improve environmental conditions for oocytes. Embryos are transferred back in a subsequent FET cycle.

**MATERIALS AND METHODS:** Patients with a PCO pattern in their ovaries were recruited. Oral contraceptives were used to plan prospectively for a day for retrieval. Letrozole was started on day 5 after stopping oral contraceptives (SOC). FSH (25 to 75U/day) was started on the 7 after SOC. Ovidrel was given on day 11 or 12 after SOC. Oocyte retrieval was on day 13 or 14 after SOC. Cycles were cancelled if all follicles were less than 8 mm or if one follicle was greater than 13 mm.

Oocyte retrieval used a Steiner-Tan needle to enable flushing and limit dead space in the oocyte collection system to 0.000004 ml. This needle is constructed from a 5 cm 19g needle attached to a 17g needle with fluid entering at the junction of these two needles. Flush fluid is simultaneously pushed into both the 19g and 17g needles. Aspiration while flushing is used to empty the 17g needle into the collection tube. Our objective was to get the oocyte into the laboratory as soon as possible after it was aspirated from the follicle. The large volume of flush also enabled the embryologist to use routine oocyte retrieval laboratory techniques to locate oocytes in the aspirate.

Sage IVM maturation media with 10% heat inactivated maternal serum and 75 mIU FSH/ml was used. Oocytes were visually evaluated for maturity at retrieval and twice a day until 48 hours after retrieval. Mature oocytes were fertilized using ICSI. Zygotes were cultured to blastocysts. Blastocysts were vitrified on day 5 or 6 after ICSI. Oral contraceptives were used as patients transitioned into our routine FET program.

**RESULTS:** Twenty patients were recruited. Two cycles were canceled for follicles that were too large. The average number of oocytes retrieved was 11. The average maturation rate was 85%. The average fertilization rate per mature oocyte was 86%. Two patients had an oocyte aspiration, which did not produce blastocysts. Thus 89% of retrievals resulted in blastocysts with an average of 3 blastocysts per patient and with 36% of fertilized oocytes becoming blastocysts. After one FET cycle, 50% of patients had a clinical pregnancy, and 38% had an ongoing or delivered pregnancy.

**CONCLUSIONS:** IVM can be adapted to not disrupt a clinical IVF lab. Better treatment of oocytes during retrieval resulted in better maturity and blastocyst production.

**IVF OUTCOME PREDICTORS - AGE**

**P-90** Tuesday, October 15, 2019 6:30 AM

**INCREASED PATERNAL AGE IS ASSOCIATED WITH DECREASED BLASTULATION AND EUPLOID RATES BUT NOT PREGNANCY OUTCOMES IN THE SETTING OF A EUPLOID SINGLE EMBRYO**



**TRANSFER.** Brent M. Hanson, MD,<sup>a</sup> Julia G. Kim, MD, MPH,<sup>a</sup> Emily K. Osman, MD,<sup>a</sup> Ashley W. Tiegs, MD,<sup>b</sup> Shelby A. Neal, MD,<sup>a</sup> Ruth B. Lathi, MD,<sup>c</sup> Richard Thomas Scott, Jr., MD,<sup>a</sup> Jason M. Fransiak, MD.<sup>a</sup> <sup>a</sup>IVI-RMA New Jersey, Basking Ridge, NJ; <sup>b</sup>Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA; <sup>c</sup>Stanford Fertility and Reproductive Medicine Center, Sunnyvale, CA.

**OBJECTIVE:** The relationship between paternal age and assisted reproductive technology (ART) outcomes is often confounded by factors arising from the female partner. In order to minimize these effects, this study utilized

preimplantation genetic testing for aneuploidy (PGT-A). To date, the role of paternal age on ART outcomes remains controversial. This study sought to determine whether increasing paternal age is associated with adverse outcomes in the setting of a single embryo transfer (SET) of a euploid embryo.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This study was performed at a large fertility practice. Included couples underwent a first cycle of in vitro fertilization (IVF) using ejaculated sperm and then underwent intracytoplasmic sperm injection (ICSI) and PGT-A followed by SET of a euploid embryo. Kruskal-Wallis testing, Chi-square analysis, and linear regression models were utilized to assess the relationship between paternal age and rates of implantation, delivery, biochemical loss, and clinical loss. The relationship between paternal age and fertilization rate, blastulation rate, and euploid rate was also analyzed.

**RESULTS:** 4367 couples met inclusion criteria. Mean male partner age was  $37.1 \pm 5.5$  years, with 87 male patients over age 50. Mean female partner age was  $34.9 \pm 4.0$  years. Among couples undergoing SET of a PGT-A tested embryo, implantation rate was 82.1% (3586/4367 embryos transferred), delivery rate was 56.8% (2480/4367 embryos transferred), biochemical loss rate was 8.8% (385/4367 embryos transferred), and clinical loss rate was 7.2% (313/4367 embryos transferred). Adjusting for female age, there was no statistically significant association between male partner age and implantation rate ( $p=0.40$ ), delivery rate ( $p=0.48$ ), biochemical loss rate ( $p=0.18$ ), or clinical loss rate ( $p=0.19$ ). A sub-group analysis evaluating men over age 50 ( $n=87$ ) also failed to demonstrate a relationship between paternal age and implantation rate ( $p=0.32$ ), delivery rate ( $p=0.19$ ), biochemical loss rate ( $p=0.08$ ), or clinical loss rate ( $p=0.42$ ).

For men over age 50, there was a significant association observed between paternal age and blastulation rate ( $p=0.01$ ) as well as euploid rate ( $p=0.03$ ) but no significant association between age and fertilization rate ( $p=0.92$ ). When using 40 years as a cutoff point, the relationship between paternal age and blastulation rate remained significant ( $p=0.0006$ ) but there was no association between age and euploid rate ( $p=0.86$ ) or fertilization rate ( $p=0.70$ ).

**CONCLUSIONS:** When couples undergo SET of a euploid embryo, increasing paternal age does not appear to detrimentally impact pregnancy outcomes, including implantation rate, delivery rate, biochemical loss rate, and clinical loss rate. However, paternal age greater than 50 negatively affected blastulation and euploid rates. Poorer blastulation was also seen in men over age 40. If a single euploid embryo is transferred, the role of paternal age is unlikely to be significant, but increasing paternal age may negatively impact a couple's ability to create a euploid embryo and thus cumulative pregnancy rate.

**SUPPORT:** None.

**P-91** Tuesday, October 15, 2019 6:30 AM

#### **ADVANCED PATERNAL AGE IS ASSOCIATED WITH AN INCREASED RISK FOR SEGMENTAL ABERRATIONS IN EMBRYOS DERIVED FROM YOUNG OOCYTE DONORS.**

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**OBJECTIVE:** According to a prior analysis conducted by our research group (unpublished data), the overall whole chromosomal (chr) aberration and mosaicism rate in embryos derived from young oocyte donors, was not associated with paternal age. The aim of the current study was to determine if there is a correlation in the rates of single, segmental, and complex chr aberrations, as well as aneuploidy mosaicism rates ( $\geq 30\%$ ), with paternal age.

**DESIGN:** A retrospective cohort study.

**MATERIALS AND METHODS:** The study had institutional REB approval. Embryos from patients undergoing in vitro fertilization (IVF) at the CReATe Fertility Centre (Toronto, Canada) derived from donated eggs, which underwent preimplantation genetic testing for aneuploidy (PGT-A), were included. Next generation sequencing (NGS) technology was used for PGT-A in all cases. A total of 3118 embryos, obtained from 437 IVF oocyte donor cycles, were analyzed. Embryos classified as aneuploid were included in the final analysis. Paternal age was divided into 3 ranges for this analysis: Group A  $\leq 39$  years ( $n=103$ ); Group B 40-49 years ( $n=66$ ); and Group C  $\geq 50$  ( $n=18$ ) years. Chi square statistical test was used to compare the rates of single, segmental and complex (two chr  $\pm$

mosaicism  $>50\%$ ) chr aneuploidies, including the total distribution of specific chr gains and losses, between these groups.

**RESULTS:** 13.8% of embryos (433/3118) were classified as aneuploid (237 (54.7%) in group A, 155 (35.8%) in group B and 41 (9.5%) in group C). Among these aneuploid embryos, 61% had a single chr aneuploidy (SCA), 24.1% had segmental aberrations and 14.9% had complex aberrations. The rate of SCA in groups A, B & C were 63.7%, 58.1%, 53.7%, respectively ( $p=0.3$ ). The most frequent SCA in group A were: trisomy (T) 16, monosomy (M) 16, M22 and M X, in group B: M X, T16, M16 and M13 and in group C: M16, T16, M X and M18. Overall, chr 16 aneuploidy was the most frequent among the 3 study groups: 13.5% in group A, 9% in group B and 17.1% in group C ( $p=0.26$ ). Mosaicism rates (aneuploid/aneuploid) in the aneuploid embryos with SCA were more frequent in group C (24.4%), as compared with groups A (5.5%) and B (5.2%),  $p=0.00004$ . Segmental aberrations were significantly more frequent in group C (36.6%), as compared with group A (19.4%) and B (27.7%) ( $A \neq C$ ,  $p=0.04$ ). More segmental losses than gains were present in all paternal age groups, however, only the difference for group C was significant (51.2% losses vs. 12.2% gains,  $p=0.05$ ). The rate of complex aberrations (CXA) in groups A, B & C was 7.3%, 16.5% and 14.2%, respectively ( $p=0.3$ ). Among SCA and CXA, gains and losses were equally distributed between the studied groups ( $p=0.7$  and  $p=0.41$ , respectively).

**CONCLUSIONS:** Our findings show that advanced paternal age  $\geq 50$ , as compared with younger paternal ages, is associated with increased rates of segmental aberrations. Also, aneuploid embryos from fathers  $\geq 50$ , had higher overall rate of chr 16 aneuploidies and higher rates of aneuploidy mosaicism with SCA. Our data adds to the general knowledge of chr integrity of preimplantation embryos and may assist counselling patients using donor eggs, particularly those with advanced paternal age.

**SUPPORT:** CReATe Fertility Centre.

**P-92** Tuesday, October 15, 2019 6:30 AM

#### **CLINICAL PREGNANCY OUTCOME ACCORDING TO AGE OF PATIENTS WITH HIGH PROPORTION OF FAILED OOCYTE MATURATION IN ICSI**



**CASES:** Yeon Sook Park, MS, Su Hyeon Kim, MS, Han Su Kim, MS, Jae Won Kim, M.D., Hee Sun Lee, M.D., Myung Hee Kim, M.D., Hyeon Jeong Jeong, M.D., Mi Kyung Chung, Ph.D. Seoul Rachel Fertility Center, Seoul, Korea, Republic of (South).

**OBJECTIVE:** The purpose of this study was *to compare* the clinical pregnancy outcomes according to age of patients with high proportion of maturation arrest oocytes in ICSI cases.

**DESIGN:** Retrospective cohort study. This study was conducted on patients with ICSI cases who had transferred embryos. Clinical outcomes were analyzed by dividing the maturation arrest rate (under 40%/over 40%) and the age of 38yrs (under 38yrs/over 38yrs).

**MATERIALS AND METHODS:** From June 2011 to December 2018, a total 2495 cycles were analyzed in this study. All the patients underwent ICSI cycle followed by fresh embryo transfer. Inclusion criteria: female age between 23 and 48yrs, use of fresh or cryopreserved sperm. Exclusion criteria: surgically retrieved sperm.

These patients were divided into group A (maturation arrest rate  $<40\%$ , Age  $<38$ yrs), group B (maturation arrest rate  $\geq 40\%$ , Age  $<38$ yrs), group C (maturation arrest rate  $<40\%$ , Age  $\geq 38$ yrs) and group D (maturation arrest rate  $\geq 40\%$ , Age  $\geq 38$ yrs). The pregnancy outcomes were compared among these 4 groups.

**RESULTS:** A total of 2495 cycles were included (group A ( $n$ ) = 1355, group B ( $n$ ) = 90, group C ( $n$ ) = 960 and group D ( $n$ ) = 54). There was no significant differences in fertilization rate between groups (group A vs. B:  $80.7\% \pm 20.0$  vs  $81.0\% \pm 24.1$ ,  $P=0.958$  and group C vs. D:  $83.2\% \pm 20.1$  vs.  $88.5\% \pm 19.6$ ,  $P=0.073$ ). But there was a significant difference in embryo quality, pregnancy rate, and miscarriage rate. More than 40% of oocyte maturation failure group had lower levels of embryo quality. There was significant difference in at least one good quality embryo transfer cycle rate (group A vs. B: 69.4% vs. 46.7%,  $P<0.001$  and group C vs. D: 63.1% vs. 55.6%,  $P<0.001$ ). And more than 40% of oocyte maturation failure group had lower clinical pregnancy rate (group A vs. B: 41.6% vs. 20.0%,  $P<0.001$  and group C vs. D: 25.7% vs. 7.4%,  $P<0.001$ ) and ongoing pregnancy rate (group A vs. B: 35.9% vs. 14.4%,  $P<0.001$  and group C vs. D: 17.0% vs. 1.9%,  $P<0.001$ ).

**CONCLUSIONS:** According to our study, high rate of oocyte maturation failure group had lower embryo quality, pregnancy rate and higher miscarriage rate. However, there was no effect on in fertilization rate. There was a similar

tendency in analysis according to age. High clinical results can be maintained only by lowering the proportion of mature failed oocyte as much as possible.

References: Lu Y, Ferrer-Buitrago M, Popovic M, Neupane J, De Vos WH, Lierman S, et al. Patients with a high proportion of immature and meiotically resistant oocytes experience defective nuclear oocyte maturation patterns and impaired pregnancy outcomes. *Reprod BioMed Online*. 2018;36:396-407.

## IVF OUTCOME PREDICTORS - EMBRYO CULTURE

**P-93** Tuesday, October 15, 2019 6:30 AM

### IS THE NUMBER OF BLASTOCYST MORPHOLOGIC EVALUATIONS (BMA) ON DAY 6 CORRELATED WITH DAY 7 EMBRYONIC COMPETENCE?

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**OBJECTIVE:** Blastocyst morphologic assessments (BMAs) are used to determine the optimal time to perform trophectoderm (TE) biopsy for preimplantation genetic testing for aneuploidy (PGT-A). Embryos that have not hatched by the morning of day 6 may be cultured to Day 7 to await TE herniation. There is concern that repeated exposure of embryos outside of incubation may induce environmental stressors that can impact embryonic metabolic activity [1]. Thus, our study sought to assess the relationship between the number of BMAs on Day 6 and development of Day 7 embryos.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The study included patients who had embryos cultured and biopsied to Day 7 for PGT-A from 2015 to 2019. Cases were separated into 2 groups: (Study Group: 2 BMAs - morning and afternoon of Day 6; Control Group: 1 BMA - morning of Day 6). The primary outcomes were the rate of blastocysts that adequately expanded for TE biopsy (BBR) and top quality blastocyst rate (TQBR) ( $\geq 4$ BB Modified Gardner). Secondary outcomes included euploid rate (ER), clinical pregnancy rate (CPR), ongoing pregnancy/live birth rate (OP/LBR), and spontaneous abortion rate (SABR). Student's t-test and chi-square tests were used for statistical analysis, with  $p < 0.05$  considered significant. Multivariate logistic regression analysis was performed to control for confounding variables.

**RESULTS:** A total of 5,034 embryos were cultured to Day 7. Within the 2 BMAs group ( $n = 1,412$ ), 478 were biopsied on Day 7. Within the 1 BMA group ( $n = 3,622$ ), 1,407 were biopsied on Day 7. TQBR, BBR, and ER were significantly higher in the 1 BMA group. When controlling for confounders, having 1 BMA was significantly associated with the number of TQBR ( $\beta = 0.73$ ,  $p < 0.0001$ ). However, our model demonstrated no correlation between the count of BMAs on Day 6 and the probability of a Day 7 blastocyst having adequately expanded for TE biopsy ( $\beta = 0.02$ ,  $p = 0.82$ ) or rate of euploid embryos ( $\beta = 0.10$ ;  $p = 0.42$ ). There were no significant differences in CPR, OP/LBR, or SABR.

**CONCLUSIONS:** Our results demonstrated that the number of BMAs on Day 6 of development for embryos cultured Day 7 did not correlate with clinical outcomes. Our data shows that a single check on Day 6 may optimally minimize environmental exposures and allow for the subsequent acquisition of critical embryonic genomic data via PGT-A without comprising outcomes of Day 7 embryos. These results may be used as a guide for other reproductive centers who are incorporating the culture of embryos to Day 7 as standard practice.

	1 BMA		2 BMA		p-value
	N	%	N	%	
TQBR	519/3622	14.33%	111/1412	7.86%	<.0001
BBR	1407/3622	38.85%	478/1412	33.85%	0.001
ER	597/1407	42.40%	176/478	36.80%	0.03
CPR	31/118	26.27%	11/49	22.45%	0.60
OP/LBR	23/118	19.49%	7/49	14.29%	0.42
SABR	8/31	25.81%	4/11	36.36%	0.51

**REFERENCE:** 1. Gardner, D.K. and R.L. Kelley, *Impact of the IVF laboratory environment on human preimplantation embryo phenotype*. *J Dev Orig Health Dis*, 2017. 8(4): p. 418-435.

**SUPPORT:** None.

**P-94** Tuesday, October 15, 2019 6:30 AM

### INFLUENCE OF POST-THAW EMBRYO CULTURE INTERVAL ON ASSISTED REPRODUCTION TECHNIQUES PREGNANCY RATES.

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**OBJECTIVE:** To compare assisted reproductive technique (ART) outcomes after frozen-thawed cleavage stage embryo transfer between embryos transferred 2 to 5 hours and transferred 18 to 24 hours after thaw.

**DESIGN:** double-blinded, randomized, control trial ([ClinicalTrials.com NCT03381001](https://clinicaltrials.com/NCT03381001)).

**MATERIALS AND METHODS:** A total of 388 patients submitted to ART treatment who had their embryos frozen on day-2 had their data analysed. All embryos were cryopreserved using the same vitrification protocol (open system) and all patients received the same endometrial priming with estradiol valerate, at 6 mg/d, taken orally, followed by vaginal progesterone started on day-0. Randomization was performed using sealed envelopes. We calculated that 286 subjects would provide 80% power for detecting over 10% absolute difference in pregnancy rate with  $\alpha = 0.05$ . The study was performed from May 2017 until December 2018.

**RESULTS:** A total of 179 patients had embryos transferred 2-5 hours after thaw (Group D2) and 209 patients had embryos transferred 18-24 hours after thaw (Group D3). The mean age in group D2 was  $36 \pm 4.4$  and  $36 \pm 5.4$  in group D3. For the D2 group vs. D3 group, respectively, the clinical pregnancy rate was 30.7% and 36.8% ( $p = 0.2$ ) and ongoing pregnancy rate was 28% and 33.5% ( $p = 0.2$ ).

**CONCLUSIONS:** ART outcomes were similar after transfer of frozen-thawed cleavage stage embryos that were kept in culture either for 2 to 5 hours or 18 to 24 hours after thaw. These results suggest that increasing the culture time of embryos in one day to improve selection before transfer does not increase pregnancy rate. More studies are necessary to confirm our results.

**REFERENCE:** None.

**SUPPORT:** Grant from CNPq (Brazilian research council) to Selmo Geber.

**P-95** Tuesday, October 15, 2019 6:30 AM

### USABLE BLASTOCYST DEVELOPMENT RATE IS INFLUENCED BY MATERNAL AGE AND ULTRA-LOW OXYGEN TENSION IN EXTENDED CULTURE.

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**OBJECTIVE:** To determine if maternal age affects usable blastocyst development rate according to extended embryo culture conditions (low or ultra-low oxygen tension).

**DESIGN:** This is a single-center retrospective cohort study performed from November 2014 to October 2018. The endpoint was to evaluate the rate of blastocyst formation as well as usable blastocyst rates as a function of maternal age and extended embryo culture conditions. All embryos were cultured in 6% CO<sub>2</sub>/5% O<sub>2</sub> from day 0 to day 3. From day 3 until day 5/6, either 5% O<sub>2</sub> tension was used (control group;  $n = 4000$  embryos,  $n = 653$  patients) or there was a switch to 2% O<sub>2</sub> (study group;  $n = 3771$  embryos,  $n = 482$  patients). The two groups were similar for age, attempt rank, mean number of retrieved oocytes.

**MATERIALS AND METHODS:** Patients were classified according to the maternal age as following: <30, 30-34, 35-37, 38-39, 40-42, and  $\geq 43$  years of age. All blastocysts were morphologically evaluated according to the standard Gardner grading system. Blastocyst and usable blastocyst formation rates were based on the number of embryos in extended culture. Usable blastocysts were considered as expanding (grade 3), fully expanded (grade 4), partially hatching (grade 5) or fully hatched (grade 6) with at least a grade A or B trophectoderm quality. In order to evaluate blastocyst yield according to embryo quality, we divided embryos as following on day 3: <6, 6-7, 8 and >8 blastomers.

**RESULTS:** Maternal age significantly affects blastocyst formation rate (58.3% for <30, 60% for 30-34, 57.4% for 35-37, 48.9% for 38-39, 43.5% for 40-42 and 36.2% for  $\geq 43$ ,  $p < 0.0001$ ); as well as usable blastocyst rate (35.7% for <30, 38.2% for 30-34, 35.4% for 35-37, 25.9% for 38-39, 23.1% for 40-42 and 14.5% for  $\geq 43$ ,  $p < 0.0001$ ). Multivariate analysis

showed that both maternal age and oxygen tension in extended embryo culture affect blastocyst formation rate, and that both maternal age and embryo quality on day 3 also affect usable blastocyst rate ( $p < 0.0001$ ). Blastocyst formation rate was significantly higher in the 2% O<sub>2</sub> group (58.3%) than in the 5% O<sub>2</sub> group (55%),  $p < 0.005$ . The extended culture under ultra-low O<sub>2</sub> tension improved for 35-37 year of age group both blastocyst formation rate: 61.1% vs 53.4% in the 2% O<sub>2</sub> and 5% O<sub>2</sub> groups respectively,  $p < 0.002$ ; and usable blastocyst rate: 38.7% vs 31.8% in the 2% O<sub>2</sub> and 5% O<sub>2</sub> groups respectively,  $p < 0.005$ . Maternal age impacts negatively on blastocyst formation rate as well as usable blastocyst rate in each group of day 3 embryo quality.

**CONCLUSIONS:** Blastocyst and usable blastocyst formation rates on day 5/6 both significantly decrease with maternal age. Beyond the age of 37, blastocyst rate is reduced by 28%. Therefore, this study leads us to question the relevance of extended culture beyond that age. Nevertheless, 2% O<sub>2</sub> tension in extended culture is associated with better blastocyst yield. In 35-37 years of age group, it also improves usable blastocyst rate. This data supports the idea that maternal age and embryo quality on day 3 are crucial criteria to be considered for the choice of extended culture strategy.

**P-96** Tuesday, October 15, 2019 6:30 AM

**THE EFFECTS OF CO-CULTURE WITH AUTOLOGOUS CUMULUS CELL ON PREGNANCY OUTCOMES BY MATERNAL AGE.**

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**OBJECTIVE:** It is known that the incidence of apoptosis in cumulus cells is associated with women age. In this study, we aimed to evaluate the influence of autologous cumulus cell co-culture on pregnancy outcomes by maternal age.

**DESIGN:** A retrospective study was performed from JANUARY 2014 to December 2018.

**MATERIALS AND METHODS:** A total of 588 cycles which underwent GnRH long or antagonist protocol with fresh embryo transfer were analyzed. The cycles with severe male factor and single embryo transfer were excluded. The cycles were divided into two groups according to maternal age; 32-36 years (Group 1),  $\geq 37$  years (Group 2). Each group had embryos cultured in defined medium with autologous cumulus cell (ACC) or without autologous cumulus cell (No ACC). The ACC was dissected from the patient's oocyte-cumulus complexes using two 29-gauge needles and washed twice. The collected ACC was directly put into the culture medium without hyaluronidase treatment. We compared the rates of clinical pregnancy, ongoing pregnancy, and implantation between ACC and No ACC by maternal age.

**RESULTS:** The woman age, man age, and Day 3 good quality embryo rate were similar in the two groups. In the Group 1 cultured with ACC, the pregnancy rates were significantly increased than No ACC (Clinical Pregnancy: 49.4% vs. 37.2%,  $P < 0.05$ ; Ongoing Pregnancy: 45.6% vs. 31.0%,  $P < 0.05$ ; Implantation: 32.1% vs. 22.1%,  $P < 0.05$ ). The clinical pregnancy and ongoing pregnancy rates were not statistically different between ACC and No ACC in Group 2 (Clinical Pregnancy: 16.5% vs. 26.3%,  $P = 0.06$ ; Ongoing Pregnancy: 13.4% vs. 19.4%,  $P = 0.21$ ). However, implantation rate was significantly decreased in ACC (9.0% vs. 15.8%,  $P < 0.05$ ).

**CONCLUSIONS:** The age of women might influence the pregnancy outcomes. These results suggested that co-culture with autologous cumulus cell, for patients under 37 years old, could improve the rates of clinical pregnancy, ongoing pregnancy, and implantation. However, it is considered that co-culture with autologous cumulus cell is not recommended to patients over 37 years old for the improvement of pregnancy rate. Further studies are needed

TABLE 1. Comparison of pregnancy outcomes between ACC and No ACC according to the age of patient.

Group (Age)	ACC				No ACC			
	Cycle (n)	Clinical Pregnancies (%)	Ongoing Pregnancies (%)	Implantation (%)	Cycle (n)	Clinical Pregnancies (%)	Ongoing Pregnancies (%)	Implantation (%)
Group 1 (32-36)	79	39 (49.4)	36 (45.6)	54/168 (32.1)	226	84 (37.2)*	70 (31.0)*	104/471 (22.1)*
Group 2 ( $\geq 37$ )	97	16 (16.5)	13 (13.4)	20/223 (9.0)*	186	49 (26.3)	36 (19.4)	63/399 (15.8)

\*  $P < 0.05$



to measure the incidence of apoptosis in autologous cumulus cells to compare the correlation between woman age and the incidence of apoptosis.

**IVF OUTCOME PREDICTORS - EMBRYO TRANSFER**

**P-97** Tuesday, October 15, 2019 6:30 AM

**LIVE BIRTH RATES AFTER BLASTOCYST TRANSFERS PERFORMED BY FELLOWS.**

Dana B. McQueen, M.D., M.A.S., Jared C. Robins, MD, John Zhang, PhD, Eve C. Feinberg, M.D. Northwestern University, Chicago, IL.

**OBJECTIVE:** To evaluate live birth rates following embryo transfer performed Reproductive Endocrinology and Infertility fellows compared to attending physicians.

**DESIGN:** Retrospective Cohort Study.

**MATERIALS AND METHODS:** Institutional Review Board approval was obtained. Women undergoing blastocyst transfer between 1/2015 and 1/2018 were reviewed. Cycle characteristics and outcomes were compared between embryo transfers performed by fellows and attending physicians. A sample size of 750 embryo transfers was required to detect a 10% difference between groups, with 80% power and alpha of 0.05.

**RESULTS:** A total of 940 blastocyst transfers were included; 254 performed by five fellows and 686 performed by ten attending physicians. There were no differences in the mean age, anti-mullerian hormone (AMH) levels or rate of preimplantation genetic testing for aneuploidy (PGT-A) testing between groups (Table). The afterload technique was utilized more frequently by fellows, 95.2% (242/254) vs. 87.8% (602/686),  $P = 0.0004$ . A stylet was used less frequently by fellows, 0.4% (1/254) vs. 4.5% (31/686),  $P = 0.0008$ . The pregnancy rate in the fellow group was not significantly different from the pregnancy rate in the attending group; 72.8% (185/254) among fellows versus 67.6% (461/686) among attending physicians,  $p = 0.27$ . There were also no significant differences in the live birth rate between groups, 51.6% (131/254) versus 49.4% (339/686) respectively,  $P = 0.61$ . After controlling for embryo transfer technique and stylet use, there remained no difference in pregnancy outcomes. The average pregnancy rate among fellows performing their first 20 embryo transfers was 67.4% (58/86), and was no different from the average pregnancy rate among attending physicians, 67.6% (461/686)  $P = 1.0$ .

TABLE 1. Group Characteristics (N=940 transfers)

	Fellow ET (N=254)	Attending ET (N=686)	p-value
Age (SD), yr	34.1 (3.4)	34.1 (3.6)	1.00
AMH (SD), ng/mL	3.9 (3.1)	3.8 (3.3)	0.68
% PGS testing	22.0% (56/254)	22.6% (155/686)	0.93
% Fresh Transfer	41.3% (105/254)	34.4% (236/686)	0.06
Afterload technique	95.2% (242/254)	87.8% (602/686)	0.0004
Stylet	0.4% (1/254)	4.5% (31/686)	0.0008
Blood on catheter tip	4.3% (11/254)	6.8% (47/686)	0.17
Embryo Retained	0% (0/254)	0.3% (2/686)	1.0
# Embryos Transferred (SD)	1.2 (0.5)	1.3 (0.5)	0.1
Pregnancy Rate	72.8% (182/254)	67.6% (464/686)	0.27
Miscarriage Rate	18.9% (48/254)	16.8% (115/686)	0.44
Live Birth Rate	51.6% (131/254)	49.4% (339/686)	0.61

**CONCLUSIONS:** Embryo transfer success rates were not different between fellows and attending physicians. Barriers to fellowship training in embryo transfer should be evaluated and addressed, as there was no compromise in pregnancy rates, even in the first twenty embryo transfers performed.

**P-98** Tuesday, October 15, 2019 6:30 AM

**PREGNANCY OUTCOME AFTER BED REST VERSUS EARLY AMBULATION FOLLOWING EMBRYO TRANSFER DURING IVF/ICSI CYCLES-A RANDOMISED CONTROLLED STUDY.**

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**OBJECTIVE:** Embryo transfer (ET) is the final and most critical step in an ART cycle. Strategies that improve success include avoidance of uterine contractility, use of soft catheters, getting rid of cervical mucus and ultrasound guided placement of embryos in mid cavity to optimise outcomes. Bed rest and immobilisation after ET has been practiced for long with the intention to improve pregnancy rates, however this is a subject of debate. Recent studies suggest that contrary to the belief bed rest does not alter pregnancy outcome after ET. Our hypothesis was that immediate mobilisation after ET improves the outcome of IVF treatment. The objectives of the study were to compare pregnancy outcomes between bed rest or early ambulation after embryo transfer (ET) in women undergoing IVF/ICSI cycles.

**DESIGN:** Prospective randomised controlled trial.

**MATERIALS AND METHODS:** Women undergoing fresh IVF/ICSI cycles with age 25-38 years, BMI 18-28 kg/m<sup>2</sup>, normal endometrial cavity, and willing to participate were included in the study. Women undergoing frozen thawed ET, any factor disturbing implantation such as uterine fibroid, adenomyosis of uterus, unilateral or bilateral hydrosalpinx not treated surgically or poor endometrium, < 6mm at time of ET were excluded from the study. A prior sample size was calculated after reviewing literature (1). Anticipating a 20% increase in live birth rate after early ambulation with 80% power and an alpha error of 5% with 95% confidence interval the sample size calculated was 90 in each group. This was done using STATA version 15. One hundred and eighty women, 90 in each group were recruited for the study. Patients fulfilling eligibility criteria were randomised to receive 15 minutes rest after ET (Group A) or early ambulation (Group B). Primary Outcome assessed was Live birth rate; secondary outcome was: Implantation rate, clinical pregnancy rate, miscarriage, ectopic pregnancy and multiple gestation rate.

**RESULTS:** The live birth rate was 20.0% (95% CI :11.7-28.3) and 26.4 % (95% CI :17.2-35.7 p value 0.310) in Group A and Group B respectively. The implantation rates were 12.7% for Group A and 13.9% for Group B (p value = 0.554). Clinical pregnancy rates were 23.3% (95 % CI: 14.26 -32.1) and 28.7 % (95 % CI :19.2-38.2) in Group A and Group B respectively (p value 0.41). Secondary outcome measures including miscarriage rates were lower in the early ambulation group however did not reach statistical significance

**CONCLUSIONS:** Pregnancy rates were comparable in both the groups even though absolute numbers were higher in the group ambulating early after embryo transfer. There is little advantage of advising bed rest after ET, rather women should be allowed early ambulation to improve pregnancy after ET in IVF cycles.

**References:** Gaikwad S, Garrido N, Cobo A, Pellicer A, Remohi J. Bed rest after embryo transfer negatively affects in-vitro fertilization: a randomized controlled trial. *Fertil Steril.* 2013; 100(3):729-35.

**SUPPORT:** None.

**P-99** Tuesday, October 15, 2019 6:30 AM

**THE CONTINUED PUSH TOWARDS ELIMINATING TWIN PREGNANCY: THE CLINICAL IMPACT OF THE 2017 ASRM EMBRYO TRANSFER GUIDELINES.**

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**OBJECTIVE:** To evaluate the differences in twin birth rates before and after implementation of the 2017 ASRM guidelines which limits the number of embryos transferred.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Prior to April 2017, ASRM guidelines allowed for the transfer of 2 embryos in women under the age of 38 after the failure of an initial single embryo transfer. The new guidelines altered that recommendation to limit the transfer of 2 embryos only after the failure of *multiple* embryo transfer cycles in good prognosis patients. Our clinic, by policy, will not transfer more embryos than the ASRM guidelines, making it an ideal data set to analyze the impact of these guideline changes. We analyzed women under 38 years old in the two years before and after the national guideline change. The primary outcome was twin live birth. Only women on their second embryo transfer cycle were included. PGT and donor oocyte cycles were excluded. Published SART data was also compared between 2016 and 2017, to assess for national changes in the twin birth rate in patients under 38 years old. Differences in twin clinical pregnancy and live birth rates were compared by chi square test. Logistic regression controlled for embryo quality. Statistical significance was assumed at  $P < 0.05$ .

**RESULTS:** 367 live births in women under 38 years old with one prior embryo transfer were analyzed. The number of embryos in these patients was significantly reduced from 1.38 per patient to 1.0 after implementation of the new guidelines ( $P = 0.001$ ). This resulted in a significant reduction in the clinical twin pregnancy rate (14.2% vs 2.5%,  $P < 0.001$ ) and twin live birth rate (12.5% vs 2.5%,  $P = < 0.001$ ). There were no higher order multiple gestations. Despite the reduction in the number of embryos transferred, there was no change in the overall live birth rate per transfer in this patient group at 46.9% before and 50.3% after the policy change ( $P = 0.31$ ). The percentage of transfers with a good quality embryo increased from 61% to 67% over this time frame. Live birth remained similar before and after the 2017 guidelines even after adjustment for embryo quality ( $P = 0.52$ ). National SART data also showed a reduction in twin live births when comparing 2016 to 2017. In over 90,000 cumulative retrieval cycles in patients under 35 years old, the twin rate decreased from 16.5% to 13.3% ( $P < 0.001$ ). In over 50,000 cumulative retrieval cycles in patients 35-37 years old, the twin rate decreased from 15.6% vs 12% ( $P < 0.001$ ). The SART data was less dramatic in the twin reduction, likely because it was not limited to patients with a prior cycle.

**CONCLUSIONS:** Implementation of the 2017 ASRM guidelines decreased the twin rate in good prognosis women in our clinic and at a national level. Good prognosis women under 38 years old benefit from single embryo transfer, even if it is not their first embryo transfer.

**P-100** Tuesday, October 15, 2019 6:30 AM

**HIGHER PREGNANCY RATES ARE OBSERVED AFTER SINGLE EUPLOID FROZEN EMBRYO TRANSFER UTILIZING TIME-LAPSE MONITORING TECHNOLOGY WHEN COMPARED TO TRADITIONAL EMBRYO INCUBATION.**

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**OBJECTIVE:** One of the challenges in assisted reproductive technology is to identify and select embryos with the highest developmental potential, with a goal of improving pregnancy and ultimately live birth rates. Preimplantation genetic testing for aneuploidy (PGT-A) with next generation sequencing (NGS) has improved pregnancy rates by transfer of euploid embryos, however room for improvement remains. Time-lapse monitoring (TLM) is a non-invasive system that allows for continuous embryo imaging, maintenance of optimal culture conditions, and detection of developmental events that may lead to improved outcomes. Our objective is to determine whether TLM at the time of embryo development leads to improved pregnancy rates over traditional embryo incubation.

**DESIGN:** Retrospective cohort study at a large, private fertility center.

**MATERIALS AND METHODS:** A comprehensive electronic chart review was performed. All patients utilizing the TLM device (Embryoscope®, Vitrolife, Sweden) at a single IVF center from 2018 through March 2019 were analyzed. All patients who underwent a frozen embryo transfer (FET) of a PGT-A analyzed day 5, 6, or 7 embryo of good or fair quality were included in the study. Statistics were performed using 2-tailed Chi-square analysis where statistical significance was set at p values < 0.05.

**RESULTS:** A total of 952 patients were identified; 83 (8.7%) used the TLM device. Of all patients, 942 (98.9%) had only a single thaw cycle and transfer. Focusing on patients with only a single thaw cycle, thereby isolating the analysis to the best overall embryo(s), the overall pregnancy rate was 644/

942 (68.4%). Of these patients, the pregnancy rate of patients using TLM was significantly higher than those using traditional incubation (59/73, 80.8% versus 585/869, 67.3%;  $p < 0.05$ ). Looking only at patients that had an elective single embryo transfer (eSET), the overall pregnancy rate was 639/869 (73.5%). Pregnancy rates were higher after transfer of embryos created with TLM technology compared to traditional incubation (54/67, 80.6% versus 585/869, 67.3%;  $p < 0.05$ ). Combining patients that had both a single thaw cycle as well as an eSET, the overall pregnancy rate was 636/933 (68.2%) and TLM embryos had a significantly higher pregnancy rate over embryos from traditional incubation (51/64, 79.7% versus 585/869, 67.3%;  $p < 0.05$ ).

**CONCLUSIONS:** TLM technology at the time of embryo development yields a statistically higher pregnancy rate as compared to embryos created with traditional incubation. This is especially true when the best embryo(s) is(are) selected for transfer as well as when only an eSET is performed. Given the significantly improved pregnancy rates, TLM incubation of embryos should be considered as an alternative to traditional incubation.

**P-101** Tuesday, October 15, 2019 6:30 AM

**DECREASING RATES OF MULTIPLE GESTATION AND NUMBER OF EMBRYOS TRANSFERRED IN GESTATIONAL CARRIER PREGNANCIES: A LONGITUDINAL ANALYSIS.** Rachel S. Mandelbaum, MD, Meghan B. Smith, MD, Jacqueline Ho, MD MS, Richard J. Paulson, MD, MS, Kristin Bendikson, M.D. University of Southern California, Los Angeles, CA.



**OBJECTIVE:** The recent national push towards elective single embryo transfer (eSET) has decreased rates of multiple gestations. However, how this translates to gestational carrier (GC) pregnancies has not been well established.<sup>1</sup> We sought to evaluate the number of embryos transferred (ET) and the incidence of multiple gestations in GC pregnancies as well as if these metrics have changed over time.

**DESIGN:** A retrospective analysis of all GC deliveries from a single agency between 2008-2019.

**MATERIALS AND METHODS:** Data from a large agency that consisted of matched GCs and intended parent (IP) couples for an index GC pregnancy were reviewed. GCs were excluded if the number ET or number delivered were not reported. Data collected for analysis included number ET, number delivered for the index GC pregnancy as well as a history of surrogacy or multiple gestations for each GC. All variables were analyzed using unpaired student's t-test and Pearson's correlation for continuous variables where appropriate and chi-squared for dichotomous variables.

**RESULTS:** Of 836 GC pregnancies, 187 (22.4%) were multiple gestations, consisting of 183 (21.9% of all pregnancies) twins, 3 (0.4%) triplets, and 1 (0.1%) quadruplet pregnancy. 116 (13.9%) GCs overall had a history of a multiple gestation prior to the current GC pregnancy, of which 53.6% were due to another prior GC pregnancy. There was a similar rate of multiple gestation in the index GC pregnancy when comparing GCs with a history of prior singleton vs multiple gestations ( $P = 0.882$ ). There was also no difference between first-time or repeat surrogates in likelihood of having a multiple gestation ( $P = 0.435$ ). In terms of number of ET, 422 GCs (61.7%) had 2 or more ET, and 14 (1.7%) had four or more. Number of ET was positively correlated with number of infants delivered in the GC pregnancy ( $r = 0.207$ ,  $P < 0.001$ ). Number of ET declined significantly over the study period; in 2008-2010, 89% of GCs had  $\geq 2$  ET compared to 30.6% in 2017-2019 ( $P < 0.001$ ). This paralleled a declining incidence of multiple gestations (29.4% in 2008-2010 vs. 11.5% in 2017-2019,  $P < 0.001$ ). Neither women with a history of twins nor repeat surrogates were more likely to have two or more ET (both,  $P > 0.05$ ).

**CONCLUSIONS:** In line with trends in autologous transfers, the number of ET and incidence of multiple gestation is declining in GC pregnancies. A GC's prior obstetric history, specifically a history of multiples or history of surrogacy, does not impact the number of ET or incidence of multiple gestation in the index GC pregnancy. To minimize obstetrical risks to GCs and maximize healthy singleton deliveries for IPs, eSET in appropriate candidates should be encouraged.

References: 1. Perkins KM, Boulet SL, Jamieson DJ, Kissin DM. Trends and outcomes of gestational surrogacy in the United States. *Fertil Steril*. 2016 Aug; 106(2): 435-442.

**P-102** Tuesday, October 15, 2019 6:30 AM

**DOES CATHETER TYPE AND/OR SONOGRAPHER IMPACT SUCCESS RATES FOR EMBRYO TRANSFER?** Emily Sarah Lin, BA pending,<sup>a</sup>

Angela Claire Thyer, MD,<sup>b</sup> Paul Chungyu Lin, MD,<sup>b</sup> Northwestern University, Evanston, IL; <sup>b</sup>Seattle Reproductive Medicine, Seattle, WA.



**OBJECTIVE:** To evaluate whether catheter type or individual sonographer differences affect the success of embryo implantation.

**DESIGN:** Retrospective chart analysis from 1/11/2016 to 4/18/2018 for all patients under age 38 that underwent an embryo transfer at a large, private in-vitro fertilization clinic.

**MATERIALS AND METHODS:** 2,537 transfers were performed over a two-and-a-half-year period by 11 physicians. Nine different catheters' and 11 sonographers' results were analyzed against implantation success. Biochemical pregnancy and clinical intrauterine gestation were both considered a positive implantation result, and not pregnant was considered a negative implantation result. In order to decipher whether implantation success was significantly affected by catheter or sonographer, a chi-squared test was performed for each factor. Given an alpha of 0.05 and 20 degrees of freedom, the value indicating significance was 10.851 for sonographers. Given alpha of 0.05 and 16 degrees of freedom, the value indicating significance was 7.962 for catheters. Once significance was found, the degree to which positive outcomes observed was more than expected ((Observed-Expected)/Expected) was combined with the degree to which negative outcomes observed was less than expected ((Expected-Observed)/Expected), giving an overall degree to which positive outcomes were seen for each factor.

**RESULTS:** Both catheters and sonographers had a significant effect on success of implantation. The chi-squared test for sonographers resulted in a total value of 13.024. A value of 10.851 was needed for significance. The chi-squared test for catheters resulted in a total value of 40.735. A value of 7.962 was needed for significance. It was found that the Wallace 18cm and the Sureview 18cm both had overall positive results with an overall positive score of 0.07 and 0.09, respectively, whereas the others did not. It was found that sonographers B, C, D, G, and H had overall positive results, with an overall positive score of 0.15, 0.18, 0.53, 0.02, and 0.17 respectively, whereas the others did not.

**CONCLUSIONS:** There is a significant impact on implantation success between catheter types. There is also a significant impact on implantation success based on which sonographer is assisting with imaging during the embryo transfer. The catheter choice demonstrated a bigger impact than the sonographer. There are limitations to this study. The physician performing the embryo transfer was not analyzed in this study. Individual physicians could have an impact on this analysis, thus potentially skewing the results.

Reference: NONE.

SUPPORT: NONE.

**P-103** Tuesday, October 15, 2019 6:30 AM

**EVIDENCE BASED QUANTITATIVE PREDICTION OF RISK OF MULTIPLE GESTATION RESULTING FROM THE TRANSFER OF MULTIPLE EMBRYOS.** Michael Awadalla, MD, Kristin Bendikson, M.D., Jacqueline Ho, MD MS, Ali Ahmady, PhD, Richard J. Paulson, MD, MS. University of Southern California, Los Angeles, CA.



**OBJECTIVE:** To develop and evaluate a quantitative model for prediction of risk of multiple gestation after transfer of multiple embryos that can be used with a mobile device application.

**DESIGN:** Cross sectional analysis of clinic based data.

**MATERIALS AND METHODS:** We developed a three-step model to quantitatively predict the outcomes of multiple embryo transfers. We used data from three years of autologous cleavage stage and blastocyst embryo transfers at a single academic fertility center. The data set consisted of 760 embryo transfers of a total of 1928 embryos.

First, a training set of data was used to develop a best fit model. The data consists of the number of live births that resulted from an embryo transfer, maternal age at oocyte retrieval (<35 years, 35-37 years, 38-40 years, or 41+ years), number of embryos, embryo stage (cleavage or blastocyst), and embryo quality determined by the embryologist (good or fair/poor). Based on the training data a rate of live birth was calculated for each of 16 embryo categories through the use of a specifically designed computer program.

Second, the same computer program was utilized to make a quantitative assessment about the likelihood that two embryos transferred concurrently will both implant more often than would be expected by chance alone. This accounts for universal factors that affect all embryos transferred concurrently.

Third, means and standard deviations of outcomes for a test sample of embryo transfers were predicted using the best fit model and random number generation. We tested the model with six groups of multiple embryo transfers (Table 1). Z-tests were used to compare actual to predicted outcomes.

RESULTS: The predicted and actual rates of multiple birth for each of six embryo transfer groups is shown in Table 1. The differences between predicted and actual rates of multiple birth were not statistically significant and the standard errors were normally distributed on a quantile-quantile plot.

TABLE 1. Predicted and actual rates of multiple birth (multiple deliveries / total deliveries)

embryos transferred		less than 38 years	38 years & greater
2 blastocysts	predicted (95% CI)	36% (23-50%)	7% (0-30%)
	actual	30% (p = 0.37)	17% (p=0.30)
2 cleavage stage	predicted (95% CI)	9% (0-25%)	16% (5-29%) *
	actual	16% (p = 0.31)	
3 cleavage stage	predicted (95% CI)	34% (18-52%)	19% (p=0.59)
	actual	29% (p=0.51)	
4 or 5 cleavage stage	predicted (95% CI)	n/a	21% (8-36%)
	actual		17% (p=0.59)

\* this group included transfer of 2 or 3 cleavage stage embryos

CONCLUSIONS: Current recommendations for number of embryos to transfer are based on expert opinion. This model can be used with a mobile device application at the point of care for evidence based quantitative prediction of risk of multiple gestation after transfer of multiple embryos.

P-104 Tuesday, October 15, 2019 6:30 AM

#### REDUCING SIZE OF TRANSFER SYRINGE IS BENEFICIAL FOR IVF PREGNANCY OUTCOMES.

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OBJECTIVE: Embryo transfer has been emphasized as one of significant factors relates to IVF pregnancy. In addition to non-traumatic technique, fluid dynamic actions during embryo transfer has studied (Ding et al, 2018). In the transfer catheter, embryos encounter sudden increase of pressure (due to push down syringe plunger) then fast decompression when out of catheter. In the mouse model, sudden pressure alteration causes blastomeres apoptosis (Grygoruk et al, 2011,2012). How the consequence of sudden pressure alteration impacts human IVF success is waiting for clarification. This study compared two types of transfer syringe (1ml or 0.2 ml) to examine potential beneficial effect by reducing the size of transfer syringe.

DESIGN: A retrospective study.

MATERIALS AND METHODS: By ethical concern, it is improper to perform embryo transfer in a Pair t design. The study retrospectively compared data in 2 periods of time with different size of transfer syringes. All frozen embryo transfers during 2016-2018 included in the study. Frozen embryos were PGTA tested euploidy. During 2016, 1 ml of air tight syringe was used for embryo transfer. During 2017-2018, 0.2 ml of microsyringe

	# FET procedure	# embryo	# sac/# embryo	# FHB/#embryo	# SAB/#embryo
2016	640	752	360 (47.8%)	326 (43.3%)	66 (8.7%)
2017-2018	763	832	460 (55.2%)	421 (50.6%)	67 (8.0%)
Analysis	n.a.	n.a.	P <0.005	P <0.005	n.s.

n.a.: not applicable; n.s.: not significant

(Embryon, Rocket) was utilized for embryo transfer. Embryo transfer catheter was Wallace Sure-Pro (PE623) or PEB623 for difficult transfer. Implantation, fetal heart beat (FHB), and spontaneous abortion (SAB) rates were compared between two examined periods.

Study data set extracted from main clinical database without identifiable patient information. Chi-square used for statistical analysis.

RESULTS: The results summarized in the following table

CONCLUSIONS: It is a fact that fluid moving from high pressure to low pressure. In the human IVF, the pressure differential generates by pushing down plunger during embryo transfer. With the same speed to push down the plunger, the higher the volume of syringe the higher the differential pressure generated. The higher pressure alteration gives higher stress to embryos/blastomeres. The consequence of higher stress reflects by the significantly lower implantation and fetal heart beat rates. The results from this study correspond to mouse embryo observations(Grygoruk et al, 2011,2012). Reducing the size of transfer syringe is beneficial for IVF success.

References: Ding D et al: Theoretical Biology and Medical Modelling 2018; 15:20-29.

Grygoruk C, et al: J Assist Reprod Genet 2011; 28:363-368

Grygoruk C, et al: Fertil Steril 2012; 97:1417-1421.

SUPPORT: None.

#### IVF OUTCOME PREDICTORS - EMBRYOS

P-105 Tuesday, October 15, 2019 6:30 AM

#### ONGOING PREGNANCY RATE OF VITRIFIED-WARMED BLASTOCYST TRANSFERS IN AUTOLOGOUS PATIENTS: SINGLE VS DOUBLE TRANSFER ACCORDING TO THE DAY OF DEVELOPMENT.

Juergen Liebermann, PhD, HCLD, Sara Sanchez-Julias, BS, Rebecca Brohammer, BS, Janna Schwab, MS, Meike L. Uhler, M.D., Jennifer E. Hirshfeld-Cytron, MD, Christopher Sipe, MD. Fertility Centers of Illinois, Chicago, IL.



OBJECTIVE: Improvement in cryopreservation techniques has led to increasing implantation rates transferring vitrified-warmed embryos. This development has supported the move to recommend single embryo transfers to a greater proportion of patients. Considering the high frequency of day 6 blastocyst formation, the associated lower implantation potential of day 6 blastocyst becomes clinically important. Therefore, in an effort to optimize the pregnancy of transferring growth-delayed day 6 blastocysts, we compared their outcome to normally-developing day 5 blastocysts, and evaluated their efficiency in regards to ongoing (oPR), implantation rate (IR), and Twin pregnancies.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: The study included a total of 3,559 day 5, and 1,740 day 6 vitrified-warmed blastocyst transfers (VBT) in autologous women of 37 years of age and younger recorded between 2004 and 2018. The day 5 group contained 1,857 single (D5sVBT), and 1,702 double (D5dVBT) transfers, whereas the day 6 group contained 680 single (D6sVBT), and 1,060 double (D6dVBT) transfers. The vitrified blastocysts were warmed about 2hrs prior to transfer. Both natural and hormone replacement cycles were used to increase receptivity of the endometrium. Progesterone was supplemented on day 15 of the cycle and blastocysts were warmed on day 5 of progesterone supplementation. Chi-square test was used for statistical analysis of oPR between single and double-vitrified-warmed embryo transfers and according to the day of development (day 5 vs. day 6).

RESULTS: The total oPR was significant lower in the day 6 group compared with those in the day 5 group (35.4% vs 51.1%). The oPR was not significantly different between the D5sVBT group and D5dVBT group (49.5 vs 52.8%). However, the oPR was significantly higher in the

	Total D5	D5sVBT	D5dVBT	Total D6	D6sVBT	D6dVBT
# embryos transferred	1.5	1	2	1.7	1	2
No. of transfers	3559	1857	1702	1740	680	1060
Implantation rate (%)	47.8 <sup>a</sup>	56.1 <sup>aa</sup>	43.3 <sup>b</sup>	33.3 <sup>a</sup>	36.2 <sup>aa</sup>	32.4 <sup>b</sup>
Ongoing pregnancy rate (%)	51.1 <sup>a</sup>	49.5 <sup>aa</sup>	52.8 <sup>aa</sup>	35.4 <sup>a</sup>	29.1 <sup>b</sup>	39.4 <sup>b</sup>
Twin rate (%)	21.9	1.7 <sup>c</sup>	42.1 <sup>c</sup>	23.1	2.1 <sup>cc</sup>	33.1 <sup>cc</sup>

Denotes statistical significance <sup>a,aa,b,bb</sup> $P < 0.01$ ; <sup>c,cc</sup> $P < 0.001$

D6dVBT group compared with the D6sVBT group (39.4 vs 29.1). The Twin PR was statistically significantly lower in both sVBT groups (1.7% vs. 2.1%) compared to both dVBT groups (42.1% vs 33.1%) regardless of the day of development.

**CONCLUSIONS:** This study showed that D5sVBT resulted in comparable oPR compared to D5dVBT, while D6sVBT resulted in significantly lower oPR compared to D6dVBT. However, in any VBT the number of embryos transferred should always carefully considered, because transferring 2 blastocysts regardless of day of development always yielded a significantly higher twin rate.

**SUPPORT:** None.

**P-106** Tuesday, October 15, 2019 6:30 AM

#### RELATIONSHIP OF EMBRYO SEX TO EMBRYO QUALITY, DAY OF BLASTOCYST TRANSFORMATION, AND IVF OUTCOMES.



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**OBJECTIVE:** While it has been theorized that the energy required for X chromosome inactivation in female embryos may have an impact on embryo development, conflicting data exists regarding the impact of embryo sex on blastocyst development and quality and subsequent IVF outcomes. The primary objective of this study is to determine if there is a relationship between embryo sex as determined by preimplantation genetic testing for aneuploidy (PGT-A) and blastocyst transformation and quality and the IVF outcomes.

**DESIGN:** Retrospective analysis.

**MATERIALS AND METHODS:** A retrospective chart review was conducted of patients age 21-47 who underwent PGT-A (n=3708) and subsequent autologous elective single embryo transfer (eSET) (n=539) from June 2007 to December 2018. Primary analyses focused on the relationship of embryo sex to day of blastocyst transformation (day of trophoctoderm biopsy) and embryo quality. Secondary analyses examined the relationship of embryo sex to morphological grade, genetic diagnosis, and rates of implantation, clinical pregnancy (CP), ongoing pregnancy (OP), chemical pregnancy, spontaneous abortion, and ectopic pregnancy. Pearson's chi-squared test was used with  $P < 0.05$  being considered significant.

**RESULTS:** There was no difference in embryo sex and day of blastocyst transformation ( $P = 0.566$ ), embryo grade ( $P = 0.057$ ), or maternal age ( $P = 0.837$ ). Similar results were observed when the analysis was repeated stratified by maternal age and being euploid. Female embryos were more likely than male embryos to be aneuploid (54.6% vs 47.2%,  $P < 0.001$ ). When embryos with sex chromosome aneuploidy were excluded, there was also no correlation between embryo sex and grade ( $P = 0.363$ ) or day of blastocyst transformation ( $P = 0.094$ ). Embryos undergoing blastocyst transformation on day 5 vs day 6 were more likely to result in implantation (71.8% vs 52.6%,  $P < 0.001$ ), CP (69.4% vs 50.9%,  $P < 0.001$ ) and OP (59.1% vs 44.7%,  $P = 0.018$ ). High-grade embryos were also more likely than mid/low-grade embryos to result in implantation (70.8% vs 60.3%,  $P = 0.018$ ), CP (69.2% vs 56.4%,  $P = 0.005$ ) and OP (59.3% vs 48.1%,  $P = 0.018$ ). Day 6 embryos were more likely to result in a chemical pregnancy (5.1% vs 1.0%,  $P = 0.004$ ). Implantation, CP, and OP rates were not different among sex embryo groups. Unlike male embryos, female embryos undergoing blastocyst transformation on day 5 vs day 6 were more likely to result in a CP (68.8% vs 52.0%,  $P = 0.012$ ) and trended towards being more likely to result in an OP (58.2% vs 45.3%,  $P = 0.062$ ).

**CONCLUSIONS:** To our knowledge, this is the largest and most comprehensive study to evaluate the potential relationship between embryo sex and quality or development and first to also look at the subsequent IVF outcomes.

Despite not finding a difference between embryo sex and embryo blastocyst development or IVF outcomes, female embryos were more likely to be aneuploid, which is likely due to an increased frequency of X chromosome aneuploidy. In addition, faster developing and higher-grade embryos were more likely to have favorable IVF outcomes.

**P-107** Tuesday, October 15, 2019 6:30 AM

#### IMPACT OF FRESH-EMBRYO PARAMETERS ON SURVIVAL AND IMPLANTATION IN VITRIFIED BLASTOCYST CYCLES: ANALYSIS OF 11936 WARMED BLASTOCYSTS.



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**OBJECTIVE:** To correlate blastocyst features with the predictive potential of survival and successful implantation in vitrified/warmed blastocyst cycles.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** The study included 11936 vitrified-warmed blastocysts transferred from January 2017 to December 2018. No PGT-A cycles were included. Pre-vitrification morphological parameters analyzed for all blastocysts were as follows: i) day of vitrification (5 vs. 6); ii) blastocyst expansion degree: cavitated (BC), fully expanded (BE) and hatching out of the zona (BHi); iii) trophoctoderm (TE) quality (A, B and C); iv) inner cell mass (ICM) quality (A, B and C); and (v) oocyte origin (donor vs. autologous). Survival and implantation rates were analyzed using a logistic regression model. Odds ratios and 95% confident intervals (CI) were calculated.  $P < 0.05$  was considered statistically significant.

**RESULTS:** Logistic regression model estimated that the day of vitrification (5 vs. 6) was the strongest predictor of embryo survival (1.71; 95% CI: 1.42 – 2.04;  $p < 0.001$ ). Additionally, the odds of survival increased in blastocysts catalogued as BC with respect to those catalogued as BHi (2.05; 95% CI: 1.48 – 2.83;  $p < 0.001$ ), and decreased in blastocysts with TE C compared to those with TE A (1.31; 95% CI: 1.07 – 1.59;  $p < 0.001$ ). However, survival was not affected by the oocyte origin. Regarding implantation, the model showed that TE quality followed by the day of vitrification were the most significant morphological predictors of success. The odds of implantation were doubled for blastocysts with TE graded as A compared to those with TE graded as C (2.03; 95% CI: 1.75 – 2.36;  $p < 0.001$ ), and were cut by half for blastocysts vitrified on day 6 compared to those vitrified on day 5 (0.51; 95% CI: 0.45 – 0.57;  $p < 0.001$ ). The odds of implantation were also increased when transferring hatching blastocysts (1.63; 95% CI: 1.41 – 1.87;  $p < 0.001$ ) and ICM was graded as A (1.35; 95% CI: 1.13 – 1.60;  $p < 0.005$ ).

**CONCLUSIONS:** Blastocysts vitrified on day 5 with top quality TE should be given priority when warming. The degree of blastocoele expansion when vitrifying is closely related to success: BC embryos showed higher survival but lower implantation rates and should be cultured after warming to allow them to expand prior to the embryo transfer. The possibility of double embryo transfer should be considered in vitrified cycles with blastocysts graded as day 6 and TE C.

**P-108** Tuesday, October 15, 2019 6:30 AM

#### DEVELOPMENT AND PRELIMINARY VALIDATION OF AN AUTOMATED STATIC DIGITAL IMAGE ANALYSIS SYSTEM UTILIZING MACHINE LEARNING FOR BLASTOCYST SELECTION.



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**OBJECTIVE:** To assess an automated static digital image analysis system's capabilities to predict a blastocyst's potential to achieve a pregnancy by analyzing morphometric features extracted from single images and computing these utilizing artificial intelligence.

**DESIGN:** Retrospective morphometric study to evaluate an automated static digital image-processing algorithm's predictive capabilities.

**MATERIALS AND METHODS:** Two balanced and high-quality embryo micrograph databases with pregnancy outcomes from single blastocyst transfers (Database A: 134 images; Database B: 87 images), were used to create a pipeline that extracts relevant morphometric features which, along with metadata, allowed us to predict pregnancy defined as beta hCG >20iu, 7 days following blastocyst transfer. Several classifiers were tested within the pipeline using cross-validation techniques to assess the generalization capabilities of the models: Bayesian, Support Vector Machines, Neural Networks, and Ada Boost. Using artificial intelligence, the probability of achieving pregnancy was then estimated.

**RESULTS:** A total of 221 images of blastocysts selected for single embryo transfers were included. The developed algorithm was successful at extracting relevant morphological features from every micrograph. Furthermore, it was successfully able to predict a positive pregnancy test in both datasets. With the use of the computed morphological features in combination, it was possible to achieve an F1 score of 0.76; accuracy of 0.75; and sensitivity of 0.77 for database A. For database B we created a predictive model with 0.74 of F1 score; accuracy of 0.67; and sensitivity of 0.78.

**CONCLUSIONS:** The proposed computational tool based on machine-learning has the capacity to link variables, extracted from single static digital images of blastocysts, to prognosis. By doing so, it allows for a new approach to embryo classification while supporting embryologists towards a more objective and accurate embryo selection process. Different from other approaches, this machine-learning tool doesn't rely on time-lapse incubators, making it a low cost candidate for easy integration into routine clinical practice. A prospective study on a larger scale is underway to replicate our initial results while, at the same time, aiming to improve predictability capabilities through automated machine-learning.

**REFERENCES:** Fishel, S., Campbell, A., Montgomery, S., Smith, R., Nice, L., Duffy, S., Jenner, L., Berrisford, K., Kellam, L., Smith, R., Foad, F., Beccles, A., 2018. Time-lapse imaging algorithms rank human preimplantation embryos according to the probability of live birth. *Reproductive BioMedicine Online* In press.

Gandomkar, Z., Brennan, P. C., Mello-Thoms, C., Jun 2018. Modern: Multi-category classification of breast histopathological image using deep residual networks. *Artificial intelligence in medicine* 88, 14–24.

Goodman, L. R., Goldberg, J., Falcone, T., Austin, C., Desai, N., 2016. Does the addition of time-lapse morphokinetics in the selection of embryos for transfer improve pregnancy rates? a randomized controlled trial. *Fertility and Sterility* 105 (2), 275 – 285e.10.

Guo, X., Yu, Q., Alm, C. O., Calvelli, C., Pelz, J. B., Shi, P., Haake, A. R., 2014. From spoken narratives to domain knowledge: Mining linguistic data for medical image understanding. *Artificial Intelligence in Medicine* 62 (2), 79–90.

Hu, Z., Tang, J., Wang, Z., Zhang, K., Zhang, L., Sun, Q., 2018. Deep learning for image-based cancer detection and diagnosis— $\text{ï}\text{z}\text{ö}\text{e}\text{ï}\text{z}\text{ö}\text{e}\text{ï}\text{z}\text{ö}\text{e}\text{ï}\text{z}\text{ö}\text{e}$  survey. *Pattern Recognition* 83, 134 – 149.

Lagalla, C., Barberi, M., Orlando, G., Sciajino, R., Bonu, M. A., Borini, A., May 2015. A quantitative approach to blastocyst quality evaluation: morphometric analysis and related IVF outcomes. *Journal of Assisted Reproduction and Genetics* 32 (5), 705–712.

Majumdar, G., Majumdar, A., Verma, I. C., Upadhyaya, K. C., Jan 2017. Relationship between morphology, euploidy and implantation potential of cleavage and blastocyst stage embryos. *Journal of Human Reproductive Sciences* 10 (1), 49–57.

Mio, Y., Mar 2006. Morphological analysis of human embryonic development using time-lapse cinematography. *Journal of Mammalian Ova Research* 23 (1), 27–35.

Rebouças Filho, P., Rebouças, E., Marinho, L., Sarmiento, R., Tavares, J., de Albuquerque, V., 2017. Analysis of human tissue densities: A new approach to extract features from medical images. *Pattern Recognition Letters* 94, 211–218.

Rocha, J., Bezerra da Silva, D., dos Santos, J., Whyte, L., Hickman, C., Laver, S., Gouveia Nogueira, M., 2017a. Using artificial intelligence to improve the evaluation of human blastocyst morphology. In: Proceedings

of the 9th International Joint Conference on Computational Intelligence - Volume 1: IJCCI, INSTICC, SciTePress, pp. 354–359.

Rocha, J., Passalia, F., Matos, F., Takahashi, M., Ciniciato, D. d. S., Maserati, M., Alves, M., de Almeida, T., Cardoso, B., Basso, A., Nogueira, M., 2017b. A method based on artificial intelligence to fully automatize the evaluation of bovine blastocyst images. *Scientific Reports* 7 (1), 7659.

Rocha, J., Passalia, F., Matos, F., Takahashi, M., Maserati, M., Alves, M., de Almeida, T., Cardoso, B., Basso, A., Nogueira, M., 2017c. Automatized image processing of bovine blastocysts produced in vitro for quantitative variable determination. *Scientific Data* 4, 170192.

Sciorio, R., Thong, J. K., Pickering, S. J., Mar 2018. Comparison of the development of human embryos cultured in either an embryoscope or benchtop incubator. *Journal of Assisted Reproduction and Genetics* 35 (3), 515–522.

Storr, A., Venetis, C. A., Cooke, S., Susetio, D., Kilani, S., Ledger, W., Jul 2015. Morphokinetic parameters using time-lapse technology and day 5 embryo quality: a prospective cohort study. *Journal of Assisted Reproduction and Genetics* 32 (7), 1151–1160.

Tang, Q., Liu, Y., Liu, H., 2017. Medical image classification via multi-scale representation learning. *Artificial Intelligence in Medicine* 79, 71–78.

Yoshida, I. H., Santos, M., Berton, C. Z., Chiarella, C. L., Tanada, M. S., Cordts, E. B., de Carvalho, W. P., Barbosa, C. P., Apr 2018. Can trophectoderm morphology act as a predictor for euploidy? *JBRA Assist Reprod* 22 (2), 113–115.

Zhang, J. J., Merhi, Z., Yang, M., Bodri, D., Chavez-Badiola, A., Repping, S., van Wely, M., 2016. Minimal stimulation IVF vs conventional IVF: a randomized controlled trial. *American Journal of Obstetrics & Gynecology* 214 (1), 96.e1 – 8.

**P-109** Tuesday, October 15, 2019 6:30 AM

### **ODDS OF EUPLOIDY ARE SIGNIFICANTLY ASSOCIATED WITH NOT ONLY AGE BUT BLASTOCYST MORPHOKINETIC PARAMETERS AND ICM/TROPHODECTODERM CHARACTERISTICS.**



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**OBJECTIVE:** Morphokinetic data from time-lapse (TL) imaging cannot diagnose euploidy with the specificity of preimplantation genetic screening but may provide valuable insight into parameters associated with a euploid diagnosis. This study analyzes the kinetics of blastocyst formation, early cleavage dysmorphisms, ICM/trophectoderm quality and the odds of the blastocyst having a normal chromosome complement.

**DESIGN:** Retrospective analysis of prospectively collected morphokinetic data from patients undergoing aneuploidy screening (PGT-A) from 2014–2017

**MATERIALS AND METHODS:** A total of 2493 zygotes were cultured in the EmbryoScope TL chamber. Embryo videos were annotated daily for the following: t2 (time to 2c), t3, t4, t5, t8, tM (morula), tSB (start of blastulation), tBL (full blastocyst), tEBL (expanded blastocyst), tHB (hatching blastocyst). Presence of dysmorphisms were also recorded. Assisted hatching was performed on day3. Blastocyst maturity stage was scored: 1=early, 2=full, 3=expanded, 4=hatching. ICM and trophectoderm morphology was graded as: 1-good, 2-fair or 3-poor, based on cell organization and number. An overall blastocyst quality score (BQS) from 1 to 6, with 6 being the best was then assigned. Trophectoderm biopsy was performed on day 5/6 and cells were sent for analysis. Euploid embryos were transferred either in the fresh cycle or subsequent frozen cycles.

**RESULTS:** Of the 1258 biopsied blastocysts, 37% (95% CI 34–40%) were diagnosed as euploid. After adjusting for age, seven variables were independently associated with odds of euploidy: For each hour increase in interval between tSB and tEB, the odds of euploidy decreased by 4.5% (OR 0.96; 95% CI 0.93–0.98; p=0.002). If tSB was <96.2, the likelihood of euploidy was 1.5 times higher (95% CI 1.2–1.9; p=0.0006). The presence of more than a single dysmorphism during early cleavage lowered the odds of the blastocyst being euploid by 49% (OR 0.51, 95% CI 0.33–0.80; p=0.003). Euploidy was also associated with blastocyst maturity stage (p<0.0001), ICM grade (p=0.002 and trophectoderm grade (p<0.0001). Percent euploidy correlated significantly to BQS score: 1=22%, 2=13.2%, 3=21.7%, 4=25.5%, 5=39.9% and 6= 47.7%; p<0.0001). A logistic regression model to enhance the probability of selecting a euploid blastocyst was then constructed. ROC analysis to determine the predictive ability of a

model using a combination of these parameters to predict euploidy gave an AUC value of 0.70. In FET cycles, a model combining cryopreservation day with TE grade was highly predictive of the euploid embryo's ability to implant (AUC=0.69). A day 5 blastocyst was three times as likely to implant (OR 2.95; 95% CI 1.57-5.73; p=0.0007). Odds of implantation for TE 1 vs TE 3 were 6-fold higher (OR 6.61 ;95% CI 2.20 -22.89; p=0.0006) and almost 2.5 fold higher with TE 1 vs TE 2 (OR2.45; 95% CI 1.24-4.92; p=0.0097).

**CONCLUSIONS:** The predictive model described here increases the probability of selecting a chromosomally normal blastocyst. Further study is needed to determine if such a model can increase odds of successful implantation in non-PGS patients .

**REFERENCES:** Desai N, Goldberg J, Austin C, and Falcone T. Are cleavage anomalies, multinucleation, or specific cell cycle kinetics observed with time-lapse imaging predictive of embryo developmental capacity or ploidy? *Fertil Steril* 2018 ; 109 (4): 665-674

Desai N, Ploskonka S, Goodman L, Attaran M, Austin C, Goldberg J and Falcone T. Delayed blastulation, multinucleation, and expansion grade are independently associated with live-birth rates in frozen blastocyst transfer cycles. *Fertility and Sterility* 106 (6) 1370-78.

SUPPORT: None.

**P-110** Tuesday, October 15, 2019 6:30 AM

**BLASTOCYST GRADE PREDICTS OUTCOME AFTER FROZEN EUPLOID TRANSFER IN PATIENTS WITH RECURRENT PREGNANCY LOSS.**



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**OBJECTIVE:** Trophoctoderm (TE) grade has been shown to be the most significant predictor of implantation and live birth after fresh untested blastocyst transfer in infertile cohorts. The goal of this study was to determine if TE grade or inner cell mass (ICM) grade are predictive of clinical outcomes in a cohort of RPL patients pursuing PGT-A.

**DESIGN:** Retrospective cohort study from a single fertility center between 2002 and 2018.

**MATERIALS AND METHODS:** Patients with 2 or more prior pregnancy losses performing PGT-A with at least one euploid embryo for transfer were included. All patients underwent ICSI and single euploid frozen blastocyst transfer (euFET). Outcome of the first euFET was recorded. Implantation was defined as beta hcg > 5 mIU/mL. Clinical pregnancy was defined as a visualized gestational sac. Pregnancy loss was defined as loss of pregnancy from implantation to twenty weeks gestation. Multivariate logistic regression analysis was used to evaluate the effect of age, TE grade and ICM grade on clinical outcomes.

**RESULTS:** 660 euFET were included, with clinical outcomes stratified by ICM and TE grade (Table 1). After adjusting for age, ICM grade is not significantly correlated with implantation rate (p=0.12, CI 0.93-1.92), miscarriage rate (p=0.18, CI 0.47-1.16), or pregnancy loss rate (p=0.21, CI 0.56-1.13) but is significantly correlated with live birth rate (p=0.03, CI 1.02-1.81). TE grade is not significantly correlated with implantation rate (p=0.32, CI 0.86-1.56) or miscarriage rate (p=0.11, CI 0.52-1.07) but is significantly

TABLE 1.

	euFET, n (%)	Implantation, n (%)	Clinical pregnancy, n (%)	Live birth/ongoing pregnancy, n (%)	Clinical miscarriage, n (%)	Pregnancy loss, n (%)
<b>ICM grade</b>						
A	217 (33%)	185 (85%)	164 (76%)	145 (67%)	18 (11%)	37 (20%)
B	405 (61%)	327 (81%)	293 (72%)	244 (60%)	47 (16%)	78 (24%)
C	38 (6%)	29 (81%)	24 (63%)	19 (50%)*	5 (21%)	10 (35%)
<b>TE grade</b>						
A	312 (47%)	259 (83%)	232 (74%)	203 (65%)	28 (12%)	52 (20%)
B	277 (42%)	228 (82%)	205 (75%)	172 (62%)	31 (15%)	53 (23%)
C	71 (11%)	54 (76%)	44 (62%)	33 (46%)*	11 (25%)	20 (37%)*

\*p<0.05, multivariate regression analysis adjusting for age

correlated with live birth rate (p=0.02, CI 1.06-1.71) and pregnancy loss rate (p=0.04, CI 0.55-0.98). 16 blastocysts were grade CC, with implantation rate 69% (n=11), clinical pregnancy rate 50% (n=8), live birth rate 31% (n=5), clinical miscarriage rate 38% (n=3) and pregnancy loss rate 55% (n=6).

**CONCLUSIONS:** Compared to embryos with grades A or B, TE and ICM grade C is correlated with lower likelihood of live birth and TE grade C is correlated with higher likelihood of pregnancy loss among RPL patients performing euFET. These results suggest that euploid pregnancy loss in the setting of RPL is still likely of embryonic origin.

**References:** 1. Hill MJ, Richter KS, Heitmann RJ, Graham JR, Tucker MJ, DeCherney AH, Browne PE and Levens ED. Trophoctoderm grade predicts outcomes of single-blastocyst transfers. *Fertil Steril* 99(5):2013.

SUPPORT: None.

**P-111** Tuesday, October 15, 2019 6:30 AM

**SURVIVAL BEHAVIOR OF EMBRYO COHORT IN CULTURE IS ASSOCIATED WITH PREGNANCY PERFORMANCE OF A SURVIVING EUPLOID BLASTOCYST AFTER SINGLE EMBRYO TRANSFER (SET): THE CANARIES IN THE COAL MINE?**



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**OBJECTIVE:** Improving metrics for embryo selection is of great interest in the field of assisted reproductive technology. Preimplantation genetic testing for aneuploidy (PGT-A) is one powerful selection tool available today. Our objective was to investigate whether the survival behavior of an embryo cohort in culture associated with 1) euploid rates of biopsied blastocysts produced from that cohort, or 2) pregnancy outcomes after subsequent single euploid embryo transfer.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** Trophoctoderm biopsies for PGT-A at a single academic center between 2010-2019 were reviewed. At our institution, grade BB (Gardner criteria) or better blastocysts are biopsied on day 5 or 6 and subsequently frozen. A "Poor Embryo Survival" (PES) subset of women was defined as those women for whom >70% of normally fertilized embryos (2PNs) did not progress to biopsiable blastocysts (i.e. dropout, corresponding to the ≥75%ile for embryo dropout). Euploid rates (euploid blastocysts / biopsied blastocysts per ovarian stimulation cycle) were compared between the PES women and the remaining 75% ("Controls"), using generalized linear models to control for age of the oocyte and account for the clustered nature of the data. Pregnancy outcomes following single euploid embryo transfer in a subsequent frozen cycle were similarly compared between PES and Control groups.

**RESULTS:** 1,400 women underwent 2,087 ovarian stimulation cycles yielding 10,087 trophoctoderm biopsies for review. Average age in PES women was 38.2y vs 37.1y in Controls. Although increasing age was associated with higher embryo dropout, euploid rates from surviving, biopsied blastocysts were no different between PES women (Table) vs Controls after adjusting for age in the model (p=0.23). On the other hand, pregnancy outcomes after euploid SET differed based on embryo cohort survival (Table). A euploid blastocyst from a PES cycle had 37% reduced odds of generating an ongoing pregnancy or live birth vs a euploid blastocyst from a Control cycle (OR 0.63, 95% CI 0.41, 0.95, p=0.03).

	PGT-A result		Pregnancy outcomes after SET of euploid blastocyst			
	Euploid	Not pregnant	Biochemical pregnancy	Clinical miscarriage	Ectopic pregnancy	Ongoing pregnancy/ Live birth*
Poor Embryo Survival (PES)	42.4%	39.4%	3.9%	7.9%	0.5%	48.3%
Control	45.5%	33.5%	3.8%	6.2%	0.3%	56.2%

\*p<0.05

**CONCLUSIONS:** Survival rates of embryo cohorts in culture do not appear to correlate with the likelihood of identifying a euploid blastocyst among blastocysts surviving to biopsy, after accounting for age of oocyte. On the other hand, high rates of embryo dropout between fertilization and blastocyst biopsy are associated with reduced odds of live birth or ongoing pregnancy following euploid SET. Embryo cohort behavior in culture may reflect non-genomic pregnancy potential of embryos emerging from these cohorts.

cida confers some level of protection during transfer, and its absence may contribute to lower clinical outcomes.

**REFERENCES:** James, R. M., Picou, A., VerMilyea, M., Hansard, L., & Werland, H. J. (2018). Transfer of completely hatched euploid blastocysts results in significantly lower pregnancy outcomes compared to euploid expanded or hatching blastocysts. *Fertility and Sterility*, 110(4), e44-e45.

Alteri, A., Viganò, P., Maizar, A. A., Jovine, L., Giacomini, E., & Rubino, P. (2018). Revisiting embryo assisted hatching approaches: a systematic review of the current protocols. *Journal of assisted reproduction and genetics*, 35(3), 367-391.

**SUPPORT:** None.

**P-112** Tuesday, October 15, 2019 6:30 AM

**FULLY HATCHED EUPLOID BLASTOCYSTS EXHIBIT LOWER PREGNANCY OUTCOMES WHEN COMPARED TO OTHER BLASTOCYST STAGES IN FROZEN SET CYCLES.**



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**OBJECTIVE:** To determine and compare the clinical outcomes of transferring a trophectoderm biopsied fully hatched blastocyst to other blastocyst stages in Single Embryo Transfer (SET) cycles.

**DESIGN:** Retrospective analysis of private clinic data outcomes.

**MATERIALS AND METHODS:** Pregnancy Rate (PR), Implantation Rate (IR) and Clinical Pregnancy Rate (CPR) of PGT-tested blastocyst SETs during 2017-2018 were analyzed. All fertilized oocytes underwent uninterrupted extended culture until the day of biopsy. Trophectoderm biopsy was performed on culture day 5, 6, or 7 using a single pulse laser breach of the zona pellucida, followed by the insertion of a beveled needle with excision of 3-5 cells. PGT testing was performed utilizing NexGen sequencing. All transfers were performed with vitrified/warmed blastocysts. PR was determined by hCG level of > 5mIU/ml. IR was determined by the number of sacs present at 3 weeks after positive pregnancy, and CPR by the presence of a positive fetal heart beat (FHB) at 7 weeks gestation. Statistical analysis was performed using Chi-square (P < 0.05).

**RESULTS:** Outcomes from 651 transfers utilizing euploid SET were analyzed. Fully hatched blastocysts (n=73) showed a significantly lower PR (42%) when compared to blastocysts with a blastocoel of more than or equal to half the volume of the embryo (n=168) (58%) (p=0.02), expanded blastocysts (n=260) with a full blastocoel (60%) (p=0.009) and hatching blastocysts (n=150) (65%) (p=0.001). SETs with fully hatched blastocysts showed the lowest IR% (29%) when compared to full blastocysts (51%) (p=0.002), expanded blastocysts (53%) (p=0.0002) and hatching blastocysts (56%) (p=0.0004). Moreover, CPR% was significantly impacted after the transfer of fully hatched blastocysts (27%) when compared to full blastocysts (47%) (p=0.004), expanded blastocysts (53%) (p=0.002), and hatching blastocysts (56%) (p=0.0006). Day of development did not influence the clinical outcomes between the different stages of blastocysts (p=0.18). Also the analysis showed no significant difference among the stages of development within the same category for neither the performing physician nor the transferring embryologist (p=0.94, p=0.65 respectively).

**CONCLUSIONS:** It has been recently reported that the transfer of fully hatched blastocysts results in significantly lower success rates when compared to other stages of blastocyst development (James, R. M et al, 2018). It has also been suggested that the complete removal of the zona pellucida increases the implantation potential. However, these studies lack evidence to support the hypothesis (Alteri, A et al, 2018). Despite the striking differences in the outcomes, other factors such as age and day of development did not influence the final result. These findings suggest that the zona pel-

**P-113** Tuesday, October 15, 2019 6:30 AM

**BLASTOCYST MORPHOLOGY CORRELATES WITH PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) RESULTS AND MAY FURTHER PREDICT PREGNANCY POTENTIAL AFTER EUPLOID SINGLE EMBRYO TRANSFER (SET).**



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**OBJECTIVE:** To 1) evaluate the relationship between blastocyst morphology and ploidy via PGT-A, and 2) determine whether morphology might further differentiate pregnancy potential among euploid blastocysts following single embryo transfer (SET)

**DESIGN:** Retrospective cohort

**MATERIALS AND METHODS:** PGT-A results from trophectoderm biopsies between 2010-2019 at a single academic center were reviewed. At our institution, Gardner grade BB or better blasts are biopsied on day 5 or 6. Expansion stage, inner cell mass (ICM) and trophectoderm grades were investigated as potential predictors of blastocyst ploidy. Euploid biopsy results were coded 1 vs 0; mixed effects generalized linear models with a logit link were used to control for age of oocyte and account for the clustered nature of the data. We used a similar approach to evaluate whether elements of morphologic grading predicted pregnancy outcomes after euploid SET. We also considered day of biopsy (5 vs 6) in the analyses.

**RESULTS:** Biopsies from 9,667 blasts produced by 1,427 women over 2,134 cycles were reviewed. There was a progressive increase in euploid rates with increasing expansion at biopsy: blastocyst 40.2%, expanded 44.4%, hatching 48.5%, hatched 52.3%, independent of age of oocyte (p<0.001). Grade A ICM blasts were more likely euploid vs grade B (49.4% vs 44.1%; p<0.001). Grade A trophectoderm blasts were more likely euploid vs grade B (56.2% vs 43.0%; p<0.001). Blasts biopsied on day 5 vs 6 were more likely euploid (50.2% vs 41.9% p<0.001). In a model containing all three components of morphology plus day of biopsy, controlled for age, all but ICM grade remained independent predictors of odds of being euploid; hatching expansion was associated with greatest increase in odds of euploid in this model (OR 2.41, 95% CI 1.99, 2.93, p<0.001). Furthermore, morphology grades correlated with pregnancy outcomes after SET of a euploid blast (n=1,101 transfers; Table). When morphologic grading plus day of biopsy were simultaneously considered in the model, Day 5 biopsy (aOR 2.19) and Grade A trophectoderm (aOR 1.47) remained independent predictors of increased odds of live birth or ongoing pregnancy (Table).

**CONCLUSIONS:** Conventional morphologic grading correlates with ploidy, however grade B blastocysts have reasonable euploid rates. In triaging among surplus euploid blastocysts for transfer, Day 5 biopsy should be prioritized, followed by Grade A trophectoderm, which may reflect non-

	Univariate OR (95% CI)	p	Multivariate aOR* (95% CI)	p
Day of biopsy				
Day 5	2.34 (1.71, 3.19)	<0.01	2.19 (1.54, 3.12)	<0.01
Day 6	Ref		Ref	
Trophectoderm				
Grade A	1.42 (1.00, 2.00)	0.05	1.47 (1.04, 2.08)	0.03
Grade B	Ref		Ref	
ICM				
Grade A	1.56 (1.14, 2.14)	<0.01	1.14 (0.82, 1.59)	0.43
Grade B	Ref		Ref	

\*Multivariate model adjusts for each component in the univariate analyses plus blastocyst expansion

genomic embryo quality and distinguish greater pregnancy potential. Grade A ICM may be considered last.

**P-114** Tuesday, October 15, 2019 6:30 AM

**INCREASED MALE LIVE-BIRTH RATES AFTER BLASTOCYST-STAGE FROZEN-THAWED EMBRYO TRANSFERS COMPARED WITH CLEAVAGE STAGE: A SOCIETY FOR ASSISTED REPRODUCTIVE TECHNOLOGIES CLINICAL OUTCOMES REPORTING SYSTEM STUDY.** Barry E. Perlman, DO,<sup>a</sup> Kavitha Krishnamoorthy, MD,<sup>a</sup> Sara S. Morelli, MD, PhD,<sup>a</sup> Patricia Greenberg, MS,<sup>b</sup> Sangita K. Jindal, Ph.D.,<sup>c</sup> Peter McGovern, MD,<sup>d</sup> <sup>a</sup>Rutgers New Jersey Medical School, Newark, NJ; <sup>b</sup>Rutgers School of Public Health, New Brunswick, NJ; <sup>c</sup>Einstein COM, Montefiore, Hartsdale, OR; <sup>d</sup>University Reproductive Associates, NJ.



**OBJECTIVE:** It has recently been described that blastocyst-stage frozen embryo transfer (FET) is associated with higher live-birth rates compared with cleavage-stage FETs, however other outcomes such as gender have not been well studied. Male-to-female sex ratio of offspring born after fresh blastocyst transfers suggest a shift towards males but whether the use of frozen-thawed embryos affects this ratio is not known. The object of this study was to investigate whether there is a difference in live-birth gender in blastocyst-stage compared with cleavage-stage FETs.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All IVF cycles reported to the Society for Assisted Reproductive Technology from 2004 to 2013 were evaluated. Patients included were those with recorded live births who underwent FETs at either the blastocyst-stage (n=56,193) or the cleavage-stage (n=42,941). Main outcome measures were live-birth gender ratios. Demographic criteria from each cycle was also collected. Statistical analysis was performed using SAS and Microsoft Excel. Chi-square analysis for bivariate associations and generalized estimating equations for adjusted associations were used for statistical analysis with p<0.05 considered as statistically significant.

**RESULTS:** There was a statistically significant increase in the number of male infant births following a blastocyst-stage FET compared with cleavage-stage FET (51.6% vs. 50%; p <0.001). After controlling for potential confounders including age (odds ratio [OR] = 1.06; 95% confidence interval [CI], 1.03, 1.08), BMI (OR = 1.08; CI, 1.04, 1.12), and male factor infertility (OR = 1.06; 95% CI, 1.03, 1.08), the increase in male live-births following blastocyst-stage FET remained statistically significant.

**CONCLUSIONS:** In patients undergoing FETs, blastocyst-stage transfers are associated with higher male gender live-birth rates when compared with cleavage-stage transfers.

**P-115** Tuesday, October 15, 2019 6:30 AM

**EMBRYO SELECTION IN DEVELOPING COUNTRIES- INNER CELL MASS TO BLASTOCYST DIMENSION RATIO AS A PREDICTOR OF PREGNANCY OUTCOME.** Deepika Krish, MS, FRM. Consultant in Rep Med, BANGALORE, India.



**OBJECTIVE:** Is there a correlation between inner cell mass (ICM) to blastocyst dimensions (diameter and area) ratio and pregnancy outcome, which can be used to select embryo for transfer in developing countries?

**DESIGN:** A prospective observational study was performed for a duration of 18 months at a tertiary level IVF centre. 185 subjects who had autologous or donor egg derived blastocyst transfers were included in the study.

**MATERIALS AND METHODS:** 385 Blastocysts were photographed at 116 hours post ICSI. ICM to blastocyst dimensions ratio was calculated using Image J software. Morphological scoring of embryos was performed according to Istanbul consensus. Predictive value of ICM to blastocyst diameter and area ratios to implantation and clinical pregnancy rate were studied by ROC curve analysis. Correlation coefficients were drawn between dimensions ratio and morphological scoring.

**RESULTS:** Mean diameter ratio of ICM to blastocyst among embryos that gave clinical pregnancy was 0.33 +/- 0.05 as against 0.30 +/- 0.09 among embryos that did not give clinical pregnancy (p value = 0.015). Mean area ratio of ICM to blastocyst among embryos that gave clinical pregnancy was 0.14 +/- 0.04 as against 0.13 +/- 0.07 (p value = 0.26). Morphological scoring was calculated from grading as per Istanbul consensus and quantified as 1/ICM score x 1/ Trophectoderm score x Expansion score. Mean morphological scoring of embryos that gave clinical pregnancy was 2.45 +/- 1.27 as against 2.04 +/- 1.08 among embryos that did not give clinical pregnancy (p value = 0.03).

Correlation coefficient between morphological scoring and mean diameter ratio of ICM to blastocyst was 0.23 (p = 0.003), showing mean diameter ratio is positively correlated with morphological scoring. Correlation coefficient between mean area ratio of ICM to blastocyst to morphological scoring was 0.14 (p = 0.06).

Area under curve (AUC) for ICM to blastocyst diameter ratio to predict clinical pregnancy was 0.586 (p=0.04), for area ratio was 0.576 (p=0.04) and for morphological scoring was 0.595 (p=0.04). The predictive ability for clinical pregnancy was highest for existing morphological scoring followed by ICM to blastocyst diameter ratio and least for area ratio.

**CONCLUSIONS:** Ratio between ICM to blastocyst dimensions would serve as a simple, cost-effective tool for selecting embryos prior to transfer in developing countries.

**SUPPORT:** NIL.

**P-116** Tuesday, October 15, 2019 6:30 AM

**DOUBLE BIOPSY, NOT DOUBLE VITRIFICATION, LEADS TO DECREASED PROBABILITY OF ESTABLISHING AND SUSTAINING A VIABLE CLINICAL PREGNANCY.** Terry Schlenker, BSc, MA, MT, Sue McCormick, BS, Robin Smith, BS, William B. Schoolcraft, MD, Mandy G. Katz-Jaffe, Ph.D. Colorado Center for Reproductive Medicine, Lone Tree, CO.



**OBJECTIVE:** Preimplantation genetic testing for aneuploidy (PGT-A) is typically performed on a trophectoderm (TE) biopsy prior to vitrification. When no PGT-A result is returned, patients can request a second biopsy and re-vitrification. Additionally, patients do request PGT-A of prior untested cryopreserved embryos which requires a single biopsy and re-vitrification. The aim of this study was to evaluate the impact of double biopsy and double vitrification on reproductive outcomes following a euploid frozen embryo transfer (FET).

TABLE 1.

	Single Biopsy/Double Vitrification FETs	Double Biopsy/Double Vitrification FET	Single Biopsy/Single Vitrification FET (Control)
Maternal Age	35.1 ± 3.7 <sup>a</sup>	37.8 ± 3.8 <sup>b</sup>	37.3 ± 3.4 <sup>b</sup>
Clinical Pregnancy with Fetal Cardiac Activity	63.8%	53.8%*	67.1%
MAB Rate	5.8%	20.0%*	4.3%
Live Birth Rate	60.1%	43.0%*	64.3%

<sup>a,b</sup> P<0.0001; \*P<0.01

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Groups were classified according to the number of rounds of biopsy and vitrification that the euploid blastocyst (≥Grade 3BB) underwent prior to FET at a single infertility clinic: Single biopsy (4-6 cells)/Double vitrification (n=326 FETs), Double biopsy (9-12 cells)/Double vitrification (n=93 FETs), and a control group of Single biopsy (4-6 cells)/Single vitrification FETs (n=93 FETs). Standard protocols for a hormone replacement FET were utilized for all patients. Statistical analysis included ANOVA and Chi-square test where appropriate, significance at P<0.05.

RESULTS: Mean maternal age was significantly lower in the Single Biopsy/Double Vitrification group (Table 1; P<0.0001). Blastocyst grade was comparable across the groups. FET reproductive outcomes revealed a significant decrease in clinical pregnancy and live birth rate, with a significant increase in MAB rate when a euploid blastocyst underwent a double biopsy and double vitrification prior to transfer (Table 1; p<0.01). There were no significant differences in reproductive outcomes between the Single Biopsy/Double Vitrification FET group and the control group with Single Biopsy/Single Vitrification (Table 1).

CONCLUSIONS: In conclusion, for transferrable quality, euploid blastocysts a single biopsy, double vitrification had comparable reproductive outcomes as a single biopsy, single vitrification, thereby supporting the efficacy of double vitrification. In contrast, a double biopsy had a significant impact on the developmental potential of a euploid blastocyst with a decreased probability of establishing and sustaining a viable clinical pregnancy. This novel study highlights the adverse impact of removing too many TE cells with a double biopsy for PGT-A.

SUPPORT: None.

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#### DEGREE OF RE-EXPANSION FOLLOWING VITRIFICATION/REWARMING OF EUPLOID BLASTOCYSTS IS INVERSELY CORRELATED WITH IMPLANTATION AND ONGOING PREGNANCY/LIVE BIRTH



RATES. Sydney Chang, MD,<sup>a</sup> Taraneh Gharib Nazem, MD,<sup>a</sup> Dmitry Gounko, MA,<sup>b</sup> Marlena Duke, MSc, ELD,<sup>b</sup> Christine Britton-Jones, PhD, HCLD,<sup>b</sup> Alan B. Copperman, MD,<sup>a</sup> Beth McAvey, MD.<sup>a</sup> <sup>a</sup>Icahn School of Medicine at Mount Sinai, New York, NY; <sup>b</sup>Reproductive Medicine Associates of New York, New York, NY.

OBJECTIVE: Routine implementation of blastocyst culture, preimplantation genetic testing, and freeze-all cycles has resulted in supernumerary cryopreserved euploid blastocysts available for frozen embryo transfer (FET). Often faced with a selection of chromosomally normal embryos, embryologists and clinicians turn to embryo morphology, morphokinetics, and timing of blastulation and cavitation to develop prognostic criteria. A recent study showed that re-expansion of vitrified/rewarmed blastocysts strongly correlated with implantation compared to blastocysts that did not re-expand.<sup>1</sup> Yet, that study did not incorporate PGT-A and was limited by small sample size. Thus, our objective was to evaluate the association between degree of re-expansion prior to FET and clinical outcomes among euploid blastocysts.

DESIGN: Retrospective, cohort study.

MATERIALS AND METHODS: The study included patients at an academic center who underwent single euploid FET cycle(s) from 2012-2019. Embryo vitrification/rewarming were performed with the Cryotop method (Kitazato). Embryos were classified into 3 groups: (1) fully re-expanded, (2) partially re-expanded, and (3) not re-expanded. Images of embryos recorded as not re-expanded after 3-4 hours post-warming were manually compared to the image taken immediately post-warming to determine

whether partial re-expansion had occurred during the culture period. Primary outcome was ongoing pregnancy/live birth (OP/LB) rate. Secondary outcomes were rates of clinical pregnancy (CP) and early pregnancy loss (EPL). Data were evaluated with T-tests, chi-square tests, and generalized estimating equations.

RESULTS: The study included 4440 single euploid FET cycles from 2968 patients. There were 118 cycles (2.7%) where embryos were not fully re-expanded 3-4 hours post-warming. Of these, 58 had partially re-expanded and 59 did not re-expand prior to FET. There was a higher proportion of day 7 embryos (27.1%) in the not re-expanded compared to the fully re-expanded cohort (2.6%). After controlling for confounders, blastocysts that did not re-expand after 3-4 hours were associated with a significant decrease in OP/LB (OR 0.19 [95% CI 0.09-0.40], p<0.0001) and CP (OR 0.19 [95% CI 0.10-0.35], p<0.0001), compared to fully re-expanded blastocysts. There was no significant difference in OP/LB or CP rates between partially and fully re-expanded groups. There was no difference in EPL rate between the 3 groups.

CONCLUSIONS: In this study assessing the contribution of embryo re-expansion after vitrification/warming in a single euploid FET model, we showed reduced CP and OP/LB rates in embryos that did not re-expand. Our findings are consistent with Coello et al. who found a lower implantation rate for embryos that did not fully re-expand at FET compared to those that did.<sup>1</sup> Though transfer of blastocysts that did not re-expand resulted in a 76% decrease in OP/LB rate, our study also found no difference in EPL. Patients can therefore be reassured that once implantation has been achieved, there is no demonstrable increase in EPL.

REFERENCES: 1. Coello A, Meseguer M, Galan A, et al. Analysis of the morphological dynamics of blastocysts after vitrification/warming: defining new predictive variables of implantation. *Fertil Steril* 2017; 108(4):659-666.

SUPPORT: None.

P-118 Tuesday, October 15, 2019 6:30 AM

#### PREDICTIVE ABILITY OF A BLASTOCYST GRADE ON REPRODUCTIVE OUTCOMES IN ELECTIVE FRESH EMBRYO TRANSFER.



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OBJECTIVE: Within Canada in 2012, 25,782 invitro fertilization cycles were initiated, and 21,054 embryos transferred (1). The clinical pregnancy rate per embryo was 36.6% (2). Transfer typically occurs day 3 at the cleavage stage or day 5 at the blastocyst stage. Allowing embryos to mature prior to transfer permits self-selection of the "best quality" embryos, leading to higher implantation and pregnancy rates. A challenge is identifying which embryo has the highest likelihood of progressing to pregnancy. In 2011, a consensus document reviewed existing evidence for grading and developed a standard guideline. The purpose of this study is to determine if blastocyst stage or grade is predictive of pregnancy(3).

DESIGN: A retrospective cohort study drawing data from the Atlantic Assisted Reproductive Therapies database and patient charts between January, 2005 and June, 2014 was completed.

MATERIALS AND METHODS: Blastocyst grade and expansion stage (ES) was determined by embryo morphology coded by an embryologist. Only fresh, single embryos transferred on day 5 of culture were included. Data was excluded if the embryo transferred was a morula, cleavage stage or complete grading details were absent. Data was excluded if corresponding

to multiple embryo transfer, frozen embryo transfer, transfer of morula or cleavage stage, was missing charted data on morphologic grading criteria, or if transfer occurred on a culture day other than 5. On selected data, logistic regression models were used to test the association between the occurrence of pregnancy and i) each blastocyst grading criteria (ES, trophectoderm [TD] and inner cell mass score [ICM]) and ii) the total embryo score stratified by blastocyst stage (early blastocyst, blastocyst, expanded blastocyst). All tests were performed at a 5% significance level. The area under the receiver operating characteristic (AUROC) curve was used to evaluate the performance of both TD and ICM in pregnancy prediction.

**RESULTS:** For each one point increase in score for ES, TD and ICM, there was a statistically significant increase in the odds of pregnancy of 1.78 (95% CI: 1.27 – 2.58), 1.38 (95% CI: 1.18 – 1.63), and 1.41 (95% CI: 1.19 – 1.69) respectively. The AUROC curve was nearly identical for both TD and ICM (0.62) and thus both discriminate similarly in predicting pregnancy. It does not appear that one offers greater predictive ability over the other.

**CONCLUSIONS:** This study suggests that ES, TD quality, and ICM quality may be useful for predicting clinical pregnancy rates amongst women undergoing transfer of an early blastocyst, blastocyst, or expanded blastocyst on culture day 5. Interestingly, it seems that TD and ICM are equivalent in terms of predictive ability. The importance of blastocyst expansion on the likelihood of pregnancy was demonstrated. When embryo score was stratified by stage, the transfer of an expanded blastocyst was associated with an increased likelihood of pregnancy. Transfer of an early blastocyst or blastocyst was not.

**REFERENCES:** (1) Canadian Assisted Reproductive Technologies Register. 2012 Pregnancy Outcomes. 2012.

(2) Wilson M, Hartke K, Kiehl M, Rodgers J, Brabec C, Lyles R. Transfer of blastocysts and morulae on day 5. *Fertil Steril* 2004 Aug;82(2):327-333.

(3) Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Human Reproduction* 2011;26(6):1270-1283.

**P-119** Tuesday, October 15, 2019 6:30 AM

**IS THERE A RELATIONSHIP BETWEEN MITOCHONDRIAL DNA CONTENT AND ABORTION RATE IN PATIENTS UNDERGOING SINGLE EUPLOID FROZEN EMBRYO TRANSFER?.**



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**OBJECTIVE:** The mitochondrial DNA (mtDNA) content of trophoctoderm cells is related to the energy supply of the blastocyst, which could affect its ability either to implant in the uterine cavity or not. While it has been demonstrated that euploid blastocysts present a lower mtDNA content as compared to aneuploid blastocysts, there are no data evaluating whether the abortion rate of euploid blastocysts could be associated with their mtDNA content. This information could help maximizing the success rate of single euploid embryo transfers in ART by selecting the blastocysts with the highest potential to achieve a live birth. The aim of this study is to determine whether the mtDNA content is related to the abortion rate in patients undergoing single euploid frozen embryo transfer (SEFET).

**DESIGN:** A retrospective cohort study of 355 SEFET cycles between April 2017 and December 2018 at a single private fertility center.

TABLE 1. Relationship between timing of transfer in different models

Outcome	Crude Model		Model I		Model II	
	$\beta$ (95%CI)	P-value	$\beta$ (95%CI)	P-value	$\beta$ (95%CI)	P-value
Timing of ET						
Fresh ET	1.0		1.0		1.0	
Frozen ET	0.68 (0.55, 0.85)	0.001	0.73 (0.58, 0.92)	0.007	0.75 (0.59, 0.95)	0.016

ET, embryo transfer, CI, confidence interval

Crude model: we did not adjust other covariants

Model I: we adjusted female age

Model II: we adjusted female age, fertilization type, infertility type, infertility duration, no. of oocyte retrieved, no. of embryo transferred and D3/D5 embryo transfer.

CI confidence interval

**MATERIALS AND METHODS:** Patients undergoing ART with pre-implantation genetic testing for aneuploidies (PGT-A) revealing the ploidy outcome and mtDNA content using Next Generation Sequencing (NGS), were included for the study. Blastocyst biopsy was performed on day 5 or 6 of development. All biopsied blastocysts were vitrified, and only euploid ones were selected for warming and subsequent SEFET using the Cryotop method and Kitazato media. Embryo transfer (ET) was performed either in a hormonal replacement therapy cycle (HRT) or in a true natural cycle (NC). Pregnancy outcome was defined 10-14 days after SEFET: a pregnancy test was considered to be positive if  $\beta$ hCG concentration in serum was > 15 IU. Ongoing pregnancy was defined as having a visible gestational sac with a fetal heart beat at 8 weeks of pregnancy while an abortion was defined as having a visible gestational sac without heartbeat until 8 weeks of pregnancy. The primary endpoint was to compare the mtDNA content between embryos leading to an ongoing pregnancy or an abortion. Unpaired two-tailed t-Student test was used to compare means of numerical variables and chi-square test for testing independence between categorical variables. A logistic regression model was performed controlling for maternal age, BMI, transfer distance from the fundus, endometrial thickness, cycle regimen and embryo quality. A p-value < 0.05 was considered statistically significant.

**RESULTS:** 355 euploid blastocysts were selected for SEFET in 314 patients with an average age of (33.7±5.55); 255 of them were biopsied on day 5 (71.8%) and 100 on day 6 (28.2%). Embryo transfer (ET) was performed in an HRT cycle (n=255; 71.8%) or a NC (n=100; 28.2%). A pregnancy rate of 66.2% (235/255) was obtained with ongoing pregnancy and abortion rates of 52.4% and 5.6%, respectively. There was no significant difference in the mtDNA content between pregnant and non-pregnant groups (p=0.095) and between the abortion and ongoing pregnancy group (p=0.15). Multivariate analysis showed the same non-significant relationship except for abortion rate and BMI (p=0.011).

**CONCLUSIONS:** Mitochondrial DNA content of the human blastocyst is unable to predict the abortion rate of implanted euploid blastocysts.

**P-120** Tuesday, October 15, 2019 6:30 AM

**FROZEN-THAWED EMBRYO TRANSFER IS BETTER THAN FRESH EMBRYO TRANSFER IN GNRH ANTAGONIST CYCLE IN NORMO-RESPONDERS: A RETROSPECTIVE COHORT STUDY.**



Xitong Liu, Resident, Haiyan Bai. Physician, Northwest women's and children's hospital, Xi'an, China.

**OBJECTIVE:** To compare the clinical outcome of frozen-thawed embryo transfer and fresh embryo transfer in GnRH antagonist protocol.

**DESIGN:** A total of 1430 normo-responder women from a single ART center (from January 2015 to January 2019) were enrolled in this retrospective cohort study. Women aged <40 years, no. of oocyte retrieved between 3 and 10, good embryo quality, underwent fresh embryo transfer or frozen-all strategy and transferred in subsequent cycle were included. Endometriosis, PGD/PGS cycles were excluded.

**MATERIALS AND METHODS:** The primary outcome was clinical pregnancy rate. A logistic regression analysis was performed to determine the variables that could be independently associated with clinical pregnancy rate. Models were adjusted for covariates including female age, fertilization type, infertility type, infertility duration, no. of oocyte retrieved, no. of embryo transferred and D3/D5 embryo transfer.

**RESULTS:** In total, 495 women were treated with fresh embryo transfer, whereas 935 patients were treated with frozen-thawed embryo transfer.

Clinical pregnancy rate(54.50% vs 63.70%,  $p<0.001$ ) were significantly higher with frozen embryo transfer compared to fresh embryo transfer. Variables that were found to be independently associated with clinical pregnancy rate were fresh/frozen embryo transfer, female age and no. of embryo transferred. After adjusting for variables, frozen embryo transfer(0.75 (0.59, 0.95),  $p=0.016$ ) was protective factor of clinical pregnancy rate.

**CONCLUSIONS:** Frozen embryo transfer is better than fresh embryo transfer in GnRH antagonist cycle in normo-responders.

**P-121** Tuesday, October 15, 2019 6:30 AM

### REGAINING OF FULL EXPANDED STAGE AT 3 HOURS POST-WARMING IS A SUPERIOR MORPHOLOGICAL MARKER FOR LIVE BIRTH RATES IN VITRIFIED-WARMED SINGLE BLASTOCYST TRANSFER CYCLES.

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**OBJECTIVE:** No study has yet univocally established significance of ICM gradation, TE gradation and degree of blastocoel expansion/re-expansion in enhancing live-births. Also, most studies have clubbed results from fresh/frozen and single/double transfer cycles. We aimed to individually assess the relevance of grades of inner cell mass, trophectoderm and degree of post-warm blastocoel re-expansion on live-birth rates exclusively in vitrified-warmed single-blastocyst transfer cycles.

**DESIGN:** Retrospective study of vitrified-warmed cycles involving women (n=380) undergoing elective single blastocyst transfer. Oocyte donation, Embryo-donation, assisted hatching and preimplantation genetic diagnosis cycles were excluded. Ethics Committee of the centre approved this study. All blastocysts were graded as per Gardner and Schoolcraft method of classification as 1-6 for degree of expansion and grades A/B/C for ICM and TE.

**MATERIALS AND METHODS:** Natural-cycle endometrial preparation was done with hormone supplementation followed by luteal-phase support with micronized progesterone. Endometrial response (thickness and homogeneity) was noted by ultrasound. Single blastocyst was transferred 3 hours after warming. Pre-vitrification grade of blastocyst was compared with the post-warmed status.  $\beta$ -hCG level measured on day8 of transfer indicated pregnancy. Positive cardiac activity at sixth week confirmed clinical pregnancy. Live-birth was the primary outcome measure.

**RESULTS:** Women were classified into Live-birth (LB; n=172) and non-pregnant (NP; n=208) groups. Age, BMI, infertility period as well as number of oocytes retrieved, rate of formation of good quality cleavage-stage and blastocyst-stage embryos and survival rates post-warming did not differ significantly between the two groups. Same brand of embryo-transfer catheter and same brand and volume of media was used for transfer. However, the degree of re-expansion was significantly higher in LB group than in NP group (Mean  $\pm$  SD:  $3.7 \pm 0.9$  v  $3.0 \pm 0.83$ ,  $P=0.0052$ ). The Fisher-Exact test odds ratio for achieving a live-birth with 3-4 degree of re-expansion was 3.0 ( $p=0.0010$ ) whereas the odds ratio was much lower (0.32) with any other degree of re-expansion. Although ICM grade was higher/better in Live-birth group than in non-pregnant group, the difference remained statistically non-significant (Odds ratio 1.23,  $p=0.82$ ). No significant difference was observed in the TE grades between the two study groups ( $p=0.2$ ). A notable difference was also observed in the endometrial echopattern ( $p=0.03$ ) although the endometrial thickness remained comparable between the two groups. Results suggest that, post-warming degree of re-expansion is the single most decisive factor for live birth rates in such cycles.

**CONCLUSIONS:** Blastocoel re-expansion to expanded 3/4 stage is a superior morphological indicator than ICM & TE grades for better live-birth rates in vitrified-warmed single blastocyst transfer cycles. However, larger multicentric trials are required to univocally establish it as a robust prognosticator of live-birth rates in vitrified-warmed ET cycles.

**SUPPORT:** None.

**P-122** Tuesday, October 15, 2019 6:30 AM

### DOUBLE EMBRYO TRANSFERS OF POOR QUALITY BLASTOCYSTS GIVE THE SAME LIVE BIRTH RATE AS A SINGLE EMBRYO TRANSFER OF A GOOD QUALITY BLASTOCYST.

Maiko Hanada, Associate, Ogikubo hospital Niji clinic, Tokyo, Japan.



**OBJECTIVE:** How can embryo transfers of poor quality blastocysts lead to good results?

**DESIGN:** A single clinic retrospective cohort study of 1379 frozen-thawed embryo transfer of blastocysts performed in 2014 -2017.

**MATERIALS AND METHODS:** Using Gardner 's blastocyst grading scale, embryos grade A or B were evaluated as good quality blastocyst and embryos containing C evaluation as poor quality blastocyst. We analyzed the live birth rate, miscarriage rate and multiple pregnancy rate of single embryo transfer of good quality blastocyst (SBT-G), single embryo transfer of poor quality blastocyst (SBT-P), double embryo transfers of good quality blastocysts(DBT-GG), double embryo transfers of good and poor quality blastocysts(DBT-GP) and double embryo transfers of poor quality blastocysts (DBT-PP).

**RESULTS:** 1379 cycles were included in the study. The mean age(SD) of the whole study population was 37.8 years ( $\pm 4.0$  years).1020 cycles in women who received SBT-G, 167 cycles in women who received SBT-P, 56 cycles in women who received DBT-GG, 74 cycles in women who received DBT-GP and 62 cycles in women who received DBT-PP. There was no significant difference in the age of each group. The live birth rate (SBT-G:21.4%,SBT-P:12.6%,DBT-GG:32.1%,DBT-GP:23.0%,DBT-PP:19.4%) was significantly higher in the SBT-G group and the DBT-GG group than in the SBT-P group. The miscarriage rate (SBT-G:28.3%,SBT-P:24.2%,DBT-GG:17.4%,DBT-GP:35.7%,DBT-PP:36.4%) tended to be lower in the DBT-GG, but with no significant difference between each group. The multiple pregnancy rate (SBT-G:2.9%,SBT-P:0%,DBT-GG:21.7%,DBT-GP:3.6%,DBT-PP:13.6%) was significantly higher in the SBT-G group and the DBT-GG group than in the SBT-P group. And the multiple pregnancy rate was significantly higher in the DBT-PP group than in the SBT-G group.

**CONCLUSIONS:** In embryo transfer using poor quality blastocysts, the live birth rates of DBT-GP and DBT-PP were almost the same. The live birth rate of SBT-G was also almost the same. From this, it was found that there is no benefit in DBT-GP, in which the live birth rate does not rise despite double embryo transfers, and only the miscarriage rate rises.In addition, although the live birth rate is low if only one poor quality blastocysts is used, the live birth rate is almost equivalent to that of SBT-G and DBT-GP by performing double embryo transfers of poor quality blastocysts. It was found that DBT-PP is an effective method when there are only poor quality blastocysts.However, Double embryo transfers raise the multiple pregnancy rate, so careful consideration is necessary.

**P-123** Tuesday, October 15, 2019 6:30 AM

### THE IMPACT OF TEMPERATURE AND RELATIVE HUMIDITY ON OUTCOMES OF OVARIAN STIMULATION AND IN VITRO FERTILIZATION USING AN OOCYTE DONATION COHORT.

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**OBJECTIVE:** To determine the effect of temperature, humidity, and precipitation prior to oocyte retrieval on ovarian stimulation outcomes among oocyte donors and early in vitro fertilization (IVF) outcomes among recipients.

**DESIGN:** Retrospective cohort study of data from a frozen donor oocyte bank from 2008 to 2015.

**MATERIALS AND METHODS:** A total of 350 oocyte donors residing in the metro-Atlanta area underwent 553 ovarian stimulation cycles with an antagonist protocol. Mature oocytes were vitrified and later thawed in individual cohorts among 989 unique recipients. Mean temperature, relative humidity, and precipitation levels were calculated for the 90 days prior to oocyte retrieval using information from the Parameter-elevation Regressions on Independent Slopes Model. The associations between these climate variables and outcomes of ovarian stimulation (e.g. estradiol level at trigger and number of total and mature oocytes retrieved) and early IVF outcomes (e.g. % fertilized oocytes and % usable embryos) were modeled using generalized estimating equations adjusted for donor age, body mass index (BMI), race, retrieval year.

**RESULTS:** The mean (standard deviation) age and BMI among oocyte donors was 25.4 (2.8) years and 22.6 (2.5)  $\text{kg/m}^2$ . Approximately 25% were racial/ethnic minorities and all were non-smokers. Donors exposed to warmer temperatures prior to oocyte retrieval had significantly higher

estradiol levels at trigger (p-trend=0.04) despite no differences in the total dose of gonadotropins. Specifically, women in the highest quartile of temperature (76.0-81.4°F) had an average estradiol level of 3761 pg/mL (95% CI 3403, 4119) compared to 3341 pg/mL (95% CI 3034, 3647) among women in the lowest quartile (38.6-49.6°F). There was no impact of temperature on oocyte counts. Lower temperatures and higher humidity prior to oocyte retrieval were associated with a slightly higher percentage of usable embryos after oocyte warming and fertilization (p-trend=0.03 and 0.04). Greater mean precipitation prior to oocyte retrieval was associated with a slightly higher percentage of mature oocytes retrieved (p-trend=0.06) but was not associated with any of the IVF outcomes..

**CONCLUSIONS:** While warmer temperatures prior to oocyte retrieval were associated with higher estradiol levels at trigger, the resulting oocytes resulted in a lower percentage of useable embryos once thawed and fertilized among recipients. Vitrified oocyte donation banks represent an excellent model to determine the impact of environmental exposure such as climate variables on IVF outcomes given that exposures experienced by the donor and recipient are uncorrelated in time and space.

**SUPPORT:** Supported in part by R00ES026648 from the NIEHS.

**P-124** Tuesday, October 15, 2019 6:30 AM

#### **TIMING OF BLASTOCYST DEVELOPMENT IS THE SOLE PREDICTOR OF POSITIVE PREGNANCY OUTCOME IN ADVANCED MATERNAL AGE PATIENTS FOLLOWING SINGLE EUPLOID BLASTOCYST TRANSFER.**

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**OBJECTIVE:** Improving embryo quality and selection is imperative to achieve a shorter time to pregnancy and successful live birth outcome, specifically for women of advanced maternal age (AMA). Preimplantation genetic testing for aneuploidy (PGT-A) allows for selection of euploid embryos, beneficial for this older population that are at increased risk for oocyte aneuploidy. The purpose of this study was to identify effective predictors of reproductive outcomes for AMA patients in conjunction with a euploid single embryo transfer (SET).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** AMA patients ( $\geq 38$  years; n=847) underwent routine oocyte retrieval, ICSI, blastocyst biopsy, PGT-A and subsequent euploid SET at a single large infertility clinic. Patients presented with various infertility diagnoses and without significantly compromised ovarian reserve. Reproductive outcomes were divided into three groups; successful live birth (n=504), positive implantation followed by pregnancy loss (ultrasound  $\pm$  FHT; n=81) and negative/biochemical (negative, or rise and subsequent direct fall of  $\beta$ hCG; n=262). Statistical analyses included Chi Square test for independence and ANOVA where appropriate, with significance at  $P < 0.05$ .

**RESULTS:** Maternal age at oocyte retrieval was significantly associated with the likelihood of an embryo transfer, as the percent of aneuploid-only cycles increased significantly with age; 38-40 years (20%), 41-42 years (46%), 43+ years (76%;  $P < 0.0001$ ). Reproductive outcomes following euploid SET were not associated with any of the following: maternal age at oocyte retrieval (mean 40.0 years), total number of oocytes retrieved, total number of MII oocytes, number of blastocysts available for biopsy, % euploid blastocysts, or the inner cell mass (ICM) and trophoctoderm grade. Pregnancy outcomes following euploid SET were only significantly associated with the timing of blastocyst development and the appearance of the ICM ( $P < 0.0001$ ). Euploid blastocysts that resulted in negative/biochemical outcomes were significantly over-represented by slower blastocyst development (52.3%) compared to those with live birth (37.5%) and pregnancy loss (38.3%) ( $P < 0.0001$ ). Remarkably, no differences were observed between successful live birth and positive implantation followed by pregnancy loss for any parameters analyzed. Interestingly, a second euploid SET for AMA patients with prior pregnancy loss resulted in 58.3% successful live births (n=36), comparable to the overall first SET live birth rate (59.5%; ns).

**CONCLUSIONS:** The timing of blastocyst development and the appearance of the ICM was the sole predictor of positive pregnancy in AMA patients following a euploid SET, and thus should be highly considered for euploid blastocyst selection to achieve the fastest time to pregnancy. Further investigations are essential to identify potential predictors of euploid pregnancy loss, and the stochastic occurrence of this adverse outcome.

**SUPPORT:** None.

**P-125** Tuesday, October 15, 2019 6:30 AM

#### **AUTOMATED HALO IDENTIFICATION: A NOVEL PREDICTIVE FEATURE FOR IVF SUCCESS IDENTIFIED THROUGH AN ARTIFICIAL INTELLIGENCE (AI) ALGORITHM.**

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**OBJECTIVE:** To identify in fertilized oocytes previously unrecognized predictive features for live birth following IVF treatment that can apparently be revealed only by an advanced novel AI algorithm.

**DESIGN:** The study used AI to analyse TL videos of embryos in their pronuclei stage.

**MATERIALS AND METHODS:** We analyzed video images of 123 fertilized embryos obtained from a time-lapse system Embryoscope. Of the 123 videos, 111 were clear enough for analysis. All embryos analyzed were graded as Top-Graded embryos and were transferred back to the uterus. Of these embryos 88 (71.5%) successfully implanted and 45 (36.6%) resulted in a live birth.

Using a machine learning algorithm, we were able for the first time to characterize a previously unrecognized feature, the pale cytoplasm creating a "halo" surrounding the nucleus of the fertilized oocyte. The measurable amount of this halo over a range of images was compared to a set threshold. The resulting yes/no decision was assessed in relation to the likelihood of the embryo to implant successfully.

We calculated a relative brightness/smoothness measure, comparing each image to a reference image of the same embryo 7 hours earlier. These measurements were compared to an internal threshold obtained experimentally, with the result reported as above threshold (significant halo identified) or below threshold (no significant halo identified).

**RESULTS:** The halo was identified in 42% of 49 videos of embryos that successfully implanted versus in only 17% of embryos that failed to implant. There was no difference in the proportion of embryos that implanted, where a halo was identified, according to whether they carried out to a live birth or miscarried.

Using the halo to predict successful implantation of a Top-Graded transferred embryo had a sensitivity of 42% and a specificity of 83%, with a positive predictive value of 85% and a negative predictive value of 46%.

**CONCLUSIONS:** An AI algorithm identified in video images of fertilized oocytes a previously unrecognized feature that is associated with a high positive predictive value for subsequent successful implantation. The Automated Halo Identification may help improve embryo selection and result in higher live birth rates.

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#### **HIGHER CUMULATIVE LIVE BIRTH RATES (CLBR) ARE EXPECTED WITH A FREEZE-ALL POLICY AS COMPARED TO A FRESH EMBRYOTRANSFER POLICY, WHEN MORE THAN TWO BLASTOCYSTS ARE AVAILABLE.**

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**OBJECTIVE:** The purpose of the study is to evaluate if there is any advantage in terms of pregnancy expressed as CLBR between patients having at least 2 blastocysts, who follow Freeze-all strategy and patients who have first a fresh ET and then subsequent frozen-thawed ETs (FET).

**DESIGN:** This is a prospective observational study, which includes two groups of patients; Group FRALL: couples which followed freeze-all Policy (no fresh ET) and up to 3 FET and Group FRESH: couples which completed one fresh ET and two subsequent FET. All couples had at least two blastocysts available for ET.

**MATERIALS AND METHODS:** Women included in the study were younger than 40 and had at least 4 blastocysts available. Exclusion criteria were: Preimplantation Genetic Testing (PGT), Testicular Sperm Extraction (TESE) cycles or poor responders (oocytes  $< 4$ ). Study was performed

between 2017 and 2018 in Assisting Nature, Centre of Assisted Reproduction and Genetics, Thessaloniki, Greece. FRALL-Group included 87 couples with a mean female age of 32.8 years, while FRESH-Group included 86 women with an average age of 33.1. The Controlled Ovarian Stimulation (COS) was based in an antagonist protocol.

**RESULTS:** The total CLBR was estimated for each group of patients, as well as for each ET separately. X<sup>2</sup> test was used to compare live birth rates between the two groups. In FRALL-Group the Live Birth Rate after the first FET was 57.5% and in FRESH-Group was 39.5%. The LBR was significantly higher in FRALL-group compared to FRESH-Group after the first ET (frozen versus fresh, p<0.05). The total CLBR for all the completed ETs was 81.6% in FRAL-Group and 71.3% in group B. Cumulatively, the live birth rates were again higher for the Freeze-all group though not statistically significant (p>0.05).

**CONCLUSIONS:** The CLBR is higher in patients who follow freeze-all strategy compared to those who undergo fresh and then FET. Our results indicate that in case of blastocyst ETs an artificially prepared endometrium (in a frozen cycle) might be superior than that after a stimulation cycle. This indicates that women considered normal or high responders have better chances of achieving live birth, if they follow Freeze-all policy. With appropriate consultation women do not argue about fresh and frozen ET, and once some criteria met, they are happy to follow our instructions. A cut-off of 2 blastocysts might look favorable into freezing all, however, higher number of cases is required in order to confirm the obtained results.

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**IMPACT OF MEIOTIC SPINDLE IMAGING ON FERTILIZATION, EMBRYO DEVELOPMENT, CLINICAL OUTCOME AND MORPHOKINETIC PARAMETERS: AN ANALYSIS OF 415 IN-VIVO MATURED AND 317 IN-VITRO MATURED HUMAN OOCYTE**



**SIBLINGS.** Yukiko Nakajo, AS,<sup>a</sup> Nobuya Aono, Ph.D.,<sup>b</sup> Hiromitsu Hattori, M.Sc.,<sup>a</sup> Yusuke Nakamura, BS,<sup>a</sup> Chiyuri Kumao, BS,<sup>a</sup> Noriyuki Okuyama, M.Sc.,<sup>c</sup> Tomoko Hashimoto, M.D., Ph.D.,<sup>c</sup> Mayumi Toya, M.D., Ph.D.,<sup>a</sup> Hideki Igarashi, M.D., Ph.D.,<sup>a</sup> Koichi Kyono, M.D., Ph.D.,<sup>c</sup> <sup>a</sup>Kyono ART Clinic, Sendai, Miyagi, Japan, <sup>b</sup>3-13-1, takanawa, Minato-ku, Tokyo, Japan; <sup>c</sup>Kyono ART Clinic Takanawa, Tokyo, Japan.

**OBJECTIVE:** To evaluate the relationship between meiotic spindle imaging of in-vivo and in-vitro matured human oocytes and intracytoplasmic sperm injection (ICSI) outcomes.

**DESIGN:** This study was a retrospective observational study conducted at Kyono ART Clinic in Japan from September 2012 to January 2019.

**MATERIALS AND METHODS:** This study included a total of 259 ICSI cycles in which were retrieved six or fewer mature oocytes and at least one immature oocyte. ICSI was performed on matured oocytes immediately after denudation. After denudation, MI oocytes were cultured for 4 hours to allow oocyte maturation. We categorized each sibling MII oocyte into an in-vivo matured oocyte group (n=415 oocytes) and an in-vitro matured oocyte group (n=317 oocytes). Both groups, the oocytes' meiotic spindles were visualized with a Polscope before ICSI. We compared fertilization rate, embryo development and clinical pregnancy rate between the two groups with or without a spindle. Furthermore, 196 embryos (96 in-vivo matured oocytes with spindle, and 7 without spindle; 39 in-vitro matured oocytes with spindle, and 44 without spindle) were analyzed for morphokinetic parameters and incidence of direct unequal cleavage (DUC) by time-lapse imaging (TLS, EmbryoScope+<sup>TM</sup>). Statistical comparisons between the experimental groups were performed through Fisher's exact test. Statistical difference was considered to be significant at P<0.05.

TABLE. Fertilization rate, embryo development and clinical outcomes

	Meiotic spindle in in-vivo matured oocytes		Meiotic spindle in in-vitro matured oocytes	
	Detected	Not detected	Detected	Not detected
No. of oocytes	325	56	70	247
Fertilization rate	73.2%* (273/373)	45.2% (19/42)	69.4%* (50/72)	49.4% (121/245)
Blastocyst rate	45.6%* (93/204)	28.6% (4/14)	34.3%* (12/35)	17.7% (17/96)
Good quality blastocyst	20.1%* (41/204)	0.0% (0/14)	8.6%* (3/35)	0.0% (0/96)
No. of vitrified oocytes	83	3	12	14
No. of transferred oocytes	63	7	10	21
Clinical pregnancy rate (fresh and vitrified)	28.2% (29/103)	25.0% (2/8)	16.7% (3/18)	8.0% (2/25)

\*P<0.05

**RESULTS:** The mean patient age was 39.0±4.1 years (range: 25-45 years). The spindle was detected in 85.3% (325/381) and 22.1% (70/317) of the in-vivo and in-vitro matured oocytes, respectively. In both groups, fertilization, blastocyst formation, and good-quality blastocyst rates were significantly higher when spindles were detected (Table). When the spindle was detected, there were no significant differences in fertilization rate or embryo development competence between in-vivo matured and in-vitro matured oocytes. Also, there was no significant difference in clinical pregnancy rate in each group (Table). In the morphokinetic parameters analysis, there were no significant differences in time points of cell division (PNf to t8), interval of cell cleavage (CC2 and S2), or incidence of DUC.

**CONCLUSIONS:** Meiotic spindle imaging may be useful for prediction in both in vitro and in vitro-matured oocyte development. When meiotic spindle is detected in matured oocytes, developmental competence may not be influenced by whether maturation occurs in vivo or in vitro.

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**CORRELATION BETWEEN BLASTOCYST STAGE OF EXPANSION AND CLINICAL OUTCOME: A RETROSPECTIVE ANALYSIS OF 810 SINGLE EUPLOID BLASTOCYST TRANSFER CYCLES AT A SINGLE IVF CENTER.**



**CDER.** Vikrant V. Reddy, MSc,<sup>a</sup> Qianying Zhao, MSc,<sup>b</sup> Odgerel Badamjav, MSc,<sup>a</sup> Jennifer Dasig, MSc,<sup>a</sup> Jeong Hee Moon, Ph.D.,<sup>a</sup> Yimin Qin, Ph.D.,<sup>a</sup> Ali Masoudi, BS,<sup>a</sup> Kenney Tuyen, BS,<sup>a</sup> Barry R. Behr, Ph.D.<sup>c</sup> <sup>a</sup>Stanford University Medical Center (LPCH), Sunnyvale, CA; <sup>b</sup>Stanford Fertility and Reproductive Medicine Center (LPCH), Sunnyvale, CA; <sup>c</sup>Stanford Fertility and Reproductive Medicine Center, Sunnyvale, CA.

**OBJECTIVE:** To determine whether a correlation exists between the stage of expansion of a euploid blastocyst and clinical outcome.

**DESIGN:** Retrospective analysis.

**MATERIALS AND METHODS:** A total of 810 PGT-A euploid single blastocyst transfer cycles between 2014 and 2018, graded using the Gardner criteria (Gardner et al., 1999), were retrospectively analyzed at a single IVF center to assess the correlation between the stage of euploid blastocyst expansion and clinical outcome irrespective of ICM and TE grade. These 810 cycles included a total of 119 fresh blastocyst transfers and 691 frozen-thawed blastocyst transfers. Clinical pregnancy was defined as a visible sac by ultrasound. All embryos were partially hatched at the pre-blastocyst stage.

**RESULTS:** Blastocyst expansion stages of 4 and 5 had a significantly higher (p < 0.001) clinical pregnancy rate (67.1% and 59.2%, respectively) compared to the expansion stages of 6 (46.4%). The expansion stages of 2 and 3 do not have a statistical significance/difference compared to the expansion stage 6. The expansion score did not have a correlation with spontaneous abortion (table). Our results contradict previously published work that found no correlation between fully hatched (grade 6) and non-hatching or partially hatched blastocysts (Rodriguez-Purata et al., 2016).

**CONCLUSIONS:** Fully hatched (grade 6) euploid blastocysts had a significantly lower clinical pregnancy rate compared to non-hatching and partially hatched (grade 4 and 5) blastocysts. There was no correlation between the expansion stage and spontaneous abortion. However, our results did indicate that blastocyst stage of expansion is an important factor for clinical success, which should be taken into consideration at the time of transfer. Our study contradicts a previously published study that found no correlation between fully hatched and non-hatched blastocysts. Artificial hatching could impact expansion grades at biopsy. Future research could focus on determining whether the implantation potential is compromised due to the biopsy procedure itself.

Expansion Stage	Euploid	Average Age	Clinical IU	p-value
Morula	1	42.2	0.00%	
1	3	36.4	0.00%	
2	3	35.4	66.70%	0.6
3	32	37.3	50.00%	0.712
4	85	37	67.10%	0.001
5	434	36.7	59.20%	0.001
6	252	37.5	46.40%	
Expansion Stage	Clinical IU	Average Age	SAB	p-value
2	2	35.4	0.00%	
3	16	37.3	12.50%	0.655
4	57	37	15.80%	0.215
5	257	36.7	10.50%	0.854
6	118	37.5	9.30%	

REFERENCES: 1. Gardner DK, Schoolcraft WB. In vitro culture of human blastocyst In: *Towards Reproductive Certainty: Fertility and Genetics Beyond1999*, Jansen R, Mortimer D (Eds). Carnforth, UK: Parthenon Publishing, 1999, 378–88.

2. Rodríguez-Purata J, Gingold J, Lee J, Whitehouse M, Slifkin R, Briton-Jones C, Copperman A and Sandler B.Hatching status before embryo transfer is not correlated with implantation rate in chromosomally screened blastocysts. *Human Reproduction*, Volume 31, Issue 11, 21 November 2016, Pages 2458–2470.

SUPPORT: None.

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### PREGNANCY OUTCOMES OF FROZEN THAWED CLEAVAGE STAGE EMBRYOS WITH OR WITHOUT EXTENDED CULTURE TO BLASTOCYST STAGE.

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OBJECTIVE: Evaluate pregnancy outcomes of frozen thawed cleavage stage embryos with or without extended culture to blastocyst stage.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Frozen embryo transfer (FET) cycles from January 2017 to April 2018 that included cleavage stage embryo transfer (D3 FET) were compared to cleavage stage embryos that underwent extended culture after thawing to blastocyst stage before transfer (EC D5 FET) and thawed blastocyst embryo transfer (D5 FET). Pregnancy outcomes such as pregnancy rate, implantation rate, abortion rate and live birth rate were compared. Patients with any of the following were excluded: endometrial thickness less than 0.7 cm, undergoing simultaneous controlled ovarian stimulation or fresh embryo transfer, only day 2/4/7 embryos available for transfer, or thawing or culture failure.

RESULTS: Total of 1182 cycles were reviewed, and, after exclusion, 843 cases were included in this study. Overall pregnancy rate for D3 FET, D5 FET and EC D5 FET were 34.84%, 50.01% and 47.87%, respectively. The implantation rate for D3 FET, D5 FET and EC D5 FET were 19.30%, 29.63% and 25.36%, respectively. The abortion rate for D3 FET, D5 FET and EC D5 FET were 27.06%, 14.23% and 17.78%, respectively. Significant statistical differences were found when comparing D3 FET pregnancy rates with D5 FET and EC D5 FET pregnancy rates. However, D5 FET and EC D5 FET pregnancy rates were comparable.

CONCLUSIONS: From our results, we have found comparable pregnancy outcomes among transfer of thawed blastocyst embryos and cleavage stage embryos with extended culture to blastocyst stage. The outcomes are also superior to thawed cleavage stage embryo transfer. This offers a new approach for patients who have many cryopreserved embryos from the past years when laboratory techniques were not yet readily available for blastocyst culturing. In addition, extended culture allows self-selection which enables identification of embryos with potential for better pregnancy outcomes.

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### FACTORS PREDICTIVE OF HAVING SUPERNUMERARY EMBRYOS IN FREEZE-ALL CYCLES; AN ANALYSIS OF SART CORS DATA.

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OBJECTIVE: The field of IVF has focused on embryo selection and PGT-A was touted as the optimal method. However, publications demonstrating

euploid live births from embryos found to be abnormal have demonstrated the limitations of PGT-A [1-3]. A selection technique only enhances the chances of success if there is a cohort of embryos from which to select. Freeze-all cycles are gaining wide acceptance due to evidence of equal or increased live birth compared to fresh transfers especially in hyper-responders [4, 5]. Therefore, we sought to identify factors predictive of having supernumerary embryos in freeze-all cycles.

DESIGN: Retrospective cohort study of women who underwent freeze-all cycles in 2014.

MATERIALS AND METHODS: Data were obtained from the Society for Assisted Reproductive Technology Registry. We defined supernumerary as having two or more embryos cryopreserved and computed the proportion of cycles resulting in this outcome. To identify predictive factors for supernumerary embryos, we first fitted a univariable Poisson regression model with a robust variance estimate. We then combined all variables with a p-value < .20 into a multivariable model.

RESULTS: Of 31,537 freeze-all cycles in 2014, 18,250 (57.9%, 95% CI: 57.3 – 58.4%) produced supernumerary embryos. On average, there were six embryos cryopreserved in cycles producing supernumerary embryos. Women with AMH > 3 or < 35 years each had on average 7 embryos cryopreserved. We included 12,173 subjects in the Poisson regression after excluding cycles missing important covariates. Factors predictive of having supernumerary embryos are presented in table 1.

CONCLUSIONS: Several factors are predictive of having supernumerary embryos. Women with AMH > 3 or younger than 35 had more opportunities for embryo transfers such that a selection technique could be applicable.

REFERENCES: 1. Fragouli, E., et al., *Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid-aneuploid blastocysts*. *Hum Genet*, 2017. **136**(7): p. 805-819.

2. Munne, S., et al., *Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected with the use of high-resolution next-generation sequencing*. *Fertil Steril*, 2017. **108**(1): p. 62-71 e8.

3. Sachdev, N.M., et al., *Diagnosis and clinical management of embryonic mosaicism*. *Fertil Steril*, 2017. **107**(1): p. 6-11.

4. Dieamant, F.C., et al., *Fresh embryos versus freeze-all embryos - transfer strategies: Nuances of a meta-analysis*. *JBRA Assist Reprod*, 2017. **21**(3): p. 260-272.

5. Roque, M., et al., *Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes*. *Hum Reprod Update*, 2019. **25**(1): p. 2-14.

SUPPORT: None.

TABLE 1.

Variable (Referent)		Adjusted Risk Ratio (95% CI)	p-value	
Age (< 35)	35 - 37	1.02 (0.99 - 1.05)	.147	
	38 - 40	0.96 (0.93 - 0.99)	<b>.017</b>	
	41 - 42	0.87 (0.82 - 0.91)	<b>&lt; .001</b>	
	> 42	0.70 (0.64 - 0.76)	<b>&lt; .001</b>	
BMI (18.5 – 24.9)	< 18.5	1.03 (0.97 - 1.09)	.340	
	25.0 - 29.9	0.96 (0.93 - 0.99)	<b>.006</b>	
	> 30	0.97 (0.94 - 1.00)	.063	
AMH (1.0 – 3.0)	< 1.0	0.90 (0.86 - 0.95)	<b>&lt; .001</b>	
	> 3.0	1.02 (0.99 - 1.05)	.135	
Nulligravida	Gravida 1+	1.06 (1.04 - 1.09)	<b>&lt; .001</b>	
	Prior fresh transfer (0)	1	0.94 (0.91 - 0.98)	<b>.001</b>
		2	0.93 (0.88 - 0.97)	<b>.003</b>
		3	0.93 (0.86 - 0.99)	<b>.037</b>
		4+	0.97 (0.90 - 1.04)	.333
Prior frozen transfer (0)	1	1.07 (1.02 - 1.13)	<b>.004</b>	
	2	1.07 (1.01 - 1.15)	<b>.034</b>	
	3	1.13 (1.04 - 1.23)	<b>.004</b>	
	4+	1.09 (0.99 - 1.21)	.084	
# eggs retrieved (0 – 3)	4 - 8	5.46 (4.56 - 6.54)	<b>&lt; .001</b>	
	9 - 13	8.42 (7.05 - 10.1)	<b>&lt; .001</b>	
	14 - 20	10.3 (8.59 - 12.3)	<b>&lt; .001</b>	
	21 - 45+	11.0 (9.18 - 13.1)	<b>&lt; .001</b>	
	Sperm source (partner)	Donor	1.05 (1.00 - 1.11)	<b>.049</b>
ICSI (no ICSI)	All mature	1.22 (1.18 - 1.27)	<b>&lt; .001</b>	
	Some mature	1.18 (1.11 - 1.26)	<b>&lt; .001</b>	

**LIVE BIRTH DATA FROM 498 ELECTIVE AND NON-ELECTIVE AUTOLOGOUS OOCYTE THAW CYCLES (2009-2018).**

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**OBJECTIVE:** We present live birth data from 498 autologous treatment cycles using frozen/thawed oocytes. We hypothesized that elective oocyte cryopreservation results in higher live birth rates (LBR) than non-elective (onco-fertility, unanticipated lack of sperm, or limited insemination).

**DESIGN:** Retrospective Cohort.

**MATERIALS AND METHODS:** We identified all autologous In Vitro Fertilization (IVF) cycles using frozen oocytes (2009-2018). Ovarian stimulation, oocyte freeze/thaw, IVF, intracytoplasmic sperm injection (ICSI), embryo culture/transfer/vitrification were performed using published protocols. Primary outcome was live birth per thaw cycle. Secondary outcomes were stratified by indication for oocyte freezing, age at oocyte retrieval and by uti-

	All	<35	35-37	38-40	>40
No. of thaw cycles	498	158	147	145	48
<i>Elective</i>	218 (44%)	18 (11%)	83 (56%)	91 (63%)	26 (54%)
<i>Non-elective</i>	280 (56%)	140 (89%)	64 (44%)	54 (37%)	22 (46%)
LBR/Fresh ET	36.7%	40.9%	36.3%	29.5%	40.7%
LBR/Frozen ET	51.5%	41.3%	62.7%	51.2%	40.0%
Cumulative LBR/Thaw Cycle	36.9%	39.2%	40.8%	31.0%	35.4%
<i>Elective</i>	41.7%*	44.4%	44.6%	37.4%	46.2%
<i>Non-elective</i>	33.2%*	38.6%	35.9%	20.4%	22.7%

\*P<0.001 age-adjusted cumulative LB per thaw.

lization of preimplantation genetic testing (PGT). Cumulative LBRs were compared using age-adjusted logistic regression.

**RESULTS:** In 498 thaw cycles involving 4,554 MII oocytes (average 9.1 oocytes/thaw), oocyte survival and fertilization rates were similar across all ages and indications for freezing (85.7% and 69.5% in aggregate). More than half of patients had a fresh embryo transfer (ET) and 48% had at least one embryo for vitrification (average 1.7 blastocysts frozen/thaw). Ten percent of thaw cycles had zero embryos for transfer or vitrification. On average, elective egg freezing patients thawed more MIIs (11.4 vs 7.4) and generated more vitrified blastocysts than non-elective (2.4 vs 1.1). Average LBR per fresh ET was 36.7% (n=300) and per frozen ET was 51.5% (n=163). Elective patients were more likely to utilize PGT compared to non-elective patients (43.6% vs 14.3%). LBR was higher when using PGT-confirmed euploid embryos. For all thaws, cumulative LBR from electively frozen oocytes was higher than from those frozen for non-elective indications (P<0.001). Across all age groups, cumulative LBR ranged from 30%-40% per oocyte thaw cycle.

**CONCLUSIONS:** We observed 30%-40% cumulative LBR after oocyte cryopreservation in all age groups and significantly higher rates when electively frozen oocytes were utilized. This underestimates the overall probability for live birth per oocyte thaw as 27% of our cohort still has unused embryos. LBR in the >40 group should be cautiously interpreted given the small cohort and higher mean numbers of MIIs thawed.

**WHAT ARE THE CHANCES OF SUCCESS FOR COUPLES PERFORMING AN IVF CYCLE WITH ONLY POOR QUALITY DAY-3 EMBRYOS CULTURED TO THE BLASTOCYST STAGE?**

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**OBJECTIVE:** In recent years, more and more IVF centers have chosen to culture all embryos until the blastocyst stage, in order to increase implantation rates. Therefore, it is important to inform couples of the strategy and to

estimate their chances of getting a good quality blastocyst; especially if the entire embryo cohort is of poor quality. The objective of this study was to evaluate the rate of usable blastocysts and the live birth rate, in couples undergoing an IVF/ICSI who obtained only poor quality day-3 (D3) embryos.

**DESIGN:** This retrospective cohort study, carried out between 2012 and 2016, analyzed 59 cycles of IVF/ICSI that resulted in at least one D3 embryo without any high quality embryos. A comparison to a control group comprising 122 cycles with D3 embryos of both good and poor quality was performed.

**MATERIALS AND METHODS:** Cycles in which all D3 embryos were of poor quality were included. All embryos were cultured until day 5 or 6 and were either transferred, cryopreserved or discarded. Exclusion criteria were egg donors, patients performing fertility preservation or modified natural cycle IVF. The embryo quality was scored according to the classification of the Istanbul consensus (Alpha / EHSRE 2011). Thus, D3 embryos were considered of poor quality if the blastomeres had a fragmentation rate > 25% (= grade 3 embryos) or if the number of cells was less than 6 (= slow-development embryos). The « usable blastocysts » rate was defined as the ratio of the number of transferred or cryopreserved blastocysts (if Gardner score ≥ 2BB) to the total number of D3 embryos. Blastulation and live birth rates were expressed as a percentage and compared between the groups by the Chi2 test.

**RESULTS:** In a total of 136 poor quality D3 embryos (from 59 patients), the blastulation rate was 23.5% (compared to a mean blastulation rate of 62% in

our laboratory), the rate of usable blastocysts was 11.0% and the live birth rate was 26.7% per embryo transfer. The rate of usable blastocysts was significantly lower if they originated from grade 3 embryos compared to slow-development embryos (6.8% vs 24.2%, p = 0.0054). The live birth rates were comparable by origin of blastocysts. Patients were statistically older and had lower anti-Mullerian hormone (AMH) levels than the control group, composed of 270 poor quality D3 embryos. Blastulation rates were statistically lower than in the control group (23.5% vs. 37.4%, p = 0.005). However, the rates of usable blastocysts and rates of live birth did not differ between the two groups. In the control group, the rate of usable blastocysts was also higher for slow-developing embryos compared to grade 3 embryos (16.1% vs 7.7%, p = 0.048).

**CONCLUSIONS:** Despite the absence of good quality D3 embryos, a cohort composed entirely of "rejected" embryos can result in a transferable blastocyst and live birth. It appears that the high fragmentation rate of blastomeres is associated with a poorer prognosis than the decreased number of cells on D3. This study could improve the counseling of couples facing this situation.

**SUITABLE TIMING TO TRANSFER BLASTOCYSTS VITRIFIED ON DAY 6 IN FROZEN-THAWED CYCLES MAY BE DAY 5, NOT DAY 6.**

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**OBJECTIVE:** To investigate the suitable timing to transfer blastocysts vitrified on day 6 in frozen-thawed cycles.

**DESIGN:** Retrospective analysis.

**MATERIALS AND METHODS:** This is a retrospective cohort study of 1788 frozen-thawed cycles of blastocysts vitrified either on day 5 or on day 6 and transferred between June 2017 and November 2018. There were 518 cycles included blastocysts vitrified on day 6 (Group A) and 1270 cycles included blastocysts vitrified on day 5 (Group B). According to the timing for blastocyst transfer which was 5 or 6 days after ovulation or progesterone use in hormone replacement therapy (HRT) cycle, the cycles in Group A were divided into two groups: cycles with blastocysts transferred on day 5 (Group C, 103 cycles) and cycles with blastocysts transferred on day 6 (Group D, 415 cycles).

**RESULTS:** Compared with Group A, the female patients in Group B was younger ( $31.37 \pm 4.42$  VS  $31.95 \pm 4.63$ ,  $P < 0.05$ ). There was no significant difference in male age, thickness of endometrium, endometrial preparation methods and the proportion of primary infertility patients between Group A and Group B. The rate of single blastocyst transfer (SBT), clinical pregnancy rate (cPR) and implantation rate in Group B were significantly higher than those in Group A (84.2% VS 65.8%, 66.0% VS 40.9%, 62.1% VS 35.1%,  $P < 0.001$ ), and the early miscarriage rate and multiple pregnancy rate in Group B were significantly lower than those of Group A (11.2% VS 17.9%, 8.9% VS 15.1%,  $P < 0.01$ ). The cPR and implantation rate in Group C were significantly higher than those in Group D (55.3% VS 37.3%, 44.8% VS 32.6%,  $P < 0.01$ ). No significant differences were found between Group C and Group D in terms of early miscarriage rate and multiple pregnancy rate. The rate of SBT, cPR and implantation rate in Group B were significantly higher than those in Group C (84.2% VS 61.2%, 66.0% VS 55.3%, 62.1% VS 44.8%,  $P < 0.05$ ), and the early miscarriage rate in Group B was significantly lower than that of Group C (11.2% VS 21.1%,  $P < 0.05$ ).

**CONCLUSIONS:** Transfer the blastocysts on 5 days, instead of 6 days after ovulation or progesterone use in HRT cycle, could improve the cPR and implantation rate of the blastocysts vitrified on day 6 in frozen-thawed cycles. The cPR and implantation rate of blastocysts vitrified on day 5 are significantly higher compared with blastocysts vitrified on day 6, and the early miscarriage rate is lower, no matter the timing to transfer blastocysts vitrified on day 6.

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**DOES THE DAY OF FINAL OOCYTE MATURATION INJECTION PREDICT OUTCOMES IN COUPLES UNDERGOING IN VITRO FERTILIZATION/INTRACYTOSPLASMIC SPERM INJECTION — AN ANALYSIS BASED ON AGE AND INDIVIDUAL CONTROLLED OVARIAN STIMULATION PROTOCOL.** Abey Eapen, MBBS DRCOG PhD, Amy E. Sparks, PhD, Yunshu Zhou, MS, Karen M. Summers, MPH CHES, Patrick Ten Eyck, MS PhD, Eyup Hakan Duran, MD University of Iowa, Iowa City, IA.



**OBJECTIVE:** Optimizing outcomes for assisted conception treatment remains a clinical challenge. Previous studies have evaluated the role of oocyte number, stage and number of embryos transferred, and the endometrium. The goal of this study was to evaluate the impact of treatment cycle duration and the influence of maternal age in individual controlled ovarian stimulation (COS) protocols on predicting live birth outcomes in in-vitro fertilization (IVF) treatment.

**DESIGN:** Retrospective study using data from a single academic center.  
**MATERIALS AND METHODS:** Demographic and outcome data for 1831 IVF cycles performed between Jan 2014 and Jun 2018 were analyzed. Cycle duration was defined as the number of days of gonadotrophin treatment until the day of final oocyte maturation injection. Live birth rate (LBR) was the primary outcome and logistic regression was used for all models. The main predictor was treatment cycle duration. Cycles were analyzed in total, and in categories of COS protocol used and maternal age ( $< 38$  and  $\geq 38$  years). Secondary outcomes included biochemical and clinical pregnancy. Cycle duration was analyzed as a continuous variable. Analyses were performed using SAS version 9.4 (SAS Institute, Cary, USA).

**RESULTS:** We included 1314 treatment cycles using autologous oocytes which resulted in fresh embryo transfers, without the use of pre-implantation genetic testing. There were 617 live births with an overall LBR of 47%. A total of 475 (36.1%) utilized a *long agonist (LA) protocol*, 346 (26.3%) utilized an *antagonist protocol with human chorionic gonadotropin (hCG) trigger*, 335 (25.4%) utilized an *antagonist protocol with gonadotropin releasing hormone (GnRH) agonist trigger*, and 158 (12%) utilized a *GnRH agonist flare protocol*. On analysis of individual protocols, increasing cycle duration was a strong negative predictor for LBR in women  $< 38$  using a *LA protocol* (OR 0.80; 95% CI [0.69-0.92],  $P = 0.001$ ) and also in women  $\geq 38$  using an *antagonist protocol with hCG trigger* (OR 0.72; 95% CI [0.54-0.96],  $P = 0.022$ ). A combined analysis of all treatment protocols and ages also suggested a significant association of increasing cycle duration with IVF outcomes (OR 0.83; 95% CI [0.78-0.89],  $P < 0.001$ ). For other types of COS treatment protocols, increasing cycle duration was not a statistically significant predictor of LBR.

**CONCLUSIONS:** Our large retrospective study suggests a relationship between IVF cycle duration, individual COS protocol and LBR. Our study while adding evidence to the existing body of evidence on detrimental effects

of prolonged ovarian stimulation, can also aid in clinical decision making on an 'optimal day' for final oocyte trigger injection based on maternal age and the individual type of COS protocol.

Statistical analysis for other age/treatment combinations: (Protocol, Age, OR; 95% CI, P value)

1. LA  $\geq 38$  - 1.02; [0.74-1.40], 0.91
2. Antagonist -hCG  $< 38$  - 0.90; [0.78-1.05], 0.17
3. Antagonist -agonist  $< 38$  - 0.87 [0.75-1.02], 0.08
4. Antagonist -agonist  $\geq 38$  - 0.88 [0.31-2.49], 0.80
5. Flare  $< 38$  - 0.98 [0.79-1.22], 0.85
6. Flare  $\geq 38$  - 0.98 [0.76-1.26], 0.88

**REFERENCES:** 1. Chuang A M, Zapanis A, Taylor M, Jindal SK, Neal-Perry GS, Lieman HJ, Polotsky AJ. Prolonged gonadotropin stimulation is associated with decreased ART success. J Assist Reprod Genet. 2010 Dec;27(12):711-7. <https://doi.org/10.1007/s10815-010-9476-6>. Epub 2010 Sep 7.

2. Ryan A, Wang S, Alvero R, Polotsky AJ. Prolonged gonadotropin stimulation for assisted reproductive technology cycles is associated with decreased pregnancy rates for all women except for women with polycystic ovary syndrome. J Assist Reprod Genet. 2014 Jul;31(7):837-42. <https://doi.org/10.1007/s10815-014-0253-9>. Epub 2014 May 28.

3. Baker VL, Brown MB, Luke B, Conrad KP. Association between oocyte number retrieved with live birth rate and birth weight: an analysis of 231,815 cycles of in vitro fertilization. Fertility and Sterility 103(4):931-8.e2, 2015. PMID: 25638421.

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**ASSISTED HATCHING: IS IT ALL IT'S CRACKED UP TO BE?.** Charis E. Ng, BHSc,<sup>a</sup> Marta Wais, MD,<sup>a</sup> Crystal Chan, MD, MSc,<sup>b</sup> <sup>a</sup>University of Toronto, Toronto, ON, Canada; <sup>b</sup>Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada.



**OBJECTIVE:** Observational studies show that blastocyst embryos must spontaneously hatch from their surrounding zona pellucida in order to implant. In IVF, assisted hatching (AH) is a laboratory procedure that intentionally breaches the embryo's zona pellucida prior to transfer. The putative benefit is to augment an embryo's ability to implant; however, there is still clinical equipoise regarding whether AH improves IVF outcomes, particularly for frozen-thawed embryos at the blastocyst stage. AH has also been associated with an increased risk of monozygotic twinning (MZT), although this is also controversial due to the small sizes of previous studies and the rare nature of this outcome. This study aims to determine the effect of AH on pregnancy outcomes in IVF patients undergoing frozen-thawed blastocyst stage embryo transfers.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All frozen-thawed embryo transfers that occurred at Mount Sinai Fertility between Jan 2013 and Dec 2017 were included. Exclusion criteria included: cancellation of cycle prior to transfer, use of pre-implantation genetics testing of the embryo, and  $\geq 2$  embryos transferred with discordant use of AH. The primary outcome was clinical pregnancy rate. Secondary outcomes included biochemical pregnancy, early pregnancy loss, live birth, and MZT rates. RR ratios, 95% CI, and p-values were calculated.

**RESULTS:** A total of 2165 transfer cycles were carried out. The AH group ( $n = 1986$ ) had similar biochemical pregnancy (38.7% vs 42.1%, aRR 0.92, CI 0.77-1.10), clinical pregnancy (29.1% vs 30.3%, aRR 0.96, CI 0.76-1.21), early pregnancy loss (43.5% vs 40.9%, aRR 1.06, CI 0.79-1.44), and live birth (19.9% vs 20.5%, aRR 0.97, CI 0.71-1.32) rates when compared to the control. MZT rates were comparable between groups (1.39% vs 1.85%, RR 0.76, CI 0.1-5.98) although the low numbers of events with this outcome limits interpretation. Interestingly, six pairs of dichorionic/diamniotic (di/di) twins resulted from single blastocyst embryo transfers. Subgroup analyses of single embryo transfers ( $n = 1599$ ) demonstrated that AH in embryos with expansion grades  $\leq 3$  was associated with a statistically significant decrease in biochemical pregnancy (32.5% vs 44.3%, aRR 0.43, CI 0.23-0.84), and clinical pregnancy (24.0% vs 32.8%, aRR 0.38, CI 0.17-0.87). There were no statistically significant differences in early pregnancy loss and live birth rate in this population, nor any pregnancy outcomes for embryos with expansion grades of 4.

**CONCLUSIONS:** This study demonstrates that AH of frozen-thawed blastocyst stage embryos resulted in similar outcomes to transfers that did not use this technique. AH was not associated with any improvement in pregnancy

outcomes including implantation, clinical pregnancy, early pregnancy loss, and live birth. The identification of di/di twins from single blastocyst embryo transfers challenges previously held notions that di/di MZT only occurs from division prior to the blastocyst stage. This study also demonstrates that AH of embryos with expansion grades  $\leq 3$  may be associated with poorer rates of beta pregnancy and clinical pregnancy.

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#### RELATIONSHIP BETWEEN THE PREGNANCY AND THE SIZE OF ARRESTED BLASTOMERE DERIVED FROM ABNORMAL CYTOKINESIS IN BLASTOCYST TRANSFER CYCLES.

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**OBJECTIVE:** From observation continual morphological changes, about 25% of normally-fertilized ova shows abnormal cytokinesis at 1<sup>st</sup> mitosis (AC). The abnormal cytokinesis is a marker to be eliminated from transfer due to chromosomal aberration and low developmental competence. However, it has been shown that a few AC embryos develop to morphologically-good blastocysts, showing implantation potential comparable to blastocysts derived from normally-cleaved embryos. Chromosome abnormality of blastocysts derived from AC-embryos is equivalent to that of blastocysts derived from normally-cleaved embryo. Some of infertility couples have only morphologically-good blastocysts developed from AC embryos. There is an urgent task to distinguish embryo with implantation potential from morphologically-good blastocysts which showed abnormal cytokinesis at 1<sup>st</sup> mitosis.

**DESIGN:** Clinical research

**MATERIALS AND METHODS:** Retrospective study of single blastocyst transfer (vitrified-warmed 415 blastocysts) between February 2018 and January 2019 were conducted. Blastocysts were separated three groups: embryos which underwent normal cytokinesis at both 1<sup>st</sup> and 2<sup>nd</sup> mitoses (control, n=262), normal cytokinesis at only 1<sup>st</sup> mitosis (1N, n=45), and abnormal cytokinesis at 1<sup>st</sup> mitosis (1A, n=108). Blastocysts developed from AC embryos were classified according to the diameter of arrested blastomere (30 and greater than 30 mm: SAB and over 30 mm: LAB). Morphological changes of embryos has been recorded using a commercial time-lapse incubator (CCM-iBIS, ASTEC). Cleavage patterns and the diameter of arrested blastomeres were determined by time-lapse data analyzing. Blastocyst quality were scored by blastocyst quality score (BQS) according to the Gardner grading system. Clinical pregnancy and miscarriage rates were compared. Tukey-Kramer, t- and chi-squared tests were used for statistical analysis.

**RESULTS:** There was no significant difference in pregnancy rates (control: 50.4%, 1N: 57.8%, 1A: 44.4%) after single blastocyst transfer and miscarriage rates (control: 22.7%, 1N: 11.5%, 1A: 22.9%) among 3 groups. The BQS (26) of control blastocysts was significantly higher than 1N (18) and 1A (19,  $P < 0.05$ ). Pregnancy rates of SAB in 1N was significantly higher than that of LAB (64.9% vs. 25.0%,  $P < 0.05$ ). Pregnancy rates of SAB in 1A was significantly higher than that of LAB (50.0% vs. 12.5%,  $P < 0.05$ ). Miscarriage rates of SAB in 1N was significantly lower than that of LAB (4.2% vs. 100%,  $P < 0.05$ ). Miscarriage rates of SAB in 1A was significantly lower than that of LAB (19.6% vs. 100%,  $P < 0.05$ ).

**CONCLUSIONS:** In some of embryos which underwent abnormal cytokinesis at 1<sup>st</sup> mitosis, abnormal cytokinesis might be occurred by fragmentation and their chromosomes normally separated. In this case, if embryos lost large volume of cytoplasm as fragmentation, their pregnancy potential would be decreased. Observing the size of arrested blastomere can predict pregnancy non-invasively in the case of morphologically-good blastocyst transfer developed from AC embryos.

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#### EMBRYO SELECT ASSAY: A NON-INVASIVE, DIPSTICK ELISA STRIP ASSAY TO IDENTIFY THE MOST COMPETENT EMBRYO FROM THE COHORT.

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**OBJECTIVE:** Identifying the single best embryo for transfer in in vitro fertilization (IVF) frozen embryo transfer (FET) is critical to improve pregnancy rates and decrease multiple gestations. To date, embryo selection has relied on embryo morphology (quality) and sometimes genetic data from pre-implantation genetic testing (PGT). Early in pregnancy, the trophoblast of the developing embryo secretes hCG which enters the maternal blood stream to signal implantation and is detectable in maternal serum about 8-12 days after embryo transfer. Previous work by Dr. Edwards and others have shown hCG levels can be identified in spent culture media (approximately 0.2 mIU/mL on day 2, 0.5 mIU/mL on day 3, and 1.4 mIU/mL on day 5). We developed a dipstick, enzyme linked immunosorbent assay (ELISA) to measure hCG in the spent embryo culture media or blastocoel fluid after the embryo is biopsied. The objective is to identify the most competent embryo with the highest reproductive potential from this cohort—rapidly, non-invasively, and cost effectively.

**DESIGN:** Experimental.

**MATERIALS AND METHODS:** The embryo select assay is a dip stick strip assay which is able to quantitatively measure small amounts of hCG using a chemiluminescent substrate and Spectromax L. We performed 2 studies to evaluate this method. The first study evaluated embryo biopsy fluid to measure the concentration of hCG using this method. The second study evaluated hCG levels from embryo spent media of individual embryos obtained from the same couples to determine if this method can determine the most competent (metabolically functional) embryo with the highest reproductive potential from its cohort. Non-parametric Kruskal-Wallis Test was used to examine differences in hCG levels by embryo grade and PGS outcome. Analyses were performed using SAS (v9.4) and p-values  $< 0.05$  are statistically significant.

**RESULTS:** For Study 1, we collected fluid from 101 embryo biopsies and measured the hCG levels. Quantitative hCG levels were detected in 60.4% of 101 samples; no hCG in 39.6%. In study 2, individual embryo biopsy fluid media from 51 embryos obtained from 15 couples were assessed to compare with current embryo selection methods (embryo quality and PGT results). 5 embryos had no detectable hCG level, which may indicate the embryos did not make hCG and would not implant. Alternatively, it may indicate that there was dropout in the assay. The other 46 samples had measurable hCG levels. There was no association between hCG levels and embryo grade ( $p = 0.19$ ) or PGT outcome ( $p = 0.14$ ).

**CONCLUSIONS:** Embryos need to make hCG in order to survive as an implanting embryo. This rapid, quantitative, novel "dipstick" assay of individual embryos provides new information regarding the embryo's metabolic function, independent of current methods (embryo morphology and PGT). Future studies of live birth outcomes using these embryos in FETs will corroborate whether this method helps to identify the best, reproductively competent embryo for transfer.

**SUPPORT:** None.

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#### SINGLE VITRIFIED-WARMED BLASTOCYST TRANSFER: WHAT ARE THE BEST PREDICTIVE FACTORS FOR SUCCESS?

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**OBJECTIVE:** To determine if the endometrial thickness, the blastocyst expansion, the inner cell mass (ICM) quality and trophoblast (TE) quality, or day of embryo freezing (5 vs 6), while controlling for the patient age at freezing, is a good indicator in predicting the clinical pregnancy outcome for single vitrified-warmed embryo transfers.

**DESIGN:** A retrospective observational study.

**MATERIALS AND METHODS:** From Jan 2016 to Dec 2018, a total of 771 frozen embryo transfers (FETs), where only a single autologous blastocyst was transferred, were analyzed. Exclusion criteria include patients over 42 years of age and cycles with preimplantation genetic testing and gestational carriers. All embryos were vitrified and warmed with the Vitrolife RapidVit™/RapidWarm™ on Rapid-i™ devices. All embryos were graded with the Gardner's scoring system immediately prior to transfers. We excluded 38 cycles (4.9%) from the analysis due to their small numbers—blastocyst expansion size-1, size-5, size-6, and embryos frozen on day 7. Continuous variables were analyzed with the student's t-test and categorical variables with the Pearson's chi-squared test. The discriminatory ability of each variable was evaluated with the multivariate logistic regression analysis.

**RESULTS:** 733 FETs were analyzed and divided into two groups: positive and negative implantation. The comparison results and the regression analysis results are shown in Table 1. For categorical variables, CPRs were compared between groups while controlling for patient age at freezing. Day 5 frozen embryos had a CPR of 50.3% vs day 6 of 41.2% ( $P = 0.12$ ). Embryos with size-4 expansion had a CPR of 51.5% vs size-3 of 44.3% vs size-2 of 32.5% ( $P < 0.003$  for

TABLE 1. Embryos with Positive and Negative Implantation and Odds Ratio:

	Positive Implantation	Negative Implantation	Odds Ratio (95% CI)
Sample Size	346	387	
Patient age (yrs)*	32.6 ± 3.3	33.9 ± 3.8	0.92 (0.88-0.96)
Endometrial thickness (mm)*	9.2 ± 2.0	8.8 ± 1.7	1.17 (1.08-1.28)
Day of Freezing			
5	70.2%	62.0%	0.90 (0.63-1.28)
6	29.8%	38.0%	
Blastocyst Expansion			
2	7.8%	14.5%	2 to 3: 1.46 (0.84-2.53)
3	28.0%	31.5%	3 to 4: 1.24 (0.88-1.75)
4	64.2%	54.0%	2 to 4: 1.13 (1.07-3.05)
ICM + TE			
AA	56.1%	38.8%	BB to AB/BA: 1.71 (1.10-2.64)
AB or BA	23.1%	23.3%	BB to AA: 2.27 (1.52-3.38)
BB	20.8%	38.0%	AB/BA to AA: 1.33 (0.90-1.19)

\*mean ± standard deviation.

size-2 vs size-4;  $P > 0.05$  for all others). Embryos with Grade AA had a CPR of 56.4% vs Grade AB/BA of 47.1% vs Grade BB of 32.9% ( $P < 0.0001$  for AA vs BB;  $P = 0.05$  for AA vs AB/BA;  $P < 0.005$  for AB/BA vs BB).

**CONCLUSIONS:** The best predictive factors for success are endometrial thickness and ICM+TE quality. The blastocyst expansion can potentially be a good predictive factor but more data are needed to determine a stronger correlation with the FET outcome.

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**MATURITY OF CUMULUS OOCYTE COMPLEX (COC) PREDICTS THE OUTCOME OF ART. ~ FOCUS ON DYSMATURE**

~. Mitsuyoshi Amita, M.D., Ph.D., Eri Ishida, M.Sc., Kuniko Tatsumi, MSc, Yoko Yoshitake, M.D., Ryosuke Akino, M.D., Takakazu Saito, M.D., Ph.D. National Center for Child Health and Development, Okura, Setagaya, Tokyo, Japan.



**OBJECTIVE:** Cumulus oocyte complex (COC) at oocyte retrieval with assisted reproductive technology (ART) can be easily classified in a visual manner by its maturity. We have classified them into three categories, mature, immature, and dysmature, for more than ten years. Dysmature COC is thought to be taken from atretic follicles. Although the classification has been generally used for over three decades, there is little study to identify whether the maturity of COC affect the results of ART, such as fertilization rate, implantation rate, and pregnancy rate. In this study, we present that the maturity of COC can be used as an indicator to predict outcome of ART.

**DESIGN:** We demonstrate that dysmature COC results in poor outcome of ART.

**MATERIALS AND METHODS:** The infertile patients who underwent in vitro fertilization (IVF) / intracytoplasmic sperm injection cycles between January 2014 and December 2017 and performed frozen / thawed embryo transfer (ET) by August 2018 participated in the study. After institutional review board approved the study and individual patients provided informed consent prior, 292 patients, 527 cycles are investigated. COCs at oocyte retrieval observed with a stereoscopic microscope were classified in three categories, mature (M group), immature (IM group), and dysmature (D group), then following fertilization rates, ET rates, and pregnancy rates of fresh ET cycles or frozen/thawed ET cycles were calculated. Statistical analysis was performed with Chi-square test.  $P < 0.05$  were considered significant.

**RESULTS:** The total number of COCs retrieved was 2924 (1611 M group, 939 IM group, and 374 D group). The fertilization rate per COC number was significantly higher in M group (63.6%), and lower in D group (36.9%). The rate of fresh ET per COC was significantly higher in M group (8.0%), and lower in D group (3.1%), and there was no pregnancy in D group with fresh ET cycles. The rate of the number of frozen embryos per COC was significantly higher in M group (42.4%), and lower in IM group (33.7%) and D group (19.5%). The pregnancy rate per thawed ET cycle was significantly higher in D group (38.9%) compared to M group (21.6%) and IM group (19.7%).

**CONCLUSIONS:** The maturity of COC at oocyte retrieval may be used as an indicator to predict outcome of ART. Dysmature COC resulted in poor fertiliza-

tion rate, ET rate and embryo freezing rate. But, frozen embryo from dysmature COC may not worse in pregnancy rate compared to those from mature COC. SUPPORT: None.

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**THE PREDICTIVE VALUE OF DAY 5 MORPHOLOGY FOR SLOW DEVELOPING**

**EMBRYOS.** Joseph Chervenak, M.D.,<sup>a</sup> Joshua Stewart, M.D.,<sup>b</sup> Nikica Zaninovic, Ph.D.,<sup>b</sup> Steven Spandorfer, M.D.,<sup>b</sup> Zev Rosenwaks, M.D.<sup>b</sup> <sup>a</sup>Department of Ob/Gyn, New York Presbyterian-Weill Cornell Medicine, New York, NY; <sup>b</sup>The Ronald O. Perleman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY.



**OBJECTIVE:** To compare pregnancy outcomes of slow-developing embryos (elective single embryo transfer of day-6 blastocysts) in frozen embryo transfer (FET) cycles based on day-5 embryo morphology.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All slow-developing embryos that became blastocysts on day 6 that were transferred in single FET cycles from January 2015 through December 2017 at an academic medical center were included. Cycles involving transfers of multiple embryos or those cycles that involved PGT were excluded. Slow-developing embryos were categorized in 3 different groups based on day-5 morphology: compacted morula, cavitating morula, early blastocyst. Live birth rate (LBR) and miscarriage rate (MR; defined as miscarriages per viable pregnancy) were calculated. Data were analyzed using Chi square and Fisher's exact t-test

**RESULTS:** Results are summarized in Table 1. Of the 474 FET cycles that met inclusion criteria, 124 were described as compacted morulae, 235 as cavitating morulae, and 115 as early blastocysts. A significantly lower LBR was achieved in the compacted morula group as compared to the cavitating morula and early blastocyst groups. This difference persisted even when limiting analysis to good-quality embryos, i.e., those of grade BB or above.

**CONCLUSIONS:** Decreased pregnancy rates have been demonstrated when transferring blastocysts on day 6 as compared to day 5 in both fresh<sup>1</sup> and frozen<sup>2</sup> cycles. However, previous data have been limited on the potential of slow-developing blastocysts based on embryo development and morphology prior to blastocyst formation and vitrification. Recent data have suggested increased pregnancy rates involving transfer of slow-developing embryos that have begun to cavitate on day 5; however, sample sizes have been small<sup>3</sup>. Our analysis reveals significantly lower LBRs and a trend toward higher MRs with the transfer of compacted embryos versus cavitating morulae or early blastocysts in day-6 single blastocyst FET cycles. These differences persisted when controlling for embryo quality. These results suggest that the developmental curve of slow-developing embryos prior to vitrification may provide helpful insight into the reproductive potential of these embryos, informing the selection of the best embryo for transfer.

References: 1. Barrenetxea G et al. Blastocyst culture after repeated failure of cleavage-stage embryo transfers: a comparison of day 5 and day 6 transfers. *Fertil Steril.* 2005;83(1):49-53.

2. Haas, Jigal, et al. "Clinical pregnancy rate following frozen embryo transfer is higher with blastocysts vitrified on day 5 than on day 6." *A Journal of assisted reproduction and genetics*12 (2016): 1553-1557.

3. Haas, Jigal, et al. "Developmental potential of slow-developing embryos: day-5 morulae compared with day-5 cavitating morulae." *A Fertility and sterility*1 (2019): 105-111.

SUPPORT: None.

TABLE 1. D6 FET outcomes by D5 morphology (all embryos)

All Embryo Transfers				
	Compacted	Cavitating	Early Blastocyst	p-value
Transfers (n)	124	235	115	
LBR (%)	23.39%	34.04%	32.17%	<0.05
MR (%)	34.09%	26.61%	30.19%	NS
Good Quality Embryos				
	Compacted	Cavitating	Early Blastocyst	p-value
Transfers (n)	110	198	103	
LBR (%)	25.45%	38.38%	35.92%	<0.05
MR (%)	33.30%	24.80%	27.50%	NS

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**ENDOMETRIAL THICKNESS IN PREDICTION OF PREGNANCY OUTCOME IN FRESH EGG DONATION CYCLES: A RETROSPECTIVE COHORT ANALYSIS.**



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**OBJECTIVE:** To analyse the relationship between endometrial thickness and pregnancy outcome in fresh oocyte donation cycles.

**DESIGN:** Retrospective cohort analysis.

**MATERIALS AND METHODS:** Single centre retrospective cohort analysis of 1928 fresh single embryo transfer oocyte donation cycles. Treatment took place at a private infertility clinic (IVIRMA Valencia, Spain) between January 1st, 2016 and December 31st, 2017. We included women under 50 years old undergoing fresh oocyte-donation treatment in the context of a hormone replacement therapy (HRT) cycle for endometrial preparation. Only women with a normal uterus on the 2D ultrasound and accepting a single transfer of a day 5 blastocyst were included. Only one good quality blastocyst according to the Spanish ASEBIR classification was transferred after 5 days of progesterone administration (Micronized Progesterone, 400 mg/12h. vaginally). We excluded cases in which an endometrial preparation under a natural cycle was performed, when more than one embryo was transferred, or any good quality blastocyst was available.

**RESULTS:** Mean age was 42.5 ± 4.8 and BMI was 23.0 ± 3.6. The overall live birth rate was 45.6%. The mean endometrial thickness was 8.7±1.7 mm, ranging from 3.0 to 17.0 mm. The distribution by percentiles is as follows: p10=6.9mm; p25=7.5mm; p50=8.5mm; p75=9.5mm; p90=11.0mm. For the purpose of the analysis, patients were categorized in to 6 groups defined by percentiles. LBR in women with endometrium ≤ p10, (≤ 6.9 mm), was significantly reduced compared to the rest of the population (36.7% vs 46.2%; p=0.015). When submitted to a multivariate logistic regression analysis in which all variables related to live birth rate were included (i.e. age, BMI, number of oocytes, number of fertilized oocytes and number of good quality blastocysts available), endometrial thickness remained as an independent factor related to live birth. An endometrial thickness ≤ 6.9 mm was associated with a significantly reduced probability of live birth compared with patients with an endometrial thickness of 7 mm or more (OR: 0.70; 95% CI: 0.50-0.97).

**CONCLUSIONS:** Our results indicate a reduction of live birth rate for more than 9 % with an endometrial thickness lower than 7 mm. This finding even remains as an independent factor after multivariate logistic regression analysis controlling for all potentially relevant confounders. To our best knowledge this study seems to represent the largest cohort investigating live birth rate in fresh oocyte donation cycles and including only single embryo transfers.

References: 1. Kasius A, Smit JG, Torrance HL, Eijkemans MJC, Mol BW, Opmeer BC, et al. Endometrial thickness and pregnancy rates after IVF: a systematic review and meta-analysis. *Hum Reprod Update* 2014;20(4):530–41.

2. Gallos ID, Khairy M, Chu J, Rajkhowa M, Tobias A, Campbell A, et al. Optimal endometrial thickness to maximize live births and minimize pregnancy losses: Analysis of 25,767 fresh embryo transfers. *Reprod Biomed Online* 2018

3. Liu KE, Hartman M, Hartman A, Luo Z-C, Mahutte N. The impact of a thin endometrial lining on fresh and frozen-thaw IVF outcomes: an analysis of over 40 000 embryo transfers. *Hum Reprod [Internet]* 2018 [cited 2018 Oct 30];33(10):1883–8.Á

4. Richter KS, Bugge KR, Bromer JG, Levy MJ. Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of in vitro fertilization with transfer of two blastocyst-stage embryos. *Fertil Steril* 2007 87(1):53–9.

5. Noyes N, Hampton BS, Berkeley A, Licciardi F, Grifo J, Krey L. Factors useful in predicting the success of oocyte donation: A 3-year retrospective analysis. *Fertil Steril* 2001;76(1):92–7.

6. Dessolle L, Darai E, Cornet D, Rouzier R, Coutant C, Mandelbaum J, et al. Determinants of pregnancy rate in the donor oocyte model: A multivariate analysis of 450 frozen-thawed embryo transfers. *Hum Reprod* 2009;24(12):3082–9.

**SUPPORT:** None.

**WHAT IS THE CLINICAL IMPACT OF THE ENDOMETRIAL RECEPTIVITY ARRAY IN PGT-A AND OOCYTE DONATION CYCLES?**



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**OBJECTIVE:** Despite the extensive investigation in the field of assisted reproduction, implantation still remains a challenge. The endometrial receptivity array (ERA) has been studied in both implantation failure (IF) and non-IF populations yielding conflicting results. We hypothesized that controlling for the embryonic factor might allow for a more accurate interpretation of the endometrial assessment.

Our aim was to evaluate the influence of the ERA test on the implantation rate (IR) and pregnancy rate (PR) in patients with previous failed euploid embryo transfers (EET) and previous failed oocyte donation embryo transfers (RET).

**DESIGN:** Single centre retrospective study, case-control study.

**MATERIALS AND METHODS:** There were 333 patients with previous failed EET or RET. Selected cases were patients with at least 1 previous failed EET (n=24) or 2 failed RET (n=32) who underwent an ERA test and a post-ERA euploid embryo transfer (EET) or oocyte donation embryo transfer (RET) between 2012-2018. Controls were patients with at least 1 previously failed EET (n=119) or 2 failed RET (n=158) who underwent EET or RET during the same period without undergoing an ERA test. Only blastocyst stage embryos were included. IR and PR were compared between the post-ERA ET and the last ET in the control group.

**RESULTS:** There were 98 clinical pregnancies (CP) among 143 EET (14CP among 24 EET in ERA group, and 84 CP among control group); and 114 CP among 190 RET (11CP among 32 ERA group, and 103 CP among control group).

There was no statistically significant difference regarding IR (55.6% [34.6%-76.5%] vs.65.0% [56.9%-73.1%]) and PR (58.3% vs.70.6%, p=0.238) in the ERA vs. control groups in the EET arm, while in the RET arm both IR (26.8% [12.3%-41.4%] vs. 57.2% [50.1%-64.3%]) and PR (34.4% vs. 65.2%, p=0.001) were significantly lower in the ERA group. Multivariate logistic regression confirmed that the performance of an ERA test did not significantly influence the PR in the EET arm and was associated with a diminished PR in the RET arm. In the ERA group, 41.1% patients were non-receptive (NR). No significant difference was found regarding IR or PR in NR vs. receptive patients in both EET and RET arms.

**CONCLUSIONS:** In our sample, the performance of an ERA test did not improve pregnancy outcomes. Future prospective studies in larger samples are needed to confirm the role of the ERA test in EET and RET.

**SUPPORT:** Non to declare.

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**INTENTIONAL ENDOMETRIAL INJURY TRYING TO IMPROVE CLINICAL OUTCOMES OF AN OOCYTE DONATION PROGRAM IN PATIENTS WITHOUT RIF. INTERYM ANALYSIS OF A RANDOMIZED CONTROLLED TRIAL.**



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**OBJECTIVE:** Oocyte donation program (OD) provides the ideal setting for investigate if endometrial scratching improves 10 % the ongoing implantation rate, as the recipient's endometrial priming guarantees the homogeneity of the endometrium and also the equality of quality of the transferred embryos and limiting the confounding factors.

**DESIGN:** A multicentric, open-label, randomized, controlled trial has been conducted in a private setting since Oct 2013. Eligible recipients were

randomly assigned in a 1:1 ratio to either ES (by pipelle biopsy in the luteal phase of the menstrual cycle prior to the embryo transfer, n=123) or no intervention (NES, n=111), through a computer-generated randomization list. At the time of interim analysis, 234 out of 600 patients were recruited (40%).

**MATERIALS AND METHODS:** Inclusion criteria: 18-44 years aged ovum recipients with preserved ovarian function, 19-29.9kg/m<sup>2</sup>, first or second OD fresh embryo transfer (ET), endometrial thickness >6 mms, and 1-2 optimal quality blastocysts transferred. Exclusion criteria: any adverse condition, and recurrent implantation failure with OD (>5 embryos transferred, including frozen transfers). The primary outcome measure was ongoing implantation rate: the number of ongoing sacs per embryo transferred. All the outcomes were analyzed on an intent-to-treat basis and per embryo transfer.

**RESULTS:** A total number of 196 recipients underwent OD-embryo transfer, 93 in scratching arm and 103 in the NES group.

No differences existed in the mean age and BMI of recipients and oocyte donors, oocytes retrieved and microinjected, fertilization rates, blastocysts transferred, endometrial thicknesses and oestradiol /progesterone levels after their endometrial priming.

The clinical pregnancy rate was 55.6% 95%CI(46.1-64.7) and 52.1% 95%CI(43.1-60.9) in the ES arm and in NES arm on the ITT analysis (OR=1.13 95%CI(0.67-1.9); p=0.59), and in the per ET analysis, 67.0% 95%CI(56.7-75.9) vs 60.4% 95%CI(50.6-69.3) respectively, OR=1.14 95%CI(0.67-1.96), p=0.34.

The implantation rate was in the per ET analysis, 63.6% 95%CI(52.5-70.9) vs 58.8% 95%CI(50.1-67.1) in the ES arm and in NES arm, OR=1.14 95%CI(0.67-1.96), and the ongoing implantation rate was 51.8% 95%CI(41.0-59.9) vs 45.8% 95%CI(35.9-53.1) in ES and NES respectively, OR=1.27 95%CI(0.75-2.16).

There were no differences in miscarriage nor multiple pregnancy rates. The endometrial biopsy procedure was well tolerated in most women.

The final decision of continuing or not the study was taken by means of applying the stochastic curtailment approach, to test the null hypothesis rejection probability given the current data available. P value of 0.7633 yielded Z=0.72, leading to keep the trial continuing.

**CONCLUSIONS:** The interim analysis shows that ES in the luteal phase of the cycle preceding the OD embryo transfer in recipients without RIF would not pose a significant benefit thus its application to all OD recipients cannot be advised at this point, although the study continues. There is not enough evidence supporting scratching to increase endometrial receptivity.

SUPPORT: None.

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### DO PATIENTS WITH A HISTORY OF CHRONIC ENDOMETRITIS BENEFIT FROM CORTICOSTEROIDS AND ANTIBIOTICS BEFORE FROZEN EMBRYO TRANSFER?



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**OBJECTIVE:** Current data suggests that use of oral antibiotics and corticosteroids (AC) prior to embryo transfer (ET) does not improve ET outcomes. We hypothesized that patients with a history of chronic endometritis (CE) may be an exception to this finding. The objective was to investigate the utility of AC prior to single thawed euploid embryo transfer (STEET) in patients with CE.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Patients who underwent STEET at an academic medical center from 1/2000 to 4/2019 were identified. Cycles prior to 1/2018 received 100 mg doxycycline bid and methylprednisolone 16 mg daily (Pre) prior to ET, and cycles performed after this date did not (Post). Cycles were evaluated for performance of endometrial biopsy (EMB) for CE, with CE defined as presence of plasma cell marker CD-138 (not by hematoxylin and eosin stain alone). Patients positive for CE were treated with 2-3 weeks antibiotics prior to ET cycle start. Outcomes were recorded as not pregnant (NP), biochemical pregnancy (BP), ectopic (E), or intrauterine pregnancy (IUP). Rates of IUP were compared to NP+E and BP. Chi-squared test was used for analysis (p<0.05).

**RESULTS:** 2774 STEET cycles were included. There were 1870 Pre and 904 Post. 462 cycles had an EMB for CE performed. Of these, 238 were positive for CE and 224 were negative. Rates of IUP versus NP, BP, and E combined were not significantly different between all Pre (n=1247, 67%) and all Post (n=628, 68%) with  $X^2(2, N=2765) = 2.24 P > .05$ . Similarly, rates of IUP

were not significantly different in patients who had not had an EMB (Untested), or in patients who were tested for CE and found to be negative. In patients with a history of CE there was a small but significant increase in IUP in Post (n=61, 54%) compared to Pre (n=46, 37%)  $X^2(2, N=238) = 9.31 P < 0.05$ .

**CONCLUSIONS:** Overall, treatment with AC was not associated with higher IUP rates. The use of AC did not improve outcomes in patients with a history of CE, and unexpectedly resulted in lower IUP rates.

TABLE 1. Pregnancy outcomes after STEET by treatment with AC (Pre) vs without AC (Post). Results were divided into 3 groups: Untested, CE positive (+CE) vs CE negative (CE-).  $X^2 =$  Chi Squared, DF = Degrees of Freedom, SIG = significant difference, NS = Non-significant difference.

All Pre N=1870	n	%	All Post N=904	n	%	X2
NP+E	439	23%	NP+E	197	22%	2.23
IUP	1247	67%	IUP	628	69%	DF = 2
BP	184	10%	BP	79	9%	NS
Pre +CE	n	%	Post +CE	n	%	X2
N=126			N=112			
NP+E	80	40%	NP+E	38	34%	9.31
IUP	46	37%	IUP	61	54%	DF = 2
BP	29	23%	BP	13	12%	SIG
Pre -CE	n	%	Post -CE	n	%	X2
N=126			N=98			
NP+E	44	35%	NP+E	36	37%	1.02
IUP	59	47%	IUP	49	50%	DF = 2
BP	23	18%	BP	13	13%	NS
Pre Untested	n	%	Post Untested	n	%	X2
N=1618			N=694			
NP+E	344	21%	NP+E	123	17%	4.23
IUP	1142	71%	IUP	518	75%	DF = 2
BP	132	8%	BP	53	8%	NS

SUPPORT: None.

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### IS THERE AN ASSOCIATION BETWEEN ENDOMETRIAL THICKNESS AT TIME OF FROZEN EMBRYO TRANSFER AND THE INCIDENCE OF SUBCHORIONIC HEMATOMA OR VAGINAL BLEEDING?



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**OBJECTIVE:** Subchorionic hematoma (SCH) is observed in 4-48% of early pregnancies.<sup>1,2</sup> Although the etiology is unknown, SCH is believed to result from partial detachment of the chorion from the uterine wall, and frequently present with vaginal bleeding (VB). Some studies have suggested that the incidence of SCH is higher in pregnancies that result from in vitro fertilization (IVF),<sup>2</sup> but the mechanism and clinical significance are unclear.

The endometrial lining proliferates under the influence of estradiol (E2) during synthetic preparation for a frozen embryo transfer (FET) cycle. Whether there is an endometrial thickness (EnT) beyond which the endometrium begins to outgrow its blood supply has yet to be discovered. Given that E2 levels are often supraphysiologic and EnT maximized during synthetic preparation, we asked whether there is an association between EnT and the incidence of SCH/VB in pregnancies achieved following single euploid FETs.

**DESIGN:** Retrospective, cohort study.

**MATERIALS AND METHODS:** The study included patients at a single, academic ART center who achieved a pregnancy following a synthetically prepared single euploid FET cycle from 2012 to 2019. Natural endometrial preparation cycles were excluded. Natural language processing was performed to identify pregnancies complicated by SCH. Blind review of the database was conducted by two independent reviewers to verify data quality. EnT was treated as a continuous variable. The primary outcomes of the study were incidence of SCH and VB. The secondary outcomes were rates of ongoing pregnancy rate/live birth (OP/LB) and clinical pregnancy loss

(CPL). Data were evaluated using T-tests, chi-square tests, and generalized estimating equations.

**RESULTS:** The study included 2515 patients who underwent 2927 single euploid FET cycles that progressed to a clinical pregnancy. The overall incidence of SCH was found to be 7.79% (n=228). Univariate analysis demonstrated a significant difference in oocyte age, body mass index (BMI), and endometrial pattern (EnP) at FET between patients with and without SCH. There was no statistically significant association between EnT at time of FET and incidence of SCH (OR 0.97 [95% CI 0.91-1.03], p=0.32) or VB (OR 0.97 [95% CI 0.93-1.06]) when controlling for oocyte age, BMI, and EnP. There was no difference between rates of OP/LB (89.04 % vs. 86.62 % p=0.30) or clinical pregnancy loss (10.96 % vs 13.38 % p=0.63) amongst patients with and without SCH.

**CONCLUSIONS:** In the largest study to evaluate the association between EnT and SCH using a single euploid FET model, we demonstrated no increase in the incidence of SCH or VB with increasing EnT in synthetically prepared FET cycles. Clinicians can be reassured that patients undergoing synthetic preparation for FET are not being placed at a higher risk for SCH or VB as a result of having a thicker endometrium. While EnT does not appear to be correlated with SCH, future studies that identify risk factors at the molecular level—such as markers of placental invasion—would offer a deeper look at the pathophysiology of SCH and help elucidate interactions at the maternal fetal interface.

**References:** 1. Zhou J, Wu M, Wang B et al. The effect of first trimester subchorionic hematoma on pregnancy outcomes in patients underwent IVF/ICSI treatment. *J Matern Fetal Neonatal Med.* 2017; 30(4):406-410.

2. Tuuli MG, Norman SM, Odibo AO, et al. Perinatal outcomes in women with subchorionic hematoma: a systematic review and meta-analysis. *Obstet Gynecol* 2011;117:1205–12.

3. Asato K, Mekaru K, Heshiki C, et al. Subchorionic hematoma occurs more frequently in *in vitro* fertilization pregnancy. *EJOG* 2014; 181:41-44.

**SUPPORT:** None.

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#### **ENDOMETRIAL PREPARATION WITH ETANERCEPT INCREASES EMBRYO IMPLANTATION AND LIVE BIRTH IN WOMEN SUFFERING FROM IMPLANTATION FAILURE DURING *IN VITRO***

**FERTILIZATION.** Karla Y. Santiago, MD,<sup>a</sup> Esther López-Bayghen, PhD.<sup>b</sup> <sup>a</sup>Ingenes México, Mexico City, DF, Mexico; <sup>b</sup>Centro de Investigación y Estudios Avanzados IPN, México, EM, Mexico.

**OBJECTIVE:** Repeated implantation failure (RIF) plague many women undergo *in vitro* fertilization (IVF). The exact cause and definition are currently under debate. Carrying out >3 failed IVF cycles with the accumulated transfer of at least 8 embryos is considered an initial definition of RIF. Typical RIF patients under 40 years with good ovarian reserve, normal endometrial morphology, normal karyotypes, antidiolipin, and normal lupus anticoagulant as well as common thrombophilias. During implantation, Tumor Necrosis Factor- $\alpha$  (TNF $\alpha$ ) stimulates MMP9 for endometrial invasion by the embryo, stimulates the expression of MUC1, gives embryo protection effects on teratogenic stress, and induces COX-2 response. Etanercept, a TNF $\alpha$  antagonist, has been shown to improve pregnancy rates in women with recurrent reproductive failure and with endometriomas. The aim of this study was to determine the effectiveness of etanercept treatment in IVF outcomes in women with RIF.

**DESIGN:** Single-arm, prospective study.

**MATERIALS AND METHODS:** Sixty-seven women suffering from RIF were recruited from the Ingenes Institute in Mexico City. All patient underwent a similar IVF protocol. Each woman received Etanercept (4 x 25 mg every 3 days) during endometrial preparation and at embryo transfer (25 mg). IVF outcomes that were assessed were embryo implantation (h-bCH >10 mg/dL at Day 14), the presence of gestational sacs at week 8 by ultrasound, and live birth.

**RESULTS:** All women reported no side-effects associated with the treatment. 70.1% of the cohort achieved embryo implantation, 67.2% developed gestational sacs; however, the live birth rate was at 44.8%. Frozen cycles (n=26) did perform better than fresh cycles (n=41) for implantation (75.6% vs 61.5%), gestational sac (73.2% vs 57.7%), and live birth rate (48.8% v 38.5%, respectively); however, these results were not significant.

**CONCLUSIONS:** Here, we showed that using Etanercept during endometrial preparation improves IVF outcomes in women suffering from RIF.

**SUPPORT:** Conacyt 231793.

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#### **CHRONIC ENDOMETRITIS SCREENING IN PATIENTS WHO EXPERIENCE EUPLOID EMBRYO IMPLANTATION FAILURE DOES NOT IMPROVE IVF OUTCOMES AFTER A SUBSEQUENT EUPLOID**

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**OBJECTIVE:** Infertile women who experience recurrent implantation failure (RIF) is commonly diagnosed with Chronic Endometritis (CE). Recent studies have shown that CE may affect endometrial decidualization and alter expression of proteins involved in endometrial-embryo receptivity. Therefore, CE is considered as a potential etiology of failed euploid embryo implantation when no other clinical cause is evident. There are limited findings to draw conclusions about the value of performing an endometrial biopsy (EB) for CE screening; especially within patients who have experienced a failed euploid embryo transfer (1). The aim of this study is to assess the clinical benefit of patients who undergo an endometrial biopsy and chronic endometritis screening following a failed euploid embryo transfer and prior to undergoing further ART treatment.

**DESIGN:** Retrospective cohort analysis.

**MATERIALS AND METHODS:** This study included infertile patients who had a failed a euploid embryo transfer and, thereafter, underwent an endometrial biopsy for CE screening, received antibiotic treatment (if indicated), and had a subsequent single, euploid frozen embryo transfer from January 2016 to December 2018. Cohorts were segregated as it follows: Group 1: patients that underwent a EB and were diagnosed with CE and received antibiotic treatment; Group 2: Patients who underwent EB and were negative for CE; and Group 3: a control group of patients without an EB. Demographic characteristics and IVF outcomes were compared among cohorts. ANOVA,  $\chi^2$  test, and an adjusted multivariate regression analysis with a GEE model were used for data analysis.

**RESULTS:** A total of 1109 patients with a failed euploid FET were included in the analysis, Group 1 (n=124); Group 2 (n=90) and Group 3 (n=985); Significant differences were found in BMI (Group 1: 24.6, Group 2: 24.5, Group 3: 23.5, p=0.01), prior number of euploid FET cycles (1.8, 1.67, 1.55, p=0.006), and days between EB and FET (Group 1: 63.1, Group 2: 94.3, p=0.001). No significant differences were found on implantation rate (69.3%, 71.1%, 64.4%, p=0.5), clinical pregnancy rate (52.4%, 54.4%, 54.8%, p=0.9), live birth rate (LBR) (45.1%, 46.6%, 42.4%, p=0.65) and clinical loss rates (7.2%, 7.7%, 11.3%, p= 0.24) among cohorts. After adjusting for age, BMI, AMH, embryo quality and day of embryo biopsy, there was no association between patients who received CE diagnosis (OR 1.5, CI95% 0.8-2.8, p=0.1), and for those who received a normal EB result (OR1.1, CI95% 0.6-1.7, p=0.6) with lower odds of LBR when compared to the control group.

**CONCLUSIONS:** Understanding the potential advantages of CE screening on embryo implantation outcome is of critical importance for modern ART specialists. To date there is no high quality evidence to support performing endometrial biopsies for CE screening in patients who experienced a failed embryo implantation.

Our study suggests that undergoing an endometrial biopsy, regardless of results, does not result in improved IVF outcomes in subsequent euploid FET as compared with patients who were not tested for chronic endometritis.

**References:** Vitagliano A, Saccardi C, Noventa M, Di Spiezio Sardo A, Saccone G, Cicinelli E, Pizzi S, Andrisani A, Litta PS. Effects of chronic endometritis therapy on *in vitro* fertilization outcome in women with repeated implantation failure: a systematic review and meta-analysis. *Fertil Steril.* 2018 Jul 1;110(1):103-112.e1.

**SUPPORT:** None.

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#### **VALUE OF ENDOMETRIAL ECHO PATTERN TRANSFORMATION AFTER HCG TRIGGER IN PREDICTING IVF PREGNANCY OUTCOME: A PROSPECTIVE COHORT STUDY.**

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**OBJECTIVE:** To investigate if the endometrial echo pattern transformation after hCG trigger affect IVF pregnancy outcome.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** In an academic center for reproductive medicine, a series endometrial echo pattern monitoring were carried out in 146 patients after hCG trigger: hCG day, from 1 through 3 days after ovum pick-up (OPU+1, OPU+2, OPU+3).The endometrial echogenicity value was obtained by ImageJ software.Patients were compared according to their pregnant status.For further analysis,endometrial echogenicity value was sorted into five groups:  $\leq 60\%$  , 61%-70% , 71%-80% , 81%-90% , and  $> 90\%$ .And Clinical pregnancy rate and embryo implantation rate were compared among the five echogenicity groups.

**RESULTS:** The endometrial echogenicity value was calculated as the ratio of the hyperechogenic endometrial area over the whole endometrial area.The endometrial echogenicity value on OPU+1,2,3 were differed markedly between clinical pregnant group and non-pregnant group ( $P < 0.001$ ) .Clinical pregnancy rate and embryo implantation rate had positive relationship with echogenicity value. The ROC curve analysis of endometrial echogenicity for pregnancy showed the area under curve was greatest on OPU+2 (OPU+1, 2, 3 were 0.738, 0.765, 0.714 respectively). Endometrial echogenicity value on the OPU+2 had the most predictive value, and the cutoff value was 76.5%.The sensitivity was 61.3% and specificity was 82.0%.

Endometrial echogenicity values after hCG trigger in the two groups.

Group	N	HCG	OPU+1	OPU+2	OPU+3
Pregnant <sup>a</sup>	65	0.45±0.14	0.75±0.12	0.78±0.12	0.81±0.11
Non-pregnant	65	0.45±0.17	0.67±0.14	0.68±0.14	0.72±0.14
P		0.888	<0.001	<0.001	<0.001

<sup>a</sup>Patients who achieved a clinical pregnancy.

**CONCLUSIONS:** The endometrial echogenicity value on OPU+2 was recommended to evaluate endometrial receptivity. When the echogenicity value  $\geq 81\%$  on OPU+2, it was highly recommended for clinicians to perform fresh ET. While it seemed appropriate to freeze the embryos from the present cycle and transfer them in a subsequent thaw cycle when echogenicity value  $\leq 70\%$  on OPU+2.More data are needed to avoid the useless and costly embryos cryopreservation in the high percentage of false positive.Further investigations are required to elaborate the key mechanism behind the regulation of endometrial secretory transformation and to adopt treatment to accelerate the appearance of ultrasonic hyperechoic endometrium after oocyte retrieval.

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#### CLINICAL UTILITY OF THE ENDOMETRIAL RECEPTIVITY ARRAY IN WOMEN WITH PRIOR FAILED TRANSFERS.

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**OBJECTIVE:** To determine the clinical utility of the Endometrial Receptivity Array (ERA) in women with  $\geq 1$  prior failed embryo transfer (ET).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This study included 214 women who underwent an ERA biopsy with a subsequent frozen ET between January 2016 and February 2019. Those with a nonreceptive endometrium were exposed to an adjusted progesterone duration in the subsequent cycle. Women were classified based on their indication for ERA biopsy: 1)  $\geq 1$  prior failed ET, defined as ET that did not result in a live birth, or 2) as a prophylactic measure due to having only a single euploid embryo or physician/patient preference. The latter group presumably did not have implantation defects and was designated as the reference group. Pregnancy outcomes included conception defined as positive hCG, clinical pregnancy defined as a gestational sac visualized on ultrasound, and ongoing pregnancy/live birth. Descriptive statistics were performed to compare the two groups in terms of ERA biopsy results and pregnancy outcomes. In a subset analysis of women with  $\geq 1$  prior failed ET, pregnancy outcomes were compared between those with receptive versus non-receptive ERA results.

**RESULTS:** Of 214 women included, 124 (58%) had  $\geq 1$  failed ET and 90 (42%) served as the reference group. Eighty-three (39%) had pre-receptive or early receptive ERA results, 111 (52%) had receptive results, and 20 (9%) had post-receptive or late receptive results, and the results did not differ significantly between the two groups ( $P=0.468$ ). 93% of subsequent frozen ET cycles were transfers of known euploid embryos. Maternal age was similar between the two groups ( $37 \pm 5.4$  vs  $38 \pm 5.6$ ,  $P=0.674$ ). Pregnancy outcomes in the subsequent frozen ET cycle were comparable between the two groups (see Table 1). Among women with  $\geq 1$  prior failed ET, the preg-

nancy outcomes for those with non-receptive ERA were not statistically different than those with receptive ERA results (results not shown).

	Prior failed ET N=124	Reference group N=90	P-value
Conception	88 (71%)	69 (77%)	0.282
Clinical Pregnancy	74 (60%)	59 (66%)	0.643
Ongoing Pregnancy or Live Birth	54 (44%)	43 (48%)	0.265

**CONCLUSIONS:** Women with  $\geq 1$  failed ET had a similar prevalence of non-receptive endometrium compared to the reference group. The pregnancy outcomes in a subsequent ET cycle between the two groups were similar. Among women with  $\geq 1$  failed ET, adjustment in progesterone exposure for those with non-receptive endometrium resulted in pregnancy outcomes comparable to those with a receptive endometrium. To further clarify the clinical utility of the ERA, studies are needed to compare outcomes in women who undergo the ERA prior to another transfer versus women who opt to proceed directly to the next transfer attempt.

**SUPPORT:** None.

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**WITHDRAWN**

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#### PROGNOSTIC VALUE OF UTERINE NATURAL KILLER (uNK) CELLS DENSITY IN PERI-IMPLANTATION ENDOMETRIUM FROM WOMEN WITH RECURRENT IMPLANTATION FAILURE.

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**OBJECTIVE:** CD56+ uterine natural killer (uNK) cells constitute major components in human endometrium and play an important role around the time of implantation. The aim of this study is to investigate the prognostic value of uNK cells density for subsequent pregnancy outcome in women with recurrent implantation failure (RIF) after IVF-ET treatment.

**DESIGN:** It is a prospective cohort study carried out in a university-affiliated IVF center.

**MATERIALS AND METHODS:** A total of 59 women with RIF participated in the study. Endometrial biopsies were obtained precisely 7 days after luteinization hormone surge in the natural cycle preceding frozen embryo transfer. Endometrial sections were immunostained for CD56 and cell counting was performed by a standardised protocol. Results were expressed as percentage of positive uNK cell/ total stromal cells.

**RESULTS:** No significance difference in uNK cell density was observed between women who did not get pregnant ( $n=31$ ; median 2.2% range 0.3-7.2%) and women who get pregnant ( $n=28$ ; median 1.9% range 0.2-8.5%). There was also no significant difference in uNK cell density between women who miscarried ( $n=9$ ; median 2.2% range 1.0-8.5%) and women who had a live birth ( $n=19$ ; mean 2.0% range 0.2-7.9%) in a subsequent pregnancy.

**CONCLUSIONS:** Uterine NK cells density in the peri-implantation endometrium is of no predictive value for subsequent pregnancy outcome in women with RIF.

**SUPPORT:** This study was supported by Hong Kong Health and Medical Research Fund.

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#### IMPACT OF ENDOMETRIAL PREPARATION IN CRYOPRESERVED-WARMED EMBRYO TRANSFER (FET) CYCLES ON PERINATAL OUTCOME.

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**OBJECTIVE:** There is clinical equipoise regarding the safety and superiority of the cryopreserved-warmed (FET) over fresh embryo transfer cycles with respect to perinatal outcomes. Prior studies suggest, that elevated estradiol ( $E_2$ ) level leads to abnormal placentation and preeclampsia (PEC). Recent data demonstrate a potential increased risk of PEC in FET (physiologic  $E_2$  level) compared to fresh embryo transfer cycles (supraphysiologic  $E_2$  level).

Specifically, the absence of a corpus luteum may play a role in disorders of placentation, however the mechanism is not well understood. The aim of this study was to evaluate the factors influencing pregnancy and perinatal outcomes in natural FET cycles (nFET) and programmed FET cycles (pFET).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All autologous FET cycles from a single academic center (N=584; Jan 2013 - Jan 2017) resulting in a positive pregnancy test were reviewed. Cycles were analyzed based on the endometrial preparation protocol: pFET (N=529) vs. nFET (N=55). Data regarding potential confounders including: maternal age, diagnosis, embryo stage at transfer, number of embryos transferred, endometrial thickness, number of delivered infants, neonatal sex, and year of transfer were collected and analyzed using t-test, Fisher exact test or  $\chi^2$  as appropriate. Random effects mixed linear regression models were used to assess the impact of endometrial preparation protocol on Human Chorionic Gonadotropin (HCG) rise.

**RESULTS:** Baseline characteristics including age, ethnicity, history of chronic hypertension, parity, history of prior preterm and full term birth, embryo stage at transfer, number of embryos transferred, endometrial thickness and preimplantation genetic testing were comparable for both groups. Patients in nFET group had lower BMI comparing to patients in the pFET group (22.9 vs. 25.2 kg/m<sup>2</sup>, p=0.002). PEC and composite of hypertensive disorders of pregnancy (PEC and gestational hypertension) rates were significantly higher in the pFET group compared to nFET group (p=0.022 and p=0.026, respectively). Notably, PEC occurred only in the pFET group (50/383 live births; 13.1%) and these pregnancies had a slower HCG rise compared to the pFET without PEC (p<0.05) and nFET (p<0.05). Interestingly, in 83% of PEC cases, E<sub>2</sub> prior to progesterone initiation was above 300 pg/mL. There was no difference in the rate of clinical pregnancy, miscarriage, biochemical pregnancy, ectopic pregnancy, live birth, fetal sex and birth weight between the groups. No differences were observed in the HCG rise by the endometrial preparation protocol (p=0.8). In singleton pregnancies, there was no difference in the rate of preterm birth, placental abruption, placenta previa, small for gestational age (SGA) and large for gestational age (LGA).

**CONCLUSIONS:** Programmed FET cycles are associated with an increased risk of PEC in singleton pregnancies compared to natural FET cycles. These findings suggest that hormonal milieu of the uterine environment in the absence of corpus luteum especially in the settings of elevated E<sub>2</sub> level may affect placentation leading to PEC.

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### **SUBENDOMETRIAL JUNCTION ZONE THICKNESS: IMPACT ON IVF OUTCOMES IN AN OOCYTE RECEPTOR PROGRAM.**

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**OBJECTIVE:** Endometrial-myometrial interface constitutes a distinct, hormone-dependent uterine compartment, denominated junction zone (JZ). Growing evidence suggests that a normal and functional JZ plays a key role in different reproductive disorders, as well as, for embryo implantation. Coronal section in three-dimensional transvaginal ultrasound (3D-TVS) of the uterus, offers accurate evaluation and measurement of JZ.

The aim of our study was to analyze the role of JZ subendometrial thickness assessment by 3D-TVS on IVF outcome in an oocyte receptor's program.

**DESIGN:** This was an observational prospective study. From January 2016 to May 2018, 58 women who met the inclusion criteria were enrolled.

**MATERIALS AND METHODS:** A total of 188 fresh or frozen embryo transfer were done. In all cases, only 1-2 good quality embryos (grade A and B) were used to be transferred.

Inclusion criteria were: nulliparous women with age range 30-50 years old and JZ thickness >3 mm. Exclusion criteria were: history of severe endometriosis, fibroids, ovarian cysts or ovarian lesions, hydrosalpinx, endometrial pathology, previous uterine surgery and severe male factor. Women underwent a detailed clinical assessment and a two-dimensional (2D) and 3D-TVS.

2D-TVS endometrial thickness and pattern were evaluated on day 11-12 of estradiol valerate treatment. Subendometrial flow was assessed by color Doppler and classified as rich, medium, poor or absent (grades III, II, I, O), respectively. 3D-TVS, subendometrial JZ was measured from basal endometrium to the internal layer of outer myometrium on coronal sections. JZ was measured in 3 locations (fundus and right/left uterus's sides).

Estrogen and progesterone were administered consecutively for endometrium preparation in a mock cycle. Estradiol valerate 2mg/8h was started on the second cycle. When endometrium reached  $\geq 8$  mm, estradiol was

maintained and vaginal micronized progesterone 200 mg/8h was started to initiate the secretory changes.

**RESULTS:** Fifty eight women were assessed. Mean age was 41,3 years old (range 30- 48). Mean JZ thickness was 4,1 mm (range 3,2-5,3). Mean endometrium thickness was 8,44 mm (range 6,5-9,6). Subendometrial color Doppler evaluation of flow was rich in 80,1% (76/94) of cases, medium in 6,4% (6/94) and poor in 12,8% (12/94).

A total of 94 (fresh or frozen embryos) were transferred. Pregnancy rate was 39,36% (37/94), clinical pregnancy rate 28,76% (27/94), chemical pregnancy rate 27,07% (10/37), abortion rate 40,74% (11/27) and live-birth rate 17,02% (16/94).

**CONCLUSIONS:** Our results showed that JZ thickness may have prognostic implications for poorer IVF outcomes. We found no differences between fresh or frozen embryo transfer, both indicating low clinical pregnancy rate, high chemical and abortion rate and, as a consequence, a very low live-birth rate. Future, well designed studies are needed to evaluate the role of the JZ in IVF.

### **IVF OUTCOME PREDICTORS - GESTATIONAL CARRIERS**

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### **CAN BIRTH WEIGHT AND GESTATIONAL AGE AT DELIVERY OF SINGLETON GESTATIONAL CARRIER PREGNANCIES BE PREDICTED BY THE GESTATIONAL CARRIER'S OWN PREVIOUS SINGLETON PREGNANCIES?.**



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**OBJECTIVE:** Gestational carriers (GC) represent a unique population in which to study the effect of assisted reproductive technology on obstetric outcomes, as they are not infertile and have usually had favorable obstetric outcomes in the past. In GC pregnancies, important questions remain regarding how perinatal outcomes are differentially impacted by the genetic parents versus the GC. This study sought to compare birth weight and gestational age at delivery between a GC's prior own pregnancies versus the current GC pregnancy.

**DESIGN:** A retrospective analysis of all GC singleton deliveries from a single agency between 2008-2019.

**MATERIALS AND METHODS:** Data from a large surrogate agency that consisted of matched GCs and intended parent couples for an index GC pregnancy were reviewed. GCs with a history of or current multiple gestation as well as with a history of or current preterm delivery were excluded. All available birth weights of the GCs own children as well as the gestational age at delivery for the GC's last own birth were collected. Both average birth weight and last singleton birth weight of the GC's prior own deliveries were correlated to the birth weight of the index GC pregnancy. Gestational age at delivery of the GC's last own delivery was compared to the gestational age at delivery for the index GC pregnancy.

**RESULTS:** Of 836 GCs, 101 were eligible for inclusion in this study. Average age of GCs at time of delivery was 34.9 years (SD 4.4) and their average BMI was 24.3 (SD 3.13). 93 GCs (76.9%) had a prior parity of  $\geq 3$ , and 27 (22.3%) had grand multiparity ( $\geq 5$ ). The average birth weight of all GC's prior spontaneously conceived singletons was 7.83 lbs (SD 3.01) and 7.68 lbs (SD 1.01) for the GC's most recent own singleton delivery. Average BW of index GC pregnancies was 7.62 pounds (SD 1.05). While average birth weight of all the GC's prior singleton children was not correlated with the birth weight of the index GC pregnancy (r=0.051, P = 0.623), the birth weight of the GC's most recent own singleton birth was significantly correlated with the birth weight of the index GC pregnancy (r=0.298, P = 0.003). Birth weight of the index GC pregnancy was not correlated with GC BMI (r = 0.083, P = 0.423). Mean gestational age at delivery was similar between the GC's last own singleton delivery and the index GC pregnancies (mean 39.1 (SD 0.993) vs. 39.1 (SD=0.983), P<0.001). Gestational age at delivery of the GC's last own singleton pregnancy was also significantly associated with the gestational age at delivery for the index GC pregnancy (P<0.001).

**CONCLUSIONS:** While birth weight and gestational age at delivery are likely multifactorial and impacted by both genetic and environmental factors, we found that in singleton GC pregnancies birth weight and gestational age are correlated with birth weight and gestational age of a GC's last own delivery. This data is of value when counseling both intended parents and evaluating candidacy for potential surrogacy.

**P-155**

**WITHDRAWN**

**PREGNANCY RATES FOLLOWING SINGLE EUPLOID EMBRYO TRANSFER ARE UNCHANGED IN GESTATIONAL CARRIER COMPARED TO NON-GESTATIONAL CARRIER IN VITRO FERTILIZATION CYCLES.**



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**OBJECTIVE:** Published data suggest that clinical pregnancy and live birth rates are higher in cycles using gestational carriers (GC) compared to non-GC IVF cycles. This data includes fresh and frozen, Day 3 and Day 5 transfers of both tested and untested embryos, preventing effective isolation and evaluation of the uterine factor.<sup>1</sup> Studies to date have not evaluated clinical outcomes between GC and non-GC euploid elective single embryo transfers (eSETs) in programmed frozen embryo transfer (FET) cycles. Our objective was to compare clinical outcomes of euploid eSETs in programmed FET cycles in GCs with non-GC cycles.

**DESIGN:** Retrospective cohort study

**MATERIALS AND METHODS:** Our study included all patients who underwent embryo transfer of a single Day 5 or Day 6 euploid embryo in a programmed FET cycle at a single IVF center in 2018. Preimplantation genetic testing for aneuploidy was performed following trophectoderm biopsy and next generation sequencing. FET cycle outcomes were compared between the GC and non-GC FET groups. Statistical analysis was performed using the student *t*-test and chi-square, where applicable.

**RESULTS:** A total of 115 GC and 428 non-GC FET cycles met inclusion criteria. There was no statistically significant difference in embryo day (63% Day 5 for GC vs 69% Day 5 for non-GC, *p*=0.22), or embryo grade (72.2% Good for GC vs 66.2% for non-GC, *p*=0.39) between the GC and non-GC cycles. Oocyte age was significantly younger in the GC compared to the non-GC group (31.2±6.6 vs 34.5±4.9, *p*<0.001), however in the setting of euploid single embryo transfer, oocyte age has been shown not to impact clinical pregnancy outcomes.<sup>2</sup> Positive pregnancy test, biochemical pregnancy, clinical pregnancy, miscarriage and ongoing pregnancy rates did not differ between GC and non-GC cycles (Table 1).

TABLE 1. Pregnancy outcomes in GC vs non-GC cycles

	GC cycles (n=115)	Autologous IVF (n=428)	p-value (chi-square)
Positive hCG	69.6%	69.9%	0.94
Biochemical pregnancy <sup>a</sup>	10.4%	9.8%	0.84
Clinical pregnancy <sup>b</sup>	60.0%	60.0%	0.99
Miscarriage <sup>c</sup>	3.5%	5.8%	0.32
Ongoing pregnancy <sup>d</sup>	56.5%	54.1%	0.64

<sup>a</sup> Transient serum b-hCG rise.

<sup>b</sup> Visualization of a gestational sac on ultrasound.

<sup>c</sup> Spontaneous loss of a clinical pregnancy at <20 weeks of gestation.

<sup>d</sup> Ongoing viable pregnancy at 10 weeks of gestation.

**CONCLUSIONS:** Our study indicates that use of a gestational carrier does not improve pregnancy rates after single euploid frozen embryo transfer when compared with an unselected infertility population undergoing autologous IVF. As equivalent pregnancy rates are seen in GC vs non-GC cycles, this may indicate an underlying benefit of euploid single FET in autologous cycles.

**References:** 1. Marugappan G, Farland L, Missmer S, Correia K, Anchan R, Ginsburg E. Gestational carrier in assisted reproductive technology. *Fertil and Steril.* 2018;109:420-428.

2. Nazem TG, Sekhon L, Lee JA, Overbey J, Pan S, Duke M, Britton-Jones C, Copperman AB, et al. In an era of euploid single embryo transfers: does oocyte age matter? *Fertil and Steril.* 2018.

**SUPPORT:** None.

**IVF OUTCOME PREDICTORS - HORMONE LEVELS**

**THE EFFECT OF SUPRAPHYSIOLOGIC ESTRADIOL LEVELS ON PREGNANCY OUTCOMES IN SINGLE EUPLOID FROZEN EMBRYO TRANSFER CYCLES.**



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**OBJECTIVE:** In the United States, frozen embryo transfers (FETs) after in vitro fertilization (IVF) cycles have increased by >80% since 2006.<sup>1</sup> Patients undergoing controlled ovarian hyperstimulation are often exposed to supraphysiologic levels of estradiol (E2), which have been associated with increased uterine contractility, dyssynchrony between endometrial and embryo development, and decreased implantation rates in fresh IVF cycles.<sup>2,3</sup> A recent study of elevated E2 levels during FET showed an association with decreased ongoing pregnancy/live birth rates (OP/LBR).<sup>4</sup> However, that study was confounded by transfer of multiple unscreened embryos and small sample size. This study evaluates the association between supraphysiologic E2 levels and pregnancy outcomes in a single euploid FET model.

**DESIGN:** Retrospective, cohort study.

**MATERIALS AND METHODS:** The study included patients undergoing single euploid FET from 2012 to 2019. Endometrial preparation was performed using oral, vaginal, transdermal E2, or a combination of these routes. Cycles involving ovum donation, natural endometrial preparation, intramuscular E2, >21 days of E2 stimulation, or an endometrial thickness <8 mm were excluded. Preimplantation genetic testing for aneuploidy (PGT-A) was performed using quantitative polymerase chain reaction, array comparative genomic hybridization or next generation sequencing. Supraphysiologic E2 levels were defined as the mean+2SD E2 of natural FET cycles occurring in the same time period. E2 exposure was measured using area under the curve (AUC). Supraphysiologic E2 was calculated as AUC E2 ≥ 7,136.4 pg/mL. The primary outcome of the study was OP/LBR. Secondary outcomes included clinical pregnancy (CP) and early pregnancy loss (EPL) rates. Data were evaluated using t-tests, chi-square tests, and generalized estimating equations.

**RESULTS:** The study included 3876 single euploid FET cycles from 2707 patients. CP and OP/LBR were 61.9% and 54.1% in the physiologic E2, and 53.9% and 47.3% in the supraphysiologic E2 groups, respectively. Univariate analysis identified BMI and embryo morphology ≥4BB as possible confounders. After controlling for these confounders, there was a decreased CP rate in cycles that had supraphysiologic compared to physiologic E2 levels (OR 0.72 [95% CI 0.52-0.99], *p*=0.04), but no difference in OP/LB rate (OR 0.78 [95% CI 0.57-1.06, *p*=0.12), or EPL rate (OR 1.16 [95% CI 0.77-1.74], *p*=0.47).

**CONCLUSIONS:** In this study evaluating the association between E2 levels and pregnancy outcomes in a single euploid FET study model, we found that CP, but not OP/LBR, is significantly lower in the presence of supraphysiologic E2 levels. This study found no difference in biochemical or clinical pregnancy loss following FET of a euploid embryo in the setting of supraphysiologic E2, suggesting that patients can be reassured that after implantation there is no demonstrable increase in EPL. Future studies might focus on pharmacogenomic markers that can identify women who will respond to equivalent doses of E2 with higher serum E2 levels, to optimize the uterine environment for FET.

**References:** 1. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Clinical rationale for cryopreservation of entire embryo cohorts in lieu of fresh transfer. *Fertil Steril* 2014;102:3-9.

2. Simon C, Cano F, Valbuena D, Remohi J, Pellicer A. Clinical evidence for a detrimental effect on uterine receptivity of high serum oestradiol concentrations in high and normal responder patients. *Hum Reprod* 1995;10:2432-7.

3. Arslan M, Bocca S, Arslan EO, Duran HE, Stadtmauer L, Oehninger S. Cumulative exposure to high estradiol levels during the follicular phase of IVF cycles negatively affects implantation. *J Assist Reprod Genet* 2007; 24:111-7.

4. Fritz R, Jindal S, Feil H, Buyuk E. Elevated serum estradiol levels in artificial autologous frozen embryo transfer cycles negatively impact ongoing pregnancy and live birth rate. *J Assist Reprod Genet* 2017; 34:1633-38.

**SUPPORT:** None.

**AN ANALYSIS OF THE CONTRIBUTION OF SERUM ESTRADIOL LEVEL ON SUBCHORIONIC HEMATOMA FORMATION RATES IN NATURAL AND PROGRAMMED FROZEN EMBRYO TRANSFER CYCLES.**



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**OBJECTIVE:** A previous study by our group<sup>1</sup> showed a higher incidence of subchorionic hematoma (SCH) formation in pregnancies from programmed

(PRG) frozen embryo transfer (FET) cycles; compared to natural (NAT) FETs where estradiol (E2) levels were lower. We analyzed whether E2 levels were associated with an increased incidence of SCH formation.

**DESIGN:** Retrospective cohort study of all single thawed euploid embryo transfer cycles resulting in clinical pregnancy from 1/2016 to 12/2018 at our center.

**MATERIALS AND METHODS:** All single euploid (by Next Generation Sequencing) FETs resulting in clinical pregnancy (presence of a gestational sac) were included. FET cycles with ploidy determined by aCGH, or cycles in which untested, mosaic, or multiple embryos were transferred were excluded. PRG cycles were defined by treatment of oral E2 daily followed by progesterone (P4); either 50-75mg intramuscular in oil or vaginal suppository. A NAT cycle, with and without letrozole, was defined by monitoring until a dominant follicle reached >18mm and ovulation was confirmed, followed by supplementation with vaginal P4 suppository. SCH was defined as a measurable clot behind the gestational sac at time of luteal ultrasound. The primary outcome was E2 levels in patients with SCH. Statistical analysis included Shapiro-Wilk test for normality for continuous variable, Mann-Whitney U and Fisher's Exact tests where appropriate. Median values are presented, as continuous variables were not parametric. A p-value <0.05 was considered significant.

**RESULTS:** 1273 cycles were identified and included; 213 NAT and 1060 PRG. Age (p=0.73), endometrial thickness (p=0.65), P4 level on cycle day (CD) 28 (p=0.82) and CD of SCH diagnosis (p=0.78) were similar between groups, though first hCG levels were lower in PRG cycles (196 vs 164 mIU/mL, p<0.001). The formation of SCH was significantly lower in NAT cycles compared to PRG (RR 0.4 (0.24 - 0.78), p < 0.001). There was no association with SCH incidence by P4 type (IM vs vaginal, p=0.40) in PRG cycles. Additionally, E2 levels were significantly higher in PRG cycles on day of P4 start (351.5 vs 268.5, p<0.001) and CD 28 (356.5 vs 249, p<0.001). However, there was no relationship between SCH formation and continuous E2 levels on day of P4 start (NAT p=0.76 PRG p=0.44) or on CD 28 (NAT p=0.71, PRG p=0.11) in either protocol. Within PRG cycles, SCH incidence was not associated with the change in E2 from day of P4 initiation to CD28 (p=0.25). E2 levels were then reclassified as high (>249pg/mL) or low based on the median E2 at day of P4 initiation (249 pg/mL). There was no association between rate of SCH formation in PRG cycles with high E2 (RR 0.75 (0.51-1.10), p = 0.09) or with high E2s on CD 28 (RR 1.10 (0.72-1.65), p = 0.38). Interestingly, in NAT FET cycles, patients with high E2 levels were more likely to have SCH formation (RR 3.23 (1.0-10.83), p<0.04).

**CONCLUSIONS:** Both SCH formation and serum E2 levels are higher in PRG FETs. However, high E2 levels was not associated with SCH formation. Further analysis is needed to determine the physiologic cause for an increased rate of SCH formation in PRG cycles and an estimation of obstetrical risk.

**References:** 1. Edison, N., Blakemore, J. K., Goldman, K. N., Hodes-Wertz, B., & Grifo, J. A. (2018). Behind the bleed: analysis of the formation of subchorionic hematomas (SCH) in single euploid embryo transfer cycles by protocol. *Fertility and Sterility*, 110(4), e260-e261.

**SUPPORT:** None.

**P-159** Tuesday, October 15, 2019 6:30 AM

#### CLINICAL MASS DATA ANALYSIS(CMDA) OF DIFFERENCE IN PREGNANCY RATE BETWEEN FROZEN-THAWED AND FRESH EMBRYO TRANSFER AT VARIOUS E2 LEVELS.

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**OBJECTIVE:** The purpose of this study was to compare the quantity and quality of oocytes according to estradiol levels in fresh embryo transfer and frozen-thawed embryo transfer, and the pregnancy rates were analyzed.

**DESIGN:** Retrospective cohort study in a reproductive center.

**MATERIALS AND METHODS:** This study included 17,601 cycles of 6,004 patients who underwent fresh embryo transfer or frozen-thawed embryo transfer between February 2014 and October 2018. All cycles were divided into 8 groups at 1,000pg/ml intervals according to serum E2 level. The quality of oocytes was analyzed by evaluating the maturity of oocytes immediately after oocyte retrieval. Fresh embryos were transferred in 10,237 cycles and frozen embryos were transferred in 6,824 cycles. Statistical analyses were performed using t-test or Chi-square test. P-values < 0.05 were considered statistically significant.

**RESULTS:** As the E2 level increased, the number of follicles increased. However, oocyte maturation rate was highest in group 1 in which the lowest number of follicles were observed under ultrasonic guidance (47.8% in fresh embryo transfer, 48.2% in FET) and lowest in group 8 in which the highest number of oocytes were observed (39.2% in fresh embryo transfer, 38.0% in FET). The pregnancy rates of respective group are shown in the table below. In fresh embryo transfer cycles, pregnancy rates were lower in group in which the lowest number of follicles were observed (group 1) or the group in which the largest number of follicles were observed (group 8) than other groups. In FET cycles, pregnancy rates of the groups in which large number of follicles were observed (group 7, 8) were higher than other groups.

**CONCLUSIONS:** The number of follicles gradually increases as the E2 level increases in both fresh ET and FET group. However, the proportion of mature oocyte has decreased. The pregnancy rate of the FET was higher than that of fresh ET in each group. This mass data analysis may be an indicator of choice for selecting fresh embryo transfer or frozen-thawed embryo transfer depending on the E2 level.

TABLE 1. Results of Follicles, Mature oocyte and Pregnancy rates in Fresh, Frozen Embryo Transfer

Groups	Fresh-ET			Frozen-Thawed ET		
	Follicles	Mature oocyte (%)	P.R <sup>a</sup>	Follicles	Mature oocyte (%)	P.R <sup>a</sup>
0~1000	6.3 ± 4.2	47	29.5	4.9 ± 4.4	48.2	41.0 <sup>2</sup>
1000~2000	11.0 ± 5.0	42	41.0	11.3 ± 5.5	41.5	44.8 <sup>2</sup>
2000~3000	14.5 ± 5.0	40	43.9	16.6 ± 5.2	39.0	47.4 <sup>2</sup>
3000~4000	17.2 ± 5.0	39	43.1	19.7 ± 5.5	37.5	46.9
4000~5000	18.9 ± 4.6	39	44.0	21.3 ± 5.2	38.3	42.9
5000~6000	19.5 ± 4.6	39	49.7 <sup>1</sup>	22.4 ± 5.1	38.1	49.6
6000~7000	21.3 ± 4.6	40	49.1 <sup>1</sup>	23.7 ± 4.8	37.7	53.6 <sup>1,2</sup>
7000~	21.5 ± 4.1	39	36.1	25.9 ± 4.0	38.0	61.1 <sup>1,2</sup>

<sup>1</sup>P<0.05; Pregnancy rates column analyzed by T-test, <sup>2</sup>P<0.05; Pregnancy rates in Fresh ET and Frozen-thawed ET analyzed by  $\chi^2$ -test, <sup>a</sup>pregnancy rates

**P-160** Tuesday, October 15, 2019 6:30 AM

#### "PREDICTIVE VALUE OF SERUM PROGESTERONE LEVEL ON DAY 4, DAY 7 AND DAY 11 AFTER BLASTOCYST TRANSFER IN A HORMONAL REPLACEMENT THERAPY CYCLE."

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**OBJECTIVE:** Recent studies have suggested that low serum progesterone (P) levels on day of embryo transfer (ET) are associated with poorer pregnancy outcome. Determination of serum P in hormonal replacement therapy (HRT) cycles reflects the absorption of exogenous P because no endogenous production exists until pregnancy week 5-6. It is of interest to know if serum P levels during mid and late luteal phase are related with the risk of miscarriage or of ongoing pregnancy.

In this study, we wanted to evaluate the predictive value of P levels from mid luteal phase (4 and 7 days after ET) to the day of the  $\beta$ -hCG check (11 days after ET) for ongoing pregnancy in HRT cycles.

**DESIGN:** Prospective cohort study performed between June 2017 and August 2018 in IVI-RMA Valencia, Spain.

**MATERIALS AND METHODS:** Eligible patients were aged between 18-42 years, with a normal uterus, and being transferred 1-2 good quality blastocysts from own or donated eggs after an HRT cycle with estradiol valerate and vaginal micronized P (400mg/12h).

Serum P levels were measured three times during the mid and late luteal phase (on the 4<sup>th</sup>, 7<sup>th</sup> and 11<sup>th</sup> day after ET).

Correlation between pregnancy results and hormonal time 2-degree polynomial fitted data was analyzed by linear model. A logistic linear model and ROC analysis were performed to assess P polynomial coefficients as a predictive test for ongoing pregnancy.

**RESULTS:** A total of 150 patients were included. Mean age was 38.1±3.9y, with a BMI of 23.4±3.6kg/m<sup>2</sup> and endometrial thickness before introducing exogenous P of 9.1±1.6mm. The overall ongoing pregnancy rate was 47.3% (95%CI=39.3-55.3).

The AUC for P exposure during the luteal phase was significantly higher in ongoing pregnancies (101.2ng/ml (95%IC=90.8-111.6)) when compared with negative  $\beta$ -hCG cases (79.4ng/ml (95%IC=66.5-92.4)), p=0.027.

On ET+11, ongoing pregnancies showed a significantly higher serum P levels when compared with negative  $\beta$ -hCG (mean difference 5.5 ng/ml (95%CI=2.6-8.3),  $p=0.001$ ).

The ROC curve showed that there is a significant predictive value of serum P levels for ongoing pregnancy rate, being the AUC (95% CI) = 0.63 (0.54-0.72) on ET+4; 0.65 (0.56-0.74) on ET+7; and 0.73 (0.65-0.81) on ET+11 with best cutoff values of 9.95, 21.3 and 11.6 ng/ml, respectively.

**CONCLUSIONS:** In HRT cycles in which vaginal progesterone is used, P levels across luteal phase days are associated with pregnancy outcome. Ongoing pregnancies showed a higher exposure to P. These results suggest that absorption to vaginal P can vary among patients and this can influence on the results.

**SUPPORT:** None.

**P-161** Tuesday, October 15, 2019 6:30 AM

**THE PREDICTIVE VALUE OF FSH BASAL LEVELS FOR ART OUTCOMES IS AGE DEPENDENT.**

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**OBJECTIVE:** Studies with robust numbers of patients to clarify the predictive value of FSH levels for ART outcomes are still needed. In this study, we examined a large cohort of ICSI patients aiming to assess across different maternal age groups the association of FSH basal levels with implantation, clinical pregnancy and abortion rates.

**DESIGN:** We performed a retrospective analysis of data collected since 2016 including 2503 autologous ICSI cycles. Each ICSI cycle represents a distinct patient. Patients were grouped according to FSH plasma concentrations measured on the second or third day of the ICSI cycle (FSH groups:  $<7.5$ ;  $\geq 7.5/\leq 10$ ;  $>10$  IU/L), and age (Age groups:  $<34$ ;  $\geq 34/\leq 37$ ;  $\geq 38/\leq 40$ ;  $>40$ ).

**MATERIALS AND METHODS:** Patients age 20 to 45 years with unexplained, male-related or tubal sub-fertility were subjected to controlled ovarian stimulation utilising a GnRH antagonist protocol, with FSH dose individually adjusted and oocyte maturation triggered with hCG 36 hours before oocyte collection. Matured oocytes were subjected to ICSI and one to three embryos were transferred fresh three days later. The effects of FSH levels on implantation, clinical pregnancy and abortion rates were assessed in different age groups by the Fisher's exact test.

**RESULTS:** Overall, FSH levels were negatively correlated with implantation and clinical pregnancy rates, but not with abortion rate. For patients with basal FSH  $<7.5$ ,  $\geq 7.5/\leq 10$  and  $>10$  IU/L, clinical pregnancy rates were 19.9% (230/1156), 19.9% (150/753) and 13.5% (80/594;  $P=0.001$ ), implantation rates were 18.3% (338/1844), 19.9% (232/1168) and 15.2% (130/853;  $P=0.026$ ), and abortion rates were 19.0% (55/289), 23.6% (47/199) and 25.0% (27/108;  $P=0.29$ ), respectively. Interestingly, when patients were stratified by age, FSH levels only significantly affected implantation and pregnancy rates in patients under 34 years. For patients under 34 years with basal FSH  $<7.5$ ,  $\geq 7.5/\leq 10$  and  $>10$  IU/L, clinical pregnancy rates were 36.9% (80/217), 28.2% (35/124) and 20.3% (14/69;  $P=0.023$ ), and implantation rates were 35.3% (110/312), 31.2% (54/173) and 19.4% (18/93;  $P=0.013$ ), respectively. As expected, implantation and clinical pregnancy rates decreased progressively with age, but did not consistently vary with FSH levels in patients older than 34. For patients older than 40 years with basal FSH  $<7.5$ ,  $\geq 7.5/\leq 10$  and  $>10$  IU/L, pregnancy rates were 9.9% (41/416), 9.2% (26/284) and 6.7% (19/283;  $P=0.345$ ), and implantation rates were 9.9% (73/741), 10.1% (48/476) and 9.5% (42/443;  $P=0.96$ ), respectively.

**CONCLUSIONS:** Higher FSH basal levels are associated with poorer ART outcomes in patients under 34 years and thus represent a useful ART prognostic tool for this age group. Interestingly, the predictive value of FSH basal levels for ART outcomes seems to fade as maternal age advances. New studies are needed to clarify the mechanisms linking higher FSH levels with impaired fertility in younger patients, and whether these mechanisms are absent, not reflected by a single FSH measurement or clinically not perceived due to their interaction with other age-related modifications in older patients.

**P-162** Tuesday, October 15, 2019 6:30 AM

**THE IMPACT OF SPONTANEOUS LH SURGE DURING A NATURAL CYCLE FROZEN EMBRYO TRANSFER.**

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TABLE 1.

Demographics/Outcomes	NC-FET at LH+6 (n=91)	NC-FET at HCG+7 (n=135)	p-value
Mean age at transfer (y)	35	37	<0.01
Mean BMI (kg/m <sup>2</sup> )	25	25	0.56
Nulliparous (n)	70 (76%)	77 (57%)	<0.01
Non-smoking (n)	87 (96%)	10 (93%)	0.36
Endometrial thickness (mm)	9.0	8.9	0.32
Mean LH value (mIU/mL)	45.0	10.5	<0.01
Mean peak estradiol value (pg/mL)	376	286	<0.01
Mean progesterone value (ng/mL)	0.8	0.4	<0.01
Pregnancy rate (n)	72 (79%)	96 (71%)	0.26
Live birth rate (n)	54 (59%)	75 (56%)	0.57

**OBJECTIVE:** This study aims to assess outcomes of natural cycle frozen embryo transfers (NC-FET) in women who had spontaneous LH surge compared to women without detected LH surge on the day of HCG trigger; we hypothesize there is no difference in pregnancy rates between the two groups.

**DESIGN:** Institutional Review Board-approved retrospective cohort study.

**MATERIALS AND METHODS:** All patients who underwent NC-FET with euploid blastocysts at a single academic institution from 5/1/2016 to 11/15/2017 were reviewed. Previous workup included confirmation of normal uterine cavity. Standard protocol for NC-FET included ultrasound monitoring and HCG trigger when the dominant follicle was  $\geq 18$  mm and endometrial lining was  $\geq 7$  mm. Patients had serum LH, estradiol, and progesterone checked on the day of HCG trigger. If LH was  $\geq 20$  mIU/mL, FET was performed 6 days after surge (LH+6), with the intent of transferring a thawed blastocyst 5 days after ovulation. If LH was  $<20$  mIU/mL, FET was performed 7 days after HCG trigger (HCG+7). Vaginal progesterone supplementation was started 3 days after spontaneous LH surge or 4 days after HCG trigger. Demographic information and pregnancy rates for both groups were recorded in a secure REDCap database and compared using t-test and chi-squared statistical analyses.

**RESULTS:** A total of 226 NC-FETs were included. Mean age at transfer was 36, and mean BMI was 25. Overall, the pregnancy rate (PR), defined by positive beta HCG, was 75% and live birth rate (LBR) was 57%. Baseline characteristics between those patients who were transferred at LH+6 (n=91) and those transferred at HCG+7 (n=135) are provided in Table 1. Women in the LH+6 group had a PR of 79% and LBR of 59%. Women in the HCG+7 group had a PR of 71% and LBR of 56% ( $p = 0.26$  and  $p = 0.57$ , respectively).

**CONCLUSIONS:** HCG triggered NC-FETs have been shown to have similar pregnancy rates as hormone replacement cycles; however, there is a paucity of data regarding monitored NC-FET timing and success in the setting of a spontaneous LH surge. In our study cohort, patients undergoing NC-FETs based on LH+6 timing had similar pregnancy and live birth rates when compared to those patients who did not have a spontaneous LH surge and transferred at HCG+7.

**SUPPORT:** None.

**P-163** Tuesday, October 15, 2019 6:30 AM

**THE RELATIONSHIP BETWEEN ANTI-MÜLLERIAN HORMONE AND ANEUPLOIDY IN REPRODUCTIVE-AGE WOMEN UNDERGOING PREIMPLANTATION GENETIC TESTING; IS THERE A**

**CORRELATION?** Monica Pasternak, MD,<sup>a</sup> Micha Thompson, BA,<sup>a</sup> Steven Spandorfer, M.D.<sup>b</sup> <sup>a</sup>Ronald O. Perlman and Claudia Cohen Center for Reproductive Medicine, New York, NY; <sup>b</sup>The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY.



**OBJECTIVE:** The primary objective of this study was to investigate whether there is a correlation between serum anti-Müllerian hormone (AMH) levels and embryo chromosomal abnormality as determined by pre-implantation genetic testing for aneuploidy (PGT-A), and whether this differs by patient age.

**DESIGN:** This is a retrospective single-institution study. Demographics of patients undergoing in vitro fertilization (IVF) with PGT-A during a 4-year period were recorded, as well as characteristics of their resultant embryos.

**MATERIALS AND METHODS:** There were 1653 IVF/PGT-A cycles performed by patients who also had a serum AMH assay measured at our

institution; patients who had AMH drawn elsewhere were not included in this study. The percent of euploid embryos per number of embryos biopsied and cryopreserved at day 5/6 of embryogenesis was calculated for each IVF cycle. Patients were separated into two age groups: less than 39 yo and greater than or equal to 39 yo. AMH values were classified as low if <1.0 and normal if  $\geq 1.0$ . Statistical analysis was performed by using the Kruskal-Wallis and Chi-square tests as appropriate.

**RESULTS:** Of the 1653 IVF/PGT-A cycles analyzed, 1266 cycles included patients who had an AMH  $\geq 1.0$ , and 387 had an AMH <1.0. We further stratified the patients by age (<39 and  $\geq 39$ yo). A total of 735 cycles included patients who were <39yo, and 531 were  $\geq 39$ yo. For women who were <39yo, there was an average of 48.2% euploid embryos per IVF/PGT-A cycle for those with an AMH  $\geq 1.0$ , compared to women <39yo with an AMH <1.0 for whom there was an average of 40.1% euploid embryos per cycle ( $p=0.01$ ). For women  $\geq 39$ yo with an AMH  $\geq 1.0$ , there was an average of 16.9% euploid embryos per IVF/PGT-A cycle, compared to those with an AMH <1.0 for whom there was an average of 15.4% euploid embryos per cycle ( $p=0.062$ ).

**CONCLUSIONS:** The relationship between serum AMH levels and embryo chromosomal abnormality in patients undergoing IVF with PGT-A has yet to be determined. Previous studies have limited data pertaining to the relationship between AMH and aneuploidy prior to embryo transfer (ET) and conception in patients utilizing IVF. Most research on this subject has been disparate in terms of how and when these serum assays were obtained, and without controlling for the use of IVF. Additionally, most studies measured AMH during a clinical pregnancy, at which time AMH levels will be suppressed physiologically. The results from our study found that in women less than 39yo, AMH is significantly correlated with percent euploid embryos per the number of embryos biopsied in a given IVF/PGT-A cycle. No significant difference was found in women greater than or equal to 39yo. Therefore, it is reasonable to expect that women of younger reproductive age to have a disparity in terms of yield of euploid embryos after IVF/PGT-A depending on ovarian reserve as determined by AMH level, and to discuss it with our patients as part of IVF/PGT-A counseling.

**SUPPORT:** NONE.

**P-164** Tuesday, October 15, 2019 6:30 AM

#### **DO PROGESTERONE LEVELS ON DAY OF EMBRYO TRANSFER CORRELATE WITH PREGNANCY OUTCOMES IN ARTIFICIAL FROZEN-THAW CYCLES?** Michelle Volovsky, MBBS (Hons),<sup>a</sup>



Cassandra Louise Pakes, MD,<sup>b</sup> Genia Rozen, MBBS (Hons), FRANZCOG,<sup>c</sup> Alex Polyakov, MBBS, FRANZCOG, MCLinEpid, MRReproMed, GradCertEBM.<sup>d</sup> <sup>a</sup>Royal Women's Hospital, Melbourne, VIC, Australia; <sup>b</sup>Royal Women's Hospital, Melbourne, VIC, Australia; <sup>c</sup>Royal Women's Hospital, Melbourne IVF, Melbourne, VIC, Australia; <sup>d</sup>University of Melbourne and Melbourne IVF, Melbourne, VIC, Australia.

**OBJECTIVE:** To investigate whether progesterone (P4) levels on the day of frozen-thaw embryo transfer (FET) to a hormonally prepared endometrium correlate with pregnancy outcomes.

**DESIGN:** This is a large single-centre retrospective cohort analysis.

**MATERIALS AND METHODS:** A standardised data set spanning 2015-2018 was analysed. This comprised of N=2010 FETs into hormonally-prepared endometria. In these cycles, P4 levels on the day of transfer were assessed in relation to pregnancy outcomes. The main outcomes measured were biochemical pregnancy (beta human chorionic gonadotropin >5), clinical pregnancy (fetal heart on ultrasound) and live birth rates. Similar to previous studies in the area, a P4 threshold of 10 ng/mL (31.8 nmol/L) was used to simulate the currently accepted level for physiological corpus luteum function [1-3]. Eight hundred and seven FETs were completed in patients with a P4 level below 10 ng/mL and 1203 FETs in patients with P4 levels at or above 10 ng/mL. Using the Chi squared test, pregnancy outcomes were then compared between these two cohorts. A multivariate logistic regression, controlling for factors such as age and embryo quality, was further used to assess the relationship between P4 levels and outcomes.

**RESULTS:** We observed no statistically significant difference in pregnancy outcomes between the two cohorts (P4 on day of embryo transfer <10 ng/mL versus  $\geq 10$  ng/mL). This was uniformly demonstrated across biochemical pregnancy rates (39.53% vs. 40.98%,  $p = 0.516$ ), clinical pregnancy rates (20.82 vs. 22.78,  $p = 0.299$ ) and live birth rates (14.25 vs. 16.21  $p = 0.233$ ). Additionally, a multivariate logistic regression also failed to demonstrate statistically significant differences in biochemical pregnancy rates (OR 1.12 CI 0.93-1.35), clinical pregnancy rates (OR 1.19 CI 0.95-1.49) and live birth rates (OR 1.23 CI 0.95-1.58).

**CONCLUSIONS:** Contrary to previous low powered studies, we demonstrated that P4 levels at or above 10 ng/mL on the day of embryo transfer are not associated with statistically significant improvements in biochemical pregnancy, clinical pregnancy or live birth rates. Therefore, this finding enhances our limited understanding of transfer day P4 levels in predicting pregnancy outcomes for universally utilized artificial FET cycles and highlights the need for further research in this area.

**References:** 1. Cedrin-Durnerin I, Isnard T, Mahdjoub S, Sonigo C, Seroka A, Comtet M, Herbemont C, Sifer C, Grynberg M. Serum progesterone concentration and live birth rate in frozen-thawed embryo transfers with hormonally prepared endometrium. *Reprod Biomed Online*. 2019; 38(3): 472-480.

2. Hull MG, Savage PE, Bromham DR. The value of a single serum progesterone measurement in the midluteal phase as a criterion of a potentially fertile cycle (ovulation) derived from treated and untreated conception cycles. *Fertility & Sterility*. 1982; 37: 355-360.

3. Jordan J, Craig K, Clifton DK, Soules MR. Luteal phase defect: the sensitivity and specificity of diagnostic methods in common clinical use. *Fertility & Sterility*. 1994; 62: 54-62.

**P-165** Tuesday, October 15, 2019 6:30 AM

#### **COMPARING FROZEN EMBRYO TRANSFER OUTCOMES WITH BASELINE LH LEVELS.** Ariel Z. Benor, M.D., Richard Grazi, M.D. Maimonides Medical Center, Brooklyn, NY.



**OBJECTIVE:** To see if, during a programmed frozen-embryo transfer (FET) cycle, an endogenous rise in the LH level prior to initiation of progesterone supplementation may influence live birth rate (LBR). In the absence of concomitant pituitary suppression, high estradiol (E2) levels will often stimulate luteinizing hormone (LH) to rise to levels commonly associated with the periovulatory LH surge. In our study, we sought to correlate the live birth rates following FET when LH rose beyond a threshold level prior to supplementation with progesterone (P).

**DESIGN:** This was a single-center, retrospective cohort study from 2016-2018.

**MATERIALS AND METHODS:** The programmed preparation of endometrium started with estradiol pretreatment for a minimum of 14 days followed by five days of P given by intramuscular or vaginal route, or both, with FET performed on day 6 of P replacement. Two groups were stratified by LH levels <15 mIU/mL and LH >15 mIU/mL. Patients who were found to have a periovulatory follicle were excluded from the analysis.

**RESULTS:** One hundred fifty-five patients who underwent a frozen embryo transfer had LH levels drawn prior to the start of progesterone supplementation (pre-P). Seventy patients had a live birth and 111 patients did not. Of the 70 with a live birth, the mean LH level was 14.5 mIU/mL and of the 111 without a live birth, the mean LH was 14.2 mIU/mL. Whether pre-P LH levels were <15 or >15 mIU/mL made no difference to the LBR ( $p>0.7$ ). There was no pre-P LH level beyond which a decrease in LBR was seen. Of those patients who had a live birth, 40% had an LH>15 (range of 15-61.5), while 60% had an LH<15 (range of 0-14.3); the mean LH level was 14.5 (95% CI, 12.0-17.0). Of those without a live birth, 63% had an LH<15 (range of 0-14.8), while 37% had an LH>15 (range of 15.2-53.8); the mean LH level was 14.3 (95% CI, 12.3-16.3). We found that regardless of what threshold level was set for LH, no level was predictive of an effect on LBR.

**CONCLUSIONS:** LH levels that exceed 15 mIU/mL prior to initiating P supplementation in a programmed FET cycle have no significant effect on LBR. In the absence of a maturing follicle, there appears to be no threshold beyond which LH levels affect LBR.

**References:** None.

**SUPPORT:** No financial support was received for this abstract.

**P-166** Tuesday, October 15, 2019 6:30 AM

#### **SERUM HCG LEVEL MEASURED 5 DAYS AFTER SINGLE THAWED BLASTOCYST TRANSFER AS A PREDICTOR OF OUTCOME.** Angela H. Liu, MD,<sup>a</sup> Ankita Raman, MD,<sup>a</sup> Carrie E. Bedient, MD,<sup>b</sup> Leah A. Kaye, MD,<sup>b</sup> Forest C. Garner, MS,<sup>b</sup> Bruce Shapiro, M.D., Ph.D., H.C.L.D.<sup>b</sup> <sup>a</sup>University of Nevada, Las Vegas, Las Vegas, NV; <sup>b</sup>Fertility Center of Las Vegas, Las Vegas, NV.



**OBJECTIVE:** Investigate serum hCG level measured 5 days after vitrified-warmed single-blastocyst transfer as a predictor of transfer outcomes

Outcome	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	RR (95% CI)	P-value
Pregnancy	89.3	100.0	100.0	70.8	3.43 (2.85-4.13)	<0.0001
Clinical pregnancy	93.2	84.0	93.5	83.4	5.63 (4.31-7.36)	<0.0001
Multiple pregnancy	83.3	6.7	0.8	97.8	0.36 (0.04-3.05)	0.3338
Ongoing pregnancy	94.2	62.4	78.2	88.2	6.62 (4.77-9.19)	<0.0001
Live birth	93.9	61.4	77.0	88.0	6.40 (4.42-9.28)	<0.0001

(pregnancy, clinical pregnancy, multiple pregnancy, ongoing pregnancy, and live birth).

**DESIGN:** Retrospective cohort study of vitrified-warmed single-blastocyst transfers performed from in a 5-year period at a private fertility center.

**MATERIALS AND METHODS:** Vitrified-warmed blastocysts were transferred after artificial endometrial preparation on the 6<sup>th</sup> day of exogenous progesterone exposure. Serum hCG levels were measured 5 and 10 days after transfer. Levels rising above 5 IU/L on any day defined pregnancy. Clinical pregnancy was defined by sonographic finding of an intrauterine gestational sac 5-7 weeks after transfer. Multiple pregnancies were those with motion of multiple fetal hearts observed at any point. Ongoing pregnancies were those with fetal cardiac activity at 10 weeks. Chi-square tests were used to determine statistical significance.  $P < 0.05$  was considered significant.

**RESULTS:** There were 932 single-blastocyst transfers in the 5-year study period. Among all 932 transfers, a day 5 hCG level 5 IU/L was predictive of each outcome except multiple pregnancy. Sensitivity, specificity, positive predictive value, negative predictive value, and P-values are shown in Table 1. The live birth rate among transfers with day 5 hCG level 5 IU/L was 77.0%; while failure to achieve that criterion was associated with a live birth rate of only 12.0%. The area under the ROC curve for day 5 hCG level as a predictor of live birth was 0.830.

**CONCLUSIONS:** Serum hCG level measured 5 days after blastocyst transfer is a useful early predictor of outcome following single thawed blastocyst transfers in artificially prepared cycles. However, the sensitivity for predicting ongoing pregnancy and live birth was only 94%, indicating that a later confirmatory test is still required. The correlation between day 5 hCG and outcome highlights the importance of early implantation and the putative peri-implantation period in artificial cycles.

**P-167** Tuesday, October 15, 2019 6:30 AM

#### A NOVEL CYTOKINE PANEL CAN BETTER PREDICT OOCYTE COMPETENCY IN OVERWEIGHT PATIENTS.

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**OBJECTIVE:** Body composition affects outcomes in fertility treatments, however there is no clear correlation between measurement of body composition (BMI, lean/fat ratio and waist/hip ratio) and outcomes. This study aimed at creating a panel of metabolites to predict oocyte quality and competency in overweight patients.

**DESIGN:** A retrospective cohort of biobanked follicular fluid (FF) samples collected between April 2017-December 2018 at a university-affiliated fertility clinic.

**MATERIALS AND METHODS:** Consented patients were included if they were undergoing IVF, did not have a female factor related diagnosis, and had a BMI  $\geq 25$ . Luminex Multiplex Bead Assays (R&D Systems) were performed on FF from 14 patients. The analytes were chosen based on an a priori literature review which highlighted metabolites previously correlated with fertility treatment outcomes in overweight patients. The tested metabolites were adipokines (resistin, leptin and adiponectin), pro-inflammatory cytokines (IL6, IL18 and TNF $\alpha$ ), the anti-inflammatory cytokine IL10, the acute inflammation marker CRP, and factors associated with fat and glucose homeostasis (insulin, prolactin and chemerin). All samples and standards were assayed in duplicate (MACS-Quant Analyzer). Absolute quantification was performed by comparing fluorescence of the samples to standard curves using Flowjo (v10). Linear regression determined the impact changes in these metabolites have on embryology outcomes, while controlling for demographic features (R v3.5.1).

**RESULTS:** Patients were similar in terms of age ( $35.6 \pm 1.1$  years), BMI ( $30.9 \pm 1.0$  kg/m<sup>2</sup>) and AMH ( $18.3 \pm 1.7$  pmol/L). The mean maturation rate was 70.6%, fertilization rate was 82.5%, cleavage rate was 99.2% and blastulation rate was 50.9%. Of all analysed factors, TNF $\alpha$  and IL18 were nega-

tively associated with BMI and positively affected fertilization rate. Notably, leptin and CRP were not associated with BMI. However, increased leptin concentration negatively affected maturation rate, and increased CRP levels showed a tendency towards decreasing blastulation rate. Interestingly, several factors were negatively associated with blastocyst quality, including: IL6, IL18, chemerin, prolactin and insulin (all  $p < 0.01$ ).

**CONCLUSIONS:** To our knowledge, this is the most comprehensive study to examine the effect multiple adipokines and cytokines have on metrics of oocyte and embryo quality in humans. While some cytokines that affect oocyte quality and embryology outcomes in fertility treatments correlate with BMI in overweight patients, others do not. Notably, IL6, IL18, TNF $\alpha$ , CRP, chemerin, prolactin, insulin and leptin all affect different aspects of oocyte and embryo development. Therefore, they should be considered as potential biomarkers to predict success in fertility treatments of overweight patients. Such biomarkers could help delineate patients of similar BMI with differing fertility potential. Further research should focus on larger-scale studies exploring these relationships in non-obese patients and determining if patients' serum can be utilized to predict fertility treatment success.

**SUPPORT:** This study was funded through reinvestment of clinical earnings by CReATe Fertility Centre.

#### IVF OUTCOME PREDICTORS - LUTEAL SUPPORT

**P-168** Tuesday, October 15, 2019 6:30 AM

#### IN PATIENTS WITH SUB-OPTIMAL ENDOMETRIAL LINING, DOES THE ROUTE OF ADMINISTRATION OF SUPPLEMENTAL ESTROGEN CORRELATE WITH FROZEN EMBRYO TRANSFER

OUTCOMES? Devora Aharon, MD,<sup>a</sup> Sass Wodoslawsky, BA,<sup>b</sup> Ariel Megan Schnur, RN, BSN,<sup>b</sup> Jordyn Banks, RN,<sup>b</sup> Melissa Bell, RN,<sup>b</sup> Margaret Daneyko, RN,<sup>b</sup> Lawrence Grunfeld, MD,<sup>b</sup> Alan B. Copperman, MD.<sup>a</sup> <sup>a</sup>Icahn School of Medicine at Mount Sinai, New York, NY; <sup>b</sup>Reproductive Medicine Associates of New York, New York, NY.



**OBJECTIVE:** Patients routinely receive supplemental oral estrogen in preparation of the endometrial lining prior to a frozen embryo transfer (FET). In patients with suboptimal growth, additional vaginal or transdermal estrogen supplementation may be prescribed in attempt to increase estrogen absorption and optimize uterine lining thickness. To date, there are limited data analyzing the clinical utility of either route. This study aims to evaluate the correlation of vaginal or transdermal estradiol supplementation with patient FET cycle outcomes.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The study included patients who underwent an autologous or donor egg FET cycle with an endometrial thickness of  $< 7$ mm on cycle day 10-15 from November 2005-April 2019. Patients were separated into groups by route of additional E2 supplementation (vaginal estradiol tablets (E2 PV group); transdermal estrogen patch (E2 TD group)). Baseline demographics and cycle characteristics were collected. Outcomes included endometrial stripe (EMS) at embryo transfer, chemical pregnancy rate, clinical pregnancy rate, and live birth rate. A sub-analysis of euploid FET was performed. A sub-analysis was performed in patients with a structural uterine factor (as identified by an initial hysterosalpingogram (HSG) or saline infusion sonohysterography (SIS)). Statistical significance was calculated using chi-square test and t-test. A p value of 0.05 was set for statistical significance.

**RESULTS:** A total of 414 patients underwent 461 FET cycles within the study, including 396 E2 T2 cycles and 65 E2 PV cycles. Baseline demographics were similar between the two groups. A statistically significant increase in EMS at transfer was seen in the E2 PV group compared to the E2 TD group, however, the absolute difference was 0.01 mm (E2 TD 8.34 (4.6-15.5, SD  $\pm 1.57$  vs E2 PV 8.35 (5.1-15.63, SD  $\pm 2.23$ ),  $p = 0.0002$ ). No statistically significant differences in chemical pregnancy, clinical pregnancy, or live birth rates were seen. In the sub-analysis of euploid FETs, EMS at transfer was significantly greater in the E2 PV compared to E2 TD group (8.66 (5.1-15.6, SD 2.06) vs. 8.32 (5.3-13.7,

SD 1.53),  $p=0.0062$ ). A significant increase in chemical pregnancy rate was seen in the E2 PV compared to E2 TD group (75% vs. 59.4%,  $p=0.05$ ). However, clinical pregnancy rates and live birth rates were similar. In the sub-analysis of patients with an initially abnormal SIS or HSG, EMS at transfer was significantly lower in the E2 PV compared to E2 TD group (7.05 (5.2-9.0, SD  $\pm 1.13$ ) vs. 8.11 (5.12-12.46, SD  $\pm 1.65$ ),  $p=0.049$ ). No significant differences were seen in clinical pregnancy, chemical pregnancy, and live birth rates.

**CONCLUSIONS:** Supplemental vaginal and transdermal estradiol were equally effective in achieving endometrial thickness  $>7$ mm, and both methods resulted in similar pregnancy outcomes. Patients can be comforted in knowing that both routes of estrogen supplementation are effective in supporting the endometrial lining prior to FET, and choice of method may be based on patient and provider preference.

Reference: None.

SUPPORT: None.

**P-169** Tuesday, October 15, 2019 6:30 AM

### NATURAL FROZEN EMBRYO TRANSFER WITH HCG BOOSTER FOR OPTIMIZATION OF CYCLE OUTCOMES: A RETROSPECTIVE COHORT STUDY.



Claire Stewart, BA,<sup>a</sup> David Reichman, MD,<sup>b</sup> Zev Rosenwaks, M.D.<sup>c</sup> <sup>a</sup>Weill Cornell Medical College, New York, NY; <sup>b</sup>Weill Cornell Medicine, New York, NY; <sup>c</sup>The Ronald O. Perleman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY.

**OBJECTIVE:** To determine whether luteal support with intramuscular injection of human chorionic gonadotropin 1-day post-luteinizing hormone (LH) surge in natural cycle frozen embryo transfers (FET) increases ongoing pregnancy rates.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All patients undergoing natural cycle FET with transfer of a single euploid blastocyst between January 2017 and December 2018 were reviewed for inclusion. Patients were divided into two groups based on whether they received one bolus dose of hCG (typical dose, 3300 IU) 1-day after identification of the LH surge. All patients received vaginal progesterone support after transfer. Groups were further stratified by embryo quality. Patients with uterine factor infertility were excluded. The primary outcome of this study was ongoing pregnancy rate. Secondary outcomes included first trimester miscarriage and biochemical pregnancy rates. Outcomes were analyzed with Chi-squared test, Fisher exact test, and logistic regression where appropriate. Odds ratios (OR) with 95% confidence intervals (CI) were calculated and adjusted for patient age at time of transfer, embryo quality assessed by blastocyst grade, BMI, gravidity, parity, and peak endometrial thickness.  $P < 0.05$  was considered statistically significant.

**RESULTS:** A total of 529 FET cycles were included. Patients receiving hCG ( $n = 146$ ) had a statistically significant higher ongoing pregnancy rate than those without treatment ( $n = 383$ ) (69.9% vs. 57.4%; adjusted odds ratio 1.72, 95% CI, 1.13-2.65). There were no significant differences observed in the rates of first trimester miscarriage or biochemical pregnancy (Table).

**CONCLUSIONS:** This study provides evidence that natural cycle FET in which the luteal phase is buttressed with both a single hCG injection after the endogenous LH surge, as well as vaginal progesterone after transfer, are associated with higher clinical success rates with minimal negative impact on the patient experience.

Pregnancy outcomes for cases and controls.

Outcome	hCG Booster (n=146)	No Booster (n=383)	P value	aOR (95% CI)
Ongoing Pregnancy	102 (.699)	220 (.574)	0.012	1.72 (1.13-2.65)
First Trimester Miscarriage	5 (.034)	14 (.037)	0.90	1.05 (0.32 – 2.96)
Biochemical Pregnancy	8 (.055)	28 (.073)	0.58	.79 (0.31 – 1.76)

**P-170** Tuesday, October 15, 2019 6:30 AM

### INTRAMUSCULAR INJECTION OF HUMAN CHORIONIC GONADOTROPIN BEFORE SECRETORY TRANSFORMATION SIGNIFICANTLY IMPROVES THE IMPLANTATION AND PREGNANCY OUTCOMES IN FROZEN EMBRYO TRANSFER CYCLES.



Ling Deng, bachelor,<sup>a</sup>

Xin Chen, PhD,<sup>b</sup> Shi-ling Chen, PhD,<sup>b</sup> De-Sheng Ye, doctor,<sup>b</sup> nf. <sup>a</sup>Southern Medical University, Guangzhou, China; <sup>b</sup>Center for Reproductive Medicine, Department of Gynecology and Obstetrics, Nanfang Hospital, Southern Medical University, Guangzhou, China.

**OBJECTIVE:** To explore the effect of intramuscular injection of human chorionic gonadotropin (hCG) before secretory transformation on pregnancy outcomes of hormone replacement treatment frozen embryo transfer cycles (HRT-FET).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Infertility patients younger than 43 years and performing HRT-FET cycles in center for Reproductive Medicine were recruited in this study. Patients suffering from adenomyosis were excluded. And a total of 904 HRT-FET cycles were analyzed, 404 cycles in the hCG group and 500 in the control group, respectively. Patients in the hCG group received intramuscular injection of 10,000 IU hCG before secretory transformation. The control group performed FET without hCG administration before secretory transformation. We compared the implantation rate (IR), clinical pregnancy rate (CPR), ongoing pregnancy rate (OPR) and live birth rate (LBR).

**RESULTS:** The basic characteristics and clinical parameters were comparable between the two groups. The CPR (58.7% vs. 49.6%; odds ratio (OR), 1.4; 95% confidence interval (CI), 1.1-1.8;  $P = 0.007$ ), OPR (50.0% vs. 41.2%; OR, 1.4; 95% CI, 1.1-1.9;  $P = 0.008$ ), LBR (47.5% vs. 38.2%; OR, 1.5; 95% CI, 1.1-1.9;  $P = 0.005$ ) and IR (43.8% vs. 34.6%,  $P = 0.000$ ) were statistically significantly higher in the hCG group as compared with the control group. After adjusting confounding factors (age at index IVF/ICSI cycles, duration of subfertility, body mass index, number of embryos transferred and good-quality embryos transferred, and cycles of previous transfer), the use of hCG was still a significant factor predictive of LBR in HRT-FET cycles (adjusted OR 1.5; adjusted 95% CI 1.1-2.1;  $P = 0.002$ ). When the analysis was restricted in the patients with age  $< 39$  years old and with at least one good embryo transferred in the included cycles, the pregnancy rates in the hCG group were more superior to the control group with statistically significant difference (CPR: 64.3% vs. 52.3%; adjusted OR, 1.6; adjusted 95% CI, 1.2-2.3;  $P = 0.001$ . OPR 56.5% vs. 45.4%; adjusted OR, 1.6; adjusted 95% CI, 1.2-2.2;  $P = 0.002$ ; LBR: 53.7% vs. 41.7%; adjusted OR, 1.7; adjusted 95% CI, 1.2-2.3;  $P = 0.001$ ).

**CONCLUSIONS:** Intramuscular injection of 10,000 IU hCG before secretory transformation statistically significantly improved IR and pregnancy outcomes in HRT-FET cycles.

**P-171** Tuesday, October 15, 2019 6:30 AM

### LUTEAL PHASE SUPPORT USING GONADOTROPIN RELEASING HORMONE AGONIST (GNRHA) VERSUS ESTROGEN AND PROGESTERONE SUPPLEMENTATION IN HIGH RESPONDERS FOLLOWING GNRHA TRIGGERING – A PROSPECTIVE RANDOMIZED CONTROLLED TRIAL.



Lilach Marom Haham, M.D.<sup>a</sup> Yariv Shlomo Gidoni, M.D.<sup>b</sup> Ohad Baruchin, MD, MHA,<sup>b</sup> Jonathan Barkat, M.D,<sup>b</sup> Michal Youngster, M.D.<sup>a</sup> Ariel Revel, M.D.<sup>c</sup> Ido Ben-Ami, M.D PHD.<sup>c</sup> <sup>a</sup>Shamir medical center, Tel Aviv university, Beer Yakov, Israel; <sup>b</sup>Shamir Medical Center, Be'er Ya'akov, Israel; <sup>c</sup>Tel Aviv university, Tel Aviv, Israel.

**OBJECTIVE:** GnRHa triggering is used as an alternative to hCG in GnRH antagonist protocols to almost eliminate the risk of OHSS. However, its main disadvantage is a significant lower pregnancy rate which is thought to be caused due to luteolysis. In order to preserve high pregnancy rates, several luteal support regimens were investigated including an intensive estrogen and progesterone supplementation and daily GnRHa treatment. However, no study, so far, compared the efficacy of these two regimens. Our aim was to compare the efficacy of GnRHa versus intensive estrogen and progesterone supplementation for luteal phase support in high responders following GnRHa triggering.

**DESIGN:** A prospective randomized controlled trial

**MATERIALS AND METHODS:** High responder patients, defined as either reaching a serum estradiol levels of  $\geq 3500$  pg/ml on the day of trigger or having  $\geq 15$  oocytes retrieved, were recruited between October 2017 until March 2019. The patients were randomly assigned to either daily intranasal GnRHa (nafarelin 200 micrograms twice daily) or a combination of estrogen and progesterone (Estrofem 4 mg twice daily, vaginal Endometrin 300 mg daily and intramuscular injection of progesterone retard 250 mg once every five days) for luteal support. The GnRH antagonist protocol using GnRHa triggering was initiated. Patients with a BMI  $> 35$  or  $< 19$ , recurrent implantation failure, moderate to severe endometriosis or hydrosalpinx were excluded. Study groups' characteristics were compared using independent t-test. Implantation rates and clinical pregnancy rates were compared using chi square test.

**RESULTS:** A total of 47 women were allocated, 23 were assigned to the GnRH $\alpha$  arm and 24 were assigned to estrogen and progesterone treatment arm. Patients' characteristics including age, BMI, gravidity, parity as well as basal FSH levels didn't differ significantly between the study groups. Treatment's characteristics including the FSH dosage, duration of stimulation, peak estradiol levels, number of oocytes retrieved, fertilization rates and number of embryo transferred also didn't differ significantly between the study groups. The implantation rate was 56.5% and 37.5% in the GnRH $\alpha$  arm and in the estrogen and progesterone arm, respectively ( $P=0.1$ ). The clinical pregnancy rate was higher in the GnRH $\alpha$  treatment group compared to the estrogen and progesterone group although the difference was not statistically significant (60.8% vs. 50%,  $P=0.45$ ). Of note, no cases of OHSS were observed in both study groups.

**CONCLUSIONS:** Luteal support using GnRH $\alpha$  alone is as effective and safe as using instead estrogen and progesterone supplementation following GnRH $\alpha$  triggering in high responders. This new approach in fresh embryo transfer in high responders after GnRH $\alpha$  triggering offer a more convenient luteal support without compromising implantation and clinical pregnancy rates.

**P-172** Tuesday, October 15, 2019 6:30 AM

### PROSPECTIVE ANALYSIS OF PROGESTERONE DURATION IN PROGRAMMED SINGLE THAWED EUPLOID EMBRYO TRANSFER CYCLES.



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**OBJECTIVE:** In the era of personalized medicine and the simultaneously increasing use of frozen embryo transfer (FET), assays of the endometrium's receptivity prior to transfer has gained popularity, especially among patients. However, the optimal timing for single thawed euploid embryo transfers (STEET) in a programmed FET has yet to be determined<sup>1</sup>. We sought to examine the outcomes of euploid FETs by length of progesterone (P4) exposure at our clinic.

**DESIGN:** Prospective cohort study of all programmed FETs of single euploid embryos between 6/1/2018 and 12/18/2018 at our center.

**MATERIALS AND METHODS:** All patients undergoing FET in the inclusion time period were asked to write down the exact time of P4 initiation and then report the start time on the day of P4 serum level check (2 days later) prior to embryo transfer. All FETs were then reviewed. Programmed FET cycles were defined as treatment of oral estradiol daily followed by either 50-75mg intramuscular P4 in oil or vaginal P4 suppository with transfer of a euploid embryo (tested by either array comparative genomic hybridization or Next Generation Sequencing). Programmed FETs with untested, mosaic or double embryo transfers, as well as natural cycles, were excluded. Cycles where exact timing data could not be determined were also excluded. Statistical analysis included ANOVA, receiver operating characteristic curve, and Spearman's Rho correlation where appropriate, with  $p<0.05$  considered significant.

**RESULTS:** 253 programmed STEET cycles met criteria and were included in the analysis. The average patient age at time of ET was  $38.0\pm 4.5$  years. 3 cycles utilized an assay for endometrial receptivity for adjusted timing. The mean duration of P4 exposure was  $112.8\pm 2.9$  hours with a range from 98.25-124.5 hours for all unadjusted cycles. Overall, 166 women had an ongoing pregnancy (OP), 25 had a spontaneous loss (SAB), 12 a biochemical pregnancy (BP) and 50 a negative pregnancy (NP) test, for a 65.6% ongoing pregnancy rate. There was no significant difference in P4 duration between outcome groups ( $112.8\pm 3.1$  OP,  $112.4\pm 4.4$  SAB,  $111.6\pm 1.7$  BP,  $113.9\pm 5.7$  NP,  $F=1.76$ ,  $df=3$ ,  $p=0.156$ ). An ROC curve assessing the ability of P4 duration to predict ongoing pregnancy (OP) had an area under the curve of 0.467 ( $p=0.38$ ). Furthermore, there was no correlation in the day 28 serum hcg value based on the duration of P4 exposure ( $rs=0.058$ ,  $p=0.399$ ).

**CONCLUSIONS:** Duration of P4 is critical to the success of a programmed FET. At our center, duration of P4 was not associated with FET outcomes. 65.6% of cycles resulted in ongoing pregnancy with our center's standard instructions, which may vary from other centers who have equivalent euploid embryo implantation rates. With growing popularity for individualized testing, these results provide evidence for patient counseling of the high likelihood of desired outcome (ongoing pregnancy) without need for personalized testing. These results also support the need for further prospective or randomized controlled study of optimal FET cycle across clinics and protocols.

**References:** 1. Mackens S, Santos-Ribeiro S, van de Vijver A, Racca A, Van Landuyt L, Tournaye H, Blockeel C. Frozen embryo transfer: a review on the optimal endometrial preparation and timing. *Hum Reprod.* 2017; 32(11):2234-2242.

**SUPPORT:** None.

**P-173** Tuesday, October 15, 2019 6:30 AM

### A NOVEL PROGESTERONE RELEASING INTRAVAGINAL RING FOR LUTEAL PHASE SUPPORT: PHARMACOKINETICS AND SAFETY IN A SHEEP MODEL.



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**OBJECTIVE:** To evaluate the in vitro release and in vivo pharmacokinetics and local tolerability of a novel, segmented ethylene-vinyl acetate (EVA) intravaginal ring (IVR) (DARE-FRT1) delivering progesterone (P) in drug-naïve female Dorset crossbred sheep. These rings are being developed to provide luteal phase support and supplementation during ART cycles and early pregnancy.

**DESIGN:** IVRs capable of releasing P at 4 mg/d, 8 mg/d and 12 mg/day were administered to female sheep to assess the pharmacokinetics and safety compared to vaginal administration of Crinone 8% gel or Prometrium (200 mg) capsules.

**MATERIALS AND METHODS:** IVRs were prepared by hot-melt extrusion to create segments of varying length and drug content. The appropriate segments were used to create segmented IVRs capable of releasing P at rates of approximately 4, 8, and 12 mg/day. Release rates of P from the three IVR formulations were measured in vitro to determine whether the target release rates had been attained. Release rates were tested using 200 mL 0.5% sodium dodecyl sulfate as a release medium, in shakers at 37°C. Sampling (2 mL) was conducted on Days 1-4, 7-11, and 14. Animals were randomized into one of six treatment groups: Group 1) Crinone<sup>®</sup> 8% gel (90 mg); group 2) Prometrium<sup>®</sup> 200 mg capsules; group 3) placebo IVR; group 4) P IVR 4 mg/day; group 5) P IVR 8 mg/d, or group 6) P IVR 12 mg/d. All IVRs were inserted on Day 1 remained in place through Day 14; the rings were removed and a new ring inserted on Day 15. The second ring remained in place until Day 29. Blood samples were taken at scheduled times for pharmacokinetic (PK) analysis. Concentrations of P in plasma were measured using a validated LC/MS/MS method. Postmortem examinations performed on all IVR groups included vaginal irritation, macroscopic and microscopic evaluations, including irritation scoring and histopathology.

**RESULTS:** Following a relatively large amount of released P on Day 1, in vitro release rates confirmed that P was released at approximately 4, 8, or 12 mg/d over Days 2 - 14. IVRs were retained over 28 days in all animals with two exceptions. Clinical observations showed no significant abnormal findings in any group. PK analysis in animals showed sustained release of P from Days 0 through 14 of ring use. PK parameters from the three different IVRs were consistent with the in vitro release rates.  $C_{avg}$  increased in a dose-related manner, with mean values of 455, 682, and 1,040 pg/mL for the 4, 8 and 12 mg/day IVR groups, respectively. The lower dose Crinone gel (90 mg P) showed substantially greater relative bioavailability compared with the higher dose Prometrium capsules (200 mg P). Irritation scores and microscopic assessments were consistent with the IVRs being well tolerated following 28 days of exposure.

**CONCLUSIONS:** The data obtained from this study demonstrate that the segmented DARE-FRT1 EVA-based IVRs are capable of sustained release of P at different rates over a 14-day period. The rings were well tolerated with minimal to mild local irritation. These results suggest the rings are suitable for evaluation in a Phase 1 clinical study in women for PK and safety.

### IVF OUTCOME PREDICTORS - OOCYTES

**P-174** Tuesday, October 15, 2019 6:30 AM

### INCREASED NUMBER OF OOCYTES RETRIEVED IN FROZEN DONOR OOCYTE CYCLES DOES NOT HAVE A NEGATIVE IMPACT ON OUTCOMES.



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Group	# Oocytes Retrieved	Median # Oocytes	# Retrievals	# Warming Cycles	Oocyte Survival	Fertilization	Blastocyst Conversion	Clinical Pregnancy from Primary Transfer
1	<13	11	43	45	89.27%	81.27%	51.90%	50.00%
2	13-24	20	321	593	92.90%	79.26%	50.33%	51.24%
3	25-32	28	211	514	93.23%	79.16%	48.18%	52.16%
4	>32	39	193	569	93.91%	78.50%	49.96%	52.50%

**OBJECTIVE:** There is mounting evidence that outcome parameters from retrievals that yield high numbers of oocytes are not negatively impacted compared to cycles where fewer oocytes are retrieved. However, the perception persists that when many oocytes are retrieved, the oocytes are of poorer quality and lead to inferior embryo development and pregnancy rates. This research aims to determine if the number of oocytes retrieved in frozen donor egg cycles is correlated with IVF outcomes as measured by the following parameters: oocyte survival after vitrification, fertilization, blastocyst development and clinical pregnancy rate.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Oocytes were retrieved from anonymous egg donors at 12 IVF clinics for use in a commercial egg bank between 2016-2018. Mature eggs were vitrified using a commercially available vitrification media from Repro Life. Retrieved, mature oocytes were divided into cohorts of 6-8 oocytes for use by multiple recipients. Egg warming was performed using Repro Life warming media at 195 different recipients' clinics. All reported warmings from the retrieval period were included. Retrievals were sorted by total egg number retrieved and divided into 4 groups: <13, 13-24, 25-32 and >32 oocytes retrieved. Outcomes were evaluated t-test between percents. For pregnancy outcomes, only primary transfers were assessed.

**RESULTS:** A total of 714 retrievals and 1721 warmings were assessed. There was no statistically significant difference between groups 2-4 for oocyte survival, fertilization, blastocyst conversion or clinical pregnancy rate. There was a statistically significant difference between group 1 compared to groups 3 and 4 for oocyte survival, but this was not considered clinically meaningful.

**CONCLUSIONS:** Analysis of the data from this study supports the hypothesis that the number of oocytes retrieved from donors does not have a negative impact on embryo or cycle outcomes. Because blastocyst conversion rates and clinical pregnancy rates are similar between all groups, it stands to reason that the groups with larger numbers of oocytes retrieved will result in more quality embryos produced which in turn will lead to more pregnancies per retrieval. Very high oocyte yields should be avoided due to concerns about safety for oocyte donors, not outcomes for donor recipients.

**SUPPORT:** None.

(ICSI) but in this case each oocyte was injected by spermatozoon together with a previous phase of buffered medium mixed with ionophore calcium (Ica) aspirating it up to filling one third of the overall length of the pipette under 40X microscope magnification. Just after, they were kept for ten minutes with Ica into benchtop incubator and finally were incubated under the same culture conditions (37°C, 6% CO<sub>2</sub>, 5% O<sub>2</sub> atmosphere) Fertilization, pregnancy, implantation and abortion rates were analysed and compared in both groups by X<sup>2</sup>, t-Student and ANOVA tests. Both live birth and neonatal outcome was also reported.

**RESULTS:** After applying AOA, we observed a significant increase in the fertilization rate (51% vs 13.1%), ongoing pregnancy rate (47% vs. 21.7%) as well as implantation rate (31.1% vs 13.1%) and lower chances of cancellation (22.7% vs 69.3%). Additionally, no adverse obstetric and perinatal outcomes effects were found after the use of AOA compare to conventional ICSI.

**CONCLUSIONS:** Our findings support the use of AOA for a specific population of patients with previous very low fertilization or fertilization failure and in consequence, the outcome was impaired. After applying AOA, the reproductive success was significantly enhanced, mainly by the significant increase in the fertilization rate, the number of embryos available for selection, reducing dramatically the cancellation rate. The safety of AOA is reflected in the current practice after confirming non-detrimental effects in the offspring.

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#### EFFECT OF REGULATION OF OOCYTE CYTOPLASMIC VOLUME DURING FERTILIZATION ON CLINICAL OUTCOMES OF SINGLE-EMBRYO TRANSFER.

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**OBJECTIVE:** Using a time-lapse monitoring system, we observed that the oocyte cytoplasmic volume altered in the duration between sperm penetration and pronuclear (PN) fading. The present study aimed to determine the oocyte cytoplasmic volume at 5 morphokinetic events during fertilization and whether its regulation influences the clinical outcomes.

**DESIGN:** Retrospective, single-center, cohort study.

**MATERIALS AND METHODS:** A total of 311 patients (311 cycles; mean age: 35.1 ± 3.7 years) that underwent minimal-stimulation in vitro fertilization (mini-IVF) followed by freshly cleaved single-embryo transfer (SET) from August 2017 to September 2018 were retrospectively analyzed. Retrieved oocytes were inseminated by intracytoplasmic sperm injection (ICSI) after meiotic spindles were confirmed. Oocytes were cultured in a time-lapse incubator (EmbryoScope<sup>+</sup>, Vitrolife) after ICSI. The oocyte cytoplasmic volume corresponded to the oocyte major axis cross-sectional area (μm<sup>2</sup>). Measurements were recorded at 5 morphokinetic events: after ICSI (tICSI), time before 2<sup>nd</sup> polar body (PB) extrusion (tPB2b), time of 2<sup>nd</sup> PB extrusion (tPB2), time before PN fading (tPNfb), and time of PN fading (tPNf). The mean areas of oocytes at the morphokinetic events were compared (Study 1). In addition, the rates of change of oocyte cytoplasmic volume from tPB2b to tPNfb (area of tPNfb / tPB2b: group A), tPB2b to tPNf (area of tPNf / tPB2b: group B), and tPNfb to tPNf (area of tPNf / tPNfb: group C) were calculated. A multivariable logistic regression analysis was performed, which includes the significant confounding factors and yields adjusted odds ratios (aORs) and 95% confidence intervals (CIs), to evaluate the correlation between oocyte cytoplasmic volume change and clinical pregnancy (gestational sac observation) after SET (Study 2). *P*-values <0.05 were considered statistically significant.

**RESULTS:** Study 1: The mean area of oocytes at tICSI, tPB2b, tPB2, tPNfb, and tPNf were 11,452, 10,826, 10,587, 10,237, and 10,308 μm<sup>2</sup>,

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#### ARTIFICIAL OOCYTE ACTIVATION (AOA) TREATMENT OFFERS A NEW OPTION IN PREVIOUS UN-SUCCESSFUL CASES DUE TO POOR FERTILIZATION HISTORY; A PAIRED COHORT STUDY.

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**OBJECTIVE:** To improve of treatment outcome in terms of fertilization, implantation and pregnancy rates as well as cancelation rate after applying AOA in the following treatments for those patients with previous very low or failed fertilization attempts. Additionally were tested both delivery rate and obstetric outcomes in children born after AOA use.

**DESIGN:** Retrospective Cohort Study.

**MATERIALS AND METHODS:** Period included between April 2013 and December 2018. The total number of oocytes included in the study was 1125 distributed in two groups: the control group formed by 509 oocytes from 66 patients who were injected without AOA, acquiring either a failure or unusually low fertilization values (<30%) and the study group consisted of 616 oocytes from the same patients (66) in a second attempt but using AOA. Injection technique with AOA is comparable to conventional injection

respectively. The oocyte areas at tPNfb and tPNf were significantly smaller than those at tICSI, tPB2b, and tPB2 ( $P < 0.05$ ). Study 2: The multivariable logistic regression analysis showed that clinical pregnancy had significant associations with group A (area of tPNfb / tPB2b, aOR: 4.8, 95% CI: 1.07–23.08,  $p < 0.05$ ) and group B (area of tPNf / tPB2b, aOR: 7.3, 95% CI: 1.22–47.58,  $p < 0.05$ ), but not with group C (area of tPNf / tPNfb, aOR: 2.08, 95% CI: 0.30–14.1,  $p = 0.4549$ ).

**CONCLUSIONS:** A significant decrease in oocyte cytoplasmic volume was observed from sperm penetration to PN fading. In addition, there were significant associations between clinical pregnancy and the degree of cytoplasmic volume change from 2<sup>nd</sup> PB extrusion to PN fading. These results suggest that the regulation of oocyte cytoplasmic volume during fertilization would influence oocyte competence, which may predict successful pregnancy after SET.

**SUPPORT:** None.

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#### **ASSOCIATION BETWEEN THE NUMBER OF OOCYTES RETRIEVED, CANCELLATION RATES, AND CLINICAL OUTCOMES IN IVF PGT CYCLES WITH SINGLE EMBRYO TRANSFER (SET) - A 2273 CYCLES REVIEW.**

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**OBJECTIVE:** The number of chromosomal aneuploidies in preimplantation embryos progressively increases with advancing maternal age. The combined effect of diminished ovarian response and increased aneuploidy rates in the older patient population is manifested in an increased proportion of IVF PGT cycles where no euploid embryos are detected. The objective of this study was to assess the correlation between the number of oocytes retrieved, cancellation rates, and clinical outcomes in IVF PGT cycles.

**DESIGN:** A retrospective study of IVF PGT cycles was conducted to identify differences in cancellation rates (no biopsy or no euploid embryos) and clinical outcomes based on the number of mature (M2) oocytes retrieved.

**MATERIALS AND METHODS:** 2273 IVF PGT cycles (26677 M2 oocytes,  $11.6 \pm 7.85$  per cycle) between January 2013 and January 2019 were included in the study (1741 patients, average maternal age –  $36.9 \pm 4.9$ ). In 242 cycles (1775 M2 oocyte,  $7.3 \pm 5.7$  per cycle) no embryos that met criteria for biopsy were developed. In 487 cycles (4058 M2 oocytes,  $8.3 \pm 7.4$  per cycle) all biopsied embryos were aneuploid. In 1544 PGT cycles at least one euploid embryo was available (20844 M2 oocytes  $13.5 \pm 8.1$  per cycle), and 1601 SETs were performed. All embryos were vitrified after biopsy, and selected embryos were subsequently thawed for SET. Clinical pregnancy rate (PR) was defined by the presence of a fetal heartbeat at 6–7 weeks of pregnancy.

**RESULTS:** Analysis of the data had shown statistically significant difference in cancellation rates in the group of young patients ( $\leq 37$  y.o.) versus older patients ( $\geq 41$  y.o.) where only 1-5 eggs were retrieved (54.4 % vs 85.9 %, respectively,  $\chi^2=15.9$ , OR = 0.21, CI = 0.09 – 0.47,  $p < 0.05$ ). The difference in cancellation rates between young and older patients in the cycles where 6-10 eggs were retrieved was 29.2 % vs 62.1 %, respectively,  $\chi^2=50.5$ , OR = 0.22, CI = 0.15 – 0.34,  $p < 0.05$ ). The biggest statistically significant difference in cancellation rates between young and older patients was found in cycles where  $>10$  eggs were retrieved (8.7% vs 51.9 %, respectively,  $\chi^2=22.72$ , OR = 0.09, CI = 0.06 – 0.13,  $p < 0.05$ ). At the same time, ongoing PR after SET was not statistically different between different age groups. Moreover, clinical PR was not statistically different between PGT cycles where 1-5 and over 10 eggs were retrieved (70.7 % (41/58) and 67.6 % (870/1288), respectively,  $\chi^2=0.25$ , OR = 1.16, CI = 0.65 – 2.06,  $p = 0.62$ ). Live birth rate (LBR) after SET in PGT cycles where 1-5 oocytes were retrieved was 66.7 % (26/39), in cycles where 6-10 oocytes were retrieved LBR was 65.1% (71/109), and in cycles where 6-10 oocytes were retrieved LBR was 61.7 % (577/936),  $\chi^2=0.4$ , OR = 1.24, CI = 0.63 – 2.45,  $p = 0.53$ .

**CONCLUSIONS:** Analysis of the data proved that the ongoing PR and LBR in PGT cycles after SET are independent of the number of eggs retrieved and maternal age. At the same time diminished ovarian response and high aneuploidy rates in the older patient population significantly increase the risk of cycle cancellation. Cycle cancellation rates should be taken

into consideration for proper patient consultation and choosing a future treatment strategy.

Reference: N/A.

SUPPORT: N/A.

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#### **IVF OUTCOME IN WOMEN WITH ENTIRE COHORT OF OOCYTES WITH COARSE GRANULATION IN PERIVITELLINE SPACE.**

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**OBJECTIVE:** The purpose of this study aims to compare embryo quality, clinical pregnancy rate (CPR) and implantation rate (IR) between the patients having all oocytes with single abnormality i.e coarse granulation in PVS (study N=52) against patients with all oocytes having normal morphology (control N=49).

**DESIGN:** This is a retrospective case control study conducted during the period from June 2015 - February 2018 at CRAFT Hospital and Research centre, Kerala, India.

**MATERIALS AND METHODS:** The study protocol has been approved by the Institutional review board (IRB).

The inclusion criteria: maternal age  $<40$  yrs and male partners with normal semen parameters were selected. Exclusion criteria: Patient with history of recurrent implantation failure, chromosomal abnormalities or uterine abnormalities and those having oocytes with coarse granularity in pvs with other morphological abnormalities were excluded to avoid the bias factor.

Controlled ovarian stimulation was done with flexible antagonist protocol with individualised dose of gonadotrophin started from day 2. On the day of ICSI the oocytes were denuded and checked for their morphology based on ISTANBUL consensus 2011 such as intracytoplasmic and extracytoplasmic dysmorphisms. Patients with entire cohort of oocytes having coarse granulation in PVS with no other oocyte abnormalities were taken into the study.

After ICSI oocytes were cultured in 6%CO<sub>2</sub> and 5%O<sub>2</sub>. On Day3 embryo morphology was assessed based on the Istanbul consensus as Grade1,2 and 3.

Grade1 embryos were vitrified on day 3 followed by endometrial preparation and frozen embryo transfer.

The primary outcome was to compare CPR. Secondary outcome was to assess day 3 grade1 embryos, IR between the two groups.

Sample Size Calculation and Statistical Analysis:

The IBM SPSS statistics version 21 was used for statistical calculations. Sample size based on the result of Clinical pregnancy rate among case (10.7%) and control (32.3%) group occurred in an earlier publication (1) and with 80% power and 95% confidence, the minimum sample size comes to 80(40 in each group).

Statistical analysis: Chi-square test was used to compare Fertilisation rate (FR), CPR, IR between study and control group.

**RESULTS:** The baseline characteristics did not differ significantly between two groups. A total of 1240 oocytes retrieved 577 oocytes belonged to study and 663 oocytes in control group. When compared between two groups, FR (67.38% vs 82.53%), CPR (18% vs 54.16%), IR (10.58% vs 33.66%), Live birth rate (14% vs 48%) showed statistically significant difference ( $p$  value  $< 0.05$ ). Though Day 3 grade1 embryo quality was not statistically significant (33% vs 40.75%) control group showed better embryo quality. Limitation of this study is that sample size is small and that it was powered to detect CPR and not live birth rate.

**CONCLUSIONS:** Coarse granulation in PVS shows low fertilisation, clinical pregnancy and implantation rate. Study intends to show that coarse granulation in perivitelline space may predict poor ART outcome and patients can be counselled regarding the same.

References: 1. Farhi J, Nahum H, Weissman A, Zahalka N, Glezerman M, Levran D. Coarse granulation in the perivitelline space and IVF-ICSI outcome. J Assist Reprod Genet 2002;19:545–549.

2. Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Reprod Biomed Online. 2011 Jun;22(6):632–46. <https://doi.org/10.1016/j.rbmo.2011.02.001>. Epub 2011 Apr 11.

SUPPORT: None.

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**THE CAUSAL EFFECT OF DYNAMIC FERTILITY TREATMENT STRATEGIES ON THE PROBABILITY OF PREGNANCY: A NOVEL APPLICATION OF MARGINAL STRUCTURAL MODELS (MSMs).**



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**OBJECTIVE:** To estimate the probability of clinical pregnancy, had all patients or subgroups of patients (based on age) followed 1 specific treatment strategy: eg, 4 cycles of ovulation induction (OI, with/without intrauterine insemination) using clomiphene or letrozole (OI Oral), 3 cycles of OI + Gonadotropin (OI Gn), or 1 cycle of assisted reproductive technology (ART) with fresh or cryopreserved embryo transfer.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** Electronic medical record data from 84,301 US patients who underwent multiple treatments (219,925 cycles) in 2009-2016 were examined. Female patients included were initially treatment-naïve; patients with an initial diagnosis of male infertility were excluded. Inverse Probability (IP) of Censoring Weighting adjusted for stopping treatment before getting pregnant and IP of Treatment Weighting adjusted for patient characteristics (eg, diminished ovarian reserve) that made them more likely to choose a treatment strategy (eg, 1 ART over 4 OI Gn cycles).

**RESULTS:** In either age group, patients needed ≥ 4 OI Gn cycles, or to switch to 1 OI Gn cycle after 3 failed OI Oral cycles to have a similar chance of pregnancy as 1 ART cycle. Chance of pregnancy was below 50% for 4-cycle treatment strategies with <1 ART cycle.

**CONCLUSIONS:** Patients ≥ 35 y may be better served by starting with or switching to ART sooner rather than repeating multiple cycles of OI. For patients <35 y, the estimated chance of pregnancy with 4 OI Oral cycles is close to 1 ART cycle. Evaluation of real-world data using MSMs to account for patient dropout and changes in patient characteristics and prognosis over time can provide important insights into treatment-decision trends and evidence for improving personalized medicine.

Reference: Robins JM, et al. *Epidemiology* 2000;11:550-60.

**SUPPORT:** Study sponsored by EMD Serono, Inc. (a business of Merck KGaA, Darmstadt, Germany), Rockland, MA, USA.

**ECTOPIC/HETEROTOPIC PREGNANCY OUTCOMES AFTER BLASTOCYST-STAGE FROZEN-THAWED EMBRYO TRANSFERS COMPARED WITH CLEAVAGE STAGE: A SART-CORS STUDY.**



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**OBJECTIVE:** It is well established that fresh embryo transfers at the blastocyst stage result in improved pregnancy outcomes compared with cleavage-stage embryo transfers. Using the Society for Assisted Reproductive Technologies Clinical Outcomes Reporting System (SART CORS) dataset, we have recently shown that blastocyst-stage frozen embryo transfer (FET) is also associated with higher live-birth rates compared with cleavage-stage FETs. However, other outcomes such as ectopic and heterotopic pregnancy rates between cleavage-stage and blastocyst-stage FETs have not been well studied. The objective of this study was to investigate whether there is a difference in ectopic/heterotopic pregnancy rates in blastocyst-stage FETs compared with cleavage-stage FETs.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All in vitro fertilization (IVF) cycles reported to the Society for Assisted Reproductive Technology from 2004 to 2013 were evaluated. Patients included were those with recorded treatment and pregnancy outcomes undergoing FETs at either the blastocyst-stage (n=118,616) or the cleavage-stage (n=137,671). Main outcome measures were pregnancy outcomes, specifically ectopic pregnancy rates and heterotopic pregnancy rates. Demographic criteria from each cycle was also collected. Statistical analysis was performed using SAS and Microsoft Excel. Chi-square analysis for bivariate associations and generalized estimating equations for adjusted associations were used with p<0.05 considered as statistically significant.

**RESULTS:** There was a statistically significant increase in pregnancy rates with blastocyst-stage FETs compared with cleavage-stage FETs (60.6% vs. 41.0%; p<0.001). Among those who became pregnant, there was a significantly lower incidence of ectopic/heterotopic pregnancy rates in blastocyst-stage FETs vs. cleavage-stage FETs (0.8% vs. 1.1%; p<0.001). Differences in ectopic/heterotopic pregnancy rates remained statistically significant after controlling for confounders such as tubal factor infertility and number of embryos transferred.

**CONCLUSIONS:** Blastocyst-stage frozen embryo transfer is associated with lower ectopic/heterotopic pregnancy rates compared with cleavage-stage frozen embryo transfer.

Estimated Cumulative Clinical Pregnancy Rate, % (95% Confidence Interval)

Initial Treatment	Cycles, n	Repeat Same Treatment		Switch to OI Gn		Switch to ART			
		Age Group		Age Group		Age Group			
		<35 y	≥35 y	<35 y	≥35 y	<35 y	≥35 y		
OI Oral	1	12.6 (12.1, 13.0)	7.8 (7.3, 8.4)	1 then 1 OI Gn	24.8 (23.5, 26.1)	16.1 (14.5, 17.7)	1 then 1 ART	58.1 (56.4, 59.8)	39.1 (36.5, 41.8)
	2	22.4 (21.8, 23.1)	14.8 (14.0, 15.6)	2 then 1 OI Gn	33.3 (32.1, 34.5)	23.6 (21.9, 25.4)	2 then 1 ART	63.7 (62.4, 65.0)	45.2 (42.9, 47.4)
	3	30.7 (29.9, 31.5)	19.6 (18.5, 20.7)	3 then 1 OI Gn	42.1 (40.9, 43.4)	28.5 (26.6, 30.4)			
	4	38.1 (37.1, 39.2)	25.0 (23.5, 25.4)						
OI Gn	1	16.5 (15.7, 17.3)	11.1 (10.5, 11.7)				1 then 1 ART	62.0 (59.9, 64.0)	38.3 (36.2, 40.4)
	2	29.8 (28.8, 30.9)	19.8 (18.9, 20.7)	2 then 1 ART	68.3 (66.7, 70.0)	49.4 (47.5, 51.4)			
	3	36.6 (35.3, 37.9)	26.5 (25.3, 27.8)						
	4	48.2 (46.7, 49.7)	33.0 (31.5, 34.6)						
ART	1	45.8 (44.8, 46.8)	29.7 (29.0, 30.5)						
	2	64.1 (63.1, 65.1)	43.7 (42.8, 44.7)						
	3	77.6 (76.7, 78.6)	57.4 (56.3, 58.5)						

**LEVERAGING A COMPOSITE OVARIAN RESERVE SCORE IN A MACHINE LEARNING MODEL OF LIVE BIRTH OUTCOMES IN IVF.**

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**OBJECTIVE:** As the number of predictive measures for IVF prognosis proliferates, there is a growing need for machine-driven tools to aid patients and their healthcare providers in navigating the complex landscape of decision making. In previous work, we identified that multiple indirect measures of ovarian reserve (baseline FSH, LH, E2, BAFC, AMH) can be combined to measure a latent variable representing a patient's overall ovarian reserve. Here, we aimed to develop a predictive model that utilizes a composite ovarian reserve score to predict cumulative live birth (LB) outcomes and risk of multiples based on the number of transferred embryos.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** 48,357 cycles were included from 34,734 patients age 25-45 undergoing autologous IVF treatment between 2010-2017 at 13 fertility centers in the US. We excluded cycles using pre-implantation genetic testing and transfer of 3 or more embryos. The dataset was divided into 2/3 for training (36,401 cycles), with the remaining 1/3 set aside as an independent validation set. We used a Bayesian approach because, in contrast to traditional regression models, this allowed us to incorporate the unobserved, but clinically relevant, composite variable of ovarian reserve as a feature of the model for cumulative LB rate and risk of multiples. Accordingly, the number of LBs per initiated cycle was modeled using a zero-inflated binomial distribution to account for patient cancellation prior to transfer, and ovarian reserve was represented in the model as a latent variable dependent on baseline levels of FSH, LH, AMH, and BAFC. To compensate for nonrandom patient dropout across multiple cycles, inverse probability of censoring weighting (IPCW) was used to more accurately predict later cycles. Considered variables in the larger model included patient age, ovarian reserve, BMI, diagnosis, number of transferred embryos, and partner semen analysis.

**RESULTS:** When our model was tested with an independent validation set, we found that it had an AUC of 0.73 for prediction of a LB and an AUC of 0.82 for prediction of multiples. The most important features of the model included patient age, number of transferred embryos, number of previous failed cycles, and BMI.

**CONCLUSIONS:** For machine-driven tools to truly augment traditionally expert-driven decisions, it is important for the underlying models to grow in sophistication with the growing list and varying importance of prognostic measures. The use of Bayesian models to better determine likely number of LBs can bring better transparency and consistency to patient care and counseling during IVF treatment.

**SUPPORT:** Celmatix.

**A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTI-CENTER, PHASE 2 TRIAL TO INVESTIGATE THE EFFECT OF BARUSIBAN ON IMPLANTATION IN IVF/ICSI PATIENTS.**

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**OBJECTIVE:** To evaluate the effect of the selective oxytocin antagonist barusiban, administered on the day of transfer, on ongoing implantation rate in IVF/ICSI patients.

**DESIGN:** Randomized, double-blind, placebo-controlled, phase 2 trial (BASIC) in 255 IVF/ICSI patients, 18-37 years, with a history of repeated implantation failures. Uterine pathology and thrombophilia disease were excluded. Patients had undergone controlled ovarian stimulation, hCG triggering, oocyte retrieval, and luteal phase progesterone supplementation.

**MATERIALS AND METHODS:** Women were randomized 1:1 on the day of transfer to barusiban (40 mg 45min pre-transfer + 10 mg 15min post-transfer) (n=130) or placebo (n=125), administered subcutaneously. Randomization was stratified by day of transfer (day 3 or 5) and number of embryos/blastocysts transferred (1 or 2). In total, 440 good-quality embryos/blastocysts were transferred (barusiban: 225; placebo: 215). Ongoing implantation (primary endpoint) and pregnancy were assessed 10-11 weeks after transfer. To adjust for imbalances in baseline characteristics between groups, the effect of barusiban was tested using a logistic regression model with treatment,

embryo/blastocyst quality, reason for infertility, and center as factors. There were more transfers of excellent-quality blastocysts in the placebo group than in the barusiban group (51% vs 29%) as well as more couples in the placebo group with male factor infertility (51% vs 35%), supporting the value of the adjusted analyses. Both unadjusted and adjusted analyses were performed, and the latter are presented.

**RESULTS:** There was no significant difference in overall ongoing implantation rate between barusiban and placebo with rates of 27.9% and 23.1%, respectively [odds ratio 1.11 (95% CI: 0.69; 1.78), p=0.663]. However, the day of transfer had a significant interaction on the primary endpoint. A significantly higher ongoing implantation rate was observed for barusiban over placebo for day 5 transfers, with 41.3% for barusiban versus 23.2% for placebo [odds ratio 2.34 (95% CI: 1.13; 4.84), p=0.022], but not for day 3 transfers (11.8% versus 17.6% [odds ratio 0.63 (95% CI: 0.30; 1.34), p=0.227]).

The overall ongoing pregnancy rates were 34.1% for barusiban and 35.1% for placebo, with 49.7% for barusiban and 33.4% for placebo for day 5 transfers, and 19.2% and 29.9% for day 3 transfers, respectively.

More mild/moderate injection site reactions were observed with barusiban than with placebo, but there was no difference in severe reactions. No serious drug reactions were reported, and neonatal outcome was comparable between groups.

**CONCLUSIONS:** The present trial was unable to demonstrate the efficacy of barusiban in patients with a history of repeated implantation failures, but it revealed a time window for a clinically relevant effect of barusiban. Barusiban increased the implantation of blastocysts when administered closer to the time of the actual implantation, but not when administered in the early luteal phase during transfer of cleavage-stage embryos. Subcutaneous administration of 50 mg barusiban was well-tolerated.

**SUPPORT:** Ferring Pharmaceuticals.

**INCREASED OCCURRENCE OF TWIN AND VERY PRE-TERM BIRTHS IN PATIENTS UNDERGOING IN VITRO FERTILIZATION (IVF) USING FROZEN DONOR OOCYTES.**

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**OBJECTIVE:** To compare pregnancy outcomes in patients undergoing IVF with elective single embryo transfer (eSET) using fresh vs frozen donor oocytes.

**DESIGN:** Retrospective cohort Society for Assisted Reproductive Technology (SART) data study.

**MATERIALS AND METHODS:** A retrospective cohort study was conducted using the publicly available data in the SART National Summary Report from 2014 to 2016. Cycle inclusion criteria were as follows: eSET, fresh donor oocytes, and frozen donor oocytes. Exclusion was use of gestational carrier. Pregnancy outcomes included live births (divided into singleton, twins, or triplets or more) and gestational age at delivery (divided into term, pre-term, and very pre-term). Term was defined as occurring after 37 weeks, pre-term as between 32 and 37 weeks, and very pre-term as before 32 weeks gestation.  $\chi^2$  test was used to compare variables between groups. A *P* value <0.05 was considered statistically significant.

**RESULTS:** A total of 8997 elective single embryo transfers were analyzed, including 6113 transfers using fresh donor oocytes and 2884 using frozen oocytes. Live birth rate in the frozen oocyte group was significantly lower compared with that in the fresh oocyte group (46.1% vs 53.4%, *P* < 0.0001, Table 1). Twin birth rate was significantly higher when using frozen oocytes compared to fresh oocytes (3.0% vs 1.1%, *P* < 0.0001). Total pre-term births (pre-term plus very pre-term) were increased in cycles using frozen oocytes,

TABLE 1. Pregnancy outcome comparison in patients using fresh vs frozen donor oocytes

	Fresh donor oocytes	Frozen donor oocytes	<i>P</i> values
Number of eSETs	6113	2884	
Live birth rate per transfer	53.4%	46.1%	< 0.0001
Singletons	98.9%	97%	
Twins	1.1%	3.0%	< 0.0001
Term delivery	84.6%	82.6%	= 0.08
Pre-term delivery	13.5%	13.7%	> 0.05
Very pre-term delivery	1.9%	3.7%	= 0.0003

but not statistically different (17.4% vs 15.4 %,  $P = 0.08$ ). There was no significant difference in percentage of pre-term births between frozen and fresh oocytes (13.7% vs 13.5 %,  $P > 0.05$ ). However, frozen donor oocytes were associated with significantly increased percentage of very pre-term births compared to fresh donor oocytes (3.7% vs 1.9%,  $P = 0.0003$ ).

**CONCLUSIONS:** The use of frozen donor oocytes in IVF treatment has become increasingly commonplace, but knowledge of related pregnancy outcome risks is currently sparse. This study using the largest SART data available so far shows that IVF patients using frozen donor oocytes had a lower live birth rate and have a significantly increased twin birth rate even with eSET when compared to fresh donor oocytes, which is likely a strong contributing factor in the significantly increased occurrence of very pre-term births and should be taken into account when counseling patients about to undergo IVF treatment.

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**WHAT INFLUENCES IMPLANTATION OF EUPLOID EMBRYOS AFTER SINGLE THAWED EUPLOID EMBRYO TRANSFER (STEET): IS IT THE STIMULATION FOR RETRIEVAL, THE UTERINE PREPARATION FOR FET, THE EMBRYO TRANSFER OR THE EMBRYO?** David H. McCulloh, Ph.D.,<sup>a</sup> Caroline McCaffrey, Ph.D.,<sup>b</sup> James A. Grifo, MD, Ph.D.,<sup>c</sup> <sup>a</sup>NYU Langone Health, New York, NY; <sup>b</sup>New York Langone Health, NYU Fertility Center, New York, NY; <sup>c</sup>NYU Langone Prelude Fertility Center, New York, NY.



**OBJECTIVE:** The use of PGT-A and vitrification to select euploid embryos for transfer has led to improved live birth success in IVF; however, some euploid embryos fail to implant. Our objective was to compare parameters from 1) the retrieval cycle (IVF) in which blastocysts were biopsied and vitrified, 2) the frozen embryo cycle (FET<sub>u</sub>) during which the uterus was prepared for transfer, 3) the embryo transfer (FET<sub>t</sub>), and 4) the embryology (Lab) records all consolidated to determine what best predicts implantation following single thawed euploid embryo transfer (STEET).

**DESIGN:** Multivariate analysis of 45 parameters from IVF, FET<sub>u</sub>, FET<sub>t</sub>, and Lab and their association with a positive pregnancy test (+hCG).

**MATERIALS AND METHODS:** Data were collected from our electronic records for patients with transfers of thawed single euploid embryos diagnosed as euploid by Next Generation Sequencing during the IVF cycle. Parameters from IVF (17), FET<sub>u</sub> (5), FET<sub>t</sub> (4), and Lab (19) were considered. All 908 cases of STEET using NGS prior to 2018 with the required fields were examined. There were 704 STEETs (77.5%) with +hCG. All +hCGs were considered implantations since 1) patients believe they are pregnant when they have a +hCG result and 2) no interfering hCG was administered to these patients. Stepwise multiple logistic regression (197 combinations of parameters) was performed using the Akaike Information Criterion (AIC) to select significant parameters.

**RESULTS:** Parameters associated with better implantation (in descending magnitude of standard partial regression coefficient) were: Thicker Endometrium (FET<sub>u</sub>); higher endogenous serum Estradiol at Start of the IVF Cycle (IVF); higher Progesterone on day of embryo transfer (FET<sub>u</sub>); more embryos vitrified (IVF); the re-expansion of the blastocyst after warming (Lab); and less expansion of blastocysts prior to biopsy (Lab). Age at retrieval, embryo grades, as well as many other parameters were not associated with implantation.

**CONCLUSIONS:** Parameters from 3 of the 4 treatment categories were associated with establishment of +hCG. Of these, some may be under our control: Thicker endometrium, higher Progesterone levels on day of embryo transfer, and less expansion of the blastocyst prior to biopsy. However, the possibility remains that these parameters may be aliases for other features such as rate of uterine proliferation, rate of blastocyst development, patient weight. FET<sub>t</sub> (embryo transfer) was the category with no parameter associated with implantation. Also notable was the lack of association between embryo grades and +hCG.

**SUPPORT:** None.

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**EFFECT OF VARIATION IN LABORATORY AND CLINICAL PRACTICES DURING THE LAST DECADE ON IVF OUTCOME.** Maria Teresita W. Lao, MSc,<sup>a</sup> Rajput Isha, M.Med Sci,<sup>b</sup> Essam S. N. Michael, M.D.,<sup>a</sup> Alex C. Varghese, Ph.D.,<sup>a</sup> Kannamannadiar Jayaprakasan, Ph.D.<sup>b</sup> <sup>a</sup>Astra Fertility Clinic, Mississauga, ON, Canada; <sup>b</sup>Department of Obstetrics and Gynaecology, Royal Derby Hospital and University of Nottingham, United Kingdom.



**OBJECTIVE:** To evaluate the effect of changes in laboratory and clinical practices on IVF outcome and to estimate trend of pregnancy rates over a period of 12 years.

**DESIGN:** A review of prospectively collected data.

**MATERIALS AND METHODS:** Review of prospectively collected data at a tertiary fertility centre. Regression analysis used to study trend in the percentage of pregnancy outcome over a period of 12 years (January 2006 to December 2018).

**RESULTS:** Total number of IVF/ICSI cycles (fresh and frozen) during 12-year period (2006-2018) was 6401. Women's age ranged from 19-51 years (mean +/-SD, 35.58 +/- 4.64). Overall clinical pregnancy rate (CPR) during this period was 34.3% (n = 2198). CPR in fresh cycles was 36.7% (1397/3806) which was similar to CPR in frozen cycles 36.5% (1092/2984) ( $p = 0.94$ ). While there was no trend ( $R^2 = 0.76$ ) observed in CPR for fresh embryo transfer (ET) over 12 years, a significant increasing trend ( $R^2 = 0.89$ ) was noted in CPR with frozen ET ( $p < 0.05$ ). In fresh ET cycles, a significant increase in CPR was noted in the year 2012 when tri-gas incubators were introduced with a CPR of 35.2% (599/1697) versus 50.6% (83/164) ( $p < 0.05$ ). On comparing the two time periods before (Jan 2006 to Dec 2011) and after (Jan 2012- Dec 2018) the introduction of tri gas incubators, the CPR showed an increasing trend albeit not significant, 35.2% (599/1697) versus 36.8% (613/1665) ( $p = 0.37$ ). In frozen ET cycles, a significant increase in CPR was noted after a switch over from slow freezing to vitrification method in 2008 ( $p < 0.001$ ). The CPRs were 18% (68/377) during slow freezing (2006-2008), 35.1% (248/706) during transition period (2009-2012) and 40.2% (671/1666) during complete switch to vitrification method (2013-2018). The overall embryo survival rate post thaw was 94.5% (3578/3786). Blastocyst (day 5 -7) transfer showed significantly favourable outcome CPR of 40.9% (1461/3568) when compared to cleavage stage embryo (day 2-4) ET with CPR of 37.08% (574/1548), in both fresh and frozen cycles ( $p < 0.005$ ). Embryo culture in sequential media in bench top incubators versus single step in time-lapse incubators showed a similar CPR 36.2% (235/649) versus 38.6% (249/645) ( $p = 0.71$ ), although there was an increasing trend.

**CONCLUSIONS:** CPR in frozen embryo transfer cycles showed an increasing trend, mainly due to successful introduction of vitrification method of cryopreservation. Use of tri-gas incubators for embryo culture and blastocyst transfer positively affected CPR. However, introduction of embryo culture in time-lapse incubators did not show any significant change, albeit an increasing trend, in outcome.

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**CONCENTRATIONS OF STROMAL CELL-DERIVED FACTOR-1 IN HUMAN FOLLICULAR FLUIDS ARE ASSOCIATED WITH CLINICAL IN VITRO FERTILIZATION OUTCOMES.** Hidetaka Okada, MD, Akemi Nishigaki, PhD Kansai Medical University, Hirakata, Japan.



**OBJECTIVE:** To investigate the association between individual concentrations of stromal cell-derived factor-1 (SDF-1) and vascular endothelial growth factor (VEGF) and sex steroid hormones in human ovarian follicles and IVF outcomes. SDF-1 and VEGF are angiogenic factors that have possible roles in ovarian function.

**DESIGN:** Prospective study.

**MATERIALS AND METHODS:** A total of 31 ICSI patients considered for blastocyst (BL) culture until day 5 were included in this study. Follicular fluid (FF) samples were obtained at the time of oocyte retrieval following ovarian stimulation from 38 year or less women with normal body mass index. Concentrations of SDF-1 and VEGF in 261 FF samples were measured with enzyme-linked immunosorbent assay. Concentrations of progesterone (P<sub>4</sub>) and estradiol (E<sub>2</sub>) were measured with a commercially available fluorescence immunoassay. IVF outcome parameters were included in fertilization rate, cleavage rate, embryo morphology on day 3, and blastocyst morphology on day 5. We calculated the number of BL forming 3 or more per total number of cleavage embryo as the rate of full BL and the number of BL forming 3BB or more as the rate of good-quality BL. Differences in the measured parameters across the different groups were statistically assessed using ANOVA followed by Dunnett's test and a level of  $P < 0.05$  was considered statistically significant.

**RESULTS:** The FF concentrations of SDF-1 and VEGF were positively correlated with P<sub>4</sub> concentrations in FF ( $r = 0.51$ ,  $P < 0.01$  and  $r = 0.71$ ,  $P < 0.01$ , respectively), but not correlated with E<sub>2</sub> concentrations in FF. Of the 261 oocytes at the MII stage, 200 were successfully fertilized; all these 200 oocytes had 2 pronuclei (PN) and developed into growing embryos. Of the 61 residual oocytes, 18 had 3 or 1 PN, 17 failed to fertilize, and 26 degenerated after ICSI. A possible relation between the concentrations of these

factor and IVF outcomes was evaluated by dividing SDF-1 (<125, 125-200, 200-275, 275-350, and  $\geq 350$  pg/ml) and VEGF (<180, 180-270, 270-360, 360-450, and  $\geq 450$  pg/ml) concentrations into five intervals creating five approximately similar sized groups. The follicular concentration of SDF-1 and VEGF were not significantly associated with fertilization and cleavage outcome, and embryo morphology. The rates of full blastocysts and good-quality blastocysts were significantly higher in follicles with an SDF-1 concentration of 275–350 pg/ml than in the follicles with SDF-1 concentrations of <200 pg/ml and  $\geq 350$  pg/ml ( $P < 0.05$ ). The follicular concentration of VEGF was not associated with the blastocyst morphology.

**CONCLUSIONS:** Our findings suggest that SDF-1 plays important modulatory roles in early luteinization and its follicular concentration may be a valuable biochemical marker of blastocyst development.

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#### **PREDICTIVE FACTORS FOR OOCYTE RETRIEVAL FAILURE IN TREATMENT CYCLES WITH ASSISTED REPRODUCTIVE TECHNOLOGY: A RETROSPECTIVE COHORT STUDY USING THE NATION-WIDE ART REGISTRY OF JAPAN.**



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**OBJECTIVE:** The purpose of this study was to evaluate the prognostic factors for oocyte retrieval failure in patients undergoing assisted reproductive technology (ART) treatment cycles.

**DESIGN:** A retrospective cohort study.

**MATERIALS AND METHODS:** The present study was approved by the Ethical Committee on human subjects. The data analyzed in this study were part of the Japanese ART registry database, which was collected by the Japan Society of Obstetrics and Gynecology from 2010 to 2012. We analyzed the data of 464,480 fresh cycles with transvaginal oocyte aspiration. The cycles with oocyte retrieval failure and those with one or more oocytes retrieved were compared to determine predictive factors for oocyte retrieval failure using multivariate logistic regression analyses.

**RESULTS:** The number of cycles with oocyte retrieval failure was 36,600 (7.9%). According to the multivariate analysis, age, cause of infertility, and controlled ovarian hyperstimulation (COH) were the independent prognostic factors for oocyte retrieval failure. The percentages of oocyte retrieval failure in the age groups of 29 years old and under, 30-34 years old, 35-39 years old, 40-44 years old, and over 45 years old were 3.2%, 4.2%, 5.9%, 10.2%, and 18.6%, respectively. The odds ratios of patients in their 30's and 40's were 1.7 and 4.0 times those of the patients in their 20's, respectively. The percentages of oocyte retrieval failure that corresponded to infertility caused by a male factor, tubal factor, endometriosis, and unknown factors were 5.6%, 7.3%, 8.0%, and 9.2%, respectively. The odds ratios for oocyte retrieval failure in the tubal factor, endometriosis, and unknown groups were 1.3, 1.4, and 1.7 times those in the male factor group, respectively. The percentages of oocyte retrieval failure in the COH cases using aromatase inhibitor or clomiphene (AI+CC), GnRH agonist, and GnRH antagonist were 10%, 1.5%, and 3.5%, respectively. The odds ratios for oocyte retrieval failure in the GnRH antagonist and AI+CC protocols were 2.4 and 5.3 times those for the GnRH agonist protocol, respectively. The percentage of oocyte retrieval failure in the natural cycles was 21%.

**CONCLUSIONS:** The predictive factors for oocyte retrieval failure might be related to a patient's age, particular causes of infertility, and COH protocols. These results provide information that may be useful for counseling patients before ART treatments.

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#### **PREDICTING CLINICAL PREGNANCY BY MACHINE LEARNING ALGORITHM USING NONINVASIVE EMBRYO MORPHOKINETICS AT AN ACADEMIC CENTER.**



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**OBJECTIVE:** To develop and examine the feasibility of a time-lapse microscopy (TLM)-based assay to predict pregnancy outcomes in patient IVF cycles.

**DESIGN:** Retrospective and prospective cohort analyses of clinical pregnancy data with embryo TLM data that were transferred at an academic fertility center.

**MATERIALS AND METHODS:** Embryos that underwent EmbryoScope™ TLM and subsequent transfer with clinical pregnancy (defined by fetal heart beat after 6 weeks) or no clinical pregnancy were included from 2014 to 2018. Machine classifiers used were morphokinetic parameters from fertilization to blastocyst formation that were annotated manually. Data were analyzed by multivariate analysis of covariance, Fisher's exact, Chi-square tests, and binomial logistic regression using R and Scikit learn, and Python software.

**RESULTS:** Learning curves were applied to a training set of 180 embryos and a validation set of 80 embryos. Supervised algorithms tested included naïve bayes classifiers, support vector machines, logistic regression, and decision trees. Highest accuracy scores were achieved with logistic regression and decision tree models. Accuracy scores of 0.6 for the two sets converged for the logistic regression model at 100 training set numbers whereas Decision Tree algorithm reached a 0.7 accuracy score converging at 180 training set numbers. Furthermore, the logistic regression prediction model (Chi square=26.3,  $p=0.010$ ), termed the Yang-Peavey Embryo Enhancement Algorithm, correctly predicted 70% of clinical pregnancies for patients under age 35. A receiver operating characteristic curve was developed and found to be significant with an area under the curve value of 0.757 (95% CI 0.667-0.848,  $p<0.001$ ). In a separate prospective cohort study, the algorithm was applied to 140 embryos that were transferred, which were manually annotated and blinded from the pregnancy outcome. The YPEEA algorithm yield 68% sensitivity and 53% positive predictive value (PPV) when applied to the full dataset.

**CONCLUSIONS:** This study demonstrates that novel Machine Learning algorithms can be used for embryo selection based on as few as 200 embryos, and be applicable to a prospective cohort of embryos. The predictive value of the algorithm is comparable or even superior in a subset of patients to that of preimplantation genetics screening and therefore is a valuable non-invasive technology to predict clinical pregnancy in IVF.

**SUPPORT:** Baylor College of Medicine Department of Obstetrics and Gynecology, the Division of Reproductive Endocrinology and Infertility, the 2016 Robert and Janice McNair Medical Scientist Training Program Scholarship.

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#### **FROZEN-THAWED EMBRYO REPLACEMENT (FER): A UK-WIDE SURVEY OF IVF CLINICS DEMONSTRATING WIDE VARIATION IN PRACTICE AND A COMPARISON OF GnRH-ANTAGONIST WITH GnRH-AGONIST PITUITARY SUPPRESSION IN MEDICATED FER.**



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Recent years have seen a dramatic rise in the number of frozen-thawed embryo replacement (FER) cycles. Between 2012 and 2016, the annual number of FETs in the UK increased by 77%, while the number of fresh cycles declined by 2%. Despite FER accounting for 30% of UK IVF workload, the optimum method of endometrial preparation for FER is unknown.

**OBJECTIVE:** This study assesses current UK trends in endometrial preparation for FER and compares the outcomes of women undergoing medicated FER with GnRH-agonist and GnRH-antagonist pituitary suppression.

**DESIGN:** The first national UK survey of practice on endometrial preparation for FER and a retrospective cohort study comparing GnRH-antagonist with GnRH-agonist pituitary suppression in FER.

**MATERIALS AND METHODS:** All 84 UK IVF clinics were asked to complete an online survey between September 2018 and January 2019.

**RESULTS:** Sixty-five clinics (77%) responded, together undertaking approximately 24,419 FERs annually. The preferred developmental stage of cryopreservation is blastocyst, favoured by 98% of clinics. In the UK 77% of FERs are medicated, 18% natural cycle, 5% modified natural cycle and <1% ovulation induction. In ovulatory women 69% of clinics favour medicated, 26% natural cycle and 5% modified natural cycle FER.

In natural cycle FER, 31% always, 44% sometimes and 25% never prescribe luteal support. Fifty-one percent of clinics transfer a thawed blastocyst on the fifth day after the predicted day of ovulation, 21% on the fourth, 14% on the sixth, 9% on the third and 5% on the seventh.

In medicated FER, 2% of clinics undertake blastocyst transfer on the third day of progesterone, 3% on the fourth, 21% on the fifth, 61% on the sixth and

13% on the seventh. Luteal support is continued from six to beyond twelve weeks' gestation, with the majority (69%) stopping at 12 weeks. The use of pituitary suppression in medicated FER varies widely. Fifty-five percent of clinics favour GnRH-agonist down-regulation, 11% GnRH-antagonist and 34% no supplementary pituitary suppression.

Consequently, we analysed all women undergoing medicated FER of one or two unbiopsied blastocysts at a UK IVF clinic between January 2014 and June 2016 comparing GnRH-antagonist with GnRH-agonist medicated FER. 578 patients (188 antagonist, 390 agonist) were included. Baseline characteristics were similar. Live birth (36.7% (antagonist) vs. 39.5% (agonist),  $p=0.519$ ), clinical pregnancy (59.5% vs. 60.5%,  $p=0.482$ ) and miscarriage rates (33.3% vs. 34.3%,  $p=0.857$ ) were similar. In the antagonist group there were less clinic visits (median (range): 2(5) vs 3(5),  $p<0.01$ ) and ultrasound scans (1(3) vs 2(5),  $p<0.01$ ).

**CONCLUSIONS:** Wide variation exists in the preferred method of endometrial preparation for FER, emphasising the need for more research to determine the optimum protocols. The survey results highlight particular inconsistency in approach to pituitary suppression in medicated FER. Our cohort study highlights potential benefits to GnRH-antagonist as an alternative to GnRH-agonist for pituitary suppression in medicated FER. However, more research is needed to confirm similar clinical outcomes.

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### IMPROVING IMPLANTATION RATE BY ADDING RECOMBINANT LH SUPPLEMENTATION TO RECOMBINANT FSH DURING CONTROLLED OVARIAN STIMULATION IN GNRH ANTAGONIST



**REGIMEN.** Edson Borges, Jr., PhD,<sup>a</sup> Daniela Paes de Almeida Ferreira Braga, PhD,<sup>a</sup> Matheus de Castro Azevedo, BSc,<sup>a</sup> Assumpto Iaconelli, Jr., MD,<sup>a</sup> Amanda Souza Setti, MSc.<sup>a</sup> <sup>a</sup>Fertility Medical Group / Sapiientiae Institute, Sao Paulo, Brazil; <sup>b</sup>Fertility Medical Group, Sao Paulo, Brazil.

**OBJECTIVE:** Gonadotropin-releasing hormone (GnRH) antagonist profoundly suppresses pituitary gland, avoiding premature luteinizing hormone (LH) surge. Consequently, recruited follicles are radically deprived of LH sustenance. The aim of this study was to investigate the effect of LH supplementation in GnRH antagonist regimen on the outcomes of consecutive ICSI (intracytoplasmic sperm injection) cycles.

**DESIGN:** Historical case-control within-subject study.

**MATERIALS AND METHODS:** Data were obtained via chart review of 228 matched cycles performed in 114 patients undergoing ICSI between 2015 and 2018, in a private university-affiliated IVF center. For all patients, recombinant follicle stimulating hormone (rFSH, Gonal-f) was used for controlled ovarian stimulation (COS) in the first ICSI cycle (rFSH group), followed by ovarian stimulation with rFSH and rLH (Pergoveris) in the next cycle (rFSH + rLH group). Pituitary suppression was achieved with GnRH antagonist (cetorelix acetate) in both groups. The sample size calculation suggested that 200 cycles would be enough to demonstrate a 20% effect with 80% power and 5% significance level considering as primary outcome implantation rate (IR). Data was analyzed by Generalized Linear Models followed by Bonferroni post hoc test.

**RESULTS:** Higher estradiol levels (1151.73 ± 194.34 pg/mL vs. 1909.11 ± 194.34 pg/mL,  $p=0.006$ ), oocyte yield (63.41 % vs. 69.78 %,  $p=0.045$ ), day-3 high-quality embryos rate (34.13 % vs. 47.71 %,  $p=0.029$ ) and IR (18.57 % vs. 26.47,  $p<0.001$ ), and lower miscarriage rate (33.0 vs. 5.0,  $p=0.031$ ) were observed in rFSH + rLH group, compared to rFSH group. In patients aged < 35 years, IR was higher in rFSH + rLH group compared to rFSH group (38.46 vs. 21.43,  $p<0.001$ ). In patients aged ≥ 35 years, higher estradiol levels (1161.80 ± 215.94 pg/mL vs. 1966.55 ± 220.13 pg/mL,  $p=0.009$ ), oocyte yield (61.28 % vs. 68.62 %,  $p=0.038$ ), day-3 high-quality embryos rate (32.01 % vs. 48.81 %,  $p=0.013$ ), and IR (17.35 % vs. 23.64 %,  $p<0.001$ ) were observed in rFSH + rLH group compared to rFSH group. In patients with low response to COS (< 5 retrieved oocytes), oocyte yield (56.82 % vs. 63.29 %,  $p=0.001$ ), mature oocytes rate (69.87 % vs. 78+12 %,  $p<0.001$ ), normal cleavage speed (62.5 % vs. 75.83 %,  $p<0.001$ ), IR (10.00 % vs. 20.45 %,  $p<0.001$ ) and miscarriage rate (100 % vs. 0.00 %,  $p<0.001$ ) were improved in rFSH + rLH group compared to rFSH group. In patients with normal response to COS (> 4 retrieved oocytes), higher estradiol levels (1725.74 ± 303.65 pg/mL vs. 2788.37 ± 281.12 pg/mL,  $p=0.010$ ), oocyte yield (75.37 % vs. 82.69 %,  $p=0.006$ ), and IR (23.33 % vs. 29.35 %,  $p<0.001$ ) were observed in rFSH + rLH group compared to rFSH group.

**CONCLUSIONS:** Ovarian stimulation with LH supplementation may prevent the decrease in estradiol levels after GnRH antagonist administration,

resulting in higher IRs, independent of maternal age and response to COS, compared to cycles stimulated with rFSH only. Improvements were also observed for ICSI laboratory outcomes and miscarriage rate when patients were stratified by age and number of retrieved oocytes.

Reference: NA.

SUPPORT: None.

**P-191** Tuesday, October 15, 2019 6:30 AM

### FRESH VERSUS FREEZE-ALL STRATEGY IN ASSISTED REPRODUCTIVE TECHNOLOGY – A COCHRANE REVIEW.



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**OBJECTIVE:** In vitro fertilisation (IVF) treatments imply a fresh embryo transfer, possibly followed by one or more frozen-thawed embryo transfers in subsequent cycles. Alternatively, one can opt to freeze all suitable embryos and transfer frozen-thawed embryos in subsequent cycles only, which is also known as the freeze-all strategy. We compared the effectiveness and safety of these treatment strategies.

**DESIGN:** We searched the Cochrane Gynaecology and Fertility Group Trials Register, the Cochrane Central Register of Studies (CRSO), MEDLINE, Embase, PsycINFO, CINAHL, and two registers of ongoing trials in February 2019 for relevant studies, and checked references and contacted study authors in the field to obtain additional data.

**MATERIALS AND METHODS:** We used standard methodological procedures as recommended by Cochrane for our search, data extraction, and analyses. The primary outcome was cumulative live birth rate (cLBR). Secondary outcomes included ovarian hyper stimulation syndrome (OHSS), pregnancy complications, and time to pregnancy.

**RESULTS:** We included six RCTs in our meta-analyses, that together reported on 4324 women. The studies compared the freeze-all strategy to IVF with fresh transfer in women with a high risk of OHSS, in 'good prognosis' women based on the number of follicles, in women with PCOS, and in young women without PCOS. The evidence was of moderate to low quality due to serious risk of bias, serious imprecision for four studies, and serious unexplained heterogeneity for one study.

For cLBR we found an OR of 1.10 (95% CI 0.97 to 1.24; 6 RCTs; 4324 women;  $I^2 = 0%$ , moderate quality of evidence) for the freeze-all strategy versus IVF with fresh transfer of embryos. These data suggest that for a cLBR of 63% following IVF with fresh transfer of embryos, the cLBR following the freeze-all strategy would be between 62% and 67%.

Women developed less OHSS after the freeze-all strategy compared to IVF with fresh transfer of embryos (OR 0.29, 95% CI 0.19 to 0.44; 4 RCTs; 4065 women;  $I^2 = 5%$ , low quality evidence). These data suggest that for an OHSS rate of 4% following the conventional strategy, the rate following the freeze-all strategy would be between 1% and 2%.

The risk of maternal hypertensive disorders and having a large for gestational age baby was increased following the freeze-all strategy (OR 2.15, 95% CI 1.42 to 3.25; 3 trials; 3940 women;  $I^2 = 29%$  and OR 1.87, 95% CI 1.43 to 2.44; 3 trials; 3119 women;  $I^2 = 0%$ , respectively, both low-quality evidence). The risk of having a small for gestational age baby was lowered following the freeze-all strategy (OR 0.68, 95% CI 0.53 to 0.89; 3 trials; 3119 women;  $I^2 = 56%$ , low-quality evidence).

One trial reported on time to conception and one trial reported on time to live birth which were both longer in the freeze all strategy.

**CONCLUSIONS:** We did not find a clear difference in cLBR between the two strategies. The freeze-all strategy lowered the risk of OHSS, increased the risk of maternal hypertensive disorders of pregnancy, increased the risk of a large for gestational age baby, and lowered the risk of a small for gestational age baby. The time to pregnancy was longer in the freeze-all strategy.

Reference: None.

SUPPORT: None.

**P-192** Tuesday, October 15, 2019 6:30 AM

### PREDICTING CUMULATIVE LIVE BIRTH RATE FOR THE FIRST CYCLE OF IN VITRO FERTILIZATION.



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University, Shanghai, China; <sup>b</sup>Wright State University, OB/GYN, Dayton, OH.

**OBJECTIVE:** To develop a prediction model to estimate the chances of cumulative live birth following the first cycle of in vitro fertilization (IVF) and cumulative embryo transfers based on female demographics and cycle stimulation characteristics.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** All women at the age of 20-50 years old who underwent their first IVF treatment in the reproductive center of Ren Ji hospital from Jan 2014 to Dec 2015 were screened. Cumulative live birth was defined as first live birth from all fresh and frozen thawed embryo transferred within 2 years after oocyte retrieval. A multiple fraction polynomial (MFP) regression model was used to predict the probability of live birth for an individual woman. Two clinical prediction models were developed: pre-treatment model using information available before starting ovarian stimulation and the post-treatment model based on additional information collected after oocyte retrieval.

**RESULTS:** After excluding cycles with PGT and oocyte freezing, 7796 women with 7796 cycles were included. In total 5146 (66.0%) cumulatively had a live birth following their first IVF retrieval. Key pre-treatment predictors of live birth were the woman's age ( $\geq 35$  vs.  $<35$  years; adjusted odds ratio 0.34, 95% confidence interval, 0.29 to 0.38), BMI ( $\geq 24$  vs.  $<24$ ; 0.82, 0.72 to 0.92), and a basal FSH ( $\geq 10$  vs.  $<10$  IU/L; 0.54, 0.46 to 0.64). Post-treatment predictors included number of fertilization (1.03, 0.99 to 1.08), basal FSH ( $\geq 10$  vs.  $<10$  IU/L; 0.89, 0.83 to 0.99), woman's age ( $\geq 35$  vs.  $<35$  years; 0.69, 0.55 to 0.84), endometrial thickness on the day of trigger ( $\geq 7.5$  vs.  $<7.5$  mm; 1.19, 1.01 to 1.49) and cumulative number of embryos transferred ( $\geq 2$  vs.  $<2$ ; 1.83, 1.40 to 2.40). A pre-treatment model of a 32 year old woman with a BMI of 21.1kg/m<sup>2</sup> and basal FSH 7.5 IU/L has a 62.9% cumulative chance of having a live birth after her first cycle. A post-treatment model for the same women with 6 fertilized oocytes and an endometrial thickness of 9 mm, has an estimated 77.6% of having a live birth.

**CONCLUSIONS:** This study provides an individualized estimate of a couple's cumulative chance of having a live birth after the first IVF cycle both before treatment and after oocyte retrieval. This may help physicians better counsel couples in preparation for their IVF journey both emotionally and financially.

**P-193** Tuesday, October 15, 2019 6:30 AM

#### **PRO CASPASE-3 AND CLEAVED CASPASE-3 GENE AND PROTEIN EXPRESSION IN HUMAN GRANULOSA CELLS CORRELATE WITH COS DURATION, LENGTH OF INFERTILITY AND PROPORTION OF MATURE OOCYTES RETRIEVED.**

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**OBJECTIVE:** To evaluate the clinical correlates of pro caspase-3, cleaved caspase-3 and other apoptosis related genes expressed in human granulosa cells (GCs) of patients undergoing controlled ovarian stimulation (COS).

**DESIGN:** Luteinized GCs obtained from *in vitro* fertilization (IVF) and fertility preservation patients were evaluated for their expression of pro caspase-3, cleaved caspase-3 and gene expression of BAX, BCL2, CASPASE3 and CASPASE8, that later were correlated with patient's clinical data, such as length of infertility, length of COS and proportion of mature oocytes.

**MATERIALS AND METHODS:** Follicular fluid (FF) samples were collected from 35 patients referred to a private clinic for couple infertility treatment and female fertility preservation between March and September 2018. The study was approved by the institutional Ethics Committee for Research on Human Subjects (COEP No 1.979.648) and the participants provided written informed consent to be enrolled in the study. Luteinized GCs were isolated from FF aspirates using a Histopaque gradient. Recovered GCs were partly fixed in 10% buffered paraformaldehyde and included in paraffin for future Hematoxylin/Eosin (HE) staining and immunocytochemical procedures, and partly kept frozen in 500  $\mu$ L of TRI Reagent until RNA isolation. Immunocytochemical staining of cellblock sections was performed for pro caspase-3 and cleaved caspase-3, according to the peroxidase reaction method with a polymerized secondary antibody for identification. Total RNA was isolated from GCs, first-strand complementary DNA (cDNA) was synthesized following Su-

perScript III reverse transcriptase Kit manufacturer's protocol, and real-time PCR was carried out for BAX, BCL2, CASPASE3, CASPASE8, and S26 as normalizer gene, using Sybr Green Master Mix Kit. Data distribution was evaluated by the Shapiro-Wilk test, clinical data and gene and protein expression linear correlations were assessed by Pearson's or Spearman's rank correlation coefficient using Prism 8 computer software.

**RESULTS:** Cleaved caspase-3 correlated positively with the length of COS ( $r = 0.445, p < 0.05$ ) and the length of infertility ( $r = 0.476, p < 0.05$ ). However, only pro caspase-3 expression presented a positive correlation with the proportion of mature oocytes collected ( $r = 0.427, p < 0.05$ ). Gene expression of CASPASE 3 and CASPASE 8 also correlated directly with the length of COS ( $r = 0.462, p < 0.05$ ;  $r = 0.420, p < 0.05$ ; respectively).

**CONCLUSIONS:** These findings suggest that pro caspase-3 is constitutively expressed in human granulosa cells and correlates with the proportion of mature oocytes retrieved, therefore it better indicates granulosa cell integrity than imminent cell death by apoptosis. Conversely, the activation of caspase-3 in granulosa cells is associated with a longer time of infertility and longer duration of COS in IVF patients.

**SUPPORT:** PRPq UFMG. Fapemig. CNPq.

**P-194** Tuesday, October 15, 2019 6:30 AM

#### **ASSOCIATION OF THE VAGINAL MICROBIOME WITH PROPHYLACTIC ANTIBIOTIC EXPOSURE AND CLINICAL OUTCOMES IN WOMEN UNDERGOING IN VITRO FERTILIZATION: A RANDOMIZED CONTROLLED PILOT STUDY.**

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**OBJECTIVE:** The objectives of this descriptive pilot study were to (1) compare the vaginal microbiome (VM) in two groups undergoing in vitro fertilization (IVF), women who received prophylactic antibiotics at baseline and those who did not, and (2) to analyze the association of the VM and clinical outcomes.

**DESIGN:** Randomized controlled pilot study within a non-inferiority trial.

**MATERIALS AND METHODS:** Women ages 18 to 43 years undergoing their first IVF cycle with a fresh embryo transfer were eligible for inclusion. Exclusion criteria were allergy to azithromycin and current use or indication for additional antibiotics. Subjects were randomized in a 1:1 ratio to either 1-gm azithromycin (AZ) at baseline (our standard of care) or no antibiotic (NA). All subjects received prophylactic cefazolin immediately following egg retrieval. Mid-vaginal swabs were obtained at 3 time points (T) (T1: baseline testing, T2: immediately prior to egg retrieval, T3: prior to embryo transfer). To survey the bacterial communities from each sample, amplicons were generated from the V4 region of the 16S rRNA gene. Illumina adapters were added during amplification, creating an indexed library for each sample. Nucleotide sequence reads were generated on the Illumina MiSeq instrument. Bacterial community structures were analyzed with R, using the Vegan and Labdsv libraries. Alpha diversity (including richness, Shannon and Simpson's diversity indices and Pielou's evenness) and beta diversity (Bray-curtis dissimilarity) were compared between AZ and NA groups and between subjects with a clinical pregnancy and those with a negative cycle outcome. Parametric and non-parametric statistics were used as appropriate.

**RESULTS:** Twenty-seven subjects were randomized, 15 to NA and 12 to AZ, and contributed 79 vaginal swabs. Results were analyzed with an intention-to-treat approach. There were no significant differences between NA and AZ groups in baseline characteristics or clinical outcomes including implantation rate (0.57 vs 0.4,  $p=0.3$ ), clinical pregnancy (60% vs 58.3%,  $p=0.93$ ) or miscarriage (0% vs 3.7%,  $p=0.44$ ) respectively. There was no difference in alpha diversity of the bacterial communities between groups. In comparing subjects with a clinical pregnancy to those with a negative pregnancy, a higher percentage of *Lactobacillus* (LB) at T3 was associated with clinical pregnancy ( $p=0.08$ ). Higher community stability from T2 to T3 was positively correlated with clinical pregnancy ( $p=0.24$ ), particularly for AZ subjects, but failed to achieve statistical significance. There was no association between the VM and implantation rate.

**CONCLUSIONS:** A higher percentage of LB at T3 and higher stability from T2 to T3 is positively correlated with clinical pregnancy. Microbial stability may be influenced by prophylactic antibiotic exposure at baseline. Future studies should be directed toward obtaining a larger sample size to determine the precise effect of antibiotic exposure on microbial stability and the VM in the immediate preconception period to optimize IVF outcomes.

**APOPTOSIS OF CUMULUS GRANULOSA CELLS IS HIGHER IN NON-PREGNANT GROUP IN PATIENTS UNDERGOING IVF/ICSI.** Yuting Fan, M.D.,<sup>a</sup>



Xiao-yan Liang, M.D., Ph.D.,<sup>a</sup> Sherman Silber, MD.<sup>b</sup> <sup>a</sup>The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China; <sup>b</sup>In-fertility Center of St. Louis, Chesterfield, MO.

**OBJECTIVE:** To evaluate apoptosis of granulosa cells in clinical pregnant group versus non-pregnant group in women undergoing in-vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI).

**DESIGN:** A prospective cohort study.

**MATERIALS AND METHODS:** A prospective cohort study was initiated at a single IVF center involving a total of 164 women undergoing IVF/ICSI cycles. Mural and cumulus granulosa cells, and follicular fluid were collected during oocyte retrieval. Annexin V-FITC/PI apoptosis staining and flow cytometry analysis were performed to evaluate apoptosis rate of mural granulosa cells and cumulus cells. Serum and follicular fluid hormones including estradiol (E<sub>2</sub>), progesterone (P), testosterone (T), anti-Mullerian hormone (AMH) were measured by ECLIA. Laboratory and clinical outcomes were analyzed.

**RESULTS:** Apoptosis of cumulus cells was significantly higher in the non-pregnant group. Follicular estradiol level was lower in clinical pregnant group. The apoptosis rate of mural cells was negatively correlated with oocyte yield, MII egg number, D3 good embryos number and blastocyst formation rate. Good blastocyst number, Blastocyst formation rate was lower in non-pregnant group.

**CONCLUSIONS:** A significantly higher apoptosis rate of mural granulosa cells was correlated with worse ovarian response, with fewer egg and embryo numbers in IVF/ICSI, as well as with age. Early apoptosis rate of cumulus cells might also have influence on clinical pregnancy.

**References:** 1. Desquiret-Dumas V, Clément A, Seegers V, Boucrot L, Ferré-L'Hotellier V, Bouet PE, Descamps P, Procaccio V, Reynier P, May-Panloup P. The mitochondrial DNA content of cumulus granulosa cells is linked to embryo quality. *Hum Reprod.* 2017 Mar 1;32(3):607-614. <https://doi.org/10.1093/humrep/dew341>.

2. Uyar A, Torrealday S, Seli E. Cumulus and granulosa cell markers of oocyte and embryo quality. *Fertil Steril.* 2013 Mar 15;99(4):979-97. <https://doi.org/10.1016/j.fertnstert.2013.01.129>.

3. Adriaenssens T, Van Vaerenbergh I, Coucke W, Segers I, Verheyen G, Anckaert E, De Vos M, Smitz J. Cumulus-corona gene expression analysis combined with morphological embryo scoring in single embryo transfer cycles increased live birth after fresh transfer and decreases time to pregnancy. *J Assist Reprod Genet.* 2019 Jan 9. <https://doi.org/10.1007/s10815-018-01398-2>.

4. Seyed Homayoon Sadraie, Hidekazu Saito, Tomoko Kaneko, Takakazu Saito, and Masahiko Hiroi. Effects of Aging on Ovarian Fecundity in Terms of the Incidence of Apoptotic Granulosa Cells. *J Assist Reprod Genet.* 2000 Mar; 17(3): 168-173.

5. Bencomo E, Pérez R, Arteaga MF, Acosta E, Peña O, Lopez L, Avila J, Palumbo A. Apoptosis of cultured granulosa-lutein A

cells is reduced by insulin-like growth factor I and may correlate with embryo fragmentation and pregnancy rate. *Fertil Steril.* 2006 Feb;85(2):474-80.

**DOES MESSAGE THERAPY IMMEDIATELY PRIOR TO EMBRYO TRANSFER IMPROVE CLINICAL PREGNANCY RATE IN IVF-PGT-A (IN-VITRO FERTILIZATION-PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY) CYCLES?** Sarah Z. Gavrizi, MD,<sup>a</sup> Amanda Skillern, MD, FACOG.<sup>b</sup> <sup>a</sup>UT Dell Medical School, Austin, TX; <sup>b</sup>Aspire Austin, Austin, TX.



**OBJECTIVE:** Prior retrospective research (1) has suggested improved implantation rate in FET (frozen embryo transfer) cycles with untested blastocysts. This purpose of this study is to evaluate whether massage therapy immediately prior to SET (single embryo transfer) of PGT-A euploid embryos improves clinical pregnancy rate.

**DESIGN:** Prospective, Double-blind, Randomized Controlled Trial.

**MATERIALS AND METHODS:** Patients undergoing SET of autologous PGT-A euploid embryo in controlled FET cycles (exogenous estrogen, intramuscular progesterone) beginning in May 2017 were considered for participation in this study. Exclusion criteria: 2 or more prior failed FETs, uterine anomaly, prior uterine surgery, and patients already undergoing massage or acupuncture therapy. A total of 65 embryo transfers were included and were randomly assigned to group by computer randomization software. Patients in the treatment group (n=31) received a standardized 20-minute massage by one therapist (GK) beginning 45 minutes prior to SET. Patients in the control group (n= 34) arrived at the same time prior to SET and received standard care without massage. Patients were blinded to group allocation until arrival for SET. One physician (AS) performed all SETs and is blinded to group allocation.

**RESULTS:** There was no significant difference in age, body mass index, underlying fertility diagnosis, number of prior embryo transfers (0 vs 1), or endometrial thickness between the two groups. All patients had trilaminar endometrium of at least 7 mm thickness by ultrasound prior to progesterone start. Implantation rate (positive quantitative beta human chorionic gonadotropin 14 days post SET) was 80.6% (n=25) in the treatment group and 64.3% (n=21) in the control group, p=0.09. The live birth rate was 64.5% (n=20) in the treatment group and 47.1% (n=16) in the control group, p=0.16.

**CONCLUSIONS:** Despite advances in modern fertility treatment, clinical pregnancy rates remain well below 100%, even in good prognosis patients. Therefore, a low-cost, low-risk intervention which may benefit this population is of great interest. The standardized massage in the study includes elements of both lower abdominal massage, which can theoretically increase blood flow to the pelvis, and head and neck massage, which may improve relaxation. Prior retrospective research (1) has suggested improved implantation rate in FET cycles with untested blastocysts. Our randomized controlled double-blind trial appears to demonstrate a clinical benefit of

Parameters	Clinical pregnant (n = 62)	Non-pregnant (n = 66)	P value
Age (yr)	29 (27.75-32)	30 (27-33)	ns
Basal serum FSH (mIU/ml)	6.57 (5.36-7.35)	6.62 (5.73-7.55)	ns
Basal serum AMH (ng/ml)	4.34 (2.14-7.46)	3.76 (1.62-6.03)	ns
MGCs early apoptosis rate (%)	0.58 (0.29-1.08)	0.63 (0.22-1.45)	ns
MGCs late apoptosis rate (%)	2.75 (0.67-6.31)	2.36 (0.06-5.68)	ns
CCs early apoptosis rate (%)	0.13 (0.032-0.86)	0.37 (0.19-0.69)	<0.05
CCs late apoptosis rate (%)	2.46 (0.47-13.4)	6.63 (1.36-14.0)	ns
CCs total apoptosis rate (%)	2.63 (0.56-14.42)	8.71 (3.81-14.61)	0.052
FF AMH (ng/mL)	3.33 (1.83-4.98)	2.91 (1.14-5.28)	ns
FF E2 (pmol/L)	602497 (468066-835750)	716750 (612033-942250)	<0.05
FF P (ng/mL)	17700 (12300-45935)	17700 (12697.5-29052.5)	ns
FF T (nmol/L)	5.26 (3.5-8)	5.29 (4.38-8.48)	ns
hCG day E <sub>2</sub> (pmol/L)	4759 (2943-7258.5)	2691 (1267-4812)	0.069
OPU egg number	13 (8-17.25)	10.5 (6-18)	ns
Fertilization rate (%)	75.00 (60.00-83.93))	67.26 (57.14-83.33)	ns
D3 good embryo rate (%)	70.71 (59.29-94.23)	70.98 (41.25-83.55)	ns
Good blastocyst number	3 (1-5)	2 (0-3.25)	<0.05
Blastocyst formation rate (%)	55.56 (50.00-83.33)	41.67 (17.21-71.63)	<0.05

massage therapy immediately prior to SET of PGT-A euploid embryos (17.4% increase in live birth). While this finding has not yet reached statistical significance, the trend continues to favor the massage group, and data collection is ongoing.

Reference: 1. Okhowat, J. "Massage Therapy Improves in Vitro Fertilization Outcome in Patients Undergoing Blastocyst Transfer in a Cryo-Cycle." *Alternative Therapies in Health and Medicine*, vol. 21, no. 2, Mar/Apr. 2015, pp. 16–20.

SUPPORT: None.

**P-197** Tuesday, October 15, 2019 6:30 AM

**WOMEN WITH PREVIOUS FAILED IVF BENEFIT FROM INTRAUTERINE INSTILLATION OF PLATELET RICH PLASMA.**



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**OBJECTIVE:** To record the improvement in the endometrial lining and pregnancy rates in Frozen Embryo Transfer (FET) cycles of women following intrauterine Platelet Rich Plasma (PRP) instillation.

**DESIGN:** A prospective case control study was carried out during the period of August 2018 to March 2019 at our centre. Women in the age group of 25 to 45 years with a history of previous cancelled cycles due to poor endometrial lining undergoing FET were included.

**MATERIALS AND METHODS:** 101 women undergoing FET at our centre were included in the study, following their consent. Intrauterine instillation of approximately 1 ml of autologous PRP was carried out on day 5, day 12 of endometrial priming and 48 hours prior to embryo transfer. The endometrial thickness was evaluated by Transvaginal Ultrasound on the days of PRP instillation and embryo transfer. Serum BhCG levels were checked 14 days after the embryo transfer.

TAB. 1. Comparison of baseline data in each group according to vitamin D status

	VitD Deficiency	vitD Insufficiency	vitD Adequacy	P
Number of patients ( n )	1384	1113	80	
Total VD ( pg/ml )	16.76 (15.03,18.55)	22.87 ( 21.22 , 25.15)	32.01 (30.88,34.16)	0.00 <sup>a</sup>
Free VD ( ng/ml )	4.50 (3.96,5.08)	4.95 (4.33,5.57)	5.46 (4.63,5.96)	0.00 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	21.34 (19.72,23.05)	21.23 (19.57,23.11)	21.49 (20.18,23.05)	0.59 <sup>a</sup>
Weight (kg)	53 (49,58)	53 (49,58)	54 (50,59.75)	0.49 <sup>a</sup>
Age (y)	29 (27,32)	29 (27,31)	30 (27,33)	0.26 <sup>a</sup>
Basic FSH (miu/ml)	5.60(4.84,6.53)	5.65(4.75,6.55)	5.65(4.83,6.28)	0.88 <sup>a</sup>
Endometrial thickness on hCG day ( mm)	13 (11.8,14.3)	13.1 (11.9,14.4)	13.2 (12.5,14.68)	0.06 <sup>a</sup>
Number of oocytes retrieved (n)	12 (9,15)	12 (9,15)	12 (8,16)	0.79 <sup>a</sup>
Number of transplantable embryos (n)	6 (3,8)	6 (3,8)	6 (3,7)	0.81 <sup>a</sup>
Number of 2PN (n)	7 (5,9)	7 (4,9)	6.5 (4,9)	0.49 <sup>a</sup>
Number of high quality embryos (n)	2 (0,5)	2 (0,5)	2 (0,5)	0.79 <sup>a</sup>
AMH (ng/ml)	5.68 (3.6,9.10)	5.74 (3.67,9.52)	6.35 (3.89,10.64)	0.30 <sup>a</sup>
Pregnancy rate,% (n)	70.95 (982)	72.42 (806)	75 (60)	0.58 <sup>b</sup>
Abortion rate,% (n)	7.08 (98)	7.19 (80)	8.75 (7)	0.85 <sup>b</sup>

**RESULTS:** 96 out of 101 women showed improvement in the endometrial lining. Of 101 women in the study, 29 women conceived (28.7%). Also, there were 10 biochemical pregnancies (9.9%). Of these 29 women, 14 had never conceived in the past and 15 women had previous pregnancy losses. Among the 45 women, who had never conceived in the past, 21 women had done multiple cycles earlier and of these 24% (n=5) got pregnant. Of the 29 pregnancies with PRP, one woman delivered, 6 women had miscarriages after cardiac activity and 15 are ongoing pregnancies. Thirty three women had past history of Genital TB. Of these, 24% (n=8) got pregnant. Of these, 5 had done multiple IVF cycles in the past and there were 3 women who had never conceived. Of the 8 pregnancies with a history of genital TB, 5 are ongoing pregnancies and 3 miscarried after cardiac activity.

**CONCLUSIONS:** Intrauterine infusion of PRP has a potential to improve the endometrial lining and clinical pregnancy rates particularly in women with multiple failed attempts and also holds promise for women with past history of Genital TB where implantation rates are low.

**P-198** Tuesday, October 15, 2019 6:30 AM

**THE ROLE OF VITAMIN D ON PREGNANCY OUTCOMES OF IVF/ICSI.**



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**OBJECTIVE:** To study the relationship between maternal vitamin D(vitD) status and pregnancy outcome of IVF/ICSI.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** A total of 2577 female patients were collected who were received IVF/ICSI treatment in our hospital from 2017.1-2018.11. Peripheral blood was collected one day before transplantation to test the total and free vitD . All patients were divided into three groups according to the level of total vitD : adequacy group(total vitD≥30pg/ml), insufficiency group(total vitD≥20pg/ml and < 30pg/ml)and deficiency group(total vitD < 20pg/ml).

**RESULTS:** There were 1384 patients in deficiency group (53.7%), 1113 patients in insufficiency group (43.2%), and 80 patients in adequacy group (3.1%). There was no significant difference in age, weight , BMI , basic FSH, LH, E2, T and AMH among the three groups. There was also no significant difference in the number of oocytes received, transplantable embryos, high quality embryos and fertilization eggs of 2PN, pregnancy rate and abortion rate among the three groups ( Tab. 1 ) . The total vitD level between pregnant group (19.62(16.59,22.83)) and non-pregnant group (19.4(16.35,22.79)) had no significant difference(p=0.45). The free vitD level between pregnant group (4.71(4.1,5.34)) and non-pregnant group (4.71(4.11,5.27)) had no significant difference too(p=0.76).

**CONCLUSIONS:** Although the pregnancy rate tended to increase with vitD level, neither total vitD nor free vitD seems was associated with IVF pregnancy rate and abortion rate.

1. Kruskal Wallis test.

2. Chi-Square test among groups.

**SUPPORT:** The company of DIAsource ImmunoAssays S.A.Â provided vitamin D kits.

**P-199** Tuesday, October 15, 2019 6:30 AM

**NON-INVASIVE OOCYTE SELECTION BASED ON CUMULUS GENE EXPRESSIONS OF CAMK1D, EFN2 AND SASH1 PREDICTS PREGNANCY: A RETROSPECTIVE STUDY IN AN ASIAN POPULATION.**



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Universiteit Brussel, Brussels, Belgium; <sup>b</sup>Quality of Laboratories, Sciensano, Brussels, Belgium; <sup>c</sup>Reproductive Medicine Research Center, Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China; <sup>d</sup>HORAC Grand Front Osaka clinic, Osaka, Japan; <sup>e</sup>Center for Reproductive Medicine, Taipei Medical University Hospital, Taipei, Taiwan; <sup>f</sup>Fertiga, Lede, Belgium.

**OBJECTIVE:** Non-invasive testing for embryo selection is not yet well established. Recently a three-gene expression model in cumulus cells was evaluated for embryo selection in a Caucasian population (JARG 2019) and showed a significant increase of clinical pregnancy rate in day 3 single embryo transfer (SET). This study investigates if the same genes, CAMK1D, SASH1 and EFNB2, are also predictive in an independent Asian ART population.

**DESIGN:** International retrospective multicentre study with individual oocyte denudation.

**MATERIALS AND METHODS:** Oocytes from 39 Asian women in three centres (China, Taiwan, Japan) scheduled for ICSI and SET underwent individual oocyte denudation after pick-up. The women were stimulated with HP-hMG (n=9) or combo HP-hMG & rFSH (n=30) and received a day 3 or day 5 fresh or frozen SET. mRNA expression analysis for three predictive genes CAMK1D, EFNB2 and SASH1 (Corona Test) and 2 endogenous control genes (UBC, B2M) was performed by QRT-PCR using the cumulus cells of the oocytes. The CC of all oocytes developing into an embryo, that was selected for transfer based on the embryo morphology, were analysed. The expression of the three predictive genes was used for multivariate stepwise regression analysis.

If a patient was pregnant, a negative control CC sample of the same patient was also analysed. These were CC of oocytes yielding an embryo but no pregnancy after transfer or oocytes which did not progress to day-3 embryos. This allowed the intra-patient ranking of oocytes of good competence (pregnancy) to low competence (arrested or no pregnancy) using the model established in the Caucasian population.

**RESULTS:** Of the 58 transferred embryos from the 39 Asian women 22 implanted and resulted in a clinical pregnancy. Thirty six embryos did not implant. The three-gene expression model separated CC samples from pregnant and non-pregnant women with an accuracy of 93%. The sensitivity was 100%, specificity 89% and the area under the curve (AUC) was 0.9848.

For 18 of the 22 pregnant women at least one oocyte with a known negative outcome (lower oocyte competence) was available. This allowed an intra-patient analysis for 18 patients with 18 fully competent and 24 low competence oocytes. In 13 of these 18 pregnant patients (72%) the expression gene model yielded the highest Corona Test score for the oocyte that resulted into a clinical pregnancy.

**CONCLUSIONS:** The multivariate analysis confirmed that CAMK1D, EFNB2 and SASH1 are also predictive for transfer outcome in Asian ART patients. The intra-patient analysis with the current Corona Test model, developed on the European Caucasian patients, proved also to be predictive for oocyte competence in an Asian ART population. Finally, our results also suggest that the three-gene expression model also works in patients stimulated with a combo protocol of rFSH and HP-hMG. This was a retrospective study and the findings need to be confirmed in a larger prospective study.

**References:** Adriaenssens T, Van Vaerenbergh I, Coucke W, Segers I, Verheyen G, Anckaert E, De Vos M, Smitz J. Cumulus-corona gene expression analysis combined with morphological embryo scoring in single embryo transfer cycles increases live birth after fresh transfer and decreases time to pregnancy. *J Assist Reprod Genet.* 2019 Mar;36(3):433-443.

**SUPPORT:** FWO Flanders, NSCF China, IOF.

**P-200** Tuesday, October 15, 2019 6:30 AM

#### **RELATIONSHIP BETWEEN NUMBER OF OOCYTES RETRIEVED FROM ANONYMOUS EGG DONORS AND SUBSEQUENT EMBRYO QUALITY AFTER IN VITRO FERTILIZATION (IVF): A RETROSPECTIVE STUDY.**

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**OBJECTIVE:** To study the relationship between number of oocytes retrieved from anonymous egg donors after controlled ovarian stimulation (COS) and embryo quality and development after in vitro fertilization (IVF).

**DESIGN:** Retrospective study of 143 IVF recipient cycles from 2013-2019 using donor oocytes from 36 anonymous oocyte donors in a private IVF center.

**MATERIALS AND METHODS:** Anonymous egg donors (n=36) 20-32 years of age were screened using strict inclusion criteria including normal range serum anti-Mullerian Hormone (AMH) levels and normal basal follicle stimulating hormone and luteinizing hormone levels on days 2-4 of the menstrual cycle. Egg donors underwent COS to optimize ovarian response. Human chorionic gonadotropin was used to trigger maturation, and oocyte retrieval was performed 36 hours later (n=47 cycles). All oocytes from each donor cycle were thawed from cryopreservation for IVF using fertile, good-quality donor sperm. Resulting blastocysts were graded according to the Society for Assisted Reproductive Technology (SART) standardized system. The percentage of oocytes that developed into good quality blastocysts after 5-6 days of culture (POB) was calculated for each oocyte retrieval. Additionally, the percentage of 2 pronuclear zygotes that developed into good quality blastocysts after 5-6 days of culture (PPB) was calculated for each IVF cycle. Data were analyzed and egg donor age, sperm donor age, and donor plasma AMH levels were eliminated as confounding variables using regression models. Oocyte retrievals were grouped by the number of oocytes collected per COS; Group Low (n=32) had <30 oocytes collected per retrieval, and Group High (n=15) had 30 or more oocytes collected per retrieval. 2-sample T-tests were used to compare POB and PPB between groups. Statistical significance was set at P<0.05.

**RESULTS:** The POB for Groups Low and High were 34.1% and 34.3%, respectively (p = 0.519). The PPB for Groups Low and High were 56.6% and 47.9%, respectively (p = 0.071).

**CONCLUSIONS:** There is debate in literature about the ideal number of oocytes retrieved from egg donors during COS, and if there are any deleterious effects on blastocyst number or quality. This preliminary study demonstrated a trend towards a significant decrease in the percentage of good quality, usable blastocysts per oocyte retrieval in cycles where 30 or more oocytes were retrieved. In conclusion, additional work is needed to further elucidate the impact of high oocyte retrieval numbers on embryo quality.

**P-201** Tuesday, October 15, 2019 6:30 AM

#### **CAN WE PREDICT WHO WILL DEVELOP A BLASTOCYST FROM AN IVF/ICSI CYCLE?**

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**OBJECTIVE:** As IVF laboratory techniques have advanced, extended culture and blastocysts transfer has become a mainstay of treatment. More and more clinics are employing blastocyst only transfer policies. Unfortunately, not all patients will have embryos that attain blastocyst stage meaning that a proportion of patients will not have an embryo transfer. The objective of this study is to identify patient or cycle characteristics predictive of blastocyst development.

**DESIGN:** We performed a retrospective database review of clinic and embryology data from all patients who had an IVF/ICSI cycle at our academic hospital-based fertility clinic.

**MATERIALS AND METHODS:** From February 1, 2012 to February 28, 2019 we looked at all cycles that had extended culture and compared patient and cycle characteristics from cycles with blastocyst development to cycles without (ie. no embryo development past cleavage stage or morula). Donor oocyte, onco-fertility, and social oocyte cryopreservation cycles were excluded. Bivariate statistical analysis was used to identify characteristics associated with blastocyst development and multivariate analysis used to create a prediction model for blastocyst development.

**RESULTS:** Of the 2474 IVF/ICSI cycles performed, 803 met inclusion criteria and had extended culture. Seventy-nine percent of patients developed blastocysts by day 5 or 6 with an average number of 2.8 blasts per cycle. Conventional IVF reduced the chance of no blastocyst development by 46% compared to ICSI (OR 0.54, 95% CI 0.33-0.89, p=0.01). The number of good quality day 3 embryos (more than 6 cells) was also associated with a better outcome; each good quality day 3 embryo reduced the chance of no blastocyst development by 14.5% (p<0.001). No other characteristics, including female age, BMI, parity, infertility diagnosis, gonadotropin dose, protocol, estradiol level, number of oocytes retrieved, and fertilization were associated with blastocyst development. The prediction model using

insemination method and number of good quality day 3 embryos was strong with a Hosmer and Lemeshow p-value of 0.71.

**CONCLUSIONS:** Use of conventional IVF and number of embryos with more than 6 cells on day 3 of culture were the only significant clinical predictor for blastocyst formation.

**P-202** Tuesday, October 15, 2019 6:30 AM

### **HUMAN GROWTH HORMONE COUPLED WITH THE CMAP ACUPUNCTURE PROTOCOL (GH-CMAP) ENHANCES BLASTOCYST FORMATION AND CLINICAL PREGNANCY RATES.**

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**OBJECTIVE:** The aim of this study was to evaluate supplemental HGH both before and during ovarian stimulation coupled with the Cridennda Magarelli Acupuncture Protocol (CMAP) (GH-CMAP Protocol) in terms of oocyte retrieved, oocyte maturity, blast formation, and clinical pregnancy rates.

**DESIGN:** This is a preliminary, prospective cohort study conducted in 2018 on 112 patients, including GH-CMAP (48) vs. antagonist protocol (A) group (64) (control).

**MATERIALS AND METHODS:** A regular antagonist protocol with 1.6mg/day HGH was given (n=48) before and during antagonist stimulation (AS) period; 64 patients were undergoing regular AS protocol (2017-2018). The CMAP protocol (3) was used with supplemental estrace, DHEA, CoQ10. Duration of ovarian stimulation GH-CMAP averaged 10 days, the control group 11 days. Normality of all variables was evaluated and number of oocyte, oocyte maturity rate and blast formation rate were log 10 transformed to become normal distribution. Multivariate regression model (JMP version 14.0) was performed to assess the effect of GH-CMAP on number of oocytes retrieved, oocyte maturity rate and blastocyst formation rate, adjusted by independent variables including age, AMH, BMI, FSH. Total number of patients N=112; GH-CMAP group n=48; Antagonist group n=64;

**RESULTS:** Co-stimulation with HGH in the GH-CMAP protocol improved blast formation rate (GH-CMAP 1.75±0.05, A 1.59±0.03; p=0.0252). Pregnancy rates trended higher in GH-CMAP group (58.8%) than in the AS protocol group (46.6%). Fewer number of oocytes retrieved (GH-CMAP 9; A13; p=0.0095) and lower oocyte maturity rate (p=0.1397) was observed in GH-CMAP group by design. There was a statistically significant association between BMI and blastocyst formation rate in that the higher BMI, the lower blastocyst formation rate (p=0.0085).

**CONCLUSIONS:** These data provide evidence that the positive effect of GH-CMAP on blast formation, consequently improve clinical pregnancy rate. The implantation rate and live birth rate are needed to be included in the further study. These results need to be confirmed by a large-scale randomized controlled trial.

References: 1. Effect of growth hormone on oocyte competence in patients with multiple IVF failures. *A Hazout et al. RBM online Vol 18. NO.5. 2009 664-670 Reproductive BioMedicine Online.*

2. The use of human growth hormone (HGH) in poor prognosis patients improves euploidy and implantation rates. A patient-controlled trial. *Fernandez et al. Fertility & Sterility O-128 Oct 20, 2015.*

3. Changes in serum cortisol and prolactin associated with acupuncture during controlled ovarian hyperstimulation in women undergoing in vitro fertilization - embryo transfer treatment. *P Magarelli et al. Fertility & Sterility Vol 92, Issue 6, p 1870-1879.*

**SUPPORT:** None.

**P-203** Tuesday, October 15, 2019 6:30 AM

### **THE EFFECT OF BODY MASS INDEX ON THE IMPLANTATION POTENTIAL OF EUPLOID EMBRYOS.**

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**OBJECTIVE:** Obesity has been associated with higher miscarriage rates after natural conception and IVF. However, the underlying mechanism, whether obesity affects egg quality or endometrial receptivity, is not well un-

derstood. Here we aim to determine the impact of body mass index (BMI) on the outcomes of frozen-thawed euploid embryo transfer cycles.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Frozen-thawed embryo transfer (FET) cycles of euploid embryos between 2013 and 2017 were included. Embryos were cultured in time-lapse incubators. Preimplantation genetic testing for aneuploidy was performed using array comparative genomic hybridization or next-generation sequencing. Cycles were divided into three groups according to the female patients' BMI: <25 kg/m<sup>2</sup>, 25-29.9 kg/m<sup>2</sup> (overweight), and ≥ 30 kg/m<sup>2</sup> (obese). The miscarriage rate and live birth rate (LBR) were compared between the three groups.  $\chi^2$  and Fisher's exact tests were used for categorical variables. Student's *t* test and ANOVA were used for parametric data. Values were expressed as mean ± standard deviation.

**RESULTS:** A total of 1011 FET of euploid embryo(s) were included: 758 with a BMI <25 kg/m<sup>2</sup>, 174 with a BMI 25-29.9 kg/m<sup>2</sup>, and 79 with a BMI ≥30 kg/m<sup>2</sup>. The women were of comparable age between the three groups (P=0.9) (Table 1). There was a trend toward a lower LBR in women with a BMI ≥30 kg/m<sup>2</sup> compared to women with a BMI <25 kg/m<sup>2</sup> (48.1% vs. 57.5%, respectively; P=0.1), but it did not reach statistical significance. The LBR for women with a BMI 25-29.9 kg/m<sup>2</sup> (54%) was comparable with the other two groups (Table 1). There was no significant difference in miscarriage rates between the three groups (8.8% for BMI <25 kg/m<sup>2</sup>, 9.6% for BMI 25-29.9 kg/m<sup>2</sup>, and 11.6% for BMI ≥ 30 kg/m<sup>2</sup>; P=0.8).

**CONCLUSIONS:** Overweight and obesity do not significantly affect the implantation potential of euploid embryos.

BMI (kg/m <sup>2</sup> )	<25	25-29.9	≥ 30	P value
Age (years)	36.3 ± 4.1	37.0 ± 4.3	36.7 ± 4.0	0.09
Live birth rate (%)	57.5	54.0	48.1	0.1
Miscarriage rate (%)	8.8	9.6	11.6	0.8

**SUPPORT:** None.

**P-204** Tuesday, October 15, 2019 6:30 AM

### **THE EFFECT OF INTERLEUKIN 6 ON CONTROLLED OVARIAN STIMULATION RESULTS AND IVF OUTCOME IN INFERTILE WOMEN WITH ADENOMYOSIS UNDERGOING IVF.**

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**OBJECTIVE:** To investigate the effect of serum interleukin 6 (IL-6) on controlled ovarian stimulation (COS) results and IVF outcome in infertile women with adenomyosis undergoing IVF.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** A total of 59 infertile women with adenomyosis who had their blood taken for analysis of serum IL-6 on the day of hCG injection in IVF cycle between May 2018 and March 2019 was included in this study. COS results and IVF outcome were compared among the three groups (group 1;<1.5 pg/ml, group 2;1.5-7.0 pg/ml, group 3;>7.0 pg/ml) according to the serum IL-6 levels. Analysis of variance (ANOVA) was used to compare the mean values among three groups. Chi-square test and Fisher's exact test were used for the comparisons of fraction. Statistical significance was defined as P<.05.

**RESULTS:** Serum IL-6 levels on the day of hCG injection was significantly higher in infertile women with adenomyosis than in patients without adenomyosis who underwent IVF during the same period (P=.01). The demographic characteristics of patients with adenomyosis were comparable among the three groups according to the serum IL-6 levels. There were also no differences in the three groups with respect to the number of oocytes retrieved, mature oocytes retrieved and fertilized oocytes. However, the number of grade 1 or 2 embryos was significantly lower in group 3 (P<.05). Clinical pregnancy rate was significantly lower in group 3, compared with group 1 or 2 (P<.001, P<.023, respectively). None of the patients with serum IL-6 levels more than 8.0 achieve pregnancy following the corresponding IVF cycle.

**CONCLUSIONS:** High serum IL-6 levels in infertile women with adenomyosis can have an adverse effect on the IVF outcome including embryo quality and clinical pregnancy rate.

**SUPPORT:** None.

**CELL FREE DNA IS AN IDEAL OVARIAN RESERVE MARKER FOR LOW OVARIAN RESPONSE FOR STIMULATION.**



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**OBJECTIVE:** Primary:

- To find the correlation of cfDNA to ovarian response in ICSI cycles.

Secondary:

- To correlate cf DNA with other markers of ovarian reserve.
- To establish a cut-off of cfDNA level for predicting poor ovarian response.

**DESIGN:** This prospective study.

**MATERIALS AND METHODS:** 65 serum samples collected at day 3 of menstrual cycle from patients undergoing ICSI procedure. FSH, Anti-Müllerian hormone (AMH) and cfDNA levels were measured in each serum sample in order to compare their predictive value for patient's ovarian response to stimulation.stati

**RESULTS:** Cell-free DNA concentrations (mean ±SD:23.88±39.78 ng/ml) were significantly and positively correlated with patient's FSH (r=0.175; p=0.053), negative correlated with AFC(r=-0.339,p=0.055\*) and AMH (r=-0.178,p=0.001) .cell-free DNA level was significantly correlated to the number of oocyte retrieved (p=0.0001).CfDNA level in were predicted in low responder (Number of oocytes retrieved < 6). ROC curve were plotted for no of oocyte retrieved and cf DNA levels in serum (AUC =0.87), which predicted cf DNA response in low ovarian reserve patient with sensitivity of 80.8% and specificity 98.6%.

**CONCLUSIONS:** Cf DNA level on 3 day of cycle in serum can predict the ovarian response to stimulation. It can independently identify ovarian response cut off more the 37.5 is used by identifying high amount of cf DNA in serum.

**IS THE 'OESTRO-ANDROGENIC' HORMONE DEHYDRO-EPIANDROSTERONE SULPHATE (DHEAS) THE INTRACRINE REGULATOR OF IMPLANTATION AND EARLY PREGNANCY?: A PROSPECTIVE STUDY IN WOMEN UNDERGOING IVF.**



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**OBJECTIVE:** Decidualization of endometrial-stroma is necessary for successful implantation. Very high levels of dehydro-epiandrosterone (DHEA), which inhibit endometrial-stromal cell differentiation via prevention of glucose-flux through pentose-phosphate-pathway, could be a probable cause for higher incidence of implantation failure among PCOS women. Contrarily, low DHEA women with diminished ovarian-reserve, when supplemented with DHEA show significant reduction in early miscarriage rates. Sulphonated-DHEA (DHEAS) is more stable than DHEA and is the most abundant circulating 'oestro-androgenic' steroid precursor for estrogen production in humans. Objective of this study was to evaluate significance of innate, endogenously circulating DHEAS during implantation in predicting implantation failure/early miscarriage in eumenorrhic women undergoing IVF.

**DESIGN:** Prospective pilot study of n=145 non-PCOS eumenorrhic, normo-responder women undergoing conventional antagonist stimulation protocol IVF. All cycles involved day5 fresh, elective single-blastocyst transfer (eSBT). Luteal phase support was provided to all women.

**MATERIALS AND METHODS:** Serum DHEAS levels in baseline as well as day7, day14 post-eSBT were measured by radio-immuno-assay using diagnostic kits. Serum estradiol, β-hCG and progesterone levels were also measured on day7/day14 post-eSBT. β-hCG measurement on d7 of eSBT was considered early indicator of pregnancy. Implantation rate, live-birth rate were main outcome measures. Cycles were classified on the basis of live-birth (LB, n=52), biochemical pregnancy (BCP, n=5), early miscarriage (EM, n=6), no implantation (NI, n=77). Statistical analysis was done using Graph-pad Prism VI software. Sample size was devised to give >80% power to the study.

**RESULTS:** Overall rates of LB, BCP, EM and NI were found to be 37.14%, 3.57%, 4.28%, and 55% respectively. DHEAS levels depicted a steady rise from baseline to d7 to d14 post-eSBT in women with LB (174±12.23 vs. 355.3±37.15 vs. 741.9±54.38 respectively). Although a rising trend was also observed in women with EM, the rise from baseline to d7 post-eSBT was rather steep (73.25±3.4 vs. 255.5±7.5 vs. 280.71±11.4). However, the rising pattern was disrupted in BCP cycles where the levels dropped from baseline to d7 and then increased on d14 post-eSBT (227±28.9 vs. 121±5.2 vs. 270±10.98); and in NI cycles where a sharp rise on d7 was followed by a decrease in levels on d14 post-eSBT (218.41±11.62 vs. 1380±131 vs. 801.7±98.8). A significant difference in the ratio of d7:baseline DHEAS levels was observed in LB vs. BCP vs. EM vs. NI cycles (2.3 vs. 0.53 vs. 3.5 vs. 6.3; p=0.0005). Similarly, the ratio of d14:d7 DHEAS levels differed significantly in LB vs. BCP vs. EM vs. NI cycles (2.1 vs. 2.23 vs. 1.1 vs. 0.58; p<0.0001). Thus, a twofold rise in DHEAS levels from baseline to d7 and d7 to d14 is critical for successful implantation leading to a live-birth.

**CONCLUSIONS:** Maintenance of a steady/balanced rise in serum DHEAS levels is an early indicator of successful implantation and predicts implantation-failure/early miscarriage in eumenorrhic women undergoing IVF.

**SUPPORT:** None.

**HIGH PINK1 EXPRESSION RELATED TO AGEING IN CUMULUS CELLS IS ASSOCIATED WITH ASSISTED REPRODUCTIVE TECHNOLOGY OUTCOME.**



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**OBJECTIVE:** Is high PINK1 expression associated with ageing in granulosa cells as well as assisted reproductive technology (ART) outcome, and what is the underlying mechanism of action of PINK1?.

**DESIGN:** Experimental laboratory study.

**MATERIALS AND METHODS:** In a prospective study, fresh granulosa cells were obtained from 48 women aged 20–40 years who underwent IVF with embryo transfer and who were divided into two groups: the diminished ovarian reserve (DOR) group (n = 20) and the control group (n = 28). Patient characteristics including age, infertility duration, body mass index, FSH, anti-Müllerian hormone (AMH) and cumulus cell PINK1 expression levels, autophagy, mitochondrial mass were analysed.

**RESULTS:** The DNML in the DOR group is activated and the PINK1 is translocated to the outer membrane of the mitochondria, and the formation of lysosomes is increased, thereby increasing the mitophagy. We also observed a significant reduction in the mass of the mitochondria in the DOR group and a severe imbalance in mitochondrial dynamics.

**CONCLUSIONS:** High PINK1 expression levels in cumulus cells were related to ageing, which may be involved in the clinical outcome of ART by promoting cell death and affecting mitochondrial function.

**PREGNANCY OUTCOMES OF PATIENTS WITH A CONGENITAL DIDEPHYS UTERUS: AN ANALYSIS OF 76 WOMEN FOLLOWING IN VITRO FERTILIZATION EMBRYO TRANSFER.**



Jingzi Xiao, Master, Xihong Li, MD./Ph.D, Yan Ouyang, MD./Ph.D, Yuyao Mao, Master Reproductive and Genetic hospital of Citic-Xiangya, Changsha, China.

**OBJECTIVE:** To evaluate the pregnancy outcomes in women with a didelphys uterus after in vitro fertilization-embryo transfer (IVF-ET).

**DESIGN:** A retrospective analysis.

**MATERIALS AND METHODS:** Seventy six women with a didelphys uterus who obtained clinical pregnancies via IVF-ET from September 2005 to December 2017 were retrospectively analyzed. The pregnancies included 56 cases of singleton pregnancies and 20 cases of twin pregnancies. In addition, there was 1 case of monozygotic twins among the twin pregnancies, Pregnancy outcomes including the rates of preterm delivery, cesarean section, live birth and perinatal mortality, birth weight, etc were analyzed.

**RESULTS:** In the patients with a didelphys uterus, the total miscarriage rate was 18.4% (14/76): the early pregnancy loss rate was 15.8% (12/76), and the late miscarriage rate was 2.6% (2/76). The rates of preterm delivery and term delivery were 27.6% (21/76) and 53.9% (41/76), respectively.

The number of babies born was 75, including 67 cases of live births and the live birth rate was 76.3% (58/76) (80.4% in singleton (45/56) and 65% in twin (13/20) pregnancies). The overall perinatal mortality was 10.7% (8/75), including 2 cases of still birth and 6 cases of neonatal death. There was a high cesarean section rate with 75.8% (47/62), and the rate of low live birth weight was 34.3% (23/67). Furthermore, the rate of very preterm birth was 11.3% (7/62) and the average gestational age at delivery was  $31.5 \pm 7.5$  weeks of gestation.

Among the twin pregnancies, there was 1 case received selective reduction, unfortunately, the women suffered a miscarriage in the 2nd month of gestation.

**CONCLUSIONS:** The pregnancy outcomes of a didelphys uterus in women who underwent IVF-ET were associated with an increased incidence of premature delivery, perinatal mortality, low live birth weight and low gestational weeks at delivery, but the live birth rate was relatively satisfactory.

TABLE. Pregnancy outcomes of didelphys uterus

Number	76
Miscarriage rate	18.4%(14/76)
early pregnancy loss	15.8%(12/76)
late miscarriage	2.6%(2/76)
Preterm delivery	27.6%(21/76)
Term delivery	53.9%(41/76)
Babies born	75
Live births	67
Perinatal mortality	10.7%(8/75)
Caesarean section rate	75.8%(47/62)
Live birth rate	76.3%(58/76)
Gestational age at delivery	$31.5 \pm 7.5$
<37week	33.9%(21/62)
20-32week	11.3%(7/62)
Live birth weight	$2550 \pm 650$
>2500g	65.7%(44/67)
<2500g	34.3%(23/67)

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#### IS FIRST TRIMESTER SUBCHORIONIC HEMORRHAGE ASSOCIATED WITH ADVERSE PREGNANCY OUTCOMES AFTER IN VITRO FERTILIZATION?

Kelsey Anderson, MD, Emily S. Jungheim, MD, MSCL, Patricia T. Jimenez, MD, Kenan Omurtag, MD, Washington University School of Medicine, St. Louis, MO.



**OBJECTIVE:** To determine the association between incidental SCH on ultrasound and pregnancy outcomes in IVF pregnancies.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** This was a retrospective cohort study of women identified from a first-trimester ultrasound database kept for IVF pregnancies from 2009 to 2017. Women with a viable first trimester pregnancy after fresh or frozen embryo transfer were included. Exclusion criteria were absence of heartbeat on ultrasound, gestational carriers, women who used donor eggs or who had a multiple gestation pregnancy. The primary outcome was live birth and secondary outcomes included spontaneous abortion, preterm delivery and infant weight at delivery. Appropriate bivariate analyses were performed followed by a multivariate regression model to further investigate associations between significant covariates and outcomes. All analyses were performed in SPSS.

**RESULTS:** 1004 women met criteria and 18.6% had a SCH. In bivariate analysis, SCH was not risk factor for decreased live birth (87.5% vs 90.2%, OR 0.7, 95% CI 0.2-1.1) or increased preterm birth (90.1% vs 85.9%, OR 0.7, 95% CI 0.4-1.2) or SAB (12.5% vs 9.3%, OR 1.4, 95% CI 0.9-2.3) There was also no difference in fetal weight with those with SCH (3334g vs 3269g,  $p=0.224$ ) and only increasing maternal age was negatively associated with live birth (32.8 vs 34.7  $p<0.001$ ). In multivariate regression analysis, all outcomes were still not statistically significant although those with SCH trended to have fewer live births (aOR 0.4, 95% CI 0.2-1.1) and higher rates of SAB (aOR 2.6, 95% CI 1.0-6.9).

**CONCLUSIONS:** Incidentally detected subchorionic hemorrhage on first trimester ultrasound is not associated with infant birth weight or probability of live birth or preterm birth after IVF. This information may be reassuring to IVF patients with SCH and otherwise viable pregnancy noted on first trimester ultrasound.

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#### PEROXIREDOXIN 4, A NEW OXIDATIVE STRESS MARKER IN FOLLICULAR FLUID MAY PREDICT IVF OUTCOMES.

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**OBJECTIVE:** For better predicting *in vitro* fertilization (IVF) outcomes, it is necessary to identify some non-invasive and sensitive markers. Studies indicated that oxidative stress status in patients was closely associated with IVF outcomes, while the results are still controversial. Prdx4 as one member in Prdx family, can catalyze the reduction of reactive oxygen species. While little data on the relationship of Prdx4 and female reproduction were demonstrated.

**DESIGN:** Our study is a prospective clinical study.

**MATERIALS AND METHODS:** All participants were recruited in the center of clinical reproductive medicine from September 2017 to December 2018. Infertile women with either tubal factor or male factor ( $n = 138$ ) undergoing controlled ovarian hyperstimulation and IVF were recruited in our study. FF samples from patients were collected on the day of oocyte collection and then centrifuged and frozen up for analysis. Prdx4 concentration in FF were measured in each participant. Furthermore, the correlation between Prdx4 level and IVF outcomes, such as clinical pregnancy rate and oocyte quality was analyzed. And subsequently, we divided all participants into three groups according to their levels of Prdx4 in FF (low, moderate and high group), then the clinical pregnancy rate and oocyte quality outcomes were all analyzed.

**RESULTS:** The pregnant women had higher levels of Prdx4 in FF than non-pregnant women. Prdx4 was positively correlated with oocyte fertilization rates ( $r = 0.326$ ;  $p = 0.013$ ) and good quality embryo rates ( $r = 0.334$ ;  $p = 0.011$ ). Furthermore, we found the pregnancy rate was positive correlated to Prdx4 level with a concentration dependent manner in three groups (pregnancy rate were 28.1%, 46.8% and 70.3% in low, moderate and high group, respectively). In the oocyte quality outcomes, the fertilization rates were significantly higher in the high group than the low group ( $p < 0.01$ ), and the good quality embryo rates of moderate ( $p < 0.01$ ) and high ( $p < 0.01$ ) groups were significantly higher than the low group.

**CONCLUSIONS:** Our results provide evidence that the upregulated expression of antioxidants in IVF patients follicular fluid (FF), such as Prdx4, tend to increase the potential pregnancy via oocyte quality mechanism.

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#### FOLLICULAR FLUID (FF) CONCENTRATION OF ANTI-MÜLLERIAN HORMONE (AMH) IN WOMEN PURSUING IN VITRO FERTILIZATION (IVF): VARIABILITY AND PREDICTORS.

Caitlin R. Sacha, MD,<sup>a</sup> Lidia Mínguez-Alarcón, PhD,<sup>b</sup> Jorge E. Chavarro, MD, Sc.D.,<sup>c</sup> Jennifer B. Ford, RN,<sup>b</sup> Patricia K. Donahoe, MD,<sup>d</sup> Irene Souter, MD,<sup>a</sup> Russ Hauser, MD, MPH, Sc.D.,<sup>b</sup> David Pépin, PhD,<sup>d</sup> <sup>a</sup>MGH Fertility Center and Harvard Medical School, Boston, MA; <sup>b</sup>Harvard T.H. Chan School of Public Health, Boston, MA; <sup>c</sup>Harvard School of Public Health, Boston, MA; <sup>d</sup>MGH Pediatric Surgical Research Laboratories, Boston, MA.



**OBJECTIVE:** To investigate the correlation of follicular fluid (FF) AMH concentrations between pre-ovulatory follicles within and between IVF cycles, and the association of FF AMH with demographics and reproductive characteristics.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** FF was analyzed from 2 or 3 pre-ovulatory follicles in 162 women (1 to 3 IVF cycles, 2-13 months apart) enrolled in the Environment and Reproductive Health (EARTH) Study at Massachusetts General Hospital Fertility Center (2010-2016). AMH concentration was quantified from a total of 217 cycles using a sandwich enzyme-linked immunosorbent assay (ELISA) method and corrected for sample volume. Spearman correlation was used to assess the correlation of FF AMH concentrations between follicles, and intra-class correlation (ICC) was calculated to

assess variability of mean cycle FF AMH concentrations between IVF cycles for each woman and between participants. Mean cycle FF AMH concentrations were then divided into tertiles (T1-T3), and Kruskal-Wallis and  $\chi^2$ -tests were applied as appropriate to explore associations of demographic and reproductive characteristics across tertiles.

**RESULTS:** The mean FF AMH concentration was 1.20 ng/ml (range=0 to 24.0 ng/ml). There was high correlation between follicles within each IVF cycle (Spearman  $r=0.78$  to  $0.86$ ), and ICC indicated low within-woman variability of mean cycle FF AMH concentrations [0.87 (95% CI 0.81 to 0.92)]. Compared to women in T1 of FF AMH concentrations (0.2 ng/mL), on average women in T3 (2.3 ng/mL) were younger (mean age in T3=33.5 vs. T1=36.0 years,  $p=0.04$ ), leaner [mean body mass index (BMI) in T3=22.4 vs. T1 24.5  $\text{kg/m}^2$ ,  $p=0.04$ ], had higher serum AMH concentrations (mean in T3=0.6 vs. T1=0.1 ng/mL,  $p=0.0001$ ), and lower day-3 follicular stimulating hormone (FSH) levels (mean T3=6.4 vs. T1= 7.0 IU/L,  $p=0.03$ ). Although most diagnoses were similar across tertiles of FF AMH concentrations, as expected, women in T3 were more often diagnosed with ovulatory disorders compared to women in T1 (T3=17% vs. T1=4%,  $p=0.30$ ). Smoking, education, and peak estradiol levels were not significantly associated with pre-ovulatory FF AMH concentrations.

**CONCLUSIONS:** We observed that pre-ovulatory FF AMH concentrations are highly correlated within an IVF cycle and that within-woman variability is low across cycles, suggesting that a dominant follicle's AMH concentration may reflect a woman's overall FF AMH concentration. Furthermore, mean cycle FF AMH concentrations were associated with other markers of ovarian reserve, suggesting a possible role in predicting future reproductive outcomes.

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#### IMPACT OF ACUPUNCTURE ON OUTCOMES FOLLOWING FROZEN EUPLOID BLASTOCYST

**ESET.** Nancy L. Bossert, PhD,<sup>a</sup> Hannah Van Der Geest, BS,<sup>a</sup> April Batcheller, MD,<sup>a</sup> William B. Schoolcraft, MD,<sup>b</sup> Jason E. Swain, PhD.<sup>c</sup> <sup>a</sup>CCRM Minneapolis, Edina, MN; <sup>b</sup>Colorado Center for Reproductive Medicine, Lone Tree, CO; <sup>c</sup>CCRM Fertility Network, Lone Tree, CO.



**OBJECTIVE:** The use of acupuncture in IVF has gained widespread acceptance, with numerous clinics offering this technique during embryo transfer. A clear consensus as to whether acupuncture improves outcomes does not exist and analysis is complicated due to confounding variables. The objective of this study was to determine if acupuncture provided at the time of frozen embryo transfers using single euploid blastocysts demonstrated any benefit compared to no acupuncture treatment.

**DESIGN:** Retrospective data analysis.

**MATERIALS AND METHODS:** Data were collected over a 4 year time period from 2015-2019. All lab conditions were the same for the duration of the study period and monthly quality control analysis confirmed no significant changes in pregnancy rate over time. Patients with single euploid blastocyst transfers were age-matched based on SART age groups and outcomes compared depending on whether the female had acupuncture treatment surrounding embryo transfer or not. All transfers were performed using a blastocyst of grade 3BB or better using a soft catheter and Embryoglu under ultrasound guidance. Data were analyzed using Fisher's Exact test,  $p<0.05$ .

**RESULTS:** No significant differences were apparent in either positive pregnancy rate or in ongoing/live birth rates between acupuncture or no acupuncture treatments in any age group examined.

**CONCLUSIONS:** Acupuncture does not appear to benefit rates of chemical pregnancy or ongoing pregnancy/live birth following transfer of a single frozen/thawed high quality euploid blastocyst. Future analysis, subdividing cycles based on day of blastocyst formation or quality of blastocyst transferred may provide additional insight. Furthermore, type of acupuncture

and specifics of the technique may have varied between patients and could be a confounding factor.

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#### FOLLICLE DIAMETER PREDICTS OOCYTE MATURITY BUT NOT FORMATION OF BLASTOCYSTS OR PLOIDY OF BLASTOCYSTS ARISING FROM MATURE OOCYTES OF DONORS.

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**OBJECTIVE:** Follicle size during controlled ovarian hyperstimulation is the only measure helpful to the clinician in deciding when to trigger final maturation. Controversy exists over whether the largest follicle(s) or the complete cohort of follicles best predicts outcome for IVF. Past efforts have concentrated on predictors of pregnancy and live birth following IVF with fresh transfer. Now there is increasing interest in retrieving oocytes for cryopreservation or for embryo production with preimplantation genetic testing for aneuploidy (PGT-A). It remains unclear whether there is a preferred size of follicles to obtain euploid oocytes or oocytes that will become euploid blastocysts.

**DESIGN:** Retrospective Analysis of Embryo Outcomes.

**MATERIALS AND METHODS:** Consented oocyte donors (N = 22) underwent retrieval of oocytes, one-by-one, from follicles with diameters measured during the retrieval. Oocytes were cultured individually, fertilized by intracytoplasmic sperm injection (ICSI) and monitored for development. Quality blastocysts, achieving Gardner grades of AA, AB, BA, BB or BC, underwent trophectoderm biopsy on days 5 or 6 and biopsies were sent to a commercial PGT-A lab in the US. Results of maturity, fertilization, development and ploidy were considered with reference to the size of the follicle from which the oocyte was retrieved. Analysis of data involved Student's T tests, and receiver operator characteristic (ROC) curves with significance determined using Mann-Whitney U test.

**RESULTS:** Oocytes were retrieved from follicles with measured diameters averaging 17.4 +/- 2.9 mm (N = 315). Of the oocytes, 80.4% had 1 polar body (MII), 9.8% had a germinal vesicle (GV) and 9.1% had neither a polar body nor a GV (MI). The sizes of the follicles from which these oocytes came were significantly different: MII, 18.3 +/- 2.2 mm; GV, 12.5 +/- 1.6 mm; and MI, 15.3 +/- 3.2 mm. ROC curves indicated that follicle diameter was a "grade A" predictor of GV oocytes (AUC = 0.96;  $P < 0.0001$ , Mann-Whitney U test) and a "grade B+" predictor of MII oocytes (AUC = 0.87;  $P < 0.0001$ , Mann-Whitney U test). Among MII oocytes, follicle size did not predict fertilization by ICSI (ROC AUC = 0.54, not significant), formation of quality blastocysts (ROC AUC = 0.53, not significant), or blastocyst ploidy (ROC AUC = 0.53, not significant).

**CONCLUSIONS:** Significant AUCs for ROC curves indicate that follicle diameter is an excellent predictor of oocyte maturity. However the diameter of the follicle from which an MII oocyte was retrieved did not predict its quality as assessed by its fertilizability with ICSI, its ability to develop into a quality blastocyst or its ploidy. Whereas follicle diameter can predict maturity of the oocytes retrieved from them quite well, follicle diameter is a poor predictor of oocyte quality including blastocyst ploidy. Since oocyte ploidy was not directly assessed, it remains unclear whether oocyte ploidy is associated with follicle diameter. However, with most embryo aneuploidy arising from errors in meiosis, we believe that follicle size is unlikely to be a good predictor of oocyte ploidy.

**SUPPORT:** None.

Age Groups	Acupuncture		No Acupuncture	
	+ $\beta$ hCG	Ongoing/Live Birth	+ $\beta$ hCG	Ongoing/Live Birth
Donor	23/28 (82.1%)	16/28 (57.1%)	18/23 (78.3%)	13/23 (56.5%)
<35 yrs old	66/76 (86.8%)	50/76 (65.8%)	73/86 (84.9%)	61/86 (70.9%)
35-37 yrs old	50/57 (87.7%)	43/57 (75.4%)	56/70 (80.0%)	48/70 (68.6%)
38-40 yrs old	51/70 (72.9%)	46/70 (65.7%)	47/50 (86.0%)	32/50 (64.0%)
>40 yrs old	17/21 (81.0%)	14/21 (66.7%)	13/20 (65.0%)	11/20 (55.0%)

**THE PREVIOUS CESAREAN DELIVERY DOESN'T AFFECT THE PROGNOSIS OF IVF-ET: A LARGE SAMPLE RETROSPECTIVE CASE CONTROL STUDY.** Shuo Yang, MD, Peking University Third Hospital, Beijing, China.



**OBJECTIVE:** To investigate whether the previous cesarean delivery would affect the treatment outcomes of multiparities accepted IVF/ICSI-ET.

**DESIGN:** Retrospective case control study of one reproductive medical center, from 1st Jan. 2009 to 31st Dec. 2015. The main outcome measures were Clinical pregnancy rate (CPR) and Live birth rate (LBR). the study group (Group 1) were patients with previous cesarean section history, the control group (Group 2) were patients with history of vaginal delivery.

**MATERIALS AND METHODS:** This is a retrospective case control study, and data collection protocol was approved by the hospital ethics. All patients were multiparities, the study group (Group 1) were patients with previous cesarean section history, the control group (Group 2) were patients with history of vaginal delivery. MatchIt package of R software was used for propensity score matching. The matching factors were age, number of oocytes retrieved and treatment time. According to 1:2 matching, the nearest neighbor matching method was used.

**RESULTS:** There were 461 cycles were included in the Group 1, and matched with 922 multiparities for the Group 2. The basic characteristics of patients refers to age, BMI, basal FSH and AFC were with no significantly difference. The initial dose of Gn was comparable between two groups, but the day of Gn injection was longer in control group and the total dose of Gn was higher too (11.3±2.4 vs. 11.9±2.7 P<0.001, 3328.5±1422.8 vs. 3595.9±1503.5, P<0.05, respectively). The number of oocytes pick-up, the rate of ICSI, MII oocyte and 2PN embryo were with no significantly difference. The cycle cancel rate was comparable between two groups. The number of embryos transferred were similar between two groups. The treatment outcomes refer to clinical pregnancy rate, implantation rate, early miscarriage rate, ectopic pregnancy rate and live birth rate. There was no uterine rupture or CS scar pregnancy in the study group.

**CONCLUSIONS:** The multiparities with history of Cesarean section accepted IVF/ICSI treatment, got similar outcomes compared with those with history of vaginal delivery.

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**OOCYTE DONOR IMPLANTATION AND PREGNANCY RATES PREDICT OOCYTE RECIPIENT PREGNANCY CHANCE IN AN EGG-SHARING DONATION PROGRAM.**



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**OBJECTIVE:** Studying oocytes from the same cohort submitted to different situations may provide greater insight into possible predictors of pregnancy in recipient cycles, allowing continuous improvement in outcomes moving forward. The objective of this study was to investigate which are the predictive factors of successful pregnancy in oocyte recipient intracytoplasmic sperm injection (ICSI) cycles in an egg-sharing donation program.

**DESIGN:** Historical cohort study.

**MATERIALS AND METHODS:** This study was performed in a private university-affiliated IVF center. Analyzed data were obtained via chart review of 1505 vitrified oocytes donated to 225 oocyte recipients undergoing 307 ICSI cycles, participating in an egg-sharing donation program, between January/2015 and May/2017. For that sample size, computed achieved post-hoc power was 100%, considering pregnancy achievement as the main outcome measure. Oocyte donors were between the age of 19 and 34 years, and recipients were between the age of 26 and 50 years. Adjusted generalized linear models were used to investigate the impact of oocyte donors and recipients characteristics on recipients' pregnancy achievement. The results are expressed as exponentiation of regression coefficient (ExpB), 95% confidence interval (CI), and p-value. A receiver operating characteristic (ROC) curve was constructed to investigate the predictive value of oocyte donor implantation rate on oocyte recipient pregnancy achievement.

**RESULTS:** Implantation rate in oocyte donor was highly correlated with pregnancy achievement in oocyte recipient cycles (ExpB: 1.181, CI: 1.138 – 1.226, p < 0.001). The ROC curve analysis demonstrated that the implantation rate in oocyte donor has a strong predictive value on the achievement of pregnancy in oocyte recipient (area under the curve: 0.98, CI: 0.95 - 0.99, p < 0.001). The achievement of pregnancy in oocyte donors and recipients were highly associated (ExpB: 54.6, CI: 28.1 – 105.8, p < 0.001), irrespective of oocyte recipient age. Oocyte donor age, body mass index, number of follicles,

retrieved oocytes, total dose of FSH administered and estradiol peak were not associated with oocyte recipient pregnancy achievement. In oocyte recipients, no association was found between the fertilization rate and the achievement of pregnancy, but the high-quality embryos rates on days 2 (ExpB: 3.397, CI: 1.635 – 7.054, p= 0.001) and 3 (ExpB: 6.629, CI: 1.185 – 37.092, p= 0.031), and blastocyst development rates (ExpB: 2.331, CI: 1.086 – 5.001, p= 0.030) were positively associated with pregnancy achievement.

**CONCLUSIONS:** Oocyte donor implantation rate and successful pregnancy, high-quality embryos rate, and blastocyst development rate predict pregnancy achievement in the oocyte recipient cycle. The strong association in pregnancy success between donors and recipients, and the lack of correlation between donor characteristics and cycles' outcomes, demonstrates the power of oocyte quality on the success of ICSI treatment.

Reference: NA.

SUPPORT: None.

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**NATURAL VERSUS MANAGED NATURAL CYCLE PRIOR TO FET: A RANDOMIZED CONTROLLED TRIAL.**



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**OBJECTIVE:** To determine whether a NC-FET is superior to a managed NC-FET.

**DESIGN:** This randomized controlled trial (RCT) included patients transferring a cleavage stage vitrified/warmed embryo in a natural cycle between January 2014 and December 2018. Women were randomized to wait for spontaneous luteinizing hormone (LH) surge (=NC) or to trigger ovulation by a single injection of human chorionic gonadotropin (hCG) (=managed NC). None of the patients received additional luteal phase support. The primary outcome was ongoing pregnancy rate (OPR). Secondary outcomes included biochemical pregnancy rate, early pregnancy loss and the number of visits, blood samples and ultrasonographic exams prior to embryo transfer.

**MATERIALS AND METHODS:** A total of 260 subjects were randomized (130 per study arm), with 229 actually starting monitoring for the study-FET (117 allocated to spontaneous LH surge and 112 to hCG injection). Seven patients needed to be switched to a hormonal replacement treatment protocol due to the absence of follicular development, 12 had no embryo available for transfer after warming and 37 had a spontaneous LH surge before hCG injection although they were allocated to the induced ovulation group (following the study protocol stating hCG injection could be performed once endometrial thickness reached 7mm and the dominant follicle 17 mm).

**RESULTS:** The study groups did not significantly differ in baseline patient characteristics, nor in relevant variables of the fresh cycle generating the vitrified cleavage embryo(s). Regarding the study-FET, circulating serum estradiol and progesterone values were similar at monitoring start in both groups, as was the last measured endometrial thickness before embryo transfer and the rate of single versus double embryo transfer. Intention-to-treat (ITT), nor per protocol (PP) analysis revealed any statistically significant difference in OPR, biochemical pregnancy rate or early pregnancy loss of NC-FET in terms of whether ovulation was spontaneous or triggered. Respectively, the primary outcome parameter OPR was 27.4% vs 25.9% (p=0.80) for ITT and 29.1% vs 30.2% (p=0.88) for PP analysis. However, patients in the managed NC-FET group had significantly fewer visits to the clinic and blood samples performed than the NC-FET group (3.03 ±1.16 vs 4.05±1.40, p<0.001).

**CONCLUSIONS:** This RCT adds new high quality evidence to the existing controversial literature concerning the performance of NC-FET versus managed NC-FET. Based on our results showing equal clinical outcomes for both protocols, we propose to by default plan patients for managed NC-FET, as this is associated to one visit less for blood sampling and is thus more patient-friendly.

Reference: NA.

SUPPORT: NA.

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**OXIDATIVE PARAMETERS IN FERTILIZATION MEDIUM OF CUMULUS - OOCYTE COMPLEX (COC) AS MEASURED BY THERMOCHEMILUMINESCENCE (TCL) MAY PREDICT TREATMENT OUTCOME IN IVF: PRELIMINARY RESULTS FROM A PROSPECTIVE STUDY.** Zofnat Wiener- Megnazi, MD,<sup>a</sup> Hadar Gluska, MD,<sup>b</sup>



Shirly Lahav - Baratz, PhD,<sup>c</sup> Idit Blais, MSc.,<sup>d</sup> Sergei Shnizer, MD, PhD,<sup>c</sup> Mara Koifman, MSc.,<sup>f</sup> David Ishai, MD,<sup>g</sup> Ido Feferkorn, MD,<sup>h</sup> Sivan Skvisky, MD,<sup>h</sup> Martha Dirmfeld, MD<sup>1</sup> <sup>a</sup>Head of Fertility and IVF Unit, Haifa, Israel; <sup>b</sup>Affiliation not provided; <sup>c</sup>Head of IVF lab., Haifa, Israel; <sup>d</sup>Carmel Medical Center, Haifa, Israel; <sup>e</sup>Carmel Diagnostics, Kiryat-Tivon, Israel; <sup>f</sup>Carmel Medical Centre IVF Lab, Haifa, Israel; <sup>g</sup>Senior consultant, Haifa, Israel; <sup>h</sup>Carmel medical center, Haifa, Israel; <sup>1</sup>Faculty of Medicine Technion, Haifa, Israel.

**OBJECTIVE:** To evaluate a possible association between oxidative parameters in COC medium as measured by Thermochemiluminescence (TCL) assay and outcome parameters in IVF.

**DESIGN:** A prospective cohort study.

**MATERIALS AND METHODS:** Sixty four women undergoing a fresh IVF cycle using conventional oocyte insemination during 2017-2019 participated in the study. COCs were incubated in a well containing 680  $\mu$ l of culture media for approximately 4-6 hours. Immediately prior to addition of semen, 20  $\mu$ l of COC culture medium were removed from each dish, examining for each sample 4 parameters: TCL amplitudes, after 50 seconds (TCLH1), 150 seconds (TCLH2), 250 seconds (TCLH3) and TCL ratio ((TCLH3-TCLH1)/100). TCL amplitudes were measured as counts per second (CPS).

**RESULTS:** We examined 97 COC fertilization media. Mean patients' age was 38  $\pm$  4.7 years. Mean number of aspirated oocytes, COCs per well and number of wells per patient were 6.2  $\pm$  3.7, 4.6  $\pm$  2.06 and 1.48  $\pm$  0.5 respectively. Of 64 IVF cycles, in one cycle (1.5%) oocytes were not fertilized, in 8 cycles (12.7%) no embryos developed and in another 8 (12.7%) cycles, all embryos were frozen. Altogether fresh embryos were transferred in 46 cycles. Twenty one pregnancies were achieved (33.3% per started cycles, or 45.9% per embryo transfer cycle). In order to find an optimal cutoff that would distinguish between TCL values that were associated with higher chances of pregnancy, Youden index was used. A discriminatory TCLH2 value of > 62.9 CPS was associated with higher chances for pregnancy (46.5% vs. 8.3%, OR=9.6, 95% CI(1.13-80.7) (p=0.03)). This value had a 95.2% sensitivity (95% CI=76.2-99.9), 32.4% specificity (95% CI=17.4-50.5), a positive predictive value of 46.5% (95% CI=31.2-62.3) and a negative predictive value of 91.7% (95% CI=61.5-99.8). No association was found between TCL parameters, regrading patient's age and number of aspirated oocytes. Multivariate analysis, correcting for age and number of aspirated oocytes, revealed that TCLH2 >62.9 was the only significant variable associated with the occurrence of pregnancy (p<0.03).

**CONCLUSIONS:** Oxidative parameters of COC medium may affect the likelihood of pregnancy. Measurement of oxidative parameters may serve as a potential aid in prediction of treatment outcome.

**P-218** Tuesday, October 15, 2019 6:30 AM

#### **IVF PREGNANCY RATES IN WOMEN UNDERGOING ACUPUNCTURE VS. CONTROLS.**

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**OBJECTIVE:** To compare IVF pregnancy rates and early pregnancy outcomes in women receiving acupuncture treatment compared to controls.

**DESIGN:** Prospective randomized study.

**MATERIALS AND METHODS:** Women ages 21 to 42 years who were seeking *in-vitro* fertilization and embryo transfer (IVF-ET) were recruited for the study. Women were excluded if they were currently using alternative therapies such as acupuncture, herbal supplements or had a contraindication to needle insertion at the acupoints. Fifty participants were enrolled and were randomized by computer to either the treatment group or control group. Those assigned to the treatment group received three sessions of acupuncture during the IVF-ET process; the control group received standard IVF treatment. The three sessions of acupuncture occurred on days 6, 7 or 8 of gonadotropin stimulation, and approximately 1 hour prior to embryo transfer and within 48 hours after the embryo transfer. Acupuncture was performed by one certified clinician following a protocol adapted from a Delphi Consensus process developed specifically for patients undergoing IVF. Differences between the two groups (acupuncture vs control) were determined using Chi-square test for the variable, pregnancy status and Mantel-Haenszel Chi-square test for the variable, pregnancy outcomes which included singleton gestation, twin gestation or early pregnancy loss. A p of < 0.05 was considered statistically significant.

**RESULTS:** We found no statistically significant differences between the acupuncture and control groups for pregnancy status ( $\chi^2 = 0.16$ , p= 0.69) and pregnancy outcomes ( $\chi^2 = 0.72$ , p= 0.53).

**CONCLUSIONS:** This study showed acupuncture based on Delphi Consensus Protocol at the above time points did not significantly affect the pregnancy rates in women undergoing IVF nor did it affect multiple birth or early pregnancy loss rates. Further studies with more subjects and/or more acupuncture sessions may be required to determine the impact of acupuncture on individuals receiving IVF treatment.

**SUPPORT:** Laura W. Bush Institute for Women's Health and University Medical Center.

**P-219** Tuesday, October 15, 2019 6:30 AM

#### **HIGH CONCORDANCE BETWEEN VAGINAL AND CERVICAL MICROBIOME ASSESSMENTS WITH INCREASING MICROBIAL DIVERSITY FOLLOWING TRANSFER OF A SINGLE EUPLOID BLASTOCYST.**

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**OBJECTIVE:** To characterize the microbiome of the vagina and cervix in infertile patients undergoing in vitro fertilization (IVF) and evaluate with respect to pregnancy outcomes following single embryo transfer (SET) of a euploid blastocyst.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** All patients initiating an autologous IVF cycle with plans to utilize preimplantation genetic testing for aneuploidy (PGT-A) and undergo SET in a frozen embryo transfer (FET) cycle were eligible for inclusion. Patients with > 1 prior failed IVF cycle and recent antibiotic use were excluded.

Ovarian stimulation, oocyte retrieval, intracytoplasmic sperm injection, extended culture, blastocyst biopsy for PGT-A, and vitrification were performed per routine protocol. Subjects with a euploid blastocyst underwent endometrial preparation for FET. Cervical and vaginal swabs were collected during the mid-proliferative phase and on day of transfer. Pregnancy outcomes were accrued.

Cervical and vaginal swabs underwent DNA isolation and next-generation sequencing of the V4 region of the bacteria-specific 16S ribosomal RNA gene using the Illumina NextSeq. The sequences were assigned to operational taxonomic units using the RDP classifier with confidence cutoffs of 0.8 in the QIIME package. All samples were assigned a Shannon diversity index (SDI) and categorized as  $\geq$  90% lactobacillus dominant (LBD) versus not (NLBD). Intra-patient correlation of cervical and vaginal specimens was assessed. Logistic regression was performed to account for age. The primary outcome of interest was ongoing pregnancy (presence of a fetal heartbeat at 8 weeks' gestation). P<0.05 was considered statistically significant.

**RESULTS:** Twenty-one subjects (mean age 35.1  $\pm$  3.9, body mass index 29.2  $\pm$  6.6, and antral follicle count 15.2  $\pm$  7.3) consented to participation and underwent oocyte retrieval. Fifteen subjects (71.4%) made at least one euploid blastocyst. Cervical and vaginal specimens were highly correlated in the mid-proliferative phase and on day of transfer, with an intra-patient correlation of 0.93.

Of the 15 FETs, there were 9 ongoing pregnancies, 1 biochemical loss, and 5 negative pregnancy tests. Increasing species diversity of the vaginal microbiome on the day of FET, as reflected by SDI, was negatively associated with ongoing pregnancy (correlation coefficient -4.3, P=0.03). There was a non-statistically significant trend towards lower ongoing pregnancy rates with a NLBD microbiome.

**CONCLUSIONS:** Increasing species diversity negatively impacts ongoing pregnancy rates following transfer of a euploid blastocyst. In addition, there is a trend towards lower ongoing pregnancy with a NLBD microbiome. Vaginal and cervical microbiome assessments were highly correlated indicating that vaginal samples alone may be sufficient. Recruitment for the study remains underway.

**P-220** Tuesday, October 15, 2019 6:30 AM

#### **RISK OF PREGNANCY FAILURE IN AN OPTIMIZED UTERINE ENVIRONMENT: LIVE BIRTH RATE FROM PGT-A EUPLOID EMBRYOS IN A PROVEN UTERUS.**

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**OBJECTIVE:** Assess the magnitude of the pregnancy failure rate from the transfer of euploid embryos after Pre-implantation Genetic Testing for Aneuploidy (PGT-A) into a proven uterine environment. An optimal uterine environment, or

proven uterus, is defined as a live birth ensuing from a multiple embryo transfer (MET) where at least one embryo results in a successful birth. While PGT-A has been shown to increase the success rate of live birth, the remaining failure rate can still be due to a multitude of factors. This study seeks to control for the uterine environment to quantify the remaining chance of failure for the cycle.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Using all completed MET cycles at our academic fertility center at Yale and from Boston IVF from 2012-2017, we identified 3,680 embryos transferred in 1,726 cycles to a proven uterus. Percentage of embryos not implanted in a proven receptive uterus utilizing PGT-A was compared to those transferred without using PGT-A. Difference of proportions analysis using a one-tailed Z-test compared the percentage lost among proven-uterus that utilized PGT-A to cycles that did not.

RESULTS: Based on the data from these two centers, forty-six of 1,726 cycles (2.7%) transferred multiple embryos after PGT-A to a proven uterus. These cycles resulted in 30/87 embryos (34.5%) failing to result in a live birth. For non-PGT-A embryos transferred to a proven receptive uterus (MET-only cycles), 1,973/3,593 embryos (54.9%) did not result in a live birth. The difference of proportion of embryos failing to result in a live birth between proven uterus cycles that utilized PGT-A was statistically significant when compared to controls without PGT-A ( $p = 0.00008$ ).

CONCLUSIONS: The false negative rate of PGT-A testing, whereby euploid embryos transferred into a receptive uterus (since in the same cycle sibling embryos had implanted and produced a live birth), is 34.5%. This study eliminated the endometrium as a cause for the failed implantation of euploid embryos and adds support to the inability of PGT-A to completely correctly identify suitable embryos for transfer. Ongoing research may help establish the false-negative rate of PGT-A and understand whether genetic mutations, non-chromosomal or developmental errors could be responsible for the lack of implantation and live birth.

SUPPORT: None.

P-221 Tuesday, October 15, 2019 6:30 AM

#### MITOCHONDRIAL REPLACEMENT THERAPY GIVE NO BENEFITS TO PATIENTS OF ADVANCED MATERNAL AGE.

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OBJECTIVE: To determine if mitochondrial replacement therapy (MRT) could increase blastulation rates, euploidy rates and pregnancy rate in patients of advanced maternal age (AMA).

DESIGN: The study period was from December 2015 to November 2018. Patients were informed and consent to possible risks and the experimental protocol was approved by ethics committee of local association of reproductive medicine. Inclusion criteria were: (1) no less than two failed previous IVF attempts, (2) low blastulation rates or recurrent embryo arrest, (3) low number or absence of euploid embryos; (4) age  $\geq 37$  years.

MATERIALS AND METHODS: 30 patients (37-47 years old, Mean age was  $42 \pm 2$  years) participated in this study. Five types of MRT (germinal vesicle transfer (GVT), MI spindle transfer (MIST), MII spindle transfer (MIIST), polar body 1 genome transfer (PB1GT) and pronuclear transfer (PNT)) were assisted by HVJ-E cell fusion kit. Intracytoplasmic sperm injection (ICSI) had been performed for all cases. If possible, reverse reconstitutions were done. Embryos obtained after reconstitution were cultured until blastocyst stage in time-lapse incubator, were biopsied for array comparative genomic hybridization (aCGH) or next generation sequencing (NGS) analysis and then were vitrified.

RESULTS: After performing various types of MRT, 109 zygotes were obtained, that resulted in 33 blastocysts (30%); 3 of which (one per patient) were euploid (2.7%). One try of elective single embryo transfer (eSET) of thawed embryo was done for each of three patients. Positive hCG level ( $> 100$  mIU/mL) and following heartbeating were confirmed only for one patient (42 y.o., PNT group). The healthy baby boy was born on 15th of March 2018 by Caesarean section. After unsuccessful attempt of MRT, one of 30 patients (41 y.o.) had an euploid embryo from conventional aCGH cycle using donor sperm and the other patient (45 y.o.) became spontaneously pregnant and gave birth to a healthy baby at full term.

Zygotic cytoplasts of woman of AMA were competent enough to support normal embryo development when carry young karyoplasts: there were 41% blastulation rates and 70% euploidy rates for reversely reconstituted zygotes.

CONCLUSIONS: Pregnancy rate after applying MRT was lower than 1%, thereby patients of AMA should be advised not to undergo such procedures in order to increase the number of euploid embryos or pregnancy rate.

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#### EFFECT OF THE VAGINAL MICROBIOME ON THE PREGNANCY RATE IN PATIENTS UNDERGOING ASSISTED REPRODUCTION TECHNIQUES.

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OBJECTIVE: Recent evidence seems to indicate that there is a relationship between the vaginal microbiome and fertility, however, it is unknown whether this effect occurs when couples undergo ART. The aim of this study is to investigate if the vaginal microbiome of the day of the transfer in couples undergo ART could affect the pregnancy rate.

DESIGN: A prospective study was performed. Patients attended to our clinic were recruited from May 2017 to April 2018. We included 31 patients performing PGT-A at blastocyst stage, elective embryo vitrification and single chromosomally normal embryo transference. Vaginal samples were collected at the moment of the transfer from the posterior sac of the vagina (patients with positive pregnancy test  $n=17$ , patients not pregnant  $n=14$ ).

MATERIALS AND METHODS: DNA was extracted using the PureLink Microbiome DNA Purification kit. Sequencing and bioinformatics analysis were performed according to Illumina Metagenomics protocol using the NexteraXT library on the Miseq instrument. The analysis of the rRNA16S V3V4 region and the bioinformatic tools QIIME2, MicrobiomeAnalyst and Phyloseq have been used to determine the microbiome.

RESULTS: We obtained the vaginal microbiome from the 31 patients which 17 achieved a positive pregnancy test and 14 not achieved. A total of 7,089,699 sequences were analyzed and 116 OTUS (97% similarity) were identified. Regarding diversity analysis, the alpha diversity index Chao1 is higher in patients who did not achieve pregnancy ( $p < 0.05$ ). As for, the beta index a lower diversity was obtained in vaginal samples from patients that achieve a pregnancy, although without reaching statistical significance ( $p=0.08$ ). Moreover, we analysed the taxonomic composition of the samples. We showed a dominance of *Lactobacillus* with predominance of *L. crispatus* (47.05%), *L. helveticus* (22.85%), *L. iners* (21.95%) and *L. jensei* (3.97%). The patients who achieved pregnancy, have higher average percentage of the genus *Lactobacillus* compared to those who do not. There is a correlation between the vaginal microbiomes dominated by *Lactobacillus* and greater reproductive success against another profile not dominated by *Lactobacillus* and with the presence of *Gadnerella*.

CONCLUSIONS: The patients who achieve pregnancy have a lower diversity than who do not achieve it. These results suggest that the presence of a low diverse vaginal microbiome predisposes to the pregnancy. Also, *Lactobacillus* in the vaginal microbiome seems to be key to embryo implantation and the genus *Gadnerella* has a negative influence on pregnancy.

#### IVF OUTCOME PREDICTORS - OVARIAN STIMULATION

P-223 Tuesday, October 15, 2019 6:30 AM

#### TRIGGER DAY FOLLICLE-STIMULATING HORMONE (FSH) "BOOST" INCREASES COSTS BUT DOES NOT IMPROVE OUTCOMES IN PATIENTS UNDERGOING IVF WITH PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A).

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OBJECTIVE: An FSH boost on trigger-day may improve outcomes in fresh transfers by enhancing folliculogenesis and endometrial receptivity. As more patients are freezing all of their embryos, the endometrial effect is less of a concern, but folliculogenesis remains relevant. Recent reports<sup>1,2</sup> conflict over the clinical effects of an FSH boost. We therefore examined the effect of an FSH boost on oocyte retrieval, quality, and development, specifically in patients undergoing PGT-A.

DESIGN: Retrospective cohort.

MATERIALS AND METHODS: Patients undergoing GnRH-antagonist IVF cycles from 1/2015 through 12/2018, were separated into two groups for comparison: those receiving only trigger injections on trigger day (NB), and those also receiving an FSH boost (B). Demographics, days of gonadotropin, #oocytes retrieved, #mature, #blastocysts, and #euploid embryos, were compared (Student's t-test or  $\chi^2$ ).

		Days of Gndtpn	#Oocytes	#Mature Oocytes	Fert Rate	#Blastocysts	#Euploid
<35	B	11.0+/-1.8*	17.1+/- 10.9	13.5+/-9.6	76.3%	5.9+/-5.1	2.8+/-2.5
	NB	9.8+/-1.9*	15.0+/-8.9	11.7+/-6.6	76.6%	6.0+/-4.3	2.5+/-2.2
35-37	B	11.3+/-1.9*	12.1+/-7.8	9.3+/-6.6	75.7%	4.2+/-4.2	2.0+/-2.3
	NB	9.7+/-1.8*	11.5+/-7.8	9.1+/-6.3	79.4%	4.2+/-3.2	1.6+/-1.9
38-40	B	11.4+/-1.7*	10.6+/-7.4	8.5+/-6.5	71.6%*	3.6+/-3.3	1.2+/-1.3*
	NB	9.6+/-1.9*	9.8+/-7.2	7.7+/-5.9	79.0%*	3.7+/-2.9	0.9+/-1.1*
41-42	B	11.1+/-2.0*	8.5+/-6.2	6.9+/-5.3	69.4%	2.5+/-2.4	0.5+/-0.8
	NB	10.2+/-1.9*	7.5+/-5.2	6.1+/-4.4	75.2%	2.5+/-2.1	0.4+/-0.6
>42	B	11.7+/-1.9*	7.4+/-6.8	5.5+/-4.3	71.2%	1.5+/-1.9	0.1+/-0.3
	NB	9.8+/-1.9*	6.7+/-5.1	5.3+/-4.1	76.7%	2.5+/-1.6	0.2+/-0.4

\* B and NB were significantly different (P < 0.05)

**RESULTS:** Both groups were stratified into SART registry age groups. Initial comparisons between the groups, without matching for trigger day estradiol levels (E2Trig), revealed a selection bias. B patients had weaker responses, with lower estradiol levels and fewer eggs. In order to examine the effect of B in each age group, we created NB comparison groups with E2Trig values indistinguishable from the B's. This was done by randomly selecting NB patients from the same age group and E2Trig stratum as B.

1394 patients were included in this matched comparison. 697 received B, and 697 did not. B patients had significantly more days of gonadotropin administration (~1 day) than NB patients. There were no consistent differences for #oocytes retrieved, #mature, fertilization rate, #blastocysts, or #euploid embryos (see table). Overall, costs associated with B amounted to \$276,923, or close to \$400 per patient.

**CONCLUSIONS:** No benefit of B was found for #oocytes retrieved, #mature, fertilization rates, #blastocysts, or #euploid embryos. There are significant cost savings associated with NB.

References: 1. Lamb JD, Shen S, McCulloch C, Jalalian L, Cedars MI, Rosen MP. Follicle-stimulating hormone administered at the time of human chorionic gonadotropin trigger improves oocyte developmental competence in in vitro fertilization cycles: a randomized, double-blind, placebo-controlled trial. *Fertility and sterility*. 2011 Apr;95(5):1655-60.

2. Juneau CR, Morin SJ, Franasiak JM, Landis JN, Molinaro TA, Scott RT. A follicle-stimulating hormone boost administered at the time of human chorionic gonadotropin trigger does not affect IVF cycle outcomes. *Fertility and sterility*. 2016;106(3):e189-e90.

treatment type, drugs used for ovulation triggering and luteal phase support) in the Cox proportional hazards models. In the FC analysis, log-binomial regression was adjusted for confounding factors by inverse probability of treatment weighting. Results for PP are presented as adjusted hazard ratios (HR) with 95% confidence intervals (CI) and for FC as relative risk (RR) with 95% CI.

**RESULTS:** 17,725 women received r-hFSH and 10,916 received u-hMG; overall 38,234 cycles were evaluated (r-hFSH, n=23,429; u-hMG, n=14,805). 77.1% of cycles were performed with a GnRH agonist. Overall, higher CP, higher OP and higher LB were observed with r-hFSH vs u-hMG. The adjusted HR (95% CI) (PP analysis) for LB was 1.10 (1.04, 1.16) and adjusted RR (95% CI) (FC analysis) was 1.09 (1.04, 1.15). For CP, the HR (PP) was 1.10 (1.05, 1.14) and RR (FC) was 1.09 (1.05, 1.13); and for OP, HR (PP) was 1.10 (1.04, 1.16) and RR (FC) was 1.09 (1.04, 1.15). Results in women receiving a GnRH agonist for LB (PP: 1.13 [1.07, 1.19]; FC: 1.14 [1.08, 1.20]), CP (PP :1.12 [1.07, 1.17]; FC: 1.09 [1.05, 1.13]), and OP (PP: 1.13 [1.07, 1.19]; FC: 1.09 [1.04, 1.15]) were similar to those in the overall population. Overall, no difference was observed for pregnancy loss per CP between r-hFSH and u-hMG (HR [95% CI] 1.07 [0.98, 1.17]); results were similar with GnRH agonist only (HR [95% CI]: 1.09 [0.99, 1.21]).

**CONCLUSIONS:** Real world data analysis of 38,234 ART treatments performed in 28,641 women showed higher LB, higher OP and higher CP after COS with r-hFSH vs u-hMG, after adjustment for confounding baseline and post-treatment variables. No difference was observed between treatments for pregnancy loss. As the majority of patients (77.1%) received a GnRH agonist, these patients were analyzed separately and similar results (higher LB, OP and CP) were observed.

**SUPPORT:** Merck KGaA, Darmstadt, Germany.

**P-224** Tuesday, October 15, 2019 6:30 AM

**EFFECTIVENESS OF RECOMBINANT HUMAN FOLLICLE-STIMULATING HORMONE (R-HFSH) VERSUS HUMAN MENOPAUSAL GONADOTROPIN (U-HMG) IN ASSISTED REPRODUCTIVE TECHNOLOGY (ART): A STUDY BASED ON GERMAN REAL-WORLD DATA.** Klaus F. Bühler, MD,<sup>a</sup> Sandra Guedes, PharmD, MSc,<sup>b</sup> Arthur Allignol, PhD, Dr,<sup>b</sup> Thomas D'Hooghe, MD, PhD,<sup>b</sup> Wilma Bilger, PhD,<sup>c</sup> Emmanuelle Boutmy, PhD, Dr,<sup>b</sup> Emilia Richter, MD, MSc,<sup>b</sup> Robert Fischer, MD,<sup>d</sup> <sup>a</sup>Centre for Gynaecological, Endocrinology, and Reproductive Medicine, Ulm and Stuttgart, Germany; <sup>b</sup>Merck Healthcare KGaA, Darmstadt, Germany; <sup>c</sup>Merck Serono GmbH, Darmstadt, Germany; <sup>d</sup>MVZ Fertility Center Hamburg GmbH, Hamburg, Germany.



**OBJECTIVE:** To compare clinical outcomes with r-hFSH (GONAL-<sup>®</sup>, Merck KGaA, Darmstadt, Germany) vs u-hMG (Menogon HP<sup>®</sup>, Ferring GmbH, Kiel, Germany).

**DESIGN:** Non-interventional study based on secondary use of data collected from 71 German IVF centers (1 Jan 2007 – 31 Dec 2012).

**MATERIALS AND METHODS:** Data were collected from RecDate, an electronic database certified by the German IVF Register. Women undergoing their first controlled ovarian stimulation (COS) cycle with r-hFSH or u-hMG were included; subsequent cycles were included if the initial treatment was used. Clinical pregnancy (CP), ongoing pregnancy (OP) and live birth (LB) were analyzed at 2 levels - per patient (PP) and per first stimulation cycle (FC). Pregnancy loss was analyzed per CP. Outcomes were also analyzed separately according to GnRH protocol received; as the rationale for GnRH antagonist use in Germany has changed since data collection (from use in poor responders to use in all) and because GnRH agonist protocols were predominantly used, only agonist findings are presented. In the PP analysis, between group differences were adjusted for using propensity scores (adjusted for age, BMI, infertility type, GnRH protocol, year of 1<sup>st</sup> cycle and IVF center) and by including potential time-dependent post-treatment confounding variables (duration of COS, ART

**P-225** Tuesday, October 15, 2019 6:30 AM

**A NOVEL FLEXIBLE PROGESTIN PRIMED OVARIAN STIMULATION PROTOCOL: COMPARISON OF PREGNANCY OUTCOMES WITH THE FLEXIBLE GNRH ANTAGONIST PROTOCOL IN AN OOCYTE DONATION PROGRAM.** Sule Yildiz, MD,<sup>a</sup> Engin Turkgeldi, MD,<sup>a</sup> Alper Eraslan, MD,<sup>b</sup> Berk Angun, MD,<sup>b</sup> Mustafa Baris Ata, M.D.<sup>c</sup> <sup>a</sup>Koc University Hospital, Istanbul, Turkey; <sup>b</sup>Dunya IVF Center, Kyrenia, Cyprus; <sup>c</sup>Koc University School of Medicine, Istanbul, Turkey.



**OBJECTIVE:** To compare a novel flexible progestin primed ovarian stimulation (fPPOS) protocol with the flexible GnRH antagonist protocol in an oocyte donation program.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Oocyte donors were started 225IU/day rFSH on cycle day 2-3, 0.25mg/day GnRH antagonist or 10mg/day medroxyprogesterone acetate (MPA) was started on stimulation day 7 or when the leading follicle reached 14mm, whichever came first. One mg leuprolide acetate was given when there were ≥ 3 follicles >17 mm. Oocytes were fertilized with the recipients' partners' sperm. Recipients were prepared in an artificial cycle, i.e. estradiol valerate 6 mg/day orally for >10 days, vaginal micronized progesterone was added 4 or 6 days before cleavage and blastocyst stage embryo transfers, respectively. Medications were continued until a negative pregnancy test or 10<sup>th</sup> gestational week. Data are defined with percentages or median (25<sup>th</sup> – 75<sup>th</sup> percentile), depending on variables. Non parametric tests and chi square test were used for comparisons.

**RESULTS:** 150 oocyte donors were included, 75 in each group. Donors in both groups were similar for age. None of them had premature ovulation and yielded similar oocyte and metaphase two oocytes with similar gonadotropin consumption. 86 women received oocytes from fPPOS and 105 women from

	MPA n=86	GnRH Antagonist n=105	P value
Median number of allocated MII oocytes (25 <sup>th</sup> -75 <sup>th</sup> percentile)	11 (11-12)	11 (10-12)	0.09
Number of fertilized oocytes (2PN) (25 <sup>th</sup> -75 <sup>th</sup> percentile)	8 (7-10)	9 (8-10)	0.25
Blastocyst transfers (%)	79/86 (91.9)	102/105 (97.1)	0.10
Number of embryos transferred			
One embryo (%)	7/86 (8.1)	2/105 (1.9)	0.12
Two embryos (%)	66/86 (76.7)	83/105 (79)	
Three embryos (%)	13/86 (15.1)	20/105 (19)	
Cleavage rate per MII oocyte % (25 <sup>th</sup> -75 <sup>th</sup> percentile)	77 (61-90)	73 (60-83)	0.16
Blastocyst rate per MII oocytes % (D5 ET)	42 (30-55)	45 (30-60)	0.22
Positive Pregnancy test (%)	67/86 (77.9)	81/105 (77.1)	0.90

GnRH antagonist cycles stimulated cycles. Recipients characteristics and outcomes are presented in the Table.

**CONCLUSIONS:** This novel fPPOS protocol seem to yield oocytes that has similar reproductive potential as oocytes from GnRH antagonist cycles. This new fPPOS protocol, is novel as progestin is not started simultaneously with gonadotropins as in prior studies of PPOS. fPPOS involves even less medication and can represent an inexpensive and patient friendly alternative when a fresh embryo transfer is not intended, e.g. oocyte cryopreservation, oocyte donation or PGT cycles, as well as anticipated over responders in whom a frozen embryo transfer would be safer and more effective.

**SUPPORT:** None.

#### P-226 Tuesday, October 15, 2019 6:30 AM

### DYDROGESTERONE FOR PREVENT LH SURGE YIELDS SIMILAR OUTCOMES TO GANIRELIX IN A SHARED OOCYTE DONOR CYCLE WITH SUBSEQUENT FROZEN-THAWED EMBRYO TRANSFER: COMPARISON OF DONORS AND

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**OBJECTIVE:** To compare pregnancy rate, clinical pregnancy rate and implantation rate of frozen-thawed embryo transfer (FET) cycles of donors and recipients.

**DESIGN:** A prospective randomized controlled trial.

**MATERIALS AND METHODS:** Between November 2017 to December 2018, we select 49 oocyte donors that would be submitted to ovarian stimulation in a shared egg donor cycle. All donors were younger than 35 years-old. Computerized randomization was conducted to assign participants into two treatment groups: hMG + dydrogesterone 10 mg 12/12h (DYG) (24 patients) or hMG + ganirelix acetate (25 patients). Oocyte maturation was triggered by administration of triptorelin. The collected mature eggs of each donor were divided half-way with a recipient and fertilized. All viable blastocysts were cryopreserved for later transfer. Only the first FET cycle was included in our study. We compared the number of oocytes and mature oocytes retrieved in the two groups. Fertilization rate (FR), number of blastocysts, pregnancy rate (PR), clinical pregnancy rate (CPR) and implantation rate (IR) of donors were compared separately from recipients.

**RESULTS:** The mean age of donors was 29.0 (DYG group) and 28.6 (ganirelix group). No cycle was cancelled because of premature LH surge. The mean number of oocytes and metaphase II (MII) were not different: 26.75 and 19.83 with DYG and 28.3 and 21.5 with ganirelix. For donors using DYG, FR was 74.1% and the mean number of blastocysts was 3.42. In ganirelix group, FR was 79.55% and 3.36 blastocysts were formed (not significant). There was also no difference in PR, CPR and IR: 79.2%, 62.5% and 63.4% for DYG and 76%, 58.3% and 55.8% for ganirelix. Analyzing recipients that received oocytes from DYG group donors, the FR was 84.3%, the mean number of blastocysts was 3.61, PR was 63.6%, CPR was 59.1% and IR, 46.5%. For recipients using oocytes from ganirelix group donors, FR was 85.9%, mean number of blastocysts was 3.6, PR was 66.7%, CPR was 54% and IR, 17%. There was no statistical significant difference between the groups for any outcomes of donors or recipients.

**CONCLUSIONS:** Ovarian stimulation using DYG for prevention of LH surge yields similar outcomes compared to ganirelix in shared oocyte donor cycle with subsequent FET in donors and recipients.

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### EFFECTIVENESS AND SAFETY OF BIOSIMILAR FOLLITROPIN ALFA IN WOMEN UNDERGOING ROUTINE OVARIAN STIMULATION WITH A GnRH ANTAGONIST: RESULTS FROM A GERMAN MULTI-CENTRE NON-INTERVENTIONAL STUDY.

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**OBJECTIVE:** To assess the effectiveness and safety of a biosimilar follitropin alfa (Ovaleap® Theramex UK Ltd) used for ovarian stimulation in routine clinical practice and in combination with a GnRH antagonist in women undergoing assisted reproduction technologies. Whilst Ovuleap demonstrated therapeutic equivalence to Gonal-<sup>®</sup> in a GnRH agonist protocol, to date there is no published data of Ovuleap's use with a GnRH antagonist.

**DESIGN:** Multicenter, prospective, non-interventional study, carried out at 34 specialized reproductive medicine centers across Germany from March 2016 to May 2017.

**MATERIALS AND METHODS:** 507 infertile women undergoing ART were screened. They were 18-40 years old, BMI <30 kg/m<sup>2</sup>, menstrual cycle duration 24 to 35 days, AMH ≥ 1 ng/mL undergoing first ovarian stimulation for IVF/ICSI and were treated with Ovuleap® using a GnRH antagonist protocol. Primary effectiveness outcomes were number of retrieved oocytes after ovarian stimulation therapy and clinical pregnancy rate. Secondary effectiveness outcomes were serum estradiol, endometrial thickness, number of metaphase-II oocytes, percentage fertilization rate, number of transferred embryos, and baby take-home rate. Maternal/fetal and neonatal adverse drug reactions (ADRs) were also collected. SAS version 9.4 was used for all statistical analyses.

**RESULTS:** 463 women received at least 1 dose of follitropin alfa were included in the final analysis. Mean age (SD) was 32.2 (4.1) and BMI was 23.4 (3.6). Mean (SD) total follitropin alfa dose was 1651.2±506.7 IU, and the median duration of administration was 9.0 days (range: 4-17 days). Mean number of retrieved oocytes was 11.7±7.2 (median=11.0; range 0-61). The mean (SD) number of transferred embryos was 1.8 (0.4). Clinical pregnancy rate/cycle was 35.6% (165/463) in the overall population and 41.4% (165/399) in women with embryo transfer. Baby take home rate was 31.8% (143/449) in women with oocyte retrieval and 36.1% (143/396) with ET. The twin and triplet pregnancy rate were 8.5% and 0.5%, respectively.

ADRs were reported for 40/463 women (8.6%). The most common ADRs were ovarian hyperstimulation syndrome (OHSS, n=23, 5.0%) and miscarriage (n=10, 2.2%). OHSS was rated mild in 14 (3.0%), moderate in 8 (1.7%), and severe in 1 (0.2%).

**CONCLUSIONS:** This real world data collected across multiple clinical sites support the existing evidence of effectiveness (number of oocytes retrieved, clinical pregnancy rate) and safety of ovarian stimulation with Ovuleap® for ART using a GnRH antagonist protocol. These results are consistent with those previously reported in randomized controlled clinical trials.

**SUPPORT:** The study was supported by Teva GmbH Germany.

#### P-228 Tuesday, October 15, 2019 6:30 AM

### THE IMPACT OF OVARIAN RESPONSE ON CLINICAL PREGNANCY AND DELIVERY RATES IN AN OOCYTE DONOR POPULATION.

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**OBJECTIVE:** In the general IVF population, it has been reported that an optimal window of ovarian response may exist, with live birth rates declining if fewer than 15 or greater than 20 oocytes are retrieved. The relationship between pregnancy outcomes and ovarian response has not been thoroughly investigated in oocyte donors. This study seeks to characterize the relationship between ovarian response, clinical pregnancy rates, and delivery rates in oocyte donors.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This study was performed at a large IVF practice. Oocyte donors who underwent their first oocyte retrieval followed by an embryo transfer in a recipient between January 1, 2012 and December 31,

TABLE 1. Clinical pregnancy rate and delivery rate based on ovarian response

	<15 oocytes retrieved	15-20 oocytes retrieved	>20 oocytes retrieved	
Clinical Pregnancy Rate	68.3%	67.8%	72.1%	Not significant (P=0.6126)
Delivery Rate	60.3%	60.6%	64.1%	Not significant (P=0.7204)

2018 were included. Ovarian response of donors was divided into three categories: fewer than 15 oocytes retrieved, 15 to 20 oocytes retrieved, and greater than 20 oocytes retrieved. Chi-square analysis was performed to assess differences in clinical pregnancy (defined as the presence of an intrauterine fetal heartbeat on ultrasound) and delivery rates based on ovarian response.

**RESULTS:** 510 donor retrieval cycles met inclusion criteria. Mean oocyte donor age was  $27.45 \pm 3.98$  years. The median number of eggs retrieved per cycle was 20 (IQR 15-28). Clinical pregnancy data was available for all patients (351 single embryo transfers, 159 double embryo transfers). Delivery data was available for 479 out of 510 patients (93.9%, 322 single embryo transfers, 157 double embryo transfers). 357 out of 510 patients (70%) achieved clinical pregnancies during the first embryo transfer cycle with use of donor oocytes. 298 out of 479 patients with complete delivery data (62.2%) achieved a live birth from their first transfer cycle with use of donor oocytes. Clinical pregnancy rates were 68.3% when fewer than 15 oocytes were retrieved, 67.8% when 15-20 oocytes were retrieved, and 72.1% when greater than 20 oocytes were retrieved. Delivery rates were 60.3% when fewer than 15 oocytes were retrieved, 60.6% when 15-20 oocytes were retrieved, and 64.1% when greater than 20 oocytes were retrieved. There were no statistically significant differences in clinical pregnancy rate ( $P=0.6126$ ) or delivery rate ( $P=0.7204$ ) based on ovarian response (Table 1).

**CONCLUSIONS:** In an oocyte donor population, the degree of ovarian response does not impact clinical pregnancy or delivery rates. These findings contradict earlier reports which demonstrated optimal outcomes when 15 to 20 oocytes were retrieved.

References: 1. SK Sunkara, V Rittenberg, N Raine-Fenning, S Bhattacharya, J Zamora, A Coomarasamy. Association between the number of eggs and live birth in IVF treatment: an analysis of 400,135 treatment cycles. *Hum Reprod.* 2011;26(7):1768-74.

2. MH van der Gaast, MJ Eijkemans, JB van der Net, EJ de Boer, CW Burger, FE van Leeuwen, BC Fauser, NS Macklon. Optimum number of oocytes for a successful first IVF treatment cycle. *Reprod Biomed Online.* 2006;13(4):476-80.

SUPPORT: None.

**P-229** Tuesday, October 15, 2019 6:30 AM

#### MILD VERSUS LONG LUTEAL AGONIST STIMULATION PROTOCOL IN NORMAL AND POOR RESPONDERS IN ASSISTED REPRODUCTION, A META-ANALYSIS OF RANDOMIZED STUDIES.



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**OBJECTIVE:** The long luteal agonist protocol is an established stimulation protocol in assisted reproduction. However, it has been criticized for its high cost, complexity and higher incidence of cyst formation and ovarian hyper-stimulation syndrome (OHSS). The mild stimulation protocol (as defined by the International Society for Mild Approaches in Assisted Reproduction, ISMAAR i.e. low dose FSH/HMG +/- oral compounds and GnRH antagonists) has been suggested as a simpler and cheaper alternative and various studies have been conducted with variable results (Nargund et al, 2007). The aim of this work was to evaluate both protocols in normal and poor responders in the light of evidence.

**DESIGN:** A meta-analysis of randomized controlled studies.

**MATERIALS AND METHODS:** A meticulous search of the literature was conducted searching the Medline database, the EMBase, the Cochrane library as well as hand searching relevant publications and proceedings of international congresses. A total of 195 studies were retrieved. The studies were evaluated independently by the first two reviewers and the differences

were settled by consensus with the third reviewer. Of the 195 studies, 5 fulfilled our inclusion criteria: 2 studies were conducted on normal responders and 3 studies on poor responders. The primary outcome measures were the clinical and ongoing pregnancy rates. The meta-analysis was conducted using the Stata/SE 11 program for windows with the Peto-modified Mantel-Haenszel method and the fixed effect model.

**RESULTS:** In normal responders, there were no significant differences between the mild and the long agonist stimulation protocols in clinical pregnancy [OR = 1.11 (95% CI = 0.82-1.49)] and ongoing pregnancy rates [OR = 1.18 (95% CI = 0.75-1.88)]. Similarly, in poor responders, there were no significant differences between the mild and the long agonist stimulation protocols in clinical pregnancy [OR = 0.97 (95% CI = 0.71-1.32)] and ongoing pregnancy rates [OR = 0.82 (95% CI = 0.57-1.18)]. However, the mean number of oocytes retrieved was lower in the mild protocol compared to long agonist protocol both in normal responders (MWD = -1.650, 95% CI = -1.893 to -1.408) and in poor responders (MWD = -0.486; 95% CI = -6.00 to -0.373). The mean amount of gonadotropins used was also significantly lower in the mild compared to the long stimulation protocol both in normal responders (MWD = -0.885; 95% CI = -1.104 to -0.666) and in poor responders (MWD = -2.109; 95% CI = -2.250 to -1.968). Mild stimulation was associated with a higher cancellation rate in poor responders [O.R = 1.96 (95% CI = 1.41-2.72)] and a lower incidence of OHSS in normal responders [O.R = 0.10 (95% CI = 0.01-0.83)].

**CONCLUSIONS:** Current evidence shows that the mild stimulation protocol (as defined by ISMAAR) is a viable alternative to the long protocol both in normal and poor responders and is associated with similar clinical and ongoing pregnancy rates. It is also associated with a lower incidence of OHSS in normal responders and a higher incidence of cancellation in poor responders.

References: Nargund G, Å Fauser BC, Å Macklon NS, Å Ombelet W, Å Nygren K, Å Frydman R, Å Rotterdam ISMAAR Consensus Group on Terminology for Ovarian Stimulation for IVF. Å The ISMAAR proposal on terminology for ovarian stimulation for IVF. Å *Hum Reprod.* Å 2007 Nov;22(11):2801-4.

SUPPORT: None.

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#### EFFECTS OF ESTRADIOL PRETREATMENT DURING FOLLICULAR PERIOD ON OUTCOME OF IN VITRO FERTILIZATION AND EMBRYO TRANSFER TREATMENT FOR POOR OVARIAN RESPONDER WITH HIGH FSH LEVEL.



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**OBJECTIVE:** TO observe the effects of estradiol pretreatment during follicular period on outcomes of in vitro fertilization and embryo transfer (IVF-ET) treatment for poor ovarian responder (POR) with high FSH level.

**DESIGN:** A prospective randomized controlled study.

**MATERIALS AND METHODS:** A total of 323 POR with high level who have undergone IVF-ET treatment were randomly divided into the pretreatment group (n=163) and non-pretreatment group (n=160) according to whether the estradiol pretreatment (oral administration with 17-β estradiol for 4mg-6mg/d at the second day to the fourth day of menstrual cycle) were conducted before super ovulation induction. General information and indices relevant to the outcome of IVF-ET treatment of two groups were compared.

**RESULTS:** In the pretreatment group, serum follicle-stimulating hormone (FSH) (13.77/14.17IU/ml, p=0.53) levels on the second day of menstruation (D2) and the fifth day (D5) were statistically significant; The D2 serum FSH in the non-pretreatment group was also statistically significant compared to that of the FSH in D5 (13.94/8.85, P=0.00). However, there was no statistical significance in comparing D2 FSH (13.98/13.94IU, P=0.51) and D5 FSH (8.79/8.85IU, P=0.45) values between the two groups. The differences of age (37.93/37.56 years, P=0.5), BMI (22.13/21.80 Kg/m<sup>2</sup>, P=0.16), AMH (0.89/0.91 ng/ml, P=0.57), basal antral follicle count (AFC) (3.57/3.59, P=0.23), the number of retrieved oocytes (2.68/2.25, P=0.70), unovulated rate (15.34/13.13, P=0.569), endometrial thickness (10.49/10.49 mm, P=0.41), the number of embryos transferred (1.60/1.63, P=0.12), transplant cancellation rate (28.21/31.90%, P=0.474) and clinical pregnancy rate (13.30/11.70%, P=0.10) were not statistically significant between the two groups.

**CONCLUSIONS:** Estradiol pretreatment in follicular phase at POR patients with high FSH level did not increase the number of MII eggs rate and clinic pregnancy rate. On the contrary, an increase in the Gn dosage and extended treatment period can impose unnecessary burden on a patient, both financially and mentally. To some extent, the level of FSH only reflects the function of the ovary. Therefore, reducing the blood FSH level cannot increase the number of eggs nor improve the clinical pregnancy outcome.

## IVF OUTCOME PREDICTORS - PROGESTERONE LEVELS

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### LONGER DURATION OF PROGESTERONE ELEVATION ADVERSELY IMPACTS PREGNANCY OUTCOMES DURING IVF IN WOMEN ≤ 40 YEARS.

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**OBJECTIVE:** The purpose of this study is to evaluate the impact the number of days of progesterone (P) elevation during an IVF cycle on the fresh embryo transfer live birth rate (LBR) at different ages. We hypothesize that the longer the duration of P exposure, the greater the likelihood for asynchronous endometrium manifested as a lower LBR for ages <35 years and 35-40 years.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** We included all patients ≤40 years who underwent fresh IVF embryo transfer between 1/2011 and 12/2017 at a large IVF clinic. Morning serum P levels were collected every 1 to 2 days during the IVF cycle starting day 4, with frequency of collection determined by the follicle size through ultrasound monitoring. We evaluated the effect of prolonged elevation of P ≥ 1.0ng/mL on livebirth rates by age group. ANOVA was used for continuous variables, and Chi-square was used for categorical data. Logistic regression was performed controlling for age, BMI, embryo stage at transfer and number of embryos transferred.

**RESULTS:** 3339 IVF cycles were included for analysis, with 1850 blastocyst transfers and 1489 day 3 embryo transfers. The LBR was lower if the day of trigger serum P was elevated (1.0-1.4ng/mL: 49.5%[330/666] and ≥ 1.5ng/mL: 43.3%[68/157]) compared to P <1.0ng/mL: 57%[585/1027] (p<0.001). Moreover, a longer duration of P elevation was associated with lower LBR (Table 1). After controlling for the potential confounding variables, prolonged duration of P elevation ≥ 1.0ng/mL (OR: 0.61; 95% CI: 0.47-0.86, p<0.001) and day of trigger P ≥ 1.0ng/mL (OR: 0.73; 95% CI: 0.63-0.84, p<0.001) were still associated with lower LBR.

TABLE 1. Live birth rate by age and number of days of P elevation ≥ 1.0 ng/mL

Age	0 days	1 day	2 days	≥ 3 days	
All embryo transfers n=3339					
Overall	49.9% (919/1841)	44.0% (335/762)	41.9% (199/475)	39.1% 102/261	< 0.001
<35 years	55.9% (578/1034)	47.0% (201/428)	44.8% (121/270)	42.8% (62/145)	< 0.001
35-40 years	42.3% (341/807)	40.1% (134/334)	38.0% (78/205)	34.5% (40/116)	0.347
Blast transfer n=1850					
Overall	57.7% (574/995)	49.4% (214/433)	46.5% (128/275)	45.6% (67/147)	< 0.001
<35 years	60.2% (374/621)	50.2% (137/273)	47.7% (83/174)	47.3% (43/91)	0.002
35-40 years	53.5% (200/374)	48.1% (83/160)	44.6% (45/101)	42.9% (24/56)	0.224

**CONCLUSIONS:** The greater the number of days of P elevation during a fresh IVF cycle, the less likely the transfer is to result in a live birth. The trend is apparent for all ages, though it was only statistically significant for those <35 years. An early rise in P warrants a timely conversation about the benefits of a freeze-all approach.

**SUPPORT:** None.

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### INCREASING LUTEAL PROGESTERONE LEVELS ARE ASSOCIATED WITH HIGHER ONGOING PREGNANCY RATES AND LOWER EARLY PREGNANCY LOSSES FOLLOWING SINGLE EUPLOID FROZEN EMBRYO TRANSFER.

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**OBJECTIVE:** Endometrial programming with exogenous estradiol (E2) and progesterone (P4) during a frozen embryo transfer (FET) cycle mimics the hormonal environment of a natural cycle, while allowing for synchroni-

zation of embryo and endometrial development. While studies have investigated the ideal timing of P4 initiation and the association of supraphysiologic E2 levels with FET and perinatal outcomes,<sup>1</sup> less is known about how the level of P4 exposure impacts implantation and placentation. Prior research has suggested that elevated P4 levels during FETs are associated with a lower ongoing pregnancy/live birth (OP/LB) rate and higher early pregnancy loss (EPL) rate.<sup>2</sup> Other studies have suggested an association between FETs and large for gestational age (LGA) and postdates infants.<sup>3</sup> Yet, there is no known mechanism for these findings.<sup>4</sup> The objective of this study is to determine whether the level of P4 exposure at time of FET and throughout the first trimester impacts FET or perinatal outcomes.

**DESIGN:** Retrospective, cohort study.

**MATERIALS AND METHODS:** The study included patients undergoing a single euploid FET at an academic center from 2012-2019. Luteal support methods other than intramuscular P4 were excluded. Serum P4 level was treated as a continuous variable. Peri-implantation P4 was defined as P4 level on the day prior to FET, and first trimester P4 was defined as average P4 from the day prior to FET until ~10 weeks of gestational age (GA). Primary outcomes were rates of OP/LB and EPL. Secondary outcomes were clinical pregnancy (CP) rate, GA at delivery, and neonatal birth weight. Small for GA (SGA)/LGA were defined using sex-specific data for the 10<sup>th</sup>/90<sup>th</sup> percentile.<sup>5</sup> Data were evaluated using univariate linear regressions with generalized estimating equations.

**RESULTS:** A total of 3773 single euploid FET cycles from 2699 patients were included. After controlling for age, BMI, endometrial thickness, embryo morphology, and days required for blastulation, there was a significant association between average P4 and OP/LB (OR 1.15 [95% CI 1.13-1.17], p <0.001), as well as EPL (OR 0.83, [95% CI 0.81-0.85], p<0.001). There was no association between peri-implantation P4 and CP rate. There was a significant decrease in GA at delivery with increasing P4 (β=-0.19 week, p<0.001). Mean first trimester P4 levels were not associated with birth weight after controlling for GA, fetal sex and BMI. There was no association between P4 and incidence of SGA/LGA infants.

**CONCLUSIONS:** In a large cohort of single euploid FETs, we showed that luteal P4 in early pregnancy is positively correlated with OP/LB rate, and inversely correlated with EPL rate. While the level of exposure to P4 is crucial for pregnancy maintenance, increasing P4 levels in the first trimester do not appear to have downstream effects on placentation. Increasing luteal P4 level

is associated with a shorter duration of pregnancy, but is not associated with differences in birth weight, or incidence of SGA or LGA infants. Future studies might focus on the pharmacogenomic profiles of women undergoing synthetic endometrial preparation with the aim of individualizing FET protocols.

**References:** 1. Sekhon L, Feuerstein J, Pan S, et al. Endometrial preparation prior to the transfer of single, vitrified-warmed, euploid blastocysts: does the duration of estradiol treatment influence clinical outcome? *Fertil Steril* 2019; in press.

2. Kofinas JD, Blakemore J, McCulloh DH, et al. Serum progesterone levels greater than 20 ng/mL on day of embryo transfer are associated with lower live birth and higher pregnancy loss rates. *J Assist Reprod Genet* 2015; 32:1395-1399.

3. Maheshwari A, Pandey S, Raja EA, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? *Hum Reprod Update* 2018; 24:35-58.

4. Pinorg A, Henningsen AA, Loft A, et al. Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique? *Hum Reprod* 2014; 618-627.

5. Duryea EL, Hawkins JS, McIntire DD, et al. A Revised Birth Weight Reference for the United States. *Obstet Gynecol.* 2014; 124:16-22).

**SUPPORT:** None.

**SERUM PROGESTERONE ELEVATION MAY ADVERSELY AFFECT EMBRYOLOGICAL PARAMETERS.**



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**OBJECTIVE:** To evaluate the association of progesterone (P) levels on the trigger day with the embryo quality in freeze all cycles.

**DESIGN:** A retrospective analysis of ICSI cycles followed by elective freezing between 2014 and 2018. The exclusion criteria were female age >37, BMI >30 kg/m<sup>2</sup>, sperm concentration <2x10<sup>6</sup>/ml, more than two failed ICSI attempts and frozen cleavage stage embryos. The primary outcomes were fertilization, blastulation, embryo quality at blastocyst stage.

TABLE I. Baseline characteristics of the women and embryo development.

	A (n=806)	B (n=475)	C (n=204)	Overall	A vs B	A vs C	B vs C
Age (years)	31.6±3.8	31.5±3.9	31.6±3.9	0,99			
BMI (kg/m <sup>2</sup> )	24.8±3.8	24.1±4.2	24.5±3.9	0,97			
Oocytes retrieved	11.6±7.7	15.0±8.9	17.0±11.1	<0.001	<0001	<0001	0.048
Total dose of rFSH/hp-hMG (IU)	2308±1198	2390±957	2330±933	0.03	0.008	0.33	0.38
Late-follicular E2 (pg/ml)	1889±1467	2624±2064	2803±2339	<0.001	<0001	<0001	0.68
No mature oocyte	9.2±6.6	11.8±7.5	13.5±9.5	<0,001	<0001	<0001	0.06
Fertilization rate%	77.1±25.1	74.4±25.2	73.1±25.2	0.004	0.009	0.007	0.46
Blastulation per started cycle%	42.3±29.1	41.6±27.9	41.8±27.3	0.68			
No of frozen blastocyst	3.1±2.9	3.9±3.5	4.4±4.1	<0.001	<0001	0.001	0.42
Day 5 frozen%	78.4	76.1	78.6	0,169			
Day 6 frozen%	21.6	23.9	21.4				

**A:** P levels on the day of triggering: ≤0.80ng/ml

**B:** P levels on the day of triggering 0.81–1.49ng/ml

**C:** P levels on the day of triggering ≥ 1.50 ng/ml

**MATERIALS AND METHODS:** A total of 1485 cycles were evaluated. Data was stratified according to P levels on the day of ovulation triggering as: ≤0.80, 0.81–1.49 and ≥ 1.50 ng/ml. Outcomes were compared among treatment arms by three-group X<sup>2</sup>, followed pairwise X<sup>2</sup> comparisons.

**RESULTS:** Baseline characteristics of the women and embryo development stratified according to the P levels were summarized in Table 1. Women in the high P level group had better ovarian response. Furthermore, inter-group comparisons showed late-follicular E2 levels (2308, 2390 and 2330 pg/ml, respectively) and the number of oocytes retrieved (11.6, 15 and 17 respectively) increased with increasing P levels. There was also a trend towards decrease in fertilization rates (77.1%, 74.4% and 73.1% respectively) among groups and it was significantly higher in the P levels ≤0.80 ng/ml group. Group with P levels ≥ 1.50 ng/ml remained associated with a significantly higher percentage of poor quality blastocysts (8.7%, 9.7% and 12.9%, respectively), albeit all groups showed similar blastulation rates.

**CONCLUSIONS:** Recent studies offers evidence that high serum P levels at late follicular phase are associated with a decrease in embryo utilization and cumulative live birth rates. Our study reports that elevated P levels may also have a detrimental effect on the embryo quality at the blastocyst stage.

**EFFECTS OF SERUM PROGESTERONE AND LH LEVELS BEFORE HCG TRIGGERING ON CLINICAL PREGNANCY OUTCOMES OF MODIFIED NATURAL FROZEN-THAWED EMBRYO TRANSFER CYCLES.**



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**OBJECTIVE:** To investigate the effects of serum progesterone and luteinizing hormone (LH) levels on the clinical outcomes of the modified natural cycle of frozen-thawed embryo transfer.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Five-hundred-and-ninety-two cycles of frozen-thawed transplantation of modified natural cycles from 2017 to 2018 were analyzed. According to the level of progesterone on human chorionic gonadotropin (hCG) days, patients were divided into two groups: group A (progesterone greater than or equal to 1 pg/ml) and group B (progesterone less than 1 pg/ml). According to LH levels, patients were divided into two groups: group C (LH greater than or equal to 20 IU/L) and group D (LH less than 20 IU/L). Pregnancy outcomes were compared and the influence of serum progesterone and LH levels on clinical outcomes on the hCG triggering day were explored, to guide the selection of the hCG triggering time in the modified natural cycle of frozen-thawed embryo transfer.

**RESULTS:** Compared with group B, group A baseline data and pregnancy rates showed no noticeable difference, but the embryo implantation rate was statistically lower in group A. There was no difference in baseline information and clinical pregnancy rates between group C and group D. The embryo implantation rate of group D was significantly higher than that of group C. Moreover, the implantation rate was significantly reduced in patients with

simultaneous elevation of progesterone and LH levels(table1).

**CONCLUSIONS:** During the modified natural cycle of frozen-thawed embryo transfer, serum progesterone and LH levels on the trigger day have an impact on clinical outcomes. We suggest that hCG induction should be selected when the LH level is less than 20 IU/L and the progesterone level is less than 1 pg/ml.

**References:** 1.Weissman A, Horowitz E, Ravhon A, Steinfeld Z, Mutzafi R, Golan A et al. Spontaneous ovulation versus HCG triggering for timing natural-cycle frozen-thawed embryo transfer: a randomized study. Reproductive biomedicine online 2011;23:484-9.

TABLE 1. Comparison of modified natural cycle with progesterone and LH increased simultaneously and overall population.

	LH>20IU/L and P>1pg/ml (n=127)	Total polulation (n=592)	P
Female age(year)	31.47±5.01	31.65±4.97	0.703
Male age(year)	32.47±5.60	33.08±5.71	0.271
HCG daily E2(ng/ml)	319.53±111.82	343.55±226.74	0.245
HCG daily oocyte number	1.00±0.22	1.01±0.18	0.467
Size of dominant follicle	17.81±1.30	17.73±1.39	0.576
Endometrium thickness	10.07±1.65	10.16±1.79	0.613
Number of transplanted embryos	1.55±0.50	1.51±0.50	0.346
Proportion of transplanted blastocysts (n)	40.9%(52)	45.44%(269)	0.320
Clinical pregnancy rate	53.5%	61.49%	0.097
Embryo implantation rate	44.16%	52.63%*	0.031

HCG day LH level is greater than or equal to 20 IU/L (397 cases) and progesterone greater than or equal to 1pg/ml, \*p<0.05

2. Veleva Z, Orava M, Nuojua-Huttunen S, Tapanainen JS, Martikainen H. Factors affecting the outcome of frozen-thawed embryo transfer. *Human reproduction* (Oxford, England) 2013;28:2425-31.

3. Kupka MS, D'Hooghe T, Ferraretti AP, de Mouzon J, Erb K, Castilla JA et al. Assisted reproductive technology in Europe, 2011: results generated from European registers by ESHRE. *Human reproduction* (Oxford, England) 2016;31:233-48.

4. Groenewoud ER, Cantineau AE, Kollen BJ, Macklon NS, Cohlen BJ. What is the optimal means of preparing the endometrium in frozen-thawed embryo transfer cycles? A systematic review and meta-analysis. *Human reproduction update* 2017;23:255-61.

5. Huberlant S, Vaast M, Anahory T, Tailland ML, Rougier N, Ranisavljevic N et al. [Natural cycle for frozen-thawed embryo transfer: Spontaneous ovulation or triggering by HCG]. *Gynecologie, obstetrique, fertilité & senologie* 2018;46:466-73.

6. Weissman A, Levin D, Ravhon A, Eran H, Golan A, Levran D. What is the preferred method for timing natural cycle frozen-thawed embryo transfer? *Reproductive biomedicine online* 2009;19:66-71.

7. Arefi S, Hoseini A, Farifteh F, Zeraati H. Modified natural cycle frozen-thawed embryo transfer in patients with repeated implantation failure: An observational study. *International journal of reproductive biomedicine* (Yazd, Iran) 2016;14:465-70.

8. Montagut M, Santos-Ribeiro S, Vos MD, Polyzos NP, Drakopoulos P, Mackens S et al. Frozen-thawed embryo transfers in natural cycles with spontaneous or induced ovulation: the search for the best protocol continues 2016;31:2803-10.

9. Groenewoud ER, Macklon NS, Cohlen BJ. The effect of elevated progesterone levels before HCG triggering in modified natural cycle frozen-thawed embryo transfer cycles. *Reproductive biomedicine online* 2017;34:546-54.

10. Requena A, Cruz M, Bosch E, Meseguer M, García-Velasco JAJRB, Endocrinology. High progesterone levels in women with high ovarian response do not affect clinical outcomes: a retrospective cohort study 2014;12:69.

11. Human Mousavi F, Dimitra K, Claire B, Etienne VDA, Georg G, Paul DJF et al. Cryopreserved-thawed human embryo transfer: spontaneous natural cycle is superior to human chorionic gonadotropin-induced natural cycle 2010;94:2054-8.

12. Irani M, Robles A, Gunnala V, Reichman DE, Rosenwaks ZJF, Sterility. Optimal parameters for determining the LH surge? in natural cycle frozen-thawed embryo transfers.

13. Baldini D, Savoia MV, Sciancalepore AG, Malvasi A, Vizziello D, Beck R et al. High progesterone levels on the day of HCG administration do not affect the embryo quality and the reproductive outcomes of frozen embryo transfers. *La Clinica terapeutica* 2018;169:e91-e5.

SUPPORT: The study was supported by National Natural Science Foundation of China(81601246,N.K).

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#### THE EFFECT OF PREMATURE ELEVATED PROGESTERONE LEVEL IN LATE FOLLICULAR PHASE ON OOCYTE QUALITY.

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OBJECTIVE: To determine whether premature elevated progesterone on day of trigger adversely affect the oocyte quality.

DESIGN: A retrospective cohort study was performed using database of Uludag University School of Medicine ART Center from 2011 to 2018. The etiology of infertility, age, BMI, stimulation protocol and trigger agent, which may affect oocyte quality, was standardized. Study inclusion criteria were; isolated male infertility etiology, women age 24-35 years, female BMI 19-30 kg/m<sup>2</sup>, 6-15 oocytes obtained GnRH-antagonist protocol cycles with 150-300 IU dose of rFSH and 250 mcg/0.5ml of rhCG.

MATERIALS AND METHODS: 421 oocytes obtained from 49 ICSI cycle were evaluated. The metaphase II oocytes were grouped according to progesterone level on the trigger day:  $\leq 0.5$ ng/ml [Grup-1 (n=103)], 0.5-1ng/ml [Grup-2 (n=204)] and  $\geq 1$ ng/ml [Grup-3 (n=76)]. Individual oocytes were evaluated based on 6 parameters: oocyte shape, oocyte size, ooplasm characteristics, structure of perivitelline space(PVS), zona pellucida(ZP) and polar

body(PB) morphology. For each oocyte, each parameter was scored as +1, 0 and -1 to determine the oocyte quality score.

RESULTS: There was no significant difference between the groups in terms of patient age (p=0.11), BMI (p=0.12), duration of infertility (p=0.48), basal FSH (p=0.28), LH (p=0.91), E2 (p=0.91), AMH (p=0.20) and AFC (p=0.60). There was a positive correlation between dose of gonadotropin and progesterone concentration on trigger day (p=0.001). There was a negative correlation between oocyte quality score and progesterone level on hCG day (p=0.001). In terms of oocyte quality score, a statistically significant difference was found between 3 groups (5.48, 4.97, 4.14, p=0.001, respectively). The quality score of the Group-3 oocytes was found to be significantly lower than both Group-1 and Group-2 oocytes (p=0.001). Also Group-2 oocyte quality score was significantly lower than Group-1 oocytes (p=0.001). There was a positive correlation between progesterone level and abnormal oocyte percentage (p=0.001). The the highest abnormal oocyte ratio was found in Group-3 (%78.9) and lowest in Group-1 (%28.2). Ooplasm (p=0.007), PVS (p=0.001) and ZP (p=0.004) abnormalities were statistically increased with higher progesterone concentration. Degenerated (p=0.55) and immature oocyte percentage (p=0.82) had no significant correlation between groups. Estradiol concentration on trigger day (p=0.001), total oocyte count (p=0.001) and mature oocyte count (p=0.001) had a positive correlation with progesterone concentration on trigger day.

CONCLUSIONS: This study comprehensively assessed the relationship between oocyte quality and progesterone. These data demonstrate that elevated progesterone levels ( $\geq 1$ ng/ml) before oocyte maturation were consistently detrimental to the oocyte. Individualization of stimulation protocols and consideration of gonadotropin dose in late follicular phase will lead to positive results in terms of oocyte quality.

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#### EFFECT OF PREMATURE SERUM LH AND PLASMA PROGESTERONE RISE ON THE CLINICAL OUTCOME OF ANOVULATORY PATIENTS TREATED WITH GONADOTROPINS.

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OBJECTIVE: To study the effect of serum LH and plasma progesterone rise on the day of HCG administration on the clinical outcome of anovulatory patients treated with gonadotropins.

DESIGN: A prospective cohort study.

MATERIALS AND METHODS: Sixty consecutive anovulatory patients attending our infertility clinic and treated for ovarian stimulation with gonadotropins were studied during their first cycle of treatment. All patients had normogonadotrophic hypogonadism (WHO group II) and had failed to become pregnant on clomiphene citrate therapy (up to 150 mg/day for 5 days). All patients were aged 20 to 38 years with a mean ( $\pm$ SD) of 26.7 ( $\pm$ 9.2) years. All male partners had normal semen parameters according to the WHO standards. Patients with hyperprolactinaemia and those with congenital adrenal hyperplasia were excluded, as well as those with other causes of infertility. The mean ( $\pm$ SD) basal (day 3) serum FSH and LH levels were 7.27 ( $\pm$ 1.82) mIU/mL and 7.57 ( $\pm$ 0.78) mIU/mL, respectively. The mean ( $\pm$ SD) basal (day 3) LH/FSH ratio was 1.09 ( $\pm$ 0.14) mIU/mL. Human menopausal gonadotropins (150 IU) were administered by daily IM injections starting day 5 of the menstrual cycle. Monitoring was effected by transvaginal ultrasound scanning of the follicles and the dose of gonadotropins adjusted accordingly. HCG (5000 IU) was administered by IM injection when 2 follicles reached 18 mm in diameter and venous blood was withdrawn on the same day and the serum/plasma kept at -20°C until the time of the LH and progesterone assay. Eighteen patients became pregnant, of whom 17 reached clinical viability (beating heart on ultrasound) and one had a miscarriage. Power calculation regarding the premature rise or otherwise of serum LH revealed that a minimum of 17 treatment cycles was needed to study in each group to achieve an 80% study power at the 5% level significance level.

RESULTS: The mean ( $\pm$ SD) of serum LH and plasma progesterone levels on the day of HCG administration were 11.10 ( $\pm$ 9.08) mIU/mL and 2.68 ( $\pm$ 0.14) ng/mL, respectively. Twenty nine patients (48.3%) had an LH rise

E/P Quartile	E/P (median, IQR)	Maturity rate (MII/total oocytes, mean±SD%)	Euploid rate (Euploid/total embryos biopsied, mean±SD%)	Positive hCG (mean±SD%)	Clinical Pregnancy (mean±SD%)	Ongoing Pregnancy (mean±SD%)
Quartile 1 (n=34)	1.75 (1.47,1.93)	79±16	56±22	82±39	79±41	71±46
Quartile 2 (n=34)	2.74 (2.40,2.87)	81±12	60±22	74±45	71±46	68±47
Quartile 3 (n=34)	3.50 (3.21,3.85)	85±13	52±20	85±36	76±43	68±47
Quartile 4 (n=34)	4.78 (3.39,7.91)	82±12	62±23	82±39	74±45	68±47

P=NS by linear or logistic regression (where appropriate) for all comparisons of outcome by quartile

of >10 mIU/mL. Of those 8 became pregnant (27.6%) compared to 10 pregnancies (32.3%) in the 31 patients with no LH rise (P =0.693). Twelve patients (20%) had a rise of plasma progesterone (=>1.5 ng/mL) and 8 patients (13.3%) had a rise of =>3 ng/mL and none of these patients became pregnant, compared to 18 pregnancies in the 48 patients (37.5%) with plasma progesterone <1.5 ng/mL (P < 0.02). The serum LH and plasma progesterone levels were 10.8 mIU/mL and 0.9 ng/mL, respectively in the patient who miscarried.

**CONCLUSIONS:** Contrary to accepted convention, the premature rise of serum LH on the day of HCG administration does not seem to affect the clinical outcome in anovulatory patients treated with gonadotropins. However, the rise of progesterone >1.5 ng/mL is detrimental to the clinical outcome in those patients. Converting the cycle to IVF in those patients and freezing all the embryos for transfer in subsequent cycle(s) is suggested.

**SUPPORT:** None.

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**EFFECT OF ESTROGEN TO PROGESTERONE RATIO AT TIME OF OVULATION TRIGGER ON SUBSEQUENT EUPLOID FROZEN EMBRYO TRANSFER PREGNANCY RATE.**

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**OBJECTIVE:** While an elevated serum progesterone level (P) prior to trigger has been associated with embryo-endometrial asynchrony and decreased pregnancy rates during in vitro fertilization (IVF) with fresh embryo transfer, few data exist in the context of a planned frozen embryo transfer. We aim to evaluate the impact of elevated P via estradiol to progesterone

ratio (E/P) at time of ovulatory trigger on clinical pregnancy rate during subsequent frozen euploid embryo transfer.

**DESIGN:** Retrospective cohort analysis

**MATERIALS AND METHODS:** All frozen embryo transfers from January-December 2018 from a high-volume private practice fertility center were included. Serum E and P levels were measured on the day of ovulatory trigger by Immulite (Siemens). E/P was calculated in an effort to control for degree of response. Embryos were cultured to the blastocyst stage for trophectoderm biopsy and vitrified. Preimplantation genetic testing for aneuploidy (PGT-A) was performed using next generation sequencing (NGS). Euploid frozen embryo transfers were performed in a subsequent natural or controlled cycle. Oocyte maturity (MII/total oocytes retrieved) and euploidy rates (euploid/ total embryos biopsied) were calculated. Clinical pregnancy and ongoing pregnancy (>10 weeks) following a first embryo transfer were examined in relation to E/P. Regression analyses were performed to analyze the impact of E/P as a continuous and categorical value (defined by quartile) on cycle outcomes.

**RESULTS:** A total of 134 women underwent a euploid frozen embryo transfer over the study period and had steroid levels at time of trigger available. Mean E at trigger was 3704±2234 pg/ml while mean P was 1.13±0.56 ng/ml for a mean E/P of 3.61±2.59. Cycle and pregnancy outcomes by quartile of E/P are listed in Table 1. There were no differences between quartiles of E/P with respect to cycle or pregnancy outcomes.

**CONCLUSIONS:** E/P ratio at the time of trigger does not appear to impact clinical outcomes in a subsequent euploid frozen embryo transfer cycle.

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**SERUM PROGESTERONE LEVEL: A PREDICTOR OF PREGNANCY IN VITRIFIED-WARMED BLASTOCYST TRANSFER.**

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CHARACTERISTICS (SD/PERCENTAGE)	S. P4 <=25.1(54)	S. P4 >25.1(76)	p VALUE
AGE [YEARS]	30.81(5.23)	31.24(4.42)	0.72
MARRIED LIFE [YEARS]	6.9(4.4)	6.0(2.9)	0.33
BMI [kg/m <sup>2</sup> ]	26.34(3.87)	25.54(3.47)	0.39
DAYS OF HRT	19.9(6.5)	20.9(6.8)	0.55
ENDOMETRIAL THICKNESS[mm]	9.69(1.45)	9.68(1.66)	0.98
ENDOMETRIAL VOLUME[mm <sup>3</sup> ]	3.41(0.95)	3.15(0.99)	0.47
ESTRADIOL LEVEL [pg/ml]	586.06(596.11)	444.27(252.60)	0.29
PROGESTERONE LEVEL [ng/ml]	0.25(0.11)	0.29(0.28)	0.47
PROGESTERONE LEVEL (ET DAY) [ng/ml]	18.59(5.10)	38.87(12.07)	<0.001
NO. OF EMBRYO TRANSFER	2.70(0.82)	2.62(0.76)	0.68
<b>PREGNANCY RATE</b>	<b>20(37.04%)</b>	<b>54(71.05%)</b>	<b>&lt; 0.001</b>
BIOCHEMICAL ONLY PREGNANCY	2(10%)	3(5.55%)	0.38
ONGOING PREGNANCY(>12 weeks) / LIVE BIRTH	15(27.78%)	48(63.16%)	<0.001
EARLY PREGNANCY BLEEDING	5(27.78%)	7(13.73%)	0.08
MISCARRIAGE	2(11.11%)	3(5.88%)	0.23
TWINS at 12 WEEKS	3(16.67%)	6(11.76%)	0.29
TRIPLET	0	1	
ECTOPIC PREGNANCY	1	0	

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**OBJECTIVE:** To know the effect of serum progesterone (P4) level on pregnancy rate in Vitrified-Warmed Blastocyst Transfer(VBT)

**DESIGN:** Interim Analysis of a Prospective observational study (IEC: CSP-MED/18/JUN/44/97)

**MATERIALS AND METHODS:** The main inclusion criteria were having a VBT with Hormone Replacement Treatment (HRT) using oral estradiol valerate and intramuscular(IM) progesterone after long acting GnRH agonist suppression. Beyond 7 mm of endometrial thickness(ET), women were started on IM micronized progesterone 100 mg once a day for 5 days. VBT was done with at least one good quality blastocyst ( $\geq 3$ AB, Gardner classification). Serum P4 level (8am - 12pm) was measured on day of embryo transfer by chemiluminescence immunoassay (Beckman coulter, UniCel DxI 800). Luteal support was given with vaginal micronized progesterone and oral dydrogesterone. Primary outcome was to observe the optimal level of Serum P4 to predict pregnancy.

**RESULTS:** Overall pregnancy rate was 56.92%(74/130). The ROC curve cut off value of serum P4 of  $> 25.1$  ng/ml could be predictive of pregnancy rate (AUC=0.68,  $p=0.01$ , Sensitivity-72.22%, Specificity-60.71%, +LR-1.84, -LR-0.46). Ongoing Pregnancy rate/Live birth rate was significantly more in serum P4  $>25.1$  ng/ml group compared to serum P4  $\leq 25.1$  ng/ml group (OR-4.40, 95%CI-2.08-9.60). In Uni variate analysis, factors which could be associated with pregnancy were; Age  $> 35$  years (44.12% vs 61.46%, OR-0.5, 95%CI- 0.22-1.10), ET  $> 8$  mm (60.2% VS 46.88%, OR-1.71, 95%CI- 0.76-3.87), Days of HRT  $> 28$  days (38.89% vs 59.82%, OR-0.43, 95%CI- 0.14-1.20), Serum P4 level  $> 25.1$  ng/ml (71.05% VS 37.04%, OR-4.12, 95%CI- 1.97-8.82)

**CONCLUSIONS:** According to this study, serum progesterone level  $>25.1$  ng/ml on day of embryo transfer can be a good predictive marker of pregnancy in vitrified-warmed blastocyst transfer.

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### PROTOCOL MATTERS:PROGESTERONE RISE ON DAY OF TRIGGER IMPACTS ANTAGONIST BUT NOT AGONIST LIVE BIRTH RATES FOR FRESH IVF CYCLES.

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**OBJECTIVE:** The purpose of this study is to determine if age and stimulation protocol influences the negative pregnancy outcome impact of progesterone rise on day of trigger during stimulated IVF-ET.

**DESIGN:** A retrospective cohort study using a large IVF database.

TABLE 2. Live birth rate by age and protocol for progesterone level

	<35 (%)		Age > 35 (%)		All (%)	
	Agonist	Antagonist	Agonist	Antagonist	Agonist	Antagonist
Low	50.5	37.4	42.5	30.68	48.1	34.85
P < 1	187/380	306/818	68/160	154/502	255/530	460/1320
Medium	54.5	34.6	41.6	21.3	50.9	29.17
P 1-1.5	158/290	191/552	47/113	82/384	205/403	273/936
High	54.6	31.4	39.0	25.8	50.42	29.27
P >1.5	95/174	98/312	25/64	51/197	120/238	149/509
p-value	0.52	0.15	0.89	<b>0.007</b>	0.67	<b>0.006</b>

**MATERIALS AND METHODS:** eIVF is a multicenter database for IVF that has collected over 122,548 patient IVF cycles between 2004 and 2018. We included all women who underwent elective fresh single blastocyst transfer and had excess embryos to freeze. Women were excluded for positive smoking status and day three follicle stimulating hormone level  $>12$  IU/L. Progesterone (P4) levels were categorized into low ( $<1$  ng/mL), medium (1-1.5ng/mL), and high ( $>1.5$  ng/mL). Age groups were divided by  $<35$  years versus  $\geq 35$  years. Gonadotropin-releasing hormone (GnRH)-Antagonist and GnRH-agonist protocols were compared separately in each age group. Statistics was analyzed using Chi-square, ANOVA, Student's t-test and logistic regression.  $P<0.05$  was considered statistically significant

**RESULTS:** 3936 cycles were included. Women in the two age groups did not differ significantly by cycle variables including BMI, AMH, FSH values. In all patients, live birth rates were lower when progesterone levels on day of trigger rose above 1 ng/ml using an antagonist suppression protocol( $p=0.006$ ). This was particularly true and significant for women  $\geq 35$  years old ( $p=0.007$ ), but not statistically significant for women  $<35$  years old. No significant difference was seen with progesterone level and live birth rate when an agonist suppression protocol was used for ovulation induction, regardless of the patients' ages. Live birth rates were higher using GnRH-agonist suppression in every progesterone group and age category ( $p<0.0001$ ).

**CONCLUSIONS:** Elevated serum progesterone levels  $>1$  ng/mL on the day of trigger is associated with reduced live birth rates following IVF/ICSI cycles in women  $\geq 35$  years when an antagonist protocol is used. Ovarian stimulation using GnRH-agonist suppression seems to protect from the adverse effect of rising progesterone and allows high pregnancy rates with fresh embryo transfer. Protocol should be considered when recommending a freeze-all cycle in the setting of elevated progesterone.

References: 1) Bosche E. al. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for in vitro fertilization: analysis of over 4000 cycles. *Human Reproduction*, Vol.25, No.8 pp. 2092-2100, 2010

2) Racal A., Santos-Ribeiro I S. et al. Impact of late-follicular phase elevated serum progesterone on cumulative live birth rates: is there a deleterious effect on embryo quality? *Human Reproduction*, Vol.33, No.5 pp. 860-868, 2018

3) Kofinas J. et al. Serum progesterone levels greater than 20 ng/dl on day of embryo transfer are associated with lower live birth and higher pregnancy loss rates. *J Assist Reprod Genet* (2015) 32:1395-1399

4) Grow D et al. A GnRH agonist and GnRH antagonist protocols: comparison of outcomes among good-prognosis patients using national surveillance data. *Reprod Biomed Online*. A 2014 Sep;29(3):299-304.

SUPPORT: N/A.

### IVF OUTCOME PREDICTORS - SPERM

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### SPERM INTRACELLULAR PH AS A PREDICTOR OF FERTILIZATION RATE IN NORMOSPERMIC INFERTILE MEN UNDERGOING IN VITRO FERTILIZATION.

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**OBJECTIVE:** To determine whether intracellular pH (pH<sub>i</sub>) of human spermatozoa can predict unsuccessful conventional fertilization outcomes in normospermic infertile men undergoing *in vitro* fertilization (IVF).

**DESIGN:** IRB approved, laboratory study of normospermic men undergoing IVF from September 2018 to present at a single institution. Couples were excluded if they used frozen sperm, had a known female factor or utilized intracytoplasmic sperm injection (ICSI) only. De-identified, normospermic fresh semen samples were also analyzed.

**MATERIALS AND METHODS:** Fresh semen was collected on the day of oocyte retrieval from normospermic ( $\geq 32\%$  progressive motility;  $\geq 40\%$  total motility;  $\geq 15 \times 10^6$  cells/ml) infertile men undergoing IVF. Sperm were subjected to standard swim up, then analyzed immediately or incubated in capacitating media (Quinn's Advantage Fertilization, CooperSurgical) at 37 °C and 5% CO<sub>2</sub> for 24 hours. pH<sub>i</sub> of spermatozoa was measured in all samples using flow cytometry (FACSCanto II TM cytometer) after incubation with pH sensitive fluorescent probe, BCECF-AM. Data were analyzed using FACS Diva and FlowJo software and included only single live sperm cells. The final sperm pH<sub>i</sub> was obtained by linearly interpolating the median fluorescence of the unknown sample in the calibration curve of known pH buffer solutions for each condition. Hyperactivated motility was measured by computer-assisted semen analysis. Standard univariate and bivariate analyses were performed, if data were not normally distributed a non-parametric test was performed.

**RESULTS:** A total of 28 fresh de-identified samples and 24 IVF samples were included in the analysis. The IVF couples included in the analysis were demographically similar. Previously, we measured pH<sub>i</sub> in capacitated fresh spermatozoa from deidentified samples and found that pH<sub>i</sub> positively correlated with the percentage of sperm that exhibited hyperactivated motility (n=17, P=0.0124). Next, we measured pH<sub>i</sub> in sperm from IVF patients before and after capacitation and found that pH<sub>i</sub> did not change (6.97  $\pm$  0.195 vs. 6.93  $\pm$  0.257). Sperm pH<sub>i</sub> positively correlated with conventional fertilization rates (number of fertilized eggs /total number of mature oocytes, n=24, P=0.0197) but not with ICSI fertilization rates (n=10, P=0.655). Sperm samples that had a conventional fertilization rate greater than 70% had a significantly higher pH<sub>i</sub> than those with a fertilization rate lower than 50% (n=10, P=0.0175). The lower 99% confidence interval of pH<sub>i</sub> in sperm from the IVF cohort was 6.77. Fertilization rates were significantly higher with sperm with pH<sub>i</sub> >6.77 than with sperm with pH<sub>i</sub> <6.77 (n=24, P=0.0027).

**CONCLUSIONS:** Sperm pH<sub>i</sub> was a stable marker within patients before and after capacitation and positively correlated with conventional fertilization rates. This measurement may be used to predict poor conventional fertilization outcomes in normospermic men undergoing IVF.

Reference: None.

SUPPORT: None.

**P-241** Tuesday, October 15, 2019 6:30 AM

### NEUROTENSIN STIMULATES THE SPERM ACROSOME REACTION AND ALTERS PERCENTAGES OF FERTILIZATION IN VITRO.

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**OBJECTIVE:** Neurotensin (NTS) is a naturally-occurring, 13-amino acid peptide which was previously reported to stimulate the acrosome reaction in mouse and bull sperm. This study determined the impact of NTS on the function of human and non-human primate sperm.

**DESIGN:** Experimental, laboratory-based research study of semen from consenting, normozoospermic human donors and cynomolgus macaques.

**MATERIALS AND METHODS:** Human semen samples from CONRAD, Norfolk, VA were filtered to obtain motile sperm. Sperm acrosome status was assessed by staining with a fluorescent lectin which binds the outer acrosomal membrane and permits microscopic visualization of the sperm acrosome (intact or reacted). Eosin-negrosin staining determined sperm viability. Computer assisted semen analysis (CASA) assessed sperm motility, progression, and velocity. For *in vitro* fertilization (IVF) studies, monkey oocytes were obtained after ovarian stimulation and follicle aspiration. Monkey sperm samples

were obtained from the Oregon National Primate Research Center. Fertilization was determined by the presence of a second polar body and 2 pronuclei.

**RESULTS:** NTS treatment of human sperm stimulated the acrosome reaction in both a dose-dependent (0.1-10  $\mu$ M) and time-dependent (5-30 min) manner *in vitro*. After a 30 min incubation, intact acrosomes decreased from 81  $\pm$  5% in untreated sperm to 46  $\pm$  5% in sperm treated with 10  $\mu$ M NTS (P<0.05, n=4 donors). NTS treatment (0.1-10  $\mu$ M for 30 min) did not alter sperm motility or progression (n=4 donors); however, there was a slight increase in proportion of viable sperm with NTS treatment (P<0.05, n=4 donors). Both a general NTS receptor antagonist (SR142948) and a NTSR1 selective antagonist (SR48692) reduced the ability of NTS to stimulate the acrosome reaction. While 92  $\pm$  2% of untreated sperm had intact acrosomes after 30 min, NTS treatment resulted in only 54  $\pm$  7% of sperm with intact acrosomes (P<0.05, n=3 donors). Incubation with NTS plus SR142948 resulted in 88  $\pm$  1% of sperm with intact acrosomes, and incubation with NTS plus SR48692 resulted in 87  $\pm$  1% of sperm with intact acrosomes (P<0.05, n=3 donors). To determine if NTS treatment compromises the ability of sperm to fertilize an oocyte, monkey sperm were treated with NTS (10  $\mu$ M for 30 min). Untreated monkey sperm had 87  $\pm$  2% intact acrosomes, while sperm treated with NTS had 50  $\pm$  1% intact acrosomes (P<0.05, n=3 separate experiments). Percentage of fertilization with untreated monkey sperm and monkey oocytes was 72%. Sperm pre-treated with NTS and then used for IVF yielded a significantly lower fertilization rate of 18% (different by Chi-squared test).

**CONCLUSIONS:** NTS effectively stimulates the acrosome reaction in human and monkey sperm. Pre-treatment of sperm with NTS significantly reduces fertilization. Therefore, the NTS pathway has potential for contraceptive development. Identification of NTSR1 as the mediator of NTS action provides a specific target for future study. This work was supported by Eastern Virginia Medical School and NICHD (HD071875 to DMD). Gonadotropins and Ganirelix were generously provided by Merck and Co., Inc., Kenilworth, NJ.

**SUPPORT:** This work was supported by Eastern Virginia Medical School and NICHD (HD071875 to DMD).

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### SPERM DNA FRAGMENTATION INDICES ARE NOT CORRELATED WITH BLASTULATION OR EUPLOIDY RATES IN PATIENTS UNDERGOING IVF WITH PGT-A.

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**OBJECTIVE:** It has been postulated that the sperm DNA integrity correlates with embryo development and implantation potential (1), also that men who suffer from high sperm DNA fragmentation experience a higher probability of sperm aneuploidy and meiotic anomalies. Theoretically, embryos from men whose ejaculates display elevated DNA fragmentation could be at a greater risk of aneuploidy following fertilization. Still, published data regarding the impact of sperm with high DNA fragmentation is highly heterogeneous and limited by small sample size, use of dated genetic testing platforms, and/ or analysis of patients with recurrent pregnancy losses. The objective of this study is to examine the correlation between indices measuring sperm DNA damage and embryo quality and euploidy rate in a diverse population of infertile couples undergoing IVF/ICSI with preimplantation genetic testing for aneuploidy (PGT-A).

**DESIGN:** Retrospective cohort analysis.

**MATERIALS AND METHODS:** All patients undergoing ICSI/PGT-A from 2012-2019 were included in the analysis. Cases in which Sperm DNA fragmentation Index (DFI) were analyzed were included. DFI was calculated using sperm chromatin dispersion, TUNEL, acridine Orange or Sperm chromatin structure assays. Patients were segregated into 2 groups: Normal DFI rate ( $\leq 30\%$ ) and Elevated DFI rate ( $\geq 30\%$ )(2) Surgical extracted or frozen/thawed semen samples were excluded of the analysis. Demographic characteristics of populations, clinical embryology parameters, and embryonic euploidy rates were compared between cohorts. T-test, Xi2, and multivariate regression with a GEE model were used for data analysis

**RESULTS:** 1108 blastocysts derived from 259 IVF/PGT-A cases were included in the study. The groups consisted of 126 cases (n= 543 embryos) with elevated DFI and 133 cases (n= 565 embryos) with normal DFI. Significant differences were found in mean male age (39.8 ±6, 37.8±5, p=0.004), female age (36.2±4, 34.8±4, p=0.007) and cases with normal morphological sperm analysis (37%, 56.3%, p=0.002) between cohorts. No differences were found in fertilization rate, zygotes achieving cleavage stage, and blastulation rates between study groups. Embryo euploidy rates were comparable (50.2% (n=273/543), 46.7% (n=264/565), p=0.24).

After adjusting for female and male patient's age, BMI, AMH, normal semen analysis and number of biopsied embryos, there were no association with elevated DFI and lower odds of embryo euploidy (OR 1.39, CI95% 0.97-2.0, p=0.07).

**CONCLUSIONS:** Although multiple studies have reported poor outcomes in patients with elevated DFI, the exact mechanism of action is unclear. Our study analysis showed no correlation between high sperm DNA fragmentation and fertilization, blastulation, or embryo euploidy rates. Our study adds to the expanding body of evidence that shows no significant relationships between elevated DNA fragmentation, embryo development, or chromosomal composition. Future studies assessing the oocyte DNA-repair mechanism following fertilization should be performed to better understand the immediate impact of sperm chromatin damage during ART intervention.

**References:** Zini A, Jamal W, Cowan L, Al-Hathal N (2011) Is sperm DNA damage associated with IVF embryo quality? A systematic review. *J Assist Reprod Genet* 28: 391±397.

Virro MR, Larson-Cook KL, Evenson DP. Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. *Fertil Steril*. 2004 May;81(5):1289-95.

**SUPPORT:** None.

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#### **CYTOGENETIC ANALYSIS BY NEXT GENERATION SEQUENCING DISCLOSED THAT EXTREMELY HIGH EUPOIDY RATE OF BLASTOCYSTS DERIVED FROM MONOPRONUCLEAR EMBRYOS WITH TESTICULAR SPERM.**

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**OBJECTIVE:** It has been reported that the blastocyst formation rate of monopronuclear (1PN) embryos was significantly lower than that of two nuclear (2PN) embryos (especially in ICSI). However, a recent study revealed that 1PN embryos contained normal chromosome copy numbers similar to those of 2PN embryos by preimplantation genetic testing for aneuploidy (PGT-A). We assessed euploidy rate of 1PN embryos derived from ICSI with testicular sperm (TESE-ICSI) comparing to ejaculated sperm-ICSI or IVF by chromosomal analysis with next generation sequencing (NGS).

**DESIGN:** Retrospective study in a single infertility center

**MATERIALS AND METHODS:** Patients who obtained 1PN embryos were asked to participate in this study, and gave written informed consent for the study between April 2016 and March 2019. All 1PN cleaved embryos were cultured until the blastocyst stage followed by chromosomal analysis with NGS. Rates of 1PN, blastulation and euploidy were compared among three groups. The number of embryos reached the blastocyst stage were 5 from TESE-ICSI, 42 from IVF and 19 from ejaculated sperm-ICSI, respectively. The average maternal age of couples provided embryos was 40.5 years. The Chi-squared tests were performed for statistical analysis and P values less than 0.05 was defined as statistically significant.

**RESULTS:** The incidence of 1PN embryos by TESE-ICSI (7.7%, 507/6562) was significantly (P<0.001) higher than that of IVF (3.8%, 845/22118) and that of ejaculated sperm-ICSI (3.7%, 1268/34547), while 2PN rate of ejaculated sperm-ICSI (72.3%) was significantly (P<0.001) higher than that of IVF (66.8%) and TESE-ICSI (51.4%). Blastocyst formation rate of 1PN embryo was 25.3% (173/684) in IVF, which was significantly (P<0.001) higher than that of ejaculated sperm-ICSI (11.4%, 110/965) and of TESE-ICSI (12.2%, 51/417). Chromosomal analysis by NGS could successfully be performed in 1PN blastocysts with similar rate among three groups; 100% (42/42) in IVF, 94.1% (19/20) in ejaculated sperm-ICSI, and 100%

(5/5) in TESE-ICSI. The euploidy rates of 1PN blastocysts were 21.4% (9/42) in IVF, 31.6% (6/19) in ejaculated sperm-ICSI and 100.0% (5/5) in TESE-ICSI. Of all 1PN blastocysts, rate of female embryos was significantly (P<0.05) higher (54.5%, 36/66) than that of male embryos (45.5%, 30/66). The male/female embryo ratio was 22/20 in IVF, 8/11 in ejaculated sperm-ICSI, and 0/5 in TESE-ICSI, respectively.

**CONCLUSIONS:** TESE-ICSI generated significantly higher incidence of 1PN embryos than ejaculated sperm-ICSI, however the euploidy rate of 1PN blastocysts in TESE-ICSI was extremely high. Since we did not perform any tests to track biparental inheritance of the 1PN embryos, uniparental diploidy owing to endoreduplication of a haploid oocyte cannot be excluded. We, for the first time, revealed that TESE-ICSI derived 1PN blastocysts bore an extremely high euploidy rate. Although several mechanisms have been proposed to account for normal 1PN embryos, we speculate premature pronuclear breakdown by immature testicular sperm might occur during the first embryonic cleavage.

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#### **SEDIMENTATION VERSUS SURGERY: TESTICULAR AND EJACULATED SPERM RESULT IN SIMILAR IVF OUTCOMES IN PATIENTS WITH**

**CRYPTOZOOSPERMIA.** Carlos Hernandez-Nieto, MD,<sup>a</sup> Joseph A. Lee, BA,<sup>a</sup> Tamar Alkon, MD,<sup>a</sup> Martha Luna-Rojas, MD,<sup>a</sup> Christine Briton-Jones, PhD, HCLD,<sup>a</sup> Natan Bar-Chama, MD,<sup>b</sup> Alan B. Copperman, MD,<sup>b</sup> Benjamin Sandler, M.D.<sup>a</sup> <sup>a</sup>Reproductive Medicine Associates of New York, New York, NY; <sup>b</sup>Icahn School of Medicine at Mount Sinai, New York, NY.

**OBJECTIVE:** There are opposing views about whether to source sperm through surgical intervention or fresh ejaculation in men with cryptozoospermia. O'Connell et al. observed ejaculated sperm to be better than testicular sperms in cryptozoospermia patients, and suggested that fertilization rate is related to sperm maturation. (1) Conversely, Cui et al. demonstrated that the use of testicular sperm achieved better embryonic quality and IVF outcomes than ejaculated sperm. That study concluded that sourcing spermatozoa via testicular extraction reduced exposure to oxygen free radicals and prevented DNA damage therefore modifying IVF clinical outcomes. (2) Our study aimed to evaluate clinical outcomes of fresh blastocyst transfers of cryptozoospermia patients with sperm retrieved through testicular extraction versus fresh ejaculated sperm.

**DESIGN:** Retrospective cohort analysis.

**MATERIALS AND METHODS:** All Cryptozoospermia patients undergoing autologous IVF/ICSI with fresh blastocyst transfers from 2005 to 2019 were included. Cohorts were separated based on the source of sperm utilized (Ejaculated vs. Testicular). Demographic, clinical embryology parameters and pregnancy rates were compared among cohorts. T-test, Chi2, and multivariate regression with GEE models were used for data analysis.

**RESULTS:** A total of 188 patients were included in the analysis (Ejaculated sperm (n= 149); Testicular sperm (n=39). Demographic characteristics were similar among cohorts. No differences were found among the ejaculated and testicular cohorts for fertilization (61.7%; 64.9%, p=0.17) blastulation rates (55.8%; 55.3%, p=0.86) and count of cryopreserved blastocysts (1.58 ±2.61; 1.10 ±1.59, p=0.15) respectively. A significant difference was found among the ejaculated and testicular cohorts for in the number of cancelled cycles due to embryos unavailable for transfer (22.8%; 7.6%, p=0.03), number of embryos transferred per cycle (1.35 ±1; 1.94±1.62, p<0.001), and mean count of good quality embryos at ET (0.75 ±0.9; 1.23±1.03, p=0.005)

After adjusting for female and male patient's age, BMI, AMH and injected oocytes, no association was found with utilizing ejaculated sperm and lower odds of fertilization (OR 1.19, CI95% 0.2-6.4, p=0.8), blastulation (OR 0.4, CI95% 0.04-4.7, p=0.5), or higher odds of cycle cancellation (OR 1.1, CI95% 0.7-1.7, p=0.6). Finally, no differences were found in pregnancy, clinical pregnancy, ongoing pregnancy, multiple pregnancy, and pregnancy loss rates among cohorts.

**CONCLUSIONS:** Our study demonstrated cryptozoospermia patients who source sperm through testicular extraction or ejaculation prior to ICSI had similar ART treatment outcomes. There does not appear to be a deleterious effect with regard to fertilization, blastulation, and embryonic quality in cryptozoospermia patients who utilize ejaculated sperm found after thorough search and sedimentation. Further prospective studies including patients undergoing single euploid embryo transfers should be performed, in order to

generate personalized and evidence based recommendations for couples facing cryptozoospermia.

References: O'Connell M, McClure N, Lewis SE. Mitochondrial DNA deletions and nuclear DNA fragmentation in testicular and epididymal human sperm. *Hum Reprod.* 2002;17:1565-1570.

Cui X, Ding P, Gao G, Zhang Y. Comparison of the clinical outcomes of intracytoplasmic sperm injection between spermatozoa retrieved from testicular biopsy and from ejaculate in cryptozoospermia patients. *Urology.* 2016. <https://doi.org/10.1016/j.urology.2016.08.071>.

SUPPORT: None.

**P-245** Tuesday, October 15, 2019 6:30 AM

**DOES MALE AGE AFFECT THE SPERM PARAMETERS AND IVF OUTCOMES?** Marta Belles, MSc, Mireia Florensa, MSc, Marga Esbert, PhD. IVI RMA Barcelona, Barcelona, Spain.



**OBJECTIVE:** Compared with the effect of the aging oocyte, the effect of male age on reproductive success has been studied in much less detail. Some studies have reported that male age declines sperm parameters but also the outcomes of IVF (In Vitro Fertilization) cycles. The mechanisms responsible for the decline in sperm fitness are not fully understood but damage by oxidative stress could be an important contributor, being responsible for the majority of sperm DNA fragmentation. Advancing paternal age also has been associated with increased risk of genetic diseases in the offspring. The main objective of this study is to assess if male age has an effect on IVF outcomes and sperm parameters.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** A total of 1898 IVF cycles performed by women younger than 35 years between 2014 and 2018 in the same clinic was analyzed. Inclusion criteria were the use of ejaculated autologous sperm, ICSI performance and single embryo transfer at D+5 without preimplantation genetic diagnosis.

We assessed if male age had an effect over sperm parameters. We also studied if male age was correlated with fertilization rate, embryo quality (measured as total blastocyst and usable blastocyst rates), pregnancy, implantation, miscarriage and live birth rates. Student's group t-test and Person's product -moment correlation analysis were used for statistical analysis and level of significance was set at  $P < 0.05$ .

**RESULTS:** Age was statistically correlated with semen volume ( $P < 0.001$ ), motility percentage ( $P < 0.001$ ), the total number of progressively motile sperm ( $P < 0.001$ ), total sperm count ( $P < 0.001$ ) and progressive motility percentage ( $P < 0.001$ ) but it was not related to sperm concentration ( $P = 0.96$ ). Global fertilization rate was 70% and it was negatively related to male age ( $P = 0.04$ ). Global blastocyst rate was 56.06% while good quality embryos rate was 46.12%. No significant differences were found on both parameters ( $p = 0.93$  and  $p = 0.94$ , respectively). Overall clinical pregnancy, implantation, miscarriage and live birth rate were 57.48%, 50.58%, 11.91% and 38.51% respectively and none of them were related to male age ( $P = 0.07$ ,  $P = 0.12$ ,  $P = 0.56$  and  $P = 0.09$  respectively).

**CONCLUSIONS:** To our knowledge, this is the largest study relating male age with IVF outcomes after a single blastocyst transfer. The fact that neither embryo quality nor clinical outcomes are affected by male age may suggest that other factors such as female age can be positively influencing the cycle results. On the other hand, the analysis of these retrospective data confirm an age-related decrease in volume, sperm motility and total sperm count as well as a lower fertilization rate by ICSI in older males.

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**HIGH RATES OF ANEUPLOIDY, MOSAICISM AND ABNORMAL MORPHOKINETIC DEVELOPMENT IN CASES OF VERY SEVERE MALE FACTOR WITH FEMALE PARTNERS  $\leq 35$  YEARS.** Semra Kahraman, Prof., Murat Cetinkaya, M.D., PhD, Yucel Sahin, MD, Hakan Kadir Yelke, MsC, Yesim Kumtepe Colakoglu, MsC, Mehmet Ali Tufekci, PhD, Mesut Yesil, MsC, Cigdem Cinar Yapan, MsC. Istanbul Memorial Hospital, Istanbul, Turkey.



**OBJECTIVE:** Male infertility is a factor in approximately 50% of ART cases. Therefore, the relationship between severe male infertility and embryo

aneuploidy has long been a subject of interest. However, most studies into this relationship were based on data obtained using FISH and there have been only a limited number of studies using comprehensive chromosomal analysis. Our study evaluates the blastocyst chromosomal status and embryo morphokinetics from the first cleavage to blastocyst stage in young women ( $\leq 35y$ ) according to severe male infertility subgroups ranging from 5million/ml to non-obstructive azoospermia (NOA).

**DESIGN:** Couples applied for ART with female age  $\leq 35$  years and presented with Severe Male Factor (SMF) indication (study group) were divided into the following 3 subgroups according to sperm concentration: 1) between five million and one million, 2) less than 1 million, 3) Azoospermia: obstructive azoospermia (OA) and Non-obstructive Azoospermia (NOA).

**MATERIALS AND METHODS:** Outcomes of the study group are compared with the control group that was composed of males with normal sperm parameters ( $>39$  million and  $>40\%$  motile sperm in the ejaculate). 543 severe male infertility cases with partners  $\leq 35y$  and 310 control cases with normal sperm parameters were studied. Initially aCGH and latterly NGS were used for PGT-A and time lapse microscope for morphokinetic evaluation.

**RESULTS:** Significantly higher chromosomal aneuploidy rates (58%) were found in couples with NOA than the other SMF groups and control groups with normal sperm parameters ( $p < 0.001$ ). Mosaicism rates were higher in all SMF subgroups than the controls but significantly so only in NOA ( $p < 0.05$ ). Higher rates of abnormality in chromosomes 2,10,11 17, 21 and sex chromosomes were observed in the most severe forms of SMF groups, NOA and less than 1m/ml groups. However, they were significantly higher only in the testicular sperm groups ( $p < 0.05$ ).

Embryo morphokinetic evaluation showed that embryos in the NOA groups reached the first cleavage significantly faster than those in the control group 26.79h vs. 27.01h, respectively;  $p = 0.048$ ). Furthermore, significantly higher rates of direct uneven cleavage (27%) and arrested embryos ( $p < 0.05$ ) from PN stage to the blastocyst stage were observed in NOA and in the less than 1m sperm groups.

**CONCLUSIONS:** Higher rates of chromosomal abnormality, mosaicism and morphokinetic abnormalities were associated in severe male factor cases particularly with testicular sperm obtained from azoospermic cases with female partners  $\leq 35$  years.

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**DOES USE OF TESTICULAR SPERM IMPROVE OUTCOMES IN NONAZOOSPERMIC COUPLES WITH PREVIOUS IVF FAILURE USING EJACULATED SPERM?**



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**OBJECTIVE:** Due to controversial evidence that testicular sperm is associated with lower sperm DNA fragmentation (SDF) and improved outcomes compared to ejaculated sperm (ES), this study evaluates intracytoplasmic sperm injection (ICSI) outcomes using testicular sperm in nonazoospermic couples with prior IVF failure using ES.

**DESIGN:** Retrospective cohort

**MATERIALS AND METHODS:** Jan 2015-Aug 2018, 64 nonazoospermic couples with  $\geq 1$  prior failed ART cycles using ES underwent testicular sperm extraction (TESE) for IVF-ICSI. Failed cycles with ES: those not progressing to clinical pregnancy. Outcomes using TESE sperm were compared to the mean values of couples' prior cycles using ES. Primary outcomes: clinical pregnancy and live birth rates (CPR & LBR). Secondary outcomes: fertilization and blastocyst conversion.

**RESULTS:** Average number of prior failed ART cycles using ES: 2.5 (range: 1-8). 71.8% of males had abnormal semen parameters. A subset of men ( $n = 28$ ) had SDF assessment (measured by sperm chromatin dispersion) of ES. Mean SDF was 39% (7-84%); 21 patients had SDF  $> 25\%$ . 88 total ICSI cycles were performed using TESE sperm (64 cycles: fresh TESE, 24 cycles: frozen-thawed). There were 52 fresh blastocyst transfers, 15 frozen blastocyst transfers, and 21 cycles without transfer (9 additional FETs using supernumerary embryos; 76 total transfers). A comparison of TESE-ICSI cycles in those couples with  $\geq 2$  prior failed ART cycles using ES yielded similar findings to the whole group.

TABLE 1. Outcomes comparing TESE couples to their prior ART cycles using ES. Blastocyst conversion=% of cycles with  $\geq 1$  blastocysts available to use. P values comparing pregnancy outcomes were not performed due to regression of the mean.

	TESE (n=88), Mean	Ejaculated (n=64), Mean	P value	Ejaculated SDF >25%: TESE (n=21; 24 transfers), Mean	Ejaculated SDF >25%: Ejaculated (n=21), Mean	P value
# oocytes retrieved	17.8	17.4	0.27	19.5	16.3	0.27
#M2's	11.7	12.1	0.72	11.6	10.8	0.74
% M2's fertilized	61.4%	59.0%	0.66	57.1%	50.6%	0.37
No blastulation	24/88 (27.3%)	39/64 (60.9%)	<0.001	9/21 (42.9%)	15/21 (71.4%)	0.02
Blastocyst conversion	61.9%	42.9%	<0.001	57.1%	28.6%	0.001
# blastocysts transferred	1.7	1.6	0.56	1.7	1.5	0.56
# blastocysts vitrified	1.6	0.6	0.003	1.2	0.4	0.006
CPR	32/76 (42.1%)	0%	—	10/24 (41.7%)	0%	—
Spontaneous abortion	8/76 (10.5%)	0%	—	1/24 (4.2%)	0%	—
LBR	27/76 (35.5%)	0%	—	9/24 (37.5%)	0%	—

**CONCLUSIONS:** In nonazoospermic couples with failed ART using ES, ICSI using TESE sperm may improve blastocyst development, number of embryos available for vitrification, CPRs, and LBRs. Testicular sperm may avoid the adverse effects of elevated SDF from ES and improve pregnancy outcomes in some patients. Randomized studies are needed to determine if such a benefit exists.

### IVF OUTCOME PREDICTORS - TRIGGER

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#### DOES OVULATORY TRIGGER CHOICE INFLUENCE MATURITY AND DEVELOPMENTAL COMPETENCE OF FROZEN-THAWED OOCYTES?

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**OBJECTIVE:** The luteinizing hormone (LH) surge stimulates resumption and progression of meiosis in oocytes from prophase to metaphase in preparation for fertilization. Given that oocyte maturity is a developmental continuum, it is unclear whether changes in the duration or level of the LH surge can have downstream effects on the microenvironment of the cumulus-oocyte complex, leading to variations in the integrity of oogenesis and early chromosomal segregation. Studies have investigated the effects of different oocyte maturation triggers—human chorionic gonadotropin (hCG), GnRH agonist (Lupron), or a combination of the two (dual)—on IVF outcomes.<sup>1,2</sup> Some evidence has suggested pregnancy rates are lower with Lupron triggers, possibly due to the shorter duration of the LH surge.<sup>3</sup>

Use of oocyte cryopreservation has increased, but most patients have yet to utilize these oocytes. Consequently, the effect of trigger type on developmental competence of frozen oocytes suspended in metaphase II is still unknown. The objective of this study was to determine whether rates of oocyte survival post-rewarming, maturation, fertilization, blastulation, and ploidy were affected by trigger type.

**DESIGN:** Retrospective, cohort study

**MATERIALS AND METHODS:** The study included patients at an academic ART center who underwent oocyte cryopreservation and subsequent re-warming for IVF/ICSI between 2010 and 2019. Patients were grouped by oocyte maturation trigger type used during their initial cycle: (1) hCG, (2) Lupron, (3) dual. Primary outcomes were thaw survival and oocyte metaphase II (MII) rates. Secondary outcomes were fertilization, blastulation, and ploidy rates. Statistical analysis was performed with the use of T-tests, chi-square tests, and multivariate linear regressions with generalized estimating equations.

**RESULTS:** A total of 182 cycles from 167 patients were included in this study. Controlling for oocyte age, AMH, and gravidity, there was no statistically significant difference in rates of thaw survival, MII, fertilization, or ploidy between groups. There was, however, a statistically significant dif-

ference in blastulation rate (Dual vs. Lupron:  $\beta=31.6$ ,  $p=0.006$ , hCG vs. dual:  $\beta=10.5$ ,  $p=0.34$ ; hCG vs. Lupron:  $\beta=-21.2$ ,  $p=0.14$ ).

**CONCLUSIONS:** Studies of the effects of oocyte maturation trigger on pregnancy outcomes are conflicting, and have focused on implantation in fresh IVF cycles. In contrast, this study examines surrogate endpoints for the efficacy of hCG, Lupron only, and dual trigger in a group of non-infertile young women. We showed that trigger type does not affect survival rates following oocyte warming, or MII rate. There appears to be an increase in blastulation rates between patients using dual trigger, compared to Lupron only. This finding is in agreement with a prior study that compared dual trigger vs. Lupron alone in high responder patients undergoing autologous IVF.<sup>4</sup> Future studies might aim to analyze oocytes and granulosa cells from follicles triggered with dual trigger vs. Lupron alone, focusing on early molecular pathways and gene networks that are integral to embryonic genome activation.

**References:** 1. Shapiro BS, Daneshmand ST, Garner C et al, Comparison of “triggers” using leuprolide acetate alone or in combination with low-dose human chorionic gonadotropin. *Fertil Steril.* 2011; 95: 2715–2717

2. Griffin D, Feim R, Engmann L, et al. Dual trigger with gonadotropin-releasing hormone agonist and standard dose human chorionic gonadotropin to improve oocyte maturity rates. *Fertil Steril* 2014; 102:405–409.

3. Kolibianakis, EM, Schultze-Mosgau, A., Schroer, A., van Steirteghem, A., Devroey, P., Diedrich, K. et al. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists. *Hum Reprod.* 2005; 20: 2887–2892

4. Werner MD, Forman EJ, Hong KH, et al. Dual trigger with GnRH agonist and varying doses of hCG increases the blastulation rate amongst high responders. *Fertil Steril* 2014; 102(3):e220.

**SUPPORT:** None.

P-249 Tuesday, October 15, 2019 6:30 AM

#### DUAL TRIGGER USING RECOMBINANT HCG AND GONADOTROPIN-RELEASING HORMONE AGONIST IMPROVE OOCYTE QUALITY AND EMBRYO GRADING FOR NORMAL RESPONDERS IN GNRH ANTAGONIST CYCLES: RANDOMIZED CONTROLLED TRIAL.

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**OBJECTIVE:** To evaluate the effectiveness of dual trigger using gonadotropin-releasing hormone (GnRH) agonist and recombinant human chorionic gonadotropin (rHCG) versus rHCG alone for normal responders in GnRH antagonist intracytoplasmic sperm injection (ICSI) cycles

**DESIGN:** Randomized, open-labeled, controlled trial (clinical trial.gov: NCT02916173).

**MATERIALS AND METHODS:** All women attended for first planned fresh embryo transfer ICSI cycles were invited to participate in the study if

they met our inclusion criteria. We included women aged less than 40 years, body mass index (BMI) ranges from 18-30 kg/m<sup>2</sup>, Anti-mullerian hormone (AMH) levels more than one ng/ml, normal, mild or moderate male factor infertility. The study participants were randomized to either group I (HCG group) triggered by 250µg of rHCG or group II (dual trigger group) triggered by 250µg of rHCG and GnRH agonist; 1 mg leuprolide acetate. The primary outcome was the number of MII oocytes in both groups. The secondary outcomes included the number of oocytes retrieved, number of Grade 1 embryos, fertilization rate, implantation rate, clinical pregnancy rate, miscarriage rate, live birth rate, the cumulative pregnancy rate per embryo transfer and cumulative live birth rate among both groups. Student's t-test and Chi-square test were used for the analysis of the outcomes.

**RESULTS:** One hundred and sixty women consented to participate and randomized (80 women in each arm). Both groups were similar in baseline demographic and clinical characteristics as mean age, BMI, duration, cause of infertility and hormonal profile. In comparison to the HCG group, women who received dual trigger had a statistically significantly higher number of retrieved oocytes (14.20±7.868 vs. 10.53±4.79, p=0.001), number of M II oocytes (10.78±6.758 vs. 8.48±4, p=0.01) and number of grade 1 embryos (5.28±3.79 vs. 4.29±2.66, p=0.04). The fertilization rate was slightly higher in the HCG group, but this did not reach a statistical significance (77.6% vs. 73.7%, p=0.442). No difference between both groups regarding the chemical (p=0.312), clinical pregnancy (0.621), implantation (p=0.731), miscarriage (p=0.523), multiple pregnancy (p=1.00) and live birth rates (p=0.725) between both groups. The dual trigger group showed significantly higher clinical pregnancy (p=0.04) and live birth rates (p=0.03) after frozen-thawed embryos transfer. No significant difference among both groups regarding the cumulative pregnancy and cumulative live birth rates (p=0.08).

**CONCLUSIONS:** Dual trigger by GnRH agonist and rHCG could improve the oocyte quality and embryo grading for normal responders in GnRH antagonist ICSI cycles.

**SUPPORT:** None.

**P-250**

**WITHDRAWN**

**P-251** Tuesday, October 15, 2019 6:30 AM

#### **TO BOOST OR NOT TO BOOST: DOES ADMINISTRATION OF RESCUE HCG IMPROVE OUTCOMES IN POOR RESPONDERS WITH LOW POST-TRIGGER VALUES?**

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**OBJECTIVE:** Ovarian hyperstimulation syndrome (OHSS) is a potential complication of ART that can be concerning to patients and a difficult therapeutic challenge to physicians. One preventative measure to minimize the risk of OHSS is to lower the dose of hCG prior to retrieval. However, there is a threshold under which final maturation of the cumulus cell-oocyte complex might not occur. Several surrogate markers may be used to determine appropriate response to trigger, including serum progesterone (P4) or hCG on day after trigger administration. When these markers suggest an inadequate response, some clinicians supplement patients with booster or "rescue" hCG. However, there is limited data on the effectiveness of rescue hCG in improving oocyte yield. Our goal was to compare outcomes between patients who did or did not receive rescue hCG in a population of patients with an inadequate response to trigger.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Our study included patients at a single academic center who underwent controlled ovarian hyperstimulation and met criteria for rescue hCG (P4 <1.0ng/dl or hCG level <40mIU/mL on day after trigger) from 2004 to 2019. Patients were separated into 2 groups based on administration of supplemental hCG (Case Group: hCG trigger 36 hours and rescue hCG 12-24 hours prior to retrieval; Control Group: hCG trigger 36 hours prior to oocyte retrieval). Patients were excluded if leuprolide acetate was used for trigger, either as a dual trigger or as leuprolide alone. A sub-analysis of poor responders to COH (Bologna criteria: age >40, antral follicle count ≤ 10 follicles total, or AMH ≤ 1ng/ml) was performed. Primary outcome was the number of oocytes retrieved. Data were

analyzed using students t-tests, chi square tests, and a multivariate logistic regression analysis, with p<0.05 considered significant.

**RESULTS:** A total of 732 patients who underwent 833 cycles were assessed. The case group consisted of 397 cycles in which both 36 hour and subsequent rescue hCG prior to retrieval were used. The control group consisted of 436 cycles in which a single hCG trigger 36 hours prior to retrieval was used. There were significant differences in age, AMH, BMI, the number of follicles ≥ 14mm visualized on day of trigger, estradiol, and progesterone on day of trigger between groups. After adjusting for the confounding variables, use of rescue hCG did not predict number of eggs retrieved ( $\beta = 0.05$ , p = 0.83). In our sub-analysis of poor responders that controlled for the same confounders, we found that the use of rescue hCG was significantly correlated with the number of eggs retrieved ( $\beta = 0.53$ , p = 0.03).

**CONCLUSIONS:** In the largest study to date evaluating the use of rescue hCG to improve oocyte yield, our data suggest an improvement in number of eggs retrieved in a subset of patients. While we did not demonstrate clinical advantage to using rescue hCG in the general study group, we found that a subset of poor responder patients benefited from supplemental hCG. Future studies would benefit from validating a threshold level for peak progesterone or hHCG that customizes the use of rescue hCG.

**SUPPORT:** None.

**P-252** Tuesday, October 15, 2019 6:30 AM

#### **GNRH-AGONIST TRIGGER IN 'FREEZE-ALL' CYCLES: IMPROVES PREGNANCY RATES AND PATIENT SAFETY.**

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**OBJECTIVE:** To evaluate whether GnRH-agonist (GnRHa) triggering improves embryo quality and live birth rates in 'freeze-all' cycles compared to human chorionic gonadotrophin (hCG).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This retrospective cohort study from January 2012 and December 2014 compared GnRHa and hCG-triggered 'freeze-all' cycles. Limiting for first cycle per patient, 396 GnRHa and 1,868 hCG-triggered cycles were included. Only cycles where embryos were available and thawed for transfer were included for live birth rates (LBR). 217 GnRHa and 509 hCG-triggered cycles were analysed for LBR. A multiple imputation approach was used to account for missing data. The primary outcome was LBR. Secondary outcomes included number of oocytes collected, embryo grade and quality, clinical pregnancy rates and the incidence of ovarian hyperstimulation syndrome (OHSS). Regression analysis was performed to adjust for confounders. P-values <0.05 were considered statistically significant.

**RESULTS:** The singleton LBR after one embryo transfer was higher in GnRHa triggered 'freeze-all' cycles compared to hCG (38.4% vs. 24.6%, p=0.001), as well as a non-significantly higher cumulative LBR (57.4% vs. 41.1%, p=0.18). There was no difference in the number of embryos thawed or transferred, and there was no difference in embryo grade or expansion. The incidence of OHSS was significantly lower in GnRHa triggered cycles (0.5% vs. 1.9%, p=0.008).

**CONCLUSIONS:** GnRHa triggering resulted in a superior LBR compared to hCG in 'freeze-all' cycles, even after adjusting for confounders. GnRHa triggering did not compromise embryo quality and significantly reduced the risk of OHSS compared to hCG. Given these findings, GnRHa triggering appears to be the way of the future for 'freeze-all' cycles.

**Reference:** None.

**SUPPORT:** None.

**P-253** Tuesday, October 15, 2019 6:30 AM

#### **HOW WE TRIGGER MATTERS: INTRANASAL GnRH-AGONIST TRIGGER MAY REDUCE OOCYTE MATURATION COMPARED TO SUBCUTANEOUS ADMINISTRATION IN ICSI CYCLES.**

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**OBJECTIVE:** To evaluate oocyte maturation and fertilisation rates of intracytoplasmic sperm injection (ICSI) cycles triggered with intranasal and subcutaneous GnRH-agonists (GnRH<sub>a</sub>).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This retrospective cohort study from May 2016 to August 2018 compared intranasal and subcutaneous GnRH<sub>a</sub> triggers in ICSI cycles. Data was extracted from 9588 ICSI cycles. A total of 781 cycles were included for analysis after excluding cycles triggered with hCG (n=8521), duplicate patient cycles (n=182), where a dual trigger was used (n=55) or where the trigger was not recorded (n=49). 214 cycles utilised the intranasal Nafarelin trigger (*Synarel; Pfizer Pty Ltd*) and 567 cycles used a subcutaneous formulation, either Triptorelin (*Decapeptyl; Ferring Pharmaceuticals Pty Ltd*) or Leuprorelin (*Lucrin; AbbVie Pty Ltd*). The primary outcome was oocyte maturation rate. Secondary outcomes included number of mature oocytes collected, number of fertilised oocytes, fertilisation rate and the incidence of ovarian hyperstimulation syndrome (OHSS). Categorical data was presented as a proportion (%) and p-values were obtained by performing a Mann-Whitney U test. Continuous and count data was presented as a mean with standard deviation and standard error of mean, and p-values were obtained by performing the student's T-test. Univariate and adjusted analyses were performed using negative binomial regression for count measures and linear regression for continuous measures. Statistical significance was defined as a p-value <0.05.

**RESULTS:** There was a trend towards higher oocyte maturation rates in patients receiving a subcutaneous GnRH<sub>a</sub> trigger compared to intranasal formulations (78.1% vs. 77.6%, p=0.059). There was a statistically significant difference in fertilisation rate in favour of the subcutaneous trigger (68.0% vs. 67.9%, p=0.016). There was no difference in the age or BMI of patients, nor was there a difference in the crude number of mature or fertilised oocytes. The incidence of OHSS was significantly lower in patients receiving the subcutaneous GnRH<sub>a</sub> triggered cycles (0.0% vs. 1.4%, p=0.004).

**CONCLUSIONS:** Subcutaneous administration of the GnRH<sub>a</sub> trigger may improve oocyte maturation and fertilisation rates in ICSI cycles, and is associated with lower rates of OHSS. Given these findings, a prospective randomised controlled trial is needed to further elucidate whether a subcutaneous formulation outperforms an intranasal GnRH<sub>a</sub> trigger.

**SUPPORT:** None.

**P-254** Tuesday, October 15, 2019 6:30 AM

**HCG TRIGGER VERSUS GNRH AGONIST TRIGGER IN PCOS UNDERGOING IVF CYCLES: FROZEN EMBRYO TRANSFER OUTCOME.** Deepika Krish, MS, FRM Consultant in Rep Med, BANGALORE, India.



**OBJECTIVE:** The use of Gonadotrophin releasing hormone agonist (GnRH<sub>a</sub>), with freeze-all strategy followed by frozen embryo transfer (FET) has been found to eliminate the risk of ovarian hyperstimulation syndrome (OHSS) in women with polycystic ovarian syndrome (PCOS) undergoing IVF cycles. However, there still has been hesitations with the use of GnRH<sub>a</sub> as a routine trigger replacing Human chorionic gonadotrophin (hCG), for concerns of compromised cycle outcome. We aimed to evaluate the outcome following transfer of embryos in FET cycles obtained from GnRH<sub>a</sub> trigger in comparison with hCG trigger in PCOS of Asian origin.

**DESIGN:** Prospective observational cohort study. 210 PCOS undergoing IVF in an antagonist protocol who were randomized in the previous study (to evaluate if GnRH<sub>a</sub> trigger is a better alternative than hCG in PCOS for prevention of OHSS; Group A: GnRH<sub>a</sub> trigger (n=92)) and Group B: hCG trigger (n=101)], were followed up in FET cycles to assess the outcome.

**MATERIALS AND METHODS:** A prospective, observational study was conducted in a tertiary care center- Milann, The fertility center, Bangalore to assess the frozen- thawed embryo transfer cycle outcome following GnRH<sub>a</sub> trigger and hCG trigger in PCOS. In the previous randomized controlled trial conducted between May 2013 and November 2015, [comparing GnRH<sub>a</sub> agonist with hCG trigger in an antagonist protocol for prevention of OHSS

with freeze-all strategy; 210 PCOS patients were randomized; 92 subjects in Group A: GnRH<sub>a</sub> trigger (n=92) and Group B: hCG trigger (n=101) included for the final analysis (Deepika et al., 2016)], were followed up prospectively over a period of three years. All participants underwent subsequent frozen-thawed embryo transfer cycles and the treatment outcome of these subjects is reported herein.

**RESULTS:** The odds of cumulative live birth rate per stimulation cycle favours GnRH<sub>a</sub> trigger as against hCG trigger [OR= 2.15; (CI 1.2-3.83); P=0.008]. A significantly higher number of mature oocytes (19.1±11.7 versus 14.1±4.3; P<0.001) and blastocysts (4.2±1.63 versus 3.26±1.22; P<0.001) were available in GnRH<sub>a</sub> group as compared to hCG group.

**CONCLUSIONS:** The cumulative live birth rate is better following transfer of frozen-thawed embryos generated from GnRH<sub>a</sub> triggered cycles compared to hCG trigger. Hence, in PCOS undergoing IVF, as a good practice point, hCG trigger should be replaced by GnRH<sub>a</sub> trigger with vitrification of all embryos followed by FET later.

**SUPPORT:** NIL.

**P-255** Tuesday, October 15, 2019 6:30 AM

**THE EFFECT OF FOLLICLE STIMULATING HORMONE ADMINISTRATION AT THE TIME OF HUMAN GONADOTROPIN TRIGGERING, IS IT IMPROVE THE OOCYTE/EMBRYO PROFILE IN IN VITRO FERTILIZATION CYCLES?** Young Sang Kim, M.D.<sup>a</sup>



Dong Soo Park, M.D.,<sup>b</sup> Mi Kyoung Koong, MD, PhD,<sup>c</sup> You Shin Kim, M.D, Ph.D.,<sup>d</sup> Myung Joo Kim, M.D.,<sup>a</sup> Ran Kim, M.D.,<sup>c</sup> Hyeok Kim, MD, PhD,<sup>f</sup> Tae Ki Yoon, M.D, Ph.D.,<sup>a</sup> Chanhong Park, M.D.,<sup>d</sup> Hannah Kim, MD.<sup>a,g</sup> <sup>a</sup>CHA Fertility Center Seoul Station, Obstetrics and Gynecology, Seoul, Korea, Republic of (South); <sup>b</sup>CHA Fertility Center, Seoul, Korea, Republic of (South); <sup>c</sup>Department of OB/GY CHA Fertility Center Seoul Station, CHA University, Seoul, Korea, Republic of (South); <sup>d</sup>CHA Fertility Center Seoul Station, Seoul, Korea, Republic of (South); <sup>e</sup>Department of OB/GY, CHA Fertility Center Seoul Station, CHA University, Seoul, Korea, Republic of (South); <sup>f</sup>CHA Fertility Center Seoul Station, OB&GY, Seoul, Korea, Republic of (South).

**OBJECTIVE:** To evaluate whether an additional follicle stimulating hormone (FSH) administration at the day of human chorionic gonadotropin (hCG) triggering can improve the oocyte/embryo quality and pregnancy rates in vitro fertilization (IVF) cycles.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** 585 patients in fresh IVF cycles with antagonist protocol divided into two groups. FSH injection at hCG triggering day (N=211) and did not (N=374). We estimate the maturation rates of retrieved oocytes, fertilization rates, top quality (Grade 1~2) embryo counts and pregnancy outcomes in two groups.

**RESULTS:** There was no significant difference between two groups in patient's demographics (age, body mass index, anti-müllerian hormone level, infertility etiology), and characteristics of fresh IVF cycles (total FSH injection dose, estradiol/luteinizing hormone/progesterone level on hCG triggering day, endometrial thickness on hCG triggering day). For outcomes of fresh IVF cycles, matured oocyte count (6.9±3.7 vs 7.1±4.0; p=0.502), fertilization rate (69.0% vs 70.3%; p=0.452), top quality embryo count (2.5±2.0 vs 2.2±1.6; p=0.086) were not significantly differ. For pregnancy outcomes of fresh IVF cycles, implantation rate (54.5% vs 48.1%; odds ratio [OR], 1.29; 95% confidential interval [CI], 0.92-1.81) and clinical pregnancy rate (42.7% vs 35.0%; OR, 1.38; 95% CI, 0.98-1.95) were not significantly differ, but ongoing pregnancy rate (38.4% vs 29.1%; OR, 1.51; 95% CI, 1.06-2.16) was significantly higher in FSH injection group.

**CONCLUSIONS:** The effect of an additional FSH administration at the day of hCG triggering did not improve the oocyte/embryo profile. Implantation rates and clinical pregnancy rates were increased in FSH injection group, but there was no significant difference between two groups. Ongoing pregnancy rates was significantly higher in FSH injection group compared with no FSH injection group.

**P-256** Tuesday, October 15, 2019 6:30 AM

**DOES POST-TRIGGER SERUM B-HCG VALUE MATTER IN PATIENTS WITH SUBOPTIMAL LH RESPONSE AFTER DUAL TRIGGER CYCLES?** Kolbe Hancock, MD,<sup>a</sup> Chelsea Canon, MD,<sup>b</sup>



Allison C. Petrini, MD,<sup>c</sup> Alexis Melnick, MD,<sup>c</sup> Zev Rosenwaks, M.D.<sup>a,4</sup> The Ronald O. Perleman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY; <sup>b</sup>New York Presbyterian Weill Cornell, New York, NY; <sup>c</sup>Ronald O. Perleman and Claudia Cohen Center for Reproductive Medicine, New York, NY.

**OBJECTIVE:** The use of dual trigger during ovarian stimulation to initiate the final maturation of oocytes has become an increasingly popular technique, as it decreases the risk of ovarian hyperstimulation syndrome. In GnRH agonist trigger cycles, there is a subset of individuals who have a sub-optimal response to GnRH agonist, and in turn decreased oocyte yield and oocyte maturity<sup>2,3</sup>. Post trigger serum luteinizing hormone (LH) levels >15 mIU/mL and ideally ≥30 mIU/mL have been associated with improved cycle outcomes<sup>2,3</sup>. In patients who received a dual trigger but had a suboptimal response to GnRH agonist trigger, we sought to investigate whether the post hCG value was correlated with oocyte maturity rate.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All patients 23-49 years old undergoing IVF stimulation cycles between 2010 and 2019 who received dual trigger with 4mg Lupron and variable doses of hCG ranging from 1,000-10,000 were analyzed for inclusion. Those whose LH level post trigger was <30 mIU/mL (LH <30) were included for analysis. The primary outcome was oocyte maturity rate. STATA Statistical Software Version 11 (StataCorp LP) was used for data analysis. A multivariate linear regression was used to assess whether the post hCG level was associated with the oocyte maturity rate in all patients with LH <30. The same regression was performed for subgroups of LH <30 in increments of 5 mIU/mL. For patients with an LH <30, multivariate linear regression was performed to assess whether the summed value of the post hCG and LH was associated with a difference in the oocyte maturity rate.

**RESULTS:** A total of 204 cycles meeting the inclusion criteria were analyzed. The average age was 36.7 ± 5.5 years, and the average BMI was 27.2 ± 6.9 kg/m<sup>2</sup>. The post hCG values ranged from 15 to 425 mIU/mL. Comparing all patients with an LH <30 and controlling for BMI and age, there was no significant correlation between post hCG and percent mature oocytes (p=0.456). Similarly, when controlling for BMI and age, there was no significant correlation between the sum of post-trigger serum LH and b-hCG and the oocyte maturity rate (p=0.38). When the post-trigger LH value was stratified by increments of five mIU/mL from 0 to 30, there was still no significant correlation between the post hCG and the oocyte maturity rate (p values range from 0.46 to 0.88). Amongst those with a post trigger LH <15 mIU/mL, there was no significant difference in the oocyte maturity rate when the post HCG was above or below 50 mIU/mL. Similarly, amongst those with a post trigger LH between 15 mIU/mL and 30 mIU/mL, there was no significant difference in the oocyte maturity rate when the post HCG was above or below 50 mIU/mL.

**CONCLUSIONS:** In patients receiving dual trigger who fail to mount an optimal response to the GnRH agonist component, post hCG level does not correlate with oocyte maturity rate. When stratified by the post trigger LH level, we have shown that there is not a specific LH value at which the post trigger hCG level had an impact on the primary outcome. There also does not appear to be an optimal summed value of post-trigger LH and b-hCG that is correlated with the oocyte maturity rate.

**References:** 1. Gunnala, Vinay, et al. "Sliding Scale HCG Trigger Yields Equivalent Pregnancy Outcomes and Reduces Ovarian Hyperstimulation Syndrome: Analysis of 10,427 IVF-ICSI Cycles." *PLoS One*, vol. 12, no. 4, 2017, <https://doi.org/10.1371/journal.pone.0176019>.

2. Chen, S.-L., et al. "Circulating Luteinizing Hormone Level after Triggering Oocyte Maturation with GnRH Agonist May Predict Oocyte Yield in Flexible GnRH Antagonist Protocol." *Human Reproduction*, vol. 27, no. 5, 2012, pp. 1351-1356., <https://doi.org/10.1093/humrep/des049>.

3. Meyer, Laura, et al. "Risk Factors for a Suboptimal Response to Gonadotropin-Releasing Hormone Agonist Trigger during In Vitro Fertilization Cycles." *Fertility and Sterility*, vol. 104, no. 3, 2015, pp. 637-642., <https://doi.org/10.1016/j.fertnstert.2015.06.011>.

**SUPPORT:** None.

**P-257** Tuesday, October 15, 2019 6:30 AM

#### EFFECT OF DUAL TRIGGER ON THE OUTCOME OF CONTROLLED HYPERSTIMULATION IN ART CYCLES.

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Parameter	Group A (N=28)	Group B (N=25)	Significance
Age	31.75 ± 4.5	31.5 ± 3.9	P=0.873
BMI	25.9 ± 4.1	26.7 ± 5.0	P=0.570
Duration of infertility	6.9 ± 4.6	5.3 ± 3.5	P=0.238
Antral follicle count (AFC)	14.07 ± 4.6	13.6 ± 4.8	P=0.391
AMH (ng/ml)	2.4 ± 0.6	2.2 ± 0.7	P=0.271
Total gonadotropin dose (IU)	3759.8 ± 1776.3	4260.0 ± 1530.0	P=0.184
Duration of stimulation	10.03 ± 1.6	10.6 ± 1.6	P=0.217
No. of oocytes retrieved	10.2 ± 4.5	9.2 ± 3.2	P=0.452
No. of mature (M-II) oocytes	7.6 ± 4.03	6.6 ± 3.1	P=0.431
Oocyte maturation rate (%)	74.9 ± 20.8	68.7 ± 21.2	P=0.288
Good & fair quality mature oocytes (%)	59.03 ± 33.0	84.9 ± 20.1	P=0.002*
Poor quality mature oocytes (%)	37.4 ± 31.8	14.6 ± 20.3	P=0.004*
Fertilization rate (%)	74.6 ± 26.7	69.1 ± 31.3	P=0.444
Pregnancy rate (%)	40 (10/11)	50 (7/14)	P=0.54
Miscarriage rate (%) in FET	27.2 (3/11)	Nil	

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**OBJECTIVE:** Dual trigger by administration of hCG with GnRH agonist, was shown to improve the outcome of ART (Assisted Reproductive Technology) cycles: oocyte maturity, ongoing pregnancy and live birth rate. The hCG and LH differ by their receptor binding and intracellular signaling, and there by not equivalent in their action. The FSH surge induced by GnRH agonist is known to positively affect oocyte maturation. The present study is aimed to know the effect dual trigger on the oocyte yield & quality, oocyte maturity, and fertilization in ART cycles.

**DESIGN:** Randomized controlled trial.

**MATERIALS AND METHODS:** All the patients who underwent controlled ovarian hyperstimulation (COH), and planned for ovulation trigger were included. Women with advanced age (>42 years), oocyte donation cycles, expected hyperresponders (AMH > 4 ng/ml) and poor responders (AMH <1.1 ng/ml) were excluded from the analysis. On the day of ovulation trigger, the study subjects were randomized to receive hCG trigger with uhCG 10,000 IU as Group A, and dual trigger with 10,000 IU of uhCG + 0.2 mg Triptorelin as Group B. COH was performed by antagonist protocol by rFSH with or without hMG. The outcome of COH such as oocyte number and maturity, oocyte quality, fertilization rate and pregnancy rate were compared between the study groups.

**RESULTS:** Of the 53 patients, 28 women received hCG trigger and 25 women received dual trigger. There was no significant difference between the number of oocytes retrieved, mature oocytes, and fertilization rate. The percentage of good & fair quality mature oocytes were significantly higher and the percentage of poor quality mature oocytes were significantly lower in the Group B compared to group A. The pregnancy rates were slightly higher in the Group B, but did not reach statistical significance.

**CONCLUSIONS:** Dual trigger is associated with increased oocyte quality in ART. The effect of dual trigger on pregnancy rate needs to be evaluated on a larger data.

**SUPPORT:** Self funded.

#### NURSING

**P-258** Tuesday, October 15, 2019 6:30 AM

**CONTENT AND ACCURACY OF FERTILITY COUNSELING VIA ASYNCHRONOUS DIGITAL WOMEN'S HEALTH CLINICS.** Lindsey N. Ulin, B.S.,<sup>a</sup> Rashmi Kudesia, MD.<sup>b</sup> <sup>a</sup>McGovern Medical School at The



University of Texas Health Science Center at Houston, Houston, TX;  
bCCRM Fertility Houston, Houston, TX.

**OBJECTIVE:** To assess the content and accuracy of fertility counseling received via asynchronous peer and professional input through a digital women's health clinic.

**DESIGN:** Quantitative and qualitative assessment of publicly available online content

**MATERIALS AND METHODS:** The fertility treatment forum of an established digital women's health clinic, consisting of posts answered asynchronously by peers and professionals, were queried for available posts. All questions and answers were transcribed and then categorized by question topic and theme, quantity and quality of responses, and credentials of respondents. Answers were reviewed for accuracy by a board-certified reproductive endocrinologist.

**RESULTS:** 87 questions were available for review, posted over a 6-month timeframe in 2018-19. Of these, 47 (54.0%) related to in vitro fertilization (IVF), 20 (23.0%) to oocyte cryopreservation (OC), and 20 (23.0%) to intrauterine insemination (IUI). A minority of posts (17, 19.5%) primarily sought emotional support. Respondents were as follows: 10 (17.2%) fellow patients, 9 (15.5%) allied health providers, 25 (43.1%) nurses or midwives, and 11 (19.0%) physicians, including 3 (5.2%) reproductive endocrinologists.

Of all 87 posts, 38 (43.7%) received no answer, 40 (46.0%) received 1 answer, and 9 (10.3%) received 2 answers. The unanswered questions (30) were mostly (78.9%) medical in nature, with 5 (13.2%) requesting emotional support and 3 (7.9%) seeking logistical clarifications. Of the 58 answers, 18 (31.0%) recommended a synchronous video follow-up appointment without offering any medical advice. Substantive answers offered a mix of the following attributes: 22 (37.9%) emotional encouragement or support, 11 (19.0%) narration of personal experiences, and 27 (46.6%) medical advice. Of those offering medical advice, 20 (34.5% of all answers) were deemed medically accurate.

**CONCLUSIONS:** Online forums and digital clinics are increasingly available and utilized for patients struggling with infertility. As access to high-quality infertility care remains limited due to cost and geography, asynchronous forums hold the potential to fill gaps in care and provide emotional support. However, in our analysis of the leading digital women's health clinic, those patients seeking answers in the infertility treatment forum received no assistance nearly half of the time, and only a third of responses were deemed medically accurate. Most responses (94.8%) were not from an individual specifically trained in reproductive endocrinology. Though further evaluation of similar sites and resources is indicated, we conclude that asynchronous digital medicine is currently a highly inaccurate and unreliable source of information for fertility patients. Further efforts are needed to ensure that women and couples can access appropriately-trained and specialized physicians and nurses to answer their detailed questions and guide treatment in a compassionate and evidence-based manner.

**SUPPORT:** None.

**P-259**

**WITHDRAWN**

**P-260** Tuesday, October 15, 2019 6:30 AM

#### **OPTIMIZING UTILIZATION OF EMOTIONAL SUPPORT DURING INFERTILITY TREATMENT.**

Sarah A. Hirsch, DO,<sup>a</sup> Pippa Simpson, PhD,<sup>b</sup> Kathryn E. Flynn, PhD,<sup>a</sup> Melodee Nugent, MA,<sup>a</sup> Abbey Kruper, PsyD<sup>a</sup> <sup>a</sup>Medical College of Wisconsin, Milwaukee, WI; <sup>b</sup>Affiliation not provided.



**OBJECTIVE:** The psychological distress of infertility influences decision-making and treatment discontinuation. Yet, only 10-34% of patients with infertility pursue counseling. Historically, barriers included logistics of scheduling appointments and sufficient coping resources. The objective of this study was to identify barriers to counseling for women with infertility in a clinic with embedded psychological support; and determine if those barriers were dependent upon screening scores for anxiety or depression.

**DESIGN:** Cross sectional retrospective chart review.

**MATERIALS AND METHODS:** Female patients presenting for initial infertility consultation were screened for anxiety and depression with the Generalized Anxiety Disorder-7 Item Scale (GAD-7) and Patient Health Questionnaire-9 (PHQ-9) as standard of care. Subjects were recruited at follow up appointments at least 3 months after initial consultation. An 11-

item survey designed to assess barriers, needs, and desires for psychological treatment was administered. Demographic data and medical history were obtained via chart review. The survey results were analyzed as a population and divided into 2 groups: those with a positive screen for anxiety or depression (score  $\geq 5$  on either scale) and those with a negative screen. Non-parametric Mann-Whitney test was used for continuous variables (reported as median and inter-quartile range) and the Fisher's Exact test was used for categorical variables. A p-value of  $< 0.05$  was considered significant.

**RESULTS:** The sample consisted of 68 participants. On a 1-5 Likert scale, emotional stress 3 (2-4) had a higher median than physical stress 2 (1-3); there was a positive correlation between emotional and physical stress ( $r=0.616$ ;  $p<0.001$ ). There were no differences in the survey items for barriers, needs, or desires between those that screened positive for anxiety/depression compared to those who did not. The primary barrier to treatment was social/emotional (65%); second was logistical (45%). The most cited barriers included alternative sources of support, scheduling conflicts, and patient perception that her stress level did not warrant treatment. Despite 50% identifying counseling as the primary preference for support, it was only utilized by 7%.

**CONCLUSIONS:** There were no significant differences in barriers to treatment for women who screened positive for anxiety/depression compared to those who did not. Also, women endorsed emotional distress associated with infertility, regardless of a positive or negative screen for anxiety or depression. Despite this, few established with embedded psychological support in the clinic, reporting social/emotional reasons over logistical barriers. Although 1/2 of women reported desiring counseling, they questioned if their distress level warranted treatment. This demonstrates that women may benefit from education and normalization of psychological support regardless of severity of mood symptoms. Universal referral or integration of emotional support into medical care may be beneficial to target all women and optimize overall outcomes.

**SUPPORT:** None.

**P-261** Tuesday, October 15, 2019 6:30 AM

#### **A NEW ERA IN MEDICINE: SOCIAL MEDIA AND PATIENT CARE.**

Anisa Hussain, MA,<sup>a</sup> Jacqueline Sehring, MA,<sup>a</sup> Elisabeth Rosen, BS, MA,<sup>a</sup> Lauren Grimm, MA,<sup>a</sup> Jody M. Esguerra, MA,<sup>a</sup> Karine Matevosian, DO,<sup>b</sup> Ruchi Kaushik Amin, MD,<sup>c</sup> Roohi Jeelani, MD,<sup>a</sup> Angeline Beltsos, MD<sup>a</sup> <sup>a</sup>Vios Fertility Institute, Chicago, IL; <sup>b</sup>Advocate Lutheran General Hospital, Park Ridge, IL; <sup>c</sup>Wayne State University, Detroit, MI.



**OBJECTIVE:** We compared physician social media goals to patient social media wants in order to optimize the physician-patient relationship in the digital world.

**DESIGN:** Anonymous survey completed by patients and physicians.

**MATERIALS AND METHODS:** An anonymous survey distributed over social media to patient and physician users investigated physician content goals and patient motivations and habits. Responses collected within a range of 0-10 were scaled as follows: 0-1=strongly disagree, 2-4=disagree, 5=neither agree nor disagree, 6-8=agree, 9-10=strongly agree. Unpaired t test was performed (GraphPad).

**RESULTS:** 219 patients and 22 physicians participated in the study. 70% of the patients were 26-45 years old. 76% of the physicians were 31-50 years old. 81% of patients looked to physicians for emotional support on social media and 63% of physicians identified emotional support as a goal of their social media activity. However, mean patient response was 4.46 (disagree) and mean physician response was 6.27 (agree),  $p=.034$ .

64% of patients looked to physicians on social media for education and 72% of physicians reported education as a social media goal. Mean patient response was 6.28 (agree) and mean physician response was 7.18 (agree),  $p=.243$ .

61% of patients looked to social media for a side of their physician beyond medicine and 81% of physicians reported using social media to bring humanity to their profession. Average patient response was 6.06 (agree) and average physician response was 7.91 (agree),  $p=.004$ .

65% of patients and 77% of physicians agreed that a doctor's role should extend beyond their physical practice. The patient mean response was 6.56 (agree), while physician mean response was 7.77 (agree),  $p=.085$ .

44% of patients felt more satisfied with their experience after following their physician on social media, while 64% of physicians surveyed reported higher satisfaction in the patients that follow them on social media. Patient mean response was 4.69 (disagree) and physician mean response was 6.18 (agree),  $p=.079$ .

**CONCLUSIONS:** Both patients and physicians agreed that social media can be a patient education tool and that the role of a physician should extend beyond the physical practice. However, patient and physician responses on using social media accounts as a tool for emotional support did not align. This may be due to the prevalence of social support groups on social media that offer extensive emotional support. Additionally, while physicians reported higher satisfaction in patients that follow them on social media, patients who did so did not report higher satisfaction. Although both patients and physicians agreed that social media is a tool for patients to see a side to their doctors beyond medicine, there was a significant difference between their responses—physicians agreed with this statement more strongly than patients. Trust is a critical component of the physician-patient relationship, and appropriate physician social media use allows to optimize this relationship in the digital age, especially when working with a younger patient population.

**P-262** Tuesday, October 15, 2019 6:30 AM

**DOES FERTILE YOGA CLASS REDUCE STRESS AND SADNESS AND PROVIDE HOPE FOR INFERTILE PATIENTS IN TREATMENT IN A PRIVATE FERTILITY PRACTICE?**



Lisa Rosenthal, BA, MA,<sup>a</sup> Hannah Shakartzi, M.D.,<sup>b</sup> Shehzeen Kamil, M.D.,<sup>b</sup> Robin Mangieri, MA,<sup>a</sup> Mark Leondires, M.D.,<sup>a</sup> Spencer S. Richlin, M.D.<sup>a</sup> <sup>a</sup>Reproductive Medicine Associates of Connecticut, Norwalk, CT; <sup>b</sup>The Stamford Hospital Dept of OB/GYN, Stamford, CT.

**OBJECTIVE:** Does Fertile Yoga reduce stress and sadness and provide hope for infertility patients undergoing treatment in a private fertility practice?

**DESIGN:** • Retrospective cohort study.

**MATERIALS AND METHODS:** Students completed an unidentified pre- and post-class questionnaire. Results were blinded from the yoga teacher. During class, two techniques were introduced: 1) one-minute mantra, “I am strong, healthy, resilient, capable, hopeful and fertile.” 2) seven movements of the spine which included forward flexion, back bend extension, lateral left bend, lateral right bend, left rotation twist, right rotation twist and axial extension. Primary outcomes were stress, sadness and levels of hopefulness before and after class using a scale of 0 to 10. 0 indicated no stress, sadness or hope and a score of 10 indicated high stress, sadness, and maximum hope. Secondary outcomes evaluated if the mantra and spine movements were helpful and likely to be used in the future. We analyzed data based on age and months conceiving. Age was subdivided into < 35, 35-37, 38-42, and > 42 years of age. Months conceiving were divided into 6-12, 12-18, 18-24, and >24 months. Statistical analysis was performed with SPSS version 25.0.

**RESULTS:** 55 patients completed pre- and post-test questionnaires. Ages were < 35 (32.7%), 35 to 37 (23.6%) 38 to 42 (29.1%) and > 42 years of age (14.5%). Months conceiving was divided into 6 to 12 (7.3%), 12-18 (27.3%), 18-24 (34.5%) and > 24 months (30.9%). There was a statistically significant decrease in stress and sadness ( $p < 0.001$ ), and an increase in hopefulness ( $p < 0.001$ ) after class (Table 1) for all age groups. All age categories felt that the mantra and seven movements were helpful during class and that they would use them in the future (ANOVA). Scheffe Test revealed that the 6 to 12-month cohort was less likely to use the mantra in the future and found that the seven movements in class were less helpful compared to the older students.

TABLE 1. pre- and post-class stress, sadness and hopefulness

Variable	Pretest		Posttest		p-value
	Mean	SD	Mean	SD	
Stress	6.96	2.14	4.00	2.15	<0.001
Sadness	5.67	2.99	3.11	2.37	<0.001
Hopefulness	6.78	2.25	7.78	1.90	<0.001

**CONCLUSIONS:** Fertile Yoga class decreased student stress and sadness and increased hope. Students reported that they were likely to continue to use the mantra and spine movements during their infertility journey. Given that many infertility patients stop treatment prematurely due to stress and feelings of discouragement, the techniques used in Fertile Yoga class could ultimately provide our patients the emotional energy and skills necessary to continue

with fertility treatment and succeed. Data is being collected to better elucidate the role of Fertile Yoga on our patients’ fertility journey.

Reference: None.

SUPPORT: None.

**P-263** Tuesday, October 15, 2019 6:30 AM

**EFFECT OF MUSIC IN REDUCING PATIENT ANXIETY DURING COLPOSCOPY: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS.**



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**OBJECTIVE:** Music therapy has been used greatly in various medical procedures to reduce associated anxiety and pain. This review aims to evaluate the evidence from published randomized clinical trials (RCTs) about the effect of music intervention in reducing patient’s anxiety during the colposcopy.

**DESIGN:** Systematic review and meta-analysis.

**MATERIALS AND METHODS:** We performed a comprehensive literature search using four electronic databases (PubMed, Cochrane library, Scopus and ISI Web of science) using the following search terms: (Music OR Symphony OR Rhythm OR Orchestra OR Song) AND (Colposcopy OR cervicoscope OR colposcope). All RCTs assessing the effect of music therapy versus no music in reducing anxiety during colposcopy were considered. Eighty-five studies were identified of which five studies deemed eligible for this review. The extracted outcomes were; anxiety, pain during and after the procedure, and satisfaction levels. Continuous outcomes were pooled as weighted mean difference (WMD) and standardized mean difference (SMD) using the Mantel-Hansel method with 95% confidence intervals (CI). All statistical analyses in this study were completed by the RevMan software package.

**RESULTS:** We included five studies with a total number of 836 patients in our final analysis. We found no effect of music therapy in reducing the anxiety levels when compared with the control group (SMD= -0.11, 95% CI [-0.36, 0.14],  $p=0.4$ ). No difference between music and control groups regarding pain during and after the procedure respectively (SMD= -0.20, 95% CI [-0.58, -0.18],  $p=0.31$ ) and (SMD=-0.10, 95% CI [-0.30, -0.10],  $p=0.33$ ). The pooled SMD showed a similarity between the music group in comparison with no music intervention group (SMD= 0.16, 95% CI [-0.02, 0.34],  $p=0.08$ ).

**CONCLUSIONS:** This systematic review suggests that music therapy has no great positive effect in reducing anxiety and pain levels and no effect in increasing satisfaction levels when compared with control groups during the colposcopy procedure.

SUPPORT: None.

**OBESITY**

**P-264** Tuesday, October 15, 2019 6:30 AM

**DIETARY PATTERNS ARE ASSOCIATED WITH OVARIAN RESERVE IN OVERWEIGHT AND OBESE WOMEN IN A REPRODUCTIVE AGE**



**COHORT.** Ashley Eskew, MD, MSCI,<sup>a</sup> Bronwyn Bedrick, BA,<sup>b</sup> Joan Riley, PhD, HCLD,<sup>c</sup> Jorge E. Chavarro, MD, Sc.D.,<sup>d</sup> Emily S. Jungheim, MD, MSCI.<sup>a</sup> <sup>a</sup>Washington University School of Medicine, St. Louis, MO; <sup>b</sup>Washington University in St. Louis, Saint Louis, MO; <sup>c</sup>Washington University School of Medicine, St. Louis, MO; <sup>d</sup>Harvard School of Public Health, Boston, MA.

**OBJECTIVE:** The objective of this study was to examine the relationship between dietary patterns and markers of ovarian reserve as measured by serum antimullerian hormone (AMH) levels and antral follicle count (AFC) in a reproductive age cohort of women.

**DESIGN:** Cross-sectional cohort study.

**MATERIALS AND METHODS:** Women aged 18 to 44 years with regular menstrual cycles were recruited for this study. Women who were pregnant, had a history of infertility, ovarian surgery or major chronic illness were excluded. AFC was determined by transvaginal ultrasound. AMH was measured with a Roche cobas e411 analyzer. A validated food frequency questionnaire (FFQ) and the Kaiser Physical Activity Survey were used to

assess diet and exercise patterns over the prior year. After assessment of physical activity and BMI, women with a caloric intake < 500 or > 5000 kcal/day were excluded. We assessed adherence to one of two dietary patterns: 1) the fertility diet (FD), characterized by a higher intake of vegetable-derived protein, increased ratio of monounsaturated to *trans*-fats, high-fat dairy, iron supplementation and a daily multivitamin, and 2) the pro-fertility diet (PFD), characterized by higher intakes of B12, folic acid, vitamin-D, dairy, and whole grains, low pesticide residue produce and soy or seafood as preferential protein sources. Adherence to a dietary pattern was defined by a factor score with higher values indicating greater adherence. Linear regression was used to control for potential confounders.

**RESULTS:** Two-hundred women were recruited and 175 were included in the analysis. Subjects were a mean age of 31.0 (±6.6) years and had a mean BMI of 27.7 (±7.0) kg/m<sup>2</sup>. After stratifying by BMI and adjusting for age, smoking and physical activity level, adherence to the PFD in overweight and obese women (BMI ≥ 25 kg/m<sup>2</sup>) was linearly associated with higher AMH concentrations. Women in the third and fourth quartiles of the PFD had mean AMH levels 1.45 ng/mL (95%CI 0.33-2.56, p=0.01) and 1.67 ng/mL (95%CI 0.60-2.74, p=0.003) higher than women in the lowest quartile respectively. The highest adherence with the PFD was also associated with a higher AFC in overweight and obese women (B=7.8, 95%CI 0.003-15.34, p=0.049). The FD was not significantly associated with AMH or AFC in overweight or obese women. Dietary patterns were not associated with markers of ovarian reserve in normal weight women.

**CONCLUSIONS:** Consumption of low pesticide residue produce and adherence to a PFD has been associated with improved reproductive outcomes in women undergoing IVF. Our study is the first to demonstrate that increased adherence to a PFD is linearly associated with AMH in an overweight and obese reproductive aged cohort of women. It is critical that further studies examine dietary patterns in at-risk populations to determine the potential impact on ovarian reserve in reproductive age women.

**References:** 1. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litten LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol.* 1992;135(10):1114-1136.

2. Yuan C, Spiegelman D, Rimm EB, et al. Validity of a Dietary Questionnaire Assessed by Comparison With Multiple Weighed Dietary Records or 24-Hour Recalls. *Am J Epidemiol.* 2017;185(7):570-584.

3. Ainsworth BE, Sternfeld B, Richardson MT, Jackson K. Evaluation of the kaiser physical activity survey in women. *Med Sci Sports Exerc.* 2000;32(7):1327-1338.

4. Gaskins AJ, Nassan FL, Chiu YH, Arvizu M, Williams PL, Keller MG, Souter I, Hauser R, Chavarro JE. *AJOG.* 2019;Feb 8, <https://doi.org/10.1016/j.ajog.2019.02.004>. [Epub ahead of print].

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**P-265** Tuesday, October 15, 2019 6:30 AM

**OBESITY CAUSES SIGNIFICANT CHANGES TO THE HUMAN SPERM PROTEOME.** Taylor Pini, PhD,<sup>a</sup>

Jason C. Parks, BS,<sup>a</sup> Monika Dzieciatkowska, PhD,<sup>b</sup> Kirk C. Hansen, PhD,<sup>b</sup> William B. Schoolcraft, MD,<sup>b</sup> Mandy G. Katz-Jaffe, Ph.D.<sup>a</sup> <sup>a</sup>Colorado Center for Reproductive Medicine, Lone Tree, CO; <sup>b</sup>Affiliation not provided.



**OBJECTIVE:** Globally, 38.5% of adult men are either overweight or obese (body mass index (BMI) ≥ 25). As sequelae of obesity have been investigated, it has become clear that excessive body weight significantly impacts reproductive capacity. In men, these impacts include poorer semen parameters (e.g. low concentration and motility), as well as lower pregnancy rates. However, the direct molecular mechanisms by which obesity impacts male fertility are not fully understood. As such, the aim was to perform a proteomic analysis to elucidate the impact of obesity on the sperm proteome.

**DESIGN:** Research study

**MATERIALS AND METHODS:** Protein lysates were prepared from spermatozoa obtained with consent during an IVF cycle from normozoospermic men with either a healthy weight (BMI < 25) or obesity (BMI > 30) (n = 5 per group, run in duplicate). Lysates were processed by FASP digestion and analyzed by liquid chromatography tandem mass spectrometry. Proteins were identified by Mascot search against the SwissProt database and IDs validated by confidence restrictions within Scaffold. A quantitative compar-

ison was performed using normalized weighted spectra (NWS), compared by Student's t-test. Differences were considered significant with a p value of < 0.05 and a fold change of < 0.5 or > 1.5. Patient data were analyzed by Student's t-test, with an α of 0.05.

**RESULTS:** There were no significant differences in patient age, semen concentration, motility, normal morphology or DNA fragmentation. Fertilization rate was borderline significant (control 94.0 ± 2.7% vs obese 76.5 ± 7.1%, p = 0.05). From a total of 2034 confidently identified proteins, 24 proteins were significantly less abundant and 3 proteins significantly more abundant in spermatozoa from obese men, compared to men of a healthy weight. Proteins with altered abundance were involved in a variety of biological processes, including oxidative stress (GSS, NDUFS2, JAGN1), inflammation (SGT1, LTA4H), translation (EIF3F, EIF4A2), protein breakdown (USP14, SKP1), and sperm function (NAPA, RNPEP, BANF2). These changes reflect obesity driven systemic inflammation and oxidative stress and highlight changes to important pathways necessary for appropriate sperm function. These changes, likely occurring during spermatogenesis, may negatively impact the function and fertility of mature spermatozoa.

**CONCLUSIONS:** These results suggest that oxidative stress and inflammation are closely tied to reproductive dysfunction in obese men. These processes likely impact protein translation and folding during spermatogenesis, leading to poor sperm function and a decline in fertility. The fact that these proteomic changes were observed in normozoospermic obese men further suggests that traditional clinical semen assessments fail to detect important biochemical changes in spermatozoa which may compromise fertility.

**P-266** Tuesday, October 15, 2019 6:30 AM

**IMPACT OF OBESITY ON OOCYTE CRYOSURVIVAL AFTER VITRIFICATION.** Luis R. Hoyos, M.D.,<sup>a</sup>

Connie Y. Cheng, M.D.,<sup>b</sup> Carrie Riesterberg, MD,<sup>c</sup> Abigail A. Armstrong, M.D.,<sup>b</sup> Molly M. Quinn, MD<sup>c</sup> <sup>a</sup>UCLA, Los Angeles, CA; <sup>b</sup>Affiliation not provided; <sup>c</sup>University of California, Los Angeles, Los Angeles, CA.



**OBJECTIVE:** Increased lipid content is thought to reduce oocyte tolerance to cryopreservation (1-3) and this has been reported to be particularly troublesome in some mammalian species (3, 4). Elevated intrafollicular insulin, triglycerides and free fatty acids have been described among obese women and incubation of mouse oocytes in human lipid-rich follicular fluid has been reported to cause oocyte lipid accumulation (5, 6). However, no human studies have described the effects of obesity on oocyte cryosurvival. Therefore, our objective was to investigate the effects of obesity, estimated by body mass index (BMI), on oocyte post-warming cryosurvival after vitrification.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Oocyte warming cycles of women who underwent oocyte cryopreservation with vitrification from January 2000-February 2018 were identified using the eIVF database (PracticeHwy, Dallas, TX). Age at retrieval, BMI, total number of oocytes warmed and oocyte cryosurvival after vitrification were extracted from the database. Outcomes for obese (BMI ≥ 30 kg/m<sup>2</sup>) patients were compared to non-obese (BMI < 30 kg/m<sup>2</sup>) patients using t-test or Wilcoxon rank-sum test where appropriate. A pre-test power calculation revealed a need for 204 subjects to detect a 25% reduction in cryosurvival in obese vs. normal subjects assuming 15% of all patients undergoing oocyte cryopreservation would be classified as obese.

**RESULTS:** A total of 535 oocyte warming cycles from 30 US clinics were identified. There were 69 obese and 466 non-obese patients with a mean BMI of 36.1 and 22.8 kg/m<sup>2</sup> respectively. There were no differences in age at retrieval (36.5±5.1 vs. 37.0±5.4 years, p=NS) between obese and non-obese

TABLE 1.

BMI (kg/m <sup>2</sup> ) Category	n	Median survival (IQR)%	Mean survival ± SD%
<20	77	100 (83-100)	87 ± 26
20 to <25	286	100 (82-100)	86 ± 27
25 to <30	103	100 (73-100)	81 ± 31
30 to <35	39	100 (71-100)	79 ± 33
35 to <40	17	100 (80-100)	83 ± 32
≥40	13	100 (50-100)	76 ± 33

subjects. There was a trend towards an increased number of oocytes warmed in obese subjects (8.1±7.5 vs. 6.3±6.9, p=0.06). There was no difference in oocyte cryosurvival after vitrification between obese and non-obese (Median IQR: 100% (70-100%) vs. 100% (80-100%), p=NS). Mean cryosurvival in the obese group was 79.5%±SD 32% vs. 85%±SD 27.5% in the non-obese group. Oocyte cryosurvival by BMI sub-group is depicted in Table 1.

**CONCLUSIONS:** Obesity does not appear to impact post-warming cryosurvival after oocyte vitrification. In humans, the intra-oocyte lipid stores that could result as a consequence of obesity may not meaningfully impact tolerance to cryopreservation unlike other mammalian species. Additional adequately powered studies are required to determine the impact of class III obesity on post-warming cryosurvival after oocyte vitrification.

**References:** 1. Seidel GE, Jr. Modifying oocytes and embryos to improve their cryopreservation. *Theriogenology*. 2006;65(1):228-35.

2. Gu L, Liu H, Gu X, Boots C, Moley KH, Wang Q. Metabolic control of oocyte development: linking maternal nutrition and reproductive outcomes. *Cell Mol Life Sci*. 2015;72(2):251-71.

3. Pereira RM, Marques CC. Animal oocyte and embryo cryopreservation. *Cell Tissue Bank*. 2008;9(4):267-77.

4. Prates EG, Nunes JT, Pereira RM. A role of lipid metabolism during cumulus-oocyte complex maturation: impact of lipid modulators to improve embryo production. *Mediators Inflamm*. 2014;2014:692067.

5. Robker RL, Akison LK, Bennett BD, Thrupp PN, Chura LR, Russell DL, et al. Obese women exhibit differences in ovarian metabolites, hormones, and gene expression compared with moderate-weight women. *J Clin Endocrinol Metab*. 2009;94(5):1533-40.

6. Yang X, Wu LL, Chura LR, Liang X, Lane M, Norman RJ, et al. Exposure to lipid-rich follicular fluid is associated with endoplasmic reticulum stress and impaired oocyte maturation in cumulus-oocyte complexes. *Fertil Steril*. 2012;97(6):1438-43.

**SUPPORT:** None.

**P-267** Tuesday, October 15, 2019 6:30 AM

**OBESITY IS ASSOCIATED WITH INCREASED QUANTITY BUT NO DIFFERENCE IN QUALITY OF OOCYTES AND EMBRYOS IN WOMEN WITH LOW ANTI-MULLERIAN HORMONE LEVEL.** Gufeng Xu, M.D., Ph.D.,<sup>a</sup> Catherine Racowsky, PhD<sup>b</sup> <sup>a</sup>Brigham and Women's Hospital, BOSTON, MA; <sup>b</sup>Brigham and Women's Hospital, Boston, MA.



**OBJECTIVE:** Both obesity and low AMH levels are associated with reduced oocyte, embryo and clinical outcomes. Whether obesity further diminishes outcomes in women with low AMH is unclear. In this study, we aimed to fill the knowledge gap by testing the hypothesis that obese women with low AMH have lower oocyte and embryo yields with reduced quality compared with women of normal weight.

**DESIGN:** Retrospective cohort of 1,542 cycles from 876 patients who underwent autologous IVF/ICSI cycles from March 2013 to October 2018.

**MATERIALS AND METHODS:** Women without PCOS and with AMH <1ng/ml were stratified by BMI: normal weight (18.5-24.9), overweight (25.0-29.9) and obese (class I: 30.0-34.9). Total, mature (MII) and two pronuclei (2PN) zygotes acted as surrogates for quantity. The % MII, %2PN/

MII, embryo scores on D5 (1=best, 6=worst), % usable (frozen+transferred) D5 embryos and No. good quality (GQ) D5 embryos acted as surrogates for quality. Implantation rate (IR) and live birth (LB) rate were assessed. We used multivariable GEE modelling with Poisson, logistic or linear regression adjusted for female age, stimulation protocol and FSH dose.

**RESULTS:** The results are shown in the table. All "quantity" parameters were increased for obese compared with normal weight women, but none of the "quality" parameters were different. Comparison of overweight vs. normal weight women revealed no differences for any quantity or quality variable except the No. of total oocytes. Decreased (not significant) trends were found for IR and LB rates among the groups (normal weight, overweight and obese, IR: 26.2, 23.9 and 25.0; LB: 27.1, 22.0 and 19.9).

**CONCLUSIONS:** Contrary to our hypothesis, obesity in low AMH women is associated with increased, rather than decreased numbers of oocytes and embryos compared with women of normal weight, with no compromise in overall quality. Underlying mechanisms remain to be uncovered but the elevated follicular androgen levels in obese women may be involved. Our findings help to reassure obese women with low AMH regarding IVF outcomes.

**SUPPORT:** None.

**P-268** Tuesday, October 15, 2019 6:30 AM

**ABC TRIAL: BODY MASS INDEX AND PERCENTAGE BODY FAT ARE NOT DIFFERENT IN POSITIVE PREDICTIVE VALUE OF MISCARRIAGE OR PRETERM DELIVERY IN PATIENTS UNDERGOING**



**IVF.** Julia G. Kim, MD, MPH,<sup>a</sup> George Patounakis, MD, PhD,<sup>b</sup> Caroline R. Juneau, MD,<sup>c</sup> Jason M. Franasiak, MD,<sup>a</sup> Scott J. Morin, MD,<sup>d</sup> Shelby A. Neal, MD,<sup>a</sup> Ashley W. Tiegs, MD,<sup>a</sup> Emily K. Osman, MD,<sup>a</sup> Brent M. Hanson, MD,<sup>a</sup> Emre Seli, M.D.,<sup>a</sup> Richard Thomas Scott, Jr., MD,<sup>a</sup> <sup>a</sup>IVI-RMA New Jersey, Basking Ridge, NJ; <sup>b</sup>IVI-RMA Florida, Lake Mary, FL; <sup>c</sup>Audubon Fertility, New Orleans, LA; <sup>d</sup>IVI-RMA Northern California, San Francisco, CA.

**OBJECTIVE:** Prior literature has suggested that maternal obesity in the infertile population increases risk of miscarriage and large-for-gestational-age (LGA) infants, and has unclear effects on gestational age. As previous studies have only investigated these outcomes in the context of body mass index (BMI), which may be an inaccurate metric for detailing body composition, this analysis also explores use of bioelectric impedance analysis (BIA) and its estimation of adiposity as a more precise method of defining obesity in patients undergoing IVF.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Patients at a single center undergoing IVF from June 2016 – March 2019 were offered utilization of the InBody 770 BIA scale at time of vaginal oocyte retrieval to determine their body composition. Participant demographics, BMI, percentage body fat (% BF), IVF outcome, pregnancy, and delivery data were recorded prospectively.

**RESULTS:** Pregnancy outcome data for 1037 females who underwent frozen embryo transfers were collected during this study period. Delivery data was obtained for 873 cycles. The positive predictive values (PPV) of BMI versus %BF were not different in investigating preterm delivery or

Adjusted oocytes and embryos outcomes of low AMH women stratified by BMI

Quantity	Normal weight (N=827)	Overweight (N=388)	Class I obese (N=160)
		Odds Ratio (95% CI)	Odds Ratio (95% CI)
No. oocytes	6.7	7.3 (1.08, 1.03 — 1.13) *	8.2 (1.22, 1.15 — 1.29) *
No. MII	5.0	5.4 (1.07, 0.98 — 1.16)	6.5 (1.29, 1.14 — 1.44) *
No. 2PN	3.4	3.6 (1.05, 0.93 — 1.17)	4.4 (1.28, 1.09 — 1.47) *
Quality			
% MII/Total	77.3	75.6 (0.91, 0.78 — 1.04)	81.4 (1.29, 0.96 — 1.61)
% 2PN/MI	68.1	66.9 (0.95, 0.78 — 1.12)	69.7 (1.08, 0.81 — 1.35)
D5 embryo grading	5.1	5.1 (-0.02, -0.22 — 0.18) #	4.9 (-0.21, -0.5 — 0.08) #
% D5 usable embryo / 2PN	59.5	56.0 (0.87, 0.70 — 1.04)	61.0 (1.06, 0.80 — 1.33)
No. GQ D5 embryos	0.4	0.4 (0.97, 0.60 — 1.34)	0.6 (1.64, 0.90 — 2.38)

# = Mean difference (OR, 95% CI)

Pregnancy Outcome	PPV of BMI $\geq$ 30	PPV of %BF $\geq$ 40%	P-value
LGA (>4000g)	12.7% (7.7-17.6)	9.45% (5.4-13.5)	0.008
Preterm Delivery (<37w)	18.5% (12.7-24.3)	15.9% (10.9-21.0)	0.058
Biochemical Loss	9.2% (6.0-12.5)	9.6% (6.5-12.7)	0.757
Clinical Loss	24.7% (18.5-31.0)	21.6 (15.9-27.2)	0.100
Loss After FH	8.7 (4.2-13.2)	7.0% (3.2-10.8)	0.283

pregnancy loss. BMI only differed from %BF in PPV of LGA infants (>4000g) where BMI was 12.7% and %BF was 9.35%.

**CONCLUSIONS:** To our knowledge, this is the first study to prospectively follow infertile patients and compare BMI to %BF in their predictive values on pregnancy outcomes. No differences were noted in the PPV of BMI versus %BF with regard to miscarriage rates or preterm delivery. BMI had a higher PPV than %BF in predicting LGA infants. Given that measurement of %BF through BIA has been previously validated in other fields of medicine, our findings suggest that BMI measures up to %BF as a successful approximation of patients' adiposity, and can be confidently used for counseling at-risk obese patients undergoing IVF.

**SUPPORT:** None.

**P-269** Tuesday, October 15, 2019 6:30 AM

### LIFESTYLE MODIFICATIONS IN MALE PARTNERS OF SUBFERTILE COUPLES IN WHICH THE SPOUSE IS OBESE IMPROVES THE CHANCES OF THE COUPLE TO CONCEIVE.

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**OBJECTIVE:** 1) To evaluate the impacts of an exposition of male partners of infertile couples to a lifestyle intervention, already targeted to their spouse with obesity, on fertility, anthropometric and lifestyle outcomes; and 2) to assess whether lifestyle and anthropometric changes in all male partners were associated to a conception.

**DESIGN:** Cross-sectional study, imbricated into a randomized controlled trial targeting the female spouses with obesity and infertility, including 97 infertile heterosexual subfertile couples.

**MATERIALS AND METHODS:** Male spouses were considered exposed to the intervention (Exp; n=41) if their spouse was randomized in the intervention group according to the randomized controlled trial, or not exposed (NExp; n=34) if the spouse was randomized in the control group. The NExp group followed the standard care for infertile, as the Exp group had access to the intervention targeting their spouse (individual sessions and group sessions). Lifestyle habits and anthropometry were assessed for both partners at 12 and 18 months, or at beginning and at 26 weeks of pregnancy. We pre-

sent mean differences between the last available evaluation visit and the initial visit. Student's *t* tests were used to compare means, chi-squared tests for proportions and Spearman's coefficients for correlations.

**RESULTS:** A total of 75 men (77%) had at least one follow-up research visit. Male partners participated little to the intervention targeting their spouse. There were no statistically significant differences for anthropometric and lifestyle changes between groups. When comparing couples with a conception and those without, independently of the exposition to the lifestyle intervention, men who conceived (n=40) had lost significantly more weight (-2.38 kg  $\pm$  4.44 vs -0.08 kg  $\pm$  4.88, *p*=0.026) and ate more fruits/day (-0.09  $\pm$  0.67 vs 0.28  $\pm$  0.65, *p*=0.016) than men who did not conceive (n=35). Weight loss remained significantly associated to a conception even after correcting for the weight loss of their spouse. Results were similar when assessing only couples in which both partners were obese (n=36). Moreover, in these couples, we found significant associations between both partners' changes in weight (*p*=0.41; *p*=0.012), and in nutritional quality (healthy eating index: *p*=0.41; *p*=0.013).

**CONCLUSIONS:** Exposing male partners to a lifestyle intervention targeting their spouse with obesity was not sufficient to improve their lifestyle. Nevertheless, our study shows that male partners of women with obesity and infertility, who lose weight or increase their daily consumption of fruits, increase the chances of their couple to conceive. Moreover, these male partners, when obese, tend to modify their weight and nutritional quality in parallel with their spouse. Therefore, engaging more actively male partners in the lifestyle intervention that is already indicated for their spouse with obesity can potentially further improve the couple's fertility.

**P-270** Tuesday, October 15, 2019 6:30 AM

### IMPACT OF BMI ON PREGNANCY OUTCOMES WITH RESPECT TO DIFFERENTIAL TSH LEVELS.

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**OBJECTIVE:** Serum thyroid-stimulating hormone (TSH) levels are routinely screened in women with infertility because thyroid disease may exert negative effects on ovulation and menstrual function. Women with clinical hypothyroidism (TSH levels > 4 uIU/mL) are treated with thyroid replacement. However, it is unclear whether subclinical hypothyroidism, defined as TSH levels > 2.5 uIU/mL (and </= 4 uIU/mL) can affect pregnancy outcome. In the present study, we evaluated the IVF treatment/pregnancy outcomes with respect to BMI in euthyroid women and in those with subclinical hypothyroidism.

**DESIGN:** A retrospective study of 1,160 IVF cases.

**MATERIALS AND METHODS:** Patients were categorized into three groups. Group 1, euthyroid, consisted of 919 women, had pre-IVF TSH levels < 2.5 uIU/mL. Group 2 included 74 women with subclinical hypothyroidism, SCI hypoT who were not treated. Group 3 included 167 women who were treated. All the patients were subgroup based on their BMI ( $\geq$  or < 30). All women underwent standard IVF protocols following usual individualized practice in our IVF clinic.

**RESULTS:** Table below shows the classification of patients with respect to their pregnancy outcomes, by TSH levels, BMI and treatment respectively.

Pregnancy Outcome	GROUP 1- EUTHYROID			GROUP 2- SCI Hypo T Untreated			GROUP 3- SCI Hypo T Treated					
	Pre, TSH<2.5, BMI <30	Pre, TSH<2.5, BMI $\geq$ 30		Pre, TSH>2.5, Non Treated BMI <30	Pre, TSH>2.5, Non Treated BMI $\geq$ 30		Pre, TSH>2.5, Treated BMI <30	Pre, TSH>2.5, Treated BMI $\geq$ 30				
Not Pregnant	177* 22.7	35.1	32 22.7	35.7	23* 36.5	36.0	3 27.3	40.3	37 26.1	35.6	8 32.0	39.3
Pregnant	601 77.2	34.6	109 77.3	36.4	40 63.5	33.3	8 72.7	35.1	105 73.9	33.8	17 68.0	29.3
Sab	92 11.8	34.8	15 10.6	39.3	9 14.3	33.6	2 18.2	38.5	14 9.9	33.4	2 8.0	33.5
BIOCHEM	69 8.9	34.4	19 13.5	36.4	7 11.1	34.0	1 9.1	32.0	13 9.2	33.3	1 4.0	21.0
DELIVERED	439 56.4	34.3	74 52.5	35.9	24 38.1	32.2	5 45.5	34.7	78 54.9	34.7	14 56.0	33.3
ECTOPIC	1 0.1	35.0	1 0.7	34.0	0 0.0		0 0.0		0 0.0		0 0.0	
Total	778 100.0	34.5+/-0.8	141 100.0	35.8+/-5.3	63 100.0	33.6+/-4.3	11 100.0	33.6+/-8.3	142 100.0	34.3+/-0.7	25 100.0	33.5+/-5.3

\* *p* = 0.013

The overall pregnancy rate was significantly lower in untreated women with SCHypoT with BMI < 30 compared to Euthyroid with BMI < 30 (p=0.013). However treated women with SCHypoT with BMI < 30 showed no significant differences compared to Euthyroid with BMI < 30 (p=0.391).

**CONCLUSIONS:** Our findings suggest that irrespective of BMI, subclinical hypothyroidism may impact IVF success and pregnancy outcomes. Low dose thyroid supplementation may be beneficial. Further studies are ongoing considering parameters such as the presence of TPO antibodies and specific treatment strategies.

**SUPPORT:** This work was supported in part by the *IVFMD, South Florida Institute for Reproductive Medicine*.

**P-271** Tuesday, October 15, 2019 6:30 AM

**THE EFFECT OF BODY MASS INDEX (BMI) ON INTRAUTERINE INSEMINATION (IUI) CYCLE SUCCESS.** Rachel M. Whynto, M.D.,<sup>a</sup>

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**OBJECTIVE:** To determine if BMI affects IUI success.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Inclusion: IUI patients from 7/2009 - 12/2018. Exclusions: if weight (wt) or pregnancy outcome unavailable, or if BMI <18.5 (due to low n), for a total of 1319 patients and 3244 IUI cycles. Primary outcome was clinical pregnancy (CP) by BMI, defined as intrauterine pregnancy (IUP) with heartbeat (HB) on ultrasound (US). Secondary outcomes were live birth (LB), multiple gestation (MG), and abnormal pregnancy (AP) (defined as +hCG without an IUP with HB at US). Chi-square was used to compare outcome data between groups. Generalized estimating equations were used to examine relationships between individual factors and outcome of CP. Initial odds ratios (OR) were calculated for all hypothesized individual factors: maternal age, smoking, gravity, parity, diagnosis, antral follicle count (AFC), cycle order, and treatment cycle type. Age and factors meeting criteria of p<0.25 were entered into regression model. Through an iterative process of variable selection, covariates were removed from model if they did not meet significance of  $\alpha=0.1$  or were not found to change any remaining parameter estimate by >15%. After the iterative process of deleting, refitting, and verifying, each variable not selected for inclusion in the original multivariate model was added back one at a time, with any significant at  $\alpha=0.1$  retained.

**RESULTS:**

TABLE 1. IUI pregnancy rates by BMI.

	BMI 18.5-24.99	BMI 25-29.99	BMI ≥ 30	p
CP (n=3329)	192/1545 (12%)	115/813 (14%)	128/886 (14%)	0.289
AP (n=3329)	52/1545 (3%)	38/813 (5%)	53/886 (6%)	0.009
LB (n=3244) <sup>1</sup>	146/1521 (10%)	72/788 (9%)	77/854 (9%)	0.876
MG (n=355) <sup>2</sup>	13/157 (8%)	6/91 (7%)	10/99 (10%)	0.683
Multiple Delivery (n=312) <sup>3</sup>	8/150 (5%)	5/77 (7%)	9/79 (11%)	0.232

<sup>1</sup>81 cases (19%) documented CP lost to follow up prior to LB.

<sup>2</sup>88 cases (20%) documented CP missing US number of fetal HB data.

<sup>3</sup>129 cases (30%) documented CP missing total birth type

Factors retained in final model for BMI and CP included: AFC (OR 1.02 (1.00-1.03)(p=0.007), smoking (OR 0.51 (0.28-0.94), p=0.030), endometriosis (OR 0.47 (0.25-0.91), p=0.026), age (OR 0.99 (0.96-1.02), p=0.514), and treatment cycle type (p=0.007). When accounting for these factors, BMI 25-29.99 were more likely to have a CP compared to BMI 18.5-24.99 (OR 1.42 (1.04-1.95), p=0.029). BMI ≥ 30 did not affect CP rates (OR 1.21 (0.88-1.66), p=0.245).

**CONCLUSIONS:** After controlling for potential confounders, patients BMI 25 - 29.99 are more likely to have CP with IUI compared to normal BMI. A BMI ≥ 30 does not have an impact on IUI CP rate or LB at a clinic requiring BMI <50. However, women BMI ≥ 30 are more likely to have AP, which is consistent with prior studies in spontaneous and in vitro fertilization pregnancies.

**SUPPORT:** None.

**P-272** Tuesday, October 15, 2019 6:30 AM

**DIET, OBESITY, AND OVARIAN RESERVE IN A HEALTHY REPRODUCTIVE AGE COHORT.** Bronwyn Bedrick, BA,<sup>a</sup> Ashley Eskew, MD,<sup>b</sup>

Jorge E. Chavarro, MD, Sc.D.,<sup>c</sup> Joan Riley, PhD, HCLD,<sup>d</sup> Emily S. Jungheim, MD, MSCI.<sup>b</sup> <sup>a</sup>Washington University in St. Louis, Saint Louis, MO; <sup>b</sup>Washington University School of Medicine, St. Louis, MO; <sup>c</sup>Harvard School of Public Health, Boston, MA; <sup>d</sup>Washington University School of Medicine, St. Louis, MO.



**OBJECTIVE:** The objectives of this study were to (1) describe dietary patterns in a cohort of healthy, reproductive-age women, (2) examine socioeconomic and demographic factors associated with adherence to these dietary patterns, and (3) assess the association between these dietary factors obesity and ovarian reserve.

**DESIGN:** Cross-sectional study of healthy women age 18-44 in St. Louis with no history of infertility, chronic disease, or ovarian surgery.

**MATERIALS AND METHODS:** A total of 185 women completed a validated food frequency questionnaire. Women with daily caloric intakes <500 kcal or >5000kcal were excluded. Principal component analysis with varimax rotation was used to combine 40 food groups into 2 independent factors, or dietary patterns, for greater interpretability. Adherence to dietary patterns was defined by each participant's factor score, such that women with higher scores for a specific dietary pattern were considered to be more adherent to that dietary pattern. Markers for ovarian reserve, serum anti-mullerian hormone (AMH) and antral follicle count (AFC) was measured via a Roche cobas e411 analyzer and transvaginal ultrasound, respectively. Logistic regression was used to examine the association between dietary patterns and obesity. Linear regression was used to assess the association between dietary patterns and markers of ovarian reserve.

**RESULTS:** Two dietary patterns were identified: a "Prudent" pattern characterized by consumption of fruits, vegetables, olive oil, and nuts, and a "Traditional" pattern characterized by consumption of meat, refined carbohydrates, and high calorie drinks. African American women and those without college degrees were more adherent to the Traditional pattern and less adherent to the Prudent pattern. Income and employment were not associated with dietary adherence. Thirty percent of our cohort was obese. On multivariate regression, odds of obesity were higher for African American women (OR 3.11, 95% CI 1.23 to 7.89) and lower for physical active women (OR 0.30, 95% CI 0.10 to 0.93) after controlling for diet, physical activity, education, and income. There was a dose-dependent increase in odds of obesity with increasing adherence to the Traditional dietary pattern, but there was no relationship between obesity and adherence to the Prudent pattern. No associations were seen between either dietary pattern and markers of ovarian reserve.

**CONCLUSIONS:** A growing body of evidence has demonstrated that diet, irrespective of maternal BMI, influences fertility, pregnancy outcomes, and newborn health. In order to identify women for wellness interventions in the preconception period, it is necessary to understand local context. In this cohort of reproductive age women in St. Louis, we describe dietary patterns that are associated with obesity, but not with markers of ovarian reserve. Future research is needed to elucidate the relationship between diet and markers of ovarian reserve.

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**A NOVEL GNRH ANTAGONIST PROTOCOL BY SWITCHING ANTAGONIST TO PROVERA TO PREVENT PREMATURE LUTEINIZING HORMONE SURGE WHEN PATIENTS TURNED OUT TO BE AT HIGH RISK OF OVARIAN HYPERSTIMULATION SYNDROME DURING OVARIAN STIMULATION.**

Ting-Chi Huang, MD,<sup>a</sup> Jiann-Loung Hwang, MD,<sup>b</sup> Kuang-Han Chao, M.D.,<sup>a</sup> Mei-Jou Chen, MD, PhD,<sup>a</sup> Chu-Chun Huang, MD,<sup>a</sup> Shee-Uan Chen, MD, PhD.<sup>a</sup> <sup>a</sup>National Taiwan University Hospital, Taipei, Taiwan; <sup>b</sup>IVF Taipei, Taipei, Taiwan.

**OBJECTIVE:** To investigate whether switching GnRH antagonist (GnRHant) to medroxyprogesterone acetate (MPA) could effectively prevent premature LH surge in GnRHant protocols when patients turned out to have a high risk of OHSS during controlled ovarian stimulation (COS) and a freeze-all strategy was chosen.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This study recruited patients (<38 years old) who received a GnRHant protocol in their first IVF/ICSI cycle. Daily rFSH and GnRHant were started on cycle day 2 or 3, and stimulation day 5, respectively. During COS, the patients turned out to be at a high risk of developing OHSS (more than 13 follicles of ≥ 11 mm in diameter) before reaching the ovulation trigger criteria. It is our policy to freeze-all in this circumstance. GnRH agonist was used to trigger ovulation. All the grade A or B embryos were vitrified on day 3 and frozen embryo transfer (FET) was performed on the subsequent cycle. In the study group (from August 2016 to July 2017) GnRHant was switched to MPA of 10mg daily till the day of ovulation trigger once freeze-all was determined (switch protocol). In the control group (from August 2015 to July 2016), GnRHant was maintained till the day of ovulation trigger as traditional GnRHant protocols. The primary outcome measure was the incidence of premature LH surge. Secondary outcome measures were the duration of GnRHant/MPA administration, duration/dose of rFSH administration, number of oocytes retrieved, number of embryos frozen, implantation and live birth rate in the first FET cycle.

**RESULTS:** A total of 401 cycles met the inclusion criteria for analysis: 205 in the control group and 196 in the study group. Premature LH surge did not occur in both groups. The characteristics of ovarian stimulation were similar between the two groups except the duration of GnRHant/MPA administration. The duration of GnRHant treatment was significantly lower in the switch protocol compared with the GnRHant protocol (3.1±1.0 days vs. 6.5±1.2 days). Majority of the patients (173/196=88.3%) received 2-4 days of GnRHant treatment before switching to MPA. Majority of the patients (185/196=94.4%) received 2-5 days of MPA treatment. The mean (±SD) duration of MPA administration was 3.6±1.1 days. No significant differences were observed in the duration (10.6±1.1 days vs. 10.5±1.2 days) and dose (1929±450 IU vs. 2005±483 IU) of rFSH administration; trigger day serum LH levels (2.0±1.4 IU/L vs. 1.8±1.1 IU/L); number of oocytes retrieved (17.0±6.4 vs. 16.9±5.9); number of embryos frozen (7.8±3.1 vs. 7.9±2.8); or live birth rate (50.5% vs. 49.8%) between switch and GnRHant protocol.

**CONCLUSIONS:** This study showed that MPA could replace GnRHant and effectively prevent premature LH surge after several days of GnRHant. This study showed that MPA could replace GnRHant and effectively prevent premature LH surge after several days of GnRHant administration in this group of patients. Switch protocol could individualize freeze-all policy in contrast to freeze-all for all in the progestin primed ovarian stimulation. It could also reduce patients' injection burden

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**ANALYSIS OF THE EFFECT OF A DELAYED SECOND DOSE OF GONADOTROPIN RELEASING HORMONE-ANTAGONIST (GNRH-A) ON OOCYTE AND BLASTOCYST QUALITY AND RISK OF OVARIAN HYPERSTIMULATION SYNDROME (OHSS).**

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**OBJECTIVE:** Use of a combination trigger (low dose hCG with GnRH-a) is commonly used as part of controlled ovarian hyperstimulation in good responders as it lowers the risk of OHSS compared to hCG alone as well as triggering oocyte maturation. We sought to determine the effects of a second

dose of GnRH-a 12 hours after the first, as part of the combination trigger on oocyte and blastocyst quality and risk of OHSS.

**DESIGN:** Retrospective cohort study of all cycles utilizing a combination trigger between 1/1/2017 and 12/31/2018 at our center.

**MATERIALS AND METHODS:** Primary outcomes were the number and maturity of oocytes retrieved and incidence of OHSS, as defined by the ASRM practice committee. A subgroup analysis of all cycles utilizing pre-implantation genetic testing for aneuploidy (PGT-A) was performed for the secondary outcomes of number of 2PN, percent of 2PN biopsied as blastocysts and number euploid. Statistical analysis included student t-test, a chi-squared test with and without adjustments for age.

**RESULTS:** 1892 cycles were included in the study, 1394 utilizing a single GnRH-a (G1) and 498 utilizing a double GnRH-a (G2) as part of the combination trigger. Demographics were not different between groups (Table 1). Cycles utilizing G2 had more oocytes retrieved (20.5 v 19.4, p < 0.03), however maturity was not different between groups. The incidence of OHSS was not different between groups, even when controlled for by age and use of cabergoline. Groups were then made by comparing low (<3500pg/mL) v high (>3500pg/mL) E2. The incidence of OHSS was higher in the high E2 group but lower with G2 (11.0% v 14.7%, p = 0.14). A subgroup analysis of cycles utilizing PGT-A was then performed comparing G1 (n=703) vs G2 (n=463). The number of 2PN zygotes was higher in G2 (12.3 v 11.2, p < 0.01) and there was a trend towards more %2pn of all eggs in G2 (60.0% v 59.8% p=0.05). The % of blastocysts biopsied from M2 was not different between groups (45.5% v 46.2%, p = 0.75), however, the total number of euploid embryos was higher with G2 compared to G1 (2.6 v 2.2, p < 0.02).

**CONCLUSIONS:** Use of G2 results in more oocytes retrieved and more euploid embryos. While there was no statistically decreased incidence of OHSS in G2 vs G1 overall, when stratified into low vs. high estradiol, there may be a clinically important reduction in OHSS for patients receiving G2 over G1.

Reference: None.

SUPPORT: None.

TABLE 1. Characteristics of G2 v G1

	Single GnRH-a (G1, n = 1394)	Double GnRH-a (G2, n = 498)	P value
Age (yrs)	35.4±4.7	35.4±4.5	p = 0.71
Maximum E2 (pg/mL)	3592.6±1451.0	3713.8±1506.8	p = 0.11
LH (mIU/mL)	101.6±47.4	101.4±50.8	p = 0.94
Oocytes Retrieved	19.4±9.6	20.5±10.5	p < 0.03
Mature Oocytes Retrieved	15.1±8.0	15.9±8.3	p = 0.06
Incidence of OHSS	10.0% (140/1394)	8.4% (42/498)	p = 0.33
Use of Cabergoline	10.8% (151/1394)	9.8% (49/498)	p = 0.61

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**WITHHOLDING LUTEAL HCG IS ASSOCIATED WITH DECREASED LIVE BIRTH (LB) IN WOMEN AT HIGH RISK FOR OVARIAN HYPERSTIMULATION SYNDROME (OHSS) DESPITE "INTENSIVE" LUTEAL SUPPORT WITH INTRAMUSCULAR PROGESTERONE (IMP).**

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**OBJECTIVE:** To determine if the absence of hCG for luteal support results in reduced LB rates from IVF with fresh embryo transfer (ET) when "intensive" luteal support with IMP is given after GnRH agonist (GnRH<sub>a</sub>) trigger.

**DESIGN:** Retrospective Cohort Study

**MATERIALS AND METHODS:** Fresh autologous IVF cycles from 2014-2017 with ≥ 24 oocytes retrieved were analyzed. Patients who did not undergo a fresh ET and those who received both hCG and GnRH<sub>a</sub> to trigger oocyte maturation were excluded. HCG trigger patients received luteal support with 100mg vaginal progesterone three times a day starting the day after retrieval. The same luteal support was used following GnRH<sub>a</sub> trigger if 1500 IU hCG was administered post retrieval. If OHSS risk was assessed as unacceptably high, hCG was held following GnRH<sub>a</sub> trigger and "intensive" luteal

support with 50mg daily IMP was administered starting the day after retrieval. All patients received 2mg twice daily oral estradiol starting the night of retrieval. Multivariable logistic regression was used to compare laboratory and pregnancy outcomes in patients receiving hCG trigger (control) to those with GnRHa trigger with and without post retrieval hCG. Adjusted models accounted for age, BMI, number of embryos transferred, embryo quality, and serum progesterone level on day of trigger. Greater than efficiency and receiver operator curves were used to determine if serum estradiol and progesterone concentrations on the day of trigger were associated with LB in each treatment group.

**RESULTS:** 984 autologous IVF cycles met inclusion criteria, distributed as follows: 235 hCG trigger, 236 GnRHa trigger with hCG post retrieval, and 454 GnRHa trigger with no hCG post retrieval. GnRHa trigger patients were older, had a higher peak estradiol level, more embryos available for vitrification, and fewer embryos transferred ( $P<0.001$ ) compared to hCG trigger. Patients without hCG exposure had lower clinical pregnancy (CP) (42% vs 52%,  $P = 0.01$ ) and LB (35% vs 44%,  $P = 0.01$ ) rates compared to those using hCG trigger in both analysis models. Patients with GnRHa trigger who received post retrieval hCG had similar CP (56%,  $P = 0.43$ ) and LB (46%,  $P = 0.42$ ) to the hCG trigger cohort. There were no statistically significant differences in biochemical pregnancy, spontaneous abortion, and ectopic pregnancy. Patients without hCG exposure had lower rates of OHSS ( $<1%$ ,  $P < 0.001$ ) compared to hCG trigger (11%) and GnRHa patients (6%). LB did not vary by peak serum estradiol in any treatment arm.

**CONCLUSIONS:** Patients receiving GnRHa trigger who did not receive hCG post retrieval had lower CP and LB rates from fresh ET despite "intensive" luteal support. This was largely due to implantation failure as pregnancy loss was similar in all treatment groups. Adjusted analysis demonstrated that higher peak serum estradiol levels in the GnRHa without hCG group did not mediate this effect. Post retrieval hCG was associated with LB outcomes equivalent to hCG trigger, but at the cost of increased OHSS relative to the no hCG group. These data suggest when hCG luteal support cannot be given due to high OHSS risk a freeze all strategy should be strongly considered.

Reference: Nadivva C, Engmann L. "Luteal phase support after gonadotropin-releasing hormone agonist triggering: does it still matter?" Fertil Steril. 2018 May;109(5):763-767.

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**MATERNAL AND FETAL OUTCOMES AFTER OVARIAN HYPERSTIMULATION SYNDROME: A ROCHESTER EPIDEMIOLOGY PROJECT (REP) STUDY.** Ajleeta Sangtani, MD, Zaraq Khan, MD, Maryama Ismail, BS, Mayo Clinic Rochester, MN.



**OBJECTIVE:** The objective of this study was to determine the effect of ovarian hyperstimulation syndrome (OHSS) on maternal and fetal outcomes.

**DESIGN:** This was a retrospective cohort design.

**MATERIALS AND METHODS:** IRB approval was obtained. Using the Rochester epidemiology project, residents of Olmsted County and the surrounding 9

TABLE 1. Demographic and Result Data of OHSS and Control Cases

Variable (mean)	Control (144)	OHSS (72)	P value
Age	32	31.9	
BMI	24.9	24.9	
Gonadotropin	1868	1860	
Days of stimulation	11.7	11.4	
Follicles	25	35	$P<0.0001$
Oocytes	15	23	$P<0.0001$
Peak E2	2243	3149	$P=0.004$
Days at delivery	264	260	
Hypertension*	33	12	
Gestational diabetes*	10	6	
Placental abruption*	2	4	
Antenatal steroid use*	12	10	
Intrauterine fetal demise*	3	2	
Number of live infants	1.3	1.3	
Birthweight	2996	2895	
1 minute Apgar	7.7	7.2	
2 minute Apgar	8.7	8.4	

\*=number of cases

counties with OHSS after in vitro fertilization were identified between 1995 and 2017. Matched controls were then screened as matches on age, parity, and cause of infertility. Two controls were identified for each patient with OHSS. Patients were included if they had a pregnancy lasting  $\geq 20$  weeks gestation after the diagnosis of OHSS. Background data and pregnancy outcomes were collected via chart review. Data was then analyzed using a t-test and ANOVA.

**RESULTS:** Patients with and without OHSS did not differ on BMI, number of stimulation days, amount of gonadotropin use. Patients with OHSS had significantly more follicles ( $p<0.0001$ ) and more oocytes ( $p<0.0001$ ) as well as a higher peak estradiol ( $p=0.004$ ) [table 1]. Rates of intrauterine fetal death, gestational diabetes, placental abruption, deep venous thrombosis, pulmonary embolism, gestational hypertension, number of liveborn infants, infant birthweight, and use of antenatal steroids did not differ between the groups. One and 5 minute Apgars did not differ between the two groups either [table 1].

**CONCLUSIONS:** The incidence of OHSS after assisted reproduction is approximately 3%. OHSS did not affect maternal or neonatal outcomes in a subsequent pregnancy in our report. Further analyses are underway to determine if outcome outcomes differ in women who have a fresh transfer after OHSS diagnosis compared to those that undergo freeze all of embryos with a planned frozen embryo transfer.

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**PREDICTION OF SEVERE OVARIAN HYPERSTIMULATION SYNDROME IN WOMEN UNDERGOING IN VITRO FERTILIZATION USING DAY 3 ESTRADIOL LEVELS, COLLECTED OVA, AND THE NUMBER OF FOLLICLES.**



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**OBJECTIVE:** Ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening iatrogenic condition that can occur during *in vitro* fertilization (IVF). The worst outcome is hydrothorax, hypovolemia, higher risk of deep venous thrombosis and oliguria. With severe OHSS patients are required to postpone embryo transfer for an undetermined amount of time. Predicting which patients are a high risk of developing OHSS is mainly based on serum estradiol (E2) levels, but other factors, such as female age, BMI, ovarian volume, antral follicle count (AFC), and polycystic ovary syndrome, are speculated to be predictive. Here, we aimed to determine if E2 levels at Day 3 and its fold change at day ten as well as antral follicle count and ova collected are predictive factors for severe OHSS.

**DESIGN:** Retrospective case-control study.

**MATERIALS AND METHODS:** Patient chart review was performed between January 2008 and December 2017 at Ingenes in Mexico City. Three hundred twenty-seven women were selected. E2  $>3000$  ng/L usually on the last day of stimulation (day 10 with three 18 mm-follicles) was defined as OHSS ( $n=151$ ). Culdocentesis was performed on a patient when upon clinical assessment patient presented features such as nausea, vomiting, oral intolerance and ascites identified by endovaginal ultrasound and abdominal ultrasound (renal and hepatic areas with visible ascites) that do not respond to conservative management ( $n=55$  severe OHSS). Predictability was evaluated by measuring the area under the receiver-operating characteristic (AUC). Differences between groups were determined by *t*-test.

**RESULTS:** The OHSS positive group, when compared to the non-OHSS group, was higher with respect to E2 Day 3 levels ( $150\pm 230$  v  $250\pm 177$  ng/L), E2 fold change ( $20.1\pm 23.8$  v  $32.2\pm 29.1$ ), AFC ( $11.6\pm 8.3$  v  $18.2\pm 9.1$ ), and Ova collected ( $10.1\pm 6.4$ ,  $21.1\pm 9.0$ ,  $p<0.001$ ). E2 Day 3 levels (AUC=0.76, 95%CI: 0.71-0.82), E2 fold change (AUC=0.71, 95%CI: 0.65-0.77), AFC (AUC=0.75, 95%CI: 0.70-0.81), and Ova collected (AUC=0.85, 95%CI: 0.81-0.89) were predictive of OHSS. For Culdocentesis, E2 Day 3 levels ( $190\pm 221$  v  $232\pm 158$  ng/L) were not different between the subjects who received culdocentesis, whereas the E2 fold change ( $24.5\pm 26.6$  v  $32.9\pm 28.8$ ,  $p=0.038$ ), AFC ( $13.7\pm 9.0$  v  $19.8\pm 8.9$ ,  $p<0.001$ ), and Ova collected ( $13.7\pm 8.9$ ,  $23.3\pm 8.1$ ,  $p<0.001$ ) were higher. Interestingly, all variables were predictive of subjects who would qualify for culdocentesis (E2 Day 3 levels: AUC=0.63, 95%CI: 0.55-0.70; E2 fold change: AUC=0.63, 95%CI: 0.55-0.71; AFC: AUC=0.74, 95%CI: 0.68-0.80; and Ova collected: AUC=0.80, 95%CI: 0.75-0.85).

**CONCLUSIONS:** Here, we demonstrate the E2 levels, as well as the ova production parameters, are indicators of IVF patients who could develop severe OHSS and may require culdocentesis.

**SUPPORT:** ConacytA 250768.

## OVARIAN STIMULATION

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### EFFECTIVENESS AND OPTIMAL DOSE OF CHLORMADINONE ACETATE (CMA) AS PROGESTIN-PRIMED OVARIAN STIMULATION.

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**OBJECTIVE:** The aim of this study was to clarify the effectiveness and optimal dose of chlormadinone acetate (CMA) as progestin-primed ovarian stimulation (PROS).

**DESIGN:** This study was a prospective study conducted at Kyono ART Clinic Takanawa in Japan from August 2018 to April 2019 and performed with the consent of the Kyono ART Clinic Ethical Committee.

**MATERIALS AND METHODS:** Study 1: The subjects were classified into two groups. In both groups, either FSH/hMG was administered on day 3. Group A comprised 32 cycles (32 patients) using 12mg CMA from day 3; group B comprised 28 cycles (28 patients) using 0.25mg GnRH antagonist when mean dominant follicle diameter reached 14 mm. All embryos were cryopreserved at the blastocyst stage for later transfer. Study 2: The optimal dose of CMA (12mg, 6mg, 4mg, and 2mg) was examined.

**RESULTS:** Study 1: Premature LH surge was not observed (0/32) in group A, whereas it was observed in 21.4% of cases (6/28) in group B; however, ovulation was not observed in either groups. Clinical outcomes in groups A and B were as follows: mean number of oocytes retrieved, 13.4±7.0 vs. 15.4±9.9; fertilization rate, 80.2% vs. 76.7%; blastocyst rate, 55.3% vs. 51.1%; good blastocyst rate, 43.6% vs. 44.0%; clinical pregnancy rate, 58.3% (7/12) vs. 60.0% (12/20); ongoing pregnancy rate, 50.0% (6/12) vs. 50.0% (10/20); miscarriage rate, 14.3% (1/7) vs. 16.7% (2/12). Thus, there were no significant differences between the two groups. Study 2: Premature LH surge was not observed: 0% (0/12), 0% (0/21), 0% (0/32), 0% (0/32) in CMA 2mg, 4mg, 6mg, and 12mg, respectively.

**CONCLUSIONS:** To our knowledge, this study is the first report of CMA worldwide. PROS using CMA completely inhibited premature LH surge and clinical outcomes equal to those of GnRH antagonist treatment. CMA is oral medicine, cheaper and effective as PROS, and 2mg CMA may be the optimal dose. Further studies are needed.

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### DOES EXTENDING CONTROLLED OVARIAN HYPERSTIMULATION DURING A GNRH ANTAGONIST PROTOCOL IN VITRO FERTILIZATION CYCLE AFFECT OOCYTE QUALITY?

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**OBJECTIVE:** The number of oocytes retrieved during an in vitro fertilization (IVF) cycle is an important determinant of success. Timing of the oocyte maturation trigger during controlled ovarian hyperstimulation (COH) must be optimized to maximize oocyte yield while avoiding hyperstimulation syndrome and impaired oocyte quality. A common protocol prescribes trigger administration when  $\geq 2$  follicles reach  $\geq 18$  mm.<sup>1</sup> Periodically, clinicians delay the trigger to allow medium-size follicles to “catch up.” A recent study segregated 200 IVF cycles into groups: delayed trigger despite the  $\geq 2$  mature follicles and trigger administration with  $\geq 2$  mature follicles, and found no difference in clinical pregnancy rate (CPR) and live birth rate (LBR).<sup>2</sup> The clinicians transferred 1-2 fresh, unselected embryos, which limits generalizability. To eliminate confounders such as multi-embryo transfer and the effect of supraphysiologic hormone levels on the endometrium, we asked whether rates of oocyte maturation, fertilization, blastulation, and euploidy were affected by prolonging COH.<sup>3</sup>

**DESIGN:** Retrospective, cohort study.

**MATERIALS AND METHODS:** The study included patients at a single academic center who underwent GnRH-antagonist IVF cycles from 2012-19.

Cycles were grouped: (1) delayed trigger despite the presence  $\geq 2$  mature follicles, and (2) administration of trigger in the presence of  $\geq 2$  mature follicles. Primary outcome was oocyte metaphase II (MII) rate. Secondary outcomes were rates of fertilization, blastulation, and euploidy. Statistical analysis was performed with T-tests, chi-square tests, and multivariate logistic regressions.

**RESULTS:** Of the 7,976 antagonist IVF cycles from 6,478 patients, trigger was administered in the presence of  $\geq 2$  mature follicles in 6521 (81.8%) cycles, 1 day beyond in 1334 (16.7%) cycles, and 2 days beyond in 121 (1.5%) cycles. Univariate analysis demonstrated differences in age, antral follicle count, peak estradiol, gravidity, and trigger type. After controlling for these confounders, no significant association was observed for continuing COH beyond visualization of  $\geq 2$  mature follicles and MII rate (OR 1.01 [95% CI 0.90-1.13]), fertilization rate (OR 0.98 [95% CI 0.88-1.10]), blastulation rate (OR 0.97 [95% CI 0.87-1.08]), or euploidy rate (OR 0.90 [95% CI 0.78-1.04]). A sub-analysis was performed for SART age group E, which also showed no differences in cycle outcomes when COH was extended.

**CONCLUSIONS:** In the largest study of GnRH antagonist protocol IVF cycles looking at oocyte developmental competence when trigger was delayed in the presence of  $\geq 2$  mature follicles, we demonstrated no significant difference in rates of maturation, fertilization, blastulation, and euploidy, even in patients  $>42$  years old. Our study suggests that continuing COH up to 2 days in select patients does not negatively affect outcomes. While reassuring, the effects of COH prolongation on genomic and non-genomic factors must be investigated. Well-controlled prospective studies assessing CPR and LBR will be needed before we can definitively quantify the limits around optimal COH duration.

### REFERENCES

1. Wirleitner B, Okhowat J, Vistejnova L, et al. Relationship between follicular volume and oocyte competence, blastocyst development and live-birth rate: optimal follicle size for oocyte retrieval. *Ultrasound Obstet and Gynecol*, 2018; 51: 118-125.

2. Awonuga AO, Wheeler K, Thakur M, et al. The value of delaying hCG administration to enable maturation of medium-sized follicles in patients undergoing superovulation for IVF/ICSI. *J Assist Reprod Genet*, 2018; 35:289-295.

**SUPPORT:** None.

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### CIRCULATING MIR-181D-5P LEVELS AS PREDICTOR OF OVARIAN RESPONSE IN WOMEN UNDERGOING CONTROLLED OVARIAN STIMULATION.

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**OBJECTIVE:** The main objective of individualization of assisted reproduction treatments is to offer every woman the best treatment tailored to her unique characteristics. However, the success of individualized controlled ovarian stimulation (COS) depends on finding a reliable method for predicting ovarian response to stimulation. Therefore, the goal for the present study was to identify potential microRNAs (miRNAs) biomarkers of the response to COS.

**DESIGN:** Cohort study.

**MATERIALS AND METHODS:** For the present study, 90 serum samples were collected prior to COS for intracytoplasmic sperm injection (ICSI). Samples were collected in a private university-affiliated IVF center, between Jan 2017 and Jan 2018, and were split into three groups, depending on the patient's response to COS: Poor Response Group:  $< 4$  retrieved oocytes (PR group, n=30), Normo Response Group:  $\geq 8$  and  $\leq 12$  retrieved oocytes (NR group, n=30), and Hyper Response Group:  $> 25$  retrieved oocytes (HR, n=30). Samples were used for two experimental sets. For the first experimental set, 5 samples from each group were pooled together and used to identify aberrantly expressed miRNAs in experimental groups, by using a large-scale microRNA expression analysis platform. For the second experimental set, 25 samples from each group were individually analyzed and the expression of specific miRNAs, determined by the first step, was investigated.

**RESULTS:** Twenty two miRNAs presented a twofold increase level in the PR or HR groups when compared with the NR group. From those miRNAs, 9 presented poor dissociation curves and were excluded from further analysis. Based on the quality of the amplification, observed in the manual analysis, the detection pattern in the experimental groups, and literature data, three miRNAs with exclusive detection in the HR group (miR-181d-5p, miR-221-3p and miR-92a-1-5p) and six miRNAs with exclusive detection in the PR group (miR-891a-5p, miR-99a-3p, miR-223-3p and

miR-200c, let-7d-3p and miR-150-5p) were selected for a subsequent validation set. The results showed that the serum levels of *miR-181d-5p* was significantly increased in the HR group when compared with LR group ( $p=0.0002$ ) and NR group ( $p=0.0091$ ). No differences were observed between NR and PR groups ( $p=0.2772$ ). Serum levels of *miR-181d-5p* was also positively correlated with the number of aspirated follicles ( $p<0.0001$ ), number of retrieved oocytes ( $p<0.0001$ ), and number of mature oocytes ( $p=0.0002$ ).

**CONCLUSIONS:** The quantification of miR-181d-5p prior to the COS may discriminate patients who will respond in an exacerbated manner to those who will respond insufficiently to the COS. The use of this tool associated with other previously described parameters may allow the individualization of the treatment, increasing treatment success while decreasing patients' risks and physical, emotional and economic burden.

**SUPPORT:** Sao Paulo Research Foundation (FAPESP, 2017/20553-5 $\hat{A}$ , 2016/08145-6 $\hat{A}$ , National Council for Scientific and Technological Development (CNPq, 405549/2016-4 $\hat{A}$ ).

**P-281** Tuesday, October 15, 2019 6:30 AM

**PHYSICIANS SHOULD AVOID CHANGING A PATIENT'S OVARIAN STIMULATION PROTOCOL FOR THE PURPOSE OF IMPROVING LABORATORY OUTCOMES.**



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**OBJECTIVE:** Providers consider a number of factors when selecting a stimulation protocol and may switch protocols when a patient has a suboptimal stimulation or laboratory outcome. We sought to determine whether providers' choice in stimulation is associated with laboratory outcomes.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** IVF cycles from 1/2010 to 3/2019 were reviewed. Cycles were categorized as: (1) E2 priming antagonist (2) Antagonists +/- OCP priming (3) Long luteal (4) Lupron stop (5) Flare. Mini-stimulations were excluded. Laboratory outcomes for first stimulations only and repeated cycles within a patient were compared. For first stimulation cycles, linear and logistic regression were used. For repeated cycles, those who completed the same stimulation were compared to those who changed, using cluster analyses for pairwise comparison. A subgroup of patients who had a low blast progression in their first cycle was also analyzed. Outcomes were adjusted for number of eggs collected and patient age.

**RESULTS:** 5209 patients underwent ovarian stimulation for IVF. When comparing between stimulation types, fertilization rate, blast progression and euploid rate were not statistically different. 2477 of these patients underwent a second cycle: 50% repeated the same and 50% completed a different protocol. The fertilization rate and blast progression were not statistically different between those who repeated the same protocol and those who changed. There was a statistically significant improvement in eggs collected, usable embryos and euploid rate for those who repeated the same stimulation, after adjustment (table). Of those with low blast progression in the first cycle, a significant improvement occurred in the second cycle, however, repeating the same protocol resulted in a slightly greater improvement (coefficient 0.03 (0.01-0.04)).

**CONCLUSIONS:** All conventional ovarian stimulation protocols result in comparable laboratory outcomes. By enlarge, the variations seen from cycle to cycle within a patient cannot be explained by stimulation type. If anything, there is a subtle benefit to staying with the same protocol, for reasons yet to be determined, but likely inherent to the patient. Until these factors are further understood, physicians should avoid changing stimulation protocols for the purpose of improving laboratory outcomes.

**P-282** Tuesday, October 15, 2019 6:30 AM

**CAN TREATMENT CHOICE AFFECT COST OF THERAPY IN PATIENTS PREDICTED TO BE HIGH-RESPONDERS? RESULTS OF AN ECONOMIC ANALYSIS OF THE MENOPUR IN GnRH ANTAGONIST SINGLE EMBRYO TRANSFER - HIGH RESPONDER (MEGASET-HR) TRIAL.**



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**OBJECTIVE:** To determine difference in treatment cost associated with cumulative live birth between highly purified-human menotropin (HP-hMG; Menopur<sup>®</sup>) and recombinant follicle stimulating hormone (rFSH; Gonal-F<sup>®</sup>) in predicted high responder women undergoing assisted reproductive technology.

**DESIGN:** Cost analysis of a multicenter, randomized, open label, assessor blind, non-inferiority trial.

**MATERIALS AND METHODS:** Ovulatory women aged 21-35y with BMI 18-30 kg/m<sup>2</sup> and serum anti-Müllerian hormone (AMH)  $\geq 5$  ng/mL (N=620) were randomized 1:1 to a 150 IU start dose of HP-hMG or rFSH in a GnRH antagonist cycle with dose adjustments allowed on day 6 of stimulation. All embryos were fertilized by intracytoplasmic sperm injection and underwent Day 5 trophectoderm biopsy for preimplantation genetic screening (PGS). Whereas morphology guided single, fresh blastocyst transfer, PGS results were only available to guide frozen blastocyst transfers. Live birth outcomes resulting from all fresh and any frozen transfers occurring within 6 months of randomization were collected. A decision tree of all per protocol trial outcomes and their associated probabilities was constructed. The resultant model was then used to perform a cost analysis using real-world trial site procedural costs and wholesale acquisition cost (WAC) of medication from when the trial began (September 2015) inflation-adjusted to 2019 cost.

**RESULTS:** Demographics for the HP-hMG and rFSH arms were similar. The primary non-inferiority end-point was met, and the cumulative live birth rate was 50.6% (157/310) for HP-hMG and 51.5% (159/309) for rFSH (difference; -0.8%; 95% CI -8.7, 7.1). Mean total dose of HP-hMG was 616 IU greater, while patients with rFSH underwent 43 more transfers, had a higher cumulative early pregnancy loss rate (-11.0%; 95%CI: -18.8, -3.1) and higher adverse event rate of ovarian hyperstimulation syndrome (-11.7%; 95% CI: -17.3, -6.1). Results of the cost analysis showed that per patient treatment cost of HP-hMG was lower at \$14,744 compared to \$15,759 with rFSH.

**CONCLUSIONS:** Cost analysis of data from the MEGASET-HR trial shows that treatment of predicted high-responders with HP-hMG may be associated with lower treatment costs compared to rFSH, despite potentially higher initial medication cost. The savings were driven by fewer embryo transfers needed and lower rate of adverse events associated with HP-hMG therapy.

**SUPPORT:** This trial was sponsored by Ferring Pharmaceuticals, Inc.

**P-283** Tuesday, October 15, 2019 6:30 AM

**A SIMULATED USE STUDY OUTLINING DIFFERENCES IN HANDLING ERRORS AND PREFERENCE BEFORE AND AFTER USE OF CURRENTLY AVAILABLE RECOMBINANT HUMAN FOLLICLE-STIMULATING HORMONE (R-HFSH) PEN INJECTORS.**



Salvatore Longobardi, MD,<sup>a</sup> Anke Seidler, MBA,<sup>a</sup> Julian G. Martins, MA,<sup>b</sup> Francois P. M. Beckers, PhD,<sup>a</sup>

Second Stimulation Cycles	Same Stimulation	Different Stimulation	Coefficient /*Adjusted Odds Ratio (CI)
# Eggs	11.8	10.7	1.0 (0.46-1.53)
Fertilization Rate	0.78	0.76	*1.6 (0.92-2.8)
# Usable Embryos	3.9	3.11	1.25 (0.79-1.72)
Blast Progression	0.51	0.48	0.03 (-0.01-0.08)
Euploid Rate	0.34	0.34	*1.39 (1.01-1.93)

**OBJECTIVE:** This study compared handling errors and preference ratings before and after use of four currently available r-hFSH pen injectors tested by women with infertility and fertility nurses.

**DESIGN:** This was a simulated use study comparing the GONAL-f<sup>®</sup> (Merck KGaA, Germany), Bemfola<sup>®</sup> (Gedeon Richter PLC, Hungary), Rekovelle<sup>®</sup> (Ferring Pharmaceuticals Ltd, UK) and Ovaleap<sup>®</sup> (Teva BV, The Netherlands) pen injectors in Germany, Poland and the UK.

**MATERIALS AND METHODS:** Injector-naïve women with infertility and injector-experienced fertility nurses tested pen injectors with masked labels in a randomized testing order. Simulated injections were made into a foam pad following the instructions for use (IFU) and injectors were rated before and after use. Handling errors were noted by the moderator during the study. After the study, errors were grouped according to severity and use-steps indicated in the IFU. Ordinal or Poisson linear mixed models were applied, adjusted for injector and testing order with an unstructured correlation matrix between measures (or with non-convergence, non-parametric or normal approximation to the Poisson methods). All analyses were exploratory by nature without any correction for multiplicity.

**RESULTS:** A total of 120 women with infertility and 60 fertility nurses participated. All participants tested GONAL-f and Bemfola injectors. Because of their similarity, participants tested either Rekovelle (71 women; 30 nurses) or Ovaleap (49 women; 30 nurses) injectors. Before simulated use, mean ratings from women with infertility were similar between the GONAL-f and other pen injectors. After use, the ratings from women were higher for GONAL-f vs other pen injectors (p<0.001 for all comparisons). Fertility nurses rated the GONAL-f injector higher than the other pen injectors both before and after simulated use, with the difference in ratings larger after simulated use (p<0.01 for all comparisons vs GONAL-f). Adjusted rates of total handling errors for both women with infertility and fertility nurses were lower with the GONAL-f pen injector (p<0.001 for all comparisons vs GONAL-f). Adjusted rates of total handling errors (95% CI) for women with infertility were 1.02 (0.84, 1.20), 1.64 (1.41, 1.87), 2.07 (1.68, 2.45) and 3.16 (2.50, 3.81) with GONAL-f, Bemfola, Rekovelle and Ovaleap pen injectors, respectively. For fertility nurses, corresponding adjusted rates were 0.31 (0.16, 0.45), 1.30 (1.00, 1.60), 1.19 (0.71, 1.66) and 1.64 (1.06, 2.21), respectively. The most difficult use-steps (i.e. during which most errors were recorded with all pen injectors) were “priming” and “giving the injection”. Significantly lower error rates were recorded during these use-steps with the GONAL-f pen injector vs the other pen injectors (p<0.05 for all comparisons).

**CONCLUSIONS:** In this study, the GONAL-f injector was rated significantly higher than other pen injectors after use. This may be a result of more handling errors, including those that may affect treatment outcomes, observed with the Bemfola, Ovaleap and Rekovelle pen injectors.

**SUPPORT:** Funded by Merck KGaA, Darmstadt, Germany.

**EFFECT OF VARIANT B LH POLYMORPHISM (RS1800447)(TREP 8 ARG) IN AN EGYPTIAN IVF POPULATION ON OVARIAN RESPONSE IN ASSISTED REPRODUCTION.** Mohamad Ghanem, MD,<sup>a</sup>

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**OBJECTIVE:** Existing evidence supports the association between specific gonadotropins and their receptor single nucleotide polymorphism (SNP) and poor ovarian response (POR)<sup>[1]</sup>. Many SNPs have been identified in the LH-β gene. The most commonly studied is rs1800447 (p. Trp8Arg) which seems to vary among ethnical groups.<sup>[2]</sup>. We aimed to assess frequency of this SNPs among our population and its association with ovarian response (OR): gonadotrophin dose and number of eggs retrieved.

**DESIGN:** retrospective

**MATERIALS AND METHODS:** We included 181 IVF females in Mansoura Integrated Fertility Centre. Ovarian stimulation was conducted using either GnRH antagonist or agonist protocol with recombinant FSH. Total gonadotropin dose and number of oocytes retrieved were evaluated. DNA was extracted from peripheral blood leucocytes followed by analysis of genetic polymorphism by PCR-RFLP technique for rs1800447 (Trp8Arg)

**RESULTS:** Patients were grouped according their codon 8 genotype into TT,TC,CC groups. The groups showed no significant differences in female age, BMI, duration and causes of infertility. Although OR was lower in the CC genotype, the differences were not significant (small subgroup numbers). POR (number of eggs retrieved <4)<sup>[3]</sup> was significantly higher in CC genotype compared to other genotypes. The frequency of homozygous genotype (CC) among poor responders 7/23 (30.7%) was three times higher than among normal responders 14/144 (9.7%) implying significantly higher risk of POR {OR 4.063, 95% CI (1.428 - 11.56) p, 0.012}

**CONCLUSIONS:** Our results showed that among a cohort of Egyptian infertile women the frequency genetic variants of V- LHB ( p.Trp8Arg , c.rs1800447) is 53.5% for all C alleles and 13.8% for CC genotype and that variant CC is significantly associated with POR compared with other genotypes with the odds ratio of developing POR 4.063, 95% CI (1.428 to 11.56) p, 0.012. This means that genotyping for this variant among IVF women can help planning ovarian stimulation protocol to circumvent the risk of POR.

**REFERENCES**

- [1] Iviggi C, Conforti A, Esteves SC, et al. Understanding Ovarian Hypo-Response to Exogenous Gonadotropin in Ovarian Stimulation and Its New Proposed Marker-The Follicle-To-Oocyte (FOI) Index. *Front Endocrinol (Lausanne)*. 2018;9:589.
- [2] Nilsson, C., Pettersson, K., Millar, R. P., Coerver, K. A., Matzuk, M. M., & Huhtaniemi, I. T. Worldwide frequency of a common genetic variant

	TT (n=84)	TC (n=72)	CC (n=25)	p
Age yrs.*	28.5±5.6	29.4±5.9	29.5±6.1	0.58 <sup>S</sup>
Infertility Duration yrs. ^	4 (0-19)	4.5 (0-18)	4 (1-19)	0.83 <sup>SS</sup>
Basal FSH m IU/mL ^	4.8 (1.2-10.5)	5 (1-11.1)	4.9 (1.9-11.9)	0.60 <sup>SS</sup>
Causes of infertility: n(%)				P=0.77 <sup>£</sup>
Male (n= 69)	33 ( 39.3)	25 (34.7)	11 ( 44)	
PCOS (n=20)	12 (14.4)	7 ( 8.3)	1 ( 4)	
Tuboperitoneal( n =29)	13 (15.4)	11 ( 12.5)	5 ( 20)	
Combined (n= 32)	14 (16. 6)	13 (16.6)	5 (2 0)	
Unexplained ( n=31)	12 ( 14.3)	16 ( 22.2)	3 (12)	
Gn Rh protocol n,(%)				0.19 <sup>£</sup>
Agonist, ( n= 81)	32 ( 38.4)	34 (47.2)	14 (56)	
Antagonist ( n=100)	52 ( 61.6)	38 (52.8)	11 (44)	
Gonadotropin dose IU*	1888.4±481.9	1958.3±526.9	2067±548.6	0.28 <sup>S</sup>
Eggs retrieved ^	10 (1-23)	9 (1-31)	7 (1-35)	0.33 <sup>SS</sup>
Ovarian response n(%)				P = 0.04 <sup>£</sup>
POR (n=23)	9 (10.7)	7(9.8)	7(28)	
Normal response (n=144)	70(83.4)	60(83.3)	14(56)	
High response (n=14)	5 (5.9)	5(6.9)	4 (16)	

\*=( mean ± SD) , ^=( median, (range) <sup>S</sup> \_Anova test, <sup>SS</sup>=Kruskal-Wallis Test, <sup>£</sup>= chi square test

of luteinizing hormone: an international collaborative research. *Fertil Steril*, 1997;67, 998-1004.

[3]. Ferraretti A.P, La Marca A., Fauser B.C.J.M., Tarlatzis B., Nargund G., Gianaroli L., on behalf of the ESHRE working group on Poor Ovarian Response Definition, ESHRE consensus on the definition of 'poor response' to ovarian stimulation for *in vitro* fertilization: the Bologna criteria, *Human Reproduction*, Volume 26, Issue 7, July 2011.

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### EFFECT OF OVARIAN STIMULATION OF OOCYTE DONORS ON IN-VITRO FERTILIZATION

**OUTCOMES.** Heather S. Hipp, MD,<sup>a</sup> Audrey J. Gaskins, ScD,<sup>a</sup> Zsolt Peter Nagy, MD, PhD,<sup>b</sup> Sarah M. Capelouto, MD,<sup>c</sup> Daniel B. Shapiro, MD,<sup>b</sup> Jessica B. Spencer, MD, MSc<sup>c</sup>. <sup>a</sup>Emory University, Atlanta, GA; <sup>b</sup>Reproductive Biology Associates, Atlanta, GA; <sup>c</sup>The University of Texas, Southwestern Medical Center, Dallas, TX.



**OBJECTIVE:** To determine the effect of ovarian stimulation in oocyte donors on in-vitro fertilization (IVF) outcomes for recipients.

**DESIGN:** Retrospective cohort study of data from a frozen donor oocyte bank from 2008 to 2015.

**MATERIALS AND METHODS:** A total of 350 oocyte donors underwent 553 ovarian stimulation cycles with an antagonist protocol. Mature oocytes were vitrified and later warmed in individual cohorts among 989 unique recipients who underwent 1745 embryo transfer cycles. The associations between ovarian stimulation characteristics and rates of oocyte warm survival, fertilization, and usable embryos (combination of number of embryos transferred and cryopreserved for future use) per oocyte warmed as well as the odds of live birth per embryo transfer cycle were modeled using cluster-weighted generalized estimating equations adjusted for donor age, body mass index (BMI), race, retrieval year, and recipient age (live birth only).

**RESULTS:** The donors were 21-32 years old with BMI <30 kg/m<sup>2</sup>. Per stimulation cycle, the median number of oocytes retrieved was 30 (range: 9-95). The majority of recipients, 78.6%, had 6-8 donor oocytes warmed. Mean (standard deviation) percentage of oocytes that survived warm, were successfully fertilized and were usable was 93.6% (11.5%), 79.8% (18.2%) and 53.9% (21.8%) respectively. Donors with more oocytes retrieved had a lower percentage of usable embryos per oocyte warmed (<15: 62.5% (95% Confidence interval (CI) 52.7-71.4), 15-30: 58.9% (95% CI 55.0-62.6), 31-50: 53.6% (95% CI 49.6-57.5), >50 52.0% (95% CI 46.0-57.9%)). Of the transfers, 856 (49.1%) resulted in a live birth. There was no difference in the probability of live birth according to number of oocytes retrieved in a donor. For example, the adjusted odds of live birth among recipients was 0.93 (95% CI 0.67, 1.31) if the donor had >50 oocytes retrieved compared to 15-30 oocytes retrieved.

**CONCLUSIONS:** Oocyte donors represent an excellent model to determine the impact of ovarian stimulation on IVF outcomes given a relatively uniform uterine environment. Although high donor oocyte yields result in more oocytes available, there are less usable embryos per oocyte warmed as number of retrieved oocytes increases. These differences in early outcomes, however, do not translate into differences in live birth rate.

**SUPPORT:** REDCap grant support at Emory was provided through UL1 TR000424.

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### THE IMPACT OF FOLLISTATIN HORMONE ON OVARIAN RESPONSE.

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**OBJECTIVE:** We aimed in this study to find the role of serum level of Follistatin in the process of folliculogenesis. Noticing the variability of Follistatin levels between women, we wanted to uncover a modifying role that Follistatin plays, resulting in a variable individual ovarian response.

**DESIGN:** A Prospective cross-sectional observation study, including 200 women undergoing an IVF program with the long stimulation protocol in the Egyptian IVF Center in Maadi, Cair, Egypt.

**MATERIALS AND METHODS:** Patients were matched regarding age, BMI, ovarian reserve (based on AMH and AFC) and HMG initial doses (150-225mIU/day). Serum Follistatin was measured in the blood sample withdrawn from the patient 12-14 days after starting the GnRHa, to test for pituitary down-regulation, prior to HMG administration. Two primary parameters were set. *Parameter 1*; the time needed to reach a satisfactory initial response (**Point A** set as: "the number of days needed by the patient to reach at least 2 follicles on each side with a minimum of 12 mm diameter"). *Parameter 2* was set as "the time needed to reach mature Graafian follicles (**Point B** set at: 20 mm follicular diameters or more and concomitant Estradiol levels corresponding to at least 200 pg/mL per follicle").

**RESULTS:** Patients were divided into four groups based on their Follistatin levels, ranging between the minimal and the maximal readings recorded in our study, using an increment of 1000 pg/ml between each group (Group 1: 200-1200, Group 2: 1201-2200, Group 3: 2201-3200, Group 4: 3201-4200 ng/ml). By 7 days of HMG stimulation 100% of Group 1 reached Point A, this was achieved in 64.1%, 38.2% and 0% in Groups 2, 3&4 respectively. By Day 9 stimulation 100% of Group 2, 91% of Group 3 and only 10.5% of Group 4 reached Point A. Point B was reached in Group 1 after 8 days in 82.1% and 100% after 10 days. At 12 days of stimulation the percentage of those who reached Point B was 92.2% in Group 2, 4.5% in Group 3 and 0% in Group 4. In that last group, 73.7% of patients needed 16 days to reach Point B, with a remaining 26.3% needed 18 days or more to reach it. Using Pearson's correlation, a strong positive correlation was found between serum Follistatin levels and Parameter 1 & 2 (r=0.899 & 0.91, respectively). The correlations between Follistatin and Age, BMI and AMH were statistically insignificant.

**CONCLUSIONS:** In this study, serum Follistatin levels had a clear effect on ovarian response. The detected inverse correlation between Follistatin levels and the ovarian response time suggests a role of serum Follistatin levels assessment prior to starting an ovarian stimulation protocol. Follistatin could act as a reliable independent predictor of the magnitude of ovarian response in cases undergoing controlled ovarian hyperstimulation for IVF. This could be used to properly tailor the dose for those patients, reducing the need for dose modification and subsequently duration of stimulation. It could equally help to predict OHSS or slow response.

**SUPPORT:** None.

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### ORAL OVULATION INDUCTION MEDICATIONS VERSUS GONADOTROPINS FOR UNEXPLAINED INFERTILITY: A SYSTEMIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS.

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**OBJECTIVE:** To compare live birth and multiple gestation in gonadotropins versus oral ovulation induction agents for patients with unexplained infertility.

**DESIGN:** Systematic review and meta-analysis

**MATERIALS AND METHODS:** A systematic review of PubMed and Embase was performed for RCTs comparing gonadotropins versus clomiphene citrate (CC) or letrozole in IUI cycles for patients diagnosed with unexplained infertility. Primary outcomes were live birth and multiple gestation. Random effects models were used for all comparisons, due to clinical heterogeneity or I<sup>2</sup> >50%. Primary meta-analysis was performed on an intent-to-treat and per patient basis, with sensitivity analyses of per protocol, per cycle, and fixed effects models performed.

**RESULTS:** Eight total trials were identified that met inclusion criteria and constituted 2,989 patients undergoing 6,590 cycles. One study reported a significant increase in live births and multiple gestations with gonadotropins in comparison to letrozole and CC. All other studies compared CC and gonadotropins. Three of these studies found no difference in live birth or multiple gestations. One study found a lower live birth rate with CC but no difference in multiple gestations. Moderate heterogeneity was suggested by the Q test (Q=0.08) and the I<sup>2</sup> index (I<sup>2</sup>=53%) for live birth comparisons. The overall likelihood of live birth was not significantly increased in patients randomized to gonadotropins (RR 1.10, 95% CI 1.00-1.21, P=0.05). Similarly, the risk of multiple gestation was not significantly increased in patients assigned gonadotropins (RR 1.09, 95% CI 0.97-1.21, P=0.15). The number needed to treat with gonadotropins was 15 to have 1 additional live birth. For every 1 additional live birth from

gonadotropins, an additional 0.88 twin pregnancies occurred. Singleton birth per cycle was similar between the two groups. The results did not change in per protocol, per cycle, or fix effect model sensitivity analyses.

**CONCLUSIONS:** Gonadotropin use in women with unexplained infertility did not increase the likelihood of live birth. For every birth gained with the use of gonadotropins, an almost identical increase in the risk of twins occurs. The randomized data do not support the use of gonadotropins for superovulation in women with unexplained infertility.

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#### **OVULATION RATE WITH LETROZOLE STAIR-STEP PROTOCOL AND IN SUBSEQUENT LETROZOLE CYCLE.**

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**OBJECTIVE:** Stair-step (SS) protocols have been successfully used for anovulatory women who fail to ovulate with the initial dose of clomiphene citrate (CC), with the additional benefit of decreased time to ovulation and increased ovulation rates compared with more traditional protocols. Letrozole is now considered first-line therapy for ovulation induction (OI). However, ovulatory rate with the letrozole SS protocol has never been reported. We sought to determine the effectiveness of the letrozole SS protocol for inducing ovulation, as well as the ovulation rate in the subsequent cycle with the letrozole dose that achieved ovulation through SS.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Anovulatory patients who underwent OI using the letrozole SS protocol at our center between 2013-2019 were included for analysis. Baseline and cycle characteristic data was collected from our electronic medical record. Ovulation was confirmed by positive result on urinary ovulation predictor kit, serum LH level >20 IU/mL or the presence of a follicle >15 mm on ultrasound. Ovulation rate was the primary outcome. Student's t-test and chi squared test was used for continuous and categorical variables, respectively. A p-value of 0.05 was considered statistically significant.

**RESULTS:** Of 108 patients who underwent letrozole SS for OI, 83.3% (90/108) of patients became ovulatory. 38.9% (35/90) patients ovulated with the 5 mg dose and 61.1% (55/90) ovulated with the 7.5 mg dose. 88.9% (80/90) of patients required one SS, whereas 11.1% (10/90) required two SS. BMI was significantly higher in women who remained anovulatory compared to those who responded to letrozole SS (37.0 ± 9.8 kg/m<sup>2</sup> v. 28.8 ± 6.7 kg/m<sup>2</sup>, respectively.) Of the ovulatory patients, 61 underwent a subsequent letrozole cycle and 91.8% (56/61) ovulated at the ovulatory dose established in the preceding SS cycle.

**CONCLUSIONS:** The letrozole SS protocol is successful in inducing ovulation in a majority of patients as well as in a subsequent cycle with the previously established ovulatory dose. Higher BMI may contribute to letrozole resistance.

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#### **SINGLE DOSE VERSUS 5 DAY DOSING OF AN AROMATASE INHIBITOR FOR OVULATION INDUCTION.**

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**OBJECTIVE:** Compare the efficacy of single dose letrozole (1D) with standard five day (5D) course for ovulation induction (OI).

**DESIGN:** A retrospective cohort study of all patients undergoing OI and intrauterine insemination (IUI) with letrozole from January 2015 through December 2017 at a single institution.

**MATERIALS AND METHODS:** All patients undergoing their first OI/IUI cycle with letrozole from January 2015 to December 2017 were included in the study. Patients either received a one time dose of 25mg letrozole (1D) on cycle day 3 or dose of 5mg daily for five days (5D) from cycle days 3-7. The primary outcome was pregnancy rate (PR). Secondary outcomes included

live birth rate (LBR), multiple gestation (MG), and miscarriage (SAB). Student's T test, chi square, and Fisher's exact statistical analysis were utilized where appropriate.

**RESULTS:** Of a total of 586 patients, the 1D group had 302 patients and the 5D group had 284 included in the study. There was no difference in smoking status, primary vs secondary infertility, or total motile concentration (TMC). Comparing 1D to 5D, there was a statistically significant, though not clinically relevant difference in both age and BMI, (31 yrs vs. 31.8 yrs, p=0.03; 26.2 vs. 27.4, p=0.02), respectively. There were no differences between 1D and 5D in PR (14.2% vs 11.6%), LBR (9.6% vs 7%), MG (16.2% vs 13.8%), or SAB (16.22% vs 13.8%).

**CONCLUSIONS:** A single dose protocol with Letrozole in an OI/IUI cycle may be considered as an alternative to standard five day dosing protocols with potential for improved compliance and similar reproductive outcomes.

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#### **PHASE 4, NON-INTERVENTIONAL, OPEN LABEL STUDY EVALUATING DOSING CHARACTERISTICS AND OVARIAN RESPONSE USING THE REDESIGNED FOLLITROPIN ALFA PEN INJECTOR IN ASSISTED REPRODUCTIVE TECHNOLOGIES (ART) TREATMENT IN ASIA: IMPROVE STUDY.**

Bum Chae Choi, MD, PHD,<sup>a</sup> Canquan Zhou, MD,<sup>b</sup> Hong Ye, MD,<sup>c</sup> Sun Yun, M.D.,<sup>d</sup> Ying Zhong, Doctor,<sup>c</sup> Fei Gong, PhD,<sup>f</sup> Nadezda Abramova, MD, PhD,<sup>g</sup> Salvatore Longobardi, MD,<sup>e</sup> Teoman Ulas, MD,<sup>h</sup> Thomas D'Hooghe, MD, PhD,<sup>e</sup>. <sup>a</sup>Creation and Love Women's Hospital, Gwang-ju, Korea, Republic of (South); <sup>b</sup>First Affiliated Hospital, Sun Yat-sen University, GuangZhou, Guangdong, China; <sup>c</sup>Chongqing Maternity and Child Healthcare Hospital, Chongqing Shi, China; <sup>d</sup>Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; <sup>e</sup>Chengdu Jinjiang District Maternal and Child Health Hospital, Chengdu Shi, China; <sup>f</sup>Reproductive and Genetic Hospital of CITIC-Xiangya, Changsha, Hunan, China; <sup>g</sup>Merck Healthcare KGaA, Darmstadt, Germany; <sup>h</sup>Merck Pte Ltd, Ascent, Singapore.



**OBJECTIVE:** Assess if use of a redesigned pen injector (RPI) for follitropin alfa (GONAL-f<sup>®</sup>, Merck KGaA, Darmstadt, Germany) with a small dose dial (12.5IU) allows greater treatment individualization, measured as reduction in the total dose (IU) of recombinant human follicle stimulating hormone (rhFSH) used per oocyte retrieved, in a subgroup of ART patients (pts) at risk for ovarian hyperstimulation syndrome (OHSS).

**DESIGN:** Phase 4, comparative study of pts receiving *in vitro* fertilization / intracytoplasmic sperm injection in an observational prospective cohort (PC) vs an historical cohort (HC).

**MATERIALS AND METHODS:** The PC included pts (20-40 yrs; BMI <30 kg/m<sup>2</sup>) using the RPI at 14 sites (Korea, Vietnam, Indonesia and China [N=1783; assessed 09/14-07/16]). The HC was from a Phase 4 study (EMR700623523) in a comparable Asian population using other injection devices (OID; N=1419; assessed 06/10-02/12). In the PC, pts followed either an agonist or antagonist protocol; rhFSH fine dose adjustment with the RPI was allowed at each stage. In the HC pts followed an agonist protocol; rhFSH dose adjustment with OID was allowed at each stage. The primary endpoint of amount of rhFSH (IU) administered per oocyte retrieved was assessed in a pt subgroup at high risk for OHSS, identified from pt characteristics (BMI, age) and biomarkers (anti-Müllerian hormone, antral follicle count, basal FSH and luteinizing hormone [LH], and estradiol [E2]) and propensity matched between the PC (N=123) and HC (N=123). The sensitivity analysis only included pts receiving agonist protocol (N=123 matched pts, each cohort). Secondary outcomes and safety (OHSS and all adverse events [AEs]) were assessed in the total population.

**RESULTS:** Pt characteristics were comparable between cohorts. In the PC, 62.5% (1115/1783) of pts received an agonist protocol, 37.0% (659/1783) an antagonist protocol (0.5% [9/1783] were on both 'other'). All pts in the HC received an agonist protocol. Mean amount (SD) of rhFSH in IU administered per oocyte retrieved (matched population) was significantly lower in the PC vs HC (132.5 [85.2] vs 332.7 [371.6]; p<0.0001; sensitivity analysis: 127.5 [81.9] vs 332.7 [371.6]; p<0.0001). LH level (SD) in IU/L between Day 6 and 8, and E2 level (SD) in pg/mL on the day of human chorionic gonadotropin administration was higher in the PC vs HC (LH: 6.71 [69.2] vs 1.63 [1.5]; E2: 4058.3 [2663.5] vs 3291.8 [2313.9]). Mean (SD) total

rhFSH dose in IU was lower in the PC vs HC (1848.4 [700.5] vs 2237.8 [772.6]); clinical pregnancy rate was improved in the PC vs HC (per embryo transfer cycle: 50.3% vs 40.7%; per initiated cycle: 35.3% vs 37.8%). OHSS incidence was significantly lower in the PC vs HC (1.5% [27/1783] vs 4.0% [57/1419],  $p < 0.0001$ ); most events were mild/moderate. 5.0% [89/1783] of patients had  $\geq 1$  AE and 1.9% [33/1783] of patients had  $\geq 1$  serious AE in the PC.

**CONCLUSIONS:** Pts using the RPI required a significantly lower rhFSH dose per oocyte retrieved vs pts using OID in this Asian population. Clinical outcomes were improved and OHSS incidence was significantly lower in the PC vs HC.

**SUPPORT:** Merck KGaA, Darmstadt, Germany.

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### USE OF LUTEINIZING HORMONE SUPPLEMENTATION FOR OVARIAN STIMULATION IN IVF/ICSI CYCLES OF WOMEN WITH GOOD OVARIAN RESERVE.



**RESERVE.** Liang Hsuan Chen, MD, Tzu-Hsuan Chin, MD, Ya-Chiung Hsu, MD, Shang Yu Huang, MD, Hsing-Tse Yu, MD, Hsien-Ming Wu, MD, PhD, Chia-Lin Chang, MD, Hong-Yuan Huang, MD, Hsin-Shih Wang, PhD, Yung-Kuei Soong, MD. Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital Linkou Medical Center, Taoyuan, Taiwan R.O.C., Taipei, Taiwan.

**OBJECTIVE:** To declare current evidence exploring the added value of LH supplementation to FSH following GnRH antagonist protocol in women with good ovarian reserve.

**DESIGN:** We conducted a retrospective analysis exploring the benefit for pregnancy achievement of LH supplementation to GnRH antagonist cycles in women with AMH level over 5ng/mL.

**MATERIALS AND METHODS:** A total of 255 women with  $AMH \geq 5$  undergoing IVF/ICSI using a GnRH antagonist protocol was included. Of these, 148 were received treatment with recombinant FSH (r-FSH) + human menopausal gonadotropin (HMG) and 107 with r-FSH alone through the ovarian stimulation.

**RESULTS:** We observed a significantly lower serum LH levels at the beginning of cycle, the day of GnRH antagonist administration and the day of oocyte triggering in the combination of r-FSH+HMG group. The treatment days and total gonadotropin dose was significantly higher in r-FSH+HMG group compared with r-FSH alone group. Nevertheless, there were no significant differences between the two groups with respect to the number of oocytes retrieved, maturation, fertilization, and blastocyst formation rate. The OHSS occurred 8% of the r-FSH+HMG group, whereas 8% OHSS developed in the r-FSH alone group. There were no difference in pregnancy outcome between the groups.

**CONCLUSIONS:** LH supplementation to r-FSH following GnRH antagonist does not seem to significantly augment serum E2 level on the trigger day and further pregnancy outcome in patient with good ovarian reserve. However, LH supplement seems to have a benefit in some normo-gonadotropic women, who developed LH deficiency following GnRH antagonist. An accurate definition of the LH threshold in GnRH antagonist cycles may

contribute to the discussion of which subgroups of women may benefit from adjuvant LH therapy.

**P-292** Tuesday, October 15, 2019 6:30 AM

### CHOOSING THE OPTIMUM MEDICATION AND DOSE IN OVULATION INDUCTION-INTRAUTERINE INSEMINATION CYCLES (OI-IUI) TO AVOID MULTIPLE GESTATION PREGNANCIES.



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**OBJECTIVE:** Does dosing or choice of ovulation induction medication impact clinical pregnancy or multiple gestation rate?

**DESIGN:** Retrospective cohort

**MATERIALS AND METHODS:** 8,911 patients underwent 15,453 oral ovulation induction-IUI (OI-IUI) cycles from 2004-2018. Primary exposure: Medication (clomiphene citrate (CC) versus letrozole (LTZ)) and dose. Primary outcome: singleton/multiple clinical pregnancy rate (CPR). Statistical associations were determined using chi square analysis and multivariable logistic regression models. Age, BMI, AMH, baseline FSH and AFC were considered as clinically relevant covariates. Generalized estimating equations were used to account for multiple cycles in the same patient. To isolate medication effect, those couples with total motile sperm counts  $< 5$  million were excluded from analysis. Cycles using gonadotropins were excluded.

**RESULTS:** When considering the overall cohort, clinical pregnancy rates were comparable between patients who received CC and LTZ (18.1% CC vs 18.7% LTZ,  $p=0.580$ ). LTZ was associated with a decreased likelihood of multiple pregnancy compared to CC among those with a clinical IUP (20.3% CC vs 12.5% LTZ  $p=0.001$ ). Increasing doses of CC or LTZ were not associated with an increased chance of pregnancy or risk of multiple pregnancy.

In ovulatory women (11,449 cycles), LTZ use was associated a similar CPR (17.2% CC vs 15.5% LTZ,  $p=0.168$ ) and a similar multiple pregnancy rate (22.0% CC vs 19.2% LTZ,  $p=0.423$ ) when compared to CC. Increased CC dosing from 50 to 100 mg decreased the chance of clinical pregnancy (CC50 (18.2%) vs CC100 (16.3%),  $p=0.014$ ) while increasing the chance of multiple pregnancy (CC50 (19.8%) vs CC100 (25.7%),  $p=0.004$ ). Increased LTZ dosing above 2.5 mg did not increase the chance of IUP ( $p=0.354$ ) but an increase from 2.5 to 5 mg did increase the chance of multiple pregnancy (LTZ 2.5 (7.7%) vs LTZ5 (22.8%),  $p=0.040$ ).

In women with ovulatory dysfunction (4,004 cycles), LTZ was associated with a similar CPR compared to CC (22.8% LTZ vs 21.0% CC,  $p=0.271$ ) with a significantly decreased risk of multiple pregnancy (6.5% LTZ vs 15.6% CC,  $p=0.002$ ). Increasing dose was not associated with increased multiples for either CC or LTZ.

**CONCLUSIONS:** To maximize clinical pregnancy rates while minimizing the chance of multiples in CO-IUI cycles, medication and dose should be chosen carefully. LTZ vs. CC had similar pregnancy rates in the overall population. In ovulatory women, consideration should be given to starting CC at 50mg, as higher doses were associated with an increased risk of multiple

Ovarian stimulation	r-FSH (n=107)	r-FSH+m-LH (n=148)	P value
Total gonadotropin dose(IU)	1498+/-386	2408+/-701	<0.001
Total FSH dose(IU)	1498+/-386	2005+/-526	<0.001
Total LH dose(IU)	-	403+/-276	
Duration of stimulation(days)	8.5+/-1.1	9.1+/-1.3	<0.001
LH on day of antagonist(IU/L)	4.1+/-4.6	2.7+/-2.9	0.005
E2 on day of antagonist(pg/mL)	807.8+/-412.3	723.8+/-483.7	0.148
LH on day of trigger(IU/L)	3.1+/-2.5	2.1+/-1.7	<0.001
E2 on day of trigger(pg/mL)	2901.6+/-1516.9	2449.1+/-1406.0	0.017
No. of oocytes	18.2+/-8.0	17.4+/-8.5	0.464
No. of metaphase II	15.7+/-8.1	14.3+/-8.2	0.176
Blastocyst formation rate(%)	57.7+/-31.7	62.6+/-54.8	0.419
No. of transferred embryos	2.0+/-0.5	2.1+/-0.5	0.836
No. of cryopreserved embryos	6.2+/-3.8	6.4+/-3.9	0.761
Ovarian hyperstimulation(%)	10 (9)	12 (8)	0.730
Pregnancy rate per ET(%)	48/82(59)	65/96(68)	0.209
Live birth rate per ET(%)	27/82(33)	45/96(47)	0.715
Cumulative live birth rate(%)	63/107(59)	91/148(61)	0.585

gestation without improvement in CPR. Patients with ovulatory dysfunction may benefit from lower multiple pregnancies with LTZ utilization.

**P-293** Tuesday, October 15, 2019 6:30 AM

**DO INFERTILE PATIENTS WHO TEST POSITIVE FOR GROWTH DIFFERENTIATION FACTOR 9 (GDF9) POLYMORPHISM C447T EXHIBIT AN ALTERED RESPONSE TO CONTROLLED OVARIAN HYPERSTIMULATION (COH)?**



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**OBJECTIVE:** GDF9 is a protein coding gene responsible for promoting granulosa cell proliferation while inhibiting FSH-induced steroidogenesis [1]. GDF9 also potentiates the final stages of follicle growth and supports metabolic cascades such as sterol biosynthesis. Single nucleotide polymorphisms (SNPs) in GDF9 are associated with an increased risk for primary ovarian insufficiency and diminished ovarian reserve [2].Fertilome®, a multigene panel test, reports GDF9 SNPs as part of a multigene targeted sequencing panel and is often suggested in poor responding patients. We sought to evaluate ovarian stimulation outcomes in patients who tested positive for the GDF9 SNP C447T.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The study included patients at a single academic center who underwent COH and Fertilome® testing from 2016 to 2018. Cases included patients who screened positive for the GDF9 SNP C447T. Control cases included patients who screened negative. Patients testing positive for a Fragile X premutation or abnormal karyotype were excluded. Our primary outcome was number of oocytes retrieved. Secondary outcomes were number of metaphase II (MII) oocytes, number of fertilized oocytes, blastulation rate, and euploidy. Data were analyzed using student's t-test, with p<0.05 considered significant.

**RESULTS:** A total of 96 patients who underwent 214 COH cycles and Fertilome® testing were assessed in the study. A total of 80 patients (170 cycles) tested positive for the GDF9 SNP C447T, while 16 patients (44 cycles) tested negative for the GDF9 SNP. Although there was a difference in BMI between groups (23.59 vs 21.42, P = 0.0005), no differences in age or AMH were observed. We demonstrated no differences in the total number of oocytes retrieved or MII oocytes. Last, there was no difference in the fertilization, blastulation, or embryo euploidy between groups.

**CONCLUSIONS:** A majority of patients who experienced poor response to IVF stimulation tested positive for the GDF9 SNP C447T. However, the presence of the SNP did not affect oocyte retrieval count or MII maturation. Thus, although the GDF9 gene may be important in follicular development and maturation, detection of SNP C447T is not associated with worse outcomes during COH. Patients can be reassured that testing positive for the SNP C447T does not translate to impaired ovarian stimulation and oocyte retrieval outcomes.

	GDF SNP positive		GDF9 SNP negative		p-value
	Mean	SD	Mean	SD	
Oocytes retrieved	12.70	7.72	11.30	6.50	0.27
MII oocytes	9.27	5.65	8.18	5.30	0.25
Fertilized oocytes	6.86	5.23	5.72	5.03	0.20
Blastocysts	4.57	4.51	3.86	4.82	0.36
Blastulation rate (%)	60.82	31.49	55.88	38.3	0.38
Euploidy (%)	45.97	36.87	47.62	39.57	0.85

**REFERENCES**

- Allen, D.T., et al., *Growth Differentiation Factor 9 (GDF9) Stimulates Proliferation and Inhibits Steroidogenesis by Bovine Theca Cells: Influence of Follicle Size on Responses to GDF91*. Biology of Reproduction, 2008. 78(2): p. 243-253.
- Wang, T.-T., et al., *G546A polymorphism of growth differentiation factor-9 contributes to the poor outcome of ovarian stimulation in women with diminished ovarian reserve*. Fertility and Sterility, 2010. 94(6): p. 2490-2492.

**SUPPORT:** None.

**P-294** Tuesday, October 15, 2019 6:30 AM

**OOCYTE RECRUITMENT OF PATIENTS SUBMITTED TO THE NEW OVARIAN STIMULATION REGIMEN USING PROGESTIN TO BLOCK THE LH SURGE.**



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**OBJECTIVE:** To evaluate the recruitment and oocyte maturity of a new low cost and easy administration ovarian stimulation regimen, which uses progesterin as an alternative to the GnRH analogue.

**DESIGN:** Cross-sectional study.

**MATERIALS AND METHODS:** It was analyzed 100 patients who underwent Assisted Human Reproduction between June 2018 to January 2019. Of those patients, 50 used the progesterin protocol as an alternative to the GnRH analogue, to suppress the premature LH surge during the follicular phase. The other 50 patients used the standard protocol with antagonist. The total number of oocytes retrieved and the classification for maturity and viability were analyzed between the groups. Variables such as age and body mass index (BMI) were considered as well. The qualitative variables were presented by absolute and relative frequency and the quantitative variables by means of a 95% confidence interval, using a normality test of the Shapiro-Wilk data (p <0.05). The Mann-Whitney test and Chi-square test were used to compare the variables according to the two induction protocols. The Chi-square test was used for the comparative analysis of the BMI. For all analyzes, the level of significance was p <0.05. The statistical program used was Stata version 11.0.

**RESULTS:** No statistically significant results were found in relation to the number of oocytes retrieved in the conventional ovarian stimulation cycles with antagonist compared to the cycles using progesterin to block the LH surge (283 versus 247, p = 0.54). Similarly, there was no difference in the degree of oocyte maturation (mature 79.72% / 77.43%, immature 13.52% / 15.68%), altered, degenerated or oocytes with ruptured zona pellucida (2.54% / 2.19%, 1.13% / 1.88%, 3.10% / 2.82%, p = 0.88). The body mass index (BMI) was also evaluated without significant differences after analysis (p = 0.07). When separated by age (up to 37 years and ≥ 38 years), the groups also did not present statistically significant differences in any of the analyzed variables.

**CONCLUSIONS:** The use of progesterin in the induction protocols to block the LH surge seems to be an option in the substitution of GnRH analogues, since it presented similar results, more accessible cost and a route of administration more comfortable for the patients.

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**COMPARISON OF TRADITIONAL AND STEP UP PROTOCOLS WITH LETROZOLE.**



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**OBJECTIVE:** To compare time to ovulation between traditional and step up protocols with letrozole.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** Patients were identified through Duke Fertility Center's Intrauterine Insemination database, which stores information about each ovulation induction cycle including patient's age, body mass index (BMI), last menstrual period, trigger date, and outcomes. The electronic medical record was used to obtain missing data points. Patients receiving ovulation induction with letrozole for ovulatory dysfunction between January 1, 2010 and March 3, 2018 were included. Patients were excluded if they received gonadotropins or if they switched to a different ovulation induction agent. In the traditional protocol, patients had an increase in letrozole dose following spontaneous menstruation or medroxyprogesterone-induced withdrawal bleed if there were no follicles at 16mm or greater on ultrasound by cycle day 20. Patients were excluded if they delayed starting a cycle with the increased dose. In the step up protocol, patients had an immediate increase in letrozole dose by cycle day 20 at the latest if no developing follicle was detected. A separate cohort of those who underwent a step up protocol with clomiphene was also included for comparison. The primary outcome was time to ovulation, defined as the number of days between the last menstrual period and the detection of a follicle at 16mm or greater on ultrasound. A secondary outcome was clinical pregnancy. Student's t-test or Wilcoxon rank sum test were used to compare variables. Statistical analyses were conducted using R version 3.5.1 (Vienna, Austria).

**RESULTS:** 49 patients were included: 21 in the traditional letrozole cohort, 15 in the step up letrozole cohort, and 13 in the step up clomiphene cohort. All cycles with traditional protocols occurred before January 2014 while step up protocols occurred after. The median age and IQR of patients in the traditional letrozole protocol cohort was 33 (31-35) vs. 28 (27-30) in the step up letrozole protocol, and the median BMI was 29 (25-36) vs. 34 (27-39). The time to ovulation was twice as long at 50.6 days for patients who underwent traditional letrozole protocol compared to the 21.9 days for those who underwent the step up letrozole protocol ( $p < 0.0001$ ). There was no difference in clinical pregnancy rate per cycle (10% [2/20] vs. 0% [0/14],  $p = 0.16$ ). In comparing letrozole step up cycles with clomiphene step up cycles, there was no difference in time to ovulation (21.9 vs. 21.1 days,  $p = 0.47$ ) or clinical pregnancy rate per cycle (0% [0/14] vs. 16.7% [2/12],  $p = 0.17$ ). No significant side effects were reported in any group.

**CONCLUSIONS:** The step up letrozole protocol allows for faster time to ovulation in initially nonresponsive patients with ovulatory dysfunction. Similar to findings seen with clomiphene, it is not essential to have menstruation prior to increasing letrozole doses for ovulation induction.

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**CORIFOLLITROPIN ALFA IN THE ULTRASHORT GONADOTROPIN-RELEASING HORMONE AGONIST (GNRHA) PROTOCOL: A NOVEL PATIENT-FRIENDLY ALTERNATIVE.**

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**OBJECTIVE:** To compare the outcomes of in vitro fertilization (IVF) and fresh embryo transfer (ET) using corifollitropin alfa in ultrashort GnRHa protocol and GnRH antagonist protocol.

**DESIGN:** A retrospective observational analysis conducted in a university-affiliated infertility center.

**MATERIALS AND METHODS:** A total of 245 unselected patients undergoing IVF/fresh ET were enrolled between January 1 and December 31, 2017, including 135 treated with ultrashort GnRHa protocol and 110 treated with antagonist protocol. The primary outcomes were the duration of stimulation, dosage of additional gonadotropin for ovarian hyper stimulation, number of total injections and outpatient department (OPD) visits before ovulation triggering, ovarian response, and ovarian hyper stimulation syndrome (OHSS) rate. The secondary outcomes were rates of pregnancy, clinical pregnancy, and live birth.

**RESULTS:** Patients treated with ultrashort GnRHa required lesser additional gonadotropin, fewer total injections, but had better ovarian responses, including more oocytes retrieved, more metaphase II oocytes, and more blastocysts than those treated with antagonist did. A premature LH surge occurred only in six patients treated with antagonist protocol. The OHSS rate was similar in the two groups. The rates of pregnancy (37.0% vs. 43.6%), clinical pregnancy (25.2% vs. 34.6%), and live birth (19.3% vs. 30.0%) did not differ significantly between the two groups.

**CONCLUSIONS:** In unselected patients using corifollitropin alfa, the ultrashort GnRHa protocol needed low dose of additional gonadotropin and fewer injections but produced similar pregnancy outcomes than antagonist protocol did, suggesting that the ultrashort GnRHa protocol could be an alternative.

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**FLEXIBLE VERSUS FIXED GONADOTROPIN RELEASING HORMONE ANTAGONIST (GNRH-ANT) STARTING DAY DURING CONTROLLED OVARIAN HYPERSTIMULATION FOR IN VITRO FERTILIZATION (IVF): A SYSTEMATIC REVIEW & META-ANALYSIS.**



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**OBJECTIVE:** To obtain the up-to-date evidence on fertility outcomes when comparing flexible and fixed start gonadotropin releasing hormone antagonist (GnRH-ant) protocols during controlled ovarian hyperstimulation for In Vitro Fertilization (IVF).

**DESIGN:** This study is a systematic review and meta-analysis of published randomized controlled trials (RCT). A systematic search of the literature, using keywords GnRH antagonist, fixed, flexible, pregnancy, and live birth, was performed across the Cochrane Library, EMBASE, and MEDLINE databases from 1996 to January 2019.

**MATERIALS AND METHODS:** Studies were selected for inclusion in the systematic review and meta-analysis if they were 1) RCTs, 2) that compared flexible versus fixed start GnRH antagonist protocols, 3) reported IVF outcomes, 4) on patients who were normo-responders. Data involving patient characteristics, IVF protocols, and fertility outcomes were extracted independently by two reviewers. Collected variables include IVF protocol used; total gonadotropin dosage; median estradiol level on day of GnRH-ant start; number of oocytes retrieved; fertilization rate; number of good quality embryos; clinical pregnancy rates; premature LH rises and cycle cancellation. Study quality assessment was performed using the Cochrane Collaboration's tool for assessing risk of bias in randomized trials.

**RESULTS:** Six hundred and thirty-eight articles were identified through database searches and five full text RCTs (701 IVF cycles) were included in our analysis. There is no statistically significant difference in clinical pregnancy rates between flexible and fixed GnRH-ant protocols (OR = 0.74, 95% CI = 0.53-1.03,  $p = 0.07$ ) with a trend towards higher clinical pregnancy rate in the fixed GnRH-ant protocol. There is no significant difference in total oocytes retrieved between the flexible and fixed GnRH-ant protocols (Pooled mean difference = 1.02, 95% CI = -0.09-2.12,  $p = 0.07$ ). There is a trend towards lower total gonadotropin dosage used in the flexible GnRH antagonist protocol (Pooled mean difference = -124.18, 95% CI = -325.36-76.99,  $p = 0.23$ ); however, the difference is not statistically significant. There is no difference in the incidence of premature LH surge between the two protocols (OR = 1.11, 95% CI = 0.56-2.18,  $p = 0.76$ ).

**CONCLUSIONS:** There is insufficient evidence to demonstrate whether flexible and fixed GnRH-ant protocols yield different IVF outcomes.

	Ultrashort (n= 135)	Antagonist (n= 110)	P value
Age (years)	37.04 ± 4.28	36.56 ± 4.26	0.382
BMI (kg/m <sup>2</sup> )	21.69 ± 2.95	22.24 ± 3.55	0.189
AMH (ng/mL)	2.58 ± 1.92	2.19 ± 2.21	0.142
Stimulation days	10.30 ± 1.52	8.88 ± 1.30	<0.001***
No. of OPD visits before triggering	2.04 ± 0.74	1.70 ± 0.64	<0.001***
Additional FSH dosage (IU)	637.78 ± 537.63	802.95 ± 435.97	0.010*
No. of shots before triggering	6.63 ± 1.88	8.62 ± 2.81	<0.001***
LH (mIU/mL) before triggering	1.12 ± 0.77	4.12 ± 6.81	<0.001***
Premature LH surge rate	0% (0)	5.45% (6)	0.006**
Max. E <sub>2</sub> level (pg/mL)	2333.87 ± 1441.06	1903.43 ± 1028.72	0.009**
No. of oocytes retrieved	12.52 ± 8.12	9.40 ± 7.03	0.002**
MII rate (%)	73.38 ± 20.45	68.40 ± 15.01	0.642
Fertilization rate (%)	71.99 ± 19.62	71.41 ± 20.69	0.821
Blastocyst rate (%)	33.81 ± 31.96	21.05 ± 31.26	0.002**
Pregnancy rate (%)	37.04	43.64	0.294
Clinical pregnancy rate (%)	25.19	34.55	0.110
Live birth rate (%)	19.26	30.00	0.051
Cumulative pregnancy rate (%)	47.24	51.38	0.527
OHSS rate (%)	1.48	0	0.200

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**IN WOMEN WITH POOR OVARIAN RESPONSE, INTRAOVARIAN INJECTION OF AUTOLOGOUS PLATELET RICH PLASMA IMPROVES OVARIAN RESERVE AND IVF OUTCOME PARAMETERS.**



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**OBJECTIVE:** Poor ovarian response (POR) is an increasingly common indication for IVF, accounting for 31% of cycles in the USA in 2016, compared to 12% in 2005. These patients are especially challenging as POR results in higher rate of cycle cancellation, lower number of embryos available for transfer, and overall lower pregnancy rates. Autologous platelet-rich plasma (PRP) is rich in growth factors and cytokines and has been used as an agent that induces tissue regeneration. PRP also promotes follicle development in vitro and two studies reported a total of 7 cases of POR, where PRP was utilized. The aim of the current study was to investigate whether intraovarian injection of autologous PRP is associated with improved ovarian reserve and IVF outcomes in patients with POR.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Reproductive age women diagnosed with POR based on Poseidon criteria and with a history of at least one prior failed IVF cycle were recruited for the study between December 15, 2018, and April 15, 2019. Antral follicle count (AFC), serum anti-mullerian hormone (AMH), and early follicular phase serum follicle stimulating hormone (FSH) levels were determined at baseline. Autologous blood obtained from peripheral vein was used to prepare PRP following standard protocols. PRP injection was performed under sedation anesthesia, using a 35 cm 17 G needle under transvaginal ultrasound guidance. On the 2-4th days of the first three menstrual cycles following the procedure, AFC, AMH, and FSH levels were re-assessed. Patients with at least one antral follicle were started on ovarian stimulation for IVF-ICSI, followed by embryo banking at cleavage stage for PGT-A. Markers of ovarian reserve (AFC, FSH, AMH) and IVF outcome parameters (number of MII oocytes, 2PN and cleavage stage embryos) were followed and compared to previous cycle.

**RESULTS:** At the time of this submission, a total of 152 patients (mean age  $\pm$  SD: 39.3  $\pm$  5.6) with the diagnosis of POR were included in the study. PRP treatment resulted in higher AFC (6.2  $\pm$  2.8 vs 2.6  $\pm$  1.6;  $p < 0.01$ ) and AMH (0.54  $\pm$  0.30 vs 0.41  $\pm$  0.28;  $p = 0.01$ , respectively), and lower FSH (17.5  $\pm$  4.7 vs 20.3  $\pm$  5.4;  $p < 0.01$ ) levels. Number of MII oocytes, 2PN and cleavage stage embryos were also increased following the PRP procedure (4.2  $\pm$  2.9 vs 2.5  $\pm$  1.9; 3.8  $\pm$  2.6 vs 2.2  $\pm$  1.7; 3.4  $\pm$  1.8 vs 2.0  $\pm$  1.6, respectively;  $p < 0.01$  for all). In 19 patients (12.5%), no changes were observed in AFC after the PRP procedure. Another 43 patients (25.3%) failed IVF due to stimulation failure, fertilization failure, or arrested embryo development. In 87 patients (60.3%), at least one cleavage embryo was obtained and embryo banking was achieved. In addition, three patients (1.9%) had spontaneous pregnancies in the first or second cycle after the PRP procedure that are ongoing.

**CONCLUSIONS:** Intraovarian injection of autologous PRP might be an alternative experimental treatment option for women with poor ovarian response to stimulation. Whether this treatment is clinically effective will need to be further investigated using a prospective randomized clinical trial design.

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**PREVENTION OF PREMATURE LUTEINIZING HORMONE SURGE IN POOR RESPONDERS WITH TWICE-DAILY GnRH-ANTAGONIST ADMINISTRATION.**



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**OBJECTIVE:** A premature luteinizing hormone (LH) surge often complicates in vitro fertilization (IVF) cycles in poor responders. We investigate the

feasibility of twice-daily GnRH-antagonist (GnRH-ant) administration in poor responders with a history of premature LH surge in a prior IVF cycle.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All patients undergoing fresh IVF with GnRH-ant based protocols were considered for inclusion. Patients were classified as poor responders according to the Bologna criteria. Of this cohort, patients with and without a premature LH surge (threshold  $> 17$  mIU/mL and  $> 2.5$ -fold rise from baseline) were identified. Demographics, baseline IVF characteristics and ovarian stimulation parameters of these groups were compared. Patients with a history of premature LH surge undergoing a subsequent IVF cycle were then treated with twice-daily GnRH-ant prospectively. In addition to any repeat events of premature LH surge, other parameters recorded included days of stimulation, total dosage of gonadotropins (IU), number of mature oocytes retrieved, fertilization rate (%), and number of embryos transferred. Pregnancy outcomes following embryo transfer (ET) were also noted.

**RESULTS:** 10,945 patients undergoing IVF with GnRH-ant based protocols were identified, of which 4,265 (38.9%) patients, with age 41 (40-43) years, met poor responder criteria. A premature LH surge was noted in 36/4,265 (0.84%) patients. These patients had higher basal follicle stimulating hormone levels [12.1 (8.61-14.8) mIU/mL vs. 10.4 (6.75-12.8) mIU/mL;  $P < 0.001$ ] and higher prevalence of combined clomiphene citrate and gonadotropin-based protocols (36.1% vs. 10.5%;  $P < 0.001$ ) compared to those without a premature LH surge. Thirty-four of the 36 patients with a premature LH surge were then treated prospectively with twice-daily GnRH-ant. No repeat events of premature LH surge were noted. Following 14 (11-16) days of ovarian stimulation with 4,890 (4,150-6,325) IU of gonadotropins, 5 (4-7) mature oocytes were retrieved, with a fertilization rate of 81.2%. With ET of 4 (3-5) embryos, the clinical pregnancy and live birth rates were 4/34 (11.8%) and 3/34 (8.82%), respectively.

**CONCLUSIONS:** Our findings suggest that poor responders undergoing IVF with combined clomiphene citrate and gonadotropin-based GnRH-ant protocols have a higher rate of premature LH surge. Twice-daily GnRH-ant administration is a feasible strategy in such patients, or any poor responder with a history of premature LH surge. Reasonable pregnancy rates are also noted following oocyte retrieval and ET in such patients.

**SUPPORT:** None.

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**TWELVE PREGNANCIES AFTER SURGICAL ACTIVATION OF FOLLICLES IN POOR RESPONDER PATIENTS.**



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**OBJECTIVE:** The success of IVF treatment in poor responder (POR) patients is low due to decreases in the number of retrieved oocytes. Recent studies demonstrated that the disruption of Hippo signaling in ovarian follicles by surgical fragmentation of ovaries induced growth of secondary follicles in mice and human. The aim of this study is to improve the clinical outcome of IVF treatment in POR patients through induction of secondary follicle growth to increase viable embryos by surgical activation.

**DESIGN:** A prospective non-randomized control study using historical controls

**MATERIALS AND METHODS:** Under ethical approval (Clinical trial registration# UMIN000028031), 66 patients who received written informed consent and met the Bologna criteria for POR were enrolled from May 2016 to October 2018. We dissected partial ovarian cortices from one side of ovary under laparoscopic surgery. The ovarian cortices were fragmented into 1-2 mm cubes followed by auto-transplantation beneath the serosa of Fallopian tubes and between cortex and medulla in ovaries. After the surgery, patients received ovarian stimulation under short protocol while maintaining normal LH levels ( $< 10$  mIU/ml) until oocyte retrieval. The primary endpoint is increase the number of growing follicles after the surgery under trans-vaginal ultrasound monitoring, whereas we evaluate the number of retrieved oocytes, fertilization rate, cleavage rate, clinical pregnancy rate, and miscarriage rate as secondary endpoints.

**RESULTS:** The median age of enrolled patients was 43.0 [36-45]. Clinical outcome was shown in Table 1.

TABLE 1. Clinical outcome of laparoscopic ovarian surgical activation

Patients (n=66)	Growing follicle number within four months until operation* (mean +SD)	Number of retrieved oocytes (mean +SD)	Fertilization rate (% , n)	D3 8cell stage rate (% , n)	Clinical pregnancy rate (% , n)	Miscarriage rate (%)
Pre-op (203 cycles)	1.34±1.03 <sup>a</sup>	1.13±0.98 <sup>b</sup>	57.7 (79/137)	25.6 <sup>c</sup> (33/129)	0 <sup>d</sup> (0/23)	-
Post-op (216 cycles)	2.81±2.02 <sup>a</sup>	1.53±1.39 <sup>b</sup>	68.2 (163/239)	40.6 <sup>c</sup> (95/234)	17.1 <sup>d</sup> (12/70)	58.3% (7/12)
					Ongoing : 1	
					Birth : 4(twins:1)	

(a-a',b-b': p<0.05, t-test, c-c',d-d': p<0.05, Chi-squared test)  
\* including cycles without operation due to small number

**CONCLUSIONS:** Our procedure significantly increased the number of growing follicles with increase in viable embryos, resulting in twelve successful clinical pregnancies and five babies. Also, it might improve embryo quality based on disruption of Hippo signaling pathway.

**P-301** Tuesday, October 15, 2019 6:30 AM

**APPLICATION OF CONTROLLED OVARIAN HYPERSTIMULATION WITH AGONIST-ANTAGONIST PROTOCOL IN POSEIDON GROUP 3 AND GROUP 4 PATIENTS WITH DIMINISHED OVARIAN RESERVE.**



Rui Yang, Doctor,<sup>a</sup> Xiaoguo Du, Master,<sup>a</sup> Lixue Chen, M.D,<sup>b</sup> Xinna Chen, Professor.<sup>a</sup> <sup>a</sup>Peking University Third Hospital, Beijing, China; <sup>b</sup>Affiliation not provided.

**OBJECTIVE:** By comparing standard antagonist regimen and agonist-antagonist protocol (AAP regimen), a combination of a microdose flare-up GnRH agonist with a GnRH antagonist in POSEIDON group 3 and group 4 patients with diminished ovarian reserve, this article aims to study if AAP regimen could improve the clinical outcomes in low prognosis patients.

**DESIGN:** This is a retrospective study.

**MATERIALS AND METHODS:** The clinical data of 646 cycles of prospective poor ovarian response POR patients (POSEIDON group 3 and 4) who received in vitro fertilization and embryo transfer (IVF-ET) in Peking University Third Hospital reproductive medical center from January 2016 to May 2018 were retrospectively analyzed. The total number of APP cycle was 323, and the control group was selected from the database with 1:1 matching of prospective low prognosis patients (POSEIDON group 3 and group 4) with similar age and approaching date of oocyte retrieval. Patients' general information, ovarian hyperstimulation indicators and clinical outcomes were studied.

**RESULTS:** AAP group had fewer antral follicle count (3.04±2.05 vs. 3.84±2.17, p<0.05) and similar AMH level (0.62±0.64 and 0.63±0.49, p>0.05) compared with control group. AAP group had shorter (9.84±2.59 vs. 10.31±2.23, p=0.015) and lower dosage (2754.18±973.37 vs. 3246.7±1044.20, p<0.05) of Gn using, and had similar number of oocytes obtained compared with control group (4.06±2.89 vs. 4.16±2.65, p=0.649). Under the same proportion of fertilization schemes (routine or ICSI methods), AAP group had higher fertilization rate (74.1% vs. 69.1%, p=0.004) and good quality embryo rate (62.6% vs. 56.9%, p=0.014), and ultimately had higher embryo implantation rate (22.3% vs. 15.8%, p=0.020) and cumulative clinical pregnancy rate (32.5% vs. 22.9%, p=0.018).

**CONCLUSIONS:** For POSEIDON patients with low prognosis and poor ovarian reserve, controlled ovarian hyperstimulation with agonist-antagonist protocol had better clinical outcomes compared with conventional antagonist regimen.

**SUPPORT:** None.

**P-302** Tuesday, October 15, 2019 6:30 AM

**CONVENTIONAL PROTOCOL VERSUS MINIMAL OVARIAN STIMULATION IN PATIENTS WITH POOR REPRODUCTIVE PROGNOSIS ACCORDING TO POSEIDON CRITERIA.**



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**OBJECTIVE:** to analyze whether minimal ovarian stimulation (MOS) is as effective as conventional controlled ovarian stimulation (COS) for patients belonging to different groups according to the Poseidon criteria.

**DESIGN:** Observational retrospective multicenter cohort.

**MATERIALS AND METHODS:** Considering that advanced reproductive age is the main factor affecting reproductive outcomes, we evaluated women from the Poseidon's group 2 and 4 (1,2) undergoing in vitro fertilization (IVF) with either MOS or conventional COS between January 2014 and October 2018. Exclusion criteria were as follows: irregular menstrual cycles, oocyte donation, severe male factor, and contraindication to pregnancy or COS. While patients from the conventional stimulation group received a GnRH antagonist protocol using high doses of gonadotropins, the MOS group received usual doses of clomiphene citrate along with low doses of gonadotropins, as previously described (3). The continuous variables were reported as mean ± standard deviation and compared using the t-Student test. Fisher's Exact test and Odds Ratio were used in order to compare reproductive outcomes, as appropriate. Statistical significance was set at p<0.05.

**RESULTS:** A total of 2,944 patients underwent 4,450 embryo transfers (MOS = 737 and COS = 3,713). Baseline characteristics of patients were similar between groups. While comparing MOS vs. conventional COS in Poseidon's group 2, there were no significant differences in biochemical pregnancy (OR 1.27, CI 0.96-1.66; p= 0.079), clinical pregnancy (OR 1.24, CI 0.94-1.63; p= 0.123), ongoing pregnancy (OR 1.1, CI 0.83-1.46; p= 0.531), and live birth rates (OR 0.98, CI 0.73-1.33; p = 0.941). Similarly, no differences were found regarding Poseidon's group 4, as shown by comparable biochemical pregnancy (OR 1.2, CI 0.98-1.48; p= 0.079), clinical pregnancy (OR 1.19, CI 0.96-1.48; p= 0.094), ongoing pregnancy (OR 1.2, CI 0.96-1.52; p= 0.117), and live birth rates (OR 1.14, CI 0.9-1.45; p=0.292). Although the number of embryos obtained was statistically higher for patients receiving conventional COS in Poseidon's group 4, also number of embryos not viable was higher.

**CONCLUSIONS:** MOS is a good alternative when conventional COS has failed or even as a first-line treatment for patients belonging to the Poseidon groups 2 and 4. Randomized controlled trials are needed before incorporating this strategy in daily clinical practice. Future studies should investigate potential benefits of minimal and mild-stimulation protocols, such as improved neonatal outcomes and lower maternal complication rates.

**REFERENCES**

1. Poseidon Group (Patient-Oriented Strategies Encompassing Individualized Oocyte Number), Alviggi C, Andersen CY, Buehler K, Conforti A, De Placido G, *et al.* A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. *Fertil Steril* 2016;105:1452-3.
2. Esteves SC, Roque M, Bedoschi GM, Conforti A, Humaidan P, Alviggi C. Defining Low Prognosis Patients Undergoing Assisted Reproductive Technology: POSEIDON Criteria-The Why. *Front Endocrinol (Lausanne)* 2018;9:461.
3. Labarta E, Marin D, Remohi J, Bosch E. Conventional versus minimal ovarian stimulation: an intra-patient comparison of ovarian response in poor-responder women according to Bologna Criteria. *Reprod Biomed Online* 2018;37:434-41.

**SUPPORT:** No financial support

**P-303** Tuesday, October 15, 2019 6:30 AM

**THE EFFECTIVENESS OF TRANSDERMAL TESTOSTERONE GEL 1% (ANDROGEL) FOR POOR RESPONDERS UNDERGOING IN VITRO**



**FERTILIZATION.** Anjali Chaudhary, MD DNB. CONSULTANT AAROGYA HOSPITAL, Delhi, India.

**OBJECTIVE:** To investigate the effectiveness of treatment with transdermal testosterone gel(TTG) 1%(androgel) before ovarian stimulation (COS) using GnRH antagonist in low responders undergoing IVF/intracytoplasmic sperm injection (ICCSI).

DESIGN: prospective randomized controlled trial.

**MATERIALS AND METHODS:** A total of 60 low responder, who were defined as patient who failed to produce <3 follicles with a mean diameter of < 16 mm with the result that <3 oocytes were retrieved despite the use of a high gonadotropin dose in a previous failed IVF/ICSI cycle from 1.1.18 to 31.3.19 (15 months). Patient were randomized into TTG pretreatment group and control group. For TTG pretreatment group, 12.5mg TTG were applied daily for 21 days in the cycle preceding COS for IVF.

**RESULTS:** There were no differences in patients characteristics between the two group. Total dose of FSH used were significantly fewer in the TTG pretreatment group than in the control group. The number of oocytes retrieved, mature oocytes, fertilized oocytes, and good quality embryos were significantly higher in the TTG pretreatment group. Embryos implantation rate and clinical pregnancy rate per cycle also were significantly higher in the women pretreated with TTG. No patient reported adverse effects attributed to TTG use.

**CONCLUSIONS:** TTG pretreatment might be beneficial in improving both response to COS and IVF outcome in low responders undergoing IVF/ICSI (fertil steril 2011;95:679-83. 2011 by American society for reproductive medicine).

**P-304** Tuesday, October 15, 2019 6:30 AM

**EFFECTS OF DIFFERENT PRETREATMENTS PRIOR TO GnRH ULTRASHORT AGONIST COMBINED WITH ANTAGONIST REGIMEN ON THE CLINICAL OUTCOMES OF EXPECTED POR**



**PATIENTS.** Xiaoguo Du, Master, Rui Yang, Doctor, Xinna Chen, Professor. Peking University Third Hospital, Beijing, China.

**OBJECTIVE:** To analyze the effect of different pretreatments on the IVF outcome of expected POR patients according to POSEIDON criteria, and to explore the appropriate treatment for POR patients.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** Analysis of the clinical data of 364 cycles of expected POR patients who received IVF-ET in the Reproductive Medicine Center of the Peking University Third Hospital from January 2016 to May 2018. According to the pretreatment prior to IVF, the cycles were divided into oral contraceptive (OCP) group (A group, N = 167), estradiol valvate group (B group, N = 56) and no pretreatment group (C group, N = 141). The clinical data, ovarian stimulation indexes, laboratory status and clinical pregnancy rate were compared among the three groups.

**RESULTS:** The age of group A [(34.8±4.9) years old] was significantly younger than that of group B [(38.0±4.9) years old] and group C [(37.9±4.7) years old] (P=0.000). The BMI of group B [(21.9 ±3.1) kg/m<sup>2</sup>] was significantly lower than that of group A [(23.5 ±3.6) kg/m<sup>2</sup>] and group C [(23.2 ±3.1) kg/m<sup>2</sup>] (P=0.014). There were significant differences in the number of antral follicles (AFC) among the group A (2.4±2.0), group B (4.1±1.9), and group C (3.5±2.0) (P=0.000). The proportion of anovulation in group A (32.9%) was significantly higher than that in group B (10.7%) and C (11.3%) (P=0.000). The number of IVF cycles in group C (3.0 ± 1.7) was higher than that in group A (2.5 ± 1.5) (P=0.017). There was no significant difference in type of infertility, duration of infertility, basal FSH and AMH among the three groups (P > 0.05). The endometrial thickness on HCG in group A [(9.4 ±1.9) mm] was thinner than that in group B [(10.6 ±1.5) mm] and group C [(10.1 ±2.0) mm] (P=0.000). The fertilization rate of group A (77.1%) and group B (77.6%) was significantly higher than that of group C (71.3%) (P=0.041). There were no significant differences in Gn dosage, days

of stimulation, number of eggs obtained, ICSI proportion, cleavage rate, number of embryos available for transfer and number of good quality embryos among the three groups (P > 0.05). The implantation rate and clinical pregnancy rate in group A (26.2%, 36.1%) and group B (26.8%, 42.0%) were significantly higher than those in group C (14.5%, 21.2%) (P=0.014, P=0.014). There were no differences in embryo transfer cycle, number of embryos transferred, proportion of blastocyst transfer cycle, abortion rate and cycle cancellation rate among the three groups (P > 0.05).

**CONCLUSIONS:** Pretreatment with OCP or estradiol valerate in luteal phase prior to GnRH ultrashort agonist combined with antagonist regimen can improve the clinical outcomes of patients with expected POR. Estradiol valerate pretreatment in luteal phase seems to be more effective.

**PREIMPLANTATION GENETIC TESTING**

**P-305** Tuesday, October 15, 2019 6:30 AM

**IMPACT OF PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) ON GESTATIONAL CARRIER (GC) CYCLES IN THE UNITED STATES.**



**STATES.** Reeva B. Makhijani, MD,<sup>a</sup> Madeline Coulter, BA,<sup>a</sup> Jeffrey Thorne, MD,<sup>a</sup> Chantal Bartels, MD,<sup>b</sup> John Nulsen, MD,<sup>a</sup> Lawrence Engmann, MD,<sup>a</sup> Claudio Benadiva, MD,<sup>a</sup> Grow R. Daniel, MD.<sup>a</sup> <sup>a</sup>Center for Assisted Reproductive Services, University of Connecticut School of Medicine, Farmington, CT; <sup>b</sup>Center for Advanced Reproductive Services, University of Connecticut School of Medicine, Farmington, CT.

**OBJECTIVE:** We analyzed the SART registry to determine the impact of PGT-A on GC in vitro fertilization (IVF) cycles in the United States.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** SART data was analyzed from 4,470 autologous IVF cycles that used a GC between 2014-2016. Cycles were excluded if donor oocytes were used, multicellular embryo(s) was transferred or embryo transfer was not attempted. The cycles were separated into 4 groups determined by use of PGT-A and number of embryos transferred as follows: (A) PGT and single embryo transfer (SET); (B) PGT and multiple embryo transfer (MET); (C) no PGT and SET (D) no PGT and MET. The primary outcome was live birth rate (LBR). Secondary outcomes were clinical pregnancy rate (CPR), clinical loss rate (CLR) and multiple pregnancy rate (MPR). One-way ANOVA or Student's t test and X<sup>2</sup> tests were used to compare continuous and categorical variables, respectively. Multivariate logistical regression was done to control for potential confounders. A p-value of 0.05 was considered statistically significant.

**RESULTS:** Groups significantly differed in terms of intended parent (IP) age, GC age, IP BMI, smoking status and parity. In MET groups, significantly fewer embryos were transferred when PGT was used (Group B: 2.0 ± 0.2 v. Group D: 2.1 ± 0.4, p<0.01). When comparing groups by number of embryos transferred (A to C, B to D), LBR and CPR were significantly higher with PGT. MPR was significantly lower with SET. After controlling for potential confounders, a significant difference in LBR remained among groups (p<0.01). Of potential confounders, only IP age was significantly predictive of live birth (OR 0.9574, 95% CI 0.9453 - 0.9697, p<0.01).

**CONCLUSIONS:** This study shows that euploid SET does not compromise LBR and significantly reduces MPR. It highlights an opportunity to increase GC safety as well as widen access to this already restricted service.

**SUPPORT:** None.

Baseline Characteristics	A: PGT SET (n=1037)	B: PGT MET (n=422)	C: No PGT SET (n=1481)	D: No PGT MET (n=1530)	p value
Mean IP age (years)	36.8 ± 4.2	36.2 ± 4.3	35.4 ± 4.9	36.2 ± 5.0	<0.01
Mean GC age (years)	32.1 ± 4.9	31.2 ± 4.9	32.5 ± 5.6	32.3 ± 5.5	<0.01
Mean IP BMI (kg/m <sup>2</sup> )	23.7 ± 5.2	24.0 ± 5.0	24.2 ± 5.6	24.9 ± 6.1	<0.01
Smoker (% , n)	39.7% (412/1037)	31.3% (132/422)	21.1% (313/1481)	13.2% (202/1530)	<0.01
Nulliparity (% , n)	39.4% (283/719)	55.1% (157/285)	38.4% (291/757)	43.5% (380/873)	<0.01
<b>Pregnancy Outcomes</b>					
CPR (% , n)	63.2% (655/1037)	74.9% (316/422)	53.1% (787/1481)	61.0% (933/1530)	<0.01
LBR (% , n)	51.2% (531/1037)	64.2% (271/422)	46.8% (693/1481)	49.5% (757/1530)	<0.01
MPR (% , n)	1.4 % (9/655)	43.4% (137/316)	1.1% (9/787)	29.9% (279/933)	<0.01
CLR (% , n)	18.9% (124/655)	14.2% (45/316)	18.0% (142/787)	18.9% (176/933)	0.28

**NGS EUPLOID EMBRYOS HAVE HIGHER DELIVERY RATES THAN THOSE DIAGNOSED AS EUPLOID BY ACGH/SNP.**

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Patty Ann Labella, BS, Melicia Clarke-Williams, BA,  
Mary Elizabeth Fino, MD, James A. Grifo, MD, PhD. NYU Langone Fertility Center, New York, NY.



**OBJECTIVE:** To review outcomes of all STEET procedures based on PGT-A platform used to determine Ploidy status.

**DESIGN:** Retrospective review of all STEET procedures over an 8 year period at a single center.

**MATERIALS AND METHODS:** More than 3200 STEET procedures performed over an 8 year period (2011 to 2018) at a single center were reviewed based on the PGT-A platform (NGS, aCGH or SNP) utilized. Our main outcome measures were: Implantation Rate (IR), Clinical Preg rate (FH) and Live Birth (LB) rate. Only embryos reported as euploid were included in the analysis- embryos reported as mosaic or those not yielding a result were omitted. Statistical significance was determined using contingency X<sup>2</sup> with 1 degree of freedom.

**RESULTS:** TABLE 1. Comparison of STEET outcomes depending on PGT-A Platform

	NGS <sup>1</sup>	aCGH + SNP <sup>2</sup>	Significance
Average age at Freeze	36.60±4.34	36.54± 4.60	NS
Implantation rate (sacs/embryo)	70.1% (1330/1897)	62.4% (858/1375)	P < 0.00001
Clinical Preg rate (FH/embryo)	66.7% (266/1897)	55.9%(768/1375)	P < 0.00001
SAB/ Clin Preg	10.3% (87/845)	12.6% (97/770)	NS
Live Births <sup>3</sup> (Live born/embryo)	61.7% (750/1216)	53.2%(657/1235)	P = 0.000022

<sup>1</sup> Only included FETs of embryos with NGS performed in the Fresh IVF cycle.

<sup>2</sup> SNP cases were included with aCGH due to low number

<sup>3</sup> Live Birth rate calculated through 2017 only (results for 2018 cycles pending)

STEET following PGT-A via NGS resulted in a significantly higher IR compared to aCGH /SNP combined (70.1% vs 62.4%). Similarly, ongoing Pregnancy rates and LB rates were significantly improved when NGS was utilized vs aCGH or SNP. SAB rates were not significantly different between platforms but all methods reduced SAB rates compared to age matched controls without PGS (18% ) (Ref 1).

**CONCLUSIONS:** STEET results in high IR, high clinical pregnancy rates and high LB rates across all age groups. However, with advances in PGT-A platforms we can continue to improve outcomes and increase safety of ART by maximizing the potential of every ET procedure. With continuing development of PGT-A platforms and interpretation methods used to determine ploidy we can further improve outcomes and safety by transferring a single embryo with the highest implantation potential every time.

**REFERENCE**

Harton et al (2013) Fert & Ster Vol. 100 (6) 1695-1703.

SUPPORT: None.

**PREIMPLANTATION GENETIC TESTING ALTERS THE SEX RATIO: AN ANALYSIS OF 44,939 CYCLES FROM THE SOCIETY FOR ASSISTED REPRODUCTIVE TECHNOLOGY DATABASE.**

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**OBJECTIVE:** Preimplantation genetic testing (PGT) is commonly used to assess for aneuploidy. Consequently, chromosomal sex is revealed, allowing couples to select the offspring's sex when multiple euploid embryos are available for transfer. The sex ratio (SER), defined as the ratio of male to female births in the population normalized to 100, is typically standard at 105. We sought to determine the extent to which the use of PGT shifts the SER in in vitro fertilization (IVF) cycles and whether this trend differs by region or parity.

**DESIGN:** Society for Assisted Reproductive Technologies (SART) database analysis.

**MATERIALS AND METHODS:** National IVF data from 2014-2016 was requested from SART including fresh and frozen transfer cycles. Women who had a singleton live birth following a fresh or frozen autologous embryo transfer of a 1) PGT blastocyst, 2) non-PGT blastocyst, or 3) non-PGT cleavage stage embryo were included. The SER for each group was calculated and compared using chi-square analysis. Subsequently, modified Poisson regression was used to model the relative risk (RR) of having a male infant compared to a female infant among PGT embryo transfers versus both non-PGT cleavage and blastocyst transfers adjusting for age, BMI, smoking status, race, parity, number of oocytes retrieved, and clinic regions. Lastly, we investigated whether the risk of having a male infant differed by parity (parous or nulliparous) or clinic region by testing the significance of the interaction term. P<0.05 was considered statistically significant.

**RESULTS:** The SER was 110 among PGT offspring compared to 106 among non-PGT blastocyst offspring (p = 0.01) and to 99 among cleavage offspring (p < 0.0001). After adjusting for covariates, the risk of having a male infant was 4% higher among PGT cycles compared to non-PGT cleavage cycles (RR 1.04; 95% Confidence Interval (CI): 1.02, 1.07). The risk was 2% higher among PGT cycles compared to non-PGT blastocyst cycles (RR 1.02; 95% CI: 1.01, 1.04). The association between PGT and infant gender did not differ by parity. The RR point estimate favored boys in all regions

(South: RR 1.03, 95%CI: 1.00, 1.05; Northeast: RR1.02, 95% CI: 0.99, 1.04; West: RR 1.02, 95% CI:1.00, 1.05) except for the Midwest (RR 1.0; 95% CI: 0.96,1.04). Differences by clinic region were not statistically significant (p=0.59).

**CONCLUSIONS:** The use of PGT shifts the sex ratio from the population standard. PGT significantly increases the risk of male offspring, and this risk does not vary by parity or clinic region. The increase in male offspring is partially attributable to extended culture and partially to PGT. Further study is needed to determine the extent to which PGT has altered the national sex ratio.

**REFERENCES**

1. "Sex Ratio at Birth (male births per female births)." Data Query. United States from 2010-2015. United Nations World Population Prospects 2017. Accessed October 29, 2018.

2. Disclosure of sex when incidentally revealed as part of preimplantation genetic testing (PGT): An Ethics Committee opinion. Ethics Committee of the American Society for Reproductive Medicine American Society for Reproductive Medicine, Birmingham, Alabama. Vol. 110. No 4. September 2018.

3. Barritt, J et al. Blastocyst embryo transfer is associated with a sex-ratio imbalance in favor of male offspring. Fert Steril 2007;87:519-23.

SUPPORT: None.

**BLASTOCYST CONVERSION RATE AND PLOIDY IN TRANSLOCATION CARRIERS.**

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**OBJECTIVE:** To determine if women intending to undergo in vitro fertilization (IVF) with preimplantation genetic testing for structural rearrangements (PGT-SR) have a poorer rate of blastocyst conversion and an increased risk of aneuploidy compared to patients undergoing IVF with PGT-A (PGT for aneuploidy).

TABLE 1. Comparison of laboratory outcomes and ploidy results following blastocyst biopsy for patients using PGT-A versus PGT-SR

Variable	PGT-A Mean ± SD	PGT-SR Mean ± SD	aRR (95% CI) Referent: PGT-A
% 2PN/MII	82% ± 18	80% ± 20	1.02 (0.98-1.06)
% Blastocysts/2PN	66% ± 24	72% ± 22	1.07 (1.01-1.13)
% D5 Biopsied/Total blastocysts	67% ± 30	69% ± 27	1.08 (1.00-1.18)
% D6 Biopsied/Total blastocysts	46% ± 33	38% ± 30	0.79 (0.65-0.96)
% Euploid blastocysts	42% ± 33	29% ± 23	0.86 (0.73-1.00)
% Blastocysts with no result	4% ± 13	2% ± 5	0.70 (0.39-1.26)

DESIGN: Retrospective cohort study.

**MATERIALS AND METHODS:** Autologous cycles with the intent of pursuing PGT-A or PGT-SR with biopsy on either day 5 or 6 were identified from all IVF cycles performed in our program from 1/2012 to 10/2018. Outcome variables assessed included fertilization rate (%2PN/MII), blastocyst conversion rate (% total blastocysts/2PN), proportion of biopsiable blastocysts (% blastocysts of adequate quality for biopsy on days 5 or 6/total blastocysts), % euploid embryos, and % embryos with inconclusive biopsy results. GEE modeling was used to control for patients with more than one cycle during the study period. Rate ratios (RR) were calculated using a Poisson regression with offset model with PGT-A cycles as the referent group, adjusted for patient age, total number of mature oocytes, BMI and intracytoplasmic sperm injection (ICSI).

**RESULTS:** 566 cycles from 388 patients were included (462 PGT-A and 104 PGT-SR cycles). Demographic information and cycle characteristics were similar between groups in terms of age, AMH, and day 3 FSH, with small differences in BMI and use of ICSI. The laboratory outcome data are shown in the Table 1.

Blastocyst conversion rate was statistically significantly higher in the PGT-SR group, although there was no difference between groups in the percentage of blastocysts biopsied on either day 5 or day 6, or the percentage of biopsies that were noninformative. Of note, in the PGT-A group, 42% of biopsied embryos were euploid, compared to only 29% in the PGT-SR group (aRR 0.86; 95% CI 0.73-1.00).

**CONCLUSIONS:** Although translocation carriers have superior blastocyst development and a similar percentage of embryos available for biopsy compared to PGT-A testers, this group had fewer euploid blastocysts available for transfer. These findings should be helpful in counseling patients with structural rearrangements.

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#### THE IDENTIFICATION OF CHROMOSOME DELETIONS IN TROPHOCTODERM BIOPSIES IS SIGNIFICANTLY REPRESENTATIVE OF THE ENTIRE BLASTOCYST.

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**OBJECTIVE:** Chromosome deletions are often random de novo events during gametogenesis and have clinically recognizable genetic syndromes with characteristics including developmental delay, intellectual disability and dysmorphic traits. They occur at a frequency of 0.5% in prenatal testing and are present in 1/700 newborns. The aim of this study was to evaluate the clinical efficacy of diagnosing chromosome deletions in blastocyst trophoctoderm (TE) biopsies.

**DESIGN:** Prospective blinded study

**MATERIALS AND METHODS:** A total of 54 transferrable quality blastocysts (≥ Grade 3BB) with chromosome deletions were donated for re-analysis with patient consent (mean maternal age = 36.7 ± 4.2 years; mean paternal age = 38.6 ± 5.1 years). Each blastocyst was separated into three distinct sections that were blinded and individually re-analyzed using the equivalent VeriSeq™ next generation sequencing (NGS) platform (Vitrolife). All analyses were performed at the same, single genetics laboratory as the original TE biopsy testing. After un-blinding, the data was compiled, identi-

fying representatives of the inner cell mass (ICM) and TE, for a complete picture of each individual blastocyst. In parallel, 21 transferrable quality euploid and 87 transferrable quality full aneuploid blastocysts (≥ Grade 3BB) identified by TE biopsy also underwent the same, blinded, sectioned re-analysis.

**RESULTS:** The overall incidence of chromosome deletions and duplications (≥ 5Mb) following TE biopsy of 34,210 human blastocysts was observed at 3.1%. There was no association observed for parental age at time of oocyte retrieval, clinical, IVF or embryo morphological parameters. Chromosome deletions were observed across the genome and equally represented on the p and q arms. Blinded re-analysis of 54 blastocysts with chromosome deletions validated the original TE diagnosis in 51 (94.4%) embryos. The chromosome deletion was observed throughout the entire embryo, including all ICM and TE sections for 39 blastocysts (72.2%). The remaining 12 (22.2%) blastocysts were concordant, displayed evidence of a diploid cell line in ≥ 1 analyzed section and also included two blastocysts showing the reverse chromosome duplication in both ICM and TE sections. Blinded sectioned re-analysis of the 21 euploid and 87 full aneuploid blastocysts also strongly validated the original TE diagnosis for 95.2% and 97.7% of the embryos, respectively. In both sets of data, the non-concordant blastocysts were identified as euploid mosaic (n=2; 4.8%) and aneuploid mosaic (n=2; 2.3%).

**CONCLUSIONS:** This study validated the strength and robustness of NGS for diagnosis of chromosomal deletions in TE biopsies. These small chromosomal deletions can result in clinically recognizable genetic syndromes, such as chromosome 1p36 and chromosome 3q29 deletion syndromes, cautioning their selection for transfer. Notably, blinded sectioned analysis also confirmed the overall high clinical efficacy of preimplantation genetic testing for aneuploidy screening from a TE biopsy and low incidence of chromosomal mosaicism.

**SUPPORT:** None.

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#### IMPACT OF DIFFERENT DEGREES OF GENETIC MOSAICISM IN THE KINETIC PROFILE OF THE HUMAN EMBRYO.

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**OBJECTIVE:** Current publications have shown that embryos classified after PGT-A as mosaics may lead to healthy live births. Some clinics transfer them under certain clinical circumstances, while others opt not to. These embryos, whose developmental potential is generally compromised compared to their euploid counterparts, still await morphokinetic characterization. The aim of this study is to examine whether embryos showing different degrees of mosaicism exhibit characteristic kinetic profiles. This may provide additional information about their viability, hence easing clinical decisions in the IVF daily practice.

**DESIGN:** Retrospective, observational study including 688 embryos from 172 patients that underwent PGT-A ICSI cycles in IVI RMA Valencia from March 2018 to April 2019 and used time-lapse technology for embryo culture.

**MATERIALS AND METHODS:** Timings of the following preimplantation events were annotated during embryo culture: time of extrusion of the PB2 (tPB2), time of PN appearance (tPNa) and fading (tPNf), time of division to 2, 3, 4, 5, 8 and >9 cells (t2, t3, t4, t5, t8, t9+), time to compaction (tSC), time to morula (tM), time to blastulation (tSB), time to blastocyst (tB), time to expanded blastocyst (tEB) and time to hatching blastocyst (tHB). At day 5-6 of development, trophoctoderm biopsies were analyzed by NGS and classified according to their relative content of aneuploid cells: euploid (<30%), low-degree mosaic (30-50%), high-degree mosaic (50-70%) and aneuploid (>70%). The annotated variables were retrospectively assessed using One-way ANOVA and Bonferroni's post-hoc with SPSS software (p < .05 was considered significant).

**RESULTS:** 55.5% (n=382) of the embryos that underwent time-lapse culture followed by PGT-A during the aforementioned period were diagnosed as aneuploid. The total mosaicism rate was 16.2% (n=112), which means that 29.3% of the embryos with >30% of aneuploid cells were mosaics to a varying extent. When examining their kinetic profile, significant differences were found in tPNf (23.12±3.02 vs. 23.92±3.47), t2 (25.70±3.09 vs. 26.54±3.56), t3 (36.29±4.08 vs. 37.26±4.39), t4 (37.95±4.37 vs. 39.09±4.94) and tSB (98.71±9.05 vs. 101.01±9.16) of euploid embryos

vs. aneuploid. However, embryos showing low and high degree of genetic mosaicism exhibited a similar kinetic behavior for all the parameters examined. Significant differences were not found for the assessed variables between euploid and low-degree mosaics, nor between high-degree mosaics and aneuploid embryos.

**CONCLUSIONS:** This preliminary study shows that mosaic embryos have a similar kinetic behavior with the cut-off values considered to establish the mosaicism degree. Our results suggest that kinetic variations between euploid and low-degree mosaics, and between aneuploid and high-degree mosaics, may be subtle or even non-existent. These findings recall the importance of a critical interpretation of any mosaics data, especially when working with limited sample sizes.

**SUPPORT:** Research supported by CDTI n. 20190022.

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#### FOUR YEARS OF PROSPECTIVE MOSAIC EMBRYO TRANSFER: A SINGLE CENTER'S

**EXPERIENCE.** Andria G. Besser, MS,<sup>a</sup> Jennifer K. Blakemore, MD,<sup>b</sup> Elizabeth J. Del Buono, MS,<sup>c</sup> Caroline McCaffrey, Ph.D.,<sup>d</sup> David H. McCulloh, Ph.D.,<sup>e</sup> James A. Grifo, MD, PhD,<sup>f</sup> <sup>a</sup>NYU Langone Fertility Center, New York, NY; <sup>b</sup>NYU Langone School of Medicine, New York, NY; <sup>c</sup>Sarah Lawrence College, Bronxville, NY; <sup>d</sup>New York Langone Health, NYU Fertility Center, New York, NY; <sup>e</sup>NYU Langone Health, New York, NY; <sup>f</sup>NYU Langone Prelude Fertility Center, New York, NY.



**OBJECTIVE:** It is now well-established that embryos diagnosed as chromosomally mosaic can result in healthy live births. Our aim was to report on our clinic's outcomes associated with mosaic embryo transfer (MET), determine which parameters predict MET success, and compare MET outcomes to single thawed euploid embryo transfer (STEET).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All STEET cycles after in vitro fertilization, trophectoderm biopsy, and preimplantation genetic testing for aneuploidy (PGT-A) by next-generation sequencing were identified as controls. Cases included all MET cycles. Statistical analysis included chi-square, with  $p < 0.05$  considered significant.

**RESULTS:** A total of 645 PGT-A frozen embryo transfer cycles occurred during the selection period. STEET occurred in 569 cycles (mean age = 35.8), and MET occurred in 70 cycles (mean age = 39.6) with 76 embryos. 47 embryos were diagnosed as segmental mosaic (SM) and 29 embryos were diagnosed as whole chromosome mosaic (WCM; including monosomies and trisomies). 28/47 (59.6%) SM embryos and 10/29 (38.5%) WCM embryos implanted, compared to 408/569 (71.7%) euploid embryos. The ongoing pregnancy/live birth rate was significantly higher in SM embryos (22/47; 46.8%) compared to WCM embryos (5/29; 19.2%;  $p < 0.01$ ). STEET resulted in significantly more ongoing pregnancies (358/569; 62.9%) than both SM ( $p = 0.03$ ) and WCM ( $p < 0.01$ ) embryos. For SM embryos, there was no significant difference in outcomes when stratified by the percentage of mosaicism in the biopsy; however, WCM embryos with 20-40% mosaicism in the biopsy had significantly higher ongoing pregnancy rates compared to those with 41-80% mosaicism ( $p = 0.03$ ). 8/8 WCM embryos with more than 1 chromosome involved failed to implant. There were no significant differences when comparing embryos with a deletion vs. a duplication or a monosomy vs. a trisomy. Maternal age at testing was not associated with differences in pregnancy outcomes in the MET or STEET groups. 15/27 fetuses conceived by MET were reported to be tested by amniocentesis (including 9 with chromosomal microarray), and none were found to have evidence of mosaicism involving the chromosome in question.

**CONCLUSIONS:** In the absence of euploid embryos, MET may be considered. While embryos diagnosed as SM have higher reproductive potential compared to those diagnosed as WCM, both categories have inferior outcomes when compared to STEET. While neonatal outcomes are reassuring, additional studies are needed to explore long-term outcomes from babies born following MET.

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#### PREIMPLANTATION GENETIC TESTING (PGT) AND FROZEN EMBRYO TRANSFER (FET) SYNERGISTICALLY DECREASE PRE-TERM DELIVERY IN PATIENTS UNDERGOING IN VITRO FERTILIZATION (IVF).

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TABLE 1. Pregnancy outcome comparison in IVF patients with PGT vs no PGT

	No PGT		PGT
	Fresh embryo transfer (ET)	Frozen embryo transfer (FET)	FET/PGT
Number of eSETs	31670	39228	33256
Live birth rate per transfer	47.0%	42.5%	52.2%
Term delivery	88.6%	89.3%	89.9%
Pre-term delivery	9.4%	8.8%	8.5%
Very pre-term delivery	2.0%	1.8%	1.5%

$\chi^2$  tests for trend analysis of term, pre-term and very pre-term delivery: FET vs ET ( $P = 0.04$ ); FET/PGT vs ET ( $P < 0.0001$ ) and FET/PGT vs FET ( $P = 0.02$ ).

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**OBJECTIVE:** To study the effects of PGT on pregnancy outcomes in patients undergoing IVF with elective single embryo transfer (eSET).

**DESIGN:** Retrospective cohort Society for Assisted Reproductive Technology (SART) data study.

**MATERIALS AND METHODS:** A retrospective cohort study was conducted using the publicly available data in the SART National Summary Report from 2014 to 2016. Cycle inclusion criteria were eSET, fresh embryo transfers (ET), and frozen embryo transfers (FET) with or without PGT. Exclusion criteria were use of gestational carriers and donor eggs. Pregnancy outcomes included live births and gestational age at delivery (term:  $> 37$  weeks, pre-term: 32-37 weeks, and very pre-term:  $< 32$  weeks).  $\chi^2$  test was used to compare variables between groups. A  $P$  value of  $< 0.05$  was considered statistically significant.

**RESULTS:** A total of 104154 eSETs were analyzed for the effect of PGT on IVF outcome and pre-term deliveries including 31670 ETs, 39228 FETs and 33256 frozen embryo transfers post PGT (FET/PGT). The main outcome was summarized in Table 1. Live birth rate in patients with FET/PGT was significantly higher than those in ET (52.2% vs 47.0%,  $P < 0.0001$ ) and FET without PGT (52.2% vs 42.5%,  $P < 0.0001$ ). FET was associated with a statistically lower pre-term and very pre-term deliveries than ET ( $P = 0.04$ ), though live birth rate was significantly lower than that in ET (42.5% vs 47.0%,  $P < 0.0001$ ). FET/PGT significantly reduced pre-term and very pre-term deliveries when compared with ET ( $P < 0.0001$ ). Pre-term/very pre-term deliveries in FET/PGT was also statistically lower than those in the FET without PGT ( $P = 0.02$ ), suggesting that PGT has an additive effect on FET in decreasing pre-term delivery.

**CONCLUSIONS:** PGT has been integrated into one of the most important roles in IVF treatment. This study using large cohort SART data demonstrates that PGT significantly improves IVF outcome. Moreover, this study shows that patients undergoing PGT accompanied with subsequent FET had significantly reduced pre-term as well as very pre-term deliveries. Lower incidence of pre-term delivery associated with PGT should be taken into account when counseling patients seeking infertility treatment.

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#### HOW MANY SINGLE EMBRYO TRANSFERS WOULD BE NEEDED TO PERFORM AN EUPLOID EMBRYO TRANSFER ACCORDING TO THE AGE OF THE PATIENT?

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**OBJECTIVE:** To estimate the number of single embryo transfers (SETs) using morphological criteria and the number of stimulation cycles needed to find a euploid embryo for transfer.

**DESIGN:** This retrospective study includes 215,723 blastocysts corresponding to 59,451 cycles of preimplantation genetic testing for aneuploidy (PGT-A) performed using Next Generation Sequencing (NGS). Results of trophectoderm biopsies performed from January 2016 to December 2018 were stratified according to the origin of the oocytes (own or donated). Ovum donors were under 30 years of age. Cycles performed with own oocytes were divided according to the female age in the following categories:

TABLE 1.

	OD	≤27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	≥44
Frequency of euploid embryos	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.4	0.4	0.3	0.3	0.2	0.2	0.2
Minimum number of blastocysts to obtain a euploid embryo	2	2	2	2	2	2	2	2	2	2	2	3	3	3	4	4	5	6	6
Mean number of blastocysts	5.7	4.9	4.8	4.8	4.7	4.7	4.5	4.2	4.1	3.9	3.7	3.4	3.2	3.0	2.8	2.6	2.4	2.3	1.9
Minimum number cycles to obtain a euploid embryo	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	3	3
Expected number of SET to transfer a euploid embryo	1.5	1.4	1.5	1.5	1.5	1.5	1.5	1.6	1.6	1.7	1.7	2	2	2.2	2.5	3	3.3	3.8	4

≤27 years, individually from 28-43 years, and ≥44 years (range: 20-45). The percentage of euploid embryos and the mean number of biopsied blastocysts at each age group were computed for the mathematical model based on the empirical data to estimate the stimulation cycles and the expected number of SETs needed to obtain a euploid embryo.

**MATERIALS AND METHODS:** The incidence of chromosomal abnormalities ranged from 34.6% to 82.5% and the mean number of blastocysts per cycle varied from 5.7 to 1.9, in the youngest versus oldest patients, respectively. To estimate the minimal number of blastocysts and cycles needed to obtain a euploid embryo at each group, these empirical data were used. To compute the expected number of SETs to be performed, a hypergeometric probability distribution was applied, using the probability-weighted average of all possible values. To obtain the final value, we applied a smooth method, computing running medians of odd span (J.H. Friedman and W. Stuetzle, Technical report, 1982). This calculation was performed starting from the minimal number of expected blastocysts at each age category.

**RESULTS:** A summary of the results is presented in Table 1 according to maternal age in years (OD means ovum donation). Each row represents the frequency of euploid blastocysts, the mean number of blastocysts per cycle, the minimum number of blastocysts and stimulation cycles needed to obtain a euploid embryo and expected number of SETs needed to transfer a euploid embryo if PGT-A would not have been performed.

**CONCLUSIONS:** This mathematical model shows the potential benefits of selecting the euploid embryo in the first SET in PGT-A, compared to standard SET in which embryo selection is performed according to morphological criteria. These data as well as the number of cycles needed to obtain an euploid embryo according to the female age are valuable information for reproductive counselling.

#### REFERENCE

Jerome H. Friedman and Werner Stuetzle (1982) *Smoothing of Scatterplots*; Report, Dep. Statistics, Stanford U., Project Orion 003.

SUPPORT: None.

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#### ARTIFICIAL VISION AND MACHINE LEARNING DESIGNED TO PREDICT PGT-A

**RESULTS.** Alejandro Chavez-Badiola, MD,<sup>a</sup> Adolfo Flores-Saiffe Farias, MSc, PhD,<sup>b</sup> Gerardo Mendizabal-Ruiz, PhD,<sup>c</sup> Andrew J. Drakeley, MD FRCOG,<sup>d</sup> Rodolfo Garcia-Sánchez, MSc,<sup>e</sup> John J. Zhang, MD, PhD.<sup>f</sup> <sup>a</sup>New Hope Fertility Center Mexico, Mexico City, EM, Mexico; <sup>b</sup>New Hope Fertility Center, Guadalajara, JA, Mexico; <sup>c</sup>Departamento de Ciencias Computacionales, Universidad de Guadalajara, Guadalajara, JA, Mexico; <sup>d</sup>Hewitt Centre for Reproductive Medicine, Liverpool Women's Hospital, Liverpool, United Kingdom; <sup>e</sup>New Hope Fertility Center Mexico, Guadalajara, JA, Mexico; <sup>f</sup>New Hope Fertility Center, New York, NY.

**OBJECTIVE:** To assess the ability of a computing tool based on artificial vision and machine learning to predict aneuploidy based on single blastocyst pictures.

**DESIGN:** Double blind, prospective, longitudinal cohort study.

**MATERIALS AND METHODS:** A self developed computing tool (CT) with artificial vision and machine learning capabilities was tested for its ability to segment images, extract features of each segment, and to predict aneuploidy on digital images of blastocysts collected between October 2018 and February 2019 from a single IVF center. All embryos were subject to embryo biopsy for PGT-A analysis with next generation sequencing (NGS). Pictures from all embryos were taken before trophectoderm biopsy. Results were assessed using a confusion matrix: PGT-A results matched against CT's predic-

tion. Technicians performing biopsy and PGT-A providers were blind to CT's predictions. Mathematicians feeding information to our CT were also blind to PGT-A results until analysis was performed.

**RESULTS:** A total of 241 blastocysts were analyzed with the use of our artificial vision and machine learning computing tool. Positive predictive value for aneuploidy was 79.5%, sensitivity of 70.1%, specificity of 73.2% and accuracy of 71.4%. F1 Score was 74.5%. Negative Predictive Value, which is the ability of the algorithm to predict euploidy, was 62.3%.

**CONCLUSIONS:** Sensitivity, specificity and accuracy with our current Artificial Vision and Machine Learning tool is not yet comparable to embryo biopsy and NGS for euploidy prediction and, at this stage, are not ready to substitute what is still considered the gold standard for aneuploidy screening. However, a positive predictive value for euploidy estimated at 72% is for now good enough to guide embryologists during the embryo selection process in those cases where PGT-A was not performed. Further studies with a larger image database are already underway aiming to improve predictive capabilities of our software.

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#### REBIOPSY OF BLASTOCYSTS REVEALS THAT NEXT GENERATION SEQUENCING PROVIDES EXCELLENT CLINICAL ACCURACY DESPITE MINOR DISCORDANCES.

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**OBJECTIVE:** PGT-A on TE biopsies (TE Bx/NGS) provides a method of selecting blastocysts with excellent prognosis for establishing clinical pregnancies, minimizing miscarriages and improving live birth rates per ART procedure. However some practitioners distrust the reliability of TE Bx/NGS because mosaicism is seen in normal placentae (derived from the TE) and small numbers of TE cells biopsied may not represent the fetus (derived from the inner cell mass). We examined rebiopsy specimens from the TE and the ICM to determine the reliability of the clinical biopsy to characterize the blastocyst. In particular, we determined concordance between the clinical biopsies and rebiopsy specimens, focusing on 1) chromosomal concordance for disomic results, aneuploid results or mosaic results in the clinical biopsy and 2) clinical concordance (whether biopsy of the ICM was concordant with the initial biopsy's result of "Euploid" vs "NOT euploid")

**DESIGN:** Rebiopsy of blastocysts with known results of clinical PGT-A

**MATERIALS AND METHODS:** Results of initial, clinical TE biopsies were obtained from Cooper Genomics. Vitrified blastocysts from patients consenting to research were selected for groups that had no aneuploidy (N = 10), aneuploidy with 1 or 2 aneuploid chromosomes (N = 4) or 1 aneuploid chromosome and 1 mosaic result (N = 18). Blastocysts were rewarmed and cells from the TE and ICM were biopsied separately, obtaining as many rebiopsy specimens as possible. Biopsy specimens were subjected to WGA and NGS in our university's core laboratory. NGS results for rebiopsies were compared with results of the clinical biopsy. Rebiopsy chromosomes were considered concordant when the same chromosomal diagnosis was observed

**RESULTS:** Chromosomal concordances were 97.0%, 74.3%, and 13.7% per chromosome, respectively, for disomic (Di), aneuploid (An) and mosaic (Mo) chromosomes in the clinical biopsy. Discordant chromosome results were predominantly mosaic results (2.6%) for Di, mosaic or complementary results (21.6%) for An, or were not seen or non-mosaic aneuploid results (74.5%) for Mo observed for the same chromosome that was seen in the clinical biopsy. These minor discordances can be considered concordant since

they mainly confirm the initial results. Counting them as concordant leads to concordances 99.6% for Di, 95.9% for An, and 88.2% for Mo per chromosome. Rebiopsies of inner cell mass were clinically concordant for 100% of the blastocysts (biopsy result of ICM agreed with the clinical result of “euploid” or “not euploid”).

**CONCLUSIONS:** Despite small number of biopsied cells (required to avoid damage to the blastocyst) and mosaicism (demonstrated by rebiopsy specimens) the excellent chromosomal concordance for rebiopsy specimens (99.6% and 95.9%) and clinical concordance for ICM biopsies (100%) indicate that TE biopsy/NGS provides excellent accuracy in its assessment of ploidy. Within this non-randomly selected subset of blastocysts, mosaics detected in the clinical biopsy outnumbered mosaics detected only by rebiopsy 2.25:1 (18:8).

**SUPPORT:** None.

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**A UNIVERSAL SINGLE TUBE PCR-BASED LIBRARY PREPARATION FOR PGT-A ALLOWING CROSS-PLATFORM NGS SEQUENCING.**

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**OBJECTIVE:** There are several methods to prepare trophoctoderm biopsy samples for Preimplantation Genetic Testing for Aneuploidy (PGT-A) by Next Generation Sequencing (NGS). Most methods use a two-step approach of Whole Genome Amplification (WGA) followed by library preparation. However, combining WGA with library preparation by utilising PCR-based library preparation approaches offers several advantages over traditional two-step methods, including protocol time efficiencies and reduced hands-on time. In addition to these advantages, a novel combined approach developed based on the PerkinElmer DOPlify® WGA kit offers the capability for cross-platform sequencing validation of a single biopsy.

**DESIGN:** Here we describe the development of a novel PGT-A approach which allows PCR-based library preparation of trophoctoderm biopsies for cross-platform NGS using either Illumina® or Ion Torrent® sequencing technology.

**MATERIALS AND METHODS:** Five-cell samples representative of trophoctoderm biopsies were manually sorted from aneuploid cell lines (Coriell Institute) and euploid lymphocytes. Cell lysis and WGA were performed using a modified DOPlify® kit protocol (PerkinElmer) followed by incorporation of Illumina®-specific adapter sequences and unique indexes in a single tube. Amplified, indexed 5-cell samples were purified, quantified then pooled before 48 sample multiplex and 1x75bp read length sequencing on the MiSeq® Instrument (Illumina). Sequencing data was analysed for correct aneuploidy calling using the PG-Find™ software (PerkinElmer).

**RESULTS:** A total of 105 5-cell samples were processed. Three samples were excluded from final analysis due to weak amplification (2.8%) with a further five samples failing to pass quality control checks (4.8%). The 48 sample multiplex generated an average of 510,000 reads per sample with 98.9% of

reads mapping to hg19. All samples that passed quality control metrics displayed the expected karyotype when analysed with the PG-Find™ software. The PCR-indexing protocol took on average 4.5 hours (2.5 hours hands on) to process 48 samples from sample receipt to NGS instrument loading.

**CONCLUSIONS:** This novel PCR-based indexing workflow provides rapid, scalable and economical sequencing for PGT-A and provides the capability for cross-platform sequencing validation of a single embryo biopsy for PGT-A. This flexible workflow allows customisable throughput and tailorable resolution to detect smaller segmental aberrations.

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**HOW IMPORTANT IS IT TO VISUALIZE 2PN IN ZYGOTES DESTINED FOR PGT-A TESTING BY NEXT GENERATION SEQUENCING (NGS)?**

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**OBJECTIVE:** To determine the incidence of euploidy in Blastocysts derived from OPN and IPN compared with 2PN embryos.

**DESIGN:** Single center retrospective review of PGT-A cases over a 4 year period (2015-2018) where a biopsy and ploidy determination was performed on blastocysts (blasts) derived from zygotes where pronuclei (PNs) were either not evident (0 PN) or only 1 pronucleus (1 PN) was evident at the time of fertilization check.

**MATERIALS AND METHODS:** At NYULMC fertilization checks are conducted ~18 hours post insemination or ICSI. The number of PN in each egg is recorded and zygotes are cultured individually. Cases where ≤50% of the mature eggs exhibit 2PN are routinely rechecked later on Day 1. In cases for PGT-A, all viable inseminated eggs excluding those with ≥3 PN remain in culture to Day 6/7. Good quality blastocysts with a distinct inner cell mass and cohesive trophoctoderm are considered for PGT-A regardless of whether they were OPN, IPN or 2PN at fertilization check. PGT-A results are shown in Table 1 along with PGT-A sex of blasts derived from each group.

**RESULTS:**

**CONCLUSIONS:** Prior to utilization of PGT-A and/or timelapse zygotes not exhibiting 2PN at fertilization check were routinely discarded. However, it is now obvious that a percentage of these, albeit small, are fertilized normally and are euploid. Though they account for only a small percentage these may be the only euploid blasts available. Implantation rates and LB rates following transfer of these blasts are similar to those for 2PN blastocysts. Of interest, ratios of XX:XY blasts derived from IPN and OPN zygotes were ~2:1 while those from 2PN zygotes were ~1:1. It should be noted that NGS cannot detect pure haploidy (23, XO) or triploidy (69, XXX) thereby possibly misdiagnosing these as euploid although our IR and LB results indicate otherwise.

**SUPPORT:** None.

TABLE 1.

	All Patient Ages	2PN	1PN	0PN	Sig
Conventional insem	Number Blasts Bx'd	11287	428	31	
	Number Euploid (% Bx'd)	3864 (34%)	113 (26%)	11 (35%)	
	Ratio XX:XY (%)	47:52	61:39	64:36	
ICSI	Number Blasts Bx'd	6553	76	147	
	Number Euploid (% Bx'd)	2189 (33%)	29 (38%)	35 (24%)	
	Ratio XX:XY	50:50	86:14	54:46	
Conventional insem+ ICSI	IR (sac/ ET) (%)	x/1809 (68%)	x/40 (60%)	x/10 (80%)	
	LB / ET with known outcome (%)	52%	44%	66%	
	LB Ratio XX:XY	362:394	11:5	4:2	

Of 11726 embs biopsied from conventional insemination, 4% developed from 1PN  
Less than 1% was from 0PN.

Of 6553 ICSI embs biopsied, 1% was from 1PNs, 2% were from 0PNs

Of the 11 XX 1PN LB N 10 (10/ 17) are from insem, 1 (1/6) from ICSI

Of the 4 XX 0PN LB N LB insem, 1 (1/1) is from ICSI

**INDIVIDUAL CHROMOSOME MOSAICISM RATES AFTER PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY: IMPLICATIONS FOR MECHANISMS RELATED TO THE EARLY STAGES OF EMBRYO DEVELOPMENT.**



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**OBJECTIVE:** Next generation sequencing (NGS) provides evidence of mosaicism in the blastocyst stage embryo. Mosaic profiles are often graded as low or high to denote levels of risk. Here we assess mosaicism as it pertains to specific chromosomes and determine rates of high and low level mosaicism for individual chromosomes.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** A total of 6525 samples were assessed during the time period of this study. Mosaicism rates were determined as a percentage of total samples, total mosaicism event and per chromosome. Additionally, we evaluated the frequency of high and low level mosaics for each chromosome.

**RESULTS:** Of the 6525 samples that underwent PGT-A testing 931 (14%) displayed whole aneuploid mosaicism. High and low level mosaicism was observed in 47% and 53% of the samples respectively. Male and female samples showed autosomal mosaicism disproportionately at 44% and 56% respectively. Mosaicism and high level mosaicism in chromosome 22 occurred at a higher rate than other chromosomes, while mosaicism rates were lowest in chromosomes 12 and 17. Data are summarized in Table 1.

TABLE 1.

Ch #	Mosaic	Total		Female	Male					
		High	Low							
1	37	4.0%	15	40.5%	22	59.5%	19	51.4%	18	48.6%
2	40	4.3%	20	50.0%	20	50.0%	22	55.0%	18	45.0%
3	35	3.8%	18	51.4%	17	48.6%	22	62.9%	13	37.1%
4	59	6.3%	32	54.2%	27	45.8%	34	57.6%	25	42.4%
5	39	4.2%	21	53.8%	18	46.2%	21	53.8%	18	46.2%
6	40	4.3%	21	52.5%	19	47.5%	22	55.0%	18	45.0%
7	33	3.5%	7	21.2%	26	78.8%	19	57.6%	14	42.4%
8	49	5.3%	20	40.8%	29	59.2%	27	55.1%	22	44.9%
9	33	3.5%	8	24.2%	25	75.8%	20	60.6%	13	39.4%
10	39	4.2%	20	51.3%	19	48.7%	25	64.1%	14	35.9%
11	34	3.7%	15	44.1%	19	55.9%	23	67.6%	11	32.4%
12	21	2.3%	8	38.1%	13	61.9%	20	95.2%	1	4.8%
13	48	5.2%	20	41.7%	28	58.3%	25	52.1%	23	47.9%
14	36	3.9%	15	41.7%	21	58.3%	18	50.0%	18	50.0%
15	39	4.2%	19	48.7%	20	51.3%	23	59.0%	16	41.0%
16	37	4.0%	17	45.9%	20	54.1%	18	48.6%	19	51.4%
17	21	2.3%	10	47.6%	11	52.4%	6	28.6%	15	71.4%
18	47	5.0%	25	53.2%	21	44.7%	26	55.3%	21	44.7%
19	56	6.0%	33	58.9%	23	41.1%	25	44.6%	31	55.4%
20	30	3.2%	11	36.7%	19	63.3%	14	46.7%	16	53.3%
21	51	5.5%	26	51.0%	25	49.0%	29	56.9%	22	43.1%
22	68	7.3%	45	66.2%	23	33.8%	42	61.8%	26	38.2%
X	33	3.5%	10	30.3%	23	69.7%	30	90.9%	3	9.1%
Y	6	0.6%	1	16.7%	5	83.3%	0	0.0%	6	100.0%

**CONCLUSIONS:** Bridging the gap between preimplantation genetics and prenatal cytogenetics has the potential to be a powerful tool for clinicians treating infertile couples. The literature has reported that mosaicism is clinically relevant. This report evaluates the rates of mosaicism for individual chromosomes providing a basis on which to correlate the incidence of preimplantation mosaicism in specific chromosomes with mosaicism observed in prenatal samples. Additionally, the data highlights the putative uneven distribution of mosaicism in male and female samples.

**RATES OF EMBRYONIC MOSAICISM ARE CONSISTENT AMONGST EMBRYOLOGISTS PERFORMING OR LOADING TROPHECTODERM BIOPSIES FOR PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY.**



Emily K. Osman, MD, Shelby A. Neal, MD,

**OBJECTIVE:** The introduction of next-generation sequencing (NGS) for preimplantation genetic testing for aneuploidy (PGT-A) has led to increased detection of mosaicism and segmental errors. It has been suggested that the incidence of such abnormalities varies between reference laboratories where the biopsy is analyzed. Additionally, the technical aptitude of the embryologist performing or handling the biopsy specimen may contribute to mosaicism, segmental errors, and “no call” outcomes including nonconcurrent or unamplified results.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** Patients undergoing in vitro fertilization (IVF) cycles with PGT-A at a single center were included. Embryos were cultured to the blastocyst stage and biopsies were performed on days 5, 6 or 7. PGT-A was performed using the NexCCS NGS platform. An embryo was designated as mosaic if the DNA copy number ranged from 0.3 to 0.7. Segmental errors were defined as chromosomal duplications or deletions that were ≥ 5 Mb. A chi-squared analysis was utilized to compare the primary outcome of mosaicism and secondary outcomes of segmental errors and “no call” results between embryologists. An alpha error <0.05 was considered significant. Given the large sample size, differences <2% were determined to be clinically irrelevant despite statistical significance.

**RESULTS:** Four embryologists performed a total of 30,899 embryo biopsies and 6 individuals loaded biopsy specimens into designated tubes. PGT-A results of embryologists performing the biopsy are listed in Table 1. Given the immense sample size, all biopsying embryologists had statistically separable differences in both primary and secondary outcomes. However, individuals performed within 1% of the mean; these differences were not of clinical significance. Similarly, differences in rates of mosaicism (5.0-5.9%), segmental errors (9.7-10.4%) and inconclusive results (1.1-2.8%) amongst different embryologists performing biopsy loading were clinically irrelevant.

**CONCLUSIONS:** Rates of mosaicism, segmental abnormalities, nonconcurrent and unamplified PGT-A results are highly consistent amongst embryologists. Variation in PGT-A results can be attributed to differences in reference laboratories. With increasing utilization of PGT worldwide, reproducible results are critical for optimizing clinical outcomes during IVF cycles.

TABLE 1. Rates of mosaicism, segmental errors and “no call” results based on embryologist performing the biopsy

Embryologist	Mosaicism		Segmental Error		“No Calls”	
	N	%	N	%	N	%
A	891/15803	5.6	1697/15803	10.7	302/15803	1.9
B	428/9969	4.3	896/9969	9.0	163/9997	1.6
C	209/3754	5.6	395/3754	10.5	43/3754	1.1
D	83/1373	6.1	136/1373	9.9	40/1373	2.9
All	1607/30899	5.2	3124/30899	10.1	548/30899	1.7

WITHDRAWN

**IN VITRO FERTILIZATION (IVF) WITH PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) IS NOT COST EFFECTIVE TO ACHIEVE A LIVE BIRTH COMPARED TO IVF ALONE IN DONOR OOCYTE CYCLES.**



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**OBJECTIVE:** The process of using donor oocyte can be costly for patients and in some cases it is not covered by insurance. For some patients, oocyte donation comes as their last resort after they have exhausted their financial limit. Optimizing every aspect of oocyte donation is important not only to improve outcomes but also reduce cost to patients. Preimplantation genetic testing for aneuploidy (PGT-A) has been shown to be cost effective in certain subpopulations of infertile patients undergoing IVF<sup>[i]</sup> <sup>[ii]</sup>. The objective of this study is to determine whether IVF with PGT-A is cost effective to achieve a live birth compared to IVF alone in donor oocyte cycles.

**DESIGN:** Cost-effectiveness study

**MATERIALS AND METHODS:** A decision analytic model was constructed using TreeAge Pro 2019 (TreeAge Software Inc, Williamstown MA) to compare the cost of IVF with PGT-A versus IVF alone to achieve a live birth. The model assumed donor oocytes were obtained from healthy females younger than 30 years old, with laboratory evidence of normal ovarian reserve, and no infertility diagnosis. The model analyzed a hypothetical single fresh oocyte donor IVF cycle with PGT-A versus IVF alone and followed the progression of a single embryo through the different decision nodes. Cost estimates of relevant clinical events and incorporated probabilities were based on data from published literature including the Society for Assisted Reproductive Technology (SART) database. Cost data was converted to 2018 US dollars. The primary outcome was the cost to achieve a live birth using IVF with PGT-A for donor egg cycles compared to IVF alone, and Monte Carlo sensitivity analyses were performed to assess for model robustness.

**RESULTS:** The model demonstrates IVF with PGT-A on average costs \$37,940 to achieve a live birth with a donor oocyte cycle across all combined age groups. In base-case analysis, IVF with PGT-A did not increase the overall effectiveness of increasing live birth rate at an additional cost of \$4650. This yielded an incremental cost-effectiveness ratio (ICER) of - \$1142.66; IVF alone with donor eggs had a net monetary benefit (NMB) of \$124,044 per live birth rate. The ICER was above the willingness to pay cost of \$40,000 for achieving one live birth assuming the live birth rate of 61.3% and \$37,940 per cycle for this patient population. Monte Carlo simulations demonstrated that IVF+PGT-A is not cost-effective in nearly all iterations at an acceptability cut off of \$40,000.

**CONCLUSIONS:** This model suggests that the addition of PGT-A to IVF in donor oocyte cycles is not cost effective compared to IVF alone over a wide range of probabilities and costs. To better understand the dynamics of cost effectiveness in this population, the willingness to pay per live birth should be refined or the motivation to pay for PGT-A needs to be further investigated.

**REFERENCES**

<sup>i</sup> Somigliana E, Busnelli A, Paffoni A, Vigano P, Riccaboni A, Rubio C, Capalbo A. Cost-effectiveness of preimplantation genetic testing for aneuploidies. *Fertil Steril*. 2019 Feb 15.

<sup>ii</sup> Collins S, Xu X, Mak W. Cost-effectiveness of preimplantation genetic screening for women older than 73 undergoing in vitro fertilization. *J Assist Reprod Genet* 2017 July 27; 34: 1515-1522

**P-322** Tuesday, October 15, 2019 6:30 AM

**THE COST-EFFECTIVENESS OF PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A): AN ANALYSIS OF 153,865 SART CYCLES.**

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**OBJECTIVE:** To determine the cost-effectiveness of PGT-A at cycle start for the treatment of infertility in the United States

**DESIGN:** Retrospective analysis of linked cycles from 1/2014–12/2016 from the Society for Assisted Reproductive Technology Clinic Outcomes Reporting System (SART CORS) applied to a decision analytic model.

**MATERIALS AND METHODS:** All first fresh autologous cycles of women undergoing IVF between 1/2014–12/2015 plus linked FET cycles from 1/2014–12/2016 were included. Banking, frozen egg, PGT-M and PGT-SR cycles were excluded. Cycles were categorized by intent to perform PGT-A.

Clinical and cost outcomes of IVF compared to IVF with PGT-A were estimated using a decision analytic model. Transitions between treatment stages relied on probability estimates from SART CORS. Patients progressed through the model until they achieved a live birth, exhausted their embryos or at one year after stimulation. Two payer perspectives were considered: patient and societal. Expected costs accounted for age-specific projections from SART CORS, such as number of embryos biopsied and total gonadotropin use.

**RESULTS:** 114,182 fresh and 39,683 linked FET cycles were included. Of fresh cycles, 18,470 (16.2%) planned PGT-A and 95,712 (83.8%) did not. Across all age groups, non PGT-A cycles used more gonadotropin, had fewer embryos, and had higher cancellation and failed fertilization rates, suggesting that patients utilizing PGT-A represent a more favorable prognosis group.

Cumulative live birth (CLBR) and twin live birth rates (TLBR) per cycle start are presented. From the patient perspective, costs incurred with PGT-A were higher in every age group when compared to IVF alone (differential \$4,551–5,137). From the societal perspective, costs incurred with PGT-A were lower in the <35 age range (-\$4,233), equivalent at age 35, and higher at every other age (\$955–6,905).

**CONCLUSIONS:** From the societal perspective, IVF with PGT-A can be cost-effective for certain ages. From a patient perspective, IVF with PGT-A is costlier at every age. Up to age 35, at which CLBR are equivalent between IVF with and without PGT-A, PGT-A incurs an additional cost to the patient of \$4,551-4,742.

**P-323** Tuesday, October 15, 2019 6:30 AM

**A COST EFFECTIVENESS ANALYSIS OF PREIMPLANTATION GENETIC TESTING FOR SICKLE CELL TRAIT COUPLES.**

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**OBJECTIVE:** Sickle cell disease (SCD) is a common autosomal recessive disease that results in significant morbidity and early mortality.

	<35	35	36	37	38	39	40	41	42
PGT-A, CLBR (%)	54.0	47.2	46.7	43.8	39.8	34.4	28.9	22.7	13.9
No PGT-A, CLBR (%)	56.6	46.3	42.9	37.7	31.8	25.7	19.6	13.0	9.9
PGT-A, TLBR (%)	6.7	6.3	5.3	5.1	4.1	3.3	1.8	1.0	1.3
No PGT-A, TLBR (%)	13.0	10.5	8.8	8.2	6.5	5.2	3.3	1.9	1.3
PGT-A cost, Patient (\$)	17,410	17,384	17,323	17,205	17,106	16,742	16,596	16,262	15,663
No PGT-A cost, Patient (\$)	12,859	12,641	12,591	12,231	12,189	11,913	11,569	11,124	11,039
PGT-A cost, Societal (\$)	37,746	36,021	34,644	33,919	31,813	29,182	26,537	23,847	20,843
No PGT-A cost, Societal (\$)	41,979	35,981	33,689	31,687	28,714	25,030	20,834	16,941	15,509

Preimplantation genetic testing for monogenic diseases (PGT-M) is the process in which embryos created via in vitro fertilization (IVF) are tested for diseases like SCD; unaffected embryos may then be selected for transfer. In the United Kingdom, this technology is available to couples with SCT; in the U.S., insurance does not routinely cover this intervention. Whether the costs of IVF with PGT-M (IVF+PGT-M) to avoid the birth of a child with SCD outweigh the lifetime medical costs of a person with SCD is unknown.

**DESIGN:** Cost effectiveness analysis.

**MATERIALS AND METHODS:** We constructed a decision analytic model using TreeAge Pro 2019 (TreeAge Software Inc, Williamstown, MA) for couples known to both have SCT, attempting to conceive with natural conception (NC) versus IVF+PGT-M. The primary outcome variable was quality adjusted life years (QALYs) for children born with or without SCD. The model incorporated probabilities and cost estimates of relevant clinical events using data from published literature. The total cost for each potential child included the cost of conception, lifetime medical care, and future potential income. We assumed all patients undergoing IVF+PGT-M also test embryos for aneuploidy (PGT-A); data were thus derived for euploid embryo transfers for all IVF+PGT-M patients. To determine whether IVF+PGT-M is cost effective, we calculated the incremental cost effectiveness ratio (ICER). Here, the ICER is defined as the ratio of the difference between the per patient per QALY costs of IVF+PGT-M compared with NC. Costs were converted to 2018 U.S. dollars. To examine the impact of changes in model input parameters, a sensitivity analysis was performed. We assumed a willingness to pay of \$30,000 which is equal to the average cost to conceive a non-SCD child in one IVF+PGT-M cycle with embryo transfer.

**RESULTS:** Healthy adults in the US have an average life expectancy of 79 years, versus 54 for individuals with SCD. By avoiding SCD, IVF+PGT-M for SCD offers a 23.27% increase in QALYs. The mean cost of SCD-related care is \$26,319 per patient per life-year, while the average cost of IVF+PGT-M is \$24,750 per non-SCD embryo transferred. The ICER for IVF+PGT-M as compared with NC was \$22,881 per QALY added to the lifespan. Therefore, the cost per QALY of conceiving a healthy child with IVF+PGT-M is \$22,881 less than the cost of SCD-related care. The ICER was less than the expected willingness to pay, and improved substantially with a decrease in PGT-M cost. Monte Carlo simulations demonstrated that IVF+PGT-M is cost effective in nearly all iterations at an acceptability cut off of \$30,000.

**CONCLUSIONS:** IVF+PGT-M is a cost effective strategy to increase QALYs for children conceived by SCT couples. These data beg for quality improvement studies to increase patient awareness of and access to IVF+PGT-M. Offering this option to SCT couples on a more widespread basis might help families avoid the financial, emotional and familial burdens of raising a child with SCD, and decrease the societal costs of SCD.

**P-324** Tuesday, October 15, 2019 6:30 AM

#### **TRANSFERRING SELECTED EMBRYOS, AFTER PGT-A DIAGNOSED AS "ABNORMAL," WHERE PATIENTS WERE REFUSED SUCH TRANSFERS AT THEIR ORIGINAL IVF CENTERS.**

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**OBJECTIVE:** To report outcomes of IVF cycles in which, by PGT-A "abnormal," embryos were transferred after such transfers had been earlier refused at the patients' original IVF centers.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Since 2014, our center has been offering couples with only chromosomally "abnormal" embryos after PGT-A ("aneuploid" or "mosaic"), selective transfers of such embryos under an experimental consent. We at ASRM 2015 reported the first healthy births following such transfers in the world.<sup>1</sup> Since our center does not recommend PGT-A, such patients uniformly had their IVF cycles elsewhere and transferred their embryos to or center after being refused transfers at their original centers. Written informed consent pointed out risks of a chromosomal abnormality, miscarriage risks, did not differentiate between "mosaic" and "aneuploid" embryos and excluded from transfer embryos with non-lethal and with >3 abnormalities. Patients also consented to early pregnancy diagnosis and termination of pregnancy, should a pregnancy be aneuploid. Number of embryos transferred followed ASRM guidelines.

**RESULTS:** Since our original report with collaborating colleagues from 2 other centers in 2015 where we reported 5 normal pregnancies,<sup>1</sup> we counselled 38 patients who moved their embryos to CHR. Among those, so far 22 have elected to have a transfer, with 7 (26.9%) achieving clinical preg-

nancy; 3/7 miscarried (42.8%); 1 was aneuploid pregnancy, 1 was 46XX, with maternal contamination ruled out and a third is currently pending a genetic results. Three pregnancies delivered normal offspring. Most IVF centers were cooperative in transferring embryos, though one transfer only occurred after the couple engaged a lawyer.

**CONCLUSIONS:** Here reported pregnancy and live birth rates are slightly lower than we reported in our initial series<sup>1</sup> and others reported,<sup>2</sup> but patients here were much older ( $44.2 \pm 4.4$  years). Considering age, the miscarriage rate was actually relatively low, confirming earlier reports. Since some of the original clinics had asked PGT-A laboratories *not* to report "mosaicism," accurate separation of "mosaic" and "aneuploid" transfers was not possible. Current definitions, based on percentages of aneuploid DNA within a single trophoctoderm biopsy, however do, anyhow, have no empiric basis.<sup>3</sup> Here presented data, therefore, suggest that, due to still excellent, pregnancy and delivery chances, embryos with alleged lethal aneuploid DNA should not be disposed. Because of downstream self-correction of human embryos, even intrauterine transfers of non-lethal "abnormalities" may have to be considered when no other embryos are available for transfer.

#### **REFERENCES**

<sup>1</sup> Gleicher et al., *Fertil Steril*. 2015; 104(Suppl), 3:e9 (ASRM, 2015).

<sup>2</sup> Munné et al., *Fertil Steril*. 2017; 108(1), 62-71

<sup>3</sup> Kushnir et al., *Reprod Biol Endocrinol*. 2018; 16(1):6

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**P-325** Tuesday, October 15, 2019 6:30 AM

#### **CLINICAL OUTCOMES OF SINGLE EMBRYO TRANSFER WITH MOSAIC EMBRYO: HIGH-GRADE MOSAIC OR LOW-GRADE MOSAIC EMBRYO, DOES IT MATTER ?.**

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**OBJECTIVE:** Preimplantation genetic testing (PGT-A) with the use of the high resolution next-generation sequencing (hr-NGS) that can detect mosaicism in excess of 20%. The 20-80% range as mosaic and transferred with caution, only in absence of euploid embryos. These cut-off level is an ongoing debate. Despite recent reports suggested low-grade mosaic embryos transfer (<50%) could result in healthy newborn as euploid embryo, very little is known about outcomes of high-grade (>50%) mosaic transfer. The aim of the study is to investigate whether high-grade mosaic embryos were capable of implanting and leading to ongoing pregnancies?

**DESIGN:** Retrospective analysis of the clinical outcome of single embryo transfer with low-grade mosaic embryos (30%-40% abnormal cells in the trophoctoderm biopsy) and high-grade mosaic embryos (50%-80%) abnormal cells in the trophoctoderm biopsy) as diagnosed with the use of hr-NGS.

**MATERIALS AND METHODS:** 108 Single embryo transfers with mosaic blastocyst were transferred at in Lee Women's Hospital from July of 2016 through December of 2017. SET cycles of 83 low-grade mosaic transfers and 25 high-grade mosaic transfers were analyzed retrospectively. Mosaic levels and clinical outcomes were evaluated in this study.

Chromosomal abnormality of biopsied trophoctoderm cells was analyzed by NGS (VeriSeq PGS-MiSeq, illumina). Before implantation, we counseled patients on the potential consequences of transferring a mosaic embryo and obtained the informed consent from each patient. After implantation, we analyzed the association between conditions of the mosaic blastocyst and pregnant outcomes of the SET for each patient. In order to confirm the condition of fetal chromosomes, NIPS or karyotyping were performed when patients were pregnant more than 10 weeks.

**RESULTS:** Our results of low-grade mosaic SET vs. high-grade mosaic SET: comparable implantation rate (51.8%, 43/83 vs 52%, 13/25;  $p=0.99$ ), ongoing pregnancy rate (47%, 39/83 vs 36%, 9/25;  $p=0.33$ ), and live birth rate (44.5%, 37/83 vs 36%, 9/25;  $p=0.45$ ) between two groups. However, significantly higher abortion rate in the high-grade mosaic SET (5.1%, 2/39 vs. 30.7%, 4/13;  $p=0.012$ ) There was also no statistical difference was observed in the average gestational weeks at delivery ( $38.3 \pm 1.6$  vs.  $38.6 \pm 1.2$  weeks;  $p=0.53$ ) and average birth weight ( $3015 \pm 507$  vs.  $3076 \pm 94$  gm;  $p=0.73$ ). Transferring of mosaic embryo with different chromosomal numbers did not affect the implantation rate and clinical outcomes. SETs of different mosaic types (whole chromosomal or segmental chromosomal abnormality) and grade of mosaicism had no obvious impact on clinical outcomes in our study. The results of NIPS or karyotyping of prenatal diagnosis in all pregnant women were normal.

**CONCLUSIONS:** The findings of this study are valuable for understanding the clinical results after SET with low/high-grade level mosaic embryos. We demonstrated that the high-grade mosaic embryos have the probability of resulting in implantation and healthy newborn but with higher abortion rate than low-grade mosaic embryos.

**P-326** Tuesday, October 15, 2019 6:30 AM

**CLINICAL EXPERIENCE FOLLOWING PGT ANALYSIS OF 38,000 CONSECUTIVE EMBRYOS USING A FAST-SEQS NGS-BASED ASSAY.**

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**OBJECTIVE:** To report our clinical experience utilizing a FAST-SeqS NGS-based PGT assay, including aneuploidy rates and outcome data.

**DESIGN:** Patients undergoing IVF may elect to pursue preimplantation genetic testing for aneuploidy (PGT-A) to identify euploid embryos, with the goal of increasing pregnancy and live birth rates while reducing multiple gestations and time to pregnancy. Preventing transfer of embryos with chromosomal abnormalities is essential to improving PGT-derived pregnancy outcomes.

**MATERIALS AND METHODS:** Trophectoderm samples were analyzed using our modified FAST-SeqS method and associated bioinformatics pipeline. FAST-SeqS can accurately detect whole chromosome and segmental aneuploidies ( $\geq 10$  MB), whole genome uniparental disomy (WG-UPiD or haploidy), all forms of triploidy, other polyploidies, and many instances of single chromosome UPiD.<sup>1-3</sup> The likelihood of transfer and aneuploidy rates, stratified by egg age, fertilization type, day of biopsy, and clinical indication were assessed.

**RESULTS:** The dataset consisted of 138,643 embryos from 29,624 cycles. Egg age ranged from 18-55 years (mean of 35). The average number of biopsy samples per case was 4.5. Of resulted samples, 56% were euploid; 77% of all cycles had at least one euploid embryo (Table 1). As previously reported, the only factors affecting aneuploidy rates were egg age and day of biopsy.<sup>4</sup>

Excluding embryos from known translocation carriers, 10% of embryos had a segmental abnormality, 32% were observed in conjunction with at least one whole chromosome abnormality. Segmental changes were seen in all chromosomes and the rate was independent of egg age.

Out of 75,726 samples analyzed with our SNP enhancement, 1.6% were polyploid. These polyploidies consisted of 924 triploid, 164 haploid/WG-UPiD and 154 tetraploids, many of which would have been misclassified as euploid or mosaic with other NGS-based assays.<sup>5</sup>

Of 1,574 transfers for which outcome data was provided, the observed clinical pregnancy rate was highly variable across clinics, with a range of 38%-80% (mean of 61%). The mean ongoing/live birth rate per transfer was 57%, with a clinic-specific rate range of 38%-72%.

**CONCLUSIONS:** The majority of patients in this dataset had at least one euploid embryo for transfer. Consistent with previous reports, an age-related decline in euploidy was observed, and segmental aneuploidy was independent of age.<sup>6,7</sup> Our data, stratified by egg age and number of embryos tested, is a valuable counseling tool for patients considering PGT-A.

**REFERENCES**

- Gole J *et al. Fertil Steril.* 2016;105(2):e25
- Umbarger MA *et al. Fertil Steril.* 2016;106(3): e152
- Kosheleva K *et al. Fertil Steril.* 2018;109(3):e54.
- Davies, J *et al. Fertil Steril.* v108, n3, e283-284, 2017
- Marin D. *et al. Curr Opin Obstet Gynecol.* 2017; 29(3):168-174
- Escudero, T *et al. Fertil Steril.* v105, n2, p. e20 - e21, 2016
- Franasiak J.M., *et al. Fertil Steril.* v101, n3, p. 656-663, 2014

TABLE 1. Likelihood of Euploid Embryos

Age	% of PGT cycles with at least 1 euploid embryo	
	1-3 embryos	4-6 embryos
Egg Donor	84%	99%
<35	81%	98%
35-37	73%	96%
38-40	57%	87%
41-42	33%	68%
>42	19%	49%
All	59%	91%

SUPPORT: Invitae

**P-327** Tuesday, October 15, 2019 6:30 AM

**PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) AND TECHNOLOGY PLATFORM: DOES PLATFORM INFLUENCE EUPLOID CALL RATES AND/OR SUBSEQUENT PREGNANCY OUTCOMES?**

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**OBJECTIVE:** With the evolution in technology platforms for PGT-A over the past decade, benefits to genetic testing companies include increased speed and reduced expense. Whether benefits have concurrently accrued to patients in terms of pregnancy outcomes is unclear. We sought to 1) compare euploid call rates by technology platform, and 2) determine whether pregnancy outcomes after single embryo transfer (SET) of a euploid blastocyst varied as a function of platform.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** Trophectoderm biopsies for PGT-A at a single academic center between 2010-2019 were reviewed. Grade BB or better (Gardner criteria) blastocysts are biopsied on day 5 or 6 at our institution. Euploid call rates (euploid results per biopsied blastocysts) were compared between three technology platforms: single nucleotide polymorphism array (SNPa), array comparative genome hybridization (aCGH) and next generation sequencing (NGS), provided by two major commercial laboratories. Total euploid blastocysts generated per cycle were also compared. Mosaicism was masked. Generalized linear models were used to adjust for age and number of eggs collected, and account for the clustered nature of the data. We similarly compared rate of live birth or ongoing pregnancy following SET of a euploid blastocyst, between technology platforms.

**RESULTS:** 8,759 blastocyst biopsies were generated from 1,253 patients over 1,873 IVF cycles. Euploid call rates were lowest among SNPa and highest among aCGH cycles (Table). Controlling for age and number of oocytes collected, compared to SNPa, aCGH cycles had 28% increased odds of euploid calls (OR 1.28, 95% CI 1.13, 1.44, p<0.001). NGS also had higher euploid call rates vs SNPa (OR 1.15, 95% CI 1.01, 1.31, p=0.03). aCGH vs NGS had similar euploid call rates (p=0.18). Total euploid blasts per cycle differed by technology platform (p=0.01), as aCGH yielded more euploid blastocysts than SNPa cycles, controlling for age and number of oocytes

	Age*	#eggs collected/ cycle**	Euploid***	Aneuploid	Undetermined	Pregnant after SET*	Live birth/ Ongoing pregnancy after SET*
SNP array	37.9 (4.0)	17.9 (9.3)	42.1%	56.0%	1.9%	64.3%	55.1%
Array CGH	37.9 (3.6)	17.7 (10.3)	48.0%	50.8%	1.2%	68.1%	52.8%
NGS	37.8 (4.0)	16.0 (8.8)	46.1%	52.7%	1.3%	67.8%	57.6%
<b>Total</b>	<b>37.9 (3.9)</b>	<b>17.4 (9.4)</b>	<b>44.2%</b>	<b>54.2%</b>	<b>1.6%</b>	<b>65.6%</b>	<b>54.9%</b>

Mean (SD) or %

\*p=NS

\*\*p=0.01

\*\*\*p<0.001

(coef 0.29, 95% CI 0.08, 0.51,  $p < 0.01$ ). Rates of live birth or ongoing pregnancy after euploid SET ( $n=987$  transfers) did not differ by platform (Table;  $p=0.83$ ).

**CONCLUSIONS:** Euploid call rates differ as a function of PGT-A technology platform after adjusting for age and eggs collected. However, these differences do not seem to translate into different pregnancy outcomes after SET of a “euploid” blastocyst. Further investigation should attempt to reconcile these differences and clarify if and how advances in PGT-A technology platforms translate to patients.

**P-328** Tuesday, October 15, 2019 6:30 AM

**PREVIOUSLY VITRIFIED EMBRYOS CAN BE SUCCESSFULLY WARMED, BIOPSIED OR REBIOPSIED, AND PROVIDE RESULTS.**

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**OBJECTIVE:** This study aimed to evaluate whether vitrified embryos could be warmed, biopsied or rebiopsied, and provide meaningful preimplantation genetic testing for aneuploidy (PGT-A) results. The vitrified and warmed embryos include both those biopsied for the first time, as well as those biopsied for a second time (rebiopsied) due to inconclusive results.

**DESIGN:** This was a retrospective study performed at a large, private IVF center which included 242 embryos being warmed and biopsied for the first time and 28 embryos being warmed and rebiopsied.

**MATERIALS AND METHODS:** Day 5 and 6 embryos of good and fair quality were vitrified for potential future use. Embryos were warmed, given time to re-expand, and biopsied. Some embryos that initially survived the warming process were unable to be biopsied due to degeneration or developmental arrest prior to re-expansion. Samples were sent to a third-party testing laboratory for PGT-A testing via aCGH, SNP array or NGS.

**RESULTS:** Two hundred forty-two embryos were warmed and biopsied for the first time. One hundred seventy-eight (74%) reached a stage and quality suitable for biopsy. One hundred ten of these embryos (62%) were euploid. When classified by age group, patients 37 years and under yielded a euploid rate of 66%, while patients 38 years and older yielded a euploid rate of 44%. Twenty-eight embryos were warmed for rebiopsy. Twenty-two (79%) reached a stage and quality suitable for biopsy. Twelve of these (55%) were euploid. When classified by age group, patients 37 years and under yielded a euploid rate of 62%, while patients 38 years and older yielded a euploid rate of 50%. Overall, 96% of thawed embryos (258/270) survived the warming process, and 78% of surviving embryos (200/258) were able to be biopsied or rebiopsied.

**CONCLUSIONS:** Embryo thaw survival rates are high, but approximately one in five embryos (22%) degenerate or arrest prior to re-expansion and cannot be biopsied. The majority of vitrified embryos can be successfully

biopsied or rebiopsied and provide meaningful PGT-A results. With informed consent, embryo thaw and biopsy or rebiopsy is a reasonable clinical option.

**P-329** Tuesday, October 15, 2019 6:30 AM

**ARTIFICIAL INTELLIGENCE: NON-INVASIVE DETECTION OF MORPHOLOGICAL FEATURES ASSOCIATED WITH ABNORMALITIES IN CHROMOSOMES 21 AND 16.**

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**OBJECTIVE:** To examine whether Artificial Intelligence (AI) algorithms and computer vision technology can non-invasively identify embryos with key morphological features associated with abnormalities of chromosome 21 and 16.

**DESIGN:** AI Analysis of embryo images in private reproductive technology programs.

**MATERIALS AND METHODS:** Approximately 2,000 static 2D images of Day 5 blastocysts with related pregnancy and pre-implantation genetic testing for aneuploidy (PGT-A) outcomes were assessed. Images were divided into three groups: training, validation, and blind test sets. Two AI models were trained, validated, and tested on embryo images by a further blind set test of 461 images with known PGT-A outcomes.

**RESULTS:** Our results show a high level of accuracy with the use of AI in detecting embryological morphological changes associated with additions to chromosome 21 or an additional full copy of the chromosome. A blind data set of 54 images achieved an accuracy of 81.5%. To expand the model to include all abnormalities of chromosome 21, we achieved an accuracy of 71% from 214 images. This reduction in accuracy is most likely the result of increased morphological variability between embryos with different (broader) abnormalities in chromosome 21. Using the same methodology, an accuracy of 73.1% was obtained when we were able to determine abnormalities of chromosome 16 in 214 images.

**CONCLUSIONS:** Embryonic chromosomal abnormalities are known to lead to implantation failure, pregnancy loss, severe chromosomal diseases (e.g. Down and ATR-16 syndromes) and have recently been associated with developmental disorders including Autism<sup>1</sup>. One of the major limitations of PGT-A analysis by traditional genetic analysis is the presence of chromosomal mosaicism within the developing embryo<sup>2</sup>. Recent advances in non-invasive embryo ploidy determination by either morphokinetic analysis by time-lapse imagery<sup>3</sup> or cell free DNA isolation from either spent conditioned culture medium<sup>4</sup> or blastocoe fluid<sup>5</sup> have shown promise, but concordance studies have shown otherwise<sup>6</sup>. This study presents, for the first time, that AI can non-invasively determine whether certain morphological features of a Day 5 blastocyst are associated with specific chromosomal abnormalities of human embryos. Additional studies and analyses are under

1 <sup>st</sup> Biopsy							
Total # of patients	Average Age	Warmed	Survival	Biopsied	Euploid	Aneuploid	Other*
48	35.3	242	232 96%	178 74%	110 62%	56 31%	12 7%

\*Other = 9 mosaic, 2 no DNA amplification, 1 no call

Rebiopsy							
Total # of patients	Average Age	Warmed	Survival	Biopsied	Euploid	Aneuploid	Other*
24	36.2	28	26 93%	22 79%	12 55%	8 36%	2 9%

\*Other = 1 mosaic, 1 no DNA amplification

way to increase specificity and explore other chromosomal abnormalities by including larger data sets

#### REFERENCES

- Weiss, L.A., Shen, Y., Korn, J.M., Arking, D.E., Miller, D.T., Fossdal, R., Saemundsen, E., Stefansson, H., Ferreira, M.A., Green, T. and Platt, O.S., 2008. Association between microdeletion and microduplication at 16p11.2 and autism. *New England Journal of Medicine*, 358(7), pp.667-675.
  - Nakhuda, G., Jing, C., Butler, R., Guimond, C., Hitkari, J., Taylor, E., Tallon, N. and Yuzpe, A., 2018. Frequencies of chromosome-specific mosaicism in trophoctoderm biopsies detected by next-generation sequencing. *Fertility and sterility*, 109(5), pp.857-865.
  - Zaninovic, N., Irani, M., Meseguer, M., 2017. Assessment of embryo morphology and developmental dynamics by time-lapse microscopy: is there a relation to implantation and ploidy? *Fertility and Sterility*, 108(5), pp.722 – 72.
  - Belandres, D., Shamonki, M. and Arrach, N., 2019. Current status of spent embryo media research for preimplantation genetic testing. *Journal of assisted reproduction and genetics*, pp.1-8.
  - Magli, M.C., Albanese, C., Crippa, A., Tabanelli, C., Ferraretti, A.P. and Gianaroli, L., 2019. Deoxyribonucleic acid detection in blastocoelic fluid: a new predictor of embryo ploidy and viable pregnancy. *Fertility and sterility*, 111(1), pp.77-85.
  - Tsuiko, O., Zhigalina, D., Jatsenko, T., et. al. Karyotype of the blastocoel fluid demonstrates low concordance with both trophoctoderm and inner cell mass. *Fertility and Sterility*, 109(6), pp.1127 – 1134.
- SUPPORT: None.

**P-330** Tuesday, October 15, 2019 6:30 AM

#### IF ANY MOSAICISM IS IDENTIFIED IN THE TROPHOCTODERM, THERE IS A 26% CHANCE OF MOSAICISM BEING PRESENT IN THE INNER CELL MASS; A CLINICAL PARADIGM, DO YOU TRANSFER MOSAIC EMBRYOS?.



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**OBJECTIVE:** To determine the correlation of mosaicism identified in the trophoctoderm (TE) to the rate of mosaicism within the inner cell mass (ICM).

**DESIGN:** Prospective

**MATERIALS AND METHODS:** 78 patients (631 embryos) underwent IVF and PGT-A was performed. All patients underwent IVF due to repeat pregnancy loss, previous unsuccessful IVF cycles, decreased ovarian reserve

or unexplained infertility between 2012 and 2016. Embryos were first biopsied at the cleavage stage and if aneuploid, remained in culture to the blastocyst stage. At the blastocyst stage of development, the ICM and TE were separated and blindly analyzed. Molecular karyotypes were performed by enhanced next generation sequencing (NGS) using a Personal Genome Machine (PGM) or S5. By deep sequencing and proprietary algorithm's, we can detect mosaicism at approximately the 10% level. This sequencing provided a minimum of over 3.5 million reads with a median sequencing fragment of 186bp.

**RESULTS:** 55% (350/631) of cleavage stage embryos were aneuploid. Of these, 37% (131/350) differentiated to the blastocyst stage. 26% (34/131) of these embryos were found to have mosaicism within both the TE and ICM.

**CONCLUSIONS:** Our results indicate that using an enhanced NGS technology, a significant percentage (26%) of embryos with detectable levels of mosaicism as determined by PGT-A in the TE population will be associated with mosaicism within the ICM as well. Clinically, an aneuploid mosaic fetus may result in a live birth with significant mental and physical deficits. Given this risk, we strongly recommend that the transfer of mosaic embryos only be considered as a last resort for very poor prognosis patients following comprehensive informed consent by a geneticist.

**P-331** Tuesday, October 15, 2019 6:30 AM

#### PRE-IMPLANTATION GENETIC TESTING (PGT-A) USING FAST-SEQS NGS OF IN VIVO CONCEIVED BLASTOCYSTS RECOVERED BY UTERINE LAVAGE.



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**OBJECTIVE:** To report the chromosomal characterization of in vivo conceived embryos utilizing a FAST-SeqS NGS-based PGT-A assay

**DESIGN:** Reported rates of euploidy per oocyte age differ amongst fertility programs. The most striking range reported, with a relatively homogenous group of oocyte donors, suggests that stimulation, culture conditions and manipulation may impact ploidy.<sup>1</sup> IVF/PGT-A reduces the transfer of abnormal embryos but may be cost prohibitive even with minimal stimulation. A preliminary report demonstrated success with retrieving in vivo created embryos for PGT-A using a patented uterine lavage system<sup>2</sup>. In vivo culture would reduce the financial burden and the potential untoward effects of the in vitro environment.

**MATERIALS AND METHODS:** Twenty women underwent ovulation induction and donor insemination, with uterine lavage 5 days later, as previously described.<sup>2</sup> The study had IRB approval and oversight by the Ministry of Health. TE biopsy was performed after lavage or following in

Study ID	Total # embryos at lavage (day 5)	Day 5 Grade <sup>6</sup> (Recovery)	Day 6 Grade <sup>6</sup> (24hr in vitro culture)	Bx Day	Interpretation	Misc Result
186	4	6 cell frag	3CC	6	Aneuploid	del(1)(q41)
187	11	4AA	6AA	6	Normal	
		4AA	6AA	6	Normal	
		9cell	3CC	6	Normal	
		4BA	5BA	6	Normal	
		6 cell vac	3CC	6	Aneuploid	Monosomy 8
192	3	12 cell, vac	4AB	6	Mosaic	trisomy 22(mos)
		8 cell, vac	2 (early)	6	Aneuploid	53,X,+1,+2,+3,+8,+9,+17,+20,+21
197	4	3AB	4AB	6	Normal	
		morula	3CC	6	Mosaic	del(4)(q32) (mos)
193	1	10 cell, vac	3CC	6	Normal	
185	2	2 (early)	6BB	6	Aneuploid	Monosomy 13
196	4	morula	3CC	6	Normal	
200		5AA		5	Normal	
		6AA		5	Normal	
		3CC		5	Normal	
202	2		6CC	5	Normal	
199	1		5BB	5	Normal	

vitro culture. Biopsies were analyzed using Invitae's FAST-SeqS NGS/bioinformatics pipeline which detects whole chromosome and segmental aneuploidies ( $\geq 10$  MB). Whole genome uniparental isodisomy (WG-UPiD or haploidy), all forms of triploidy, and most single chromosome UPiD are also leveraged from SNP data.<sup>3-5</sup>

**RESULTS:** Thirty-five viable embryos were recovered from 15 patients. Five blasts were biopsied on day 5 and 13 biopsied on day 6 from 10 patients total. Mean egg age for the resulting biopsied embryos was 26 (range 21-30). In all ( $n=18$ ), 12 embryos (67%) were euploid, 4 were aneuploid (22%) and 2 mosaic (5.7%). Of interest, all 5 embryos biopsied on day 5 were euploid regardless of grade; the day 6 aneuploidy rate was 46%.

**CONCLUSIONS:** In this small sample, the higher aneuploidy rate in day 6 biopsies was consistent with Invitae's previous report<sup>7</sup>. Additionally, the euploid rate for egg age ( $\leq 30$ ) and the mosaic rate are consistent with Invitae's internal data (68% and 5.7%, respectively). Uterine lavage is an effective alternative to IVF for the recovery of viable embryos for PGT-A. Additional studies are planned to confirm these findings.

#### REFERENCES

1. Munne, S. et al., Human Reproduction 2017;32:743-749
2. Munne, S. et al., ESHRE Abstract, 2018;0261
3. Gole J et al. Fertil Steril. 2016;105(2):e25.
4. Umbarger MA et al. Fertil Steril. 2016;106(3): e152.
5. Kosheleva K et al. Fertil Steril. 2018;109(3):e5.
6. Gardner DK, et al. Fertil Steril 2000;73:1155-8.
7. Davies, J et al. Fertil Steril 2017;108(3) e283-284.

**SUPPORT:** FINANCIAL SUPPORT: Previvo Genetics, Invitae

Protocol Registration and Results System (PRS) **Trial Registration Number** and Name: Punta Mita Study TD-2104: Clinical Trials NCT03426007

\*Previvo Uterine Lavage System, Generation 1.9, Previvo Genetics, Inc., San Carlos, California

**P-332** Tuesday, October 15, 2019 6:30 AM

### PGT-A (PREIMPLANTATIONAL GENETIC SCREENING) IN PATIENTS WITH PARTIAL X MONOSOMY USING OWN OOCYTES: IS A SUITABLE INDICATION?.

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**OBJECTIVE:** Evaluate the reproductive outcome of preimplantational genetic diagnosis (PGT-A) in patients with mosaic Turner's syndrome (MTS) using own oocytes, compared to mosaic and pure Turner syndrome (PTS) using ovum donation (OD)

**DESIGN:** Retrospective cohorts study from January 2011 until December 2017, scrutinizing >120,000 IVF cycles from 14 infertility clinics in Spain, searching for pure or mosaic TS, confirmed by the karyotype

**MATERIALS AND METHODS:** University-affiliated private-infertility centre. 67 PGT-A in MTS patients (FISH/arrays-NGS), on which 65 controlled ovarian hyperstimulation cycles (COH) were performed, and embryo transfers (ET) performed in 32. As well, 165 women belonged to the OD-MTS or PTS group, with 157 cycles and 156 ET

**RESULTS:** Mean age and body mass index was 38.1y(37.3-39) vs.37.8y(37-38.7), 24.6kg/m<sup>2</sup>(23.4-25.8)vs.23.6kg/m<sup>2</sup>(22.9-24.2.1) for PGT-A (MTS) and OD (MTS/PTS) respectively, without significant differences found.

The mean number of oocytes MII retrieved/received, for PGT-A in MTS and OD (MTs and PTS) respectively were 10.56 (9.84-11.27) and 9.32 (8.43-10.22); embryos transferred 1.5(1.3-1.7) and 1.79(1.69-1.89); implantation rate per ET 22.58% (8.5-36.65) in PGT-A and 35.19%(28.52-41.86) in OD, not reaching statistical significance, but showing differences.

Pregnancy rates tended to be higher but not significant ( $p=0.27$ ) in OD 52.6% 95CI(60.4-44.7) vs. PGT-A 41.9% 95CI(24.8-59.0), while miscarriage rates remained statistically comparable, although with a noticeable higher rate when using donated oocytes, being OD 42.3% 95CI(31.6-52.3) vs. PGT-A 10.3% 95CI(0.0-.26.8), resulting in live-birth rates of OD 28.84%95CI (7.1-21.7), higher than observed for PGT-A 21.87%95CI(7.5-36.1).

**CONCLUSIONS:** The retrospective nature of this study may be a reason for caution. Despite being the largest sample size ever reported with PGT-A

in MTS the number of patients included is still low. Subsequently, the conclusions reached should be taken carefully until a larger body of evidence will be available

Oocyte donation (OD) seems to be the best reproductive option in female who are missing one of the X chromosomes, with or without mosaicisms present.

Nevertheless, based on the previous data, PGT-A is a valid therapeutic option in patients with mosaic Turner's syndrome (MTS) using own oocytes and OD should not necessarily be recommended directly as the treatment of choice.

#### REFERENCES

- Hovatta O. Pregnancies in women with Turner's syndrome. Ann Med 1999; 31:106-10.
- Pasquino AM, Passeri F, Pucarelli I, Segni M, Municchi G. Spontaneous pubertal development in Turner's syndrome. Italian Study Group for Turner's J Clin Endocrinol Metab 1997; 82:1810-3.
- Abir R, Fisch B, Nahum R, Orvieto R, Nitke S, Ben Rafael Z. Turner's syndrome and Å fertility: current status and possible putative prospects. Hum ReprodUpdate 2001;7:603-10.
- De Braekeleer, M., Dao, T.N.. (1990) Cytogenetic studies in copules experiencing repeated pregnancy losses. Hum Reprod, 5, 519-528.
- Gianaroli, L., Magli, M.C., Ferraretti, AP., Munnè, S. (1999) Preimplantation diagnosis for aneuploidies in patients undergoing *in vitro* fertilization with a poor prognosis: identification of the categories for which it should be proposed. Fertil Steril, 72, 837- 844.
- Kahraman, S., Bahce, M., Samli, H., Imirzalioglu, N., Yakisn, K., Cengiz, G., Donmez, E. (2000) Healthy births and ongoing pregnancies by preimplantation genetic diagnosis in patients with advanced maternal age and recurrent implantation failure. Hum Reprod, 15, 2003-2007.
- Onalan G, Yilmaz Z, Durak T, Sahin FI, Zeyneloglu HB. Successful pregnancy with preimplantation genetic diagnosis in a woman with mosaic Turner syndrome. Fertil Steril. 2011 Apr;95(5):1788.e1-3.
- Tarani L, Lampariello S, Raguso G, Colloridi F, Pucarelli I, Pasquino AM, et al. Pregnancy in patients with Turner's syndrome: six new cases and review of literature. Gynecol Endocrinol 1998;12: 83-7.
- Ogata T, Matsuo N. Turner syndrome and female sex chromosome aberrations: deduction of the principal factors involved in the development of clinical features. Hum Genet 1995;95:607-29.
- Landin-Wilhelmsen K, Bryman I, Hanson C, Hanson L. Spontaneous pregnancies in a Turner syndrome woman with Y-chromosome mosaicism. J Assist Reprod Genet 2004;21:229-30.
- Lutjen P, Trounson A, Leeton J, Findlay J, Wood C and Renou P. The establishment and maintenance of pregnancy using *in vitro* fertilization and embryo donation in aÅ patient with primaryÅ ovarian failure. Nature 1984; 307,174-175
- Press F, Shapiro HM, Cowell CA, Oliver GD. Outcome of ovum donation in Turner's syndrome patients. Fertil Steril. 1995 Nov;64(5):995-8.
- Khastgir G, Abdalla H, Thomas A, Korea L, Latache L, Studd J. Oocyte donation in Turner's syndrome: an analysis of the factors affecting the outcome. Hum Reprod. 1997 Feb;12(2):279-85.
- Foudila T, Söderström-Anttila V, Hovatta O. Turner's syndrome and pregnancies after oocyte donation. Hum Reprod. 1999 Feb;14(2):532-5. PubMed PMID: 10100005.
- Alvaro Mercader B, Imbert R, Demeestere I, Englert Y, Delbaere A. Pregnancy outcome after oocyte donation in patients with Turner's syndrome and partial X monosomy. Hum Reprod. 2011 Aug;26(8):2061-8.
- Deligeorgiou E, Stergioti E, Dimopoulos KD, Karountzou V, Prapas Y. Pregnancy outcome after oocyte donation in patients with Turner's syndrome: Clinical experience and management. J Obstet Gynaecol. 2016 May;36(4):504-7.

**P-333** Tuesday, October 15, 2019 6:30 AM

### EMBRYO CULTURING CONDITIONS AND THE DEVELOPMENT OF NON-INVASIVE PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY DETECTION (NI-PGT-A).

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**OBJECTIVE:** The absence of standardised culturing conditions or molecular testing methodologies, including whole genome amplification (WGA) used for non-invasive preimplantation genetic testing of spent embryo culture media for aneuploidy (NI-PGT-A) may explain the variable rates of

concordance reported between the spent embryo culture media and embryo biopsy results to-date. Culture conditions impact the accumulation of embryonic and contaminating DNA in spent embryo culture media and optimisation of either the culturing conditions, molecular testing methodologies, or both, should yield the highest level of concordant results for NI-PGT-A.

**DESIGN:** This study examined rates of ploidy concordance between spent embryo culture media and embryo biopsies to evaluate the impact of culturing conditions on NI-PGT-A results.

**MATERIALS AND METHODS:** Spent embryo culture media was collected from single embryo culture droplets following biopsy of the embryo for PGT-A then stored at -20°C, with ethics approval. Equivalent volumes of spent embryo culture media samples from 10µl-60µl culture droplets, from either continuous (n=4 labs) or two-step cultures (n=4 labs) were whole genome amplified using DOPlify® kit reagents (PerkinElmer). WGA DNA yield was assessed by gel electrophoresis and high sensitivity quantification using a Qubit® instrument (Thermo Fisher® Scientific). Next generation sequencing libraries were generated according to the PG-Seq™ kit 48 sample protocol and sequencing was performed on a MiSeq® instrument (Illumina®). Data was bioinformatically aligned to hg19, and WGA DNA yield, NGS metrics, and whole chromosome aneuploidy concordance with the PGT-A result for the embryo biopsy were determined.

**RESULTS:** Whole genome amplification using the DOPlify® kit reagents resulted in the amplification of 78-100% of spent embryo culture media samples (WGA failure rate 0-22%). Ploidy concordance with the embryo biopsy ranged from 29-75% for autosomal chromosomes and 47-94% for sex chromosomes using a single-step culturing system (n=4), compared with concordance rates of 67-90% and 50-97% respectively when media was changed during the 5-6 day culture (n=4). DNA yield was not affected by embryo culture media droplet volume, or continuous or two-step culture. Sex chromosome concordance varied between individual labs, suggesting that embryological processes are important in NI-PGT-A testing. Further statistical analysis during the ongoing larger scale collaborative study will determine quality control parameters for acceptance of NI-PGT-A results.

**CONCLUSIONS:** Successful NI-PGT-A using spent embryo culture media will possibly require specific culturing conditions and/or specialised molecular methodologies for accurate and representative amplification and testing of the embryonic DNA. In a step toward this, we identified that renewing culture media during IVF improves overall concordance rates between the embryo biopsy and spent embryo culture media for NI-PGT-A.

**P-334** Tuesday, October 15, 2019 6:30 AM

#### **APPLICATION OF WHOLE GENOME NEXT GENERATION SEQUENCING (NGS) ANALYSIS OF PRODUCTS OF CONCEPTION (POC) AFTER SINGLE EMBRYO TRANSFER.**

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**OBJECTIVE:** Genetic assessment of tissue from products of conception (POC) can elucidate the reason for miscarriage in approximately 50-70% of first trimester miscarriages. Assessment of the fetal chromosomal composition may be very helpful in counselling and management of patients experiencing miscarriages, especially after IVF, or in patients with recurrent pregnancy losses. However, obtaining fetal tissue from early miscarriages is often compromised by maternal cell contamination (MCC). Here we present the results from assessing early POC samples (<10 GW) after IVF single embryo transfer, controlling for MCC, using whole genome NGS at the CReATe Fertility Centre.

**DESIGN:** A retrospective study.

**MATERIALS AND METHODS:** POC samples (n=294) (Jan, 2016-Apr, 2019) from early pregnancy losses after IVF treatment were obtained by suction D&C collection. Four representative samples of fetal tissue and/or chorionic villi were separated from decidual tissue and blood using a dissecting microscope. A maternal/paternal blood sample was obtained for DNA extraction to test for MCC. MCC was determined using analysis of short tandem repeats-STRs (AmpFLSTR Identifier Plus kit) of maternal and fetal DNA (fDNA). After confirmation of fetal DNA origin, whole genome NGS was carried out using VeriSeq kit. GeneMapper (Applied Biosystems) and BlueFuse Software v4.4 (Illumina) were used to analyze the STR and NGS data.

**RESULTS:** In total, we analyzed 294 POC samples (8.45±1.8 weeks) from patients undergoing IVF. Overall, mean maternal age was 36.8±8.5 years. fDNA confirmed by STR MCC analysis was obtained from 45.6% (n=134) of the samples. NGS analysis for chromosomal aberrations showed that 49.3% (66/134) of the POC samples were euploid (46,XX n=37; 46,XY n=29). 14.2% of fDNA samples (19/134) were from euploid embryos tested by NGS for aneuploidy at blastocyst stage. All these 19 POC samples were confirmed to be euploid and 100% sex concordant with preimplantation result. Aneuploidy was detected in 46.3% (62/134) [trisomy- T16,12.9%; T21, 12.9%; T22, 11.3%; T15,9.7%; T20, 6.5%; T14, 4.8%; T3 and T18, 3.2%; 1.6% of each T9,T12,T12+14,T13, T15+16,T8+15, T15+22, T17; monosomy - X, 17.7%], and 4.8% were triploid (69,XXY). Mosaicism was detected in 4.5% (6/134): (-X,30%,1/6);(+X,60%, 1/6); (-Xp11.1-q21.33, 36Mb, 60%, -Xq21.33-qter, 40%, 1/6); (-Y,60%,1/6); (+8,50%,+9,50%, 1/6);(+12q,30%, 1/6).

**CONCLUSIONS:** MCC is high in POC samples from early pregnancies and controlling for it is warranted. NGS results from euploid embryo transfers were fully concordant with PGT-A results. Establishing STR MCC and NGS analysis of POC on a larger scale would improve diagnostic accuracy, and could aid in patient counselling and management.

**SUPPORT:** Create Fertility Centre.

**P-335** Tuesday, October 15, 2019 6:30 AM

#### **RETROSPECTIVE EVALUATION OF PGD-HLA CASES FOR DIVERSE GENETIC DISEASES.**

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**OBJECTIVE:** The combination of preimplantation genetic diagnosis (PGD) with human leukocyte antigen (HLA) matching has appeared as a remarkable tool for the therapy of single-gene or acquired diseases in affected individuals. The technique (PGD-HLA) provides the parents who have affected child with disease-free and HLA-matched embryos that are compatible with the affected child, offering an unaffected newborn and a donor for affected child.

**DESIGN:** The present retrospective evaluation covers 64 couples who had undergone 131 PGD cycles in total for both HLA matching and elimination of the mutation(s) associated with different diseases, including acute lymphoblastic leukemia (n=2), aplastic anemia (n=1), Diamond-Blackfan anemia (n=2), Fanconi anemia (n=2), Griscelli syndrome (n=2), Hermansky-Pudlak syndrome (n=1), hyper IgE syndrome (n=1), hyper IgM syndrome (n=3), myelodysplastic syndrome (n=1), Morquio syndrome (n=1), severe combined immunodeficiency (n=1), thalassaemia (n=55), Wiskott-Aldrich syndrome (n=1), chronic granulomatous disease (n=1) and amyotrophic lateral sclerosis (n=1).

**MATERIALS AND METHODS:** Oocytes were picked up by antagonist protocol. After in vitro fertilization (IVF), eight blastomere cells were analyzed for wild type cells by the gene- and HLA-linked STR markers as well as linkage analysis at day three, and normal and HLA-compatible cells were transferred to the mother via frozen embryo transfer (FET).

**RESULTS:** Amongst the total embryos (n=1217), 250 embryos (20%) were wild type in terms of scanned mutation(s) and 206 embryos (17%) were found to be HLA-matched. 125 embryos in total were transferred and 33.8% clinical pregnancy rate per transfer was achieved.

**CONCLUSIONS:** The present study underlines the importance and efficacy of PGD-HLA method in the treatment of related diseases.

**P-336** Tuesday, October 15, 2019 6:30 AM

#### **DOES PGT WITH FRESH EMBRYO TRANSFER AFFECT PERINATAL OUTCOMES?: AN ANALYSIS OF THE 2014 AND 2015 SART DATA.**

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**OBJECTIVE:** Clinical studies have shown a difference in perinatal outcomes following fresh versus frozen embryo transfer. With the advent of pre-implantation genetic testing (PGT), many clinics have moved towards “freeze-all” cycles in conjunction with PGT, though there do remain many clinics who perform PGT before fresh embryo transfer. This study aims to assess the differences in perinatal outcomes of autologous fresh embryo transfer using embryos that underwent biopsy for PGT versus those that did not.

**DESIGN:** Retrospective cohort study

**MATERIALS AND METHODS:** The Society for Assisted Reproductive Technology (SART) database was used to identify fresh embryo transfer cycles that did and did not undergo PGT from 2014 and 2015. Cycles in which embryos were transferred on days 5, 6, or 7 were included. Log binomial regression models were used to assess for associations between embryo biopsy and pregnancy and perinatal outcomes. Models were adjusted for covariates including maternal age, race, BMI, smoking, prior IVF cycles, prior preterm/full-term births and cause of infertility. Low birth weight (LBW) was the primary outcome.

**RESULTS:** The mean age of the no biopsy patients (N=52,754) and biopsy patients (N=1,003) was 33.9 and 35.2 years, respectively (P<0.01). Compared to patients whose embryos were not biopsied, patients whose embryos were biopsied were significantly more likely to have a clinical pregnancy (61.2 vs. 57.3%, adjusted relative risk (aRR) 1.16, 95% confidence interval (CI) 1.07, 1.26) and live birth (54.4 vs. 48.4%, aRR 1.17, 95% CI 1.07, 1.29). Of the live births (N=25,462 no biopsy, N=543 biopsy), the incidence of large for gestational age (LGA) neonates was significantly higher in the biopsy group compared to the non-biopsy group (12 vs. 10.3%, aRR 1.45, 95% CI 1.04, 2.02). There were no differences in the incidence of LBW (24.9 vs. 28.8%, biopsy vs. no biopsy, aRR 0.83, 95% CI 0.61, 1.12) or preterm delivery (PTD) (20 vs. 22.6%, aRR 0.84, 95% 0.6, 1.16).

**CONCLUSIONS:** Evaluating the subset of patients whose had fresh embryos transferred following PGT allows for the assessment of the effects of PGT itself without the confounding effects of embryo cryopreservation. While there was a difference seen in the incidence of LGA babies, there were otherwise no differences seen in perinatal outcomes between fresh transfer with and without embryo biopsy. Future studies should assess potential etiology for the observation of an increase in LGA babies following fresh transfer after embryo biopsy.

**SUPPORT:** None.

**P-337** Tuesday, October 15, 2019 6:30 AM

**ACCURACY OF CELL-FREE EMBRYONIC DNA TESTING FOR EUPLOIDY AND ANEUPLOIDY IN COMBINED SPENT EMBRYO CULTURE MEDIUM AND BLASTOCOEL FLUID SAMPLES WITH OR WITHOUT USING A CELL LYSIS STEP BEFORE AMPLIFICATION.** Valeriy Kuznyetsov, PhD,<sup>a</sup> Svetlana Madjunkova, MD,<sup>b</sup> Rina Abramov, MSc,<sup>b</sup> Ran Antes, PhD,<sup>b</sup> Iryna Kuznyetsova, PhD,<sup>a</sup> Clifford Lawrence Librach, MD<sup>c</sup>. <sup>a</sup>CReATe Fertility Centre, Toronto, ON, Canada; <sup>b</sup>CReATe fertility centre, Toronto, ON, Canada.



**OBJECTIVE:** Blastocoel fluid (BF) and spent embryo culture medium (SEM) both contain cell-free embryonic DNA (cfeDNA). Attempts to use cfeDNA for non-invasive preimplantation genetic testing (NIPGT), brings to light several factors that could affect the accuracy of this approach. These include maternal contamination by cumulus/corona cells and DNA degradation. The objective of this study was to determine the accuracy, efficacy and reliability of whole genome amplification (WGA) to determine ploidy status (euploidy/aneuploidy) of the blastocyst using combined SEM+BF samples with or without using a cell lysis step before WGA.

**DESIGN:** Controlled prospective study. Two NIPGT samples (SEM+BF) from each culture medium droplet were used for amplification and subsequent testing of chromosomal abnormalities. Results were compared with the corresponding trophectoderm (TE) biopsy sample used as control.

**MATERIALS AND METHODS:** Laser zona opening was performed on Day 4, and embryos were transferred to fresh 20µl droplets of Global HP medium with HSA. Thirty nine human blastocysts were collapsed by a laser pulse, and then both SEM and BF samples were collected together as one

D4-D5/6 NIPGT sample. WGA products (SurePlex kit) of NIPGT-1 (WGA with Cell lysis step), NIPGT-2 (WGA without Cell lysis step) and TE biopsy samples were assessed with Qubit 3.0 Fluorometer and VeriSeq™ PGS kit on the MiSeq system (Illumina). Results were statistically evaluated using Chi Square/Fisher exact tests where appropriate with significance at P < 0.05.

**RESULTS:** The mean amount of amplified DNA was higher for NIPGT-1 samples (39.3 ± 3.0 ng/µL, range 15.7 - 85.9 ng/µL) than for NIPGT-2 samples (31.9 ± 2.5 ng/µL, range 10.2 - 72.7 ng/µL), however the difference was not significant. Informative PGT-A results were obtained for 94.9% (37/39) TE biopsies, for 92.3% (36/39) NIPGT-1 and for 89.7% (35/39) NIPGT-2 samples (P>0.05). Euploidy/aneuploidy concordance rate per sample for whole chromosome copy number for NIPGT-1 vs. TE, NIPGT-2 vs. TE and NIPGT-1 vs. NIPGT-2 samples was 97.1% (33/34); 97.0% (32/33) and 100% (35/35), respectively; with one false negative result for a single chromosome trisomy. NIPGT-A correctly determined the gender in all NIPGT-1 and NIPGT-2 samples and correctly determined (excepting one sample) the whole and segmental chromosome aneuploidies in NIPGT-1 and NIPGT-2 samples.

**CONCLUSIONS:** NIPGT-1 and NIPGT-2 samples showed a similar high concordance rate with the corresponding TE biopsy samples for euploidy and aneuploidy. Amplification of cfeDNA without using “cell lysis step” has potential to reduce the risk of maternal contamination of NIPGT samples by residual cumulus/corona cells.

**SUPPORT:** CREATE FERTILITY CENTRE.

**P-338** Tuesday, October 15, 2019 6:30 AM

**MOSAIC EMBRYO DIAGNOSIS CORRELATED WITH ABNORMAL 15Q DUPLICATION SYNDROME IN OFFSPRING.** Emily L. Mounts, MS, CGC,<sup>a</sup> Shihui Olive Zhu, MSc,<sup>a</sup> Rebecca K. Sanderson, PhD,<sup>b</sup> Alison Coates, PhD,<sup>a</sup> John S. Hesla, MD,<sup>a</sup> <sup>a</sup>ORM Fertility, Portland, OR; <sup>b</sup>CooperGenomics, Los Angeles, CA.



**OBJECTIVE:** Outcome data from the practice of mosaic embryo transfer has to date not suggested an association with adverse postnatal outcomes. The objective of this case review was to determine whether a chromosomal duplication syndrome, discovered in a phenotypically abnormal child who was the product of euploid embryo transfer, could be retrospectively identified in full or mosaic form using an updated PGT-A platform.

**DESIGN:** Case report.

**MATERIALS AND METHODS:** A 29yo G1P1 female patient with ovarian factor infertility and her male partner, who had oligospermia, underwent IVF/PGT-A via next-generation sequencing (Veriseq PGS, Illumina Inc). 5/7 embryos were diagnosed as euploid and FET of two euploid male embryos resulted in the birth of healthy twin boys. By 8 months of age one twin had failed to meet his developmental milestones, developed marked obesity, and had abnormally low growth hormone and insulin levels.

**RESULTS:** Multiplex ligation-dependent probe amplification (MLPA) and methylation testing was performed to evaluate for Prader-Willi syndrome (caused by 15q11.2-13 paternal deletions) on the affected boy. This testing incidentally diagnosed an extra paternal copy of 15q11.2-q13. Chromosomal microarray [Dian Diagnostics] confirmed a 5.76MB duplication at 15q11.2-q13.1. The boy was diagnosed with 15q duplication syndrome, a highly variable condition associated with developmental delays, autism spectrum disorders, and a phenotype influenced by parental origin of the duplication (maternal vs paternal). Retrospective analysis of his PGT-A results was requested per clinic protocol. Review of archived profile images of the original NGS data [BlueFuse Multi, Illumina] from the two male embryos reaffirmed the original interpretations of euploid males. Preserved amplified DNA from both embryos was reanalyzed using NGS and PGTai [CooperGenomics proprietary algorithm]. This algorithm detected a high-level mosaic (57%) 6Mb duplication on 15pter-q13.3 in one embryo, demonstrating that this finding was detectable by PGT-A using an updated platform with a revised algorithm and increased resolution. Additionally, two separate and larger segmental mosaic abnormalities were identified upon reanalysis of preserved amplified embryonic DNA from the affected boy and his healthy twin brother, respectively; the former was not confirmed on postnatal CMA. The

patient's three remaining euploid embryos were also then reanalyzed via PGTai and 2/3 embryos were reaffirmed to be euploid. The third embryo was found to have the same 15pter-q13.1 duplication as the affected boy, also in the mosaic range (78%), suggesting that the variant may be inherited.

**CONCLUSIONS:** This may be the first case in which correlation between a mosaic PGT-A result and the same finding postnatally, in non-mosaic form and likely resulting in an abnormal phenotype, has been made. This case is highlights the significant challenges of predicting mosaic embryo transfer outcomes. Transfers of mosaic result embryos may not always result in binary outcomes, an essential consideration for genetic counseling of families considering this option.

**P-339** Tuesday, October 15, 2019 6:30 AM

#### **OUTCOMES OF A SIMPLIFIED MOSAIC RANKING SYSTEM.**

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**OBJECTIVE:** Investigating outcomes of transferring mosaic embryos according to a simplified classification system that replaced our more complex 1-9 ranking system.

**DESIGN:** Following previous implementation of a comprehensive mosaicism classification system, a review of our mosaic embryo outcome data, together with that of recent literature (Spinella et al 2018, and Munne et al 2017) was conducted. The goal was to introduce a more simplified classification system – using an A-D grading – which ranks mosaic embryos for clinical use (as per table below). The study included 158 single transfers of embryos (between April 2016 to February 2019) which exhibited a mosaic shift (ranging from 20-79%) using NGS technology.

**TF-A = NAD** (no abnormality detected) – first choice for transfer

**TF-B = Noisy result/low level mosaicism**  $\leq 40\%$  – second choice

**TF-C: MOSAIC** (significant mosaicism detected  $>40\% < 80\%$ ) -third choice, further stratified by:

**C1:** -segmental

-low risk chromosomes

**C2:** -high risk chromosomes **TF-D:** ABN (abnormal) – not available for transfer

**MATERIALS AND METHODS:** Mosaic embryos were separated into the above categories dependent on the percentage of mosaicism present in the sample and the chromosome involved, with only levels over 40% being reportable findings. This study retrospectively compared the positive bHCG and fetal heart (FH) outcomes for the TF-A, B and C groups. We also analyzed outcomes for whole chromosome mosaics compared to segmental mosaic findings.

**RESULTS:** We found that overall, TF-A group had the highest positive bHCG rates (62.9%) and FH rates (53.7%) followed by TF-B bHCG (57.6%) and FH rates (47.6%). TF-C had lower positive bHCG rates outcomes (44%) and FH rates (36%). Analysing TF-C (40-80% mosaics) in more detail we found that segmentals had higher bHCG and FH rates (69.2% and 61.5%) compared to whole chromosome mosaics (20% and 10%). Furthermore, irrespective of the mosaic percentage, all single segmentals (in the 20-80% range) had clearly better outcomes than multiple segmentals (bHCG; 63.3% vs 44.4% and FH; 48.3% vs 33.3%). In comparison low level segmental mosaics ( $\leq 40\%$ ) had higher bHCG (58.9%) and FH (42.9%) rates compared to low level whole chromosome mosaics (44.8%) and (36.2%).

**CONCLUSIONS:** Overall the positive bHCG and fetal heart outcomes support the simplified classification system and the concept of preferentially transferring low level before high level whole chromosome mosaics in the absence of NAD embryos. Single segmental mosaic embryos have improved clinical outcomes compared to whole chromosome mosaic embryos and should be considered for preferential transfer ahead of other types of mosaic findings. Whole chromosome mosaics should be considered last choice.

**P-340** Tuesday, October 15, 2019 6:30 AM

#### **EVALUATION OF THE IMPACT OF THE PULLING AND FLICKING TROPHOCTODERM BIOPSY PROCEDURES ON THE INTEGRITY OF THE BIOPSIED CELLS AND THEIR CORRELATION TO PGT-A RESULTS.**

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**OBJECTIVE:** Blastocyst biopsy is currently the gold standard in PGT-A. However, because trophoctoderm (TE) cell excision is technically challenging, results can vary depending on how the procedure is performed. To ensure successful results, it is important not only to avoid harming the blastocyst during the biopsy procedure, but also to ensure a minimal damage on the biopsied cells. In this study, we aimed to evaluate the impact of two different TE biopsy techniques (pulling and flicking) on the integrity of the biopsied cells and to correlate their status with the chromosome screening results of the two methods.

**DESIGN:** This is a retrospective observational study that includes the data analysis of 268 TE biopsies performed on blastocysts from 83 patients (mean age – 39.1 y/o) that underwent a PGT-A cycle between October 2018 and April 2019. Chromosome screening analysis were carried out by an associated genetics laboratory. Indications for PGT-A cycles included advanced maternal age, recurrent implantation failure, recurrent miscarriage and/or severe male factor.

**MATERIALS AND METHODS:** Trophoctoderm biopsies were performed with the assistance of a dynamic laser. Assisted hatching was performed on Day 3 and blastocysts with herniating cells or completely hatched were biopsied with the “pulling” or “flicking” techniques. In the pulling method, blastocysts were held firmly with the holding pipette and the biopsy needle used to pull TE cells away from the blastocyst, while laser pulses were applied. In the flicking method, laser pulses were used to allow TE cells to be drawn inside the biopsy pipette and subsequently the TE cells were excised with a quick movement of the biopsy pipette against the holding pipette. Biopsied cells were then photographed and classified according to their integrity status as follows: intact (A); partially lysed (B); completely lysed (C). After biopsy, the cells were washed and transferred into PCR tubes to be processed for chromosome screening by NGS. Statistical significance was assessed by Students *t*-test or Fisher's exact test.

**RESULTS:** A total of 118 blastocysts were biopsied with the pulling method and 150 with the flicking technique and no differences were found in terms of mean age of the patients ( $39.5 \pm 1.1$  and  $38.5 \pm 3.2$ , respectively) or average number of laser pulses used ( $4.2 \pm 1.1$  vs  $3.9 \pm 0.9$ , respectively). Overall, the pulling technique resulted in higher ( $p = 0.0009$ ) percentage of pieces graded as A (74.6%,  $n = 88$ ) than the flicking method (54.7%,  $n = 82$ ), but no differences were found among the two groups in terms of euploidy rates (28% and 36%, respectively). Regardless of the technique used, all cells graded as C were majorly (80-100%) diagnosed as chromosomally abnormal compared to those that were classified as morphologically intact (43.9-62.5%) or partially lysed (52.4-62.5%).

**CONCLUSIONS:** These results indicate that the pulling and flicking methods do not differ in terms of rates of chromosomally normal blastocysts as long as both procedures are applied correctly. The integrity of the cells seems to affect the results of aneuploidy rates, which might depend on blastocyst morphology.

**SUPPORT:** Institutional funding.

**P-341** Tuesday, October 15, 2019 6:30 AM

#### **DEVELOPMENT OF A NEXT GENERATION SEQUENCING METHOD (PGT-SR PLUS) TO DETERMINE CARRIER STATUS OF BALANCED TRANSLOCATION PATIENT EMBRYOS.**

Hua Jin, PhD, Hui Zheng, MA, Alysha Nicole Salbato, BS, Robert Snyder, BS, ManLi, MD, PhD, Lian Liu, MD. PacGenomics, Agoura Hills, CA.



**OBJECTIVE:** Currently, PGT-SR is the only PGT option in the United States for patients with a balanced translocation. While PGT-SR does

reliably screen for chromosomal copy number normal embryos and avoid the transfer of unbalanced translocation embryos, PGT-SR cannot determine the carrier status of embryos. Our objective is to develop a generic genome-wide next generation sequencing method (PGT-SR Plus) that can determine the carrier status of balanced translocation patient embryos, regardless of the type or location of translocation. This will allow patients the option to transfer embryos without the structural chromosomal abnormality.

**DESIGN:** Feasibility and validation study.

**MATERIALS AND METHODS:** The feasibility of the PGT-SR Plus method has been tested on 10 cases involving various chromosomes, including both Robertsonian and reciprocal translocations. Parental blood was collected to determine the balanced translocation allele. Then, PGT-SR was performed on all embryos to identify those with a normal chromosomal copy number. These identified embryos, along with one unbalanced embryo, were then tested with PGT-SR Plus. The unbalanced embryo is used as a reference for phasing the parental carrier's balanced translocation allele. The carrier status of each embryo was then determined based on whether or not the embryo carries the parental balanced translocation allele.

**RESULTS:** All 10 translocation cases that have been tested with PGT-SR Plus have had definitive results for the carrier status of the balanced translocation embryos. 7 of these 10 cases were also tested on the illumina karyomapping microarray platform and the same carrier statuses were identified, indicating that our generic next generation sequencing method and bioinformatic analysis pipeline are encouraging for screening structural chromosomal abnormalities.

**CONCLUSIONS:** The next generation sequencing-based PGT-SR Plus is a promising method for determining the carrier status of balanced translocation patient embryos. As a generic method, it does not rely on the design of patient specific primers. It is applicable to all currently identified structural chromosomal abnormalities and is therefore very affordable.

**P-342** Tuesday, October 15, 2019 6:30 AM

**IMPACT OF TROPHECTODERM BIOPSY FOR PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) ON EARLY BETA-HCG TRENDS IN SINGLE FROZEN EMBRYO TRANSFERS (FET) RESULTING IN LIVE BIRTH.**



Laura Perez Soriano, BA,<sup>a</sup> Joshua Stewart, M.D.,<sup>b</sup> Steven Spandorfer, M.D.,<sup>b</sup> Zev Rosenwaks, M.D.<sup>b</sup>, <sup>a</sup>Weill Cornell Medical College, New York, NY; <sup>b</sup>The Ronald O. Perleman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY.

**OBJECTIVE:** Newer techniques in PGT-A allow blastocyst biopsy removing 6-10 trophectoderm cells for genetic analysis. As syncytiotrophoblasts produce beta-hCG, our objective was to determine the effect of trophectoderm biopsy for PGT-A on early serum beta-HCG trends in pregnancies resulting in a singleton live birth.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All patients undergoing an FET cycle of a single blastocyst between January 2015 to December 2017 were analyzed. Cycles were divided into those with PGT-A and without. For PGT-A cycles, only euploid embryos were included. Inclusion criteria: 2 serum BhCG results obtained 2 days apart, delivery of a live singleton. Exclusion criteria: cycles utilizing donor oocytes, multiple gestation pregnancies. Primary outcomes were initial serum BhCG and 2-day increase in BhCG. Secondary outcomes were serum estradiol (E2) and progesterone (P4). Groups were further stratified by FET protocol, natural cycle or medicated.

**RESULTS:** 487 cycles met inclusion criteria, 279 with PGT-A and 208 without. There was no difference in mean initial BhCG or second serum BhCG levels between the cycles with PGT-A and those without PGT-A despite controlling for age and protocol. The median 2-day increase in BhCG was significantly higher in the PGT-A group versus the cycles without PGT-A (247.9% vs 238.9%, respectively, p=0.02). There was no difference in the 2-day rise of serum E2 or P4 levels between the groups.

**CONCLUSIONS:** BhCG is commonly used as a marker of trophoblast differentiation and to assess pregnancy viability, but little is known about the impact of trophectoderm biopsy on BhCG trends. Our results reveal no difference in initial BhCG between cycles with PGT-A and without. While there was a significantly greater 2-day increase in BhCG in the PGT-A group, the clinical relevance of this minimal difference is unclear. However, it is clinically reassuring that trophectoderm biopsy does not impair BhCG rise. This contributes to previous studies that suggest trophectoderm biopsy does not affect implantation or early pregnancy steroidogenesis, adding to the overall safety of PGT-A to achieve healthy pregnancies.

**REFERENCES**

<sup>1</sup>A Penzias, A., Bendikson, K., Butts, S., Coutifaris, C., Falcone, T., Fossum, G., ... & Mersereau, J. (2018). The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. *Fertility and Sterility*, 109(3), 429-436.  
<sup>2</sup>A Shamonki, M. I., Frattarelli, J. L., Bergh, P. A., & Scott, R. T. (2009). Logarithmic curves depicting initial level and rise of serum beta human chorionic gonadotropin and live delivery outcomes with in vitro fertilization: an analysis of 6021 pregnancies. *Fertility and sterility*, 91(5), 1760-1764.

Demographics	PGT (n= 279) Mean ± SEM	No PGT (n=208) Mean ± SEM	P
Age	36.4 ± 0.2	33.5 ± 0.2	< 0.001
BMI	23.3 ± 0.2	23.0 ± 0.2	ns
AMH	3.7 ± 0.2	4.5 ± 0.3	0.03
<b>Mean Serum Hormone Levels (mIU/mL)</b>			
1 <sup>st</sup> BhCG	275 ± 9.4	266 ± 12	ns
2 <sup>nd</sup> BhCG	678 ± 25	640 ± 30	ns
1 <sup>st</sup> E2	302 ± 11	311 ± 16	ns
2 <sup>nd</sup> E2	332 ± 12	332 ± 14	ns
1 <sup>st</sup> P4	26.7 ± 0.6	25.3 ± 0.7	ns
2 <sup>nd</sup> P4	26.5 ± 0.7	24.6 ± 0.7	ns
<b>2-day % Increase in Hormone, Median (IQR)</b>			
BhCG	244 (220 – 277)	237 (209 – 260)	0.02
E2	112 (96 – 129)	112 (95 – 127)	ns
P4	100 (88 – 114)	97 (86 – 126)	ns

Differences calculated by Paired Student's T-test or Wilcoxon rank sum tests.

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**ANTHROPOMETRIC, HORMONAL AND HEMATOLOGICAL CHARACTERISTICS OF WOMEN UNDERGOING IN VITRO FERTILIZATION.**



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**OBJECTIVE:** To describe the anthropometric, hormonal and hematological characteristics of women undergoing in vitro fertilization (IVF).

**DESIGN:** Prospective.

**MATERIALS AND METHODS:** We report on a subgroup analysis of 10 women undergoing IVF from subjects prospectively enrolled in an IRB approved study of hemostatic balance. Samples were longitudinally collected during the follicular phase prior to the IVF cycle (V1), prior to commencing gonadotropins (V2), 30-90 minutes prior to oocyte retrieval (V3), and 14 days after oocyte retrieval (V4) with a subsequent fresh embryo transfer. Complete blood counts, progesterone (P4) and estradiol (E2) were assessed at all visits, body composition was evaluated by Dual Energy X-ray Absorptiometry at V1. Pregnancy was detected by serum hCG 14 days after retrieval and luteal support, V4. Women who conceived were compared with those who did not using two-sample t-tests and Wilcoxon tests; data presented as mean ± standard deviation with the significance threshold set at  $p < 0.05$ .

**RESULTS:** The mean age of subjects at retrieval was  $32 \pm 3.7$  years, mean BMI  $25.5 \pm 3.9$  kg/m<sup>2</sup>, with no significant differences between women based on conception status. Half of the women conceived with the initial fresh IVF cycle. Women who conceived had a lower waist:hip circumference ratio ( $0.77 \pm 0.05$ ) compared to those who did not ( $0.9 \pm 0.04$ ),  $p=0.003$ . There were differences in android tissue fat and android:gynoid fat ratio based on conception status (not statistically significant). There were no other differences in body composition or bone mineral density age matched Z scores between groups.

E2 and P4 during the IVF cycle are shown in the table below. The mean P4 was higher in the not pregnant group at V3, and both E2 and P4 were significantly lower at V4. Total white blood cell, neutrophil and lymphocyte counts were higher in the pregnant group at V4. Higher E2 ( $p=0.02$ ), P4 ( $p=0.02$ ), lymphocyte % ( $p=0.02$ ) and neutrophil % ( $p=0.02$ ) were found at V4.

Means (SD)	Pregnant (n=5)		Not Pregnant (n=5)	
	E2 pg/mL	P4 ng/mL	E2 pg/mL	P4 ng/mL
V2	13 (1)	0.3 (0.1)	16 (11)	0.2 (0.1)
V3	1316 (474)	6.7 (2.5)	1291 (807)	10.1 (5.5)
V4	1334 (885)	151 (42.2)	21 (9)	30.6 (9.9)

**CONCLUSIONS:** Hematological differences exist between women who successfully conceive following a fresh IVF cycle, as demonstrated by increases in neutrophil and lymphocyte counts in women who had a positive hCG versus those who did not conceive. There are also differences in estradiol and progesterone early when pregnancy is diagnosed. Waist hip ratio was inversely associated with pregnancy in this small sample, but there may also be differences in body composition not detected with this sample size. Our future studies may elucidate further metabolic and hematologic factors related to successful fresh IVF cycles.

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SUPPORT: New England Fertility Society REI Fellows Research Grant.

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**HUMAN MURAL GRANULOSA CELLS CULTURED IN SERUM-FREE CONDITIONS CAN BE USED TO STUDY MOLECULAR MECHANISMS OF HUMAN GRANULOSA CELL DIFFERENTIATION IN**



**VITRO.** Sarah Baumgarten, MD, PhD,<sup>a</sup> Nicola Winston, PhD,<sup>b</sup> Michelle Fierro, BS,<sup>c</sup> Humberto Scoccia, MD,<sup>c</sup> Carlos Stocco, PhD.<sup>c</sup> <sup>a</sup>Mayo

Clinic, Rochester, MN; <sup>b</sup>University of Illinois at Chicago, Chicago, IL; <sup>c</sup>UIC, Chicago, IL.

**OBJECTIVE:** This study aimed to establish an innovative system in which the molecular mechanisms of FSH-induced granulosa cell differentiation during ovarian follicle maturation could be studied.

**DESIGN:** *In vitro* studies of gene and protein expression were conducted in primary cultures of mural granulosa cells collected from women undergoing *In vitro* fertilization (IVF) at the University of Illinois Hospital.

**MATERIALS AND METHODS:** Mural granulosa cells collected from IVF patients were cultured in phenol-red-free media containing 2% serum for 0, 24, 48, or 72h. At these time-points, the cells were serum starved for 24h and then treated with FSH for 48h. Total RNA was isolated from these cells, and gene expression of key granulosa cell differentiation markers including *Cyp19a1*, *Star*, and *P450scc* was measured by quantitative RT-PCR. Additionally, total protein was isolated from these experimental groups and the expression of CYP19A1, StAR, and P450SCC at the protein level was measured by Western blot. Differences between the means of different groups were analyzed by ANOVA or *t*-test and considered statistically significant at  $p < 0.05$ .

**RESULTS:** After culturing cells in serum-containing media for at least 24h before serum starvation and FSH treatment, the expression of steroidogenic genes *Cyp19a1*, *Star*, and *P450scc* were reduced significantly, suggesting that under the culture conditions used mural granulosa cells de-differentiate and resemble undifferentiated granulosa cells. After treatment with FSH, the expression of *Cyp19a1*, *Star*, and *P450scc* increased significantly by 3.5, 2.5, and 3-fold respectively. The protein expression of CYP19A1, StAR, and P450SCC was also increased significantly by 3, 4, and 1.5-fold, respectively.

**CONCLUSIONS:** Mural granulosa cells from IVF patients cultured in serum for 24h followed by serum starvation respond to FSH with an increase in steroidogenic gene and protein expression, suggesting that cell cultured in this manner can be used as an experimental approach to study the molecular mechanisms of human granulosa cell differentiation in response to FSH *in vitro*.

**SUPPORT:** This work was supported by NIH grant number R01HD057110 (COS); SCB was supported by NIH training grant number T32HL07692.

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**ATYPICAL VAGINAL TEMPERATURE PATTERNS MAY IDENTIFY SUBTLE, NOT YET RECOGNIZED, CAUSES OF INFERTILITY.**



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**OBJECTIVE:** To determine if averaged nocturnal vaginal temperature measurements recorded during non-menstruation by use of the OvuSense system (OS), could describe atypical patterns potentially associated with reduced fertility.

**DESIGN:** Retrospective, longitudinal, comparative, observational study.

**MATERIALS AND METHODS:** 10,463 ovulatory cycles from 6,647 OS users aged 20 to 52 (if age provided), with cycle length 11 to 190 days (90% 22 to 47 days). Participants used OS vaginally at night to monitor core body temperature (temp), having voluntarily been asked to provide date of birth and identify how long they had been trying to conceive before OS use. OS produces a representative temp for each night of recordings taken every 5 minutes, which are then assessed with a proprietary moving averaged calculation to produce a "smooth" analysis curve. The main outcome measures were: proportions of normal and atypical OS temp patterns as classified by observation of the smooth curve and applied mathematical criteria, frequency of their occurrence, and associations between patterns.

**RESULTS:** Three novel atypical temp patterns were identified: (a) "Crash To Baseline" = first nightly averaged temp falls by  $>0.2$  degrees Celsius ( $^{\circ}\text{C}$ ) to lowest cycle temp point (baseline) - in 1,481 cycles (14.2%) from 1,352 OS users (20.3%), (b) "False Start" = rise of  $>0.1$   $^{\circ}\text{C}$  did not result in ovulation but instead a return to baseline temp followed by ovulation two or more days later in the cycle - 981 cycles (9.4%); 939 users (14.1%), (c) "Crash After Ovulation" = final temp  $>0.2$   $^{\circ}\text{C}$  lower than the post ovulatory peak temperature - 1,259 cycles (12.0%); 1,062 users (16.0%). Additionally, Short Luteal Phase (SLP) (d) was noted with menstruation 9 or fewer days post-ovulation - 871 cycles (8.3%); 793 users (12.0%). SLP occurred combined with pattern (a), (b), or (c) 237 cycles (2.3%); 231 users (3.5%). SLP co-existed with (a)

133 cycles; 128 users, with (b) 155 cycles; 153 users, with (c) 7 cycles; 7 users. SLP co-existed with pattern (a) + (b) 33 cycles; 32 users, and as in low frequency with (a) + (c) 1 cycle; 1 user, and (b) + (c): 2 cycles; 2 users. Therefore 3,721 cycles exhibited one or more 'atypical' patterns (a), (b), or (c) = 35.6%.

**CONCLUSIONS:** It is likely OS continuous vaginal temp patterns closely reflect luteal progesterone changes, hence describe subtle progesterone secretion or metabolism anomalies, which not yet have been recognised. (a) suggests high progesterone early in the cycle, (b) suggests a small progesterone rise which does not result in a sustained ovulatory rise, but is followed by an ovulatory rise later in the cycle. (a) and (b) would be expected to occur in women with PCOS, and further studies are planned to examine this within the OS population. (c) suggests that progesterone may fall sharply in some women before onset of menses, and it is possible that fertility may be impaired in these cycles. Relatively strong correlation between SLP and patterns (a), (b), and/ or (c) indicates vaginal, core-body temp monitoring may represent a promising method of identifying previously undetectable causes of infertility in women with "normal" ovulation.

**References:**

- Papaioannou S, Delkos D, Pardey J (2014) Vaginal core body temperature assessment identifies pre-ovulatory body temperature rise and detects ovulation in advance of ultrasound folliculometry. European Society of Human Reproduction and Embryology 30th Annual Conference.
- Papaioannou S, Aslam M (2012) Ovulation assessment by vaginal temperature analysis (Ovusense Fertility Monitoring System) in comparison to oral temperature recording. American Society for Reproductive Medicine 68th Annual conference.

**SUPPORT:** This study was financially supported by Fertility Focus Ltd.

**P-346** Tuesday, October 15, 2019 6:30 AM

**IGF-1 AND IGFBP-3 SERUM CONCENTRATIONS IN PATIENTS UNDERGOING PROGRAMMED FROZEN-THAWED EMBRYO TRANSFER OF EUPLOID PGT-A EMBRYOS.**

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**OBJECTIVE:** IGF-1 has been shown to induce embryonic development in vitro, but at high concentrations exhibits toxic effects by decreasing embryonic glucose uptake. IGFBP-3 binds IGF-1, modulating its bioavailability and is essential to its function. Prior reports have associated elevated follicular phase IGF-1 with pregnancy loss in frozen-thawed embryo transfer in natural cycles (n-FET), but an association in programmed (p-FET) cycles or in the luteal phase has not been analyzed. We sought to determine whether serum levels of IGF-1 and IGFBP-3 correlate with the outcome of p-FET cycles.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Patients who underwent p-FET of single, good quality (Grade  $\geq 2BB$ ), PGT-normal embryos were included in the study. GnRH-agonist suppression was started in the preceding luteal phase, overlapped with estradiol patches, and stopped with the start of progesterone. Serum samples were collected on cycle day 2 (CD2), the day of progesterone start (CDP4), and cycle days 28 (CD28) and 30 (CD30). Embryo transfer occurred on the 7<sup>th</sup> day of P4 administration. Serum levels of IGF-1 and IGFBP-3 were compared between those who did not achieve pregnancy, those who had a live birth, and those who had a pregnancy loss. Serum IGF-1 and IGFBP-3 levels were measured by chemiluminescent immunoassays using the Immulite 2000 Xpi. Statistical analysis was performed using Chi-square and Fisher's exact test.  $P < 0.05$  was deemed statistically significant.

**RESULTS:** A total of 102 patients who underwent p-FET of single euploid embryos over 2 years were analyzed. The mean age at retrieval was  $35.7 \pm 4.1$  years, BMI  $24.2 \pm 4.8$  kg/m<sup>2</sup>, gravity  $1.8 \pm 1.6$ , parity  $0.4 \pm 0.6$  and peak endometrial thickness  $9.7 \pm 2.0$  mm. 76.5% of patients were pregnant and

	p-FET, Delivered	p-FET, Pregnancy loss	<i>p</i>
IGF-1 CDP4 ( $\geq$ mean value)	63.6%	33%	<b>0.044</b>
IGF-1 CD 28 ( $\geq$ mean value)	58%	14%	<b>0.044</b>
IGF-1 CD28 ( $\geq$ 160 ng/ml)	70.8%	14%	<b>0.007</b>

78.2% of those had a live birth. Among women who conceived, those who had a subsequent pregnancy loss had significantly higher serum IGF-1 levels on CDP4 and CD28 compared to those who achieved live birth when analyzing patients whose serum IGF-1 levels were above the mean value of IGF-1 level on CDP4 (136 ng/ml,  $p = 0.044$ ) and CD28 (138 ng/ml,  $p = 0.007$ ), and between patients with CD28 IGF-1 levels one standard deviation above the mean ( $\geq 160$  ng/ml,  $p = 0.007$ ). There was no significant difference in the serum levels of IGFBP-3 in any of the treatment cycle days.

**CONCLUSIONS:** In p-FET cycles with transfer of a single, euploid, high quality embryo, IGF-1 serum levels on day of progesterone start and CD28 are significantly higher in patients who subsequently had a pregnancy loss compared to those who had a live birth. This is in contrast to the findings of prior studies in n-FET.

Reference: None.

SUPPORT: None.

**REPRODUCTIVE BIOLOGY - BASIC**

**P-347** Tuesday, October 15, 2019 6:30 AM

**UPREGULATION OF THE LONG NON-CODING RNA TUG1 INHIBITS GRANULOSA CELL APOPTOSIS AND AUTOPHAGY IN POLYCYSTIC OVARY SYNDROME BY REGULATING ERK/MAPK PATHWAY.**



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**OBJECTIVE:** Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility in women of reproductive age, and its etiology remains poorly understood. Evidence has indicated that the increase in granulosa cell (GC) proliferation is associated with PCOS. Altered activities of long non-coding RNAs (lncRNAs) have been associated with human diseases and development. Taurine upregulated gene 1 (TUG1), an evolutionarily conserved lncRNA, has been shown to play an oncogenic role in various cancers. However, little is known about the role of TUG1 in PCOS. Therefore, the aim of this study is to explore the potential role of TUG1 in the pathogenesis of PCOS.

**DESIGN:** We measured TUG1 lncRNA expression levels in GCs from 58 PCOS patients and 58 controls. Also, TUG1 was knocked down in a human GC tumor-derived cell line, KGN, to investigate the role of TUG1 and its molecular mechanism in cell apoptosis and autophagy.

**MATERIALS AND METHODS:** GCs were collected from women with or without PCOS undergoing IVF or ICSI treatment. The PCOS diagnosis was based on the Rotterdam revised criteria, and control patients were limited to male factor or tubal disease and had a normal ovarian reserve. Quantitative real-time PCR was used to measure the differential expression levels of TUG1 between PCOS patients and controls. The receiver operating characteristic (ROC) curve was drawn to evaluate the diagnostic values of TUG1 in PCOS. In the KGN cell line, TUG1 was knocked down with locked nucleic acid GapmeRs. Cell counting kit-8 assays, ethynyl-2-deoxyuridine assays and flow cytometry were used to study the role of TUG1 in cell proliferation and apoptosis, and western blotting was performed to detect the potential underlying mechanism.

**RESULTS:** We first found that TUG1 lncRNA was significantly upregulated in PCOS GCs and was associated with the antral follicle count ( $R = 0.264$ ,  $P < 0.01$  versus control). The ROC curves illustrated strong separation between all the PCOS patients and the control group (AUC: 0.627; 95% CI: 0.526–0.728;  $P = 0.017$ ). TUG1 was primarily localized in the nuclei of GCs. TUG1 knockdown in KGN cells inhibited cell proliferation and promoted cell apoptosis. In addition, TUG1 knockdown induced an increase in the protein levels of bax, bak, cleaved caspase-3, caspase-9, cleaved caspase-9, LC3B and phosphorylated ERK (p-ERK), and a decrease in the protein levels of bcl-2 and p62. Furthermore, inhibition of the ERK/MAPK pathway with U0126, the upregulation of p-ERK, bax, bak, cleaved caspase-3, caspase-9, cleaved caspase-9, LC3B, and the downregulation of bcl-2 and p62 by the knockdown of TUG1 were all attenuated. Therefore, downregulation of TUG1 may promote cell apoptosis and autophagy by activation of the ERK/MAPK pathway.

**CONCLUSIONS:** Our study first reported that the expression of TUG1 was significantly higher in the PCOS group than that in the control group. TUG1 may inhibit cell apoptosis and autophagy in GCs through inhibition of the ERK/MAPK pathway and contribute to excess antral follicles.

TUG1 has potential diagnostic value in PCOS. Therefore, analysis of TUG1 and its molecular mechanisms of action provide new insights into the pathogenesis of PCOS.

SUPPORT: This work was supported by the National Natural Science Foundation of China (grant number: 81671524).

**P-348** Tuesday, October 15, 2019 6:30 AM

**EFFECT OF ADRIAMYCIN, BLEOMYCIN, VINBLASTINE AND DACARBAZINE (ABVD) TREATMENT ON FEMALE MICE REPRODUCTIVE FUNCTION.** Yubing LIU, Sr., Ph.D.<sup>a</sup> Xinmei LU, Sr., Master,<sup>b</sup> Xiaocan LEI, Sr., Ph.D.<sup>c</sup> Richeng Chian, Sr., Ph.D.<sup>a</sup> <sup>a</sup>Shanghai 10th People's Hospital of Tongji University, Shanghai, China; <sup>b</sup>Zhongshan Hospital, Fudan University, Shanghai, China; <sup>c</sup>Zunyi Medical University, Zhunyi, China.



**OBJECTIVE:** Adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) combined treatment is the standard first-line treatment for early stage Hodgkin lymphoma (HL). With this treatment, over 90% of early-stage patients achieve long-term remission and can be considered cured. It is clinically believed that ABVD combined treatment has little effect on fertility and no risk of POI, but there is serious gonadal toxicity when adriamycin treatment alone. Researchers even found an increase in the number of primitive follicles in the ovaries of patients after ABVD treatment. Only limited data is available on long-term female gonadal toxicity following ABVD combined treatment, and most of them are descriptive clinical studies. The purpose of this study is to investigate the effects of ABVD treatment on reproductive function of female mice.

**DESIGN:** Experimental study on mouse model of ABVD treatment.

**MATERIALS AND METHODS:** Eight weeks female mice were injected i.p. with vehicle or ABVD once weekly for 4 weeks. Estrous cycles were monitored daily after first injection (n=5 each). Body weight, ovary weight, number of follicles at each stage and serum AMH were analyzed after finishing treatment and estrous cycles recovered to normal (n=5 each). Natural mating trials were undertaken when the estrous cycles recovered (n=5 each). Offspring data (number of pups per litter and pup weight at postnatal Day 2) were recorded.

**RESULTS:** Most mice were completely lost estrous cycle after 1 week of first ABVD injection. After 4 weeks treatment, the body weight change (3.95±1.52 vs. 0.42±2.05g,  $P<0.01$ ), the gross ovarian weight (11.68±1.89 vs. 3.48±2.41mg,  $P<0.01$ ), the ovary organ index (38.67±5.50 vs. 12.51±8.56,  $P<0.01$ ) were significantly lower than the control group. Follicle counts revealed a significantly decrease in the number of primordial follicles (363.4±59.97 vs. 138.3±17.88,  $P<0.01$ ), secondary follicles (61.74±5.08 vs. 46.36±12.81,  $P<0.05$ ), antral follicles (51.46±20.46 vs. 16.32±3.81,  $P<0.05$ ), mature follicles (26.36±16.03 vs. 4.06±1.63,  $P<0.05$ ) and total follicles (753.1±109.5 vs. 401.1±42.63,  $P<0.01$ ) after 4 weeks treatment. Estrous cycles returned to normal after 4 weeks ABVD removed. Then there were no differences on the gross ovarian weight (7.93±3.11 vs. 7.35±3.44mg,  $P=0.87$ ) and the ovary organ index (26.11±10.81 vs. 27.94±13.34,  $P=0.88$ ) compared with the control. Interestingly, serum AMH (170.70±16.60 vs. 215.70±5.32 ng/L,  $P<0.01$ ) and number of primordial follicles (121.5±15.12 vs. 364.1±133.1,  $P<0.05$ ), primary follicle (130.7±18.14 vs. 312.4±101.5,  $P<0.05$ ) and total follicles (527.6±22.3 vs. 913.1±269.2,  $P<0.05$ ) were significantly increased. The number of offspring in ABVD group was less than that in control group (12.2±1.79 vs. 9.4±0.55,  $P<0.05$ ), but there was no significant difference in offspring weight between ABVD group and control group (1.64±0.13 vs. 1.64±0.19,  $P=0.95$ ).

**CONCLUSIONS:** ABVD treatment can affect the estrus cycle of mice, but the reproductive function can be restored and the fertility reserve ability can be recovered following drug withdrawal.

References: 1. Borchmann S, von Tresckow B, Engert A. Current developments in the treatment of early-stage classical Hodgkin lymphoma. *CURR OPIN ONCOL* 2016;28:377-83.

2. Schaapveld M, Aleman BM, van Eggermond AM, Janus CP, Krol AD, van der Maazen RW *et al.*. Second Cancer Risk Up to 40 Years after Treatment for Hodgkin's Lymphoma. *N Engl J Med* 2015;373:2499-511.

3. McLaughlin M, Kelsey TW, Wallace WH, Anderson RA, Telfer EE. Non-growing follicle density is increased following adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) chemotherapy in the adult human ovary. *HUM REPROD* 2017;32:165-74.

4. Barr RD. Risk of premature menopause after treatment for Hodgkin's lymphoma. *J Natl Cancer Inst* 2014;106.

5. Eelink CM, Incrocci L, Witte BI, Meurs S, Visser O, Huijgens P *et al.*. Fertility and sexual function in female Hodgkin lymphoma survivors of reproductive age. *J CLIN NURS* 2013;22:3513-21.

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**P-349** Tuesday, October 15, 2019 6:30 AM

**THE IMPACT OF BOTULINUM TOXIN A (BOTA) TREATMENT ON ENDOMETRIAL BLOOD FLOW.** Youn-Jung Kang, Ph.D.<sup>a</sup> Sooyeon Kim, MD,<sup>b</sup> Siwon Lee, MD,<sup>c</sup> Hwang Kwon, M.D., Ph.D.,<sup>d</sup> Jung-Jae Ko, Ph.D.<sup>a</sup> Kyung-A. Lee, Ph.D.<sup>a</sup> Hwa Seon Koo, MD.<sup>a</sup> <sup>a</sup>CHA University, Seongnam-si, Korea, Republic of (South); <sup>b</sup>CHA Bundang Medical center, Seongnam-si, Korea, Republic of (South); <sup>c</sup>Department of obstetrics and gynecology, Mound Sinai Medical Center, Miami Beach, FL; <sup>d</sup>Fertility Center, Seongnam-si, Gyeonggi-do, Korea, Republic of (South).



**OBJECTIVE:** Most embryos produced *in vitro* fail to produce live offspring after transfer. There is a dearth of research activity addressing this problem despite the significant population of women suffering repeated failure of implantation after transfer of high-quality embryos. We hypothesize that a proportion of these failures arises due to failure of construction of functional endometrium with the proficient blood flow. We have investigated the impact of treatment with Botulinum toxin A (BoTA), which is widely used in the field of plastic and reconstructive surgery with the specific purpose of enhancement in wound healing, to induce endometrial angiogenesis to improve the endometrial blood flow and increase the vessel formation at the site of uterine cavity.

**DESIGN:** *In vitro* assessment of impact of BoTA treatment on endometrial epithelial and stromal cells using various types of cell-based assay. *In vivo* effect of intrauterine injection of BoTA on endometrial angiogenesis by measuring CD31 expression.

**MATERIALS AND METHODS:** **i) *in vitro*:** BoTA (0.5, 2, 10 IU/ml) was exposed to human endometrial epithelial carcinoma (Ishikawa) cells and stromal (CRL4003) cells in culture condition for 24h and 72h. Proliferation and migration of the 2 cell types were observed in response to BoTA treatment. Quantitative RT-PCR was used to quantify the expression levels of HIF1 $\alpha$  and VEGF $\alpha$ , well-known surrogates of angiogenic effects. Data were normalized to  $\beta$ -actin mRNA and analyzed using the ordinary one-way ANOVA with Tukey's multiple comparisons. **ii) *in vivo*:** BoTA was injected to the intrauterine cavity of female mice and uterine tissues were harvested at day 3 and 8. Changes in endometrial histology and CD31 immunoreactivity in response to BoTA treatment were examined to assess the levels of endometrial angiogenesis.

**RESULTS:** BoTA treatment enhances the capacity of proliferation of wound healing of both endometrial epithelial and stromal cells. QRT-PCR results revealed that soluble BoTA treatment induced integrin  $\beta$ 3 (~3 fold) and IL-8 (~2 fold) mRNA in both endometrial epithelial (Ishikawa cells) and stromal cells (CRL4003). The expression levels of HIF1 $\alpha$  (~1.5 fold,  $p<0.001$ ) and VEGFR2 (~4 fold,  $p<0.001$ ) were significantly increased in BoTA-treated Ishikawa cell compared to untreated group. In CRL4003 cells, Vimentin (~1.5 fold,  $p<0.001$ ) and IL-6 (~2.5 fold,  $p<0.001$ ) were significantly higher in groups with BoTA treatment compared to control group. Of note, little impact was observed in 10 IU BoTA-treated cells and no toxic effect was induced by BoTA treatment. Significantly, intrauterine injection of BoTA induced higher expression of CD31 in uterine tissues compared to saline-treated group displaying higher numbers of blood vessel formation near uterine cavity.

**CONCLUSIONS:** Our findings indicate that BoTA treatment has a beneficial effect on reconstruction of functional endometrium prior to embryo implantation by increasing endometrial blood flow near the uterine cavity suggesting BoTA treatment as a potential therapeutic strategy for *in vitro* fertilization-embryo transfer (IVF-ET) cycles.

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**WITHDRAWN**

**P-351** Tuesday, October 15, 2019 6:30 AM

**HORMONE SECRETION IN A MICROFLUIDIC OVARY-ON-A-CHIP PLATFORM USING ENGINEERED FOLLICLES.** Young Bin Won, M.D.,<sup>a</sup> Inha Lee, M.D.,<sup>a</sup> Jae Hoon Lee, M.D.,<sup>a</sup> SiHyun Cho, M.D., Ph.D.,<sup>b</sup> Hee Dong Chae, M.D., Ph.D.,<sup>c</sup> Byung Seok Lee, M.D., Ph.D.,<sup>a</sup> Young Sik Choi, M.D., Ph.D.<sup>a</sup> <sup>a</sup>Yonsei University College of Medicine, Severance hospital, Seoul, Korea, Republic of (South); <sup>b</sup>Yonsei University



College of Medicine, Gangnam Severance Hospital, Seoul, Korea, Republic of (South); <sup>c</sup>Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of (South).

**OBJECTIVE:** Although organ-on-a-chip platforms to reproduce physiological functions have been developed in a variety of tissues, there have been only a few reports on ovary-on-a-chip platforms. The human ovarian follicle is the functional unit of an ovary which consists of granulosa and theca cells interacting in an intimate relationship to produce reproductive hormones such as estradiol and progesterone. The aim of this study was to develop a dynamic microfluidic ovary-on-a-chip platform comprising of multilayered engineered follicles that could demonstrate ovarian endocrine function *in vitro*.

**DESIGN:** *In vitro* animal study.

**MATERIALS AND METHODS:** Granulosa and theca cells were isolated from the ovaries of 3-5 week old rats. After aggregation of cells into a spheroid shape, the engineered follicles were placed in a PDMS platform for structural support and dynamic microfluidics was constructed in a three-dimensional network of gelatin hydrogels fabricated with thermo-responsive sacrificial poly(N-isopropylacrylamide) microfibers. Two types of engineered follicles were crafted through forced aggregation of theca and granulosa cells; Bi-layered follicle with inner granulosa cells surrounded by outer theca cells (BF), and tri-layered follicle with a 5% matrigel basal membrane between the two cell layers (TF). Three dimensional static and dynamic cultures were observed for 30 days. The dynamic culture medium was continuously perfused at a flow rate of 7  $\mu$ L/min. Hormone secretion of 17 $\beta$ -estradiol, progesterone, and testosterone were measured by ELISA. Spheroid circularity was assessed to determine the effect of morphological factors on hormone secretion. F-actin staining to assess the overall shape and structure of the cells and live/dead assay to assess the cell viability were performed.

**RESULTS:** The structure and viability of engineered follicles were maintained for both the static and dynamic cultures up to the observed 30 days. The circularity of TF was maintained better than that of BF in both static and dynamic culture. The dynamic TF produced 17 $\beta$ -estradiol for longer without decrease as opposed to the dynamic BF which tapered off starting from day 15. The same was true for progesterone production. Progesterone secretion peaked at day 21 and remained elevated longer for dynamic TF compared to the dynamic BF. Hormone production, both 17 $\beta$ -estradiol and progesterone, remained uniformly stagnant without increasing during static culture and significantly lower compared to that of the dynamic culture. Statistically significant differences in testosterone levels were not observed among all static and dynamic cultures.

**CONCLUSIONS:** This microfluidic ovary-on-a-chip platform using engineered follicles with a matrigel basal membrane yielded better hormone secretion results. This platform may provide an opportunity to research ovarian physiology and to establish a novel *in vitro* disease model.

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#### **LOW MOLECULAR WEIGHT HYALURONAN INDUCES INFLAMMATORY GENE EXPRESSION IN OVARIAN STROMAL CELLS AND IMPAIRS GAMETE DEVELOPMENT *IN VITRO*.**

Jennifer E. Rowley, MS,<sup>a</sup> Farners Amargant Riera, PhD,<sup>a</sup> Michele T. Pritchard, PhD,<sup>b</sup> Francesca E. Duncan, PhD,<sup>a</sup> <sup>a</sup>Center for Reproductive Science, Northwestern University, Chicago, IL; <sup>b</sup>Department of Pharmacology, Toxicology, & Therapeutics, Kansas University Medical Center, Kansas City, KS.



**OBJECTIVE:** Female reproductive aging is characterized by a decline in gamete quantity and quality. We recently identified that the ovarian stroma becomes inflamed with age. The ovarian stroma is the microenvironment in which gametes develop, and we predict that this pro-inflammatory milieu impacts gamete quality. In other tissues, age-associated inflammation is partially driven by extracellular matrix (ECM) degradation products, such as low molecular weight hyaluronan (LMW-HA). HA is a major component of the ovarian ECM, and enzyme expression data suggests that ovarian HA is increasingly fragmented into LMW-HA with age. Thus, we hypothesized that LMW-HA fragments stimulate an inflammatory response in the ovarian stroma and impair gamete quality.

**DESIGN:** Two tightly controlled *in vitro* mouse model systems to examine the effect of LMW-HA on the stroma and follicle compartments of the mammalian ovary.

**MATERIALS AND METHODS:** Isolated ovarian stromal cells or secondary ovarian follicles were treated with physiologically relevant (10  $\mu$ g/mL or 100  $\mu$ g/mL) concentrations of 200 kDa LMW-HA. Ovarian stromal cells were treated for 6 hours and expression of 84 inflammatory genes was

analyzed using a qPCR array. Isolated follicles were cultured with LMW-HA for 12 days. Follicle survival, growth, morphology, estradiol production and markers of gamete quality were assessed using brightfield microscopy.

**RESULTS:** Primary ovarian stromal cells treated with both concentrations of LMW-HA exhibited differential expression of 16 pro-inflammatory genes. Most notably, eotaxin receptor *Ccr3* (4.07 and 3.57-fold change following 10  $\mu$ g/mL or 100  $\mu$ g/mL treatment, respectively) and a suite of *Ccr3*-related, eosinophil activation genes ( $p = 0.044$ ) were significantly regulated by LMW-HA. Interestingly, these findings were consistent with an age-dependent increase in ovarian stromal expression of *Cc111*, a major CCR3 ligand (1.86-fold change,  $p = 0.0002$ ). In follicle cultures, LMW-HA treatment did not affect follicle survival, growth, or morphology but the 100  $\mu$ g/ml condition did significantly reduce estradiol production ( $p = 0.0098$ ). With respect to gamete quality, follicles grown in 10  $\mu$ g/mL LMW-HA produced a higher proportion of morphologically abnormal gametes relative to controls (50.7% vs. 17.1%,  $p = 0.0035$ ). Strikingly, only 48.1% of morphologically normal gametes reached a mature metaphase-II (MII) stage (versus 90.0% of control normal gametes,  $p = 0.0213$ ) and MII eggs had significantly smaller diameters ( $p = 0.0023$ ). Follicles cultured in 100  $\mu$ g/mL LMW-HA produced more severely impaired gamete morphology, as 97.4% of gametes were abnormal (vs. 8.8% of controls,  $p < 0.0001$ ). This was primarily due to premature oocyte meiosis resumption by day 10-12 of culture, ultimately leading to *in vitro* aging of the resulting MII eggs ( $p = 0.026$ ).

**CONCLUSIONS:** Our data demonstrate that bioactive LMW-HA fragments may contribute to reproductive aging by driving an inflammatory stromal milieu while also reducing gamete quality and impairing granulosa cell function. Further, these data suggest a novel role of eosinophils in ovarian inflammation.

**SUPPORT:** NICHD, R01HD0937263.

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#### **THE IMPACT OF MULTIPLE-DOSE PACLITAXEL ON FERTILITY IN MICE AND THE PROTECTIVE EFFECT OF GONADOTROPIN RELEASING HORMONE-AGONIST.**

Mengge Cui, Master Department of gynecological oncology, Tongji Hospital, Wuhan, China.



**OBJECTIVE:** Chemotherapeutic agents have numerous side effects. However, whether paclitaxel, the best-selling agent, causes premature ovarian failure is still controversial. In addition, most of articles only used single-dose paclitaxel, which was far from clinical dosage, to show the findings. Thus, further studies should be made on multiple doses. Besides, with the increase in incidence and the decrease in fatality of some cancers, the demand for ovarian protection is soaring. Gonadotropin releasing hormone agonists (GnRH<sub>a</sub>) are the most widely used agents for ovarian protection during chemotherapy. However, the available evidence is insufficient. And, the protective effect of GnRH<sub>a</sub> to ovaries from paclitaxel remains unclear.

Our study is going to demonstrate the phenomena and duration of the gonadotoxicity caused by multiple-dose paclitaxel and to investigate the protective effect of GnRH<sub>a</sub> meanwhile via mimicking the clinical dosages and intervals in NCCN guidelines.

**DESIGN:** Three subsequent doses of 30 mg/kg paclitaxel (1 dose per 3 days) or an equal volume of vehicle was given intraperitoneally to 7-week-old female ICR mice. These mice were given 1 mg/kg GnRH<sub>a</sub> (every day) or normal saline for one estrous cycle before, during and another estrous cycle after chemotherapy. On the 1st, 6th, 11th or 16th day after the multiple-dose paclitaxel, the mice were managed in several ways: follicle counting ( $n=5$ /group/time point), acquisition of oocytes ( $n=5$ /group/time point) and immunofluorescence.

**MATERIALS AND METHODS:** 1. Seven-week-old female ICR mice 2. GnRH<sub>a</sub> (triptorelin acetate, Ferring AG, Switzerland), paclitaxel (Hospira Australia Pte Ltd) 3. Histology and follicle count 4. Oocyte collection 5. Immunofluorescence 6. Statistical analysis.

**RESULTS:** The follicle counting showed that paclitaxel only destroyed antral follicles for 2 estrous cycles after chemotherapies and induced increasing atretic follicles without affecting follicles in other stages. Add GnRH<sub>a</sub> to paclitaxel significantly reduced the amount of atretic follicles (30.60 $\pm$ 5.00 versus 63.80 $\pm$ 4.00,  $P < 0.05$ ). Moreover, the ovarian stimulation was also performed to determine the duration of the gonadotoxicity after multiple-dose paclitaxel. The acquisition of MII oocytes in paclitaxel-only group was extremely less on the 1st and 6th day after the last dose of the treatment (D1: 1.00 $\pm$ 0.00 versus 30.40 $\pm$ 5.27,  $P < 0.01$ ; D6: 17.20 $\pm$ 4.25 versus 31.33 $\pm$ 4.67,  $P < 0.05$ ). Compare to the control, mice, with the protection of GnRH<sub>a</sub>, ovulated even more MII oocytes on the 6th day after chemotherapies (46.80 $\pm$ 3.44 versus 31.33 $\pm$ 4.67,  $P < 0.05$ ). And

up to 2 estrous cycles after the last dose, the quantity of MII oocytes in all groups showed no statistical difference. Meanwhile, the morphology of oocytes was observed by immunofluorescence.

**CONCLUSIONS:** These results indicated that paclitaxel mainly impacted antral follicles and the gonadotoxicity lasted no more than 2 estrous cycles of mice. The protective effect of GnRH $\alpha$  on ovaries was significant. This study provides a laboratory evidence for the impact of paclitaxel and the effectiveness of GnRH $\alpha$  in clinical practice.

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**ESTABLISHMENT OF DECREASED OVARIAN RESERVE MOUSE MODEL BY CONSECUTIVE SUPEROVULATION.** Xiaowei Nie, M.D., Daorong Hou, Ph.D. Embryologist, NANJING, China.



**OBJECTIVE:** This study investigated the effect of consecutive superovulation on the ovaries and established a decreased ovarian reserve (DOR) model in mice.

**DESIGN:** One hundred fifty C57BL/6 female mice aged 7–8 weeks and thirty C57BL/6 female mice aged 44 weeks were used. The mouse POF model was induced by 5–15 consecutive superovulation treatments with pregnant mare serum gonadotropin (PMSG), human chorionic gonadotropin (HCG) and prostaglandin F $_{2\alpha}$  (PGF $_{2\alpha}$ ). Normal adult mice were compared with mice displaying natural ovarian aging.

**MATERIALS AND METHODS:** The following serum biochemical parameters were measured: including follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P), estradiol (E2), inhibin B (INH B), malondialdehyde (MDA), total superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) levels. Follicles were counted using H&E staining. Levels of 8-hydroxyguanosine (8-OHdG), 4-hydroxynonenal (4-HNE), Nitrotyrosine (NTY), anti-Mullerian hormone (AMH) and CDKN2A/p16 (p16) were detected using immunohistochemical staining. Reactive oxygen species (ROS) levels were measured using dihydroethidium (DHE) staining. Cell apoptosis was detected using an in situ TUNEL fluorescence staining assay. Levels of proteins involved in ROS-related pathways and the p16 protein were detected using Western blotting. Sod1, Sod2 and Sod3 mRNA levels were detected using quantitative polymerase chain reaction (Q-PCR). Oocyte quality was evaluated using in vitro fertilization (IVF) and zygote culture.

**RESULTS:** Consecutive superovulation groups presented lower P, E2, SOD, GSH-Px and INH B levels, significantly higher FSH, LH, MDA and ROS levels, and significantly fewer primordial follicles compared with the control group. Consecutive superovulation groups presented significantly increased levels of Sod2, 8-OHdG, 4-HNE, NTY, significantly increased levels of the SIRT1 and FOXO1 proteins, significantly increased levels of the senescence-associated protein p16, as well as decreased AMH, Sod1 and Sod3 levels and increased granulosa cell apoptosis compared with the control group.

**CONCLUSIONS:** Consecutive superovulation significantly decreased ovarian function and oocyte quality and increased oxidative stress and apoptosis in the ovary via a mechanism involving the p16 and SIRT1/FOXO1 signaling pathways. These findings suggest that consecutive superovulation may be used to establish a mouse model of ovarian aging.

**References:** 1. Zhang J, Fang L, Shi L, Lai Z, Lu Z, Xiong J, Wu M, Luo A, Wang S: Protective effects and mechanisms investigation of kantai capsule on the ovarian function of a novel model with accelerated aging ovaries. *Journal of Ethnopharmacology* 2017;195:173-181.

2. Jeelani R, Khan SN, Shaeib F, Kohan-Ghadir HR, Aldaheri SR, Najafi T, Thakur M, Morris R, Abu-Soud HM: Cyclophosphamide and acrolein induced oxidative stress leading to deterioration of metaphase ii mouse oocyte quality. *Free Radic Biol Med* 2017;110:11-18.

3. Ben-Meir A, Yahalomi S, Moshe B, Shufaro Y, Reubinoff B, Saada A: Coenzyme Q-dependent mitochondrial respiratory chain activity in granulosa cells is reduced with aging. *Fertil Steril* 2015;104:724-727.

4. Benadiva C, Engmann L: Luteal phase support after gonadotropin-releasing hormone agonist triggering: does it still matter? *Fertil Steril* 2018;118:30074-30078.

5. Zhang JQ, Shen M, Zhu CC, Yu FX, Liu ZQ, Ally N, Sun SC, Li K, Liu HL: 3-nitropropionic acid induces ovarian oxidative stress and impairs follicle in mouse. *PLoS one* 2014;9:e86589.

6. Ding C, Zou Q, Wang F, Wu H, Wang W, Li H, Huang B: HGF and bFGF secretion by human adipose-derived stem cells improves ovarian function during natural aging via activation of the SIRT1/FOXO1 signaling pathway. *Cell Physiol Biochem* 2018;45:1316-1332.

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**CHARACTERIZING MEIOTIC AND MITOTIC ERRORS IN THE INNER CELL MASS AND TROPHOCTODERM OF POOR QUALITY PREIMPLANTATION EMBRYOS.** Christine Briton-Jones, PhD, HCLD,<sup>a</sup>



Lucky Sekhon, MD,<sup>a</sup> Ethan Ellis, BS,<sup>b</sup> Joseph A. Lee, BA,<sup>a</sup> Eric E. Schadt, PhD,<sup>b</sup> Robert P. Sebra, PhD,<sup>c</sup> Alan B. Copperman, MD,<sup>b</sup> <sup>a</sup>Reproductive Medicine Associates of New York, New York, NY; <sup>b</sup>Icahn School of Medicine at Mount Sinai, New York, NY; <sup>c</sup>Sema4, a Mount Sinai Venture, Stamford, CT.

**OBJECTIVE:** Human blastocysts that undergo trophectoderm (TE) cell biopsy for pre-implantation genetic testing for aneuploidy (PGT-A) are capable of achieving normal morphological development despite having gains or losses in chromosome copy number. Occasionally in clinical embryo culture we see nonviable blastocysts with morphology of few or no inner cell mass (ICM) cells and good quality TE cells. Also the reverse is seen, a blastocyst with good quality ICM but few elongated TEs. These embryos are not suitable for biopsy or clinical use. However, these morphologically abnormal blastocysts provide a novel glimpse at the very earliest stages of human cell differentiation. The aim of the study was to compare rates of meiotic and mitotic errors resulting in loss, gain or mosaicism of chromosomes in cells from poor quality blastocysts.

**DESIGN:** Experimental study on human embryos donated for research.

**MATERIALS AND METHODS:** The study included embryos donated by patients from fresh cycles between January and June of 2016. Embryos reaching the blastocyst stage of development but ineligible for TE biopsy (<4CC, Modified Gardner's), were biopsied and 5-6 cells were evaluated for aneuploidy by NGS. Ploidy status including mosaicism was identified based on bioinformatical interpretation of chromosome copy number falling within disomic (between 1.8 - 2.2), aneuploid thresholds (less than 1.2 and more than 2.8) and mosaic (between 1.2-1.8 and 2.2-2.8). The mean number of monosomy, trisomy and mosaic calls was determined and compared for each study group. Kruskal Wallis was used to determine statistically significant differences, where  $p < 0.05$ .

**RESULTS:** Of the 15 blastocysts, with isolated poorly graded ICM (n=9) or trophectoderm (n=6), that underwent NGS. Of the embryos with poor ICM and good TE: 7 were euploid; 2 were mosaic and 1 was aneuploid. Of the embryos with good ICM and poor TE grade: 2 were euploid and 4 had complex aneuploidy. Blastocysts with good ICM grade but poor TE grade had significantly higher incidence of mosaicism and aneuploidy ( $p < 0.0001$ ).

**CONCLUSIONS:** As embryo development reaches the blastocyst stage, the incidence of aneuploidy is significantly reduced. The reason for this reduction in incidence of aneuploid calls between cleavage stage and blastocyst stage embryos is believed to be that the burden of aneuploidy leads to embryonic arrest. This study showed that embryos with many copy number variants are still capable of growing a morphologically normal ICM. In contrast the blastocysts with morphologically normal trophectoderm had fewer aneuploid calls, despite having none or few ICM cells present. Our study's findings suggest that the consequence of aneuploidy is less severe in ICM cells compared to TE cells, as at this specific time point in embryonic development, ICM cells are more closely related to the cleavage stage blastomeres than the differentiated trophectoderm. Our current work is focused on identifying differential gene expression in these embryos, which allow a unique opportunity to study the roles of ICM and TE cells largely independent of each other.

**Reference:** None.

**SUPPORT:** None.

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**3D MODELING REVEALS CHROMOSOMES EXHIBIT NONRANDOM SEGMENTAL RADIAL ORGANIZATION AND UNIQUE HAIRPIN-LOOP CONFIGURATIONS IN SPERM NUCLEI.** Helen G. Tempest, PhD,<sup>a</sup> Dimitrios Ioannou, PhD,<sup>b</sup> <sup>a</sup>Florida International University, Miami, FL; <sup>b</sup>Embryologist, Miami.



**OBJECTIVE:** Genomes are non-randomly organized within the interphase nucleus; and spermatozoa are proposed to have a unique hairpin-loop

configuration, which has been hypothesized to be critical for the ordered exodus of the paternal genome following fertilization. This model describes centromeres clustering in the center (chromocenter), with p- and q-chromosome arms stretching toward the nuclear periphery. However, we recently proposed a refined segmental model of sperm chromatin organization; to further investigate these findings and their relationship to the hairpin-loop model we examined the 3D configurations of chromosomes in human sperm nuclei.

DESIGN: Transversal laboratory study.

MATERIALS AND METHODS: This study was approved by the local IRB, five normozoospermic males were recruited. Three-color fluorescence in-situ hybridization (FISH) was utilized to target the centromeres, and chromosome p- and q-arms of eight chromosomes (2, 3, 6, 8, 10, 12, 16, and 18). Wide-field fluorescence microscopy and 3D modeling was employed to image and visualize sperm cells and FISH probes in 3D. The radial organization of each targeted loci was established by measuring the distance of the geometric center of each loci to the nearest nuclear periphery. Nonrandom organization of was established using the Chi-squared goodness-of-fit test ( $p < 0.05$ ). Furthermore, hairpin-loop configurations were determined by the angle created between the p- and q-arms. A minimum of 30 cells per subject, per chromosome were studied.

RESULTS: Distinct reproducible chromosome-specific patterns of organization emerge. All chromosomes were found to possess nonrandom radial organization ( $p < 0.05$ ), with the exception of the chromosome 12 centromere. Chromosome arms were found to form discrete hairpin-loop configurations. However, different chromosomes were observed to preferentially form narrower or wider hairpin loops that were largely reproducible between the five subjects enrolled. We did not find evidence to support the existence of a centralized chromocenter(s) with 68.3% of investigated centromeres being more distally localized within the sperm nucleus than one (30.5%) or both (37.8%) of their respective chromosome arms.

CONCLUSIONS: We report reproducible nonrandom hairpin-loop organization of chromosomes that partially supports the proposed hairpin-loop model of organization. However, our findings do not support the existence of a centralized chromocenter. This provides further evidence to support a more segmented chromosome organization in the human sperm nucleus, which could result in specific genomic regions being exposed, remodeled and activated first, following fertilization. The sequential exodus and remodeling could impact patterns of gene activation observed within the early embryo, perturbations in which, could negatively impact fertilization and early embryogenesis. Further research is warranted to evaluate the functional relevance of the nonrandom hairpin-loop organization of chromosomes in sperm observed in this study, and how this may impact spermatogenesis, fertilization and embryogenesis.

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#### THE UBIQUINONE MITOCHONDRIA-TARGETED ANTIOXIDANT AMENDS THE EFFECT OF MATERNAL AGE ON OOCYTE SPINDLE FORMATION AND DEVELOPMENTAL COMPETENCE.

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OBJECTIVE: To examine the effect of maternal age on mitochondrial function and spindle formation in maturing oocytes, and to investigate whether in vitro treatment with mitochondria-targeted antioxidants (AOs) can reverse the impact of aging on oocyte quality.

DESIGN: Preclinical models of oocyte quality were used for this study. In experiment 1, oocytes were obtained from young (1-month-old) mice and treated with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to induced oxidative stress or with H<sub>2</sub>O<sub>2</sub> and mitochondria-targeted AOs. In experiment 2, oocytes were collected from young and old (>12 months) mice and treated with mitochondria-targeted AOs during in vitro maturation (IVM). End point assays in both experiments were stage of maturation reached, mitochondrial function and spindle organisation.

MATERIALS AND METHODS: Cumulus-free oocytes were cultured in vitro for 14 h in M2 or M16 medium, or in the same medium containing H<sub>2</sub>O<sub>2</sub> (25 μM), with or without AOs (experiment 1). In a separate experiment, oocytes from young and old mice were matured in vitro in the presence and absence of mitochondria-targeted AOs. At the end of the culture period mito-

chondrial membrane potential (MMP) was measured by ratioing the fluorescence intensities of Tetramethylrhodamine methyl ester (TMRM), and MitoTracker green (MTG). Oocytes were fixed to label the microtubules and DNA. ImageJ software was used to analyse spindle dimensions and chromosome alignment. Student *t*-test and ANOVA were applied to compare between groups and a P value below 0.05 were considered statistically significant.

RESULTS: We find oxidative stress causes a decrease in MMP ( $P < 0.001$ ) and an increase in the frequency of disrupted spindles and misaligned chromosomes ( $P < 0.001$ ). Co-treatment with H<sub>2</sub>O<sub>2</sub> and AOs reversed the MMP and spindle disruption to control levels. Oocytes from old mice matured to the MII stage in vitro also show decreased MMP and disrupted spindles. These age-related phenotypes were completely reversed by incorporating antioxidants in the culture media. Furthermore, for the first time we have performed live-cell ratiometric imaging of TMRM and MTG for the full time-course of maturation in young and old eggs. This study reveals that MMP increases significantly during IVM in young oocytes ( $P < 0.001$ ) but not in old oocytes.

CONCLUSIONS: Oxidative stress and maternal age are both associated with decreased MMP and spindle disruption and chromosome misalignment. Time-lapse imaging suggests that mitochondria in young oocytes undergo an adaptive increase in MMP during IVM and that this capacity is lost in mitochondria of old oocytes. The compromised mitochondrial function in maternal aging and the ability of the mitochondria-targeted AOs treatment to mitigate against aging and oxidative stress-induced mitochondrial and spindle disruption, suggests that mitochondria may be a useful therapeutic target for improving oocyte quality.

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#### LOSS OF MITOCHONDRIAL FUSION PROTEIN MFN2 RESULTS IN A REPRODUCTIVE AGING PHENOTYPE WITH TELOMERE SHORTENING, REDUCED FERTILITY, AND ACCELERATED DEPLETION OF FOLLICULAR POOL.

Man Zhang, M.D., Ph.D.,<sup>a</sup> Muhammed Burak Bener, B.S.,<sup>a</sup> Zongliang Jiang, Ph.D.,<sup>b</sup> Tianren Wang, M.D., Ph.D.,<sup>c</sup> Ecem Esencan, M.D.,<sup>a</sup> Richard Scott III, B.S.,<sup>d</sup> Emre Seli, M.D.,<sup>a</sup> <sup>a</sup>Yale School of Medicine, New Haven, CT; <sup>b</sup>Louisiana State University, Baton Rouge, LA; <sup>c</sup>Foundation for Embryonic Competence, Basking Ridge, NJ; <sup>d</sup>Foundation of Embryo Competence, Basking Ridge, NJ.

OBJECTIVE: Mitochondria change their shape through fusion and fission in order to adapt to their metabolic milieu and respond to environmental stress. Mitofusin-2 (MFN2) is a key regulatory protein in this process, mediating mitochondrial fusion and interaction with endoplasmic reticulum. The aim of the present study was to determine the role of MFN2 in female reproductive competence using a mouse model with oocyte-specific deletion of *Mfn2*.

DESIGN: Experimental study.

MATERIALS AND METHODS: *Mfn2*<sup>fllox/fllox</sup> mice were crossbred with *Zp3-Cre* mice to produce mice with oocyte-specific *Mfn2* deletion (*Mfn2*<sup>-/-</sup>). To evaluate fertility, mature (8-weeks-old) *Mfn2*<sup>-/-</sup> and wild type (WT) female mice were mated with adult WT males of proven fertility for 12 weeks. Follicle development was assessed in serial ovarian sections stained with hematoxylin and eosin. Ability to generate oocytes (germinal vesicle [GV] and metaphase II [MII]), 2-cell embryos, and blastocysts was assessed after injection with PMSG (5IU) or PMSG and hCG (5IU) and mating with WT males as indicated. RNA sequencing analysis was performed using pooled *Mfn2*<sup>-/-</sup> and WT GV oocytes and secondary follicle enclosed oocytes (SFOs) (n=3 mice per group). Protein and mRNA expression were assessed using immunofluorescence and qRT-PCR, respectively. Telomere length was assessed using quantitative real-time PCR.

RESULTS: Mature female *Mfn2*<sup>-/-</sup> mice exhibited reduced fertility compared to WT females (5.21 ± 0.39 vs 7.63 ± 0.31 pups per litter,  $p < 0.001$ ). They had decreased number of antral follicles (9.33 ± 2.33 vs 30.67 ± 1.67,  $P < 0.01$ ), and generated a significantly lower number of GV oocytes (16.33 ± 1.20 vs 29.33 ± 0.67,  $p < 0.001$ ), MII oocytes (10 ± 0.58 vs 21.33 ± 1.20,  $p < 0.01$ ), 2-cell embryos (8 ± 0.58 vs 20.33 ± 0.88,  $p < 0.001$ ) and blastocysts (6.33 ± 0.88 vs 13 ± 0.58,  $p < 0.001$ ). RNA-seq analysis revealed 363 and 1041 genes that were differentially regulated in *Mfn2*<sup>-/-</sup> GV oocytes and SFOs, respectively. Affected pathways included telomere signaling in GV oocytes and cell death (apoptosis) signaling in SFOs ( $p < 0.01$ ). Pro-apoptotic protein caspase 6 and ceramide were significantly increased in *Mfn2*<sup>-/-</sup> SFOs. Telomere length in *Mfn2*<sup>-/-</sup> GV oocytes was shorter compared to WT with decreased expression of telomere protective protein TRF1. When we assessed changes in follicular pool across mouse reproductive lifespan, we found *Mfn2*<sup>-/-</sup> ovaries to have significantly lower number of

primordial, secondary, and antral follicles at 6 months ( $p < 0.05$ ), and dramatically decreased number of follicles at all stages at 12 months ( $p < 0.001$ ), compared to WT.

**CONCLUSIONS:** Targeted deletion of *Mfn2* in oocytes results in female subfertility associated with impaired oocyte maturation and follicle development. Oocytes lacking MFN2 show shortened telomeres and increased apoptosis, resulting in compromised oocyte quality and accelerated follicular depletion, consistent with a reproductive aging phenotype.

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### THE ROLE OF AKAP13 INHIBITORS AND ACTIVATORS AND MATRIX STIFFNESS IN HIPPO PATHWAY SIGNALING FOR PRIMORDIAL FOLLICLE ACTIVATION.

Jacqueline Yano Maher, MD, MA, Md, Soriful Islam, PhD, Szu-Chi Su, MS, James Segars, MD. Johns Hopkins University, School of Medicine, Baltimore, MD.



**OBJECTIVE:** Signaling pathways of primordial follicle activation are incompletely understood. Disruption of Hippo pathway signaling promotes gonadotropin independent follicle activation in granulosa cells. F-actin formation increases primordial follicle activation through Hippo signaling inhibition, and A-Kinase Anchoring Protein-13 (AKAP13) possesses a Rho-guanine exchange region (GEF) that promotes actin nucleation. Our objective was to test whether pharmacologic manipulation with AKAP13 inhibitor (A13) or AKAP13 activator (A02) affected Hippo signaling in a human granulosa cell line. Second, we tested whether activation of RhoA by manipulation of substrate stiffness affected Hippo signaling.

**DESIGN:** Translational research using COV434 cells, derived from a human granulosa cell tumor.

**MATERIALS AND METHODS:** Downstream Hippo signaling targets, Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ), bind to the Tea Domain Family of transcription factors (TEAD). Since TEADs mediate nuclear YAP/TAZ function, we used a TEAD luciferase reporter (TEAD-luc) to assess gene activation by YAP/TAZ. We previously reported endogenous AKAP13 levels in COV434 cells as determined by immunoblot. A13 and A02 are small molecules previously identified by virtual screening for molecules that altered Rho-GEF activity of AKAP13 (Diviani et al., 2016). COV434 cells were plated at 200K/well x 1 day and serum starved the second day. The third day, cells were transfected with 500ng TEAD-luc and either a control vector, an AKAP13 expression construct, 10uM A13, or 10uM of A02. In some experiments, four hours later, FSH was added as a treatment. After 24 hours, cells were lysed, assayed for luciferase activity and normalized with an MTS assay. Next, we assessed changes in TEAD-luc activity among stiff polystyrene (2-4 GigaPascals) vs. 3 different soft silicone Flexcell® plates: untreated, laminin-coated, or pronectin-coated. Student's t-tests were used to determine statistical significance.

**RESULTS:** Addition of AKAP13 did not augment TEAD-luc reporter activity, possibly due to high levels of endogenous AKAP13 in COV434 cells, as detected by immunoblot. Of note, treatment with A13 reduced TEAD-luc activity by 69% ( $p < 0.0001$ ) and A02 increased TEAD-luc activity by 73% ( $p < 0.0001$ ). FSH treatment or vehicle control did not affect TEAD-luc reporter activity. In the second series of experiments, there was a significant decrease in TEAD-luc activity between the polystyrene plate and all 3 silicone Flexcell type plates ( $p < 0.0001$ ).

**CONCLUSIONS:** These data indicate that TEAD-luc reporter activity in COV434 cells could be decreased or increased by inhibition or activation of AKAP13 activity, respectively. If supported by in vivo data, these data suggest that pharmacologic manipulation of Hippo signaling might represent a new strategy for follicle activation.

Reference: Diviani D, Raimondi F, Del Vescovo CD, Dreyer E, Reggi E, Osman H, Ruggieri L, Gonano C, Cavin S, Box CL, Lenoir M, Overduin M, Bellucci L, Seeber M, Fanelli F. Small-Molecule A Protein-Protein Interaction Inhibitor of Oncogenic Rho Signaling. Cell Chem Biol. 2016 Sep 22;23(9):1135-1146.

**SUPPORT:** Howard and Georgeanna Jones Endowment & Edward E. Wallach Research Award.

**P-360** Tuesday, October 15, 2019 6:30 AM

### CULTURE MEDIA WITH AND WITHOUT EXPOSURE TO HUMAN PREIMPLANTATION EMBRYOS CONTAIN EXTRACELLULAR VESICLES OF COMPAREABLE SIZE.

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**OBJECTIVE:** Extracellular Vesicles (EV) are cell-derived particles with a lipid bilayer membrane ranging in size from 30 to more than 1000 nm in diameter. EVs carry a variety of biomolecules and play pivotal roles in intercellular communication. It has been evidenced that EVs secreted by mammalian preimplantation embryos play a central role in the embryo-endometrium crosstalk during implantation and could therefore become potential biomarkers for embryonic reproductive potential. This study aimed to develop and optimize an EV isolation protocol for spent culture media (SCM) of human IVF preimplantation embryos so as to characterize the embryonic EV population and their potential as reproductive biomarkers.

**DESIGN:** Experimental study.

**MATERIALS AND METHODS:** SCM microdrops (50 uL) were collected following 48 hours of embryo culture from day 3 to day 5 of development. Microdrops incubated under the same conditions in the IVF lab without embryo exposure were used as negative controls. Two methods were tested for EV isolation. 1) Size exclusion chromatography (SEC): 70 SCM microdrops were pooled for each sample (2 samples exposed to ~188 embryos each) and concentrated to ~180 uL using centrifugal filters prior to SEC (Izone). After SEC, 21 fractions of 200 uL were obtained from each sample. Fractions 3, 4, and 5, the most enriched in EVs, were further pooled and re-concentrated to ~180 uL. 2) Differential centrifugation: 30 SCM microdrops were pooled for each sample (3 samples exposed to ~100 embryos each). The 3 pooled SCM samples and other 3 non-pooled single embryo culture microdrops were subjected to three rounds of centrifugation at different g forces before a final 90 min step of ultracentrifugation at 110000 x g. Finally, transmission electron microscopy imaging was performed in all processed samples obtained using the 2 methods in order to visualize and analyze EV. Negative controls were processed similarly.

**RESULTS:** Using both SEC and differential centrifugation, spherical and highly electrodense particles ranging from 22 to 159 nm were observed in both embryo SCM and embryo unexposed media samples. Size of nanoparticles isolated from pooled SEC fractions (3, 4 and 5) were comparable between SCM and negative control (Size: SCM =  $57.05 \pm 0.06$  nm, NC =  $44.47 \pm 14.23$  nm). In addition, differential centrifugation allowed for isolation and visualization of EVs from single embryo culture microdrops, as well as from media unexposed to embryos.

**CONCLUSIONS:** SEC and differential centrifugation successfully isolated EVs from pooled and single embryo culture samples of human embryo SCM. Interestingly, presence of EVs was also evidenced from culture medium microdrops unexposed to embryos, which presented a size distribution comparable to the ones found in embryo SCM. These findings urge to conduct further research in order to shed light on the origin and possible effects of non-embryonic EVs in culture media, as well as the role of embryo-derived EVs during implantation and their potential as biomarkers.

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### EFFECTIVENESS OF PLATELET RICH PLASMA ON PREVENTION OF CHLAMYDIA INDUCED HYDROSALPINX IN A MURINE MODEL.

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**OBJECTIVE:** To test whether oviduct delivery of platelet rich plasma (PRP) can attenuate chlamydia induction of hydrosalpinx in a mouse model

**DESIGN:** Intravaginal inoculation of CBA/J mice with *C. muridarum* can induce almost 100% bilateral hydrosalpinx, which was used as a hydrosalpinx induction model for comparing the effect of PRP on hydrosalpinx development.

**MATERIALS AND METHODS:** A total of 16 CBA/J mice were infected intravaginally with a standard dose of *C. muridarum*, then PRP was instilled into one oviduct and a sham instillation with phosphate buffer solution was performed on the contralateral oviduct at the same time. Oviduct instillation was performed in four groups of mice occurring on day 7 (D7), day 7 plus day 21 (D7/21), day 21 (D21), or day 14 plus day 21 plus day 28 (D14/21/28) after infection. Vaginal and rectal shedding were monitored in all mice. Mice were then sacrificed, and pathologic evaluation performed. Statistical analysis was performed using the Mann-Whitney test.

**RESULTS:** Oviduct instillation of PRP on day 21 with or without additional instillations was associated with a 41.5% reduction in degree of hydrosalpinx compared to sham instillation with an average hydrosalpinx score of

1.62 and 2.77 respectively ( $p=0.15$ ). Instillation of PRP on D14/21/28 was associated with a 43% reduction of hydrosalpinx, average score 1.14 and 2 for sham ( $p=0.56$ ). Oviduct instillation of PRP on D21 alone was associated with a 50% reduction in degree of hydrosalpinx compared to sham instillation with an average score of 2 and 4 respectively. The average grade of inflammation on histopathology was 1.57 with any day 21 instillation vs 1.77 sham instillation ( $p=0.54$ ). PRP instillation on D7 was not associated with reduction in degree of hydrosalpinx or grade of inflammatory infiltrate. No differences were observed in vaginal or rectal shedding of *C. muridarum* amongst the four groups.

**CONCLUSIONS:** Our results suggest that oviduct instillation of PRP was associated with a reduction in the degree of *C. muridarum* induced hydrosalpinx in CBA/J mice; however, this reduction was not statistically significant.

References: 1. Á Chen J, Á Zhang H, Á Zhou Z, Á Yang Z, Á Ding Y, Á Zhou Z, Á Zhong E, Á Arulanandam B, Á Baseman J, Á Zhong G. *Chlamydia* induction of a hydrosalpinx in 11A strains of mice reveals multiple host mechanisms for preventing upper genital tract pathology. *PLoS One*. 2014 Apr 15;9(4):e95076.

2. Á Oz M, Á Cetinkaya N, Á Bas S, Á Korkmaz E, Á Ozgu E, Á Terzioğlu GS, Á Buyukkagıncı U, Á Akbay S, Á Caydere M, Á Gungor T. *A randomized controlled experimental study of the efficacy of platelet-rich plasma and hyaluronic acid for the prevention of adhesion formation in a rat uterine horn model*. *Arch Gynecol Obstet*. 2016 Sep;294(3):533-40

3. Á Zhang H, Á Zhou Z, Á Chen J, Á Wu G, Á Yang Z, Á Zhou Z, Á Baseman J, Á Zhang J, Á Reddick RL, Á Zhong G. *Lack of long-lasting hydrosalpinx in A/J mice correlates with rapid but transient chlamydial ascension and neutrophil recruitment in the oviduct following intravaginal inoculation with Chlamydia muridarum* *Infect Immun*. 2014 Jul;82(7):2688-96.

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**WITHDRAWN**

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### THE REGULATION OF ENDOPLASMIC RETICULUM STRESS IMPROVES THE DEVELOPMENT OF POST-OVULATORY AGED MOUSE OOCYTES.

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**OBJECTIVE:** Endoplasmic reticulum stress (ER stress) is closely associated with several ageing-related diseases, such as neurodegenerative disorders, diabetes mellitus, arteriosclerosis and cancer. Similarly, ER stress in oocytes and preimplantation embryos may be involved in oocyte aging and affect embryo development. The aim of this study is to clarify the relationship of ER stress with the quality of aged oocytes and whether the regulation of ER stress improves the embryo development of aged oocytes.

**DESIGN:** Animal model study.

**MATERIALS AND METHODS:** Animals were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals, as approved by the Animal Care and Use Committee of Yamagata University. In this study, mouse oocytes released from the oviduct at 14 hours and 20 hours post-hCG were designated as “fresh” and “aged” oocytes, respectively. We compared embryo development and GRP78 expression (a chaperone protein increased by ER stress) in fresh oocytes, aged oocytes and preimplantation embryos. To evaluate the regulation of ER stress on embryo development of aged oocytes, aged oocytes were treated with salubrinal, a specific inhibitor of PERK pathway on ER stress, for 1 hour before IVF. Embryo development, expression of GRP78 and phospho-eIF2a (phosphorylated via the PERK pathway) and the rate of dead blastomeres in blastocysts were compared between aged oocytes and salubrinal-treated aged oocytes. Lastly, embryo transfer of salubrinal-treated aged oocytes was performed to examine the safety of salubrinal.

**RESULTS:** Aged oocytes showed lower fertilization rate and poor embryo development like ER stress-induced oocytes. Although GRP78 expression was significantly higher in aged oocytes than in fresh oocytes, salubrinal significantly lowered GRP78 and phospho-eIF2a levels and improved embryo development via decrease of dead blastomeres. Salubrinal treatment

had no adverse effect on pups' birth weight and the presence of congenital malformation, as well as no significant effect on the rate of live births. Pregnancy rate, however, was significantly higher in the salubrinal-treated group than in the aged group.

**CONCLUSIONS:** Present results show that ER stress contributed to oocyte aging and suppression of the ER-related PERK pathway by salubrinal significantly improved embryo development in post-ovulatory aged oocytes. Hence, regulation of ER stress might represent a promising therapeutic strategy to overcome poor oocyte quality.

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### ABNORMAL PHOTOPERIOD EXPOSURE BEFORE PREGNANCY AFFECTS OFFSPRING LIPID METABOLISM IN SD RATS.

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**OBJECTIVE:** Exposure to constant light or shift work impairs endogenous circadian rhythm, which can lead to metabolic diseases. Previous animal and human studies demonstrated that circadian rhythm disruption during pregnancy affects the long-term health of their progeny. But circadian rhythm disruption before pregnancy would have any effect on their offspring is not thoroughly studied. This study is designed to investigate the effects from maternal circadian disruption.

**DESIGN:** Randomized animal study.

**MATERIALS AND METHODS:** Fifteen 6-8 week-old adult female SD rats exposed to abnormal photoperiod (18 h:16 h light/dark cycle) and control photoperiod (12 h:12 h light/dark cycle) for 4 months. Thereafter, rats were housed in control photoperiod, mated, gestated and reared their offspring. At the age of 20 weeks, offspring were sacrificed every 8 hours. Tissue and plasma were harvested. Data were analyzed with t-tests.

**RESULTS:** Exposure to abnormal photoperiod results in prolonged and irregular estrous cycles with pregnancy rate decreased ( $p<0.05$ ). Their ovary weight decreased ( $p<0.01$ ), less corpus luteum and more expanded follicles in ovary H&E stain slides. The offspring from abnormal photoperiod group not only had significantly body weight gain (male +43.1%,  $p<0.01$ ; female +7.8%,  $p<0.05$ ) but also higher body fat rate (female +10.1%,  $p<0.01$ ). The circadian genes expression pattern in the liver were not consistent with control group. Serum LDL-c and HDL-c of female offspring elevated ( $p<0.05$ ). Serum cholesterol and HDL-c of male offspring decreased ( $p<0.05$ ).

**CONCLUSIONS:** Abnormal light-dark cycle induced maternal circadian rhythm disruption have an effect on offspring lipid metabolism disorder in rats. As shift work, artificial night lighting, jet lag are becoming increasingly prevalent. Our finding may have the implications for people to conceive and pay attention to offspring health.

**SUPPORT:** Grant support was provided by the National Key Research and Development Program of China (2018YFC1005003).

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### CYTOKINE PROFILING REVEALS A UNIQUE INFLAMMAGING SIGNATURE IN HUMAN FOLLICULAR FLUID AND THE OVARY.

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**OBJECTIVE:** Reproductive aging in the ovary is characterized by a decrease in oocyte quality and quantity that leads to adverse reproductive outcomes such as infertility, miscarriages, and birth defects. Aging is associated with a general increase in damaging chronic inflammation termed “inflammaging.” The goal of our study was to determine how inflammaging impacts the ovary.

**DESIGN:** Translational.

**MATERIALS AND METHODS:** To examine whether inflammatory cytokines increase in the human ovary with age, we obtained human follicular fluid aspirated from the first follicle from the right or left ovary from 30 participants ranging in age from 27.7-44.8 years old undergoing oocyte retrieval. We performed cytokine antibody arrays on the follicular fluid which measured 80 unique cytokines. Cumulus cells that would have otherwise been discarded were obtained from women undergoing oocyte retrieval for

a non-cancerous diagnosis at Fertility and Reproductive Medicine (FRM) who were  $\geq 18$  years old and  $\leq 30\text{kg/m}^2$ . We performed immunoblot analysis on cumulus cells with a TGF $\beta$ 3-specific antibody and normalized expression to the GAPDH signal. To investigate TGF $\beta$ 3 expression in ovarian tissue, we generated a human ovarian Tissue Microarray (TMA) using samples from two reproductive research archives: the National Physician's Cooperative (NPC) and the Northwestern University Reproductive Tissue Library (NU-RTL). The array contained cortical tissue samples from 60 participants in two cohorts of females: 22 months-20 years old and 39-58 years old. We performed immunohistochemistry on this array with the TGF $\beta$ 3 antibody and quantified expression based on age and tissue sub-structures.

**RESULTS:** Of the 80 cytokines measured in the follicular fluid on the cytokine antibody array, 61 were above threshold. We plotted the cytokines by both chronologic age (years) as well as reproductive age (AMH) and found that six cytokines; IL-3 IL-7, IL-15, TGF $\beta$ 1, TGF $\beta$ 3, and MIP-1 showed a positive correlation with chronologic age but were negatively correlated with AMH. Thus these cytokines represent a unique inflammatory aging signature in the ovary. To validate these follicular fluid findings, we focused on TGF $\beta$ 3 which is part of the transforming growth factor beta (TGF $\beta$ ) family of proteins that has unique immunoregulatory properties. To validate that TGF $\beta$ 3 expression increases with age, we examined two cellular compartments – the cumulus cells immediately surrounding the oocyte and the ovarian tissue microenvironment. We did not observe an age-associated increase in TGF $\beta$ 3 expression in the cumulus cell samples, suggesting that the age-associated increase in this cytokine in follicular fluid was attributable to a different cellular source. Within the human ovary, TGF $\beta$ 3 localized throughout the stroma, vasculature, and within follicles. Interestingly, we observed a significant age-associated increase in TGF $\beta$ 3 expression in the ovary, specifically in samples enriched in stroma and vasculature.

**CONCLUSIONS:** Inflammaging is a hallmark of reproductive aging in human follicular fluid and ovarian-derived TGF $\beta$ 3 is a central component of this signature.

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WITHDRAWN

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**PREINCUBATION TIME CAN BE EFFECTIVE ON THE QUALITY AND FERTILIZATION POTENTIAL OF MOUSE MII OOCYTES.** Fatemeh Mohammadi, PhD, student, Zahra Zandieh, PhD, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran (Islamic Republic of).



**OBJECTIVE:** It is demonstrated that non-optimal preincubation time in IVF/ICSI (in vitro fertilization/intracytoplasmic sperm injection) cycles can lead to reduction in the oocyte quality, regarding to oxidative stress condition and mitochondrial alteration, and consequently can decrease the oocyte fertilization potential. Nevertheless, there is not any explanation of standard preincubation time in ART (assisted reproductive technology) guidelines. Myo-inositol, as an antioxidant, exists naturally in the follicular fluid and is a marker of good quality in the oocytes. This study evaluated the oxidative stress condition, mitochondrial alterations and fertilization potential in mouse MII oocytes following 0, 4 and 8 hours preincubation time in the simple and myo-inositol supplemented media.

**DESIGN:** This was a basic experimental study that included 50 adult (6-8 weeks-old) female NMRI mice which underwent hormonal superovulation from 2018 to 2019.

**MATERIALS AND METHODS:** Cumulus Oocyte Complexes (COCs) which were retrieved from 6-8 weeks-old superovulated female NMRI mice were pooled and divided randomly in five experimental groups: (1) control (2) 4 hours preincubation in simple medium (3) 4 hours preincubation in 20 mmol/l of myo-inositol supplemented medium (4) 8 hours preincubation in simple medium (5) 8 hours preincubation in 20 mmol/l of myo-inositol supplemented medium. COCs in each group were denuded and intracellular

Reactive Oxygen Species (ROS), glutathione (GSH), Mitochondrial Membrane Potential (MMP) and mitochondrial distribution were measured by a fluorometric assay. ATP content of oocytes also was measured using the ELISA method. Pronucleus formation was assessed for evaluation of oocytes fertilization potential.

**RESULTS:** Results showed that intracellular H<sub>2</sub>O<sub>2</sub> and glutathione levels, mitochondrial distribution, mitochondrial membrane potential, ATP content, as well as fertilization rate were different between groups. Nonetheless, myo-inositol supplementation could improve levels of H<sub>2</sub>O<sub>2</sub>, glutathione, mitochondrial distribution, ATP content and fertilization rate. Unlike other variables, mitochondrial membrane potential of oocytes was not reduced after 4 hours of preincubation in either simple or supplemented medium, but 8 hours of preincubation time could decrease it significantly. Addition of myo-inositol to the medium could not ameliorate mitochondrial membrane potential in oocytes preincubated for 4 and 8 hours. While, ATP content did not decline in oocytes preincubated for 4 and 8 hours, supplementation of myo-inositol in medium could increase it in both groups.

**CONCLUSIONS:** Finally, the analysis addressed that 4 hours or more preincubation time can influence the oocyte quality related to alternation in H<sub>2</sub>O<sub>2</sub>, glutathione, mitochondrial integrity and mitochondrial membrane potential which ultimately leads to reduced oocyte fertilization potential. Supplementation of myo-inositol in medium improves the oocyte quality in comparison to the simple medium and saves 4 hours for preincubated oocytes.

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**COLLAGEN AND HYALURONAN MATRICES UNDERGO AGE-RELATED CHANGES IN THE HUMAN OVARY.** Sharron L. Manuel, MD, PhD, MS,<sup>a</sup>

Elena Antonova, PhD,<sup>b</sup> Jessica E. Hornick, PhD,<sup>c</sup> Farners Amargant Riera, PhD,<sup>d</sup> Jian-Jun Wei, MD,<sup>e</sup> Mary Ellen Pavone, MD, MSCI,<sup>a</sup> Michele T. Pritchard, PhD,<sup>f</sup> Francesca E. Duncan, PhD.<sup>a,g</sup> <sup>a</sup>Northwestern University, Chicago, IL; <sup>b</sup>Biological Imaging Facility Northwestern University, Evanston, IL; <sup>c</sup>Research Associate Professor, Dept Molecular Biosciences Northwestern University, Evanston, IL; <sup>d</sup>Center for Reproductive Science, Northwestern University, Chicago, IL; <sup>e</sup>Northwestern University Department of Pathology, Chicago, IL; <sup>f</sup>Department of Pharmacology, Toxicology, & Therapeutics, Kansas University Medical Center, Kansas City, KS.



**OBJECTIVE:** Female reproductive aging is characterized by a decrease in gamete number and quality, which contributes to infertility. The ovarian microenvironment in which gametes grow likely influences their development and quality, and we recently demonstrated a significant increase in age-associated fibrosis in the mouse ovarian stroma. Whether such stromal changes are conserved and occur in the human ovary is unknown. The objective of this study was to examine how collagen and hyaluronan (HA), two major extracellular matrix (ECM) components, change in the human ovary with age.

**DESIGN:** Translational.

**MATERIALS AND METHODS:** To examine age-associated collagen and HA content changes in the human ovary, we generated a tissue microarray (TMA) consisting of 1 mm human ovarian cortex cores from 60 individuals in four age cohorts, ranging in age from 1.8 – 58 years old. Sequential sections of the TMA were processed for hematoxylin & eosin (H&E) staining to assess tissue architecture, picrosirius red (PSR) staining to assess collagen I and III, and fluorescent-tagged HA binding protein (HABP) -mediated staining to assess HA levels. The PSR stained tissue was imaged by both light and polarized light microscopy, while HA was imaged using fluorescence microscopy. The percent area that was PSR or HA positive as well as the mean intensity (MI) were determined.

**RESULTS:** The amount of cortical collagen decreased between the 1.8 – 10-year-old cohort and the 11 – 20-year-old cohort ( $p = 0.0045$ ) perhaps related to puberty onset. Collagen then increased between the 11 – 20-year-old cohort and the  $\geq 51$ -year-old cohort, likely reflecting increased fibrosis ( $p = 0.0009$ ). In contrast to collagen, there was an overall decrease in ovarian HA content between the young participants (1.8 – 20-year-olds) and the older participants (39 – 58-year-olds) ( $p < 0.0001$ ). The ovarian cortex cores revealed considerable heterogeneity with some samples containing follicles, vasculature, and/or stroma. Therefore, we stratified our analyses by structural category. In the  $\geq 51$  year old cohort, we observed significant age-associated increased fibrosis in blood vessel-containing cores ( $p = 0.011$  by percent area and  $p = 0.027$  by MI) and stroma ( $p = 0.019$  by MI) when compared to the 11 – 20-year-old cohort. Although fibrosis

increased and overall HA levels decreased with age, there was no correlation between HA and collagen content on an individual core basis.

**CONCLUSIONS:** These studies demonstrate that the human ovarian cortical ECM undergoes significant changes with age, and that ovarian stromal fibrosis is a conserved mammalian aging hallmark. Cortical ovarian collagen content is high at age extremes, and likely reflects normal stromal composition during early development but age-related pathology with advanced age. HA shows an opposite pattern and decreases with advanced reproductive age. The precise interplay between the collagen and HA matrices is currently under investigation.

**SUPPORT:** *Supported by:* National Institute of Child Health and Human Development (R01HD093726).

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#### **THE EFFECT OF AUTOPHAGY AFTER MOUSE OOCYTE ACTIVATION TEST.**

Atsushi Yamamoto, MD, PhD, Naoki Yoshikawa, Bachelor of Medicine, Sae Onozuka, Bachelor of Agriculture, Akiyoshi Osaka, Bachelor of Medicine, Shin Oonota, Bachelor of Medicine, Toshiyuki Iwahata, MD, PhD, Yoshitomo Kobori, MD, PhD, Kouhei Sugimoto, MD, PhD, Hiroshi Okada, MD, PhD, Dokkyo Medical University Saitama Medical Center, Koshigaya, Japan.



**OBJECTIVE:** Autophagy is a lysosome-mediated intracellular process for protein degradation and is induced in the situation of amino acid starvation and several biological stimulations to maintain the cytoplasmic homeostasis. And previous studies in the reproductive field have shown that autophagy after fertilization is essential in embryogenesis. Though in the male infertility field, there is the test named mouse-oocyte-activation test (MOAT) to check the human sperm function after fertilization, there are no reports about the relation between MOAT and autophagy induction and we check the relations.

**DESIGN:** Experimental Research.

**MATERIALS AND METHODS:** To collect MII oocytes, 8-10 weeks old female mice (C57BL/6) were superovulated. Oocytes were fertilized by intracytoplasmic sperm injection (ICSI) using 1 (or 2) human sperm; MOAT or mouse sperm. After 5-hour incubation, embryos in paraformaldehyde and immunostained by microtubule-associated protein 1 light chain 3 alpha (LC3) which is the marker of autophagy. Then they were analyzed by fluorescence microscopy and LC3 puncta in each embryo were counted.

**RESULTS:** LC3 puncta were significantly detected in a human sperm injection more than in a mouse sperm injection. The number of puncta was almost the same in 1 sperm injection as in 2 sperm injections. The size of puncta was bigger in 1 sperm injection than in 2 sperm injections.

**CONCLUSIONS:** Autophagy was induced by xenogenic sperm fertilization. The reason why autophagy was induced strongly in human sperm injection may be that the removal reaction of xenogenic proteins occur strongly, or that the volume of autophagy inducing factor is more in the human than in mice. LC3 puncta was bigger in two-sperm injection because the proteins to remove may be much more than in one-sperm injection. Though we have to check the phenomenon quantitatively and analyze the difference autophagic induction between normal and infertile man, to detect autophagic function individually by MOAT may lead to a new evaluation of male infertility therapy.

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#### **FUNCTIONAL ACTIVITY OF MITOCHONDRIA IN AGED OOCYTES IS ASSOCIATED WITH CYTOSKELETON STABILITY IN MICE.**

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**OBJECTIVE:** Dysfunctional mitochondria are strongly associated with oocyte quality and aging. However, cannot fully explain the decrease of mitochondrial activity in oocytes. Here, we studied dysfunctional mitochondria and assessed whether their functionality in aged oocytes was associated with cytoskeleton stability.

**DESIGN:** Experimental animal study.

**MATERIALS AND METHODS:** We performed time-lapse confocal live microscopy of mitochondrial motility in both young and aged oocytes. We then examined the association between cytoskeleton stability and mitochondrial motility with young oocyte, aged oocytes and 150 $\mu$ M cytochalasin B (CB)-treated young oocytes and analyzed the relationships between mitochondrial motility and functional activity including ATP production ratios.

**RESULTS:** Young oocytes showed dynamic mitochondrial motility and high ATP production levels during maturation, whereas aged oocytes did not. Cytoskeleton destabilization in CB-treated young oocytes led to a significant decrease in motility of mitochondria and to maturation ratios comparable to those of aged oocytes. Young oocytes present well development with microtubule formation in the ooplasm. But old oocytes have less development and thin microtubule formation in the ooplasm. Besides, 150 $\mu$ M cytochalasin B (CB)-treated young oocytes showed a lot of disconnected microtubule formation in the ooplasm like disassembly microtubule formation look like microtubule formation in the aged oocytes. It was found that CB disturbed cytoskeleton formation in the oocytes. Therefore, low mitochondrial motility was associated with low ATP production ratios in CB-treated young oocytes and in aged oocytes.

**CONCLUSIONS:** In this study, aged oocytes showed a loss of motility and poor ATP production ratios compared to young oocytes. These findings may be related to cytoskeleton stability with a loss of motility and poor ATP production ratios of mitochondria as observed in aged oocytes. Mitochondrial motility along the cytoskeleton may play an important role for the determination of oocytes quality, depending on age.

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#### **ROLE OF VOLTAGE DEPENDENT N AND P/Q TYPE CALCIUM CHANNEL IN MOUSE EGG FERTILIZATION.**

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**OBJECTIVE:** During mammalian fertilization, phospholipase C zeta (PLCZ), induces repetitive changes termed Ca<sup>2+</sup> oscillations. Ca<sup>2+</sup> oscillation triggers egg activation, including cortical granule (CG) exocytosis, resumption of second meiosis, block to polyspermy, and initiating embryonic development to the blastocyst stage. The sources of Ca<sup>2+</sup> ion elevation during Ca<sup>2+</sup> oscillations are Ca<sup>2+</sup> release from endoplasmic reticulum through inositol 1,4,5 tri-phosphate receptor and Ca<sup>2+</sup> ion influx through Ca<sup>2+</sup> channel on the plasma membrane. Ca<sup>2+</sup> channels have been characterized into voltage-dependent Ca<sup>2+</sup> channel (VDCs), ligand-gated Ca<sup>2+</sup> channel, and leak-channel. VDCs expressed on muscle cell or neuron is specified into L, T, N, P, Q, and R type VDCs by their activation threshold or their sensitivity to peptide toxins isolated from cone snails and spiders. It has been shown that plasma membrane potentials of mammalian oocyte are changed according to their maturational stage from germinal vesicle stage to meiosis II stage that could be fertilized by sperm. Also, addition of VDCs blockers inhibits mammalian oocyte maturation or embryo development.

**DESIGN:** The present study was aimed to investigate that localization pattern of N and P/Q type voltage dependent calcium channel in mouse oocytes and the role in fertilization.

**MATERIALS AND METHODS:** Five to six week old C57BL/DBA F1 female mice were superovulated. Cumulus-enclosed eggs were retrieved from oviduct. Ca<sup>2+</sup>-imaging: Oocytes were loaded with Fura 2 AM, transferred into TL-HEPES microdrops placed on a monitoring. Porcine sperm factor or adenophostin were injected by a picoinjector. 10mM SrCl<sub>2</sub> were applied in Ca<sup>2+</sup> free medium. Oocytes were monitored simultaneously using a fluorescence microscopy. Fluorescence intensities of [Ca<sup>2+</sup>]<sub>i</sub> changes were estimated every 20 sec with 340/380. Zona pellucidae were removed from eggs with acid-Tyrode's solution and fixed in 4% PFA. Eggs were incubated with an antibody against N-type or P/Q type voltage dependent Calcium channel antibody (Alomone labs). Counter staining were performed with Alexa Flour 555, antin phalloidin (FITC) and DAPI.

**RESULTS:** Ca<sup>2+</sup> oscillation were observed in Ca<sup>2+</sup>-contained medium with sperm factor or adenophostin A injection. The oscillations were disappeared in Ca<sup>2+</sup> free medium. Actin filament disruptor, latrunculin A abolished Ca<sup>2+</sup>-oscillation by SrCl<sub>2</sub> or Ada microinjection. Ca<sup>2+</sup>-influx was

decreased by Lat A treatment. N or P/Q type VDCC specific inhibitor,  $\omega$ -Conotoxin CVIB induced abnormal Ca<sup>2+</sup> oscillation profiles in SrCl<sub>2</sub> treatment. N or P/Q type VDC were distributed on plasma membrane, not in cytoplasm in cortical cluster form.

**CONCLUSIONS:** Ca<sup>2+</sup>-influx is essential for Ca<sup>2+</sup>-oscillation during mammalian fertilization. This Ca<sup>2+</sup>-influx may be controlled through the N or P/Q type voltage dependent Ca<sup>2+</sup> channel. Abnormal VDCs expression of eggs could be tested in fertilization failure or low fertilization eggs in sub-fertility women. This research was supported by a grant from Republic of Korea NRF-2017 RID1A1B03028155.

**SUPPORT:** This research was supported by a grant from Republic of Korea NRF-2017 RID1A1B03028155.

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### THE CHANGING OF CELL MODULATION VIA EPIDERMAL GROWTH FACTOR RECEPTOR IN HUMAN DECIDUAL STROMAL CELLS.

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<sup>a</sup>St.luke Clinic, Oita, Japan; <sup>b</sup>Department of Obstetrics and Gynecology, Faculty of Medicine, Oita University, Yufu, Japan.



**OBJECTIVE:** Human endometrial stromal cells (ESCs) undergo morphological and functional changes by growth factors and/or steroid hormones. Decidual cells are thought to be involved in the maintenance of pregnancy. The purpose of the present study was to clarify the physiological role of epidermal growth factor receptor (EGFR) in the regulation of the endometrial secretion of chemokines (interleukin (IL)-8, monocyte chemoattractant protein (MCP)-1), matrix metalloproteinase (MMP)-1 and vascular endothelial growth factor (VEGF) in decidual cells. Among the various genes that modulate decidualization, the homeobox transcription factor homeobox A10 (HOXA 10) is indispensable for the decidual transformation of endometrial stromal cells. In an attempt to clarify the mechanism by which HOXA 10 would govern decidualization, HOXA10 mRNA expression was investigated.

**DESIGN:** Laboratory study with the use of human endometrium.

**MATERIALS AND METHODS:** Normal endometrial specimens were obtained from premenopausal patients who had undergone hysterectomies for subserosal leiomyomas. Normal ESCs were separated from endometrial tissue fragments by collagenase digestion. Decidualization of ESCs (DSCs) was induced by incubating subconfluent cells in media containing medroxyprogesterone acetate (MPA) and db-cAMP for 16 days. To investigate the regulation of EGFR in cultured ESCs/DSCs, the expression of EGFR mRNA and protein production were evaluated. The secretion of chemokine, MMP-1, and VEGF in response to epiregulin (ER) was also evaluated. The effects of ER on the motility with ESCs/DSCs were assessed by an *in vitro* wound repair assay. To investigate the expression of HOXA 10 in cultured ESCs/DSCs, the expression of HOXA 10 mRNA was evaluated.

**RESULTS:** According to the real-time quantitative PCR (RT-PCR) analysis, EGFR mRNA expression levels on day 4 after decidual stimulation appeared to be higher than those on day 0. At 8 days after stimulation, the

production of EGFR protein was higher than those on day 0. The productions of IL-8 and MMP-1 increased in the DSCs with the addition of ER. The wound repair of the DSCs was significantly enhanced compared to that of the ESCs. When ER was added, the wound repair was more enhanced. According to the RT-PCR analysis, HOXA 10 mRNA expression levels on day 12 decidual stimulation appeared to be higher than those on day 0. However, the downregulation of HOXA 10 in the DSCs were expressed on day 16.

**CONCLUSIONS:** Our results suggest that cell function is changed by decidualization in association with increasing EGFR expression. The up-regulation of EGFR accompanied with decidualization may contribute to have some influence on maintenance of pregnancy.

Reference: None.

SUPPORT: None.

### REPRODUCTIVE GENETICS

P-374 Tuesday, October 15, 2019 6:30 AM

### THE TP73 GENE (rs3765730) G > A POLYMORPHISM IS ASSOCIATED WITH OVARIAN RESPONSE DURING IVF/ICSI TREATMENT.

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**OBJECTIVE:** To investigate a possible association between a TP73 gene polymorphism and ovarian response after IVF/ICSI.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** This study included 137 women submitted to IVF/ICSI cycles.

The enrolled individuals met the following inclusion criteria: age  $\leq$  37 years; normal karyotype; having two ovaries as evinced in ultrasound examination; no history of ovarian surgery, endometriosis, hydrosalpinx, infection, or endocrine disorders.

DNA extracted from peripheral blood was sequenced on MiSeq (Illumina) to find single nucleotide polymorphisms (SNPs) in the TP73 gene. SNPs were identified using the TruSeq Custom Amplicon (TSCA) Panel (DesignStudio Illumina).

The findings from sequencing were associated with age, anti-Müllerian hormone (AMH) levels, antral follicle counts (AFC), total dose of recombinant FSH (r-FSH), follicle size, number of retrieved oocytes, and clinical outcome of IVF/ICSI cycles.

**RESULTS:** The TP73 (rs3765730) G>A SNP were identified. Although no difference was observed in ovarian reserve indicators (AMH and AFC), women with the AA genotype had significantly better ovarian response to rFSH. No difference was observed in clinical outcomes. Table 1 presents a summary of the results.

**CONCLUSIONS:** The TP73 rs3765730 polymorphism apparently affected ovarian response to rFSH and the clinical outcomes of IVF/ICSI

TABLE 1. Results

	TP73 (rs3765730) Genotypes			
	GG	GA	AA	P
n	51.1%(70/137)	37.2%(51/137)	11.7%(16/137)	
Cycles	47.7%(94/197)	42.6%(84/197)	9.7%(19/197)	
Age (years)	33.3 $\pm$ 3.1	33.8 $\pm$ 2.5	33.4 $\pm$ 3.0	0.92
AMH (ng/ml)	2.1 $\pm$ 3.4	2.6 $\pm$ 3.4	2.9 $\pm$ 2.8	0.26
AFC (n)	12.6 $\pm$ 9.1	16.0 $\pm$ 11.4	18.6 $\pm$ 12.6	0.10
Total dose rFSH (UI)	2309 $\pm$ 1036 <sup>a</sup>	2282 $\pm$ 1299 <sup>b</sup>	1674 $\pm$ 808 <sup>a,b</sup>	<sup>a</sup> 0.01; <sup>b</sup> 0.04
Follicles (n):Total	10.8 $\pm$ 7.7 <sup>a</sup>	10.8 $\pm$ 9.4 <sup>b</sup>	16.8 $\pm$ 9.9 <sup>a,b</sup>	<sup>a</sup> 0.01; <sup>b</sup> 0.003
Follicles (n): $\geq$ 18 mm	3.4 $\pm$ 2.2 <sup>a</sup>	3.5 $\pm$ 2.7 <sup>b</sup>	4.8 $\pm$ 2.3 <sup>a,b</sup>	<sup>a,b</sup> 0.01
Retrieved oocytes (n):Total	7.7 $\pm$ 5.7 <sup>a</sup>	7.3 $\pm$ 6.6 <sup>b</sup>	12.5 $\pm$ 7.2 <sup>a,b</sup>	<sup>a</sup> 0.01; <sup>b</sup> 0.001
Retrieved oocytes (n):Metaphase II	5.5 $\pm$ 4.4 <sup>a</sup>	5.9 $\pm$ 5.4 <sup>b</sup>	9.6 $\pm$ 6.1 <sup>a,b</sup>	<sup>a</sup> 0.006; <sup>b</sup> 0.005
Fertilization rate	65.8%	68.8%	68.5%	0.55
Implantation rate	29.4%	24.1%	28.0%	0.53
Pregnancy rate/patient	62.9%	58.8%	68.8%	0.74
Pregnancy rate/transfer	40.7%	35.7%	42.3%	0.75

Values within rows with the same superscript letter were significantly different

cycles. Homozygosity of the A allele was associated with significantly better results. The identified SNP may provide an additional tool to test patients for ovarian response and thus help in the individualization of ovarian stimulation protocols. To the best of our knowledge, this is the first study associating this SNP and ovarian response.

SUPPORT: Merck Grant for Fertility Innovation (GFI-2014).

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**SEQUENTIAL CLINICAL MANIPULATIONS OF EMBRYOS RESULTS IN ALTERATIONS IN EXPRESSION OF GENES INVOLVED IN INNATE IMMUNITY, APOPTOSIS, AND MITOCHONDRIAL FUNCTION.**



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**OBJECTIVE:** Although data do not suggest a decreased clinical pregnancy rate with single blastocyst (blast) vitrification (vit) or blast biopsy, vit before and after biopsy has been shown to compromise embryo survival, suggesting a greater impact of cryoprotectants following trophectoderm biopsy. There are also little data on the long-term safety of embryo vit. Clinical studies suggest differences in perinatal outcomes between babies born following fresh and frozen embryo transfer (FET), with higher rates of preterm birth (PTB) and pre-eclampsia (PEC) after FET, and higher rates of low birthweight (LBW) after fresh transfer. In this study, we aim to identify specific genes affected by blast vit and biopsy that may account for these phenotypic differences.

**DESIGN:** Laboratory research.

**MATERIALS AND METHODS:** Female mice were superovulated with 5 IU PMSG and 5 IU hCG and mated with male mice. Blasts were flushed on E3.5 and divided into 4 groups: no manipulation (g2), single vit/thaw (g3), double vit/thaw (g4), and single vit/thaw plus biopsy and re vitrified and thawed (g5). 3 sets of 15 blasts per group were pooled for RNA extraction. Low input libraries were made using Takara SMART-Seq v4 and Illumina Nextera XT kits. RNA-Seq was performed on an Illumina NextSeq 550 (75 base pair, paired-end, 30 x 10<sup>6</sup> reads/sample). Differentially expressed genes (DEGs) were determined by two group t-tests (P ≤ 0.05) and organized into top enrichment pathways by P value (P ≤ 0.05) via gene set variation analysis in R Bioconductor. Our sample size achieves a power of 80% with an alpha of 0.05 to detect a >2.5 fold change in transcript expression.

**RESULTS:** Analysis of DEGs revealed significant alterations in multiple pathways. These differences were seen in multiple comparisons between all groups, with greater effect seen with increasing manipulations. For example, 3,340 DEGs were found between g2 and g5. STRING network analysis showed clustering in innate immunity, apoptosis, and mitochondrial function pathways. Several DEGs with plausible mechanisms for the outcomes of interest were identified, including *Clqa* (log fold change 2.9), *Tlr2* (logFC 5.44), and *Tnf* (logFC -2.98).

**CONCLUSIONS:** In this pilot study, multiple genes involved in innate immunity exhibited altered expression with increasing levels of manipulation. *Clqa*, a complement system component, is significant to trophoblast invasion, and KO mice also exhibit a PEC phenotype. Decreasing levels of *Clqa* expression were seen with increasing manipulation in our study, sug-

gesting a possible mechanism for the increased risk of PEC with FET. *Tlr2* is a common mediator of apoptosis, and its activation has been found to trigger PTB. Our data shows decreasing *Tlr2* expression with increasing manipulation, which may represent a plausible mechanism for the increased rates of PTB following fresh transfer. *Tnf* has been shown to be increased in placentas from PEC pregnancies; increased *Tnf* early in pregnancy also suppresses trophoblast invasion. We found increasing levels of *Tnf* expression with increasing manipulation, which may represent another mechanism for the increased risk of PEC following FET.

SUPPORT: Prelude Scientific Advisor Board Grant.

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**THE MATRIX METALLOPROTEINASE-9 (MMP9) P. Gln279Arg POLYMORPHISM IS ASSOCIATED WITH OVARIAN RESERVE AND OVARIAN RESPONSE DURING IVF/ICSI TREATMENT.**



Laura D. Vagnini, B.Sc.,<sup>a</sup> Claudia G. Petersen, Ph.D.,<sup>b</sup> Ana Lucia Mauri, B.Sc.,<sup>b</sup> Adriana Renzi, Ph.D.,<sup>a</sup> Bruna Petersen, B.Sc.,<sup>b</sup> Mariana Mattila, B.Sc.,<sup>c</sup> Juliana Ricci, R.N.,<sup>c</sup> Felipe Dieamant, M.D.,<sup>b</sup> Joao Batista A Oliveira, M.D., Ph.D.,<sup>b</sup> Ricardo L. R. Baruffi, M.D.,<sup>b</sup> Jose G. Franco Jr., M.D., Ph.D.<sup>b</sup> <sup>a</sup>Paulista Center for Diagnosis, Research and Training, Ribeirao Preto, Brazil; <sup>b</sup>Center for Human Reproduction Prof. Franco Jr/ Paulista Center for Diagnosis, Research and Training, Ribeirao Preto, Brazil; <sup>c</sup>Center for Human Reproduction Prof. Franco Jr, Ribeirao Preto, Brazil.

**OBJECTIVE:** To investigate a possible association between an MMP9 gene polymorphism and ovarian response after IVF/ICSI.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** This study enrolled 135 women submitted to IVF/ICSI cycles.

The enrolled individuals met the following inclusion criteria: age ≤ 37 years; normal karyotype; having two ovaries as evinced in ultrasound examination; no history of ovarian surgery, endometriosis, hydrosalpinx, infection, or endocrine disorders.

DNA extracted from peripheral blood was sequenced on MiSeq (Illumina) to find single nucleotide polymorphisms (SNPs) in the MMP9 gene. SNPs were identified using the TruSeq Custom Amplicon (TSCA) Panel (DesignStudio Illumina).

The findings from sequencing were associated with age, anti-Müllerian hormone (AMH) levels, antral follicle counts (AFC), total dose of recombinant FSH (r-FSH), follicle size, number of retrieved oocytes, and clinical outcome of IVF/ICSI cycles.

**RESULTS:** The MMP9p.Glutamine(Gln)279Arginine(Arg)(rs17576) polymorphism was identified. Women with the Gln/Gln genotype had significantly poorer ovarian reserve indicators (lower levels of AMH and AFC), poorer ovarian response to rFSH, and poorer clinical outcomes (implantation rate). Table 1 presents a summary of the results

**CONCLUSIONS:** The MMP9 p.Gln279Arg polymorphism was associated with ovarian reserve and seemed to have affected ovarian response to rFSH and the clinical outcomes of IVF/ICSI cycles. Homozygosity of the Gln allele was associated with significantly poorer results. The identified SNP might provide an additional tool to test patients for ovarian response and thus help in the individualization of ovarian stimulation protocols.

SUPPORT: Merck Grant for Fertility Innovation (GFI-2014).

TABLE 1. Results

	MMP9 p.Gln279Arg Genotypes						
	Gln/Gln	Gln/Arg	Arg/Arg	P	Gln/Gln	Gln/Arg+Arg/Arg	P
n	59.3%(80/135)	31.8%(43/135)	8.9%(12/135)		59.3%(80/135)	40.7%(55/135)	
Age(years)	33.6±2.8	33.3±3.0	33.5±3.6	0.86	33.6±2.8	33.4±3.1	0.57
AMH(ng/ml)	2.1±3.4 <sup>a</sup>	2.6±2.2 <sup>a</sup>	3.7±5.6	<sup>a</sup> 0.04	2.1±3.4	2.8±3.2	0.04
AFC(n)	12.4±9.9 <sup>a,b</sup>	16.7±8.4 <sup>a</sup>	22.3±17.5 <sup>b</sup>	<sup>a</sup> 0.001; <sup>b</sup> 0.02	12.4±9.9	17.9±11.1	0.0004
Total dose FSH(UI)	2385±1173 <sup>a</sup>	1787±950 <sup>a</sup>	1854±1006	<sup>a</sup> 0.004	2385±1173	1802±954	0.002
Follicles(n):Total	10.8±7.7 <sup>a</sup>	16±10.6 <sup>a</sup>	15.8±10.5	<sup>a</sup> 0.01	10.8±7.7	15.9±10.5	0.01
Follicles(n):≥ 18mm	3.3±2.3 <sup>a,b</sup>	4.6±2.8 <sup>a</sup>	4.8±2.5 <sup>b</sup>	<sup>a</sup> 0.01; <sup>b</sup> 0.04	3.3±2.3	4.6±2.7	0.004
Retrieved oocytes:Total	7.8±5.3	11.2±8.2	10.6±7.0	0.12	7.8±5.3	11.1±7.9	0.04
Retrieved oocytes:MII	5.9±4.0	8.1±6.8	8.2±6.0	0.58	5.9±4.0	8.1±6.6	0.21
Fertilization	66.6% <sup>a</sup>	65.1% <sup>b</sup>	81.9% <sup>a,b</sup>	<sup>a,b</sup> 0.001	66.6%	68.9%	0.49
Implantation	26.4% <sup>a</sup>	40.2% <sup>a</sup>	30.8%	<sup>a</sup> 0.02	26.4%	38.2%	0.04
Pregnancy/patient	46.3%	62.8%	58.3%	0.89	46.3%	61.8%	0.08
Pregnancy/	39.8%	51.9%	50%	0.34	39.8%	51.5%	0.14

**TOWARDS PERSONALISED REPRODUCTIVE MEDICINE: SCREENING FOR GENETIC VARIANTS AND ITS INFLUENCE IN CONTROLLED OVARIAN STIMULATION.**



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**OBJECTIVE:** The most studied polymorphism to assess the ovarian stimulation was N680S-FSHR, however, others genes related to follicular growth could also play an important role in determining the ovarian response. The aim of this work was to evaluate the association between ovarian stimulation and the genetic variants present in genes involved in ovarian function.

**DESIGN:** Oocyte donors are the most adequate model to evaluate ovarian stimulation because are young women with normal ovarian function. This prospective randomized study includes 124 healthy, normoovulatory, caucasian egg donors genotyped for six SNPs present in ESR1, AMH, AMHR2, GDF-9 and LHCGR and four STRs present in ESR1, SHBG, CYP19A1 and AR. All donors followed a standard ovarian stimulation protocol using a daily dose of 225UI of either uFSH or rFSH.

**MATERIALS AND METHODS:** SNPs were analysed by TaqMan allelic-discrimination assays (rs2234693-ESR1, rs10407022-AMH, rs2002555-AMHR2, rs10491279/rs254286-GDF-9, rs2293275-LHCGR) and the STR-polymorphism in the ESR1, SHBG, CYP19A1 and AR genes by fluorescent-PCR. The genotypes obtained were compared to the ovarian stimulation.

**RESULTS:** The mean age of the oocyte donors included in the study was 23.9±3.5y. The mean AMH level was 45.44±23.5pmol/ml and the mean number of antral follicles count was 14.2±2.8. We performed a linear regression, taking into consideration confounding factors such as age, smoking, BMI and AMH. Regarding the number of retrieved oocytes, we found statistically significant differences for the ESR1 SNP (19.3±8.9 for TT vs 15.3±6.2 for CC/CT, p=0.027) and ESR1 (TA)n STR (19.1±8.3 for <17repeats vs 14.7±6.2 for >17repeats, p=0.020). When we combined both genotypes, the haplotype analysis showed that women that carries CC or CT in the ESR1 gene at position -397T>C (rs2234693) with a number of repeats in the ESR1 (TA)n polymorphisms higher than 17 retrieved lower oocytes (14.0±5.6) than the other genotypes (p=0.001). Regarding AMHR2 we observed an association with the length of stimulation (9.1±1.4 for AA vs 9.7±1.3 for AG/GG, p=0.021) and gonadotropin received (2050±319 for AA vs 2188±299 for AG/GG, p=0.017). No significant association among genotype, retrieved oocytes and ovarian stimulation was observed for LHCGR, CYP19A1, AMH, SHBG, AR and both of GDF-9 SNPs (p>0.05).

**CONCLUSIONS:** We reported that polymorphisms in the ESR1 and AMHR2 genes showed a clear association with the number of retrieved oocytes and the stimulation data, respectively. Therefore, our results suggest that polymorphisms in the genes for key reproductive hormones receptors could be used to predict the ovarian response and to personalized and adjust the stimulation drugs prior the overtaken treatment. Such pharmacogenetics approach will facilitate the selection of the optimum protocol for each patient.

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**WHERE DOES EXTRA X IN KLINEFELTER SYNDROME COME FROM?**



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**OBJECTIVE:** It has been reported that the incidence of sperms with disomic XY is higher in the testis tissue smear of KS patients and these

sperms are the cause of KS. However some papers report the maternal origin. So we performed this study to investigate the origin of extra X in Klinefelter Syndrome (KS).

**DESIGN:** Cytogenetic analysis in KS patients and their parents.

**MATERIALS AND METHODS:** Blood samples from 29 KS patients were used for X-chromosome short tandem repeats (STR) analysis. The STR analysis also included data of the parents of the KS patients (24: both parents, 5: mother only, 0: father only) from January 2015 to March 2019. This study was conducted with the informed consent of all participating patients and approved by The Institutional Review Boards of the Saint Mother Obstetrics and Gynecology Clinic and adhered to JCMJER criteria UMIN Clinical Trial Registry was UMIN000024542.

Blood samples of 29 KS patients and one or both of their parents were used to determine the origin of the extra X chromosome using X-chromosome haplotype markers (short tandem repeats of 12 loci), according to the method by Shrivastava et al. With DNA extracted from the samples, multiplexed PCR amplifications of the 12 X-STR loci and AMELOGENIN were conducted using an Investigator Argus X-12 QS Kit (Quigen, Germany). The data obtained was analyzed with GeneMapper ID software.

**RESULTS:** X-chromosomal STR DNA profiles were compared among KS patient and their parents. In 13 of the 29 KS patients, both two X chromosomes were maternal origin, showing that an extra X chromosome was left in an oocyte as a result of chromosomal non-disjunction at the 1<sup>st</sup> (4/13) or 2<sup>nd</sup> (9/13) meiotic division. In 15 patients, X-chromosomes were inherited from parents, suggesting that fertilization of XY-sperm is the cause of KS.

**CONCLUSIONS:** Although the sample number applied for X-chromosomal STR DNA profiling is not enough, the present data may indicate that contribution of XX oocyte to the production of XXY embryos is greater than XY sperm. Namely, a XX oocyte penetration by a Y sperm is the main cause of KS. Cytogenetic analysis with smear of testicular cell mixture that was used in the studies may overestimate chromosomal abnormality.

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**BREAKPOINT MAPPING UNCOVERING ABOUT 1.32% OF APPARENT BALANCED RECIPROCAL TRANSLOCATION (ABRT) CARRIERS EXISTING CRYPTIC COMPLEX CHROMOSOMAL STRUCTURAL VARIATIONS IN PREIMPLANTATION GENETIC TESTING (PGT).**



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**OBJECTIVE:** To identify precise breakpoints, evaluate the reproduction-related risks and guide the following PGT treatment, high resolution breakpoint mapping was performed in ABRT carriers indicated by G-banding.

**DESIGN:** A single-center, descriptive research.

**MATERIALS AND METHODS:** A large sample of 833 cases with ABRT who planned to accept PGT treatment were recruited in this study. For these patients, the approach of the next-generation sequencing following microdissection (MiroSeq) of the junction region in the derivative chromosomes, and linkage analysis of the adjacent single nucleotide polymorphisms (SNPs) were performed to distinguish the carriers from noncarriers in balanced embryos. For some cases with unbalanced chromosome rearrangement in the breakpoint region, SNP-array and fluorescence in situ hybridization (FISH) techniques were further used to determine the accurate karyotype.

**RESULTS:** In the 833 cases with ABRT, we found 11 cases (1.32%) carried cryptic complex chromosomal structural variation, including 3 unbalanced chromosome rearrangements and 8 balanced ones in which 2 cases carried both inversion and translocation. In these 11 cases, 5 cases related to 3 chromosomes with 4 to 21 breakpoints and 6 cases involved 2 chromosomes with 3 to 6 breakpoints. It is noteworthy that there were two cases exhibited rare chromoanagenesis, including chromothripsis and chromoplexy. Fortunately, two couples have been both successfully transplanted a normal

TABLE 1. The result of X-chromosome STR analysis (a case of maternal origin extra-X)

Patient no. 09KY						
Marker	DXS10148	DXS10135	DXS8378	DXS10079	DXS10074	DXS7132
Father	20	22	10	18, 21	17	16
Mother	20, 22.1	21, 22	10	17, 18, 20	16, 18	14, 15
Patient	22.1	22	10	18, 20	18	14
Marker	HPRTB	DXS10101	DXS10103	DXS10134	DXS10146	DXS7423
Father	12	31.2	18	35	24, 40.2	14
Mother	13, 14	29, 31.2	17, 19	36, 37.3	26, 32	15, 16
Patient	14	29, 31.2	17, 19	36, 37.3	26, 32	15, 16

embryo and given birth to a healthy child, and the remaining nine cases are undergoing PGT treatment.

**CONCLUSIONS:** In this large-scale analysis of ABRT, high resolution breakpoint mapping precisely characterized these breakpoints and uncovered 1.32% of the ABRT carriers existed cryptic complex chromosomal structural rearrangement. These data suggests that high resolution breakpoint mapping used in PGT can improve the accuracy of evaluating the reproduction-related risks and avoid genetic risks for the ABRT carriers.

**SUPPORT:** This study was supported by the National Key R&D Program of China 2018YFC1003100 (L.H.) and 2016YFC1000206 (G.L.), National Natural Science Foundation of China 81873478 (L.H.) and Merck Serono China Research Fund for Fertility Experts.

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#### **SINGLE CELL GENE EXPRESSION OF HUMAN PUBERTAL TESTIS DEVELOPMENT.**

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**OBJECTIVE:** We investigated the molecular mechanism underlying human testis development during puberty by single cell RNA-seq profiling.

**DESIGN:** We derived single cell suspensions using the testicular biopsies from 4 juvenile donors (7-14), and performed single cell RNA-seq profiling and analysis.

**MATERIALS AND METHODS:** We performed scRNA-seq profiling of whole testis tissues from juvenile donors (two technical replicates for each donor): one 7-year old (~3000 cells), one 11-year old (~3000 cells), one 13-year old (~3000 cells), one 14-year old (~3000 cells). This yielded a dataset composed of ~12000 single cell transcriptomes. We compared the current dataset with the single cell transcriptome from the young adult (~25 years old) and infant (~1 year old) male donors described in our previous work. We performed dimension reduction and clustering analysis using SEURAT and SDA programs, and utilized known markers to help decode cell identities. We further performed differential gene expression and gene ontology analysis to study the gene expression programs that display differential gene expression dynamics.

**RESULTS:** We found that spermatogonial stem cells (SSCs) commit to spermatogenesis in two sequential phases: mitotic differentiation (involving proliferation and metabolic changes) followed by subsequent commitment to meiosis, which may be induced by testosterone and activin signals. Remarkably, the early SSCs (marked by PIWIL4, TSPAN33 and many other genes) were pre-determined during infancy (~1 year old), and persisted in adults. Regarding the somatic niche, we identified a common pre-pubertal cell precursor for Leydig and Myoid cells, and revealed pathways for pubertal differentiation, including the insulin signaling pathway. We have confirmed critical roles of testosterone in promoting germ cell differentiation both *in vivo* and *in vitro*. Importantly, we have developed a culturing system that maintains human seminiferous tubule (STs) growth and spermatogonial replication *in vitro* for three weeks.

**CONCLUSIONS:** The current study provided the first single cell transcriptional cell atlas for pre- and peri-pubertal testis development, and uncovered many important signaling pathways that may regulate both germ cell and somatic cell maturation during human puberty, which could be critical for initiation and maintenance of spermatogenesis. This can be applied to an *in vitro* culture system to help drive and maintain *in vitro* spermatogenesis using testicular tissues from prepubertal boys undergoing cytotoxic chemotherapy.

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#### **PRE-SURROGACY OBSTETRICAL RISK ASSESSMENT FOR POTENTIAL GESTATIONAL CARRIERS.**

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**OBJECTIVE:** Develop an obstetrical risk scoring system for the evaluation of women wishing to become gestational carriers.

**DESIGN:** Prospective Cohort Study

**MATERIALS AND METHODS:** Every pregnancy is associated with some maternal risk. In gestational surrogacy, the involvement of a third-party calls for caution as well-defined criteria in the evaluation of gestational carrier candidates

(GCC's) are lacking. We created a novel scoring system, the Gestational Carrier evaluation of Obstetrical Risk (GCOR) scale to rate the risk of severe maternal morbidity (SMM). Severe maternal morbidity (ACOG, Obstetrical care consensus #5, 2016) includes complications such as hysterectomy, transfusion of > 4 units of PRBC, ICU admission and stroke. The first step was the identification risk factors for SMM, from obstetrical and medical history. Examples include history of pre-eclampsia, prior cesareans, diabetes, hypertension and obesity. Based on the review of more than 75 published studies looking at individual risk factors for SMM, a score of 1 to 10 was assigned for each risk factor. Concurrently, ninety three patients were sent to a single Maternal-Fetal Medicine (MFM) provider for a pre-surrogacy evaluation. For each GCC, obstetrical and medical records were reviewed and a face-to-face, Skype or phone interview was conducted to complement the information from the records. Risk factors were abstracted and a GCOR score was assigned by adding all the risk factors scores. For each GCC, an MFM consultation report with a GCOR score was sent to referring providers and their satisfaction was recorded. A GCOR score > 10-20 suggests a high risk for SMM. The evaluation of risks of less severe maternal outcomes was also provided in the MFM report.

**RESULTS:** Pre-surrogacy evaluations were requested by IVF clinics (n=73) or by surrogacy agencies (n=20). Most referrals were for maternal risk concern(s) and some were for concurrent fetal/neonatal perinatal risk concern(s). Common indications for the requests were: underlying medical condition (n=54), history of pre-eclampsia (n=16), history of preterm delivery (n=16), prior cesareans (n=34), grand multiparity (n=12) and prior postpartum hemorrhage (n=6). The underlying medical conditions were varied, ranging from obesity (n=61), diabetes (n=12), thyroid disease (n=16), hypertension (n=14), corrected cardiac defect, grade 3 uterine prolapse and multiple sclerosis.

The mean GCOR score was 7.2 +/- 5.2 (range 2-22). Thirty three patients had a GCOR score ≥ 15. High GCOR scores were most often due to prior pre-eclampsia, prior postpartum hemorrhage and morbid obesity. All (100%) referring provider viewed the GCOR scoring system as simple and were satisfied with the reports.

**CONCLUSIONS:** This pilot study suggests that GCOR scoring combines objectivity and simplicity in assessing risk of SMM in gestational carriers candidates. We will continue to evaluate GCC's to refine our scoring system and analyze data prospectively with inclusion of postpartum and perinatal outcomes. We plan to create a similar scale for fetal/neonatal risks and to adapt the GCOR scoring for planned multifetal pregnancies.

**SUPPORT:** N/A.

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#### **LUTEAL PHASE-DERIVED OOCYTE-CUMULUS COMPLEXES: GENE EXPRESSION AND MITOCHONDRIAL DNA COPY NUMBER.**

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**OBJECTIVE:** The double stimulation (DuoStim) became a new approach of poor responder management. However, luteal phase stimulation-derived (LPS) oocytes require further investigation. One of the methods to determine oocyte quality is investigation of the cumulus cells (CCs), which surround the oocyte and are pivotal in determining oocyte developmental competence. Several studies have revealed certain CCs genes that are correlated with oocyte competence and embryo development; also there are data, which shows that mitochondrial DNA (mtDNA) copy number is positively linked with embryo quality. However, gene expression and mtDNA quantification in CCs of LPS derived oocytes after the DuoStim approach still has not been investigated.

**DESIGN:** A total of 39 patients with a reduced ovarian reserve were included in the study. Inclusion criteria: age <43 years; AMH <1.2 ng/ml; AFC <6; basal FSH ≥ 11 IU/ml. Exclusion criteria: uterine fibroids ≥ 4 cm, deep endometriosis, cancer, BMI ≥ 29 kg/m<sup>2</sup>, smoking, severe male infertility. Gene expression was assessed in a total of 169 CCs. 20 CCs were excluded: 4 due to mRNA impairment and 16 due to immature oocytes. A total of 149 CCs were divided into two groups: group 1 included 55 follicular phase-derived oocytes from 15 patients and group 2 included 94 LPS -derived oocytes from 24 patients.

**MATERIALS AND METHODS:** The expression levels of *HAS2*, *VCAN*, *ALCAM*, *PTGS2*, *GREMI*, *ITPKA*, *TRPM7*, *SDC4*, *CALM2*, *SPSB2*, *TP53I3*, *PGR*, *PFKP* and mtDNA were assessed using quantitative polymerase chain reaction. Statistical analysis – the Mann-Whitney test, t-test, the chi-squared test; p<0.05 was considered to be statistically significant.

**RESULTS:** CCs gene expression was similar between the groups. However, a significant increase in the mRNA levels of *VCAN* (15.542±6.8 vs.

20.353±10.58,  $P = 0.001$ ), *SDN4* (1.016±0.65 vs. 1.318±0.97,  $P = 0.013$ ), and *TP53I3* (0.185±0.09 vs. 0.270±0.11,  $P < 0.001$ ) were found in CCs from LPS-derived oocyte. The quantification of mtDNA copy number were comparable between the groups (355.87±112.97 vs. 361.79±131.56 in group 1 and 2, respectively,  $p > 0.05$ ). No significant differences in the embryological and clinical outcomes were found between the groups (Table).

**CONCLUSIONS:** The DuoStim approach did not affect mtDNA copy number and CCs gene expression in luteal phase-derived oocytes, except for *VCAN*, *SDN4*, and *TP53I3* expression levels, which could lead to changes in the follicle environment.

TABLE

	1 group (n=15)	2 group (n=24)
Age (y)	36.5±5.0	37.0±4.2
AMH (ng/ml)	0.7± 0.4	0.93± 0.3
Retrieved oocytes	5.57±2.3	5.7±2.7
MII oocytes (n)	2.1±2.1	4.9±2.2
Blastocysts (n)	1.3±1.4	2.7±2.2
TOP-blastocysts (n)	1.3±1.4	1.0±1.2
Positive hCG per ET	7/13 (53.8)	7/22 (31.8)
Clinical pregnancy	5/13 (38.5)	7/22 (31.8)

References: 1. Vaiarelli A, Cimadomo D, Ubaldi N, Rienzi L, Ubaldi FM. What is new in the management of poor ovarian response in IVF? *Curr Opin Obstet Gynecol*. 2018;

2. Ubaldi FM, Capalbo A, Vaiarelli A, Cimadomo D, Colamaria S, Alviggi C, et al. Follicular versus luteal phase ovarian stimulation during the same menstrual cycle (DuoStim) in a reduced ovarian reserve population results in a similar euploid blastocyst formation rate: new insight in ovarian reserve exploitation. *Fertil Steril* 2016;

3. Fragouli E, Wells D, Iager AE, Kayisli UA, Patrizio P. Alteration of gene expression in human cumulus cells as a potential indicator of oocyte aneuploidy. *Hum Reprod [Internet]* 2012 [cited 2018 Sep 23];27(8):2559–68;

4. Adriaenssens T, Wathlet S, Segers I, Verheyen G, De Vos A, Van der Elst J, et al. Cumulus cell gene expression is associated with oocyte developmental quality and influenced by patient and treatment characteristics. *Hum Reprod [Internet]* 2010 [cited 2018 Sep 23];25(5):1259–70;

5. Ogino M, Tsubamoto H, Sakata K, Oohama N, Hayakawa H, Kojima T, Å et al. Mitochondrial DNA copy number in cumulus cells is a strong predictor of obtaining good-quality embryos after IVF. *J Assist Reprod Genet*. 2010 Mar;33(3):367–371.

SUPPORT: None.

**P-383** Tuesday, October 15, 2019 6:30 AM

#### PREVALENCE OF GENETIC DISEASE CARRIERS AMONG PATIENTS SEEKING FERTILITY TREATMENT.

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**OBJECTIVE:** This study explores the prevalence of autosomal recessive and X-linked genetic disease carriers among patients presenting to care in the fertility setting as compared to the prevalence in the general prenatal screening population.

**DESIGN:** Disorder level carrier screening positivity rates for patients from reproductive medicine (RM) clinics were compared to patients from non-RM clinics referred for testing at a large, clinical testing laboratory. Patients were screened for 3-220+ autosomal recessive or X-linked genetic disorders via a genotyping assay.

**MATERIALS AND METHODS:** A retrospective review of expanded carrier testing results for 330,329 samples was conducted. Fisher's exact test and a Logistic Regression model factoring in ethnicity were utilized to determine significant differences between positivity rates observed in the RM vs non-RM populations. Correcting for multiple hypothesis testing was done using Benjamini-Hochberg method.

**RESULTS:** 330,329 carrier testing results were reviewed. 14,258 patient samples were ordered by a RM clinic, and 316,071 samples were ordered by a non-RM clinic. Differences in positivity rates were observed (table 1), however, rates were statistically different for 3 disorders (corrected  $p$  value  $< 0.05$ ), very long chain acyl-CoA dehydrogenase deficiency (VLCAD), glucose-6-phosphate dehydrogenase deficiency (G6PD), and familial Mediterranean fever (FMF). When correcting for ethnicity, G6PD was no longer statistically significant, thus only 2 conditions

were significantly different. The RM cohort consisted of significantly more male samples (20.02% vs 3.45%). Within the RM cohort, significantly more patients self-reported Asian, Caucasian/White, and other or mixed ethnicity, while significantly less patients self-reported African American/Black and Hispanic ethnicities.

**CONCLUSIONS:** Our results indicate that while differences in positivity rates were observed, only two disorders were statistically significant when factoring patient reported ethnicity differences between the cohorts. Most notably, we did not observe differences in Fragile X and cystic fibrosis as previously reported. This data adds to previous literature characterizing the population seeking fertility treatments and is the first publication to describe a difference in positivity rates for VLCAD and FMF. The effect of these disease processes in carrier states as related to fertility is unknown, but may be of interest for future investigations. Future studies investigating clinical indication for seeking care in a RM clinic may further delineate differences in positivity rates within this unique patient population.

SUPPORT: None.

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#### A "TANDEM-REFLEX" STRATEGY MINIMIZES RESULTS DELIVERY TIME FOR COUPLES UNDERGOING CARRIER SCREENING.

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**OBJECTIVE:** To determine the impact of the "tandem reflex" strategy on turnaround-time and test utilization.

Expanded carrier screening aims to detect couples at risk for having children with severe and profound Mendelian disorders. As many couples are pregnant or actively trying to conceive while undergoing carrier screening, obtaining screening results in a timely manner is important. Reproductive partners are typically tested in a sequential manner: the female partner is tested first and if she is a carrier, her partner is tested for the condition(s) for which she was found to be a carrier. In current practice, this commonly necessitates a subsequent visit to a physician for submission of the partner's sample, such that the time to receive a combined couple report is roughly double the time it takes to receive an individual carrier screening report. This need for a secondary sample submission imposes workflow challenges to the clinic and patients, reduces the likelihood of the partner getting screened, and thus may hamper detection of at-risk couples. To minimize turnaround-time and maximize the detection of at-risk couples, we implemented a "tandem reflex" strategy wherein both partners submit samples in tandem, but are tested sequentially, with the second partner's sample tested only if the first partner was found to be a carrier.

**DESIGN:** Retrospective data analysis.

**MATERIALS AND METHODS:** The time between a reproductive couple's submission of its first sample to the delivery of a combined report was measured before and after implementation of the "tandem reflex" strategy. Samples submitted and tested simultaneously were also analyzed for unnecessary partner testing.

**RESULTS:** Before implementing the "tandem reflex" strategy, the average time for a sequentially tested couple to receive a full couple-based carrier screening report was approximately 34 days (95th percentile 70 days,  $N = 11,434$  couples). After implementing the tandem-reflex strategy, the average wait time for a couple to receive their combined report was reduced to approximately 15 days (95th percentile 25 days,  $N = 383$  couples), with individual reports returned within 11 days (95th percentile just under 21 days). Among 9,718 couples for which samples were submitted and tested simultaneously, 41% of females were negative for all tested conditions; in this scenario, the tandem reflex strategy would not have triggered testing of the male partner.

**CONCLUSIONS:** The "tandem reflex" strategy decreased by half the turnaround-time for receiving a combined carrier screening report compared to sequential testing, resulting in the timely receipt of crucial information for reproductive and pregnancy management.

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#### CARRIER SCREENING IN 2019: IS SCREENING FOR MORE GENES THE NEW STANDARD OF CARE?

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**OBJECTIVE:** To report ordering patterns within and outside of ACOG carrier screening guidelines between medical specialties.

TABLE 1. Summary of Ordering Patterns &amp; Positive Rates

Panel	REI	ObGyn	MFM	GC	Other	% of all orders	Positive Rate
288 gene panel with 13 add-on genes	16%	21%	0%	18%	9%	15%	77%
288 gene panel	36%	27%	66%	53%	53%	41%	65%
46 gene panel	13%	10%	9%	3%	16%	12%	44%
3 gene panel	12%	24%	14%	3%	13%	14%	15%
Other: ACOG/ACMG ethnicity-specific genes	14%	7%	4%	2%	4%	9%	22%
Other: All other combinations	9%	11%	7%	21%	6%	9%	Varies
Total	7729	3106	446	903	4275	16459	51%

DESIGN: ACOG recommends universal carrier screening for cystic fibrosis (CF) and spinal muscular atrophy (SMA) and ethnicity-based screening when appropriate. ACOG acknowledges that expanded carrier screening (ECS) has many benefits but states ECS panels should only include high impact disorders (well-understood, severe, and common.)<sup>1,2</sup>

Our laboratory offers carrier screening for up to 301 genes. These genes are available in pre-curated panels (3, 46, or 288 genes), or they can be ordered as customized panels. Thirteen additional genes (common, variable, and/or adult onset) are available as an add-on to any panel. All combinations are offered at the same out-of-pocket cost.

MATERIALS AND METHODS: Testing for up to 301 genes was performed by NGS. Ordering patterns by clinician type and positive rates were assessed.

RESULTS: In a ten-month period, 16,459 patient samples from 1,390 clinicians were tested. Almost half of all orders came from REIs and 9% of orders identified the patient/partner as pregnant. The largest pre-curated panel was ordered most frequently (n=6,699). Concurrent testing was performed for 62% of opposite-sex couple orders. Guideline-based testing accounted for 23% of all orders with ObGyns having the highest adherence to guideline-based ordering (31%) and genetic counselors (GCs) having the lowest (5%). MFMs were the only group that did not order all available genes (Table 1).

Of all tests, 31% were positive for 1 disorder, 21% were positive for 2 or more, and 48% were negative. The most common autosomal recessive disorders at-risk couples screened positive for (add-on genes excluded) include *CFTR*-related disorders, *GJB2*-related non-syndromic hearing loss, *HBB*-related hemoglobinopathies, Smith-Lemli-Optiz syndrome, SMA and phenylalanine hydroxylase deficiency (PKU).

CONCLUSIONS: Despite current guidelines, our data shows that 56% of clinicians preferred a large panel ( $\geq 288$  genes), even including frequent/variable disorders and only 12% ordered the 46 gene panel with only high impact disorders. Additional investigation is needed to understand the decision tree within and between practices including the role insurance coverage and cost plays on carrier screening ordering.

References: 1. ACOG Committee Opinion # 690. Obstet Gynecol. 2017 Mar;129(3):e35-e40.

2. ACOG Committee Opinion # 691. Obstet Gynecol. 2017 Mar;129(3):e41-e55.

SUPPORT: Invitae.

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### THE PROLONGED DISEASE STATE OF INFERTILITY IS ASSOCIATED WITH BLASTOCYST IMPRINTED EPIGENETIC DYSREGULATION.

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OBJECTIVE: Epidemiological studies suggest that the disease state of infertility may play a role in the observed increased incidence of rare imprinting disorders in children born following infertility treatment. Imprinting disorders frequently arise from epigenetic dysregulation at imprinting control regions (ICRs). Examples include loss of imprinted DNA methylation at the *KvDMR* ICR in ~50% of children with Beckwith-Wiedemann Syndrome, and loss of methylation at the *H19* ICR in ~45% of children with Russell-Silver Syndrome. The purpose of this study was to examine the association between duration of infertility and DNA methylation at four ICRs in euploid blastocysts.

DESIGN: Research study.

MATERIALS AND METHODS: Surplus cryopreserved euploid blastocysts of transferrable quality (grade  $\geq 3BB$ ; n=58) were donated with IRB approval and patient consent. Blastocysts were subdivided into four groups based on duration of infertility, classified as number of months of reported primary infertility prior to the oocyte retrieval that resulted in a live birth [Fertile Control: 0 months,

donor oocyte/donor sperm (n=14); Infertile Short: 12-24 months (n=14); Infertile Intermediate: 36-48 months (n=14); Infertile Long:  $\geq 60$  months (n=16)]. Female age was restricted to  $\leq 39$  years. Infertility diagnoses were equally varied among the test groups. Euploid blastocyst DNA was isolated (QIAamp DNA Micro Kit; Qiagen) and bisulfite converted (EZ DNA Methylation-Direct Kit; Zymo Research) prior to PCR amplification and pyrosequencing (PyroMark Q24 Advanced system; Qiagen). Statistical analysis included Student's t-test and one-way ANOVA where appropriate, with significance at  $p < 0.05$ .

RESULTS: Extended durations of infertility  $\geq 36$  months (Infertile Intermediate + Infertile Long; mean=65 months) showed significant alterations in blastocyst imprinted DNA methylation, with a decrease in methylation marks when compared to short durations  $\leq 24$  months (Fertile Control + Infertile Short; mean=10 months). The ICRs for *KvDMR* (39% Extended Infertility vs. 48% Short Infertility;  $p < 0.05$ ), *H19* (29% Extended Infertility vs. 41% Short Infertility;  $p < 0.05$ ), and *MEST* (40% Extended Infertility vs. 49% Short Infertility;  $p < 0.05$ ) showed significant hypomethylation, while *SNRPN* trended downward without significance. Infertility diagnoses, blastocyst grades, and total doses of recombinant follicle stimulating hormone during ovarian stimulation were comparable across the groups.

CONCLUSIONS: This novel study is the first to report evidence that altered blastocyst imprinted methylation correlates with prolonged infertility. The prevalence of ICR hypomethylation was significant in euploid blastocysts derived from patients with an extended duration of infertility  $\geq 36$  months. Ongoing studies will investigate whether the underlying infertility leads to epigenetic errors, or if the methylation alterations themselves are perpetuating the duration of infertility? Our results contribute towards the identification of a mechanistic link between imprinted epigenetic dysregulation and infertility as a prolonged disease.

SUPPORT: None.

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### ARE THERE ANY SIMILARITIES IN GENE EXPRESSION BETWEEN EUPLOID EMBRYOS AND ANEUPLOID EMBRYOS COMPATIBLE WITH LIFE?

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OBJECTIVE: Examples of human aneuploidies compatible with life include (but are not limited to) Down, Edwards, Klinefelter and Turner syndromes. Why or how are these unique aneuploid embryos able to implant and develop to term while other aneuploid embryos fail to implant or result in miscarriage? ART with PGT-A provides an opportunity not only to identify these aneuploidies but also to analyze blastocoel fluid contents. Blastocoel fluid is known to contain cell-free DNA, mRNA, extracellular vesicles and proteins, therefore comparison of the fluid components from various embryos of known ploidy status may provide insight into why some aneuploidies are compatible with life. Apoptotic remnants (i.e. mRNAs) that reside within the embryo's blastocoel fluid may vary in relation to the embryo's ploidy status. This study compared apoptotic gene expression in blastocoel fluid-conditioned media using Real-Time PCR from a euploid embryo resulting in a term birth, embryos harboring aneuploidies compatible with life, and aneuploid embryos incompatible with life.

DESIGN: Retrospective analysis of day-5 euploid and aneuploid blastocoe fluid apoptotic gene expression.

MATERIALS AND METHODS: Blastocoe fluid-conditioned media (25µL) was collected following trophectoderm (TE) biopsy of ICSI-generated day-5 blastocysts. Biopsied TE cells were sent for preimplantation genetic testing for aneuploidies using NGS. The blastocoe fluid conditioned media from 12 embryos were each subjected to DNase I treatment prior to cDNA synthesis before assessing gene expression via RT-PCR using TaqMan Fast Array-Human Apoptosis plates (assessing 92 apoptosis associated genes).

RESULTS: Of the 92 apoptotic genes analyzed, NOD1, NOD2 and TRADD expression were detected only in aneuploid embryos compatible with life. CASP5 gene expression was detected only in autosomal aneuploidies (n=4 Down syndrome and n=1 Edwards syndrome), while LTB was detected only in sex chromosome aneuploidies (n=1 Klinefelter syndrome and n=2 Turner syndrome). Aneuploid embryos (n=3) incompatible with life did not reveal any unique gene expression profiles. The euploid embryo (resulting in a term birth) analyzed did not show expression of any of the aforementioned genes.

CONCLUSIONS: This is the first report of gene expression differences detected in blastocoe fluid between survivable and non-survivable aneuploidies and an euploid embryo resulting in a term birth. While differences in apoptotic gene expression were observed among the embryos analyzed, we saw no similarities between an euploid embryo and survivable aneuploidies. Interestingly, we detected CASP5 and LTB expression unique to autosomal aneuploidies and sex chromosomal aneuploidies, respectively. Further studies will elucidate the processes by which certain aneuploidies can result in a live birth.

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#### DEFINING A CLINICAL VALIDITY FRAMEWORK FOR PHARMACOGENOMIC BIOMARKERS OF IVF TREATMENT RESPONSE AND OUTCOMES.

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OBJECTIVE: To define the landscape of clinically valid genetic associations with IVF treatment response and outcomes.

DESIGN: The Clinical Genome (ClinGen) Resource has defined a scoring framework to evaluate the strength of the evidence linking variations in a particular gene to a phenotype or disease. Here, we applied this framework to analyze the genetic and experimental evidence linking genes to reproductive phenotypes within the context of IVF treatment.

MATERIALS AND METHODS: We optimized natural language processing algorithms to identify relevant studies published before September 18<sup>th</sup>, 2018 that examined a statistical and/or functional gene-phenotype relationship in the context of IVF treatment. We used a scoring algorithm designed to implement principles from the ClinGen framework, including seven semi-quantitative measures of study quality, to assign a score to each study, generating a cross-study clinical validity score (CVS) between 0 and 18 for each gene-phenotype combination. We then leveraged our multi-omics Reproductive Atlas platform to characterize reported genes for their role in reproduction and more general physiological and cellular functions.

RESULTS: Within 9,454 studies identified, we found 55 IVF-related phenotypes with at least one report of an association with one of 115 genes. 97 of these genes had sufficient published evidence to quantify a CVS. Of the resulting 128 gene-phenotype combinations, 8 had 'strong' evidence (CVS: 12-18), 26 'moderate' (CVS: 7<12), 39 'limited' (CVS: 0>7), and 55 'none' (CVS = 0). Our study demonstrated that *FSHR* and *LHCGR* were the most extensively studied genes (examined in 34% (n=99/291) of assessed genetic studies), with strong or moderate evidence of association with 'ovarian reserve', 'ovarian response to stimulation', 'implantation', and 'oocyte to embryo transition' phenotypes. However, our analysis also highlighted genes with functions other than gonadotropin regulation: *TUBB8*, *PADI6*, and *TLE6*, which regulate oocyte cytoskeletal structure, had strong and moderate relationships with oocyte maturation (CVS=13.5 for *TUBB8*) and embryo development phenotypes (CVS=11 and 8.5 for *PADI6* and *TLE6* respectively). Six genes in our analysis (*BDNF*, *HTR2A*, *HTR2C*, *ITGB3*, *SLC64A*, and *TPHI*) are well characterized for their role in serotonin signaling and had evidence of a clinically valid association with 'implantation and early development', 'implantation failure', and 'pregnancy loss after IVF'.

CONCLUSIONS: We have established a machine-driven framework for rapidly analyzing and establishing the degree of clinical validity at a given point in time for a gene association with an IVF-related reproductive phenotype. Hundreds of new reports enter the evidence base annually. A framework and semi-automated workflow like the one designed in this study can help evaluate genetic

biomarkers that show the best promise for leveraging pharmacogenomic and genetic insight to optimize IVF treatment protocols and outcomes.

SUPPORT: Financial support for this project was provided by Celmatix Inc. and Ferring Pharmaceuticals, Inc.

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#### ZP1 PATHOGENIC VARIANTS CAUSE 'GENUINE' EMPTY FOLLICLE SYNDROME: EVIDENCE FOR THE EXISTENCE OF AN INTACT OOCYTE AND A ZONA PELLUCIDA IN FOLLICLES UP TO EARLY ANTRAL STAGE.

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OBJECTIVE: Empty follicle syndrome (EFS) is the complete failure to retrieve oocytes from mature follicles after ovarian stimulation for *in vitro* fertilization. "Genuine" (GEFS) occurs without any human or pharmaceutical error during the ovarian stimulation process and its existence has been a question in the field until *LHCGR* and *ZP3* were identified as causative genes. Even so, it is still unclear what happens to these patients' oocytes, and the pathogenesis of GEFS remains obscure. For most GEFS cases, additional  $\beta$ -hCG or repeated controlled ovarian hyperstimulation (COH) by different protocols dose not succeed in oocyte recovery, and use of donor oocytes have been proposed as the only viable alternative choice. We sought to identify novel pathogenic variants (PVs) causing EFS and dissect follicular development in EFS patients.

DESIGN: COH, genetic analysis, and ovarian immunohistochemistry (IHC).

MATERIALS AND METHODS: Five unrelated infertile women with clinical manifestations of GEFS were included in this study, as approved by the Ethics Committee of our hospital with patient consent. We performed exome sequencing in two unrelated consanguineous families with EFS and female infertility. PV screening of *ZP1* was also performed in three unrelated patients. Follicular development and zona pellucida (ZP) assembly were assessed by IHC using ovarian serial sections.

RESULTS: Six novel PVs and one known PV in *ZP1* were identified. Studies in CHO cells showed that these PVs, except for two splice site variants, resulted in either the degradation or truncation of *ZP1* protein. IHC staining demonstrated that all preantral follicles had normal architecture, with a thin ZP lacking *ZP1* present surrounding growing oocytes. However, this thin ZP was defective in normal cumulus-oocyte complex organization during antral folliculogenesis, leading us to speculate this might lead to oocyte degeneration or increased fragility of the oocyte during follicular puncture, ultimately resulting in what presents as EFS. Our findings are complementarity to previous studies, in which oocytes lacking ZP were retrieved from patients carrying biallelic *ZP1* truncating mutations that were speculated to prevent the ZP formation. For these cases, we suspect that a thin ZP might actually exist within ovarian follicles, but is degraded before ovulation, or is lost during granulosa cell removal. In addition, we report a new phenotype of human ZP in the absence of *ZP1* protein, which is similar to our earlier study showing that human oocytes carrying homozygous *ZP2* truncating PVs form a thin ZP without *ZP2*.

CONCLUSIONS: We identified several novel *ZP1* PVs causing EFS and female infertility in a recessive genetic mode, and for the first time present morphological evidence showing the normal preantral folliculogenesis with abnormal ZP assembly in EFS patients carrying biallelic *ZP1* PVs. Our data provides a better understanding of the biological functions of *ZP1* in human ZP assembly and folliculogenesis, and gives new insights into the pathogenesis of EFS, potentially being of great inspiration for therapeutic developments.

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#### ANALYSIS OF ACCESSIBLE CHROMATIN LANDSCAPE IN THE INNER CELL MASS AND TROPHECTODERM OF HUMAN BLASTOCYSTS USING ATAC-SEQ.

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OBJECTIVE: The chromatin accessibility landscape during the early-stage embryo development, especially the early lineage specification, has not yet been delineated in human preimplantation embryos, mainly due to the assay limitation. We optimized the Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) for low DNA input with the aim of exploring the chromatin

remodeling pattern in human preimplantation embryos and revealing the epigenetic regulation of inner cell mass (ICM) and trophoblast (TE) differentiation.

DESIGN: Experimental study.

**MATERIALS AND METHODS:** The whole ICM and partial TE were biopsied from eight aneuploid embryos. DNA obtained from lysed samples was tagged using Nextera Tn5 transposase and purified by phenol-chloroform extraction. PCR was conducted using Phusion high-fidelity PCR master mix (NEB) with customized index adapter oligos. AMPure XP magnetic beads (Beckham Coulter) were used for Library purification. Sequencing was performed on Illumina NextSeq 550 with paired-end 150 bp reads. Sequencing reads were aligned to human genome reference Hg19 using Bowtie2. All PCR duplicates, mitochondrial, unmapped and non-uniquely mapped reads were removed. Peaking calling was conducted using MACS2 and visualizations of the peaks in a genomic context were generated. ChIPseeker was used for peak annotation and differential ATAC-seq peaks analysis was conducted by DiffBind. The ATAC peak distribution differences were analyzed using both Fisher's exact test and Chi-squared test.

**RESULTS:** The assay for ATAC-seq was optimized and validated to obtain high-quality data using small sample input (10-30 cells). The ATAC-seq result of each sample for both ICM and TE groups showed a highly reproducible pattern. A large fraction of the ATAC seq peaks were located in the promoter and distal intergenic regions in both ICM and TE, which is consistent with previously published data from animal models. Transcription factor binding sites (TFBS) are often accessible in the active genes and not uniformly distributed over the promoter region. Our data showed that ATAC peak distributions of the promoter regions (<1kb) and distal regions versus other regions were significantly different between ICM vs TE samples ( $P < 0.01$ ). We detected that higher percentage of accessible binding loci were located within 1kb of the transcription start site in ICM compared to TE ( $p < 0.01$ ). However, higher percentage of accessible regions were detected in the distal region of TE compared to ICM. In addition, 8 differential peaks with the  $FDR < 0.05$  between ICM and TE were detected and these 8 accessible locations were identified in ICM samples.

**CONCLUSIONS:** This is the first study to compare the landscape of the accessible chromatin between ICM and TE of human preimplantation embryos, which unveiled chromatin-level epigenetic regulation of cell lineage specification in early embryo development.

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**AN OVARIAN COMPONENT INVOLVED IN SUBFERTILITY OF THE NSMF KO MOUSE.** Erica Loudon, M.D. Ph.D., Lynn Chorich, B.S., M.S., Lawrence Layman, M.D. Augusta University, Augusta, GA.



**OBJECTIVE:** Genetic approaches in humans with gonadotropin releasing hormone (GnRH) deficiency causing normosmic hypogonadotropic hypogonadism (nHH)/Kallmann syndrome (KS) have been important to better understand normal reproduction. *NSMF* (NMDA receptor synaptonuclear signaling & neuronal migration factor), formerly known as *NELF* (nasal embryonic LHRH factor), gene mutations have been identified in humans with either nHH/KS. However, the phenotype of the *Nsmf* knockout (KO) mouse is less severe than the human. The *Nsmf* KO females have reduced numbers of GnRH neurons and delay in vaginal opening, but normal puberty and subfertility. We previously showed *Kiss1* mRNA expression was increased in the hypothalami of KO animals and that pituitary gonadotropin responses were not different in wild type (WT) vs *Nsmf* KO mouse. Our objective in this study was to identify cell types that express *Nsmf* in the ovary and determine if the subfertility in the female *Nsmf* KO mouse has a gonadal component.

**DESIGN:** NSMF protein cellular localization was determined in the WT mouse ovary. *Kiss1* and *Kiss1r* mRNA expression was characterized in the KO vs WT 8 week old mice, and ovarian responses to gonadotropins were studied in 3 week old *Nsmf* KO mice in the diestrus phase.

**MATERIALS AND METHODS:** Heterozygous *Nsmf* mice were bred to homozygosity. Ovaries from KO vs WT mice were sectioned and prepared for immunohistochemistry (IHC) using a monoclonal anti-NSMF antibody. RNA extracted from ovaries of KO and WT animals were subjected to RT-qPCR for *Kiss1* and *Kiss1r* expression. The  $\Delta\Delta Ct$ , cycle of threshold, method was used to calculate relative gene expression of *Nsmf* KO vs control using *Gapdh* expression for normalization. To determine the ovarian response to gonadotropins, WT and KO mice 3 weeks of age were superovulated using PMSG and hCG. Mice were sacrificed and oocytes were removed from the oviducts and counted. Differences were analyzed using the Mann-Whitney U test. 8-week old mice also had serum gonadotropins before and after ovariectomy.

**RESULTS:** Our preliminary findings demonstrate *Nsmf* mRNA expression in the ovary, and IHC studies and serum gonadotropins after ovariectomy are

ongoing. *Kiss1r* expression is unchanged in *Nsmf* hypothalamus and ovary, but *Kiss1* was upregulated in the hypothalamus and the ovary. Preliminary data suggests that oocyte numbers were modestly decreased in the KO (~18/ovary) vs WT (30/ovary), but maturity has not been assessed yet.

**CONCLUSIONS:** A hypothalamic component appears to be involved in the subfertility of the *Nsmf* KO mouse, as demonstrated by a decreased number of GnRH neurons as well as our finding of increased *Kiss1* expression in the hypothalami of *Nsmf* KO mice, which we hypothesize is a compensatory increase secondary to deficient NSMF. Therefore we sought to characterize *Kiss1* and *Kiss1r* expression in the ovary. *Kiss1r* expression was unchanged, but there was a significant increase in *Kiss1*, which is known to be expressed in granulosa cells in mice. We also demonstrated *Nsmf* expression in the WT ovary. The reduced number of oocytes in the *Nsmf* KO mouse supports an ovarian role for NSMF in the subfertility of the *Nsmf* KO mouse.

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**PRO-APOPTOTIC GENE EXPRESSION IN BLASTOCOEL FLUID FROM EUPLOID DAY-5 EMBRYOS IS ASSOCIATED WITH NEGATIVE PREGNANCY OUTCOMES.** Deepti M. Athavale, B.S.,<sup>a</sup> Alyssa Barré, B.S.,<sup>a</sup> Allison C. Kranyak, B.S.,<sup>a</sup> Arnav Lal, na,<sup>a</sup> Jonathan L. Blalock, BS,<sup>a</sup> Shawn Zimmerman, PhD, HCLD,<sup>b</sup> T. Arthur Chang, PhD, HCLD, ELD,<sup>c</sup> Randal D. Robinson, MD,<sup>d</sup> J. David Wininger, PhD, HCLD,<sup>c</sup> William E. Roudebush, PhD,<sup>a</sup> Renee J. Chosed, PhD.<sup>a</sup>



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**OBJECTIVE:** The identification of molecular markers for use during selection of embryos for intrauterine implantation can enhance in vitro fertilization-embryo transfer success rates. Assessing apoptotic gene expression in blastocoel fluid-conditioned media from human embryos with known ploidy and implantation status provides the opportunity to study patterns and processes occurring during early embryo development. Apoptosis occurs during preimplantation development and may serve to selectively eliminate aneuploid cells from the developing embryo thereby enhancing implantation potential. Therefore, apoptotic remnants (i.e. mRNAs) may reside within the embryo's blastocoel fluid and vary in relation to the embryo's implantation potential. This study compared apoptotic gene expression in blastocoel fluid-conditioned media using Real-Time PCR from euploid embryos with known implantation outcomes.

**DESIGN:** Retrospective analysis of day-5 euploid blastocoel fluid apoptotic gene expression and implantation outcome.

**MATERIALS AND METHODS:** Blastocoel fluid-conditioned media (25 $\mu$ L) was collected following trophoblast (TE) biopsy of ICSI-generated day-5 blastocysts. Biopsied TE cells were sent for preimplantation genetic testing for aneuploidies using NGS. The blastocoel-fluid conditioned media from 10 euploid embryos (6 that implanted; 4 that did not implant) were each subjected to DNase I treatment prior to cDNA synthesis before assessing gene expression via RT-PCR using TaqMan Fast Array-Human Apoptosis plates (assessing 92 apoptosis associated genes).

**RESULTS:** Of the 92 genes analyzed, CASP7 and MCL1 gene expression were only detected in euploid embryos that successfully implanted. Conversely, expression of TNFRSF25 and BCL2L11 genes were only detected in euploid embryos that failed to implant. Several other apoptotic genes (BAD, BCL2L13, BCAP31, NOD1 and CARD18) were expressed more often in embryos that failed to implant versus those that successfully implanted.

**CONCLUSIONS:** This study poses that specific apoptotic remnants (mRNAs encoding apoptotic genes) may represent a molecular indicator of euploid embryo future implantation potential. Specifically, we detected the expression of seven pro-apoptotic genes associated with negative implantation outcomes. Apoptosis is initiated within the developing embryo in response to the presence of aneuploidies and/or ROS-induced damaged cells. Our results suggest that altered cells may still reside within some euploid blastocysts, thus initiating apoptosis. Evidence of apoptotic cell elimination may be detected by expression of pro-apoptotic gene products found within the blastocoel fluid.

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**FRAGILE X CARRIER SCREENING ACCOMPANIED BY GENETIC CONSULTATION HAS CLINICAL UTILITY IN POPULATIONS BEYOND THOSE RECOMMENDED BY GUIDELINES.** Katie Johansen Taber, PhD,<sup>a</sup>



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MD.<sup>a</sup> <sup>a</sup>Myriad Women's Health, South San Francisco, CA; <sup>b</sup>Myriad Genetics, Salt Lake City, CA; <sup>c</sup>Myriad Genetics, Salt Lake City, UT.

**OBJECTIVE:** To determine the clinical utility of FXS carrier screening by analyzing actions among *FMR1* premutation carriers who do and do not meet American College of Medical Genetics and Genomics (ACMG) or American College of Obstetricians and Gynecologists (ACOG) criteria for testing.

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability, with 1:151 women carrying an *FMR1* premutation that confers elevated risk for FXS in offspring. ACMG and ACOG recommend *FMR1* carrier screening only in women with a family history of FXS, intellectual disability suggestive of FXS, or fragile X-related disorders. Screening is also recommended for those undergoing fertility evaluation. Offering screening for *FMR1* to all women who are pregnant or considering pregnancy has been resisted in part due to questions about the clinical utility of screening. Concerns have also been raised about the ability to adequately counsel large numbers of screened women about the complex inheritance patterns and the wide range of phenotypes associated with FXS.

**DESIGN:** Retrospective survey of couples at increased risk for a pregnancy affected by FXS.

**MATERIALS AND METHODS:** *FMR1* premutation carriers identified by expanded carrier screening (ECS) between September 2015 and December 2017 were invited to respond to a survey about their actions following receipt of screening results.

**RESULTS:** A total of 122 *FMR1* premutation carriers responded to the survey. Providers recommended screening for 77% of patients, while 23% of patients had requested screening themselves. 79% of screening occurred in females that did not meet the ACMG/ACOG family history criteria, and 52% occurred in those who did not meet the ACMG/ACOG fertility evaluation criteria. 99% of those screened had received post-test genetic consultation.

Among 73 *FMR1* premutation carriers screened preconceptionally, 74% planned or pursued actions that reduce the risk of having an affected pregnancy, including in vitro fertilization with preimplantation genetic testing for monogenic conditions (52%), prenatal diagnosis when pregnancy occurred (25%), use of a gamete donor (6%), avoiding pregnancy (6%), and adoption (4%). A family history of FXS increased the likelihood of pursuing risk-reducing actions, but undergoing fertility evaluation did not. Among 49 *FMR1* premutation carriers screened prenatally, 41% planned or pursued prenatal diagnosis. Neither family history nor undergoing fertility evaluation had a significant effect on the decision to undergo prenatal diagnosis.

**CONCLUSIONS:** Providers recommended, and patients desired, FXS carrier screening regardless of whether the patient met current ACMG/ACOG screening criteria. Patients who did not meet screening criteria took action to reduce the risk of having an affected pregnancy to nearly the same extent as those who did meet criteria. Nearly all patients made reproductive and pregnancy management decisions informed by genetic consultation. These results support offering FXS carrier screening to all women who are pregnant or considering pregnancy.

**SUPPORT:** This analysis was fully funded by Myriad Women's Health.

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#### THE EXPRESSION OF HUMAN ENDOGENOUS RETROVIRUS SYNCYTIN IN HUMAN ANEUPLOIDY ARE INSUFFICIENT COMPARED TO EUPLOIDY.

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**OBJECTIVE:** Retrotransposons are a group of abundant, repetitive sequences, which originated from ancient retroviral infections of our ancestral genomes. They are silenced by epigenetic marks throughout most of life, but become activated during early embryo development, with reprogramming of the epigenome. Some retrotransposons play regulatory roles during early development. Non-Long Terminal Repeat (LTR) retrotransposons (e.g. LINE-1) regulate gene expression during early mouse embryo development. LTR retrotransposons, such as the human endogenous retrovirus HERV-W and HERV-FRD (Syncytin-1 and Syncytin-2), mediate placentation<sup>1</sup>. We hypothesized that aneuploidy, which disrupts implantation, would affect expression of retrotransposons during early human development.

**DESIGN:** Prospective laboratory study.

**MATERIALS AND METHODS:** Blastocysts donated by patients who underwent IVF/PGT-A at NYU Langone FC were thawed, stripped of zona pel-

lucidae by laser (to remove sperm or cumulus) and their genomic DNA and mRNA separated by the G&Tseq protocol<sup>2</sup> with modifications. mRNA expression and gene copy number were measured by RT-qPCR and qPCR using Bio-Rad CFX96 thermocycler and iQ SYBY Green mix, and the  $\Delta\Delta Cq$  method was used to express their levels relative to GAPDH and 5S RNA, respectively. Data were analyzed by Mann Whitney U test and Student's T-test with GraphPad Prism 8 software.

**RESULTS:** Syncytin-1 was expressed in all human embryos, but its expression in euploid embryos (n=2) was significantly higher than in aneuploid embryos (n=6) (median 1.943 vs 0.316,  $P=0.0019$ ). Expression of Syncytin-2 also was extremely higher in euploid compared to aneuploid embryos (median 2.155 vs. 0.1124,  $P=0.0003$ ). The copy number of ALU sequences in aneuploid embryos was greater than in euploid embryos (mean  $0.943\pm 0.067$  vs  $0.807\pm 0.0716$ , T-test  $P=0.0486$ ), but LINE1 copy number or expression did not differ between euploid and aneuploid embryos (mean  $0.787\pm 0.069$  vs  $0.904\pm 0.094$ , T-test  $P=0.1778$ ).

**CONCLUSIONS:** We compared expression of a number of retrotransposons between euploid and aneuploid human blastocysts, and discovered that the human endogenous retrovirus, Syncytin-1 and Syncytin-2, are markedly decreased in aneuploid compared to euploid embryos. Given the crucial role of Syncytins in formation of human placenta, our data provide a possible mechanism of implantation failure in euploid embryos, and suggest a possible biomarker for implantation.

**References:** 1. Soygur B, Moore H. Expression of Syncytin 1 (HERV-W), in the preimplantation human blastocyst, embryonic stem cells and trophoblast cells derived in vitro. Hum Reprod. 2016 Jul; 31(7):1455-61.

2. Macaulay IC, Teng MJ, Haerty W, Kumar P, Ponting CP, Voet T. Separation and parallel sequencing of the genomes and transcriptomes of single cells using G&T-seq. Nat Protoc. 2016 Nov; 11(11):2081-103.

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**A STEP TOWARD GENE REMODELING OF MAMMALIAN SPERMATOZOA BY CRISPR-Cas9.** June Wang, B.A., Alessandra Parrella, M.Sc., Philip Xie, B.S., Zev Rosenwaks, M.D., Gianpiero D. Palermo, M.D., Ph.D., The Ronald O. Perleman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY.



**OBJECTIVE:** To identify the optimal conditions to carry out genomic remodeling of mammalian spermatozoa using CRISPR-Cas9.

**DESIGN:** Mouse spermatozoa with and without in vitro decondensation were transfected with a CRISPR ribonucleoprotein (RNP) targeting exon 10 of the Stra8 gene in chromosome 6. The cleavage site was designed for noninvasive gene repair by homologous recombination. The success of editing at the target site was measured by mismatch cleavage assay.

**MATERIALS AND METHODS:** Epididymal spermatozoa were retrieved from one B2D6F1 mouse. Half of the sample was resuspended in mHTF, and the remaining was incubated with 46 uM heparin and 10 mM GSH at 37°C and 5% CO2 for 30 minutes to decondense the DNA. Purified Cas9 and a custom Stra8 gRNA were pre-complexed for 10 minutes. In both the decondensed and untreated conditions, a CRISPR group was electroporated with the RNP and a negative control was electroporated without. The Neon Transfection System (ThermoFisher Scientific) was used at 1100 volts, 30 milliseconds, and 1 pulse, based on our preliminary research. After 30 minutes at room temperature, samples were prepared for analysis by the GeneArt Genomic Cleavage Detection kit. In brief, DNA was extracted from the cells and a 500-bp region around the CRISPR target site was amplified by PCR. The presence of mismatches at the cleavage site due to indels causes two fragments to appear in gel electrophoresis as opposed to one band of uncleaved DNA.

**RESULTS:** The raw epididymal sample yielded a concentration of 30 million, 0.75 ml, and 76% motility. An aliquot was processed by microfluidic selection for a final sample of 8 million, 1.0 ml, and 89% motility. After the decondensation process, motility decreased to 72%, compared to 85% in the untreated sample. Immediately after electroporation, the motility of the negative controls dropped to an average of 60%, while that of the CRISPR samples decreased to an average of 37%. Finally, the gel analysis revealed 11% cleavage efficiency in the CRISPR population without decondensation, and 18% in the decondensed sample.

**CONCLUSIONS:** Our results indicate that we were able to cleave genomic DNA in the final exon of the Stra8 gene in up to 18% of spermatozoa. The addition of a brief decondensation treatment proved to be beneficial. While this assessment took into consideration the entire population of electroporated spermatozoa, it would be interesting to observe the proportion of successful transfection in spermatozoa that retained motility.

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**THE EXTRACELLULAR CUMULUS MATRIX DOUBLES THE SPERM ZONA-ADHESION IN NORMOZOOSPERMIC PATIENTS.**

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**OBJECTIVE:** The aim of this study was to observe the effect of the cumulus extracellular matrix on the sperm zona-adhesion rate in healthy fertile men.**DESIGN:** Comparison of the zona-adhesion rate between spermatozoa treated with cumulus extracellular matrix and non-treated spermatozoa.**MATERIALS AND METHODS:** The cumulus matrix proteins used in this study were isolated from 150 cumulus complexes that were obtained from 16 donors during oocyte retrieval procedures. The cumulus cells and their extracellular matrix were separated by pipetting followed by centrifugation. The protein content in the pool of isolated cumulus matrixes (CM) was measured by Bradford method. Semen samples were obtained from 30 normozoospermic donors. After sperm washing, the motile spermatozoa were isolated by swim-up and diluted to  $0.5 \times 10^6$  cells/ml. Each sample was divided into four aliquots and incubated with (1) 0.5 mg/ml CM, (2) 1.25 mg/ml CM, (3) 2.5 mg/ml CM and (4) wash medium for 30 min at 37°C. The zona-adhesion rate was evaluated by counting the adhered spermatozoa to immobilized acid-solubilized zonae pellucidae from healthy donors. Results are presented as number of adhered spermatozoa per  $1 \text{ mm}^2$  of the immobilized surface ( $\text{sp}/\text{mm}^2$ ). Statistical analysis was performed with paired t-test using IBM SPSS Software ver.21.**RESULTS:** The zona-adhesion rate of the untreated spermatozoa was  $81 \pm 17 \text{ sp}/\text{mm}^2$  (Mean  $\pm$  SD) and ranged between  $54 \text{ sp}/\text{mm}^2$  and  $116 \text{ sp}/\text{mm}^2$ . CM treatment of the spermatozoa dose-dependently and significantly increased the zona-adhesion rate in every patient ( $p < 0.05$ ). When spermatozoa were treated with 2.5 mg/ml CM, 1.25 mg/ml CM and 0.625 mg/ml the mean sperm zona-adhesion was  $128 \pm 28 \text{ sp}/\text{mm}^2$ ,  $107 \pm 37 \text{ sp}/\text{mm}^2$  and  $99 \pm 27 \text{ sp}/\text{mm}^2$ , respectively.**CONCLUSIONS:** The results from this study show the important role of the cumulus matrix in the preparation of the spermatozoa before meeting the oocyte and confirm that the cumulus effect should be considered during sperm processing for ICSI.

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**VARICOCELE DIMINISHES SPERM CAPACITATION FUNCTION AND THE CHANCES OF GENERATING A PREGNANCY.**Philip Xie, B.S.,<sup>a</sup> Alessandra Parrella, M.Sc.,<sup>a</sup> Alexander J. Travis, VMD, PhD,<sup>b</sup> Zev Rosenwaks, M.D.,<sup>a</sup> James A. Kashanian, MD,<sup>c</sup> Gianpiero D. Palermo, M.D., Ph.D.<sup>a</sup>  
<sup>a</sup>The Ronald O. Perleman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY; <sup>b</sup>Cornell University, Ithaca, NY; <sup>c</sup>Weill Cornell Medicine, Department of Urology, New York, NY.**OBJECTIVE:** To determine whether varicocele can adversely affect sperm capacitation and therefore the probability of generating a pregnancy (PGP).**DESIGN:** In 8 consenting men with grade 2 varicoceles or larger, we assessed functional semen characteristics by using Cap-Score™ to measure the percentage of sperm that can capacitate, and calculated the related PGP calculation. Ten men with normal semen parameters, no varicoceles, and proven fertility served as a control. Cap-Score was determined in a blind fashion regarding presence/absence of varicocele.**MATERIALS AND METHODS:** Semen analyses were performed on fresh ejaculates of 18 consenting men. Those men in the control group did not have a history of varicocele nor pertinent urological issues. Presence of a varicocele was determined by physical exam in the standing position. All men in the varicocele group were diagnosed with a unilateral or bilateral varicocele of grade 2 or higher. Sperm capacitation was measured by Cap-Score™ assay (Androvia LifeSciences) with a normal threshold of  $>27.6\%$ . To quantify the actual number of capacitated spermatozoa, Cap-Score  $\times$  volume  $\times$  concentration was calculated. PGP was determined by the corresponding Cap-Score™, with a threshold of  $>32.7\%$  considered normal. Semen parameters, Cap-Score™, total capacitated spermatozoa and PGP were compared between control and varicocele group using unpaired *t* tests at 0.05, with 0.05 considered significant.**RESULTS:** Men in the control group ( $n=10$ ) and those with varicocele ( $n=8$ ) were of comparable age ( $34.7 \pm 2.8$  years and  $36.0 \pm 7.0$  years, respectively). Semen parameters including volume ( $2.9 \pm 0.9$  ml), concentration ( $69.8 \pm$  $26.3 \times 10^6/\text{ml}$ ), motility ( $47.7 \pm 2.8\%$ ) and normal morphology ( $3.1 \pm 0.7\%$ ) in the control also did not differ from those men with varicocele (volume of  $3.1 \pm 1.1$  ml, concentration of  $45.4 \pm 30.9 \times 10^6/\text{ml}$ , motility of  $45.6 \pm 1.0\%$  and normal morphology of  $2.8 \pm 1.0\%$ ). The control group had an average Cap-Score™ of  $31.4 \pm 4.8\%$  while that of the varicocele group was  $26.4 \pm 3.7\%$  ( $P = 0.03$ ). There was a significantly higher number of capacitated spermatozoa in the control group ( $67.1 \pm 40.1 \times 10^6$ ) when compared to the varicocele group ( $32.8 \pm 22.4 \times 10^6$ ) ( $P = 0.04$ ). In the control group there was an average PGP of  $39.7 \pm 9.1\%$ , while men with varicocele yielded a PGP of  $31.0 \pm 6.0\%$  ( $P = 0.03$ ).**CONCLUSIONS:** Scrotal varicocele is known to induce male infertility by impairing sperm production and inducing sperm chromatin fragmentation. While the impact of varicocele on actual sperm function is unclear, in this study, we demonstrate for the first time that venostasis may be responsible for lessening one of the prominent sperm functions such as capacitation.

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**IMPACTS OF TEST (TES AND TRIS) YOLK BUFFER AND COOLING ON THE ABILITY OF HUMAN SPERM TO CAPACITATE.**G. Charles Ostermeier, PhD,<sup>a</sup> Cristina Cardona, PhD,<sup>a</sup> Melissa A. Moody, MS,<sup>a</sup> Alana J. Simpson, BS,<sup>a</sup> Romeo Mendoza, MT,<sup>a</sup> Alexander J. Travis, VMD, PhD.<sup>b</sup> <sup>a</sup>Androvia LifeSciences, Mountainside, NJ; <sup>b</sup>Cornell University, Ithaca, NY.**OBJECTIVE:** Studies across several mammalian species show that  $G_{M1}$  localization patterns are indicative of capacitation at the single cell level. The Cap-Score™ Male Fertility Assay reports the proportion of sperm displaying  $G_{M1}$  localization patterns consistent with capacitation. Using clinical pregnancy outcomes, Cap-Score was previously shown to prospectively predict a man's fertility and the relationship between Cap-Score and a man's probability of generating a pregnancy was established. TEST (TES and Tris) yolk buffer (TYB) can prolong the fertilization capacity of sperm. Here, we evaluated whether incubation in TYB overnight at a cool temperature affected human sperm capacitation.**DESIGN:** To evaluate the impact of semen extension with TYB and cooling on sperm capacitation, ejaculates were split into control and test samples for a repeated measure design.**MATERIALS AND METHODS:** Studies approved by WIRB (20152233). Semen was collected, liquefied and split into control and test samples. Control samples were processed normally for Cap-Score. Test samples were extended in TYB at 1:1 ( $n=5$ ), 1:6 ( $n=7$ ) or 8:5 ( $n=5$ ; volume ratio of semen:TYB) and cooled overnight in a Styrofoam box with an ice pack. The next day, samples were washed, exposed to non-capacitating (NC) or capacitating (CAP) conditions for 3 hrs, and then fixed overnight before Cap-Score determination. Test-samples were compared to controls using paired t-tests.**RESULTS:** In all experiments, Cap-Score was greater for control-CAP when compared to control-NC ( $p < 0.05$ ). No differences were observed between the control-CAP and the test-CAP for any dilution (1:1 ratio:  $39.7 \pm 0.04$  vs  $40.0 \pm 0.02\%$ ;  $p=0.87$ ; 1:6 ratio:  $32.0 \pm 0.04$  vs  $34.0 \pm 0.03\%$ ;  $p=0.33$ ; 8:5 ratio:  $36.0 \pm 0.02$  vs  $34.2 \pm 0.01\%$ ;  $p=0.5$ ).**CONCLUSIONS:** A good capacitation response was observed in the controls for all experiments, suggesting proper stimulus by the CAP condition. The ratios of semen:TYB were chosen to mimic typical ejaculate volumes, such that a constant volume of extender could potentially be utilized in an at home semen collection kit that maintains sperm capacitation ability. Addition of a fixed volume of TYB to varying ejaculate volumes would limit user input. Similar Cap-Score values between the control-CAP and test-CAP, no matter the ratio, indicates that ejaculates can be maintained overnight in varying concentrations of TYB with minimal impact on next-day function. At home sample collection could lessen the burden of processing samples at clinics with limited resources. It could also encourage pursuit of workup by men whose main barrier is privacy in producing samples at clinics or bringing them to clinics. It could also broaden the geographical availability of sperm function tests to those living far from clinics, and reduce financial burdens associated with travel and time away from work.**SUPPORT:** Androvia LifeSciences.

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**RELATIONSHIP AMONG INTRACELLULAR SUPEROXIDE DISMUTASE ACTIVITY, GLUTATHIONE PEROXIDASE ACTIVITY, MOTILITY AND MORPHOLOGY IN HUMAN SEMEN.**

Luchezar Vasilev Jelezarski, PhD, Dimitar Parvanov, PhD, Vilyana Georgieva, MSc, Rumiana Ganeva, MSc,



Georgi Stamenov Stamenov, MD/PhD. Nadezhda Women's Health Hospital, Sofia, Bulgaria.

**OBJECTIVE:** Oxidative damage by reactive oxygen species (ROS) is one of the main causes for sperm dysfunction. Important components of the anti-oxidative defense systems are the superoxide dismutase (SOD) and glutathione dismutase (GPx). Therefore, our objective was to examine the relationship among sperm SOD activity, sperm GPx activity, sperm motility and morphology in human spermatozoa.

**DESIGN:** Prospective study.

**MATERIALS AND METHODS:** Sixty four patients aged between 26 and 39 years were selected. Samples were collected by masturbation after sexual abstinence for 3-5 days. After semen liquefaction, semen analysis was performed (concentration, progressive motility and non-strict morphology) according to WHO 2010 guidelines. Sperm SOD and GPx activities were determined using Ransod and Ransel diagnostic kits (Randox Laboratories Ltd., Antrim, UK). An aliquot of the corresponding sperm suspension ( $20 \times 10^6$  sperm/mL) was centrifuged at  $600 \times g$  for 5 minutes and the supernatant was discarded. The remaining pellet was treated with 0.5 mL of 0.1% Triton X-100 in PBS and vortex-mixed three times for 20 seconds followed by centrifugation at  $1,000 \times g$  for 5 minutes. Aliquots of the supernatant were added to the wells of the microplate and the assay was performed according to the manufacturer's instructions. The supernatant was discarded and the pellet was treated with 0.5 mL of 0.1% Triton X-100 in PBS, vortex-mixed three times for 20 seconds, and centrifuged at  $1,000 \times g$  for 5 minutes. Aliquots of the supernatant were added to the wells of the microplate and the assay was performed according to the manufacturer's instructions. Statistical analysis was performed by Spearman's correlation test using SPSS v.21 (IBM Corp., Armonk, NY, USA). Descriptive parameters and patient characteristics were reported as mean  $\pm$  SD and median.  $P < 0.05$  was considered statistically significant.

**RESULTS:** The determined SOD activity ranged between 0 and  $1415 \text{ U}/10^9$  spermatozoa with a mean of  $131.82 \pm 242.11 \text{ U}/10^9$  spermatozoa and a median of  $56.64 \text{ U}/10^9$  spermatozoa. The observed GPx activity ranged from 0.2 to  $111.57 \text{ U}/10^9$  spermatozoa with a mean of  $5.12 \pm 14.57 \text{ U}/10^9$  spermatozoa and a median of  $1.67 \text{ U}/10^9$  spermatozoa. There was a significant but low positive correlation between sperm SOD and GPx activities ( $R=0.27$ ;  $p=0.04$ ). Sperm SOD activity did not correlate significantly with sperm motility and morphology. In contrast, sperm GPx activity showed a significant negative correlation with the progressive motility ( $R=-0.48$ ;  $p < 0.01$ ) and negative correlation with the sperm morphology ( $R=-0.49$ ;  $p < 0.01$ ).

**CONCLUSIONS:** Intracellular sperm GPx activity seem to be linked more strongly to the sperm motility and morphology parameters rather than the sperm SOD activity. Among the studied group the lower sperm motility and poor sperm morphology were associated with relatively high GPx activity.

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#### VARIATION IN THE PERCENTAGES OF Y-CHROMOSOME BEARING SPERM IN EJACULATES.

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**OBJECTIVE:** To assess the variation in the percentages of Y-chromosome bearing sperm in ejaculates for the purpose of determining an underlying etiology for sperm-based sex selection (inconsistent) outcomes.

**DESIGN:** Ejaculates from 50 randomly selected men testing for routine semen analysis (RSA) were analyzed for percentage of Y-bearing sperm using a real-time polymerase chain reaction (qPCR) with unique primers. Institutional Review Board exempted this study under 45 CFR 46.101(b).

**MATERIALS AND METHODS:** Ejaculates obtained by self-masturbation were frozen following RSA. The frozen sperm samples were thawed and extracted through a modified Qiagen Genra Puregene Kit (Hilden, Germany) protocol for DNA purification from body fluids. Dithiothreitol (DTT) was added to the Cell Lysis Buffer provided in the kit to a final concentration of 80mM. 250  $\mu\text{L}$  of this modified buffer was then added to 50  $\mu\text{L}$  of the thawed sperm sample. The rest of the protocol for DNA purification from body fluids was then followed as specified by the manufacturer.

A quantitative PCR was developed using sex-determining region Y (SRY) on the Y-chromosome as the target, and the CFTR primer pair or PPIH as the reference. The primer sequences for both SRY and PPIH were obtained from Integrated DNA Technologies (Coralville, IA): SRY – F: TGGCGATTAAGTCAAATTCGC; R: CCCCTAGTACCCTGACAATGTATT (ampli-

con = 137 bp); CFTR – F: GAAGAGAACAAAGTCCGGCAG; R: TTGCCGGAAGAGGCTCCT (amplicon = 69 bp); and PPIH – F: CAGTCATGGTAAACTGGAAAG; R: AGGTGCTTCCTTTGTATCCTATT (amplicon = 109 bp). All reactions were performed on Applied Biosystem's (Foster City, CA) ViiA 7 Real-Time Thermal Cycler in triplicate at 95C for 10 minutes, followed by 40 cycles of 95C for 15 seconds and then 60C for one minute, followed by melt curve analysis. Data were analyzed on the same instrument. Samples with high standard deviation were repeated. 9 samples with the highest variation from normal were repeated, demonstrating consistency in these high-variation specimens, and supporting the validity of the method.

**RESULTS:** The mean percentage of Y-chromosome-bearing sperm for each individual sample was calculated. There was a significant difference in the overall mean  $\pm$  SD between the proportion of Y-chromosome-bearing sperm and X-chromosome-bearing sperm ( $45.36 \pm 7.88$  vs.  $54.42 \pm 7.88$ ). More importantly, a high level of variation in the percentages of Y-chromosome-bearing sperm was observed among the ejaculates of the 50 individuals. Summarizing the results, 17 ejaculates had more than, and 14 ejaculates had less than, the 99% confidence interval of the overall mean of the Y-chromosome-bearing sperm ( $45.58 \pm 2.87$ ; Figure 1).

**CONCLUSIONS:** Y-chromosome-bearing sperm content in ejaculates varies considerably among men. Inconsistency in sperm-based sex-selection outcomes appears to be a function of differences in the ejaculates themselves, rather than the reliability with which Y-chromosome-bearing sperm are identified.

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#### DURAMYCIN DISRUPTS SPERM MOTILITY AND *IN VITRO* FERTILIZATION (IVF).

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**OBJECTIVE:** The aim of our study was to investigate effects of phosphatidylethanolamine (PtE) on mouse epididymal sperm and to evaluate the effect of Duramycin, a broad-spectrum antibiotic commonly used in animal husbandry and a compound used to detect PtE in cell membranes, on sperm progressive motility and fertilization capacity in IVF.

**DESIGN:** Capacitated caudal epididymal sperm were isolated from mice and either left untreated or incubated with Duramycin. Sperm progressive motility and sperm cell death were assessed. Additionally, sperm were used for IVF and the percentage of resultant two-cell embryos was analyzed.

**MATERIALS AND METHODS:** Caudal epididymal sperm were isolated from  $> 10$  week-old C57BL/6 mice. To detect PtE, capacitated sperm were incubated with biotinylated Duramycin for 30 minutes, followed by streptavidin conjugated with Texas Red, mounted, and analyzed via fluorescent microscopy. Sperm progressive motility was assessed after a 30 minute incubation with  $0.1 - 2 \mu\text{M}$  Duramycin or control DMSO. The effect of Duramycin on sperm death was evaluated with 7AAD (necrosis) and staining of cleaved caspase-3 (CC3; apoptosis) by immunofluorescent microscopy. During IVF, sperm (untreated or incubated with  $2 \mu\text{M}$  Duramycin) were used to inseminate oocytes isolated from super-ovulated C57BL/6 female mice. After 24 hours, the percentage of resultant 2-cell embryos was analyzed.

**RESULTS:** PtE exposure was detected exclusively on the midpiece of mouse sperm. The fertilization rate of oocytes inseminated with untreated sperm was  $\sim 75\%$ , while it was completely abolished (0%) when sperm were pre-incubated with  $2 \mu\text{M}$  Duramycin. Sperm progressive motility was completely disrupted by  $0.25 - 2 \mu\text{M}$  Duramycin and dramatically reduced with  $0.1 \mu\text{M}$  Duramycin (% of motile sperm – Control:  $\sim 56\%$ ; Duramycin  $4.2\%$ ). Sperm death increased after incubation with Duramycin (% of 7AAD+ sperm – Control:  $2.3 \pm 2.3$ , Duramycin:  $13.8 \pm 4.4$ ), while CC3+ cells were not detected.

**CONCLUSIONS:** Duramycin significantly impaired sperm motility even at very low concentrations. This may explain the incapacity of the Duramycin treated sperm to fertilize oocytes. Duramycin did induce cell necrosis on a fraction of sperm; however, this cannot explain the complete disruption of sperm motility. Since Duramycin binds the sperm midpiece where PtE is exposed, it is possible that Duramycin disturbs mitochondrial activity depleting sperm energy and leaving the sperm immotile but alive. Environmental toxins have been implicated as a powerful contributor to the published widespread decline in semen parameters. The frequent use of Duramycin in agriculture portends frequent human exposure with unknown health and fertility consequences. Future studies are needed to examine the presence of Duramycin in our food chain.

**THE EFFICACY OF OXIDATION REDUCTION POTENTIAL(ORP) IN MALE INFERTILITY AND ITS RELATIONSHIP WITH SEMINAL LEUKOCYTE CONCENTRATION.** Shinnosuke Kuroda, M.D., Tepei Takeshima, M.D., Yasushi Yumura, Ph.D, Yokohama City University, Medical Center, Yokohama, Japan.



**OBJECTIVE:** Reactive oxygen species (ROS) in semen has been reported to have negative effect to male fertile capacity, and recent studies reported the efficacy of oxidation-reduction potential (ORP) which reflects the balance of oxidants and antioxidants in semen. The source of ROS in semen is considered as immature spermatozoa and seminal leukocytes, but the detail is still unknown. The aim of this study is to evaluate the relationship between the concentration of seminal leukocytes and oxidative stress level using ROS, ORP.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** Between April 2018 and March 2019, 29 infertile males who visited Reproduction Centre of Yokohama City University Medical Center were enrolled. All patients underwent semen analysis and measurement of ROS and ORP levels. The ROS level in semen was measured using Monolight 3010™ Luminometer and the ORP level was measured using MiOXSYS System™. The concentration of peroxidase-positive leukocytes were evaluated using myeloperoxidase staining (Endtz test). The relationship between ROS levels, ORP levels, the concentration of leukocytes and semen parameters were evaluated using correlation analysis.

**RESULTS:** The sperm concentration and motility were  $39.6 \pm 36.6 \times 10^6/\text{ml}$ ,  $30.0 \pm 18.9\%$ , respectively. The total ROS level was  $9328.9 (\pm 18851)$  Relative Light Units, and the ORP level was  $46.1 (\pm 38.7)$  mV. The ROS level was significantly correlated with ORP level ( $r = -0.79$ ,  $p < 0.01$ ). The concentration of leukocytes measured by Endtz test was positively correlated with both ORP level ( $r = 0.46$ ,  $p = 0.023$ ) and ROS level ( $r = 0.82$ ,  $p < 0.01$ ). ORP level was negatively correlated with sperm concentration ( $r = -0.41$ ,  $p = 0.026$ ), while ROS level didn't show significant correlation with every semen parameters.

**CONCLUSIONS:** To our knowledge, this is the first study that showed the significant correlation between ORP levels and seminal leukocytes concentration. Our study suggested that peroxidase-positive leukocyte is one of the main source of ROS in semen. Although ROS was strongly correlated with leukocyte concentration, ORP level is considered to have better potential to reflect the sperm quality.

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WITHDRAWN

## BASIC REPRODUCTIVE RESEARCH

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**ANDROGENS NEGATIVELY AFFECT CILIARY FUNCTION AND ALTER GENE EXPRESSION IN THE HUMAN FALLOPIAN TUBE.** Tia Jackson-Bey, MD MPH,<sup>a</sup> Angela Russo, Ph.D.,<sup>b</sup> Alexandria N. Young, B.S., B.A.,<sup>b</sup> Joanna E. Burdette, Ph.D.<sup>b</sup> <sup>a</sup>University of Illinois at Chicago, College of Medicine, Chicago, IL; <sup>b</sup>University of Illinois at Chicago, College of Pharmacy, Chicago, IL.



**OBJECTIVE:** To evaluate the impact of androgen exposure on human fallopian tube epithelium in relation to ciliary function and gene expression.

**DESIGN:** Translational research

**MATERIALS AND METHODS:** We exposed human fallopian tube epithelium to either a hormonally physiologic (low testosterone) or hyperandrogenic (high testosterone) culture media for 7-14 days. The hyperandrogenic media was characterized by twice the concentration of testosterone (2nM) than in the physiologic media (0.8 nM). After 7 days, cilia were imaged with spinning confocal microscopy to capture ciliary beating. The cilia beating frequency was then quantified using Fiji Image J software. After 14 days, gene and protein expression was assessed via immunohistochemistry staining, qualitative PCR, RNA sequencing and ELISA. Parallel experiments were conducted in static conditions, with the tissue on porous wells partially submerged in culture media that was exchanged every 2-3 days, as well as in microfluidic "organ on a chip" devices, in which fresh media

is dynamically circulated through, and waste removed from, wells containing the human fallopian tube epithelium in culture media.

**RESULTS:** After 7 days, a difference was seen in the rate of ciliary beating frequency as detected by spinning disk confocal microscopy. Human fallopian tube epithelium exposed to high testosterone had a decreased rate of cilia beating compared to human fallopian tube epithelium exposed to the low testosterone media. Further, at differing testosterone concentrations, the ciliary beating frequency exhibited a dose-response decrease as concentration of testosterone increased. Changes in genes that regulate cilia structure and function were found after 14 days. RNA sequencing showed that amongst other genes, FOXJ1, SAA2, and DNAH5 were down-regulated and KIF5C, MAP2 and NTN4 were up-regulated respectively in the high testosterone group. These genes play major roles in ciliary motility and structure. Genes involved in hormonal signaling, including ZBTB16, were also found to be elevated in the human fallopian tube epithelium exposed to high testosterone on RNA sequencing. Immunohistochemistry staining showed that the androgen receptor was up-regulated and became localized to the nucleus in the high testosterone group, while estrogen receptor expression was reduced. qPCR also showed androgen and estrogen receptors to be up and down regulated in the high testosterone environment, respectively. In addition, qPCR showed down-regulation of OVGPI, an estrogen regulated epithelial glycoprotein important for reproductive function, and up-regulation of ZBTB16, an androgen target gene involved in cell cycle regulation. ELISA showed decreased VEGF in the high testosterone conditions.

**CONCLUSIONS:** These novel findings demonstrate that elevated androgen exposure alters cilia expression and function in the human fallopian tube. These ex-vivo experiments may add to our understanding of the mechanisms of subfertility and reproductive health risks in women with living with hyperandrogenic disorders, such as PCOS, obesity and androgen producing tumors.

**SUPPORT:** The study PI is part of a NIEHS UG3 ES029073

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**MIR-297 REPRESSES THE EXPRESSION OF PROGESTERONE RECEPTOR AND DECIDUALIZATION IN EUTOPIC ENDOMETRIUM IN INFERTILE WOMEN WITH ENDOMETRIOSIS.** Wei Huang, Ph.D, M.D., Tingting Liu, M.D. West China Second University Hospital of Sichuan University, Chengdu, China.



**OBJECTIVE:** Progesterone resistance is one of the epigenetics affecting the decreased endometrial receptivity and implantation failure in endometriosis-associated infertility. Altered miRNAs expression plays an important role in the pathophysiology of endometriosis. Our previous study demonstrated that miR-297 was overexpressed in the mid-secretory eutopic endometrium in the endometriosis group compared with control. We performed our study to explore the regulation of miR-297 on the aberrant progesterone receptor expression and impaired decidualization in the endometrial stromal cells from eutopic endometrium of women with minimal or mild endometriosis.

**DESIGN:** Human tissue study.

**MATERIALS AND METHODS:** We performed our study to explore the regulation of miR-297 on the aberrant progesterone receptor expression and impaired decidualization in the endometrial stromal cells from eutopic endometrium of women with minimal or mild endometriosis. Eutopic endometrial tissues from infertile endometriosis patients ( $n = 20$ ) and normal patients ( $n = 19$ ) were collected in vitro analysis. Endometrial stromal cells were isolated and transfected with miR-297 mimic or miR-297 inhibitor or the respective controls. Gene expression regulation was examined by real-time-quantitative PCR, Western blot and luciferase reporter assay. Artificial decidualization assay was performed to investigate the role of miR-297 during decidualization in vitro.

**RESULTS:** Eutopic endometrial tissues from infertile endometriosis patients ( $n = 20$ ) and normal patients ( $n = 19$ ) were collected in vitro analysis. Endometrial stromal cells were isolated and transfected with miR-297 mimic or miR-297 inhibitor or the respective controls. Gene expression regulation was examined by real-time-quantitative PCR, Western blot and luciferase reporter assay. Artificial decidualization assay was performed to investigate the role of miR-297 during decidualization in vitro. The expression of progesterone receptor especially progesterone receptor B were decreased after transfected with miR-297 mimic and increased by miR-297 inhibition. Moreover, overexpressed miR-297 inhibited the decidualization of endometrial stromal cells in vitro.

**CONCLUSIONS:** Our study demonstrated the regulation of miR-297 on the blunted PR expression is direct.

**SUPPORT:** National Natural Science Foundation of China (No.81370693)

### INHIBITION OF BOTH DNA METHYLTRANSFERASES AND HEDGEHOG SIGNALING SUPPRESSES THE PHENOTYPE OF HUMAN UTERINE LEIOMYOSARCOMA CELLS.

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**OBJECTIVE:** Uterine leiomyosarcoma (LMS) is the most common of uterine sarcoma, it is a rare and aggressive tumor with poor prognosis. Our group described previously that the hedgehog (HH) signaling was activated in LMS, which contributed to its aggressive phenotype. However the mechanism by which HH activation in LMS is largely unknown. The objective of this work was to characterize the genetic and epigenetic mechanism in HH signaling and evaluate the anti-HH effect of DNA methyltransferase inhibitor (DNMTi) alone or in combination with GLI inhibitor (GLIi) on LMS.

**DESIGN:** Laboratory research studies using human uterine smooth muscle (UTSM), LMS cells and LMS patient samples.

**MATERIALS AND METHODS:** LMS cells were used to evaluate the mRNA and protein expression of DNMT1, 3a, 3b, *PTCH1*, *SMO* and *SUFU* mutations were evaluated in 7 LMS patients from 3 different Brazilian institutions (CEP 477/15) using next generation sequencing Ion AmpliSeq (Thermo Fisher Scientific). The percentage of *PTCH1* methylation was determined by EpiTect Methyl II PCR array (Qiagen) in LMS cells. Proliferation, migration, invasion and apoptosis assays were performed to evaluate the inhibitory effect of DNMTi (2  $\mu$ M of 5-aza-2'-deoxycytidine) alone or in combination with GLIi (15  $\mu$ M of Gant61) during 72 hours. The statistical analysis was performed using GraphPad Prism 5. Significance was accepted for  $p < .05$ .

**RESULTS:** No hot spot mutations on *PTCH1*, *SMO* and *SUFU* sequences were detected in LMS patient samples. Upregulation of *DNMT1*, *3a* and *3b* mRNA and protein was observed in LMS compared to UTSM cells. The percentage of *PTCH1* DNA methylation in LMS was 2.3%. Treatment with DNMTi decreased the expression of *DNMT1*, *3a* and *3b* and DNA methylation of *PTCH1* to 1%. Although inhibition of DNMT did not change *PTCH1* gene expression, significant downregulation of *GLI1* was observed in LMS cells ( $p < .05$ ). The DNMTi in combination with GLIi (Gant61) exhibited decreased *SMO* and *GLI1* protein expression ( $p < .05$ ), and suppressed *GLI1* nuclear translocation. Moreover, the combination treatment showed more inhibitory effects on proliferation, migration, invasion and induced apoptosis in LMS cells ( $p < .05$ ).

**CONCLUSIONS:** Our study demonstrates for the first time that although genetic mutations of key HH members are not observed, DNA methylation is tightly linked with LMS phenotype via HH signaling. Notably, a combined treatment of DNMTi and GLIi exhibits a more robust inhibitory effect on LMS phenotype. Further understanding the mechanism of HH pathway in LMS may lead to development of a novel treatment strategy for this aggressive cancer.

**SUPPORT:** Support: FAPESP 2017/24448-1, 2015/23482-6, 2015/21068-8; RO1 ES028615; U54 MD007602

## CLINICAL ART

P-805 Tuesday, October 15, 2019 6:30 AM

### WHAT IS FERTILITY FRAUD, WHAT ARE COURTS AND LEGISLATURES DOING ABOUT IT, AND HOW MIGHT IT IMPACT REPRODUCTIVE MEDICINE?.

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**OBJECTIVE:** To describe the recent fact pattern associated with "fertility fraud" cases, describe new judicial rulings and legislative developments related to fertility fraud, and describe how these cases may impact ART practice.

**DESIGN:** 1) Qualitative, semi-structured interviews with former patients who had been inseminated without consent with the sperm of their fertility physicians, and the donor-conceived children who were conceived through these procedures. Cases include 6 U.S. cases, 1 Canadian case, and 1 European case. 2) Review of existing "fertility fraud" case law and new 2019 state legislation.

**MATERIALS AND METHODS:** 25 individuals were interviewed concerning a) the circumstances under which the illicit inseminations took

place; b) their decisions to seek criminal charges against these physicians and initiate civil suits; and c) their efforts to seek state legislative reforms. Moreover, authors conducted legal analyses of court proceedings, judicial opinions, and state legislation. In these cases, women undergoing IUI (primarily in the 1970s-1990s) were told that they would be inseminated sperm from the husband, anonymous donor, or a mixture of the two, and conceive. Decades later, those children, now adults, undergo direct-to-consumer genetic testing, only to learn that they have many half-siblings, and usually a genetic relationship to the doctor or his relatives. Upon contacting half-siblings, the children learn that the parents of each underwent treatment from the same physician.

**RESULTS:** Former patients feel physically violated and assaulted, and often feel as if seeking accountability implies a rejection of a beloved child. Donor children experience profound disruptions to personal identity, particularly if they did not know they were conceived through donor sperm IUI. Many become estranged from families after they decide to pursue relationships with new half-siblings or seek accountability from physicians. In 2019 legislative sessions, state legislatures in Indiana and Texas have taken significant steps to pass "fertility fraud" legislation. Spurred by the Donald Cline case, Indiana legislation creating a civil and criminal cause of action for fertility fraud was unanimously passed in both the House and Senate and awaits the Governor's signature. Texas legislation that creates a criminal cause of action for fertility fraud under the state's sexual assault law has passed the Senate and a House committee unanimously, and awaits a final vote in the House; the Governor has commented on social media approvingly about this bill.

**CONCLUSIONS:** Although most fertility fraud cases are decades in the past, their contemporary fallout can have important implications for today's practitioners.

**SUPPORT:** None

**References:** Against seminal principles: ethics, hubris, and lessons to learn from illicit inseminations Madeira, Jody et al. Fertility and Sterility, Volume 110, Issue 6, 1003 - 1005

P-806 Tuesday, October 15, 2019 6:30 AM

### INTRAUTERINE INSEMINATION CYCLES: CHARACTERISTICS ASSOCIATED WITH LIVE BIRTH AND THRESHOLDS FOR INEFFECTIVE AND FUTILE CARE.

Alessandra J. Ainsworth, MD,<sup>a</sup> Emily P. Barnard, DO,<sup>b</sup> Sarah Baumgarten, MD, PhD,<sup>a</sup> Camden Lopez, MS,<sup>a</sup> Amy Weaver, MS,<sup>a</sup> Zaraq Khan, MD<sup>a</sup> <sup>a</sup>Mayo Clinic, Rochester, MN; <sup>b</sup>University of Pittsburgh School of Medicine, Pittsburgh, PA.



**OBJECTIVE:** This study aimed to identify intrauterine insemination (IUI) cycle characteristics associated with live birth and to define ineffective and futile care guidelines.

**DESIGN:** This retrospective cohort study evaluated couples pursuing IUI at Mayo Clinic from 1/2005 to 9/2017. Couples using fresh partner ejaculate were included. Female age, ejaculate and inseminate parameters, and ovarian stimulation type were evaluated for association with live birth, defined as birth after 24 weeks gestation. Outcomes were evaluated per cycle, rather than per patient.

**MATERIALS AND METHODS:** Univariate and multivariable logistic regression models were fit to evaluate the association of cycle characteristics with probability of live birth. Models were fit using generalized estimating equation methodology with an exchangeable correlation structure to account for the correlation between cycles involving the same patient. Ineffective and futile care were defined as live birth <5% and 0%, respectively, consistent with ASRM guidelines.

**RESULTS:** A total of 2912 IUI cycles were included for 1117 women. No live births were reported in women 43 years of age or older. Initial analysis identified a threshold of live birth >5% for inseminate motility of 70%. Multivariable analysis restricted to women under 43 years of age, type of ovarian stimulation, age, and inseminate motility were significantly associated with higher odds of live birth. Rates of live birth per combination of aforementioned factors are presented in Table 1. Categories with less than 10 subjects were considered inconclusive, rather than ineffective.

**CONCLUSIONS:** Female age and inseminate motility were found to be primary contributors to live birth rate. Ineffective care was noted with low motility (<70%) in the inseminate even for women aged 35-37. Increasing female age, above 37, compounded by low motility met criteria for futile care. Both pre- and post-treatment components should be reviewed for counseling and appropriately directed care.

Age group (years)	Motility of sperm in inseminate	Type of stimulation	% of each row with a live birth	Ineffective or Futile	
<35	<70%	Injectable	2/33	6.1%	Ineffective
		Oral	10/204	4.9%	
		Natural	0/7	0%	
	≥70%	Injectable	53/372	14.3%	
		Oral	141/1354	10.4%	
		Natural	4/38	10.5%	
35-37	<70%	Injectable	1/9	11.1%	Ineffective
		Oral	1/33	3.0%	
		Natural	0/1	0%	
	≥70%	Injectable	24/95	25.3%	
		Oral	18/308	5.8%	
		Natural	1/9	11.1%	
38-40	<70%	Injectable	2/24	8.3%	Futile
		Oral	0/25	0%	
		Natural	0/1	0%	
	≥70%	Injectable	9/102	8.8%	
		Oral	4/141	2.8%	
		Natural	0/3	0%	
41-42	<70%	Injectable	0/7	0%	Ineffective
		Oral	1/7	14.3%	
		Natural	0/1	0%	
	≥70%	Injectable	3/34	8.8%	
		Oral	1/44	2.3%	
		Natural	0/3	0%	
43+	<70%	Injectable	0/5	0%	Futile
		Oral	0/4	0%	
		Natural	0	-	
	≥70%	Injectable	0/35	0%	
		Oral	0/10	0%	
		Natural	0/3	0%	

**P-807** Tuesday, October 15, 2019 6:30 AM

**OOCYTE DISPOSITION PREFERENCES: PLANNING FOR THE FUTURE.** Anne Hutchinson, M.D., Rafael Confino, BS, John Zhang, PhD, Angela K. Lawson, Ph.D., Mary Ellen Pavone, MD, MSCI Northwestern University, Chicago, IL.



**OBJECTIVE:** To characterize the frozen oocyte disposition preferences of patients undergoing medical and social fertility preservation

**DESIGN:** Descriptive Study

**MATERIALS AND METHODS:** This descriptive study was performed using data collected between 2011 and 2018 in the Division of Reproductive Endocrinology and Infertility at Northwestern University. Demographic and cycle information was collected for each patient. Medical diagnosis was collected for each medical fertility preservation patient. Medical and social fertility preservation (FP) patients were distinguished based on documentation in their initial consult note in the electronic medical record. Disposition options included: disposal, donation to research, or donation to a specified third party which was decided at the time of initial consent and scanned into the patient chart. The demographic parameters were compared between the two groups using chi-squared analysis.

**RESULTS:** 578 oocyte vitrification cycles were identified between 2011 and 2018. 15 cycles were noted to have no documented disposition preference and were excluded from the analysis. 143 cycles corresponded to medical FP patients and 435 to social FP. Medical FP patients were more likely to be under the age of 35, have a higher BMI, and have had a prior live birth. In both groups, the most commonly selected option was donation to research (48.3% social, 48.3% medical), followed by donation to a specified third party (27.4% social, 28.7% medical) and finally disposal of oocytes (22.8% social, 17.5% medical).

**CONCLUSIONS:** Our data shows that oocyte disposition choices are similar in patients undergoing oocyte vitrification for medical and social indications. Both groups most commonly elect donation to research, followed by donation to a specified third party. Disposal of oocytes was the least common disposition choice for both groups. While oocyte vitrification is a relatively new technology, utilization of these frozen gametes is low. Disposition preferences will

become increasingly important as this patient population ages and meets their reproductive goals. Formalized research protocols need to be established to accommodate this anticipated increase in oocytes available for research.

**P-808** Tuesday, October 15, 2019 6:30 AM

**ASSOCIATION OF BMI WITH POST-OPERATIVE MORBIDITY IN PATIENTS UNDERGOING MYOMECTOMY: AN ANALYSIS OF THE AMERICAN COLLEGE OF SURGEONS' NATIONAL SURGICAL QUALITY IMPROVEMENT PROGRAM (ACS NSQIP).**



Lauren M. Kendall Rauchfuss, MD,<sup>a</sup> Tana Kim, MD,<sup>b</sup> MacKenzie P. Purdy, MD,<sup>b</sup> Elizabeth B. Habermann, PhD,<sup>c</sup> Katherine A. Bews, BA,<sup>c</sup> Amy E. Glasgow, MHA,<sup>c</sup> Zaraq Khan, MD<sup>a</sup> <sup>a</sup>Mayo Clinic, Rochester, MN; <sup>b</sup>Division of Reproductive Endocrinology and Infertility, Rochester, MN; <sup>c</sup>Robert D. and Patricia E. Kern Center for the Science of Health Care Delivery Surgical Outcomes Program, Mayo Clinic, Rochester, MD, Rochester, MN.

**OBJECTIVE:** To study the post-operative outcomes among different BMI classes after undergoing myomectomy.

**DESIGN:** A retrospective cohort study, comparing 30 day surgical outcomes after myomectomy among patients with low and normal, overweight, obese (class I and II), and morbidly obese (class III) BMI ranges.

**MATERIALS AND METHODS:** Following IRB approval, the ACS NSQIP was utilized from years 2010-2016. Current Procedural Technology codes were used to identify patients undergoing myomectomy. Pre-operative demographics and 30 day surgical outcomes were obtained. Primary outcomes were any wound complications and serious surgical complications which included wound disruption, sepsis, deep venous thrombosis, pulmonary emboli, acute kidney injury, and hospital readmission. Univariate analyses were performed using Chi-Square, t-test, Mann-Whitney U-test and ANOVA as appropriate. Multivariable logistic regression models were used to identify demographic factors independently associated with primary outcomes.

**RESULTS:** A total of 3,407 women underwent a myomectomy procedure. Univariate analyses comparing low-normal BMI patients to morbidly obese patients are shown. (table1)

Morbidly obese patients were more likely to develop wound complications after adjusting for confounders (adjusted OR 4.1;95%CI; 1.5-11.3). Similarly, morbidly obese patients had a trend towards higher risk of serious systemic surgical complications, (adjusted OR 1.5; 95%CI; 0.6-3.8).

**CONCLUSIONS:** In conclusion, morbid obesity was a significant risk factor for 30 day post-operative complications in patients undergoing myomectomy. Further research is needed to identify interventions to improve post-operative morbidity after myomectomy.

TABLE 1. Comparison of patients across low-normal BMI to Morbid obesity (BMI Class II)

	Low-Normal BMI n= 1042	Morbidly Obese (BMI>40) n =280	p-value
<b>Pre-operative Demographics</b>			
Black race	266 (25.5)	168 (60.0)	<0.001 <sup>a</sup>
Diabetes with pharmacotherapy	12 (1.51)	30 (10.7)	<0.001 <sup>a</sup>
Hypertension with pharmacotherapy	37 (3.6)	81 (28.9)	<0.001 <sup>a</sup>
ASA Class III/IV	32 (3.1)	119 (42.5)	<0.001 <sup>a</sup>
<b>Surgical and post-operative characteristics</b>			
Abdominal Myomectomy	472 (45.3)	160 (57.1)	0.002 <sup>a</sup>
Inpatient Recovery	498 (47.8)	169 (60.4)	0.002 <sup>a</sup>
Total Surgery Time (min)	131.9 ± 76.7	157.3 ± 84.9	<0.001 <sup>b</sup>
Length of Hospital stay	1.0 (0.0-14.0)	2.0 (0.0-31.0)	<0.001 <sup>3</sup>
Unplanned Hysterectomy at time of Myomectomy	30 (2.9)	20 (7.1)	0.0009 <sup>a</sup>

<sup>1</sup> Chi-Square n(%)

<sup>2</sup> t-test mean(Standard deviation), Mann-Whitney U-test Median(Range)

**DETECTION OF THE FERTILE WINDOW USING A WEARABLE MEDICAL DEVICE AND THE CALENDAR METHOD: A COMPARATIVE STUDY.**

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**OBJECTIVE:** While many women rely on the calendar method to detect their fertile window and prevent or aid conception, recent advances in wearable sensor technology and artificial intelligence suggest a wrist-worn medical device could provide women with an accurate, individualized prediction. In this study, we compare the accuracy and precision of these two methods in identifying the six-day fertile window.

**DESIGN:** Retrospective analysis of data from a clinical sample

**MATERIALS AND METHODS:** Thirty-four conception-seeking women enrolled in a trial to test the performance of a wrist-worn medical device in detecting physiological changes across the menstrual cycle. Participants wore the Ava Fertility Tracker nightly while sleeping for up to a year. Via three sensors, the Ava Fertility Tracker measures seven different biophysical parameters every 10 seconds including skin temperature and heart rate. Participants synced their bracelet each morning with the complementary smartphone app, which relies on a machine learning algorithm to predict and detect the real-time fertile window. Participants also completed a daily diary entry about their activity in the last 24 hours and recorded whether they had received a positive urinary luteinizing hormone (LH) test each morning. Women had to be older than 18 years old, not be currently taking hormonal birth control, and have regular cycles (defined as 24-35 days in length) in order to be included in our analyses. For each subject and each cycle, we retrospectively calculated the fertile window as would have been predicted by three different calendar methods: the Standard Days method, the Rhythm Method, and the Alternative Rhythm Method. Using the LH test result as an objective measure of ovulation, we compared the accuracy and precision of each calendar method to the algorithm-identified fertile window. We defined precision as the fraction of days which the method reported as fertile that aligned with the LH-detected fertile window and accuracy as the percentage of correctly classified fertile or infertile days overall.

**RESULTS:** The accuracy in identifying the fertile days for the wearable device was 88.1% (Standard deviation [SD]=9.1%) compared to 76.8% (SD=5.1%) for the Standard Days method, 69.2% (SD=15.6%) for the Rhythm Method, and 67.6% (SD=16.1%) for the Alternative Rhythm Method. Furthermore, the wearable fertility tracker had the highest precision of any of the methods analyzed (70.3%, SD=21.9% v. 42.7%-47.7% for the calendar methods [SDs=7.6%-13.0%]).

**CONCLUSIONS:** Despite the ease of use and straightforward calculations driving the calendar method, using a wrist-worn medical device that records multiple physiological parameters simultaneously provides a more accurate and more precise estimation of the fertile window. Our findings have implications for women across the reproductive lifespan; whether women are trying to best time conceptive sex or minimize the number of days requiring back-up contraception, wearable technology represents a significant step forward in individualized, AI-driven healthcare.

**SUPPORT:** Funding for this study was provided by a grant from the Swiss Commission for Technology and Innovation (CTI) and Ava AG.

**CRYO TANK ISSUES**

P-810 Tuesday, October 15, 2019 6:30 AM

**CRYO TANK QUALITY CONTROL: HOW TO DETECT A TANK THAT IS 'FAILING'.**

James R. Graham, MS ELD,<sup>a</sup> Cristina L. Applegate, MS,<sup>b</sup> Seamus R. Graham, HSDG,<sup>c</sup> Michael J. Tucker, PhD<sup>b</sup> <sup>a</sup>SG Fertility, Rockville, MD; <sup>b</sup>Shady Grove Fertility, Rockville, MD; <sup>c</sup>Student, North Potomac, MD.

**OBJECTIVE:** Cryo tank failure at several US and Canadian IVF clinics has been reported in the news media recently. Hundreds of patient specimens were lost. Current Quality Control (QC) measures are typically to assess liquid nitrogen (LN<sub>2</sub>) consumption and re-fill on a strict schedule. We wished to see what happens when a properly QC'd tank fails (defined as vacuum loss) to judge whether the 'measure & fill' approach was adequate.

**DESIGN:** Observational

**MATERIALS AND METHODS:** 12 retired various model cryogenic dewars (35L+Taylor-Wharton and 47L MVE) were filled with LN<sub>2</sub> to the neck of the tank per QC protocol. Each tank was fitted with temperature sensors at mid-level and the bottom of the canister. Furthermore a temperature probe was placed in the lid of the tank as well as the shoulder of the tank (near handle). Imm holes were drilled either into the shoulder of the tank (external breach), or into the neck of the tank (internal breach). Temperatures were then taken from the time of the breach, and every 15 minutes thereafter until the tank was considered 'failed' (internal mid tank temperature rose above -150C.)

**RESULTS:** Boiling of the LN<sub>2</sub> was evident within 60 seconds of the breach regardless of tank model.

LN<sub>2</sub> vapor was visible within 60 seconds of breach of each tank regardless of tank model.

Upon vacuum breach of each tank the sound of vacuum loss was audible for up to 4 hours with external breaches.

Temperature of lid due to escaping cold LN<sub>2</sub> gas went below 5C in the first 15 minutes of the breach regardless of model, and stayed below this level until the tank was considered failed (above -150C)

Ice "crowning" of the lid was only obvious with external breaches, but not so with internal breaches.

Time for tank to fail was dependent on tank model and type of breach.

Temperature of the external probe on the tank showed a drop in surface temperature, but this was dependent on type of breach and varied in time from when breach had occurred.

Time to tank failure depended on tank model and breach type, but for a minimum of 12hrs internal temperature were maintained above -150C for all tanks tested.

Lid temperatures below 5C were consistently observed until a tank's internal temperature went above -150C regardless of model or breach type.

**CONCLUSIONS:** Tanks that experience vacuum loss will almost immediately experience LN<sub>2</sub> boiling and emit a steady stream of cold LN<sub>2</sub> gas. Monitoring lid temperature twice daily could identify tanks that have lost vacuum. Using an infra-red gun to take the lid temperature of a cryo tank twice daily (a.m. & p.m.) would be a quick and convenient way to identify tanks that have lost vacuum, and that are compromised. Tank lid temperatures that deviate more than 20% from the mean of other tanks in the storage area should be evaluated for failure. Additionally tanks can be fitted with remote alarmed lid temperature sensors to detect temperature drops due to LN<sub>2</sub> boiling that would occur when a tank loses vacuum.

Weekly and even daily 'measure & fill' QC protocols appear inadequate without cryo-tank lid temperature monitoring.

P-811 Tuesday, October 15, 2019 6:30 AM

**THE ANATOMY OF LIQUID NITROGEN (LN<sub>2</sub>) CRYO DEWAR TANK FAILURES.**

Mitchel C. Schiewe, MS, PhD,<sup>a</sup> Shane Zozula, B.S., T.S. (ABB),<sup>a</sup> Erica J. Behnke, PhD,<sup>b</sup> Jason Cowles, BA,<sup>c</sup> Rob Manchise, BS,<sup>c</sup> John B. Whitney, BS<sup>a</sup> <sup>a</sup>Ovation Fertility, Newport Beach, CA; <sup>b</sup>Ovation Fertility, Cincinnati, OH; <sup>c</sup>Trust Gnosis, Brea, CA.

**OBJECTIVE:** The key factor in averting the catastrophic loss of precious gametes and embryos rests in the comprehensive implementation of quality management practices and the early detection of an unexpected failure event. The goal of our investigation was to simultaneously evaluate, interrupt and understand weight and temperature changes of induced dewar tank failures under continuous video surveillance over a 24h interval.

**DESIGN:** A prospective, observational study assessed a variety of induced tank failure events monitored by real-time video, weight determination and temperature measurements following an external or internal breach of their insulating vacuum. Our aim was to characterize the nature of different tank failures and determine what alarm indicators may best provide an early warning of potentially catastrophic outcomes.

**MATERIALS AND METHODS:** Using a novel Wi-Fi based, pressure sensitive weight cart devices (TrustGnosis; Brea, CA) and a hard wired temperature-based continuous monitoring alarm system (Xiltrix, Netherlands), we prospectively correlated 'failure' characteristics of several aged (>18 years old; n=6) 35-36L Taylor-Wharton dewar LN<sub>2</sub> storage tanks and one 'recalled' new Biocane 73L TS/Chart tank. We initially drilled (1/16") the vacuum port of the 73L dewar and two smaller tanks (35HC, 36VHC). In phase 2, we increased the external drill (ED) opening to 1/8" and 3/16" on two 35HC tanks, while two others (35HC) where drilled (1/4") through their inner base seam (ID) into the vacuum space. An ANOVA regression model was used to correlate the relationship between weight and LN<sub>2</sub> levels.

**RESULTS:** The intentional destruction of all external drilled dewar tanks created an aspiration noise as room air initially warmed the interstitial space outside the inner tank. Conversely, internal drilled tanks displayed overt bubbling of its inner liquid chamber and immediate LN vaporization (within 10 sec). LN vapor streaming outside the cap and neck of the external drilled tanks was also evident within 30 sec. An external thermocouple registered 50C within 3 min. Ice was seen on the cap surface by 3 min, while gradual frost build-up occurred over several hours. Icing and condensation on the tank walls was apparent early on and throughout failure. A 20% evaporation detected by weight took about 4h, while the first internal temperature alarm at -194oC did not occur until 5.5-6.5h. The ID tanks reached -170oC sooner (+14-15h) with 65-75% evaporation compared to >95% evaporation for the ED group (+18-19h), prior to the rapid rise of temperature for both treatments (subzero by +24-25h). The ED-treated 73L tank responded similar to the 35HC tanks, but at twice the evaporation rate. The external drill size did not affect evaporation rates.

**CONCLUSIONS:** Tank quality and type of vacuum breach can influence the rate of failure. In all cases overt physical signs of pending failure were continuously visible for >14h before critical temperatures were reached. Overall, external quality measurements and device systems represent a promising future offering greater precision, labor efficiency, and improved specimen security/safety.

**SUPPORT:** None

**P-812** Tuesday, October 15, 2019 6:30 AM

### USEFULNESS OF REMOTE, CONTINUOUS WEIGHT DETERMINATION FOR THEA ROUTINE QUALITY MANAGEMENT OF CRYO DEWAR TANKS.

Mitchel C. Schiewe, MS, PhD,<sup>a</sup> Shane Zozula, B.S., T.S. (ABB),<sup>a</sup> Tannia Ochoa, BA,<sup>a</sup> Jason Cowles, BA,<sup>a</sup> Rob Manchise, BS,<sup>b</sup> John B. Whitney, BS<sup>a</sup> <sup>a</sup>Ovation Fertility, Newport Beach, CA, <sup>b</sup>Trust Gnosis, Brea, CA.



**OBJECTIVE:** The quality management of small volume (30-73L) LN<sub>2</sub> dewar cryostorage tanks have historically been maintained by routine (i.e., at least weekly) internal dipstick measurements and re-filling. Meanwhile, alarm systems, if used, have been based on a designated internal temperature threshold (<-180°C) or LN<sub>2</sub> level set point (e.g., upper canister level). The goals of our investigation were to evaluate the prospective value of real-time pressure sensitive, weight measurements of mobile dewar tanks for operational qualification (OQ) and performance qualification (PQ).

**DESIGN:** Real-time weight measurements were correlated to changes in LN<sub>2</sub> volume and temperature under new tank validation (i.e., OQ) and standard tank use (i.e., PQ). Evaporation usage rates were calculated at time of fill up (t<sub>0</sub>) minus measurement prior to next fill (t<sub>1wk</sub>; usage rate=t<sub>0</sub>-t<sub>1wk</sub>), based on weight (E<sub>w</sub>) or volume level (E<sub>L</sub>). An evaporation rate index (E<sub>vap</sub>) was calculated for in-use tanks (T) using new tanks as the control (C) group (E<sub>vap</sub> =T0-T1/C0-C1). Differences in E<sub>vap</sub> were compared among tanks to determine if an objective measure for cryotank retirement was possible?

**MATERIALS AND METHODS:** Using a novel Wi-Fi based weight cart device system (TrustGnosis; Brea, CA) several new (n=6) and aged (>18 years old; n=5) 35-36L Taylor-Wharton/Worthington dewar LN<sub>2</sub> storage tanks were routinely monitored for 3-6 weeks to correlate pre- and post-fill dipstick measurements to weight changes. All tanks were hardwired into our Xilatrix (Netherlands) biphasic temperature/canister level continuous monitoring alarm system. All tanks were filled weekly, and weekly usage rates determined by weight recordings and dipstick measures prior to filling. Manual and remote online data were correlated by ANOVA and the E<sub>vap</sub> indexing calculated to assess OQ/PQ determinations.

**RESULTS:** The mean LN<sub>2</sub> usage rate (E<sub>w/L</sub>) of new VHC-35 tanks was 3.1 lbs/3 cm, yielding a direct correlation of 1.0. In contrast, aged tanks varied in their E<sub>w</sub> (6.4 to 9.8 lbs) and E<sub>L</sub> (4-10cm). The evaporation rate of aged tanks (19 to 27% E<sub>w</sub>/week) was greater (p<0.05) than Group 1 new tank controls (8.5 to 10% E<sub>w</sub>/week). The E<sub>vap</sub> index of the aged tanks by levels ranged from 2.0 to 3.0. Greater (p<0.05) precision was verified using weight measurements (E<sub>vap</sub> index=2.4-2.9).

**CONCLUSIONS:** Remote monitoring of LN<sub>2</sub> dewar tank weights can be an effective and more precise method to measure daily and weekly usage/evaporation rates. Manual dipstick measures are subject to user error and complacency in QC practices, whereas remote weight measurements are not. Additionally, a weight-based E<sub>w</sub> threshold alarm may represent an improved early warning alarm system for the potential detection of a failure scenario. Overall, external quality measurements and device systems represent a promising future offering greater precision, labor efficiency, and improved specimen security and safety. Furthermore, our data shows that new tank validations (OQ) and weekly performance (PQ) can be objectively

evaluated by weight and used to formulate a useful threshold measure assessing dewar tank retirement.

**SUPPORT:** None

### ART LAB - EMBRYO CULTURE

**P-403** Wednesday, October 16, 2019 6:30 AM

### INFLUENCE OF COMMERCIAL EMBRYO CULTURE MEDIA ON IN VITRO DEVELOPMENT, PREGNANCY, AND PERINATAL OUTCOMES AFTER IVF: A SINGLE-CENTER RCT.

Masao Murakami, PhD,<sup>a</sup> Keiko Tanaka, MS, Hitomi Otsubo, BS, Shigetoshi Mizumoto, Ph.D., Yojo Nagao, MS, Takeshi Kuramoto, M.D., Ph.D., Kuramoto Women's Clinic, Fukuoka, Japan.



**OBJECTIVE:** Numerous embryo culture media can now be used for IVF, raising the question whether any medium is superior to others. Their ability to yield embryos *in vitro* does not necessarily mean that the embryos are viable. Previously, in animals, serum was commonly added to culture media to yield blastocyst stage (BS) embryos, but this impaired embryonic, fetal, and offspring health. In humans, the medium composition and culture duration as well as cryopreserved ET reportedly affect treatment efficacy and the offspring phenotype. Given the importance of media in clinical outcomes, well-designed RCTs are needed, but existing relevant data are insufficient. Here, we provide updated data on an RCT conducted to compare clinical outcome between three embryo culture media widely used in IVF.

**DESIGN:** A single-center RCT.

**MATERIALS AND METHODS:** This study included 795 healthy patients undergoing their first IVF cycle at our clinic from February 2016 to August 2017. They were randomized by computer-generated tables into three groups and underwent our standard oocyte retrieval and IVF/ICSI procedures. Embryos were cultured in G1/G2 Plus (Vitrolife) (A), Global Total (LifeGlobal) (B), or Sequential Cleav/Blast (Origio) (C) media. Thirty-seven patients with no 2PN oocytes 18 h after insemination were excluded from the study. During embryo culture, for cycles where the patients had only one good-quality (GQ) embryo by D3, the embryos were vitrified on D2/3 (cleavage stage, CS). When the patients had ≥ 2 GQ embryos by D3, ≤ 2 GQ embryos were vitrified on D2/3, the culture of the remaining embryos was extended, and all GQ BS embryos were vitrified on D5/6. Data for vitrified ET performed until the end of March 2019 were analyzed.

**RESULTS:** Total numbers of vitrified CS (A: 1.35 ± 0.05, B: 1.38 ± 0.04, C: 1.35 ± 0.04) and BS (A: 1.71 ± 0.14, B: 2.08 ± 0.17, C: 2.05 ± 0.15) embryos/cycle did not differ, but the number of vitrified D5 BS embryos was fewer (P < 0.005) in Group A (0.84 ± 0.09) than in Groups B (1.55 ± 0.15) and C (1.30 ± 0.12). After vitrified CS/BS ET, the clinical pregnancy rate (CPR) was lower, albeit non-significantly (P = 0.062), in Group C than in Group B (implantation rates (A: 41.2%, B: 43.2%, C: 37.2%), CPRs (A: 49.8%, B: 53.5%, C: 45.0%), ongoing/delivered PRs (ODPRs) (A: 41.0%, B: 43.0%, C: 36.7%)). There were 314 live-born children (286 singletons and 28 twin children). Perinatal data for singletons were similar, except for the birthweight adjusted for gestational age and gender (z-score) (A (CS: -0.17 ± 0.20, n = 25; BS: 0.21 ± 0.13, n = 71), B (CS: 0.20 ± 0.22, n = 18; BS: 0.26 ± 0.10, n = 88), C (CS: 0.40 ± 0.22, n = 13; BS: 0.47 ± 0.12, n = 71) (A (CS) vs. C (BS): P < 0.01, A (CS) vs. B (BS): P = 0.061).

**CONCLUSIONS:** A culture system yielding fewer BS embryos tended to have lower birthweight z-score, while the ODPR was comparable to or slightly better than those of other systems. Differentiation of the ability of media to support *in vitro* development with its ability to yield viable embryos may partly be important for better outcome. Further studies with more participants, including follow-up on the health of children born from embryo culture, are required to clarify the effects.

**P-404** Wednesday, October 16, 2019 6:30 AM

### EXPLORING NEW COMPLEX PROTEIN SUPPLEMENT SOLUTIONS FOR CLINICAL EMBRYO CULTURE MEDIA.

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**OBJECTIVE:** The objective of this research was to investigate the effectiveness of a novel protein supplement (GroPro; consisting of antioxidants,

growth factors, and fatty acids), to support preimplantation embryo development *in vitro*. GroPro was designed to act as a reliable complex protein supplement alternative for clinical ART, in order to better support embryo development and quality compared to human serum albumin (HSA) alone.

**DESIGN:** Basic Research Study. Media supplementation with GroPro, our standard recombinant HSA (AlbIX, Novozymes), or Vitrolife HSA were compared for the ability to support mouse blastocyst development, cell differentiation (inner cell mass (ICM) and trophoctoderm (TE)), mitochondrial DNA (mtDNA) copy number, as well as blastocyst implantation potential and subsequent fetal development.

**MATERIALS AND METHODS:** *In vivo* matured oocytes were obtained from outbred CF1 mice and subsequently fertilized *in vitro*. Zygotes were randomly assigned to sequential embryo culture medium containing AlbIX (2.5mg/ml), Vitrolife HSA (5.0 mg/ml), or GroPro (5.0 mg/ml) (4 replicates, n=525). After 112h of culture, development was assessed and blastocysts were either fixed and immunostained to visualize ICM and TE cells (n=153), or flash frozen individually to determine mtDNA copy number using qPCR relative to genomic DNA (n=30). D3.5 blastocysts cultured in Vitrolife HSA and GroPro were surgically transferred into recipients (n=258). Statistical analysis was performed using one-way ANOVA and Pearson's Chi-Square (ET results); significance was determined at p<0.05.

**RESULTS:** There were no differences in blastocyst development (AlbIX 52.0% ± 6.5%, Vitrolife HSA 57.3% ± 5.3%, GroPro 69.0% ± 1.6%) or hatching (AlbIX 47.4% ± 5.4%, Vitrolife HSA 50.0% ± 3.9%, GroPro 58.1% ± 1.6%) between treatments. Embryos cultured in Vitrolife HSA contained significantly (p<0.01) more TE cells and total cells, while embryos cultured in AlbIX contained significantly (p<0.05) less ICM cells compared to every other treatment; the ratio of ICM to TE was not different between treatments. There was significantly lower relative mtDNA copy number in embryos cultured in AlbIX compared to embryos cultured in GroPro (p<0.05). No difference in implantation potential (Vitrolife HSA 51%, GroPro 57%) or fetal development (Vitrolife HSA 28%, GroPro 22%) was observed.

**CONCLUSIONS:** These findings demonstrate that in the mouse, additional antioxidants, growth factors and fatty acids do not provide any additional benefit over HSA alone, although they are not detrimental to embryo development or quality. This novel protein supplement may be a viable alternative for the culture of human embryos, in which complex protein supplements such as SPS and SSS support better blastocyst development than HSA alone.

**SUPPORT:** None.

**P-405** Wednesday, October 16, 2019 6:30 AM

**COMPARISON OF HUMIDIFIED VERSUS NON-HUMIDIFIED INCUBATION WITH SEQUENTIAL CULTURE MEDIA IN A TIME-LAPSE INCUBATOR USING SIBLING OOCYTE SPLITS.**

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**OBJECTIVE:** Many modern embryo culture incubators are non-humidified. Initial studies indicate that evaporation of culture media may occur in non-humidified culture environments, even under mineral oil. This evaporation may negatively impact embryo development and quality. Controlling for other variables in the culture system while trying to study the impact of humidity can be difficult. The objective of this study was to compare outcomes following sibling zygote splits in identical culture conditions, within the same incubator, utilizing a time-lapse system that permits both dry and humidified culture.

**DESIGN:** Prospective randomized trial.

Oxygen level	Time points (hrs)												
	t2	t3	t4	t5	t6	t7	t8	tSC	tM	tSB	tB	tEB	tHB
6% O <sub>2</sub> (n=106)	0.0 ± 0.0	19.7 ± 2.4	21.0 ± 2.7	29.5 ± 1.9	29.9 ± 2.0	30.9 ± 2.5	30.9 ± 2.1	35.2 ± 2.9 <sup>a</sup>	41.5 ± 3.7 <sup>b</sup>	56.7 ± 3.8 <sup>c</sup>	63.3 ± 5.9 <sup>d</sup>	69.7 ± 7.6 <sup>e</sup>	79.7 ± 8.6 <sup>f</sup>
2% O <sub>2</sub> (n=108)	0.0 ± 0.0	20.0 ± 1.8	21.0 ± 1.7	29.4 ± 1.6	29.8 ± 2.0	30.9 ± 2.4	31.6 ± 2.9	36.1 ± 3.1 <sup>a</sup>	42.9 ± 3.3 <sup>b</sup>	59.3 ± 5.9 <sup>c</sup>	68.6 ± 7.7 <sup>d</sup>	77.6 ± 9.8 <sup>e</sup>	86.0 ± 8.6 <sup>f</sup>

<sup>a,b,c,d,e,f</sup> P<0.05. Time points are expressed as Mean ± SD.

**MATERIALS AND METHODS:** A time-lapse incubator with individual chambers (Geri, Serono) was utilized to culture all embryos. Three chambers were humidified by placing the supplied water chambers inside (~60% humidity) and the 3 other chambers were non-humidified. Room humidity was ~35%. All chambers were gassed using the same gas supply using 6.5% CO<sub>2</sub> and 5% O<sub>2</sub>. pH was verified to be similar in all chambers. All embryos were grown in the supplied Geri dishes with the wells filled with 80µl Sage sequential media with 10% v/v SPS under 4 mL Paraffin oil (Life Global). Sixteen patients with >6 MIIs at ICSI had half of their ICSI'd oocytes placed into a humidified and non-humidified chamber. All embryos were treated identically except for the presence or absence of humidity in the respective chambers. Embryos were observed and media exchanged/refreshed following 24h, 72h and 120h. Data were analyzed using Fisher's Exact Test.

**RESULTS:** Use of a humidified chamber yielding significantly more good quality blastocysts on day 5, 6 and overall by day 7 than non-humidified chambers.

	Humidified	Non-Humidified
Oocyte #	105	110
% Fertilization	79.0%	81.8%
% Good Cleavage Rate	79.5%	76.7%
% Total Blasts on D5	48.2%	43.3%
% Blasts >= 3BB D5	24.1% <sup>a</sup>	14.4% <sup>b</sup>
% Total Blasts D6	62.7%	54.4%
% Blasts >= 3BB D6	49.4% <sup>a</sup>	25.6% <sup>b</sup>
% Total Blasts D7	62.7%	60.0%
% Blasts >= 3BB D7	50.6% <sup>a</sup>	31.1% <sup>b</sup>

**CONCLUSIONS:** Humidified culture in the Geri time-lapse system yielded more good quality blastocysts on day 5, day 6 and overall on day 7 and than non-humidified culture. No differences in fertilization, good quality cleavage or total blastocyst development was apparent. Under the culture conditions used, evaporation may have occurred to compromise blastocyst quality, though this seems unlikely with media exchanges at 48h intervals based on prior studies within our laboratory. Results may vary with fewer media changes, in laboratories using single step media in an uninterrupted fashion, or if using differing amounts or types of mineral oil overlay.

**P-406** Wednesday, October 16, 2019 6:30 AM

**EFFECT OF ULTRA-LOW OXYGEN (2%) ENVIRONMENT ON MOUSE EMBRYO MORPHOKINETICS AND BLASTOCYST DEVELOPMENT.**

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**OBJECTIVE:** To determine if culture of mouse embryos in an ultra-low oxygen environment could enhance blastocyst development in terms of time-lapse morphokinetics.

**DESIGN:** Prospective study.

**MATERIALS AND METHODS:** A total of 214 commercially obtained frozen mouse embryos (B6D2F1 & B6C3F1 hybrid) were thawed and cultured in One-Step medium with 10% Serum Protein Substitute using an EmbryoScope time-lapse incubator at 37°C, 5.5% CO<sub>2</sub>, and either (1) 6.0% O<sub>2</sub> (n=106) or (2) 2.0% O<sub>2</sub> (n=108). Embryo images were recorded every 10 minutes for 6 days of culture. Time-lapse videos were annotated for the following time points: 2cell (t2), 3cell (t3), 4cell (t4), 5cell (t5), 6cell (t6), 7cell (t7), 8cell (t8), start of compaction (tSC), morula (tsM), start of blastulation (tSB), blastocyst (tB), expanded blastocyst (tEB) and hatching

Media	Time points (hrs)												
	t2	t3	t4	t5	t6	t7	t8	tSC	tM	tSB	tB	tEB	tHB
One-Step (n=105)	0.0 ±0.0	19.7 ±2.4	21.0 ±2.7	29.5 ±1.9	29.9 ±2.0	30.9 ±2.5	30.9 ±2.1	35.2 ±2.9	41.5 ±3.7	56.7 ±3.8	63.3 ±5.9	69.7 ±7.6	79.7 ±8.6
Insulin (n=99)	0.0 ±0.0	20.0 ±1.7	21.0 ±1.9	29.6 ±1.9	30.1 ±1.8	30.8 ±1.8	31.0 ±2.2	35.2 ±2.3	42.0 ±3.0	57.6 ±3.5	62.9 ±4.3	69.3 ±6.4	80.4 ±8.7
IGF-1 (n=101)	0.0 ±0.0	20.0 ±1.4	20.9 ±2.1	29.7 ±2.0	30.0 ±2.1	30.9 ±2.3	31.0 ±2.3	35.2 ±2.7	41.9 ±3.1	56.7 ±3.7	62.3 ±4.6	68.8 ±6.6	79.6 ±7.5

Time points are expressed as Mean ± SD.

blastocyst (tHB). The 2-cell stage was considered as time zero since the exact time of insemination was unknown. Time points were statistically compared between the two groups. Blastocyst development rates for both culture environments were also compared.

**RESULTS:** There were no statistically significant differences between the two groups in any of the time points measured up to the 8-cell stage. However, after the 8-cell stage, the 2% O<sub>2</sub> group showed significantly slower embryo development for each time point up to the hatching blastocyst stage. There was no difference in the blastocyst development rate between the 6% O<sub>2</sub> and 2% O<sub>2</sub> environments (99.1% vs 95.4%, P=0.099).

**CONCLUSIONS:** Culture of mouse embryos in a 2% oxygen environment did not show any improvement in blastocyst development. However, embryos cultured in 2% oxygen took significantly longer to reach the blastocyst stage than those cultured at 6% oxygen, and this delay became prominent after the 8-cell stage.

**P-407** Wednesday, October 16, 2019 6:30 AM

#### DOES SUPPLEMENTATION OF MEDIA WITH INSULIN OR INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) ENHANCE MORPHOKINETICS OF MOUSE EMBRYO DEVELOPMENT?



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**OBJECTIVE:** To evaluate if adding either insulin or insulin-like growth factor 1 (IGF-1) to culture medium improves mouse embryo development and time-lapse morphokinetics.

**DESIGN:** Prospective study.

**MATERIALS AND METHODS:** A total of 305 commercially obtained frozen mouse embryos (B6D2F1 & B6C3F1 hybrid) were thawed and cultured in (1) One-Step medium only, (2) One-Step medium with 100ng/mL insulin, and (3) One-Step medium with 100ng/mL IGF-1, using an EmbryoScope time-lapse incubator at 37°C, 5.5% CO<sub>2</sub>, and 6.0% O<sub>2</sub>. The EmbryoScope was set to record images of each embryo every 10 minutes for 6 days of culture. The following time points were annotated: 2cell (t2), 3cell (t3), 4cell (t4), 5cell (t5), 6cell (t6), 7cell (t7), 8cell (t8), start of compaction (tSC), morula (tM), start of blastulation (tSB), blastocyst (tB), expanded blastocyst (tEB) and hatching blastocyst (tHB). The 2-cell stage was considered as time zero since the exact time of insemination was unknown. All time points were statistically compared between each of the three groups with a P-value of <0.05 considered to be significant. Blastocyst development rates for each group were also compared.

**RESULTS:** A total of 304 blastocysts developed from the 316 embryos cultured, yielding an overall blastocyst development rate of 96.2%. When comparing the blastocyst development rate between the 3 groups, there were no significant differences between the One-Step and IGF-1 media groups (99.1% vs 96.2%). However, the insulin group showed significantly lower blastocyst rates when compared to the controls (93.3% vs

99.1%; p=0.02). There were no statistically significant differences in any of the time points measured between the One-Step, insulin and IGF-1 groups.

**CONCLUSIONS:** No beneficial effects were noted by adding insulin or IGF-1 to culture media for mouse embryo development. Mouse embryos cultured with insulin showed a lower blastocyst development rate compared to unsupplemented One-Step media. No differences were seen in any of the time-lapse morphokinetics parameters by supplementing media with either insulin or IGF-1. Ongoing studies using a different more sensitive strain of mouse embryos are underway to see if any subtle changes in morphokinetics may be detected.

**P-408** Wednesday, October 16, 2019 6:30 AM

#### INCREASING THE EFFICACY OF MOUSE EMBRYO ASSAYS FOR QUALITY CONTROL IN THE IVF LABORATORY.



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**OBJECTIVE:** Since the strain of mouse embryos commonly used for quality control in IVF laboratories almost always have >90% blastocyst development, possibly due to improved culture conditions, it is very difficult to detect negative effects of media or contact materials that would otherwise affect human embryo development. The aim of this study was to evaluate a different strain of mouse embryos that would be more sensitive to culture changes and therefore be a better marker for quality control as well as for research studies.

**DESIGN:** Prospective study.

**MATERIALS AND METHODS:** One-cell embryos from B6D2F1 & B6C3F1 Hybrid mice (Strain 1) and from C57BL-6N mice (Strain 2) were used for this study. Embryos were thawed and cultured in different types of media: (1) One-Step medium, (2) One-Step medium with IGF-1, and (3) One Step medium with insulin. All embryos were cultured in an EmbryoScope incubator at 37°C, 5.5% CO<sub>2</sub> and 6% O<sub>2</sub> for 6 days. The blastocyst formation rate was calculated as the number of blastocysts per embryos cultured for each group and compared statistically using a Chi-squared test.

**RESULTS:** The data showed that Strain 2 embryos had significantly lower blastocyst development rates than Strain 1 embryos in One-Step medium routinely used in IVF culture (Table 1). Furthermore, when testing the effect of adding IGF-1 or insulin to the media, Strain 2 embryos showed more pronounced changes in blastocyst development rates compared to Strain 1. The insulin-supplemented media resulted in significantly lower blastocyst development rate for both strains.

**CONCLUSIONS:** The C57BL-6N strain of mouse embryos is more sensitive than the B6D2F1 & B6C3F1 Hybrid and appears to be a better model for detecting subtle changes in culture conditions. Ongoing studies are underway to determine if there are any changes in time-lapse morphokinetics between these two strains.

TABLE 1. Blastocyst development rates between two strains on mouse embryos cultured in One-step media, IGF-1 and insulin

Culture media	Strain 1 (B6D2F1 & B6C3F1 Hybrid)	Strain 2 (C57BL-6N)	P-value
One-Step (n=238)	99.1% (n=106) <sup>a</sup>	80.3% (n=132) <sup>b</sup>	P<0.0001
IGF-1 (n=249)	96.2% (n=105)	74.3% (n=144)	P<0.0001
Insulin (n=249)	93.3% (n=105) <sup>a</sup>	68.8% (n=144) <sup>b</sup>	P<0.0001
P-value	<sup>a</sup> P=0.02	<sup>b</sup> P=0.02	

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**DEEP CONVOLUTIONAL NEURAL NETWORKS (CNN) FOR ASSESSMENT AND SELECTION OF NORMALLY FERTILIZED HUMAN**

**EMBRYOS.** Irene Dimitriadis, MD,<sup>a</sup> Charles L. Bormann, PhD,<sup>a</sup> Manoj Kumar Kanakasabapathy, MS,<sup>b</sup> Prudhvi Thirumalaraju, BS,<sup>b</sup> Raghav Gupta, BTech,<sup>b</sup> Rohan Pooniwala, BTech,<sup>a</sup> Irene Souter, MD,<sup>c</sup> Sarah T. Rice, MS,<sup>c</sup> Pragati Bhowmick, MD,<sup>c</sup> Hadi Shafiee, PhD<sup>b</sup>  
<sup>a</sup>Massachusetts General Hospital, Harvard Medical School, Boston, MA; <sup>b</sup>Brigham and Women's Hospital, Harvard Medical School, Boston, MA; <sup>c</sup>MGH Fertility Center and Harvard Medical School, Boston, MA.

**OBJECTIVE:** To evaluate whether an artificial intelligence (AI) framework can be used to classify between normally (2PN) and abnormally (non-2PN) fertilized embryos at the pronuclear (PN) stage.

**DESIGN:** Historical Prospective Cohort Study.

**MATERIALS AND METHODS:** Embryo images from a retrospective dataset recorded by multiple optical systems at 18 hours post-insemination (hpi) were utilized. The deep convolutional neural network (CNN) model was trained and tested, with a total of 3,469 embryos, to classify between 2PN (n=2,893) and non-2PN (n=576) embryos.

The training set contained 2,366 images (6.33 2PN:1 non-2PN) while the validation set contained 154 images (0.97 2PN:1 non-2PN) with a distribution aimed at minimizing training bias. During training, the dataset was augmented through randomized rotations of the images ranging from 0 to 359 degrees, which was done using OpenCV libraries (ver. 3.1.0). In each training batch, we used 16 unique images per class supplemented by augmented data for the training class. When all the images of a specific embryo class were used for CNN training, the same embryo class was shuffled randomly to create different batches, making every batch unskewed by repeating and augmenting the embryo images.

For the independent test set, we used 949 non-overlapping images (4.42 2PN:1 non-2PN), which were obtained from 100 patient cohorts.

**RESULTS:** Using annotated data of 2,366 inseminated oocytes, the CNN was trained and validated to categorize oocytes based on their fertilization outcomes. The ability of the CNN in classifying zygotes based on their fertilization status at 18 hpi was evaluated using a test set of 949 pronuclear stage embryos from which two completely out-of-focus images were removed. The accuracy of the algorithm in 2PN and non-2PN embryo classification using the 947 embryos test set was 91.86% (CI: 89.94% to 93.53%). A t-Distributed Stochastic Neighbor Embedding (t-SNE) was performed to visualize the separation of dataset by the network in a 2D space. With the observation of good separation between the two classes, the network was further probed by mapping the final activation layers to visualize the saliency for the identification of the pixels that are being utilized by the network. It was confirmed that the network focused on features pertaining to the embryo in its decision-making process.

For the given test set, the sensitivity and specificity of the algorithm in identifying 2PN embryos were 93.26% (CI: 91.21% to 94.96%) and 86.83% (CI: 81.42% to 91.14%), respectively. The positive predictive value and negative predictive value of the CNN were 96.24% (CI: 94.74% to 97.33%) and 78.07% (CI: 73.04% to 82.39%), respectively. The area under the curve (AUC) value, established through a receiver operating characteristic (ROC) analysis, was 0.90 (CI: 0.88 to 0.92).

**CONCLUSIONS:** Here, we report the development and evaluation of an AI-based approach for automated human embryo assessment and selection of normally fertilized embryos at the pronuclear stage with high accuracy.

**SUPPORT:** This work was partially supported by the Brigham Precision Medicine Developmental Award (Brigham Precision Medicine Program, Brigham and Women's Hospital) and R01AI118502, R01AI138800, and R21HD092828 (National Institute of Health).

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**DEEP LEARNING CAN IMPROVE DAY 5 EMBRYO SCORING AND DECISION MAKING IN AN EMBRYOLOGY LABORATORY.**

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**OBJECTIVE:** To evaluate whether an artificial intelligence (AI) network could improve the consistency of morphologic embryo grading at the blastocyst stage and ultimately aid embryologists in embryo disposition decision making.

**DESIGN:** Prospective double blinded trial using a retrospective dataset.

**MATERIALS AND METHODS:** Using a dataset comprising of 3,469 embryos, the deep convolutional neural network (CNN) model was trained and tested to primarily classify between non-blastocysts and blastocysts using images of embryos captured at 113 hours post insemination (hpi). Using a blinded 742 embryo image dataset, we evaluated the grading tendencies of 7 embryologists qualitatively classifying day 5 blastocysts on a 5-grade system (poor, fair, good, great, and excellent). A coefficient of variation (%CV) was calculated to evaluate the variability across the 7 embryologists. Furthermore, we used a blinded 56 embryo image dataset to evaluate the disposition decisions (biopsy/cryopreservation (HQB) vs. discard (non-HQB); HQB criteria: >3CC) of 10 embryologists after rotating the embryo image 90 and 180 degrees. Consistency was defined as the percentage of cases where the disposition decision was unaffected by the rotation. For both tasks, we compared the degree of variability in the embryologists' assessments to that of the CNN.

**RESULTS:** When qualitatively classifying day 5 blastocysts into a 5-grade system, embryologists exhibited a high degree of variability (%CV: 44.98%), implying significant variation in embryo quality assessment between the embryologists. When selecting day 5 blastocysts for biopsy or cryopreservation, embryologists had an average consistency of 52.14% (CI: 40.99% to 63.29%) and 57.68% (CI: 47.39% to 67.97%), respectively. The CNN outperformed the embryologists with a consistency of 83.95% and 83.92% (P<0.05 for both), respectively. Chronbach alpha analysis revealed an alpha coefficient of 0.5982 (CI: 47.39 to 67.97) for the embryologists and 1.00 (lower CI: 1.00) for the CNN. Of note, the recommended internal consistency range should be higher than 0.9 alpha coefficient in clinical settings.

**CONCLUSIONS:** The results of our study show a high degree of inter- and intra-embryologist variability in scoring day 5 blastocysts, likely due to the subjective nature of traditional morphology grading. This may ultimately lead to less precise disposition decisions and the discarding of viable embryos. The application of a deep CNN, as shown in our study, can introduce improved reliability and high consistency during the process of embryo selection and disposition, potentially improving outcomes in an embryology laboratory.

**SUPPORT:** This work was partially supported by the Brigham Precision Medicine Developmental Award (Brigham Precision Medicine Program, Brigham and Women's Hospital) and 1R01AI118502, R01AI138800, and R21HD092828 (National Institute of Health).

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**PREDICTING BLASTOCYST FORMATION OF DAY 3 EMBRYOS USING A CONVOLUTIONAL NEURAL NETWORK (CNN): A MACHINE LEARNING APPROACH.**

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**OBJECTIVE:** To assess the accuracy of a convolutional neural network (CNN) in identifying progression from a day-3 embryo to the blastocyst stage compared to traditional morphologic assessment by an embryologist.

**DESIGN:** Historical prospective cohort study.

**MATERIALS AND METHODS:** A retrospective dataset of 1,190 previously annotated embryo images were used to train a CNN to predict development of embryos to blastocyst stage using time-lapse images. Using a set of 748 embryos, embryos were assessed by the CNN and five embryologists at 70 hours post insemination (hpi). Images captured from 113 hpi were available to assess blastocyst formation. A t-test was used to compare accuracy between both methods.

**RESULTS:** The accuracy of the CNN in selecting an embryo, which developed into a blastocyst by 113 hpi for single embryo transfer at 70 hpi was 83.5% while, the average accuracy of the embryologists (n=5) was 80.2% (95% CI: 77.7-82.7%). A one sample t-test revealed that the CNN performed significantly (P<0.05) better than the embryologists in selecting a single cleavage stage embryo for transfer that will eventually develop into a blastocyst. When the CNN and the embryologists selected two embryos for transfer at 70 hpi of which at least one developed into a blastocyst by 113 hpi, the CNN performed with an accuracy of 96.9% while the embryologists performed with an average

accuracy of 94.0% (95% CI: 92.4-95.7%). A one sample t-test revealed that the system performed significantly ( $P < 0.05$ ) better than the embryologists in selecting two embryos for transfer among which at least one will eventually form a blastocyst. The accuracy of the CNN in selecting an embryo at 70 hpi, which developed into a high-quality blastocyst (HQB) for a single embryo transfer (SET), was 63.9% that is significantly higher ( $P < 0.05$ ) than the average accuracy of the embryologists (52.8%, 95% CI: 48.6-57.0%). The accuracy of the CNN in selecting an embryo at 70 hpi, which developed into HQB for a double embryo transfer (DET), was significantly higher (79.4%,  $P < 0.05$ ) compared to the embryologists with an average accuracy of 72.4% (95% CI: 70.7-74.0%).

**CONCLUSIONS:** Here, we reported an artificial intelligence-based approach for predicting the developmental fate of cleavage stage embryos. Our study shows that the developed CNN outperforms an embryologist's morphologic assessment at 70 hpi in predicting blastocyst formation. Additionally, we demonstrated that this technology might be used to select embryos with the highest in-vitro developmental potential. Utilization of artificial intelligence (AI) technologies in human IVF practices may allow for more objective/standardized methods for improving embryo selection.

Reference: None.

**SUPPORT: Financial Support:** This work was partially supported by the Brigham Precision Medicine Developmental Award (Brigham Precision Medicine Program, Brigham and Women's Hospital) and 1R01AI118502, R01AI138800, and R21HD092828 (National Institute of Health).

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### THE APPLICATION OF MACHINE LEARNING METHODS TO EVALUATE PREDICTORS OF LIVE BIRTH IN PROGRAMMED THAW CYCLES.



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**OBJECTIVE:** The utilization of frozen embryo transfers is increasing annually. The objective of this study was to investigate the utility of machine learning (ML) methods to weight predictors for positive pregnancy and live birth rate (LBR) in programmed thaw cycles.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All first, autologous programmed thaw cycles (January 2014 to October 2017) were reviewed. Data was collected from both stimulated and subsequent frozen cycles. Each patient received estrogen replacement until endometrial thickness was deemed adequate (usually  $\geq 8$ mm). Progesterone was prescribed with embryo transfer after 5 doses of progesterone supplementation.

For data analysis, we normalized the numerical variable and one-hot-encoded the categorical variables. For each outcome, a logistic regression model was fitted with LASSO regularization. The model test ROC was evaluated and averaged across 10 random training/test data splits. For each outcome, the top variables, most predictive across the 10 random splits are presented using regression coefficients (RC).

**RESULTS:** A total of 1726 cycles were available for analysis with 129 variables evaluated. The median age of the cohort was 34.3. The positive pregnancy rate among our cohort was 70% and the LBR was 47%. Top predictors for both models are shown in Table 1. The ROC for model fit for positive pregnancy and LBR was 0.65 and 0.73 respectively. Interestingly, both increasing age at oocyte retrieval and anti-mullerian hormone (AMH) level were weaker predictors for live birth (RC -0.5, 0.6 respectively) than those listed. Transfer of a euploid embryo was a weaker predictor for LBR in our cohort (RC =0.2), as were blastocyst alphanumeric grades.

**CONCLUSIONS:** The abundance of measurements related to infertility treatment is well suited for the application of ML. A clinician makes decisions based on knowledge and past experience which may bias the process and impact clinical

TABLE 1. Top predictors, with regression coefficients, for positive pregnancy and LBR

Top predictors for Clinical Pregnancy	Regression Coefficient	Top predictors for live birth	Regression Coefficient
# Stimulation cycles	-1.8	# Stimulation cycles	-3.1
Embryo age at transfer (blastocyst transfer)	1.6	Embryo age at transfer (blastocyst transfer)	1.1
# of blastocysts in correlating fresh cycle	0.8	# of blastocysts in correlating fresh cycle	1.1
Endometrial thickness at transfer	0.6	Endometrial thickness at transfer	0.8
# Miscarriages < 20 weeks	-0.8	# Miscarriages < 20 weeks	-0.8

ical outcomes. In our work, we already find that factors considered by clinicians to predict the outcome are not identical to those considered by our model. Validation and further development of ML models is ongoing.

SUPPORT: None.

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### DEEP LEARNING FOR AUTOMATIC DETERMINATION OF BLASTOCYST EMBRYO DEVELOPMENT STAGE.



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**OBJECTIVE:** To build and to validate a computational tool aimed at reducing the subjectivity inherent to current embryo classification methods.

**DESIGN:** Data augmentation for building and initial validation of a neural network architecture using an embryo pictures' bank.

**MATERIALS AND METHODS:** An Inception V3 deep convolutional neural network architecture was built and trained using a dataset containing 1,204 pictures of blastocyst obtained from 2 IVF centers and classified by an expert embryologist into three categories according to its development stage: (i) expanding, (ii) hatching and (iii) hatched. The dataset was increased to a total of 15,000 images using data augmentation techniques to assure that the network model is robust to translations and rotations. 12,000 images were employed training and the remaining 3,000 for validation through the computation of the weights of the neural network.

**RESULTS:** Once the network was trained, we used it to classify 56 images never seen before by the network. All 54 images were correctly classified by the network.

**CONCLUSIONS:** Results indicate the feasibility of employing deep learning techniques for the automatic and objective classification of blastocyst development stage which will pave the way for building computational tools that will aid the expert embryologist to define a ranking based on quantitative information.

SUPPORT: Darwin Technologies LTD.

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### ARTIFICIAL NEURAL-NETWORK ANALYSIS COMBINED WITH TIME-LAPSE IMAGING PREDICTS EMBRYO ABILITY TO DEVELOP TO THE BLASTOCYST STAGE.



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**OBJECTIVE:** To assess the potential of machine learning algorithms, implemented for image analysis at early developmental stages, to predict embryo development to the blastocyst stage.

**DESIGN:** The ANN approach was undertaken to assess retrospectively the ability of human embryos to develop to the blastocyst stage. The analysis focused on 113 embryos generated in 32 IVF cycles, carried out between October 2015 and May 2018. Female age was  $36.3 \pm 4.9$  years. To minimize possible patient-based bias, cycles were recruited ensuring to have in the same cohort both embryos able to develop to the blastocyst stage and arresting at earlier stages.

**MATERIALS AND METHODS:** Embryos were subject to time-lapse assessment to monitor development and perform trophectoderm biopsy for preimplantation genetic testing of aneuploidy. Fertilisation was achieved by ICSI. Time-lapse monitoring started immediately after ICSI, with a 15 min interval between consecutive observations. Of 113 embryos analysed, 55 reached the blastocyst stage (BL-group) and 58 arrested sometime after the 2-cell stage (NoBL-group). ANN analysis was performed, at this stage, only during the first two cell divisions (175 frames; 2,625 min).

**RESULTS:** We developed a classification platform consisting of three main steps: 1) collection of time-lapse images of preimplantation embryos; 2) evaluation of time-lapse sequence images of each embryo by a particle image velocimetry software that detects cytoplasmic movements; 3) finally, analysis of cytoplasmic movement patterns through an ANN that predicts developmental competence. Specifically, cytoplasmic movements of single embryos development were measured as multivariate time series and used to train and test a Long-Short Term Memory (LSTM) neural network. LSTM displayed the capacity to learn "long-term" temporal dependences of both BL- and NoBL-group and provide a classification when challenged blind. Following a ten-fold cross validation of the training set, the specific LSTM selected was trained with 90% of data and tested on the remaining. Thus, based on the analysis of the cytoplasmic movement occurring during the first two cell divisions of single blind embryos (test set), the trained LSTM reached an 82% classification accuracy in the prediction of development to the blastocyst stage.

**CONCLUSIONS:** This study represents an initial attempt to build up a robust system of classification of the quality of human preimplantation embryos totally automatized from input to output. A three-steps workflow, combining time-lapse imaging, particle image velocimetry and artificial neural network (ANN) classification, predicts with high accuracy embryo ability to develop to blastocyst stage. Further refinement of the approach is expected to impact embryo assessment ability and improve efficiency in assisted reproduction treatments.

Reference: None.

SUPPORT: None.

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#### **A MASSIVE EMBRYO MORPHOKINETICS COMPARISON SYSTEM IS ABLE TO SELECT EMBRYOS WITH HIGH IMPLANTATION POTENTIAL ENHANCING SINGLE EMBRYO TRANSFER POLICY.**



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**OBJECTIVE:** To analyze the abilities of DANA automatic embryo selection software to interpret embryo morphokinetic parameters by massive comparison with a database of embryos with known implantation potential and generate a transfer order ranking in a single embryo transfer policy.

**DESIGN:** DANA compares each cohort of embryos with a data cloud of KID (Known Implantation Data). This data cloud was performed from a retrospective analysis of 1021 KID embryos. For that, timings of embryo cleavage and cell cycle lengths were included in the DANA software. Morphokinetic parameters were arranged on a 2D graph and the software analysed the unit average distance (UAD) of the embryos to the centre of the cloud. We defined a category of TOP embryos with an UAD  $\leq$  0.5.

**MATERIALS AND METHODS:** The percentage of twin gestation was calculated in our double embryo transfer cases comparing whether 1 or 2 of the transferred embryos had a high score in the Dana ranking. A total of 357 fresh cycles from infertile couples undergoing oocyte donation were included; 1562 embryos were analyzed from which 536 were transferred, and 371 embryos achieved the status of KID embryos.

**RESULTS:** Therefore, we compared cases in which the two blastocysts transferred were TOP, compared to those cases in which only 1 being TOP. The twin gestation rate was significantly higher in those cases in which the number of TOP embryos (UAD  $\leq$  0.5) transferred was two 52% vs. 25% in one top group (P < .001).

**CONCLUSIONS:** In cases in which a double embryo transfer was performed, the twin gestation rate was significantly higher when the two embryos were classified as TOP by the software, reaching very high values. In consequence, the selection method here presented, is a relevant strategy, to encourage single embryo transfer at least when two TOP blastocyst are available.

**SUPPORT:** The development of this publication was financially supported by CDTI research project IDI-20170310 from Spanish Government of Economy and competitiveness, a research grant from the Spanish Society of

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#### **ART LAB - EMBRYOS**

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#### **EFFECTS OF MEIOTIC SPINDLE IMAGING IN HUMAN OOCYTES FOLLOWING PIEZO-ICSI ON OOCYTE FERTILIZATION AND EMBRYO DEVELOPMENT.**



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**OBJECTIVE:** Recent studies using polarized light microscopy have revealed a correlation between meiotic spindle imaging in human oocytes following intracytoplasmic sperm injection (ICSI) and fertilization rate. However, these studies have only assessed conventional-ICSI, in which a beveled and spiked micropipette is used to aspirate the cytoplasm and break the membrane before sperm are injected. To our knowledge, no studies have yet elucidated the relationship between meiotic spindle imaging in human oocytes following Piezo-ICSI, and fertilization or embryo development. In Piezo-ICSI the membrane is broken by applying a Piezo pulse, which produces ultra-fast submicron forward momentum using uniquely-shaped flat-tipped micropipettes with no bevel or spike. The objective of this study was to investigate the effect of meiotic spindle imaging in human oocytes following Piezo-ICSI on fertilization and embryo development.

**DESIGN:** Retrospective, case control.

**MATERIALS AND METHODS:** We retrospectively investigated 529 oocytes with the first polar body retrieved from 124 infertile couples (147 cycles; women's average age, 37.8  $\pm$  4.8; partner's average age, 39.7  $\pm$  4.8; expressed as the mean  $\pm$  SD) who attended the Piezo-ICSI program at the Kameda IVF Clinic Makuhari between May 2016 and December 2018. Of these, 489 oocytes (92.4 %) with visible meiotic spindle comprised the Spindle (+) group, while 40 oocytes (7.6 %) not observed meiotic spindle comprised the Spindle (-) group. Meiotic spindle imaging was performed using polarized light microscopy, and the rates of oocyte survival, fertilization, good-quality day-3 embryos, blastocysts, and good-quality blastocysts were evaluated for both groups. Categorical values were compared using Fisher's exact test. A P-value of < 0.05 was considered significant.

**RESULTS:** The fertilization rate of the Spindle (+) and Spindle (-) oocytes was 92.0 % (450/489) and 70.0 % (28/40), respectively. The rate of good-quality day-3 embryo formation by the Spindle (+) and Spindle (-) oocytes was 62.9 % (283/450) and 35.7 % (10/28), respectively. The rate of blastocyst formation by the Spindle (+) and Spindle (-) oocytes was 53.7 % (205/382) and 32.1 % (9/28), respectively. The rate of good-quality blastocyst formation by the Spindle (+) and Spindle (-) oocytes was 29.8 % (114/382) and 3.6 % (1/28), respectively. Significantly higher rates of fertilization, good-quality day-3 embryos, blastocysts, and good-quality blastocysts were obtained in the Spindle (+) group than in the Spindle (-) group.

**CONCLUSIONS:** To the best of our knowledge, this is the first study to evaluate the effect of meiotic spindle imaging in human oocytes following Piezo-ICSI on fertilization or embryo development. Spindle imaging (i.e. identifying oocytes with visible or not observed meiotic spindle) does influence the outcome of Piezo-ICSI in human oocytes, including fertilization and embryo development. Our results demonstrate that the combination of meiotic spindle imaging and Piezo-ICSI can increase the fertilization of viable oocytes without oocyte loss in human assisted reproductive technology.

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#### **EFFECT OF DIFFERENT 6-DIMETHYLAMINOPURINE (6-DMAP) TREATMENTS ON REVERSIBLE ARRESTING OF MONO- AND TRIPRONUCLEAR EMBRYOS AT THE PRONUCLEAR STAGE.**



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**OBJECTIVE:** To determine the optimal concentration of 6-DMAP to synchronize human zygotes at the pronuclear stage (presumably at the G2-phase of the cell-cycle) without compromising subsequent development to blastocyst, as possible pre-treatment to enhance the natural DSB repair pathways in CRISPR-Cas9 technology.

**DESIGN:** This study used mono- (MPN; n=580) and tripronuclear (TPN; n=261) human embryos. They were incubated for 6hrs in different 6-DMAP concentrations, in order to assess the arresting rate. After 6-DMAP treatment, zygotes were cultured to the blastocyst stage, in order to assess the effect of 6-DMAP on subsequent developmental competence.

**MATERIALS AND METHODS:** MPN and TPN zygotes were incubated in 0mM (control), 0.24mM, 0.48mM or 0.60mM 6-DMAP, in GEMS medium (Genea Biomedx) for 6h at 37°C, 6%CO<sub>2</sub> and 5%O<sub>2</sub>. Arresting rate was calculated as percentage of zygotes, blocked at PN stage when 6-DMAP treatment had finished. Then, MPN/TPN were cultured in a time-lapse incubator in 20µL GEMS for 5 days. Blastocyst rate was calculated as a percentage of blastocysts per number of pronuclear-arrested zygotes. Morphokinetic variables included the precise occurrence time of pronuclear fading and cleavage (6h after 6-DMAP treatment, t0).

**RESULTS:** Concerning MPN zygotes, higher arresting rates were observed in 0.48mM and 0.60mM 6-DMAP groups (averaged: 86.1%) than in 0.24mM (44.4%; p= 0.004). In 0.24mM and 0.48mM 6-DMAP groups, some zygotes exhibited an anomalous pronuclear fragmentation at the end of 6-DMAP treatment (27.8% and 7.1%, respectively). This event was never observed in 0.60mM or control groups. Morphokinetic analysis showed that regardless 6-DMAP concentration, PNF and cleavage occurred at comparable timings (averaged: 3.9h and 8.3h, respectively). Regardless of 6-DMAP concentration, arrested MPN cleaved (78.3%) and progressed to the blastocyst stages (18.2%) at comparable rates to controls (77.8%; p=0.3 and 18.6%; p=0.96, respectively).

As regards TPN zygotes, they were arrested at the pronuclear stage efficiently (averaged, 92.3%), regardless 6-DMAP concentration. No PN fragmentation was observed at any 6-DMAP concentration or controls. However, at 0.24mM and 0.48mM concentrations pronuclei faded significantly later than 0.60mM group did (2.8-8.0h vs. 1.6-4.0h; p=0.03). Concerning developmental competence, TPN zygotes cleaved (83.0%) and progressed to the blastocyst stage (33.6%) at comparable rates to control (81.7%; p=0.34 and 33.6%; p=0.6, respectively), regardless 6-DMAP concentration.

**CONCLUSIONS:** MPN and TPN zygotes, incubated in 0.6mM 6-DMAP for 6h did efficiently arrest the first cell-cycle at the G2-phase without compromising subsequent development. This finding could have a potential applicability in CRISPR-Cas9 technology due to DSB repair pathways are dependent of the cell-cycle stage.

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#### **DEEP LEARNING-ENABLED PREDICTION OF FERTILIZATION BASED ON OOCYTE MORPHOLOGICAL QUALITY.**

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**OBJECTIVE:** Failure to fertilize oocytes can be associated with both the male and female factors. However, for certain women especially with premature ovarian failure, diminished ovarian reserves or genetically transmittable diseases, donor egg may be the only available option in giving birth to a healthy child. Addition of donor eggs to a cycle significantly increases the patient's out-of-pocket costs. Obtaining premium quality eggs that have a high chance of success may help reduce the uncertainty in patients, while potentially improve rates of pregnancy. Currently, there is no objective system that can evaluate oocyte quality and predict its developmental potential. In this work, we have developed a deep learning-based approach that evaluates oocytes based on their morphology and predicts their fertilization potential.

**DESIGN:** Human oocytes and embryos imaged with an Embryoscope were used as retrospective data in this study. A deep neural network was developed and trained to predict fertilization in embryos at 18 hours post insemination (hpi) by evaluating the respective oocytes before fertilization.

The developed network was evaluated using another independent set of oocyte images with known fertilization outcomes. The system differentiated fertilized embryos primarily based on the number of pro-nuclei (2PN vs non-2PN). We probed if a deep neural network was able to identify enough features in oocytes to sufficiently differentiate between the developmental outcomes through a receiver operator characteristic (ROC) analysis. We also evaluated the networks predictive power.

**MATERIALS AND METHODS:** We developed deep convolutional neural network that was trained with 2123 images at the oocyte stage and 18 hpi. The network was trained to classify between oocytes that eventually fertilized with 2PN or abnormally fertilized at 18 hpi. We evaluated the ability of the trained network in predicting the developmental outcome with 712 oocyte images. The predictions were compared with the actual developmental outcomes and a ROC analysis was performed with an area under the curve (AUC) baseline of 0.5 and alpha of 0.05 to verify its ability to separate the groups.

**RESULTS:** The network was able to differentiate between the fertilization outcomes with an accuracy of 67.0% (95% CI: 63.4% to 70.4%). The AUC of 0.6133 obtained through a ROC analysis confirmed that the network was able to differentiate between the outcomes with a reasonable degree of accuracy. After establishing that the neural network was able to differentiate oocytes based on their fertilization potential, we tuned the network to conservatively identify oocytes with the highest fertilization potential. In our evaluations, the network achieved a maximum predictive power of 86.0% (95% CI: 77.3% to 92.4%).

**CONCLUSIONS:** Our results suggest that a neural network can be used to help identify the highest quality oocytes objectively based on their fertilization potential. The high predictive power of the trained network can carefully select the oocytes with the promise of improving the patient prognosis.

**SUPPORT:** This work was partially supported by the Brigham Precision Medicine Developmental Award (Brigham Precision Medicine Program, Brigham and Women's Hospital) and R01AI118502, R01AI138800, and R21HD092828 (National Institute of Health).

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#### **USE OF A SPECIFIC GRAVITY DEVICE TO PREDICT BLASTOCYST SEX.**

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**OBJECTIVE:** Previous research has demonstrated that a Specific Gravity Device (SGD) is useful in providing a noninvasive means of assessing embryo quality at various stages of development from zygote to blastocyst. Preliminary data suggested the system might also be useful for predicting embryo sex. The objective of the present study was to assess the predictive value of the SGD in determining embryo sex using a bovine model.

**DESIGN:** Lab-based trial of the SGD in predicting embryo sex.

**MATERIALS AND METHODS:** Bovine oocytes were collected from *in vivo* ovaries and fertilized *in vitro*. Six hundred embryos developed into grade 1 or 2 blastocysts and were individually assessed in SGD. Embryo descent times were measured and recorded in seconds and then used in an Embryo Prediction Algorithm (EPA) to predict embryo sex. Sex of each embryo was also confirmed individually by Polymerase Chain Reaction (PCR). Comparisons were then made between EPA prediction and PCR values to assess the ability of the SGD to predict embryo sex.

**RESULTS:** PCR data were obtained on 463 of the 600 embryos and available for comparison with SGD predictions. The EPA demonstrated significant differences between male and female embryos (P<0.05). Further, the EPA demonstrated 65.3-78.4% accuracy selecting for female embryos. These data suggest, with refinement, the SGD might provide a noninvasive means of predict sex of preimplantation embryos.

**CONCLUSIONS:** The SGD can detect embryo sex based on differences in embryo buoyancy. Theoretically, the differences in the buoyancy of mammalian blastocyst embryos would be a reflection of differences in the chromosomal weight of X and Y chromosomes or developmental differences of male and female embryos. Data demonstrate a high degree of correlation between SGD and the PCR results suggesting the technology can provide a noninvasive means to differentiate female pre-implantation embryos without the use of pre-implantation genetic testing or sexed semen. On-going studies are assessing if improvements in the EPA will allow predictive values for male embryos as well. Identifying the sex of an embryo is important for family balancing or for patients with a known sex-linked genetic disorder.

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### A CLINICAL MODEL PREDICTING SUPERNUMERARY EMBRYOS IN WOMEN UNDERGOING FREEZE-ALL CYCLES UTILIZING SART CORS DATA.

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**OBJECTIVE:** The field of IVF has focused on embryo selection technology and multiple methods have been utilized including metabolomics, time lapse imaging and PGT-A. PGT-A was touted as the optimal method but it has recently come under scrutiny due to some evidence of euploid births from embryos found to be aneuploid on testing [1-3]. A selection method can only enhance the chances of success if we have a cohort of embryos to select from and yet, we have inadequate counseling tools for patients on their chances of having supernumerary embryos for a selection method to be applicable. We have identified factors predictive of having supernumerary embryos in freeze-all cycles. Therefore, we sought to create a clinical prediction model using those identified factors for clinical counseling.

**DESIGN:** Retrospective cohort study of women who underwent freeze-all cycles in 2014.

**MATERIALS AND METHODS:** Data were obtained from the Society for Assisted Reproductive Technology. We defined supernumerary as having two or more embryos cryopreserved. We utilized previously identified predictors and entered them into a logistic regression model presenting a receiver operating characteristic curve (ROC) for all predictors. Any predictor that did not alter the area under the curve for the ROC was removed from the prediction model. We then utilized methods described by Sullivan and colleagues [4] to modify the final model into a risk index. The number of points assigned to each significant covariate equaled its regression coefficient divided by the parameter estimated in the model with the smallest value rounded to the nearest whole number. The accuracy of the prediction model was then tested using an ROC.

**RESULTS:** Of 31,537 freeze-all cycles in 2014, 18,250 produced supernumerary embryos. We included 16,395 cycles into the logistic regression model after excluding cycles with missing AMH as this was a very strong predictor of the outcome. Table 1 demonstrates the points assigned to each necessary predictor. The area under the curve (AUC) for the ROC was 0.84.

**CONCLUSIONS:** Age, AMH and number of eggs retrieved are necessary predictors for the model. The AUC for the ROC is considered excellent discrimination and therefore, this model can be used to counsel patients undergoing freeze-all cycles on their probability of having supernumerary embryos for a selection method to be applicable.

TABLE 1. Points assigned to each significant covariate

Variable (Referent)*		Points
Age (<35)	35 - 37	0
	38 - 40	-1
	41 - 42	-2
	> 42	-4
AMH (1.0 – 3.0)	<1.0	-1
	>3.0	+1
# eggs retrieved (0 – 3)	4 - 8	+8
	9 - 13	+12
	14 - 20	+15
	21 - 45+	+18

\*Referent category is assigned zero (0) Points

Score = sum of all Points in a given individual.

\*Scores = Score + 5 (automatically sets the minimum summative score to zero).

References: 1. Fragouli, E., et al., *Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid-aneuploid blastocysts*. Hum Genet, 2017. **136**(7): p. 805-819.

2. Munne, S., et al., *Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected with the use of high-resolution next-generation sequencing*. Fertil Steril, 2017. **108**(1): p. 62-71 e8.

3. Sachdev, N.M., et al., *Diagnosis and clinical management of embryonic mosaicism*. Fertil Steril, 2017. **107**(1): p. 6-11.

4. Sullivan, L.M., J.M. Massaro, and R.B. D'Agostino, Sr., *Presentation of multivariate data for clinical use: The Framingham Study risk score functions*. Stat Med, 2004. **23**(10): p. 1631-60.

SUPPORT: None.

### BLASTOCYST AND EMBRYO SCREENING SELECTION TOOL (BESST): AN ULTRAFAST NON-INVASIVE EMBRYO SELECTION TEST FOR USE IMMEDIATELY PRIOR TO EMBRYO TRANSFER.

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**OBJECTIVE:** Development and implementation of a novel embryo selection workflow immediately prior to transfer during routine fertility cycles

**DESIGN:** A scoring algorithm for the selection of embryos based on a mass spectral profile of culture media has been developed previously. Here the algorithm was adapted and optimized to be integrated into the current routine practices of a fertility centre in the USA. The applicability and advantages of using this workflow were analyzed retrospectively using post implantation data outcomes from the cohort.

**MATERIALS AND METHODS:** A total of 1190 embryo cell culture media were collected and frozen prior to implantation from a single IVF clinic in the USA between March 2014 and March 2018. Samples were stored in 50µl aliquots and subsequently 1 µl was applied directly onto prepared stainless-steel plates with α-Cyano-4-hydroxycinnamic acid matrix. After drying on a hot plate, the sample plate was loaded to a Shimadzu benchtop MALDI-ToF mass spectrometer. To score embryos, we used the Blastocyst and Embryo Screening and Selection Tool (BESST) software, previously installed on the instrument. This tool provides an embryo score from mass spectral features of between 0 and 5, with 5 being the best chance of implantation and ongoing pregnancy and 0 being the least.

**RESULTS:** The time responses of our workflow were monitored starting from sample preparation to embryo scores reporting for candidate embryos. The total time obtained ranged between 6 to 8 minutes, which is a reasonable time to give an informative response before embryo implantation. In each case, scores showed substantial differences between embryos from the same cycle; indicating that some embryos had a greater chance of success when compared to others. Statistically, embryos selected with higher scores (>4) correlated with more cases of successful implantation and ongoing pregnancy with a positive predictive value of 76.9%. In comparison, those embryos with low scores (<1.5) poorly correlated with ongoing pregnancy outcome, predicting the chance of ongoing pregnancy of 35.7% or lower. In unsuccessful pregnancies, the tool was able to identify embryos from the same cycle with higher scores in comparison to the embryo transferred. This suggests that relying on BESST in these cases could have resulted in an implanted embryo and an ongoing pregnancy.

**CONCLUSIONS:** We have successfully implemented a fast scoring system for embryo selection that can be applied in any fertility clinic immediately prior to transfer. We further demonstrated that the integration of this workflow on current practices in fertility clinics may provide advantageous information that increases the chances of successful embryo implantation and ongoing pregnancy.

### QUALITY EVALUATION OF DIRECT CLEAVAGE EMBRYOS AT THE FIRST DIVISION USING A COMBINED EARLY CLEAVAGE WITH AN EMBRYO MORPHOLOGICAL GRADING METHOD.

Yumi Nagata, M.D., Ph.D.,<sup>a</sup> Hiroyuki Tomari, Ph.D.,<sup>a</sup> Saki Gondo, M.D.,<sup>b</sup> Kensuke Saito, M.D.,<sup>b</sup> Kou Honjo, M.D.<sup>b</sup> <sup>a</sup>IVF Nagata Clinic, Fukuoka, Japan; <sup>b</sup>Affiliation not provided.



**OBJECTIVE:** Several studies have reported a clear correlation between the occurrence of DC (divided into three or more cells) during the first division of an embryo and impaired embryo development potential in humans. In addition, it has also been reported that the pregnancy rate due to transfer of DC embryos that developed to blastocysts is similar to that of normal cleavage embryo transfer. However, only few studies have reported on methods for assessing DC embryo quality. In this study, we evaluated the quality of DC embryos using early embryo two-step evaluation (ETE) methods combining early cleavage and morphological grading.

**DESIGN:** This prospective observational study was performed in a single *in vitro* fertilization (IVF) center between 2015 and 2017. This study

included patients undergoing IVF or intracytoplasmic sperm injection. All study participants provided informed consent and the study design was approved by the ethics committee of the IVF Nagata Clinic, Fukuoka, Japan.

**MATERIALS AND METHODS:** We analyzed 1,242 DC embryos with normal fertilization using a time-lapse incubator. Ex.1: We compared blastocyst formation rates between 3- and  $\geq 4$ -cell groups during the first division. Ex.2: The two groups from Ex.1 were classified using ETE methods. The blastocyst formation rates of each group were compared. Embryos were evaluated for EC at 27 hours after insemination and morphology was scored on day2 (poor,  $\geq 4$  cells with  $\geq 50\%$  frag.; fair,  $\geq 4$  cells with  $<50\%$  and  $\geq 20\%$  frag.; good,  $\geq 4$  cells with  $<20\%$  frag. and equal blastomere).

**RESULTS:** Ex. 1: Among the 1,242 DC embryos, 669 were in the 3-cell group and 573 were in the  $\geq 4$ -cell group. The blastocyst and high-quality blastocyst formation rates were significantly higher ( $p<0.01$ ) in the 3-cell group than in the  $\geq 4$ -cell group (53.5% vs. 32.7%, 28.0% vs. 14.1%, respectively). Ex. 2: Among the 669 embryos in the 3-cell group, 211 were in the EC-fair embryos, 141 were in the EC-poor embryos, 75 were in the late cleavage (LC)-fair embryos, and 242 were in the LC-poor embryos. Among the 573  $\geq 4$ -cell group, 102 were in the EC-fair embryos, 127 were in the EC-poor embryos, 90 were in the LC-fair embryos, and 254 were in the LC-poor embryos. The blastomeres of DC embryos were unequal and no embryo was evaluated as good. The blastocyst and high-quality blastocyst formation rates were significantly higher ( $p<0.05$ ) in the EC-fair embryos of the 3-cell group than in the other groups (71.6% vs. 18.5%–57.8%, 44.1% vs. 5.1%–33.3%, respectively).

**CONCLUSIONS:** The EC-fair embryos of the 3-cell group had high blastocyst development ability for DC embryos. The results suggest that the detailed evaluation of DC embryos at the early embryonic stage is not only predictive of embryogenic potential, but also useful for the selection of embryos for early embryo transfer.

#### ART LAB - ICSI

P-423 Wednesday, October 16, 2019 6:30 AM

#### IDENTIFICATION OF THE INDICATORS FOR RESCUE ICSI: THE EFFICACY OF TIME-LAPSE IMAGING FOR THE SIGNS OF FERTILIZATION IN IVF.

Rie Matsunaga, M.A., Hiromi Morita, M.A., Rui Hasegawa, B.A., Kana Isobe, B.A., Megumi Miura, M.A., Yuki Kobayashi, B.A., Minako Kamihata, B.A., Shinichi Watanabe, DVM, Tomoko Maeda, Ph.D., Hiroshi Makino, Ph.D., Masanori Ochi, Ph.D., Toshitaka Horiuchi, Ph.D. OCHI YUME CLINIC NAGOYA, Nagoya, Japan.

**OBJECTIVE:** We previously reported how the presence of the fertilization cone (FC) and cytoplasmic wave (CW) can act as indicators for the necessity of early-rescue ICSI after short-term insemination (ESHRE 2018). However, FC is present for only short durations. In addition, in some eggs it is difficult to identify CW due to cytoplasmic texture. Consequently, such determinations, when based upon one observational time point under an inverted microscope, can sometimes be difficult. Thus, we investigated whether a time-lapse incubator (TL) that allows the observation of embryos over time could be useful for identifying FC and CW.

**DESIGN:** We analyzed 6,704 mature eggs from 2,212 cycles of 1,438 individuals that were collected and then subjected to IVF between 2014 and 2017.

**MATERIALS AND METHODS:** We performed insemination at 12:00, removed cumulus cells 5 hour after insemination, and then checked for the presence of the second polar body (2PB), FC, and CW under an inverted microscope. We repeated the observation when FC or CW was not identified, even in the presence of 1PB and 2PB. In a conventional-method group, we performed the second and subsequent observations for fertilization signs every 1-hour under an inverted microscope. Meanwhile, in the TL group, we performed observations for fertilization signs every 1-hour using time-lapse imaging. In both groups, we considered the observation of 2PB extrusion, or the observation of either FC or CW, to be a sign of fertilization. When no fertilization sign was identified by 19:00, we conducted rescue ICSI. We then compared the proportions of positive FC and CW identification, as well as differences in the accuracy of determination between the conventional-method and TL groups.

**RESULTS:** A 2PB was observed in 2963 oocytes in the conventional-method group and 2548 oocytes in the TL group. The proportions of positive fertilization signs by FC were 13.0% and 19.9% in the conventional-method and TL groups, respectively, indicating that the proportion was significantly higher in the TL group. Meanwhile, those by CW were 70.1% and 62.2% in the conventional-method and TL groups, respectively, indicating that the pro-

portion was lower in the TL group. The infertility rates of the embryos determined to have FC and CW were 3.1% and 1.0%, respectively, in the conventional-method group, and 0.2% and 0.1%, respectively, in the TL group; thus, the rate of infertility was significantly reduced in the TL group.

**CONCLUSIONS:** Observation over time improves the accuracy of diagnosis for FC and CW, which makes time-lapse observation very useful for determining fertilization signs.

Reference: None.

SUPPORT: None.

P-424 Wednesday, October 16, 2019 6:30 AM

#### SPERM DNA FRAGMENTATION REDUCES EMBRYO DEVELOPMENT AND ONGOING PREGNANCY IN COUPLES WITH NON-MALE FACTOR INFERTILITY UNDERGOING INTRACYTOPLASMIC SPERM INJECTION CYCLES.

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**OBJECTIVE:** Nearly 15% of infertile men have semen parameters within normal reference ranges, which underlines that there must be others subcellular or nuclear factors, which are not identifiable by conventional semen analysis, that may contribute to male infertility. The value of SDF testing to improve the determination of the reproductive status of men that has neither altered seminal parameter nor history of male factor infertility still has to be determined. The objective of this study was to investigate the possible implications of sperm DNA fragmentation (SDF) for the outcomes of intracytoplasmic sperm injection (ICSI) in couples with non-male factor infertility.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** This study included data from 475 non-male factor infertility ICSI cycles, performed from June/2016 to June/2017, in a private university-affiliated IVF center. The sample size calculation suggested that 416 cycles would be enough to demonstrate a 20% effect with 80% power and 5% significance level. Semen samples were evaluated for sperm count, motility, morphology and SDF. Sperm DNA Fragmentation was measured using a Sperm Chromatin Dispersion (SCD) test. Cycles were divided according to SDF index into two groups: low fragmentation index ( $\leq 30\%$  SDF, n= 433) and high fragmentation index ( $>30\%$  SDF, n= 42). Laboratorial and clinical outcomes were compared between groups using generalized linear models with linear distribution followed by Bonferroni post hoc test, with adjustment for potential confounders.

**RESULTS:** Fertilization rate was similar between groups ( $\geq 30\%$  SDF: 85.28 $\pm$ 1.06 vs.  $<30\%$  SDF: 90.68 $\pm$ 3.61%,  $p=0.153$ ). Significant lower rates of normal cleavage speed ( $\geq 30\%$  SDF: 61.12 $\pm$ 4.21% vs.  $<30\%$  SDF: 72.53 $\pm$ 1.24%,  $p=0.010$ ), high-quality embryos on day three ( $\geq 30\%$  SDF: 23.07 $\pm$ 5.56% vs.  $<30\%$  SDF: 36.41 $\pm$ 1.53%,  $p=0.021$ ), blastocyst development ( $\geq 30\%$  SDF: 39.09 $\pm$ 2.73% vs.  $<30\%$  SDF: 58.83 $\pm$ 7.59%,  $p=0.016$ ) and high-quality blastocyst rate ( $\geq 30\%$  SDF: 11.97 $\pm$ 1.22% vs.  $<30\%$  SDF: 30.09 $\pm$ 2.39%,  $p<0.001$ ) were observed in cycles with higher SDF. Implantation rate was significantly reduced in the SDF  $\geq 30\%$  group (33.24 $\pm$ 1.66% vs.  $<30\%$  SDF: 46.40 $\pm$ 4.61%,  $p<0.001$ ), despite the similar pregnancy rates ( $\geq 30\%$  SDF: 30.40% vs.  $<30\%$  SDF: 32.40%,  $p=0.862$ ). A 2.5-fold increase in the miscarriage rate was observed in cycles with SDF above the established cutoff ( $\geq 30\%$  SDF: 42.8% vs.  $<30\%$  SDF: 16.8%,  $p=0.018$ ).

**CONCLUSIONS:** High SDF index leads to poor embryo development, and reduced implantation and ongoing pregnancy in couples with non-male factor infertility. Sperm DNA fragmentation testing may reveal hidden sperm abnormalities in men who have been categorized into idiopathic infertility based on apparently normal standard sperm parameters, bringing additional information to sperm quality evaluation in men with unknown history of infertility.

Reference: NA.

SUPPORT: None.

P-425 Wednesday, October 16, 2019 6:30 AM

#### EFFECT OF SPERM SELECTION TECHNIQUES ON HUMAN NEONATAL GENDER RATIO IN PATIENTS UNDERGOING ICSI.

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Ashok Agarwal, PhD.<sup>a,d</sup> Ganin Fertility Center, Cairo, Egypt; <sup>b</sup>Ganin IVF lab Director, Cairo, Egypt; <sup>c</sup>University of the Western Cape, Bellville, South Africa; <sup>d</sup>Cleveland Clinic, CLEVELAND, OH.

**OBJECTIVE:** To investigate the effect of commonly used sperm selection techniques, density gradient centrifugation (DGC), physiological ICSI (PICSI), and magnetic activated cell sorting (MACS), on the neonatal gender ratio of ICSI outcome.

**DESIGN:** Retrospective cohort study comparing the effect of sperm selection on gender ratio in three groups through statistical data analysis. [ClinicalTrials.gov Identifier: NCT03922568](https://doi.org/10.1186/1745-7256-3-2568).

**MATERIALS AND METHODS:** A total of 529 babies of known gender born out of 388 ICSI cycles between August 2016 and May 2018 at Ganin Fertility Center, Cairo, Egypt, were investigated for the gender ratio and then divided into three groups according to the sperm selection technique used before performing sperm injection: DGC (237 neonates out of 173 ICSI cycles), PICSI (147 neonates out of 109 ICSI cycles), and MACS (145 neonates out of 106 ICSI cycles). In PICSI and MACS groups, the sperm samples were processed by DGC prior to sperm selection. All embryos transferred were at the blastocyst stage. Power analysis was done by comparing the sex ratio of the neonates between DGC, PICSI and MACS. The chi-squared test for independent samples was chosen to perform the power analysis with  $\alpha$ -error level at 0.05. P values less than 0.05 were considered statistically significant. All statistical calculations were done using IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

**RESULTS:** Sperm selection using DGC, PICSI and MACS leads to different male ratios. The highest male ratio was observed in the MACS group (62.7%) compared to the DGC group (46.4%) ( $P=0.002$ ) with statistical power of (76.3%). In contrast, there was no difference ( $P=0.2$ ) between the PICSI group with a male ratio of (53.1%) and the DGC group (46.4%). The PICSI and MACS groups also did not differ significantly ( $P=0.09$ ). Moreover, there was neither a significant difference in female age (Mean $\pm$ SD) between DGC (29.9 $\pm$ 5.2 yrs.), PICSI (29.9 $\pm$ 4.5 yrs.), and MACS (30.6 $\pm$ 4.8 yrs.) ( $P=0.45$ ), nor in the male age of DGC (34.99 $\pm$ 6.4 yrs.), PICSI (36.2 $\pm$ 6.2 yrs.) and MACS (36.2 $\pm$ 7.7 yrs.) ( $P=0.22$ ).

**CONCLUSIONS:** The use of MACS as sperm selection technique significantly alters the neonatal sex ratio at birth in favor of male offspring. Further investigations should be made on phospholipid phosphatidylserine externalization which is the marker of apoptotic sperm during MACS sperm separation and its possible association with sex chromosome may provide some evidence for an association between semen quality and sex ratio of the offspring. To verify the outcome of higher male ratio in the PICSI group is needed, future studies with larger number of subjects are needed to compare PICSI with DGC.

**SUPPORT:** N/A.

**P-426** Wednesday, October 16, 2019 6:30 AM

#### **CERTAIN MORPHOLOGICAL CHARACTERISTICS OF THE SPERMATOZOON USED FOR INTRACYTOPLASMIC SPERM INJECTION (ICSI) ARE ASSOCIATED WITH A HIGHER LIKELIHOOD OF DEVELOPMENTAL ARREST AND EMBRYO DEGENERATION.**

Magdalena Vasileva, MSc, Dimitar Parvanov, PhD, Kristina Nikolova, MSc, Rumiana Ganeva, MSc, Georgi Stamenov Stamenov, MD/PhD. Nadezhda Women's Health Hospital, Sofia, Bulgaria.

**OBJECTIVE:** Around 25% of in-vitro cultured human embryos arrest their development and degenerate during the first 5 days. The functional and morphological characteristics of the spermatozoa, selected for the intracytoplasmic sperm injection (ICSI) could account for the subsequent embryo development failure. The objective of the present study was to assess the relationship between the morphological characteristics of the spermatozoon used for ICSI and the increased risk of developmental arrest and embryo degeneration.

**DESIGN:** Prospective observational study.

**MATERIALS AND METHODS:** A total of 83 spermatozoa from 68 men used in ICSI procedures were studied. Inclusion criteria were: 1) patient age < 39 years, 2) patient body mass index (BMI) < 25 kg/m<sup>2</sup>, 3) excellent quality oocytes. To find out which sperm morphological features could be responsible for the embryo degeneration the morphology of each individual spermatozoon used for ICSI was recorded. The morphology of all individual spermatozoa was evaluated using high magnification ( $\times 6100$ ) immediately before the injection. Seven sperm morphological parameters and the presence of sixteen head, neck and tail abnormalities were evaluated. All 83 embryos were cultured in-vitro in Global one-step medium. The frequencies of occurrence of sperm morphological defects were compared between the re-

sulted viable and degenerated embryos. Data was analyzed using the IBM SPSS software, v.21.0.

**RESULTS:** Among 83 ICSI performed, 63 embryos (75.9%) had successful development and 20 embryos (24.1%) arrest their development before day 5. The incidence of pyriform head, thick midpiece and cytoplasmic residues among the spermatozoa that resulted in embryos with developmental arrest were significantly higher in comparison to spermatozoa that led to successfully developed embryos (20% vs. 13%, 40% vs. 21% and 20% vs. 6%, respectively,  $p < 0.05$ , Chi-square test). In addition, the spermatozoa resulted in embryos with successful development had significantly shorter heads and tails in comparison with those that resulted in embryo development arrest (4.46 vs. 4.63  $\mu$ m and 41.42 vs. 43.65  $\mu$ m,  $p < 0.05$ , Student t-test, respectively). When receiver operating curve (ROC) analysis was performed to find the optimum cut-off value to predict embryo development sperm head length had the best AUC (AUC = 0.66, CI 95%: 0.51–0.76). The optimum cut-off value for head length was 4.25  $\mu$ m (sensitivity 89.5%, specificity 60.3%). The recorded spermatozoa tail length had lower AUC (0.63, CI 95%: 0.49–0.77) and the optimal cut-off value of 42.5  $\mu$ m (sensitivity 63.2%, specificity 42.5%).

**CONCLUSIONS:** Specific sperm morphological characteristics, such as head length and tail length and the presence of certain sperm abnormalities (pyriform head, thick midpiece and residual cytoplasmic droplets) are associated with an increased risk of developmental arrest and embryo degeneration. Selection of human spermatozoa with head length < 4.25  $\mu$ m, tail length < 42.5  $\mu$ m and without pyriform head, thick midpiece and cytoplasmic residues significantly increase the chance for successful embryo development.

**P-427** Wednesday, October 16, 2019 6:30 AM

#### **DAY 2 ICSI DOES RESULT IN GOOD QUALITY BLASTOCYST DEVELOPMENT AND PREGNANCY.**

Rebecca Kile, MS,<sup>a</sup> Haleigh Silz, MS,<sup>a</sup> Sue McCormick, BS,<sup>a</sup> William B. Schoolcraft, MD,<sup>a</sup> Rebecca L. Krisher, PhD.<sup>b</sup> <sup>a</sup>Colorado Center for Reproductive Medicine, Lone Tree, CO; <sup>b</sup>CCRM, Lone Tree, CO.



**OBJECTIVE:** Poor prognosis patients are often faced with negative IVF cycle outcomes, in which no or very few blastocysts are produced. Utilizing immature eggs recovered at oocyte retrieval may increase their chance of success. The aim of this study was to determine the efficacy of in vitro maturation of immature oocytes recovered in a standard IVF cycle, matured in vitro and fertilized with ICSI (D2, or rescue, ICSI), with respect to good quality blastocyst yield and establishment of pregnancy.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** After oocyte retrieval, cumulus oocyte complexes (COC) were denuded of cumulus cells. Mature oocytes (MII) were fertilized by ICSI (D1); immature oocytes at the germinal vesicle (GV) or metaphase I (MI) stage were placed into Oocyte Handling Medium for Maturation (OHM Mat) and incubated overnight. ICSI (D2) was performed on all oocytes that matured to MII. Zygotes (2PN) were cultured in sequential culture medium for 5-7 days when good quality blastocysts were biopsied for PGT-A and vitrified.

**RESULTS:** A total of 165 patient IVF cycles in 2018 in which D2 ICSI was performed were reviewed (average age 37.8 yr $\pm$ SEM, range 27–47). There were 2,101 oocytes retrieved; 1,325 (63.1%) were MII, 363 (17.3%) were MI, and 408 (19.4%) were GV. After IVM, 527/771 oocytes matured (68.4%). ICSI on D1 resulted in higher ( $P < 0.01$ ) normal fertilization (63.3%) than on D2 (56.5%), and improved ( $P < 0.01$ ) cleavage (D1, 101.2%; D2, 85.9%). In total, 36 patients (21.8%) that underwent D2 ICSI produced a good quality blastocyst from eggs that were immature at retrieval. Total good quality blastocyst ( $\geq$  grade 3BB) development (per 2PN) for Day 2 ICSI was 20.8% across all patients. Within patients that had blastocyst development from D2 ICSI eggs, there was no difference ( $P > 0.05$ ) in total blastocyst production per 2PN between D1 (52.0%) and D2 (53.0%), or in euploid blastocysts (D1, 47.5%; D2, 38.9%). Three D2 ICSI euploid blastocysts have been transferred into three individual patients, resulting in 1 negative hCG, 1 biochemical pregnancy, and one ongoing pregnancy. For two of these patients, no D1 euploid blastocysts were produced.

**CONCLUSIONS:** Retaining immature oocytes and performing Day 2 ICSI can yield good quality euploid blastocysts capable of supporting a pregnancy, although fertilization and embryo cleavage is reduced. Although the percentage of patients that may ultimately benefit from D2 ICSI is low, for poor prognosis patients these rescued immature oocytes may produce the only euploid blastocysts available for FET. Thus, incorporating D2 ICSI into the treatment protocol gives poor prognosis patients the best chance at ART success.

**SUPPORT:** None.

**DO DIFFERENT SPERM SELECTION TECHNIQUES HAVE AN IMPACT ON EMBRYOLOGICAL FINDINGS AND CLINICAL OUTCOMES OF ABNORMAL SPERM DNA FRAGMENTATION PATIENTS COMPARED TO NORMAL ONES; A RETROSPECTIVE COHORT STUDY.**



Manar Mohamed Hozyen, MSc, Eman Mohamed Hassanen, BSc, Yasmine sayed Azzouz, BSc, Hanaa Ahmed Alkhader, MBBCh, Hosam Zaki, MBBCh, Msc. FRCOG, Ganin Fertility Center, Cairo, Egypt.

**OBJECTIVE:** To determine the effect of sperm selection techniques for abnormal sperm DNA fragmentation (SDF) patients on the blastocyst grading, implantation and pregnancy rates compared to normal SDF.

**DESIGN:** Retrospective cohort study included 501 couples who underwent ICSI in Ganin Fertility Center from January 2017 to January 2019.

**MATERIALS AND METHODS:** Cases were assigned to normal SDF (125 couples) using ejaculated sperm processed by density gradient centrifugation (DGC) using Isolate and abnormal SDF group (376 couples) which subdivided to; 70 cases as ejaculated sperm processed by (DGC), 128 cases as physiological ICSI (PICSI) using ejaculated sperm selected by hyaluronan binding PICSI dishes, 107 cases as ejaculated sperm selected by magnetic activated cell sorting columns (MACS) using Annexin V microbead labeling followed by column separation and 71 cases using testicular sperm (TESTI). All included cases reached the blastocyst stage, female age was ≤37 years old and male with ≥ 5 millions of sperm count. SDF test was done by TUNEL assay and bench-top flow cytometer, cutoff value was 20%. Blastocyst morphological assessment was carried out by experienced embryologists, high-quality blast is defined as ≥ 3BB Grade according to Gardner's criteria. The data were collected and results were analyzed using statistical Software. The difference is considered significant if *P* value is ≤ 0.05.

**RESULTS:** There were no significant differences in male age, female age, number of MII oocytes or number of embryos transferred between the groups.

**CONCLUSIONS:** PICSI and MACS has superiority over TESTI as sperm selection techniques for patients with abnormal SDF and could improve the embryological and clinical parameters to the normal level. These findings should be confirmed by larger prospective randomized studies.

References: 1- Itai Gat, Katelynn Tang, Kevin Quach, Valeriy Kuznyetsov, Ran Antes, Melissa Filice, Khaled Zohni, Clifford Librach; Sperm DNA fragmentation index does not correlate with blastocyst aneuploidy or morphological grading, PLoS ONE, 2017.

2- Artur Wdowiak and Iwona Bojar; Relationship between pregnancy, embryo development, and sperm deoxyribonucleic acid fragmentation dynamics, Saudi J Biol Sci. 2016 Sep; 23(5): 598–606.

3- Alvarez Sedó C, Bilinski M, Lorenzi D, Uriondo H, Noblía F, Longobucco V, Lagar EV, Nodar F. Effect of sperm DNA fragmentation on embryo development: clinical and biological aspects. JBRA Assist Reprod. 2017 21(4):343-350.

**SUPPORT:** None.

**IDENTIFICATION OF THE OPTIMAL PUNCTURE POSITION IN PIEZO-ICSI USING IMAGE ANALYSIS: A PILOT STUDY.**



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**OBJECTIVE:** Oocyte degeneration may take place in Piezo-ICSI as a result of unintentional membrane rupture in the puncturing process. Identifying the appropriate puncturing position may decrease the likelihood of membrane rupture and thus degeneration. Therefore, it was evaluated using image analysis whether it was possible to identify the optimal puncture position.

**DESIGN:** Retrospective image analysis during ICSI procedure.

**MATERIALS AND METHODS:** Image feature analysis is generally used to represent the useful features such as color, brightness, and contour. Among capturing image features, Local Binary Patterns (LBP) can efficiently summarize the local structures of images, and it has been applied in texture analysis in various fields including face recognition and moving image analysis in real-time. We employed this methodology to analyze the moving images of 131 oocytes following ICSI. These oocytes were categorized as either unintentional rupture (UR: n = 101) or no rupture (NR: n = 30). An image of the oocyte before puncture and of the puncture position was acquired from the moving images, and LBP values were calculated in the analysis region centered around the puncture position. In order to select an effective pattern for evaluation of rupture from the 256 types of shape patterns acquired by LBP, median values for the UR and NR groups were calculated, and the patterns with little difference to the median were eliminated. Data was classified by hierarchical clustering method using the three effective patterns for evaluation. We employed the Ward's hierarchical cluster analysis method, and calculated the Euclidean distance between the barycenter of the cluster and each data point in order to define an index indicating the implausibility of membrane rupture. A t-test was used for statistical analysis.

**RESULTS:** Two clusters, Cluster A and B, were classified from hierarchical clustering. Following ICSI, 2 out of 27 oocytes from Cluster A and 28 out of 104 from Cluster B happened to have UR. When Cluster A represented the NR group and Cluster B the UR group, the sensitivity was 0.93. A significant difference between the UR and the NR group was reported from the Euclidean distance calculations between the barycenter of Cluster A and each data point (*P* = 0.001), where data showed a longer distance from the barycenter amongst the UR group and a shorter distance in the NR group.

**CONCLUSIONS:** Through image feature analysis, the presence or absence of membrane rupture was evaluated from the shape feature of the oolemma. The distance from the barycenter of Cluster A was associated with the likelihood of unintentional rupture. From this, visualizing shape features of the oolemma in real-time can contribute to the decrease in ICSI degeneration rate. From now on, it will be necessary to analyze more sample images and establish a visualization technique.

	Normal SDF %	DGC %	PICSI %	MACS %	TESTI %	<i>P</i> values of Normal vs. DGC	<i>P</i> values of Normal vs. PICSI	<i>P</i> values of Normal vs. MACS	<i>P</i> values of Normal vs. TESTI
SDF	14.3	28.6	29.3	29.3	29.6	0.00	0.00	0.00	0.00
Fertilization rate	78.5	78.1	75.9	76.5	71.7	0.88	0.07	0.29	0.01
Blastulation rate	65.6	58.1	59.6	59.3	53	0.01	0.02	0.04	0.00
High quality blast rate	54.6	48.9	56.1	54	42.6	0.09	0.60	0.85	0.01
Good TE*	18.9	17.1	21.6	17.3	15.5	0.24	0.73	0.16	0.10
*Trophectoderm									
Fair TE	34.7	30.2	32.1	35.2	24.3	0.09	0.35	0.63	0.00
Poor TE	7.6	6.3	9.5	8.4	12.4	0.51	0.28	0.84	0.66
Good ICM*	32.8	30.9	35.1	31.2	29.4	0.21	0.61	0.50	0.18
*Inner cell mass									
Fair ICM	26.5	21.1	27.5	28.2	21.6	0.03	0.97	0.64	0.02
Poor ICM	1.5	1.7	0.9	0.7	2.2	1.00	0.49	0.21	0.79
Pregnancy rate	66.3	51.6	61.4	61.1	54.3	0.06	0.46	0.44	0.11
Ongoing pregnancy rate	62.2	41.9	59.6	58.9	50.0	0.01	0.70	0.63	0.11
Implantation rate	47.9	33.7	48.0	42.2	38.6	0.02	0.99	0.31	0.14
Miscarriage rate	2.0	8.1	3.0	2.1	4.3	0.11	1.00	1.00	0.65

**HIGHER PREGNANCY RATES AFTER ZONA PELLUCIDA SPERM SELECTION.** Rumiana Ganeva, MSc, Dimitar Parvanov, PhD, Magdalena Vasileva, MSc, Kristina Nikolova, MSc, Georgi Stamenov Stamenov, MD/PhD Nadezhda Women's Health Hospital, Sofia, Bulgaria.



**OBJECTIVE:** To investigate the effectiveness of immobilized acid-solubilized zonae pellucidae in the selection of spermatozoa for intracytoplasmic sperm injection (ICSI).

**DESIGN:** A prospective sibling oocytes study.  
**MATERIALS AND METHODS:** In this study were included 113 couples who fulfilled the inclusion criteria: 1) unexplained infertility factor; 2) good quality oocytes; 3) fertilization failure for 3-5 consecutive ICSI procedures; 4) at least one oocyte at germinal vesicle stage (GV) and 5) at least four metaphase II oocytes retrieved during follicular puncture. Zonae pellucidae were isolated from the patient's own GVs. Zonae were acid solubilized and diluted in carbonate buffer (pH 9.6) for air dry immobilization on glass petri dishes. The partner's semen was washed and placed in the dishes. The spermatozoa that adhered on the immobilized surface were used for ICSI in the half of the retrieved oocytes from each woman. The other half of the oocytes was fertilized by conventional ICSI. In total, 312 oocytes were injected with zona-selected spermatozoa (zona-selection group) and 366 oocytes were injected with conventionally-selected spermatozoa (control group). The resulted embryos from the zona-selection and the control group were used in 43 and 50 single embryo transfers, respectively. Main outcomes were fertilization rate, embryo quality, implantation rate and pregnancy rate. Statistical analysis was performed using SPSS Software ver.21.

**RESULTS:** Slightly higher fertilization rate was observed among the oocytes injected with zona-bound spermatozoa in comparison to the conventional ICSI group (75.6% vs. 72.3%, p=0.38). Also no significant differences were observed in the embryo quality and in the implantation rates between the zona-selection and the control group (p=0.24 and p=0.59, respectively). However, the pregnancy rate was considerably higher in the zona-selection group when compared with the control group (34.8% vs. 16.4%, p=0.02). Moreover the miscarriage rate also differed significantly (7% in zona-selection vs. 18% in control group, p=0.03).

**CONCLUSIONS:** The use of patient's zona pellucida immobilized proteins in selection of spermatozoa for ICSI increases pregnancy rates and reduces the risk of miscarriage in couples with unexplained infertility and good quality oocytes.

**IMPACT OF A MODIFIED ICSI INJECTION PROCEDURE IN ART OUTCOMES: PRELIMINARY FINDINGS.** Claudia G. Petersen, Ph.D.,<sup>a</sup> Ana Lucia Mauri, B.Sc.,<sup>a</sup> Mariana Mattila, B.Sc.,<sup>b</sup> Laura D. Vagnini, B.Sc.,<sup>c</sup> Adriana Renzi, Ph.D.,<sup>c</sup> Bruna Petersen, B.Sc.,<sup>a</sup> Andreia Nicoletti, R.N.,<sup>b</sup> Felipe Dieamant, M.D.,<sup>a</sup> Joao Batista A. Oliveira, M.D., Ph.D.,<sup>a</sup> Ricardo L. R. Baruffi, M.D.,<sup>a</sup> Jose G. Franco Jr., M.D., Ph.D.<sup>a</sup>



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**OBJECTIVE:** The objective of this study was to evaluate if several pushes in oolema membrane at the moment of injection together with the deposition of spermatozoa under the oocyte's cortex impact ICSI outcomes.

TABLE 1. Results

	Standard ICSI	Modified ICSI	P
n	114	64	
Age(years)	37.8±3.8	37.8±4.2	0.4
MII oocytes(n)	5.2±3.2	5.8±3.7	0.5
Fertilization(%)	62.2±27.1	64.9±32.0	0.2
Fertilization failure(%)	13.2%	7.8%	0.3
Embryos transferred(n)	2.0±0.8	2.2±0.7	0.2
Implantation rate(%)	14.7%	25%	0.01
Clinical pregnancy rate/cycle(%)	23%	42%	0.01
Clinical pregnancy rate/transfer(%)	26%	46%	0.01

**DESIGN:** Matched case-control study.

**MATERIALS AND METHODS:** A total of 178 patients who were submitted to ICSI procedure were include. At the moment of ICSI procedure, two differs injection procedures was performed:

- Group 1/standard ICSI(n:114)-patient who in all their MII oocytes ICSI was performed with standard injection procedure. Standard injection procedure included those oocytes, in which at the moment of the injection, the injecting needle was pushed once against the oocytes and introduced till the center of the oocyte, where the spermatozoa was deposited.
- Group 2/modified ICSI(n:64)-patients who in all their MII oocytes ICSI was performed with a modified injection procedure. Modified injection procedure included those oocytes at the moment of the injection, the injecting needle was pushed at least 3 times against the oocytes and introduced till the cortex of the oocyte, where the spermatozoa was deposited.

The following parameters were evaluated in each group: patient age, number of oocytes in metaphase II retrieved, fertilization rate, number of embryos transferred, implantation rate and pregnancy rate.

**RESULTS:** Patients who had their oocytes injected by a modified injection procedure showed an adequate fertilization (64.9%), and higher implantation rate (25%), pregnancy rate/cycle (42%) and pregnancy rate/transfer(46%) compared with those patients in which a standard ICSI injection procedure was performed (fertilization rate:62.2%, implantation:14.7%, pregnancy rate/cycle:23%, pregnancy rate/transfer:26%). A decrease in fertilization failures was also observed in the group 2 (modified ICSI), however not significant. Table 1 shows the results.

**CONCLUSIONS:** The modified injection ICSI procedure seems to be useful for improve implantation and clinical pregnancy rates, with satisfactory results. Additional date will be important to provide more information.

**CONSIDERATION OF LOW-INVASIVE ICSI (NBP-ICSI) USING A NON BEVEL PIPET.** Satoshi Akimoto, Bachelor, Ayumi Hamaki, Bachelor, Wakana Bekku, MD, Jun Matsukawa, MD, Miwa Sato, MD, Shuichiro Hara, MD, PHD, Hiroto Tajima, MD, PHD, Hironori Asada, MD Shinyurigaoka general hospital, Kawasaki-city,Kanagawa, Japan.



**OBJECTIVE:** During intracytoplasmic sperm injection (ICSI), degeneration of punctured ovum is often encountered due to the high probability of a weakened egg membrane. In response to such cases, low-invasive ICSI using a Non Bevel Pipet (NBP-ICSI) is performed at this hospital. Consequently at this time, achievement of this procedure was considered.

**DESIGN:** Cases with a history of weakened egg membrane and high rate (10% and higher) of degeneration were divided in two periods, a period when NBP-ICSI was implemented, and a period when conventional-ICSI (C-ICSI) was implemented. Culture achievement between the two groups was retrospectively considered.

**MATERIALS AND METHODS:** NBP-ICSI : ①ã€€€An incision is made into a section of the zona pellucida using a PZD pipet, during hyaluronidase treatment. ② During ICSI, a Non Bevel Pipet manufactured for use in PIEZO-ICSI is processed and used. The Non Bevel Pipet is inserted from the slit made in ①, and ICSI is performed.

C-ICSI : ICSI is performed using a regular ICSI pipet. Evaluation items, including fertility rate for each ICSI method, degeneration rate after ICSI, blastocyst formation ratio, favorable blastocyst ratio at Day 5, embryo use ratio, were compared and considered.

**RESULTS:** Regarding culture achievement for NBP-ICSI, fertility rate:69.2%(110/159), degeneration rate after ICSI:8.2%(13/159), blastocyst formation ratio: 49.4%(43/87), favorable blastocyst ratio at Day 5(19/87) 21.8%, and embryo use ratio: 36.4%(40/110)were obtained. Culture achievement for C-ICSI was fertility rate:67.0%(126/188), degeneration rate after ICSI:19.1%(36/188), blastocyst formation ratio: 50.0%(42/84), favorable blastocyst ratio at Day 5(16/84)19.0%, and embryo use ratio: 39.7%(50/126). From the results, NBP-ICSI showed a significant lower degeneration rate after ICSI. No significant difference was observed in other evaluation items.

**CONCLUSIONS:** Achievement of low-invasive ICSI using NBP-ICSI showed the same level of achievement as PIEZO-ICSI which has been reported by many facilities, indicating the possibility of reducing the degeneration rate in cases which have a higher degeneration rate after ICSI. However, since no difference was observed for the evaluation items other than the degeneration rate, basic problems during fertilization and development due to a weakened egg membrane in ICSI still remain and are not thought to be resolved even if invasiveness in ICSI is reduced. In the consideration at this time, the fact that this method has lower invasiveness in ICSI could be

clarified, and the same level of achievement as PIEZO-ICSI or even better can be expected. In the future, expanding the application range of this method to cases with normal egg membranes, along with further consideration as to whether NBP-ICSI can improve culture achievement or not, is desired.

## ART LAB - SPERM

P-433 Wednesday, October 16, 2019 6:30 AM

### WHAT IS THE BEST SPERM SOURCE AND METHOD OF SPERM SELECTION IN CASES WITH ABNORMAL SEMINAL OXIDATION-REDUCTION POTENTIAL (ORP) LEVELS ON THE DAY OF ICSI?



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**OBJECTIVE:** To investigate whether PICSU or TESA is better for the selection of sperm in cases of abnormal seminal ORP levels for ICSI patients.

**DESIGN:** Prospective randomized trial, which included 74 patients undergoing ICSI at a busy Fertility Clinic, Cairo, Egypt, from January 2018 to January 2019. [ClinicalTrials.gov](https://clinicaltrials.gov) ID: NCT03360526.

**MATERIALS AND METHODS:** A total of 74 patients with sperm counts of more than  $5 \times 10^6$ /mL and an ORP of more than 1.42 mV/ $10^6$ /mL were included in the study. Male partners were examined for infertility and seminal ORP was measured using the MiOXSYS analyzer. PICSU® dishes (Origio, Knardrupvej, Denmark) were prepared by hydrating the hyaluronan microdots with medium followed by incubation for sperm binding at 30°C. Sperm were checked for the hyaluronan binding capacity, immobilized and injected into mature oocytes. TESA was done by testicular tissue aspiration followed by sample processing and oocyte injection. Seminal ORP was tested in the same ejaculate that was used for ICSI and patients with abnormal ORP were randomized into two arms, PICSU (n=40) and TESA (n=34). Embryological parameters included: fertilization, cleavage, blastulation and good quality blastocyst rates were recorded. Pregnancy was followed up after 15 days of embryo transfer and pregnancy rate calculated. All statistical calculations were done using SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

**RESULTS:** There were no significant differences in the female age ( $30.1 \pm 4.58$  vs.  $29.2 \pm 4.16$  yrs.) ( $P=0.3569$ ), male age ( $36.4 \pm 5.87$  vs.  $34.6 \pm 6.44$  yrs.) ( $P=0.2041$ ), seminal ORP values ( $5.8 \pm 6.22$  vs.  $5.8 \pm 7.99$  mV/ $10^6$  sperm/ml) ( $P=0.9808$ ) and the number of mature injected oocytes ( $15.7 \pm 7.8$  vs.  $16.8 \pm 7.7$ ) ( $P=0.5631$ ) between the PICSU and TESA groups, respectively. The blastulation rates between PICSU and TESA showed a significant difference (60.2% vs. 48.4%;  $P=0.0114$ ). In contrast no difference in ORP levels was seen between PICSU and TESA, for fertilization (79.8% vs. 80.7%), cleavage (73.4% vs. 73.8%), high quality blastocyst (57.2% vs. 51.9%), pregnancy (67.8% vs. 50%), implantation (41.6% vs. 36.6%), and ongoing pregnancy rates (94.7% vs. 84.6%). There were also no correlations between ORP levels and fertilization ( $P=0.1523$   $R=-0.1792$ ), cleavage ( $P=0.1475$ ,  $R=-0.1724$ ), blastulation ( $P=0.1763$ ,  $R=-0.1623$ ), and the percentage of high quality blastocyst formation ( $P=0.0902$ ,  $R=-0.2055$ ). The mean ORP level for the pregnant group was  $6.36 \pm 6.98$  mV/ $10^6$  sperm/ml as compared to  $6.39 \pm 8.81$  mV/ $10^6$  sperm/ml in the non-pregnant group ( $P=0.9891$ ).

**CONCLUSIONS:** The use of PICSU as a sperm selection method in patients with abnormal seminal ORP levels may result in better selection of sperm and improved blastulation rate. Thus, contrary to reports in the literature that TESA-retrieved sperm are unexposed to seminal reactive oxygen species, our study failed to show the advantage of TESA over PICSU dishes.

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### INVESTIGATION OF DEEP LEARNING BASED DETECTION OF SPERM MORPHOLOGICAL DEFECTS.



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Japan; <sup>b</sup>Kameda IVF Clinic Makuhari, Chiba, Japan; <sup>c</sup>Kameda Medical Center, Kamogawa, Japan.

**OBJECTIVE:** Sperm selection in intracytoplasmic sperm injection (ICSI) is generally performed by embryologists' subjective visual inspection, and developing the method of an objective sperm selection and evaluation is necessary. In this study, we focused on the evaluation of sperm morphology and aimed to investigate the method to detect morphological abnormalities by computer analysis using deep learning models and to evaluate their performances.

**DESIGN:** We extracted still images of sperms from the videos, which were recorded during ICSI, and embryologists inspected the sperm morphology. We constructed models from these still images and embryologists' inspection results. We evaluated the accuracy to detect morphological defects and visualized the important region for prediction of abnormality by these models.

**MATERIALS AND METHODS:** We used a set of 1,095 images of morphologically normal sperms, which succeeded in fertilization, and another set of 475 images of morphologically abnormal sperms, which were not used for ICSI. Embryologists visually inspected these sperms and identified their morphologically abnormal sites. We conducted 2 kinds of classification. The first is whether the sperm has morphological defects. The second is which portion has morphological defects among 3 classes of the head only, both head and neck, and none. These images were analyzed with 2 kinds of convolutional neural network (deep learning) models, which were a simple model with 9 hidden layers and the VGG16 model with 22 hidden layers and pre-trained parameters in the transfer learning. We compared these model performances and examined the accuracy improvement in image size and class weight adjustment in inverse proportion to imbalanced data.

**RESULTS:** The discrimination accuracy on the morphological abnormality of the sperm in the VGG16 model was 95.6% (AUC 0.988) in 224 pixels square images, and this was better than that of the 9 hidden layers model (Accuracy 83.2%, AUC 0.959). The abnormal site classification accuracy in the VGG16 model was 87.1% (AUC 0.958). The class weight adjustment could improve the accuracy in neither the VGG16 model nor the 9 hidden layers model. On the other hand, we got similar accuracy using 64 pixels square images but we found that the models learned background noises in images through visualization of the important region.

**CONCLUSIONS:** We confirmed that the deep learning models on sperm morphology can properly identify morphological defects at high accuracy. This suggested that these models will be able to support in selecting objectively morphologically normal sperm in the future. Our models could work well even in class-imbalanced data, and the class weight adjustment was not necessary for imbalanced data. However, if the image resolution is insufficient for appropriate learning, these models could not learn well even if the accuracy of the model was high. Therefore, the visualization of important region is needed for validation of learning models. In this study, the number of samples is limited at a single facility, and we are going to add much more samples in multiple facilities to validate our method.

**SUPPORT:** Miraca Research Institute G.K.

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### DEFECTS IN SPERM CAPACITATION AND FERTILIZING ABILITY ARE HIGHLY PREVALENT IN MEN UNDERGOING FERTILITY WORKUPS, EVEN IF NORMOSPERMIC.



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Cap-Score (%)	PGP (%)	% of men having fertility exams (n=1610)	% normospermic men having fertility exams (n=948)	% men having fertility exams >10M TMC (n=1489)	% fertile men (n=76)
≤ 18	≤ 19	8	6	7	1
19 - 25	20 - 29	28	27	28	9
26 - 31	30 - 39	32	32	32	14
32 - 36	40 - 49	17	19	18	36
37 - 42	50 - 59	9	10	9	24
> 42	≥ 60	6	6	6	16

**OBJECTIVE:** Semen analysis (SA) fails to evaluate fertilizing ability and best identifies extreme infertility cases. Cap-Score™ functionally assesses sperm capacitation/male fertility and prospectively predicts pregnancy. Here, we examine the association of SA, Cap-Score, and Cap-Score's relationship with the probability of generating pregnancy in 3 cycles (PGP; Schinfeld et al., 2018), in men having fertility exams vs fertile men.

**DESIGN:** Correlation study: Cap-Score, PGP and SA metrics were compared in 1610 men questioning fertility vs 76 fertile men (pregnant partner or recent father).

**MATERIALS AND METHODS:** Semen was collected from men having SA because of fertility concerns (9 clinics; 10/2016 to 3/2019). Volume, concentration and motility were assessed (WHO criteria; morphology omitted due to variable methods). Fixed samples were shipped to Androvia for Cap-Score and PGP determination. Fertile men were assessed previously (WIRB 20152233). **Table 1** was designed with even PGP bins and evaluated by Chi-square.

**RESULTS:** 59% (948/1610) of men having SA were normospermic (volume, concentration, motility). Compared to fertile men ( $p < 0.001$ ), more men having fertility exams had Cap-Scores  $\leq 31$  (PGP bins of  $\leq 19$ , 20-29 and 30-39). Fewer than expected had Cap-Scores  $\geq 32$  (PGP bins of 40-49, 50-59 and  $\geq 60$ ). This distribution revealed a high prevalence of reduced capacitation/fertilizing ability in men having fertility exams. Defects in sperm function were equally prevalent regardless of passing any single or multiple SA metrics, or those having  $> 10$  million total motile cells (TMC;  $p = 0.990$ ).

**CONCLUSIONS:** Of normospermic men having fertility exams, 65% (616/948) had Cap-Scores  $\leq 31$  (PGP  $\leq 39\%$ ); in contrast, only 25% of fertile men (19/76) scored in this range. Conversely, only 35% (332/948) of normospermic men questioning their fertility had Cap-Scores  $\geq 32$ , in contrast to 75% of fertile men. These data support reports that reduced sperm function/fertilizing ability is common in men questioning their fertility and cannot be detected by traditional SA, contributing to the high percentage of men diagnosed with idiopathic infertility. In men having fertility exams, reduced Cap-Scores were detected equally in normospermic men vs all men examined. These data show that a test of sperm capacitation offers a powerful complement to traditional SA, capable of identifying normospermic men with reduced sperm fertilizing ability.

Reference: Schinfeld et al. *Cap-Score™ prospectively predicts probability of pregnancy. Molecular Reproduction and Development.* 2018; 85 (8-9), 654-664

SUPPORT: Androvia LifeSciences LLC.

**P-436** Wednesday, October 16, 2019 6:30 AM

#### PATERNAL CONTRIBUTION TO EARLY EMBRYONIC DEVELOPMENT IN SEVERE MALE FACTOR PATIENTS.

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**OBJECTIVE:** Current evidence suggests that the maternal genome is primarily responsible for embryonic development until the cleavage stage, at which time, expression of paternal genes occurs along with activation of the embryonic genome [1]. Theoretically, sperm could influence earlier post-fertilization events, since defects in the sperm centrosome have the potential to compromise early cell division. Additionally, sperm DNA damage has been shown to adversely affect embryo quality as early as day 2 of devel-

opment [2]. Evidence regarding the association between severe male factor infertility and embryonic development, embryonic aneuploidy, or clinical outcomes within in vitro fertilization (IVF) cycles utilizing intracytoplasmic sperm injection (ICSI) is contradictory [3]. Thus, we sought to assess the relationship between severe male factor infertility and early embryonic development in an IVF model that includes ICSI and preimplantation genetic testing for aneuploidy (PGT-A).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Our study included patients at a single academic center who underwent IVF-PGT-A cycles from 2011 to 2019. ICSI was used in all study cases. Patients were divided into 2 cohorts: severe oligospermia ( $< 5$  million/mL), and normal semen analyses (SA) ( $\geq 5$  million/mL). The primary outcome was cleavage rate (CR). Secondary outcomes were fertilization rate (FR), blastulation rate (BR), euploid rate (ER), ongoing pregnancy/live birth rate (OP/LBR), and clinical loss rate (CLR). Student's t-test, chi-squares, and multivariate logistic regression analyses were used for statistical analysis, with  $p < 0.05$  considered significant.

**RESULTS:** A total of 3,029 patients underwent 3,488 IVF-PGT-A cycles during the study period, leading to 4,716 single, euploid frozen embryo transfers. In our unadjusted analysis, the FR and CR were significantly lower in the severe oligospermia group compared to the normal SA group (FR 82.30% vs 77.78%,  $p < 0.0001$ ; CR 99.25% vs 98.23%,  $p = 0.007$ ). There were no significant differences in BR, ER, or clinical pregnancy outcomes between the groups. After performing an adjusted analysis that controlled for confounding variables, a significant difference in CR between the oligospermia group and the normal SA group ( $\beta = 0.99$ ,  $p = 0.03$ ) remained.

**CONCLUSIONS:** In the largest study to date evaluating the association between the paternal genome and embryonic development, we demonstrated that oligospermic samples are associated with impaired early embryo development. Our results provide new insight into the role of the paternal genome in embryonic development prior to activation of the embryonic genome. Future studies should aim to examine more closely paternally-derived genomic actions, including epigenetic factors such as paternal centrosome function, chromatin packaging, or histone modification, which impact successful cell division and growth prior to the cleavage stage in severe male factor patients. Our findings may lead to a better understanding of the ways in which maternal-paternal genomic interactions drive early embryonic development.

References: 1. Schultz, R.M., *The molecular foundations of the maternal to zygotic transition in the preimplantation embryo.* Hum Reprod Update, 2002. 8(4): p. 323-31.

2. Simon, L., et al., *Paternal influence of sperm DNA integrity on early embryonic development.* Hum Reprod, 2014. 29(11): p. 2402-12.

3. Mazzilli, R., et al., *Effect of the male factor on the clinical outcome of intracytoplasmic sperm injection combined with preimplantation aneuploidy testing: observational longitudinal cohort study of 1,219 consecutive cycles.* Fertil Steril, 2017. 108(6): p. 961-972.e3.

SUPPORT: None.

**P-437** Wednesday, October 16, 2019 6:30 AM

#### DOES THE USE OF MICROFLUIDIC SPERM SORTING FOR THE SPERM SELECTION IMPROVE IVF SUCCESS RATES IN MALE FACTOR INFERTILITY?

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TABLE 1. Sperm parameters and cycles characteristics

Variables	Group I (n=71)	Group II (n=68)	P value
Sperm count (million/ml)	15,49±17,47	30,94±27,14	<0,01*
Ejaculate volume (ml)	3,68±1,52	3,92±1,90	0,67
Morphologically normal spermatozoa (%)	1,08±1,16	2,04±1,65	<0,01*
TMSC	5,8±38,45	8,29±74,83	0,05*
Total dosage of gonadotropins (IU)	3145±1000	2852±920	0,06
Maximum estradiol levels (pg/mL)	2074±1154	1979±1375	0,35
Duration of stimulations (day)	9,5±1,48	9,27±1,61	0,18
Endometrial thickness on hCG day (mm)	9,8±1,9	10±2,28	0,79
Total number of oocytes retrieved	9,81±6,46	11,1±6,86	0,23
Number of mature oocytes retrieved	6,69±4,31	7,76±5,16	0,28
Number of PN	5,32±3,54	4,38±3,37	0,06
Pregnancy rate	50,7% (n=36)	27,9% (19)	<0,01*

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**OBJECTIVE:** IVF success rate may improve with the selection of viable, motile, and morphologically intact sperm.

**DESIGN:** This multicentric prospective RCT was designed to evaluate the clinical outcome of ART cycles in an male factor infertility, where the spermatozoa were selected using either a conventional gradient-density centrifugation technique or microfluidic sperm sorting.

**MATERIALS AND METHODS:** A total of 139 patients who underwent IVF because of male factor infertility at Bezmialem and Yeditepe University Hospital were included in this study. All patients were randomly divided into two groups according to the sperm selection method: group I (n=71): microfluidic sperm-sorting chip; group II (n=68): density-gradient centrifugation. Data collected included male and female age, type of infertility, duration of infertility, previous IVF attempts, total dosage of gonadotropins, maximum estradiol levels, duration of stimulations, endometrial thickness on hCG day, total number of oocytes retrieved, number of mature oocytes retrieved, number of PNs, sperm count, ejaculate volume, morphologically normal spermatozoa, total motile sperm count, and clinical PR.

**RESULTS:** There was a statistically significant improvement in clinical pregnancy rates in the microfluidic sperm-sorting chip when compared to other group (50,7% vs. 27,9%; p<0.01). In group I, sperm count, morphologically normal spermatozoa, total motile sperm count were significantly lower (Table 1) (p <0,01, p <0,01 and p = 0.05, respectively). The number of PNs was also higher in group I although it did not reach statistical significance (5,32±3,54 vs 4,38±3,37, p = 0.06).

**CONCLUSIONS:** Microfluidic devices, “labs-on-a-chip”, are a disposable, easy to use, and inexpensive method for sperm sorting. Our results show that IVF success rates may improve with the use of a microfluidic sperm-sorting chip for sperm selection in male factor infertility.

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**WHICH IS THE BETTER CHOICE FOR THE AZOOSPERMIC PATIENTS WITH AZFC MICRODELETIONS, TESTICULAR SPERM OR DONOR SEMEN (AID) SPERM?** Li Zhang, Ph.D.<sup>a</sup> Jiaming Mao, MD,<sup>b</sup>



Ping Liu, MD; Ph.D.<sup>a</sup> Jie Qiao, MD; Ph.D.<sup>c</sup> <sup>a</sup>Peking University Third Hospital, Beijing, China; <sup>b</sup>Peking University Third Hospital, Beijing, China; <sup>c</sup>Peking University Third Hospital, Beijing, China.

**OBJECTIVE:** We performed a retrospective study to investigate either testicular or AID sperm is the better choice for azoospermic patients with AZFc microdeletion.

**DESIGN:** Comparing the outcomes of aPAZFcM with patients with unexplained idiopathic non-obstructive azoospermia (iNOA) undergoing ICSI with testicular and ejaculated sperm versus azoospermic patients undergoing ICSI with AID sperm was conducted after excluding infertility caused by female factors and female older than 35.

**MATERIALS AND METHODS:** We analyzed outcomes of 148 patients with AZFc microdeletions undergoing 205 cycles with ejaculated and testicular sperm, 176 iNOA patients undergoing 265 cycles with ejaculated and testicular sperm and 177 azoospermic patients with AID sperm undergoing 284 cycles between September 2015 and September 2018. Experiment groups: group A, testicular sperms for ICSI in aPAZFcM; group B, ejaculated sperm for ICSI in patients with AZFc microdeletions; group C, testicular sperm for ICSI in iNOA patients; group D, ejaculated sperms for ICSI in iNOA patients. Control group (group E): AID sperm for ICSI in azoospermic patients. The parameters were fertilization rate (FR), 2PN cleavage rate (2PNCR), blastocyst formation rate (BFR), implantation rate (IR), cumulative pregnancy rate (CPR), cumulative live-birth rate (CLBR), cumulative miscarriage rate (CMR) and Cancelled Cycle Rate (CCR). Analysis of categorical variables was evaluated with  $\chi^2$  or Fisher’s exact tests. A level of P<0.05 was considered statistically significant.

**RESULTS:** Comparing group A, group B has shown better ICSI outcome with statistically significant differences in FR, BFR, CPR, CLBR and CCR between the two groups (all p values were less than 0.02), while iNOA patients had similar ICSI outcomes either with testicular or ejaculated sperm. The group B, D and E had similar outcomes. The group E has exhibited much better ICSI outcome than group A with statistically significant differences in FR, BFR, IR, CPR, CLBR and CCR between the two groups(all p values were less than 0.005), while it was just little better than group C. The CCR is the highest in group A, and the FR is the highest in group E among all five groups.

Parameter	Group A	Group B	Group C	Group D	Group E
FR(%)	28.12	58.46	52.55	57.72	70.02
2PNCR(%)	99.01	96.56	98.71	97.81	98.55
BFR(%)	21.83	40.48	34.89	28.26	34.45
IR(%)	21.49	30.00	32.28	26.61	36.04
CPR(%)	21.28	47.56	45.93	52.94	64.32
CLBR(%)	12.77	41.46	31.4	37.25	51.76
CMR(%)	3.19	3.66	9.88	3.92	5.53
CCR(%)	22.77	10.58	8.16	2.9	1.76

**CONCLUSIONS:** Our results suggest that ICSI with ejaculated sperm is a more optimal treatment for patients with AZFc microdeletions while iNOA patients didn’t like that. ICSI with AID sperm is a better treatment for azoospermic patients with AZFc microdeletion.

Reference: No.  
SUPPORT: No.

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**SPERM SELECTION WITH HYALURONIC ACID (PICSI) IMPROVES EFFICIENCY OF IVF CYCLES.** Lucia Alegre, Ph.D.<sup>a</sup> Irene Hervás, Ph.D, student,<sup>b</sup>



Lorena Bori Amal, Ph.D.<sup>a</sup> Alberto Tejera, Sr., Ph.D.<sup>c</sup> Tamara Viloria, Ph.D.<sup>a</sup> Jose Alejandro Remohi, MD, Ph.D.<sup>d</sup> Marcos Meseguer, Ph.D.<sup>c</sup> <sup>a</sup>IVIRMA Global, Valencia, Spain; <sup>b</sup>Affiliation not

provided; <sup>c</sup>Embryologist, Valencia, Spain; <sup>d</sup>IVIRMA Valencia, Valencia, Spain; <sup>e</sup>IVIRMA Global, Valencia, Spain, Tel Aviv, Israel.

**OBJECTIVE:** Sperm immaturity is linked with sperm anomalies caused by spermatogenesis defects. The sperm selection technique PICSi (physiologic intracytoplasmic sperm injection) avoids immature spermatozoa selection before microinjection. Our purpose is to demonstrate explicitly the utility of PICSi technique in IVF cycles. We monitored from the first zygote obtained by PICSi technique until the fresh or vitrified embryo transfers derived from the treatment compared with ICSI routine technique.

**DESIGN:** This is the first reported study, up to now, where all transferred embryos were blastocyst stage and only couples undergoing oocyte donation were included, avoiding the oocyte factor bias. PICSi technique can identify mature spermatozoa from a sperm sample to select through HA (hyaluronic acid) receptors binding ability. Single centre analysis, prospective, randomized and triple-blinded trial were undertaken. In the project a total of 277 infertile couples were recruited, 142 in the PICSi group and 135 in the control.

**MATERIALS AND METHODS:** Spermatozoa were incubated in AH drops for selection before microinjection in PICSi samples. In both groups, zygotes were cultured in a time-lapse incubator (Geri, Genea or Embryoscope, Vitrolide). The study involved a total of 3104 mature injected oocytes, 2433 zygotes, 1144 viable embryos obtained (Transferred + Vitrified), 348 embryos for fresh transfer, 140 for vitrified transfer and 203 live births took place. According to the sperm concentration and motility, 4 groups were created. The possible differences between fresh and vitrified outcomes were taken into account with the cumulative pregnancy rates by performing survival curves analysis.

**RESULTS:** Blastulation rate was similar for PICSi and ICSI groups. Nevertheless, the proportion of good quality embryos in Day 5-6 was higher in PICSi. Significant differences were found in morphokinetic parameters (in hours after ICSI) between groups: tPNa (8.80h vs. 9.14h), tSB (101.55h vs. 102.60h) and BHi (114.77h vs. 110.91h) PICSi group and Control, respectively. The implantation rate was comparable between PICSi and ICSI group. The pregnancy rate was higher in PICSi group, (but non-significant) 74% vs. 70% PICSi group and Control, respectively. No differences were found comparing PICSi-ICSI in fresh or vitrified transfer cycles. PICSi group showed a higher pregnancy rate (but non-significant) when patients presented lower sperm count. No differences were observed in ongoing pregnancy rate or live birth rates between PICSi-ICSI. However, after 4 cycles of embryo transfers the cumulative pregnancy rate in PICSi was significantly higher 88%, while in ICSI group was 71% (LogRank and Tarone-ware Test <0.05).

**CONCLUSIONS:** No differences between embryo quality and development potential were found between PICSi and ICSI groups; nevertheless, the global efficiency of PICSi cycles was higher. The use of the PICSi technique could be a competitive advantage for patients undergoing oocyte donation especially in those cases in which pregnancy is not successfully accomplished after first cycle.

**SUPPORT:** PI14/00523. Spanish Ministry of Economy and Competitiveness. Instituto de Salud Carlos III program.

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#### **SELECTING SPERMATOZOA WITH INTACT CHROMATIN MAY REDUCE EMBRYO ANEUPLOIDY.**

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**OBJECTIVE:** We tested a novel approach for treating couples with complete and persistent embryo aneuploidy. Using a microfluidic device, we selected spermatozoa with the highest progressive motility and genomic integrity, capable of generating euploid embryos.

**DESIGN:** From October 2016 to April 2019, 13 consenting couples with male partners with high sperm chromatin fragmentation (SCF) in their ejaculate and a history of embryo aneuploidy and/or recurring implantation failure underwent a new ICSI cycle in which semen specimens were processed by microfluidics sperm selection (MFSS) and density gradient centrifugation (DGC).

**MATERIALS AND METHODS:** Consenting men had their ejaculates screened by standard semen analysis according to WHO 2010 criteria. Spec-

imens were processed by DGC and MFSS. SCF was measured by TUNEL utilizing a commercial kit (In Situ Cell Death Detection Kit, Roche). At least 500 spermatozoa were counted under fluorescent microscopy, with an established threshold of 15%. Fertilization and clinical pregnancy rates were assessed and compared between the two preparation methods.

Preimplantation genetic testing for aneuploidy (PGT-A) was performed on the resulting embryos. Embryo implantation and pregnancies after replacement of thawed euploid blastocysts were recorded.

**RESULTS:** A total of 13 men with an average age of 41.5±10 years had the following average semen parameters: concentration of 40.5±44 x10<sup>6</sup>/mL, 26±19 motility, and 2.2±1% morphology. After DGC and MFSS, the sperm concentration was 27±41 and 4.4±7 x10<sup>6</sup>/mL, with 47.7±43% and 97.1±4% motility, respectively (*P*<0.0001). The average SCF decreased from 29% in the raw samples to 20% following DGC (*P*=NS), and dropped to 2.2% after MFSS processing (*P*<0.0001). These couples (female partner, 39±6 years) underwent 15 ICSI cycles with DGC-selected spermatozoa and achieved a fertilization rate of 71.3% (97/136), which generated 64.5% (31/48) morphologically good-quality embryos; of these, 12.5% (6/48) were determined by PGT-A to be euploid. Two of these euploid embryos were transferred and did not yield a pregnancy. In a subsequent ICSI cycle with MFSS processing, a fertilization rate of 73.2% (104/142) resulted in 61.8% (34/55) good-quality embryos. Of these, 36.3% (20/55) were identified as euploid and were cryopreserved (*P*<0.05). Seven couples received a thawed single euploid blastocyst and all 7 became pregnant (*P*<0.0001), resulting in the delivery of two healthy babies, with 71.4% (5/7) still ongoing.

**CONCLUSIONS:** Dysfunction of the male genital tract increases both single-strand (ss) and double-strand (ds) DNA nicks and breaks, resulting in spermatozoa that impair embryonic development. Because dsDNA breaks in the male gamete can be responsible for embryo aneuploidy, the use of MFSS processing to select spermatozoa with the highest motility and genomic integrity may enhance the chances of obtaining a euploid conceptus for transfer.

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#### **ASSESSING AND RESTORING GAMETE FERTILIZING ABILITY.**

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**OBJECTIVE:** To successfully achieve ICSI fertilization in couples with a history of complete fertilization failure due to a lack of sperm cytosolic activating factor.

**DESIGN:** In a prospective controlled manner, consenting couples (IRB 0712009553) with a history of ICSI fertilization failure were included. Various tests were carried out on the male partners' ejaculates to confirm sperm-related activation deficiencies. Following the utilization of a proprietary gamete treatment method in a subsequent ICSI cycle, embryology and clinical outcomes were recorded and compared with same-patient history cycles.

**MATERIALS AND METHODS:** Spermatozoa were assessed by standard semen analysis. According to the initial morphological evaluation, subsequent tests were performed. These included an in-house PLCζ assay to screen for the presence of sperm cytosolic activating factor, and aniline blue staining to assess protamine content. Transmission electron microscopy (TEM) and mouse oocyte activation test (MOAT) were also used to identify structural and functional deficiencies. A proprietary gamete treatment method was performed with ICSI by pre-treatment of spermatozoa and post-injection oocyte activation.

**RESULTS:** A total of 22 couples (maternal age, 35.8±5 yrs; paternal age, 40.1±6 yrs) were included. Prior to undergoing cycles with gamete treatment, these couples underwent a total of 29 ICSI cycles, resulting in a fertilization rate of 10.6% (23/216). However, no couples received a conceptus due to poor embryo development.

Ejaculated spermatozoa were assessed by semen analysis, which yielded an average concentration of 63.8±47x10<sup>6</sup>/ml, 40.6±19% motility, and an overall normal morphology of 1.1±0.5%, with >90% head defects. Confirmatory TEM revealed 93.3±12% occurrence of round heads in patients (n=5) diagnosed with globozoospermia. Both the in-house PLCζ assay

and MOAT indicated a lack of sperm cytosolic activating factor and compromised fertilizing aptitude. The aniline blue assay also showed a sperm chromatin condensation deficiency, particularly in the globozoospermic patients, with a sperm chromatin fragmentation of 16.8%, corroborated by a 1.9% FISH aneuploidy.

All couples underwent a total of 37 ICSI cycles with gamete treatment, resulting in a 41.2% (120/291) fertilization rate and a 33.3% (8/24) clinical pregnancy rate ( $P < 0.05$ ). Of the 8 couples who achieved a clinical pregnancy, 4 delivered a healthy baby.

**CONCLUSIONS:** In couples with recurrent and complete fertilization failure, the application of a battery of bioassays can help to assess sperm activating factor dysfunction and compromised fertilizing ability. In these couples, gamete treatment in a subsequent cycle enhances the chances of fertilization and successful pregnancies. The achievement of healthy offspring indicates that gamete treatment to overcome fertilization failure appears safe.

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#### **SECOND EJACULATION: A SIMPLE, COST FREE MECHANISM TO DEAL WITH HIGH SPERM DNA FRAGMENTATION.**

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**OBJECTIVE:** High sperm DNA fragmentation is a controversial subject. However, many physicians test for DNA fragmentation and feel it is important. If high, methods of dealing with DNA fragmentation include testicular sperm aspiration, Anexin sperm wash and ICSI. These procedures add cost, pain after surgery and are of undetermined value. Sperm DNA fragmentation is felt to occur in the epididymis while waiting to be expelled. This study was undertaken to determine if a second ejaculation 3-hours after the first could improve sperm DNA fragmentation, by limiting time in the epididymis.

**DESIGN:** A prospective cohort study where males were requested to wait 3-days without an ejaculation at which point a semen analysis and DNA fragmentation was performed and repeated 3-hours later on a 2<sup>nd</sup> specimen.

**MATERIALS AND METHODS:** 112 subjects underwent the two semen analysis protocol as part of the fertility evaluation. All ejaculations were performed at the fertility center. DNA fragmentation was evaluated using the halo test. Data was compared by intra-subject t-test. Data is presented as % or mean $\pm$ SD. Power analysis suggested  $\geq 73$  subjects were required for an 80% power and an alpha of 5% with a 2 unit mean difference with SD of 6 units. High DNA fragmentation is  $>35\%$ .

**RESULTS:** Male age was 36 $\pm$ 7 years (range 29-65). DNA fragmentation decreased from 34.6 $\pm$ 19.4 to 23.7 $\pm$ 16.0% ( $p < 0.0001$ ) in the 1<sup>st</sup> and 2<sup>nd</sup> specimen respectively. Average percentage improvement 23% $\pm$ 30%. Among subjects with high fragmentation 22/49 (45%) failed to improve into the normal range. Regarding subjects with initial DNA fragmentation  $>35\%$ , comparison of 1<sup>st</sup> and second 2<sup>nd</sup> fragmentation were 52 $\pm$ 16% & 36 $\pm$ 17% ( $p < 0.0001$ ), respectively. Greatest improvement was 97%-28% DNA fragmentation. 7/112 had worse DNA fragmentation in the second specimen and of those only 2 fell above the normal range, both with a first specimen above the normal range as well. Among semen parameters volume went from 3.1 $\pm$ 3.3ml to 1.9 $\pm$ 0.8ml,  $p = 0.0001$ , concentration from 41 $\pm$ 39 to 32 $\pm$ 31 million/ml,  $p = 0.001$  & progressive motility increased from 57 $\pm$ 21% to 60 $\pm$ 21%,  $p = 0.06$ . In none of the cases where total motile sperm count was greater than 5 million did the quality of the second semen specimen convert the subject to ICSI. The first 10 subjects had both 1st and 2nd DNA fragmentation confirmed with the TUNEL assay and equivalent improvements were seen  $r = 0.97$  ( $p < 0.05$ ), this was not continued due to cost assumed by the clinic.

**CONCLUSIONS:** High DNA sperm fragmentation can often be managed with a second ejaculation 3 hours after the first. Changes in sperm quality are not clinically significant and none of the ICSI specimens from ejaculation 1 would have required ICSI based on the ejaculation 3 hours later. 55% improve into the normal range. Therefore, a second ejaculation represents a safe, cost free mechanism to deal with this issue in many patients.

**SUPPORT:** None.

**P-443** Wednesday, October 16, 2019 6:30 AM

#### **SPERM-BORNE mRNAs AS A BIOMARKER FOR HUMAN SPERM QUALITY.**

Yunge Tang, Master degree,<sup>a</sup> Ying Zhang, MD, PhD,<sup>b</sup> Wenzhong Zhao, PhD,<sup>b</sup> Xinzong Zhang, MD, PhD,<sup>b</sup> Weibing Qin, MD, PhD,<sup>b</sup> Shunmei Deng, MD,<sup>b</sup> Jiabao Wu, BSc,<sup>b</sup> Mengyuan Zhang, PhD,<sup>b</sup> Wei Yan, M.D., Ph.D.<sup>c</sup> <sup>a</sup>Family Planning Research Institute of Guangdong Province, Guangzhou, China; <sup>b</sup>Affiliation not provided; <sup>c</sup>University of Nevada, Reno School of Medicine, Reno, NV.



**OBJECTIVE:** Although the World Health Organization (WHO) criteria for semen quality are widely followed, a significant proportion of sperm samples provided by sperm banks around the world fail to lead to successful pregnancies, highlighting the needs for better biomarkers that allow for identification of truly fertile sperm.

**DESIGN:** Laboratory study using human sperm samples.

**MATERIALS AND METHODS:** We profiled and compared mRNAs in sperm samples with higher (5 pregnancies out of  $<20$  attempts,  $>25\%$ ;  $n = 10$ ) and lower ( $<1$  pregnancy out of 30 attempts,  $<3.3\%$ ;  $n = 10$ ) pregnancy rates using RNA-Seq. Among numerous differentially expressed genes (DEGs) identified between sperm with high (HPR) and low (LPR) pregnancy rates.

**RESULTS:** We selected 23 spermatogenesis-related genes and 10 energy metabolic genes as potential biomarkers for sperm quality because these showed the greatest difference in abundance in the two groups. Further optimization by examining their expression levels in 30 HPR and 30 LPR sperm yielded a list of 9 genes that were selected as biomarkers because they could distinguish sperm samples with extremely high ( $>40\%$ ) or extremely low ( $<1\%$ ) pregnancy rates. We then re-tested all of the 60 samples in a blinded manner (i.e., no sample information provided to the examiner) and our results showed that these 9 genes can reliably distinguish the two extreme groups.

**CONCLUSIONS:** Our data suggest that sperm-borne mRNAs can be excellent biomarkers for predicting the fertility potential of sperm in addition to the current motility- and morphology-based methods. We are exploring other RNA species as well as epigenetic markers as potential biomarkers for human sperm quality.

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#### **DEEP LEARNING-ENABLED SMARTPHONE-BASED SYSTEM FOR AUTOMATED EMBRYO ASSESSMENTS AND EVALUATION.**

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**OBJECTIVE:** Traditionally, embryos are visually assessed by embryologists and the selection process has been shown to be highly subjective. Commercially available time-lapse imaging (TLI) systems have provided a standardized imaging platform and they provide automated and uninterrupted continuous imaging of embryos over the course of in-vitro embryo development. Recent reports of artificial intelligence (AI) systems make use of data obtained from such time-lapse systems<sup>1, 2</sup>. However, these systems are large and prohibitively expensive. Here, as proof-of-concept, we report for the first time, the development and evaluation of an inexpensive smartphone-based system that can perform embryo evaluations using deep-convolutional neural networks (CNN) on-phone.

**DESIGN:** We have developed an inexpensive ( $< \$5$ ) smartphone imaging system that can be used to image embryos during in-vitro culture. The smartphone-based system automatically evaluates embryos based on their morphology using an AI algorithm. We used a depthwise convolutional deep neural network and transfer-learned with retrospective embryo images captured at 113 hours post insemination (hpi) that was annotated by a total of 10 embryologists. We evaluated the system to differentiate 50 embryos based on their blastocyst status.

**MATERIALS AND METHODS:** Our device consisted of a 3D-printed housing that contained the objective lenses extracted from DVDs, a light

source, and batteries. A smartphone application was developed which performed the analysis locally. The AI utilized by the application was transfer-learned, trained, and validated with 1790 embryo images. To test our system, 50 embryos donated by patients were imaged using the smartphone system at 113 hpi. Images were automatically analyzed by our developed network without the need for any image processing. Performance metrics were calculated for the smartphone system and the overall performance of the smartphone system with the performance of deep-learning based approach that used Embryoscope data was compared.

**RESULTS:** The accuracy of such a system in classifying 50 embryos based on their blastocyst status was 96% (CI: 86.29% to 99.51%). Its sensitivity and specificity were 93.55% (CI: 78.58% to 99.21%) and 100% (CI: 82.35% to 100%), respectively, while its positive and negative predictive values were 100% and 90.48% (CI: 71.32% to 97.32%), respectively. A chi-squared analysis comparing the performance of an Embryoscope-based deep-learning approach with our smartphone system-based deep-learning approach revealed an insignificant difference of 5.03% ( $P=0.33$ ,  $P>0.05$ ).

**CONCLUSIONS:** The results reported here demonstrate that combined with the use of an AI-empowered imaging system, automated embryo analysis is not limited to only expensive time-lapse hardware and inexpensive (<\$100) systems can be developed for use at fertility centers without loss in performance. The overall impact of our AI-empowered system is significant since it enables integration into clinical practices at resource-limited settings at very minimal costs.

References: I. I. Dimitriadis, C. L. Bormann, P. Thirumalaraju, M. Kanakasabapathy, R. Gupta, R. Pooniwal, I. Souter, J. Y. Hsu, S. T. Rice, P. Bhowmick and H. Shafiee, *Fertility and sterility*, 2019, **111**, e21.

2. P. Thirumalaraju, J. Y. Hsu, C. L. Bormann, M. Kanakasabapathy, I. Souter, I. Dimitriadis, K. A. Dickinson, R. Pooniwal, R. Gupta, V. Yogesh and H. Shafiee, *Fertility and sterility*, 2019, **111**, e29.

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#### **CHARACTERIZATION OF GLOBOZOOSPERMIA AND THE EFFICACY OF ASSISTED OOCYTE ACTIVATION (AOA) IN AFFLICTED PATIENTS.**

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**OBJECTIVE:** To characterize the phenomenon of globozoospermia using various biomarkers and analyze reproductive outcomes in afflicted patients.

**DESIGN:** In 5 consenting men with globozoospermia, we assessed protamine content, sperm chromatin fragmentation (SCF), sperm aneuploidy, ultrastructural details by TEM, and epigenome. ICSI cycles with or without AOA were performed on 3 couples, and outcomes were compared.

**MATERIALS AND METHODS:** Semen analyses were performed on ejaculates of 5 consenting men. Protamine content was measured by Aniline Blue assay on 200 spermatozoa, with a  $\leq 20\%$  normal threshold. SCF scored by TUNEL assay examined 500 spermatozoa, with a  $\leq 15\%$  normal threshold. Aneuploidy rate assessed by FISH was performed on 1000 spermatozoa, with a  $< 1.6\%$  normal threshold. Confirmatory TEM allowed observation of sperm ultrastructural details to confirm the extent of globozoospermia. The transcriptome of 1 man was profiled and compared to donor specimen with proven fertility. AOA was performed by exposing post-ICSI oocytes to calcium ionophore. ICSI outcomes were compared using chi-square, with 0.05 considered significant.

**RESULTS:** Men ( $34.1 \pm 4$  years) had an average concentration of  $39.6 \pm 33 \times 10^6/\text{ml}$ , motility of  $31.8 \pm 23\%$ , and normal morphology of  $0.1 \pm 0.3\%$ . Men were considered globozoospermic when standard morphology assessment showed that  $>70\%$  of their spermatozoa had round heads. Concurrent testing revealed abnormal protamine content of  $40.3 \pm 8\%$ , borderline normal SCF of  $14.4 \pm 2\%$ , and an aneuploidy rate of  $4.8 \pm 4\%$ . Confirmatory TEM found  $93.3 \pm 12\%$  occurrence of round heads. Complete globozoospermia was confirmed in 3 men. Epigenetic analysis of one man elucidated 2 under-expressed genes: MMP14 ( $P < 0.05$ ) and

AHNAK2 ( $P < 0.05$ ). MMP14 encodes for matrix metalloproteinase involved in reproduction and embryo development, and AHNAK2 encodes nucleoprotein associated with calcium signaling and inferential oocyte activation. Three couples (male age,  $35.3 \pm 4$  years; female age,  $33.7 \pm 2$  years) underwent ICSI cycles ( $n=9$ ). AOA cycles ( $n=4$ ) resulted in a  $59.2 \pm 15\%$  fertilization rate,  $77.8\%$  cleavage rate,  $33.5 \pm 17\%$  embryo transfer (ET) rate, and  $25\%$  clinical pregnancy rate (CPR) and delivery rate. Cycles without AOA ( $n=5$ ) yielded  $11.6 \pm 15\%$  fertilization rate,  $100\%$  cleavage rate,  $7.6 \pm 9\%$  ET rate, and  $0\%$  CPR. AOA cycles had higher fertilization rates ( $P<0.0005$ ). In 2 couples with complete globozoospermia, all cycles without AOA resulted in fertilization failure, while AOA cycles reported a fertilization rate of  $59.2 \pm 15\%$ .

**CONCLUSIONS:** This study reported 2 novel genes related to globozoospermia, which can cause spermiogenic abnormality and hinder oocyte activation. We also found that AOA can greatly enhance ICSI fertilization of globozoospermic men and is paramount in those with complete form.

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#### **173 BABIES BORN AFTER ROUND SPERMATID INJECTION INTO OOCYTES: SURVEY OF THEIR DEVELOPMENT FROM FERTILIZATION UP TO 2 YEARS OLD.**

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**OBJECTIVE:** To compare physical and cognitive development of 173 babies born after round spermatid injection (ROSI) with those born after natural conception.

**DESIGN:** Physical and cognitive development of ROSI babies recorded by parents in government-issued Mother-Child Handbook was checked and verified by attending pediatricians.

**MATERIALS AND METHODS:** 967 men participated in ROSI. 173 ROSI babies were followed up 2 years for their physical and cognitive development. Controls were 1818 naturally born babies.

Physical and cognitive development of ROSI babies (e.g., body weight increase, response to parents and understanding and speaking simple language) were comparable to those of naturally born babies.

**RESULTS:** Of 173 ROSI babies three had congenital aberrations at birth, which corrected spontaneously (ventricular septa) or after surgery (cleft lip and omphalocele).

Body weights at 12 and 18 months of age in ROSI group were significantly lower than those of natural babies. Furthermore, the body mass index at 18 months in the ROSI babies was significantly lower than in the natural group. Nevertheless, it should be noted that there was no statistical significant difference of childhood growth between two groups at 24 months of age.

No statistical differences with respect to their cognitive development were found, however a part of the response at 1 year old in the ROSI group was significantly lower than in natural babies.

**CONCLUSIONS:** This study showed that there were no significant differences between ROSI and naturally born babies in either physical or cognitive development during the first two years after birth.

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#### **A NEW SPERM PREPARATION SOLUTION IMPROVES THE OUTCOME OF HUMAN CONVENTIONAL IN VITRO FERTILIZATION WITH HYPERACTIVATED SPERMATOZOA.**

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**OBJECTIVE:** Sperm preparation in human *in vitro* fertilization (IVF) requires not only good sperm motility but also sperm physiological function. We examined the effect of ORIGIO® Gradient (OG) Series™, a sperm preparation solution that mimics the physiological environment of the spermatozoa *in vivo*. The results showed that OG improved the fertilization and early embryonic development rates in conventional (c) IVF cycles (ASRM 2017). The purpose of this study was to clarify the effect of OG on pregnancy rate after embryo transfer (ET) in c-IVF cycles and to

evaluate sperm motility function using a sperm motility analysis system (SMAS).

**DESIGN:** A prospective quasi-randomized controlled study was performed in a single IVF center between January 2016 and December 2017.

**MATERIALS AND METHODS:** Patients who undertook c-IVF were randomly allocated to two groups: for the control group, sperm preparation was performed using 80% Percoll solution (Sigma) with Sperm Washing Medium (Irvine Scientific); for the test group, sperm preparation was performed using 80% OG with ORIGIO® Sperm Wash (Origio). Sperm preparation was performed using density gradient centrifugation (25 min at 500 ×g) with a subsequent swim-up (30 min). We examined 47 cycles of fresh ET and 99 cycles of vitrified-wormed ET. Clinical pregnancy and implantation rates after ET were compared between the two groups. We evaluated the sperm motility function after sperm preparation over time using SMAS (DITECT) between the two groups. We also evaluated the fractal dimension, which is one indicator of hyperactivated spermatozoa.

**RESULTS:** There were no significant differences in patient characteristics between the two groups. Among the 47 fresh ET cycles, 26 were in the control group and 21 were in the test group. Clinical pregnancy and implantation rates in the test group were higher than in the control group (24% vs. 15%, 18% vs. 12%, respectively). Among the 99 vitrified-wormed ET cycles, 44 were in the control group and 55 were in the test group. Clinical pregnancy and implantation rates in the test group were higher than in the control group (27% vs. 18%, 21% vs. 13%, respectively). There were no significant differences in sperm motility function (straight line velocity, curvilinear velocity, average path velocity, flagellar beat cross frequency, and amplitude of the lateral head) over time between the two groups. The fractal dimension of the test group was significantly higher ( $p < 0.05$ ) than that of the control group after 5 hours ( $1.50 \pm 0.05$  vs.  $1.36 \pm 0.06$ , respectively).

**CONCLUSIONS:** Our results showed that the new sperm preparation solution improves clinical outcomes in human c-IVF programs, and suggest that the increase and maintenance of hyperactivated spermatozoa may contribute to the improvement of outcomes in c-IVF.

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**YOUNGER FEMALE AGE MAY COMPENSATE THE HIGH SPERM DNA FRAGMENTATION IN THE ART PROGRAMS.** Anastasia Kirillova, PhD,<sup>a</sup>



Irina Vjacheslavovna Ushakova, PhD,<sup>a</sup> Maria Farnakovskaya, PhD,<sup>b</sup> Yulia Kiseleva, PhD,<sup>c</sup> Olga Golubeva, PhD,<sup>c</sup> Tatiana Volodjaeva, MSC,<sup>c</sup> Nona Mishieva, PhD,<sup>d</sup> Aydar Abubakirov, PhD<sup>d</sup> <sup>a</sup>Embriologist, Moscow, Russian Federation; <sup>b</sup>National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov of Ministry of Healthcare of Russian Federation, Moscow, Russian Federation; <sup>c</sup>Embryologist, Moscow, Russian Federation; <sup>d</sup>Reproductive endocrinology, Moscow, Russian Federation.

**OBJECTIVE:** The impact of sperm DNA damage on the outcomes of IVF cycles remains controversial. The aim of our work is to determine if maternal age affects the outcomes of ART programs with high levels of partner's DNA fragmentation.

**DESIGN:** This retrospective study included 287 couples, undergoing IVF treatment (n=86), ICSI (n=98), ICSI-PGT-A (n=103) with evaluation of functional semen parameters and sperm DNA fragmentation in 2 years (2016-2018). 287 women enrolled in the study were distributed according to their age as followed: under 30 y.o. (n=78); 31-34 y.o. (n=79); 35-40 y.o. (n=89); older than 40 (n=41).

**MATERIALS AND METHODS:** Sperm DNA fragmentation was evaluated using the TUNEL assay. Fertilization and embryo culture according

to the manufacturers recommendations (COOK, Australia). Array CGH (Agilent, USA) was performed for 24-chromosome embryonic genome analysis. The fertilization rate, rates of blastocyst formation, and implantation rates were evaluated.

**RESULTS:** Our results showed that there are lower fertilization rates (72.3% vs. 84.3%;  $p < 0.05$ ) and significantly lower rates of blastocyst formation (31.8% vs. 54.2%;  $p < 0.05$ ) in the group with high values of sperm DNA fragmentation in comparison with the group with normal values of this parameter. Other results are presented in the table.

Our data demonstrates that for couples with a female partner under 30 there is no significant difference in the clinical pregnancies rates between the group with high values of sperm DNA fragmentation and the group with normal values of this parameter (39% vs 40% (IVF), 36% vs 34% (ICSI), 60% vs 59% (ICSI-PGT) respectively). On the contrary, for couples with female partners older than 31 the clinical pregnancies rates were higher for groups with normal values of sperm DNA fragmentation compared to the groups with high values of this parameter.

**CONCLUSIONS:** Our study shows that high values of sperm DNA fragmentation do not influence the outcomes of the ART programs only when a female partner is younger than 30 y.o. Thus, we can speculate that oocytes of younger women have the ability to compensate the spermatozoa damaged DNA.

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**ICSI OUTCOMES USING SPERMATOZOA WITH OPTIMAL GENOME CHARACTERISTICS.**



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**OBJECTIVE:** To select spermatozoa with superior chromatin integrity, capable of increasing implantation and clinical pregnancy rates with ICSI.

**DESIGN:** From October 2016 to April 2019, semen specimens from consenting men (N=47) with prior ICSI failure due to high DNA fragmentation in their ejaculate were simultaneously processed by density gradient centrifugation (DGC) and microfluidic sperm selection (MFSS). TUNEL was carried out on the raw specimens and on the differently selected aliquots. In men treated by ICSI with their female partners, clinical outcomes were compared between the two sperm-selection methods.

**MATERIALS AND METHODS:** Fresh ejaculate specimens from consenting men were analyzed according to WHO 2010 criteria. DGC and MFSS were used to isolate spermatozoa based on cell motility and fluid dynamics. Sperm chromatin fragmentation (SCF) was assessed by TUNEL on at least 500 spermatozoa under a fluorescent microscope utilizing a threshold of  $\geq 15\%$ .

**RESULTS:** A total of 47 men with an average age of  $40 \pm 9$  years had the following average semen parameters: concentration of  $46.9 \pm 38 \times 10^6/\text{mL}$ ,  $32.8 \pm 14$  motility, and  $2.3 \pm 1\%$  morphology. After DGC or MFSS, the sperm concentration was  $33.0 \pm 27$  and  $11.6 \pm 12 \times 10^6/\text{mL}$ , with  $62.0 \pm 31\%$  and  $97.7 \pm 2\%$  motility, respectively ( $P < 0.0001$ ).

The morphology of the raw sperm sample improved from  $2.3 \pm 1\%$  to  $3.6 \pm 1\%$  after MFSS, while it remained at  $2.4 \pm 1\%$  after DGC. The average SCF decreased from 24% in raw samples to 15% following DGC, and fell to 1.7% after MFSS processing ( $P < 0.0001$ ).

Couples (n=16) who underwent ICSI had an average SCF in their raw sample of 27.1%, which became 19% after DGC selection and only 1.6% after MFSS ( $P < 0.0001$ ). These couples (female age,  $38 \pm 5$  years; male age,  $40 \pm 8$  years) underwent 38 cycles with DGS sperm selection, achieving a fertilization rate of 67%. The implantation rate was only

TABLE. Rates of clinical pregnancies, %

Age of women	IVF (<30)	ICSI (<30)	ICSI-PGT (<30)	IVF (31-34)	ICSI (31-34)	ICSI-PGT (31-34)	IVF (35-40)	ICSI (35-40)	ICSI-PGT (35-40)	IVF (>40)	ICSI (>40)	ICSI-PGT (>40)
Level of DNA fragmentation <15%	39%	36%	60%	34%	33%	52%	29%	31%	33%	11%	14%	23%
Level of DNA fragmentation >15%	40%	34%	59%	28%	24%	41%	18%	19%	21%	0%	0%	2%

3.5% (2/57) with a clinical pregnancy rate of 7.6% (2/26), resulting in one delivery and one pregnancy loss. Subsequently, these couples underwent 21 ICSI cycles with MFSS and achieved a fertilization rate of 65%. The clinical pregnancy rate rose to 61.5% (8/13;  $P < 0.05$ ), with a pregnancy loss of 12.5% (1/8) and an implantation rate of 36% (9/25;  $P < 0.0001$ ).

**CONCLUSIONS:** According to our study, SCF appears to be linked to the kinetic characteristics of the sperm cell. MFSS yielded the highest portion of progressively motile sperm with the highest DNA integrity. A microfluidic sperm selection system may serve to identify spermatozoa with the greatest potential to enhance embryonic developmental competence.

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#### **FROM FERTILIZATION TO BLASTOCYST: A COMPARATIVE STUDY OF TESTICULAR TO EJACULATED SPERM IN INTRACYTOPLASMIC SPERM INJECTION (ICSI) TREATMENT CYCLES.**



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**OBJECTIVE:** To explore the effect of sperm source on high quality blastocyst development.

**DESIGN:** Retrospective case control.

**MATERIALS AND METHODS:** Data for retrospective analysis was gathered from patients who received In vitro Fertilization (IVF) treatment from January 2007 to May 2017. We examined how many 2 pro-nuclear embryos progressed to high quality blastocyst in 1) ICSI cycles with 132 Testicular biopsy/aspiration compared with 2)  $n = 132$  ICSI cycles with ejaculated sperm with an initial Total Motile Count (TMC)  $> 0$  and  $< 5$  million and 3)  $n = 132$  ICSI cycles with ejaculated sperm with a TMC  $\geq 5$  million using analysis of covariance (ANCOVA) testing. Gardner's embryo grading scale<sup>1</sup> was used for blastocyst grading. 4BB or better was considered a high quality blastocyst. High quality day 2 embryos were defined as 4-6 cells with  $\leq 10\%$  fragmentation. Female age, follicle stimulating hormone, body mass index (BMI), sperm analysis parameters, the number of fertilized 2 pro-nuclei, day 2 embryos, day 5 blastocyst data, and day 6 blastocyst data were compared.

**RESULTS:** No significant difference was found in regard to maternal age, ovarian reserve and BMI in all groups. Significant baseline differences in the number of 2pns were found when assessing day 2 embryo development ( $F(6,361) = 341.26, p = 0.20$ ). Sperm source was not found to have a significant effect ( $F(6,361) = 0.53, p = 0.4652$ ) on high quality day 2 embryo development; means were 2.9 embryos (SD = 3.0) in the testicular biopsy group, 3.6 embryos (SD = 3.4) in the high TMC ejaculated sperm group and 4.0 embryos (SD = 3.7) in the low TMC ejaculated sperm group. Sperm source was found to have a significant effect on day 5 blastocyst development ( $F(6,361) = 5.40, p = 0.0207$ ); the means were 0.3 blastocysts (SD = 0.7) in the testicular biopsy group, 0.5 blastocysts (SD = 1.3) in the high TMC ejaculated sperm group, and 0.8 blastocysts (SD = 1.9) in the low TMC ejaculated sperm group. When considering the number of baseline 2pns, it was found that the effect was greater on day 5 blastocyst development ( $F(6,361) = 52.44, p = 0.0$ ). With regard to day 6 blastocyst development, the number of 2pns had the greatest effect ( $F(6,361) = 1.19, p = 0.2764$ ), and sperm source was not found to have a significant effect ( $F(6,361) = 75.14, p = 0.0$ ). The means of high quality day 6 blastocyst development were 0.4 blastocysts (SD = 0.9) in the testicular biopsy group, 0.8 blastocysts (SD = 1.3) in the high TMC ejaculated sperm group and 0.7 blastocysts (SD = 1.1) in the low TMC ejaculated sperm group. The testicular biopsy group showed an effect of day 5 blastocyst development when controlling for BMI, FSH levels and maternal age, but no other significant effects were seen on day 2 embryo and day 6 blastocyst development were seen. Differences in the baseline number of 2pns seemed to have the greatest impact.

**CONCLUSIONS:** This study showed that testicular biopsy sperm produces poorer day 5 blastocyst embryo development when compared to ejaculated sperm ICSI cycles. Male factor infertility may have a negative effect not only on high quality blastocyst development but also the method of sperm production may have an impact as well.

Reference: 1. <sup>1</sup> Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. In: Jansen R, Mortimer D, eds. Towards reproductive certainty. Carnforth: Parthenon Press, 1999: 378–388.

SUPPORT: None.

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#### **SPERM RETRIEVAL RATES AND CLINICAL OUTCOMES WITH TESTICULAR SPERM EXTRACTION IN RELATION TO THE ETIOLOGY OF AZOOSPERMIA.**



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**OBJECTIVE:** Sperm retrieval rates (SRR) and clinical outcomes after intracytoplasmic sperm injection (ICSI) in testicular sperm extraction (TESE) cases in relation to the etiology of azoospermia have not well been investigated yet. Here we report our latest five-year experience in TESE.

**DESIGN:** Retrospective clinical analysis.

**MATERIALS AND METHODS:** This study investigated SRR of conventional TESE in obstructive azoospermia (OA) and microdissection TESE in cryptozoospermia and non-obstructive azoospermia (NOA) patients between September 2013 and December 2018 (1455 TESE attempts with 1222 patients). The etiologies of NOA were categorized as unexplained, Klinefelter's syndrome (KS), post chemotherapy, post orchiopexy, and microdeletion of azoospermia factor (AZF) c on the Y chromosome. A total of 473 couples had 1128 TESE-ICSI cycles (136 couples and 332 cycles with OA and 337 couples and 796 cycles with NOA) were evaluated with respect to fertilization, embryonic development and clinical pregnancy rates (CPR).

**RESULTS:** SRR of patients with first TESE attempts (49.9%) was significantly higher than that of patients who previously failed sperm retrieval (32.5%) ( $P < 0.001$ ). In the first TESE cases, SSRs were 100% (155/155) in OA, 21.2% (102/482) in unexplained NOA, 50.5% (54/107) in KS, 47.8% (22/46) in post chemotherapy, 75.0% (33/44) in post orchiopexy, and 87.1% (27/31) in AZFc microdeletion, respectively. SRR of OA was significantly higher, while that of unexplained NOA was lower than any other groups. Normal fertilization rates in OA (62.0%) and post chemotherapy (63.4%) were significantly higher, but that of AZFc microdeletion (39.7%) was significantly lower than any other groups. Blastocyst development rate and good-quality blastocyst rate in AZFc microdeletion (27.4% and 9.3%) were significantly lower than any other groups and the rates in KS (40.5% and 15.1%) were lower than in OA (51.3% and 22.1%), post chemotherapy (50.6% and 23.6%), and post orchiopexy (52.1% of blastulation). CPRs per embryo transfer were lower in unexplained NOA (29.7%), and AZFc microdeletion (27.5%) than in OA (39.9%). We have had a total of 243 newborns so far with comparable congenital anomaly rate comparing to those with ejaculated sperm-ICSI.

**CONCLUSIONS:** The success of sperm recovery, fertilization and pre- and post-implantation development was significantly influenced by the etiology of azoospermia. However, the offspring with testicular sperm was as healthy as that with ejaculated sperm at least in our experience.

Reference: None.

SUPPORT: None.

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#### **FACTORS WHICH PREDICT IMPROVEMENT IN DNA FRAGMENTATION ON A SECOND SEMEN SPECIMEN 3 HOURS AFTER THE FIRST.**



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**OBJECTIVE:** High sperm DNA fragmentation is felt by many physicians to be important. Sperm DNA fragmentation occurs in the epididymus while waiting for ejaculation, so shortening the time since last ejaculation to 3 hours from 3 days improved DNA fragmentation results by an average of 23%. This study was undertaken to determine what factors predict at least a 30% improvement in sperm DNA fragmentation when comparing a first ejaculate after 3 days of abstinence and a second 3 hours after the first.

**DESIGN:** A prospective cohort study was performed on semen analysis. Males waited 3 days without an ejaculation at which point a DNA fragmentation was performed and was repeated on a 2<sup>nd</sup> specimen 3 hours latter.

**MATERIALS AND METHODS:** 112 subjects underwent the 2 semen analysis protocol. All ejaculations were at the fertility center. Analysis were part of the initial work up. DNA fragmentation was evaluated with the halo test. Data was compared by intrasubject t test. Data is presented as % or mean±SD. Power analysis suggested ≥73 subjects were required for an 80% power and an alpha of 5% with a 2 unit mean difference with SD of 6 units. Stepwise multivariate logistic regression was used to model predictors of ≥30% improvement in DNA fragmentation in the second specimen.

**RESULTS:** Male age was 36±7 years (range 29-65). DNA fragmentation decreased from 34.6±19.4 to 23.7±16.0% (p<0.0001) in the 1<sup>st</sup> and 2<sup>nd</sup> specimen respectively (23%±30%). 58/112 subjects demonstrated a >30% improvement in sperm DNA fragmentation in the 2<sup>nd</sup> specimen compared to the 1<sup>st</sup>. 7/112 had worse DNA fragmentation in the 2<sup>nd</sup> specimen. Two factors predicted at least a 30% improvement in DNA fragmentation in the second specimen; male age (95% CI 0.84-0.99, p=0.03) and use of a multivitamin (95% CI 1.25-19.8, p=0.02). 1<sup>st</sup> ejaculate volume (CI 0.84-2.65), 2<sup>nd</sup> volume (CI 0.23-1.39), 1<sup>st</sup> concentration (CI 0.98-1.005), 2<sup>nd</sup> concentration (CI 0.99-1.03), 1<sup>st</sup> motility (CI 0.97-1.03), 2<sup>nd</sup> motility (CI 0.98-1.04), smoking (CI 0.28-15.7), cannabis use (CI 0.10-2.45) and fathering previous pregnancies (0.19-2.9) failed to predict improvement. Initial DNA fragmentation trended towards being a predictor of improvement (CI 1.0-1.06, p=0.06).

**CONCLUSIONS:** High DNA sperm fragmentation can often be managed with a 2<sup>nd</sup> ejaculation 3 hours after the first. Younger men and those taking a sperm improvement vitamin supplement were more likely to have at least a 30% improvement in DNA fragmentation on the second specimen. All men should be proscribed such a vitamin who will undergo this protocol. Those male with extremely high DNA fragmentation may be less likely to show a 30% improvement, likely due to the greater change in absolute number needed.

**SUPPORT:** None.

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#### **SPERM DNA FRAGMENTATION INDEX IS NOT ASSOCIATED WITH RECURRENT IVF/ICSI FAILURE.**

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**OBJECTIVE:** To assess whether DNA Fragmentation Index (DFI) or High DNA Stainability (HDS) as measured by Sperm Chromatin Structure Assay (SCSA), was predictive of recurrent in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) failure

**DESIGN:** We performed a retrospective cohort study of couples undergoing IVF, ICSI and frozen embryo transfer (FET) cycles between 2009 – 2018 performed at a large volume fertility center. SCSA was performed for all males prior to IVF/ICSI cycles.

**MATERIALS AND METHODS:** All couples between 2009 to 2018 who underwent ≥ 2 IVF/ICSI cycles, with maternal age ≤ 40 were included in our analysis. Patients having undergone prior IVF/ICSI at outside centers were excluded. Recurrent IVF/ICSI failure was defined as ≥ 2 failed IVF/ICSI cycles in couples with maternal age ≤ 40. Success was defined as a cycle that led to live birth.

**RESULTS:** A total of 393 couples with 1215 cycles were included in the analysis with a pregnancy success of 36.9% and an average live birth of 20.6%. The average (±standard deviation) female age of 34.0 ± 3.6 and an average total motile sperm count of 68.1 ± 76.7 million sperm. DFI and HDS were not predictive for achieving a pregnancy (p=0.76 & p=0.96, respectively), nor was DFI predictive of spontaneous abortion (p=0.92). However, HDS was found to be predictive of spontaneous abortion, with higher rates of HDS seen in live births vs spontaneous abortion (12.4% vs 9.3%, p=0.003). DFI and HDS were not associated with recurrent IVF failure (p=0.43, p=0.14, respectively), nor were they predictive of IVF success, defined as live birth, in those with normal values of DFI and HDS when controlling for female age.

**CONCLUSIONS:** We found that neither DFI or HDS, as assessed by SCSA, were predictive of recurrent IVF failure in patients with high DFI or HDS when controlling for female age and total motile sperm count. This finding suggests that SCSA does not predict recurrent IVF failure.

**P-454**

**WITHDRAWN**

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#### **MULTI-SITE, BLIND PROSPECTIVE TRIAL ASSESSING WHETHER SEMEN OXIDATION REDUCTION POTENTIAL (SORP) ASSESSMENT CAN BE USED TO PREDICT LOW FERTILISATION WITH CONVENTIONAL IVF: INTERIM ANALYSIS.**



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**OBJECTIVE:** To identify if static oxidation-reduction potential (sORP) can be used clinically at the time of insemination to predict low fertilisation.

**DESIGN:** Multi-site prospective control blind study involving 6 independent clinics. Interim analysis assessing data from the first 10 patients enrolled in the study. Primary outcome for the interim analysis: rate of low fertilisation (<25% 2PN/MII), normal fertilisation (2PN per MII). Secondary outcomes: overall fertilisation rate (2+PN per MII), 1PN and 3PN rates per MII, daily embryo quality, morphokinetic parameters. Inclusion Criteria: patients undergoing IVF with at least 4 follicles ≥ 10mm, and 4 mature oocytes collected.

**MATERIALS AND METHODS:** While the semen sample was prepared for IVF treatment, 30ul was analysed using Mioxsys. Samples were divided into two groups based on the reading obtained from the Mioxsys, above and below a pre-established threshold. The scientists collecting data from the Mioxsys machine were not aware of the cut-off value to determine treatment, or the outcome of the treatment at the time of data entry. Embryos were cultured in the Embryoscope.

**RESULTS:** Out of the first ten patients (134 mature oocytes), nine had normal sORP (0.12-0.93, 121 oocytes, Control), and one patient had high sORP (1.69, 13 oocytes, Treatment). Interestingly, the only patient with a low fertilisation rate (2/13, 20%) was in the Treatment group, whilst normal fertilisation rates were all normal in the Control group (ranging from 50-100%, overall 88/121=73%). Compared to Control, Treatment group had a lower 2PN rate (Control vs Treatment: 88/121=73% vs 2/13=15%, p<0.01), higher polyploidy rate (5/121=4% vs 5/13=38%, p<0.001). Difference in overall fertilisation rate approached significance (93/121=77% vs 7/13=54%, p=0.07). 1PN rate (7/121=6% vs 0/13, NS), median number of cells on day 2 (4 vs 3, NS) and day 3 (7 vs 6.5, NS), did not differ between Treatment and Control. Cleavage embryos with more day 3 fragmentation (sORP 0.66+ 0.37) or more unevenness (sORP 0.59+0.36, n=49) were associated with higher sORP than embryos with lower day 3 fragmentation (sORP 0.25+0.22 respectively, p<0.0001) or more evenness (sORP 0.38+0.4, n=25, p=0.03). However, good embryo quality rate on days 2 (45/88 vs 1/2, NS), and 3 (32/74 vs 1/2, NS) did not differ between Treatment and Control. Morphokinetics was not significantly affected by treatment.

**CONCLUSIONS:** Out of 10 patients undergoing IVF, sORP assessment correctly identified the 1 case where low fertilisation occurred. With 25% of normospermic samples leading to low fertilisation, new diagnostic tools are required to ascertain whether IVF is the correct treatment. This is the first multi-site prospective study assessing whether sORP can be used this way. Although preliminary, our results are encouraging and in line with other single-centre publications.

### THE EVALUATION OF SEMINAL OXIDATION REDUCTION POTENTIAL CAN PREDICT NORMAL SPERM PARAMETERS.

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**OBJECTIVE:** The standard semen analysis is the most popular laboratory test in diagnosis of male fertility. However, it is well-known that normal results of semen analysis can not exclude men from the causes of couples infertility. One of the most important parameters of sperm, in its fertilizing potential is Sperm DNA integrity that has direct positive correlation with Assisted Reproductive Techniques (ART). The most common cause of sperm DNA damage is Oxidative Stress (OS). The evaluation of seminal oxidatives stress have a crucial role in the identification of patients who may benefit from treatments. The aim of our study was to use MioXSYS System to evaluate OS and to correlate this evaluation sperm parameters, DNA fragmentation and chromatin decondensation.

**DESIGN:** This is a prospective comparative study that was performed between January 2018 and Mars 2019 includes patients with primary or secondary infertility ( $\geq 3$  years). Human semen samples were obtained from 200 patients performing a complete exploration of semen parameters at a private ART clinic. Sperm parameters were evaluated according to World Health Organization 2010 guidelines. Exclusion criteria included azoospermia and samples with a concentration  $< 1 \times 10^6$  sperm/mL.

**MATERIALS AND METHODS:** In each semen sample, in addition to conventional sperm parameters the following parameters were measured: (i) Spermatozoa with DNA strand breaks were assessed by TUNEL (cut-off value  $< 30\%$ ), (ii) Abnormal chromatin condensation using Aniline Blue assays (cut-off value  $< 20\%$ ), (iii) Oxidative stress was measured by MioXSYS Analyzer. The study subjects were grouped into two groups referring to a cut-off value of  $1.36 \text{ mV}/10^6$  sperm/mL of Seminal Oxidation reduction potential (sORP): group 1 with low level of sORP and group 2 with high level of sORP. We identified 2 subgroups in each group: groups (1A and 2A) had all normal criteria of sperm quality and groups (1B and 2B) failed to meet one or more criteria of sperm quality.

**RESULTS:** Comparing to patients of group 1, patients of group 2, had a significantly lower mean sperm count ( $14.73$  vs  $64.72 \times 10^6$  sperm/mL) progressive motility ( $24\%$  vs  $38\%$ ), and vitality ( $52\%$  vs  $68\%$ ). Conversely patients of this group had significantly higher levels of DNA fragmentation and chromatin decondensation. This results confirm that sORP, DNA fragmentation and chromatin decondensation were inversely associated with normal sperm parameters. When subgroups of patients were investigated according to normal or abnormal semen parameters we identified 2 subgroups in each group: a subgroup containing 25% of patients ( $n=29$ ) of group 1B failed to meet one or more criteria of sperm quality and a second subgroup contain 93% ( $n=74$ ) of group 2B. For these two subgroups we identified a negative correlation between sperm parameters and level of these two parameters: DNA fragmentation and chromatin hypocondensation ( $p < 0.001$ ).

**CONCLUSIONS:** The combination of conventional sperm parameters with the advanced sperm function test should be included in assessment of male infertility because they can have prognostic implications for couples undergoing ART.

### USEFULNESS OF A NEW SPERM TRANSPORT CONTAINER "TRANSPORTER-S" FOR INFERTILITY TREATMENT.

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**OBJECTIVE:** The container for storage and transport of ejaculated semen is "clean and wide-bore glass or plastic container" in the guidelines in usual, and it is generally used that a cylindrical container with a height of about 8 cm is used. Since this container has a large volume relative to the amount of semen, which is 100 to 200 ml, it is difficult to completely remove it when collecting it for examination. The new container (Transporter-S: TS) is less likely to be exposed to air and has excellent liquid stability and is suitable for storage and transport of ejaculated semen compared with conventional products.

**DESIGN:** Prospective study.

**MATERIALS AND METHODS:** <Examination 1>

TS is characterized in that stored samples are less susceptible to temperature changes than conventional containers, and the effects of storage environment on samples were compared between TS and conventional containers. As a substitute for semen in TS and conventional containers, put 5 ml of distilled water at  $37^\circ \text{C}$  and leave each container in an environment at room temperature ( $25^\circ \text{C}$ ) and the ambient temperature in winter (estimated at  $10^\circ \text{C}$ ) for 15 hours. It compared about the temperature change for every minute.

<Examination 2>

Sperm tests were performed on each of the 14 healthy volunteers at the same abstinence period. At that time, with respect to sperm parameters and sperm DNA fragmentation index (DFI) in seminal fluid stored in a conventional container and transporter S, place them at room temperature  $25^\circ \text{C}$ . and change over time (0 hours 2 hours 4 hours 6 hours) Measurement survey.

<Examination 3>

The volunteers who provided ejaculated semen were asked about the feeling of using TS and compared with the conventional container.

The contents of the questionnaire were evaluated by comparing the conventional container and TS: very good 5 · good 4 · normal 3 · bad 2 · very bad 1 of 5 stages.

**RESULTS:** Compared with conventional containers, TS has a slower change in sample temperature and is less susceptible to low ambient temperature and, it was more difficult to be affected when the outside temperature was low. In semen that was stored using TS, the decline in exercise rate and survival rate over time became slower than in conventional containers.

(Motor rate changes are significantly different after 4 hours and 6 hours. Sperm survival rates are also significantly different after 6 hours.) The sequestration using TS became 1 very good, 4 good, 5 normal, 1 bad, 1 bad. The average value of the questionnaire results was  $3.07 \pm 0.92$ , which was comparable to conventional containers. We think that TS use is effective in infertility treatment including the use that is not stable in the climatic area and the patient who takes time after preparation.

**CONCLUSIONS:** It is considered that TS is less likely to be exposed to air and has excellent liquid stability and is suitable for storage and transport of ejaculated semen, as compared with conventional containers. In the future, it is necessary to conduct further examinations by changing semen and storage temperature with relatively poor findings such as OAT cases.

Reference: None.

SUPPORT: None.

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### THE TIME-COURSE FROM GERMINAL-VESICLE BREAK DOWN (GVBD) TO FIRST POLAR BODY EXTRUSION (PBE) IN RESCUED *IN VITRO* MATURATION (R-IVM), A PROSPECTIVE STUDY ON TIME LAPSE IMAGING.



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**OBJECTIVE:** To access the time-course and associated factors of oocyte maturation from GVBD to PBE in r-IVM.

**DESIGN:** Non-comparative; Prospective.

**MATERIALS AND METHODS:** Patients underwent intracytoplasmic sperm injection and had at least one GV oocyte after denudation were included. After denudation, GV oocytes were cultured in G-IVF® media and placed into a time-lapse incubator. Images were taken every 10 mins for 144 hours. The GVBD and PBE time were counted. Patient's age, protocol, base and hCG day luteinizing hormone (LH), base follicle stimulation hormone (FSH), base and hCG day estradiol (E2), immaturation rate, and big follicle acquisition rate (BFA,  $BFA = [N \text{ of retrieval oocyte}] / [N \text{ of follicles} \geq 12\text{mm}]$ ) were recorded for univariable clustered Cox regression [1,2]. Variables ( $p < 0.3$ ) were chosen for multivariable analysis. Hazard ratio (HR) with 95% confidence interval (95%CI) were reported.

**RESULTS:** There were 36 patients (79 GV oocytes) recruited. 12 GV oocytes did not mature. The overall time-course was 23.2h (95%CI 21.3-24.4h). The baseline and analysis results are shown in Table I. The  $BFA < 1$  means GV oocytes were from big follicles. GV oocytes in group  $BFA < 1$  showed shortened time-course in both univariable (HR 2.43, 95%CI 1.49-4.35,  $p < .001$ ) and multivariable analysis (HR 2.38, 95%CI 1.32-4.35,  $p = 0.004$ ). The adjusted chance of maturation in group base E2 concentration  $> 50$  was revealed to be two times greater (HR 2.00 95%CI 1.06-3.81,  $p = 0.034$ ).

**CONCLUSIONS:** We first demonstrate a precise time-course of GVBD to PBE in stimulated cycles, which can contribute significantly to catching the fertilization window in r-IVM as well as traditional IVM. We found that GV oocytes from big follicles or patient with higher base E2 have higher chance of maturation. These findings give clues for oocyte and follicle development, but further studies are needed to confirm.

References: [1] Moore, D. F. (2016). Applied survival analysis using R, Springer.

[2] Glidden, D. V. and E. Vittinghoff "Modelling clustered survival data from multicentre clinical trials." Statistics in medicine 23(3): 369-388. (2004)."

TABLE I.

Characteristics	Baseline	Univariable analysis			Multivariable analysis		
		HR	95%CI	P-value	HR	95%CI	P-value
Age (year)	35.89±3.21						
≥35 (n [%])	24 (66.67)	R			R		
<35(n [%])	12 (33.33)	0.95	0.83-1.1	0.505	1.16	0.53-2.52	0.715
Stimulation protocol							
Long (n [%])	9 (25.00)	R			R		
Antagonist (n [%])	27 (75.00)	0.76	0.46-1.27	0.296	0.66	0.42-1.03	0.068
Base FSH (IU/l)	6.40 (5.55-7.98)	0.98	0.88-1.1	0.761	NC		
Base LH (IU/l)	4.55 (4.05-6.68)	1.00	0.91-1.11	0.965	NC		
LH on hCG day (IU/l)	3.35	0.98	0.91-1.06	0.655	NC		
Immaturation rate (%)	23.41±11.21	0.97	0.96-0.99	<b>0.010</b>	0.98	0.96-1.01	0.198
Base E2 (pmol/l)	104.2±48.28						
<50 (n [%])	4 (11.10)	R			R		
≥50 (n [%])	32 (88.90)	1.37	0.92-2.05	0.124	2.00	1.06-3.81	<b>0.034</b>
E2 on hCG day (pmol/l)	11550 (8433.50-15167.00)	0.78	0.49-1.26	0.317	NC		
BFA (%)	108.01 (81.77-132.35)						
<1 (n [%])	21 (58.30)	R					
≥1 (n [%])	15 (41.70)	2.43	1.49-4.35	<b>&lt;.001</b>	2.38	1.32-4.35	<b>0.004</b>

R=Reference.

NC=Not Chosen.

### A NOVEL TECHNIQUE OF USING MECHANICAL GRIPPERS TO IMMOBILIZE EMBRYO DURING BIOPSY.

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**OBJECTIVE:** Blastocysts undergo a lot of localised mechanical stress during a biopsy. Especially near the holding pipette region. Is there a way to avoid this situation and make biopsy less traumatic for the blastocyst?

**DESIGN:** The study comprises a combination of mechanical modelling, finite-element simulation of the blastocyst, and the estimation of the stress developed during biopsies of the blastocysts due to the means of mechanical manipulation—holding pipette-based aspiration vs. gripper-based mechanical locking. The study also involves blastocysts that are biopsied, size of the blastocysts, deformation pattern that they undergo during a biopsy.

**MATERIALS AND METHODS:** Two sets of blastocysts (Day-5, 25 each) are biopsied. One of the sets using micropipette-based aspiration technique and the other using the novel gripper-based technique. The procedure of the biopsy is video-graphed and images are extracted for the simulation. The mechanical stress that the blastocyst undergoes in each of the cases is simulated using a nonlinear contact-based finite element analysis. For the simulation, the blastocyst is considered as an elastic body with a heterogeneous distribution of modulus. Further, the size and the extent to which the blastocyst undergoes the deformation in each of the cases are noted and used for computing the stress-distribution. For the sake of commonality, the number of finite-elements or the mesh distribution is kept constant for all the cases of the blastocysts. The blastocyst model for the simulation is divided into 200 mesh elements. Each mesh element is akin to a cell within the blastocyst. Each of the blastocysts is subjected to the pipettes of # in diameter at the holding part and # diameter at the biopsy-end which are commonly used in the case of aspiration-based technique. In the case of gripper-based technique, the biopsy pipettes utilized are similar to those used for aspiration-based techniques while the dimension of the arms of the gripper and the cusp to accommodate the blastocyst remains the same. The peak stress for each are noted and further, the distribution of the stresses are compared.

**RESULTS:** The mechanical stress (peak) that a blastocyst undergoes with micropipette-based aspiration technique is 76% higher than that of the gripper-based technique. Also, the stress distribution in the case of gripper-based technique is observed to vary between 0.012 MPa and 0.024 MPa as against the aspiration-based technique which varies between 0.03 MPa and 0.09 MPa. This distribution indicates that the stress in case of the gripper-based technique is far more distributed than the aspiration technique

**CONCLUSIONS:** 1. The novel gripper-based technique is less traumatic to the blastocysts during a biopsy. 2. It is also observed to be easier to perform a biopsy using a gripper than a holding micropipette where the aspiration has to be dynamically changed depending upon the pressure of aspirating the biopsy.

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**DAY 2 LASER ASSISTED HATCHING (AH) SIGNIFICANTLY IMPROVES IMPLANTATION RATES IN FRESH BLASTOCYST TRANSFERS.**

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**OBJECTIVE:** The efficacy of assisted hatching has been widely debated. The variable results reported for AH are confounded by the numerous methods of performing AH, including mechanical partial zona dissection, acid tyrode's, and more recently by laser assistance. Assisted hatching has most commonly been performed on Day 3 embryos. This study assesses if Day 2 laser assisted hatching can improve implantation rates for fresh blastocyst transfers.

**DESIGN:** Prospective observational cohort.

**MATERIALS AND METHODS:** On the morning of Day 2 of culture, all embryos were sorted into groups (<4-cell, 4-cell and >4-cells). Those in the AH group underwent laser assisted hatching using an Octax laser (4.0ms) on the morning of Day 2 at the time of embryo check. All embryos were then cultured undisturbed until assessment on the morning of Day 5 when the highest quality embryo(s), based on morphology, were selected for embryo transfer. A total of 446 fresh Day 5 transfers between Jan 2016 - Mar 2019 were analyzed (244 transfers with AH and 202 transfers without AH). A total of 682 embryos were transferred in the 446 cycles (363 embryos in the AH group and 319 embryos in the non-AH group). Because 206 of the 446 transfers involved transfer of more than 1 embryo, a mixed model accounting for both fixed and random effects (i.e. repeated measurements) was used, with SAC modeled as a function of the fixed effects assisted hatching (AH), age, and body mass index (BMI).

**RESULTS:** Day 2 assisted hatching is associated with successful implantation (p=0.036). As expected, age was negatively associated with implantation rate (p<0.0001). BMI was not. The R-square for the model was 0.52, and the variance component of the random effects (owing to multiple embryo transfers) was 0.082 (95% CI [0.046-0.11]).

	No AH	With AH	Total
# Fresh Day 5 Transfers	202	244	446
Mean Maternal Age	35.6	34.4	35.0
Mean BMI	26.8	27.0	26.9
Mean # Embryos Transferred	1.58	1.49	1.53
# Embryos	319	363	682
# Sacs	137	194	331
Implantation Rate (%)	42.9%	53.4%	48.5%

**CONCLUSIONS:** Large data sets such as the SART database do not allow for evaluation of the impact of specific AH techniques. Certainly, any embryo handling and exposure has the potential to be detrimental to blastocyst development and implantation rates. Here, we show that Day 2 laser AH can lead to a significant increase in implantation rates from 42.9% without AH to 53.4% with AH. Day 2 embryos generally have larger perivitelline space which may allow for reduced peripheral laser exposure to the blastomeres. Further studies are needed to confirm if Day 2 laser AH confers benefit over Day 3 laser AH.

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**INNOVATIVE HIGH THROUGHPUT SCREEN IN EMBRYONIC STEM CELLS REPORTS STRESS-FORCED IMBALANCED DIFFERENTIATION, IMPORTANT TO ANALYZE STRESS IN IVF AND DRUG DEVELOPMENT; ANALYSES USING BULK AND SINGLE CELL RNASEQ.**

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versity, Detroit, MI; <sup>b</sup>Reproductive Stress 3M, Inc., Grosse Pointe Farms, MI; <sup>c</sup>Wayne state university, Detroit, MI.

**OBJECTIVE:** To validate a high throughput screen (HTS), Rex1-promoter- stemness- fluorescent reporter embryonic stem cells (ESCs) were tested for stress-forced override in stemness and changes in metabolic, developmental, epigenetic and proliferative states.

**DESIGN:** Laboratory study.

**MATERIALS AND METHODS:** ESC were tested by bulk or single cell (sc) RNAseq after 72hr exposures to 0-300mM hyperosmotic sorbitol (with stemness-maintaining Leukemia Inhibitory Factor, LIF) to quantify stress-forced differentiation. Controls for normal stemness were LIF+ and normal differentiation were LIF-. RNA was isolated by RNAeasy lysis or 10XGenomics Dropseq, RNA quality was checked by Agilent TapeStation, cDNA was synthesized using Lexogen's QuantSeq library kit, and barcoded, multiplexed and sequenced by Illumina NovaSeq 6000. Data were demultiplexed by CASAVA software and FC expression was compared between conditions. In triplicate experiments, significant fold change (FC) genes (FC ≥ 2; FDR ≤ 0.05, P < 0.05) identified affected pathways. Proliferation or death were assayed by Hoechst staining or Trypan blue staining, respectively. Validating studies including qPCR, immunoblot and immunofluorescence.

**RESULTS:** Stress forces dose-dependent responses; 6 Warburg anabolism/stemness transcripts and proliferation decreased. Compared with normal differentiation, stress-forced, dose-dependent range of highest up- and down-regulated mRNA increased 3.5 fold, but total transcript types decreased 10% in ESCs exposed to 0-300 mM sorbitol. Stress forced 5FC increases in 8 1<sup>st</sup> lineage mRNA, but 5FC decreases in 3 2<sup>nd</sup> lineage transcripts and 85% of later lineages transcripts. The most significant effects on later lineages were increased neural toxicity through suppression. Increases occurred in checkpoint genes, genes mediating epigenetic DNA/Histone methylation, stress response/heat shock genes. Genes that would mediate invasion after implantation decreased. All of the effects reported here were significantly high for FC and low for false discovery rate (FDR)/P value.

**CONCLUSIONS:** Stress decreases proliferation, stemness, and Warburg anabolism resulting in fewer stem cells. It compensates for the fewer cells with increase essential, prioritized 1<sup>st</sup> lineage differentiation compared with decreased 2<sup>nd</sup> and later lineages. This should predict teratogenesis with highest predictive values for neurotoxicity. Stress adaptation is mediated by high FC in few transcripts. This HTS assay should identify IVF culture conditions leading to optimal implantation and exposures of new drugs that could harm the implanting embryo and its stem cells.

**SUPPORT:** NIEHS-A 1R41ES028991-01.

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**AUTOMATED COMPUTER ANALYSIS OF HUMAN BLASTOCYST EXPANSION FROM EMBRYSCOPE TIME-LAPSE IMAGE FILES.**

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**OBJECTIVE:** To develop a rapid, quantitative, and automated analysis of human blastocyst expansion from Embryoscope time-lapse image files of zona-ablated embryos using artificial intelligence (AI).

**DESIGN:** A retrospective observational study comparing time-lapse images of blastocyst expansion in zona-ablated embryos measured either manually using Embryoscope software tools versus automatically using a customized neural network to perform semantic segmentation (SemSeg) on exported Embryoscope image files.

**MATERIALS AND METHODS:** Manual expansion measurements of the trophoctoderm (TE) enclosed cavity was performed using the Embryoscope's elliptical measurement tool (ET) at 2.0-hr intervals for the first 10.0 hours of expansion after initial blastocyst formation in 46 laser-ablated human blastocysts. Manual measurements (in μm<sup>2</sup>) were compared to values calculated using semantic segmentation (SemSeg) to files of approximately 30 consecutive time-lapse images/embryo over the same 10.0 hr period. All embryos had been laser-ablated to enable subsequent biopsy; thus, the total area of blastocyst expansion was defined as the sum of 1) the TE-enclosed area within the zona plus 2) the TE-enclosed area herniating irregularly from the ablation slit.

**RESULTS:** Compared to manual measurement using the Embryoscope's elliptical tool, the automated approach using SemSeg demonstrated many

advantages. Although the ET could accurately measure the TE within the uniformly elliptical zona pellucida, it less accurately traced the irregular TE cell surfaces herniating from the ablation slit; the final measurements were less consistent or reproducible between operators. In contrast, such irregular cellular outlines were more accurately demarcated by SemSeg with the neural network, which had an accuracy of > 99%, even in areas abutting embryo well boundaries. While the average discordance between the two approaches was 3.2-3.4% at both the beginning and end of the assay, some individual embryo measurements varied by more than 10% at 10.0 hours due to the limitations in the elliptical tool's accuracy at embryo well edges and boundaries. The averaged median initial and final expansion areas were 12639  $\mu\text{m}^2$  and 20717  $\mu\text{m}^2$  (using the ET) versus 13055  $\mu\text{m}^2$  and 21439  $\mu\text{m}^2$  (using SemSeg). Using either approach, subsequent rank ordering of individual embryos within cohorts revealed an enrichment for euploidy among embryos most rapidly expanding. However, the greatest advantage of SemSeg is to enable an automated, objective analysis of large-scale data sets by machine learning platforms.

**CONCLUSIONS:** This is the first report of the successful application of automated image analysis to the dynamic process of trophectoderm epithelium expansion in the human blastocyst from stock time lapse files in embryos that will undergo biopsy. This approach now enables the inclusion of this important morphokinetic information in machine learning applications aimed at the non-invasive identification of euploidy.

**SUPPORT:** This work was supported by the Division of Research of Department of Obstetrics and Gynecology and Women's Health of the John A. Burns School of Medicine and an intramural grant from the Pacific IVF Institute.

## ART OFFSPRING

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**NEONATAL OUTCOMES OF SINGLETON LIVE BIRTHS WITH VANISHING TWIN SYNDROME FOLLOWING TRANSFER OF DOUBLE EMBRYOS IN ASSISTED REPRODUCTIVE TECHNOLOGY: A RETROSPECTIVE COHORT STUDY.** Junfang Yan, Master, The Third Affiliated Hospital of Zhengzhou University, Zhengzhou, China.



**OBJECTIVE:** To compare neonatal outcomes in singleton live births between groups with and without VTs following transfer of double embryos.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Anonymised data on all cycles performed in the China were obtained from the Reproductive medicine department of the Third Affiliated Hospital of Zhengzhou University, involving 6220 singleton live births (2772 fresh embryos transfer (ET) cycles and 3448 frozen embryos transfer (FET) cycles). We analyzed the obstetric outcomes of gestation age, PTB, SGA (small for gestation age), birthweight, LBW, congenital malformation, pediatric admission and NICU admission in cycles of fresh ET and FET. Logistic regression analysis was performed adjusting for confounders, including age of women, BMI, value of AMH, infertile years, current cycle, antral follicles, cause of infertility, number of oocytes retrieved, endometrial thickness at the day of transplantation, number of high-quality embryos, embryo stage.

**RESULTS:** In the fresh ET cycles, the birthweight and gestational age in the study group were lower than in the control group, (2962.4 $\pm$ 563.1 vs. 3104.9 $\pm$ 498.5,  $p=0.000$ ) and (262.8 $\pm$ 8.4 vs. 268.9 $\pm$ 13.9,  $p=0.000$ ), respectively. There was a significantly higher risk of PTB (adjusted odds ratio (aOR) 2.45, 95% CI: 1.98-3.03) and LBW (aOR 2.11, 95% CI: 1.67-2.65) in the study group than in the control group. And there was a higher risk of pediatric admission (aOR 2.55, 95% CI: 2.07-3.13) and NICU admission (aOR 1.98, 95% CI: 1.32-2.96) in the study group than in the control group, and in the FET cycles, the gestational age and birthweight in the study group were lower than in the control group, (263.0 $\pm$ 15.7 vs. 273.0 $\pm$ 10.5,  $p=0.000$ ) and (3099 $\pm$ 662.1 vs. 3352 $\pm$ 671.5), respectively. There is a significantly higher risk of PTB (aOR 2.45, 95% CI: 2.23-3.43) and LBW (aOR 2.67, 95% CI: 2.13-3.34) in the study group than in the control group. And there was a higher risk of pediatric admission (aOR 2.62, 95% CI: 2.14-3.21) and NICU admission (aOR 2.22, 95% CI: 1.43-3.46) in the study group than in the control group.

**CONCLUSIONS:** There was a higher risk of LBW, PTB, pediatric admission and NICU admission between the study groups and control groups in fresh ET and FET cycles. However, no increased risk of SGA and congenital malformation was observed in singleton live births in both fresh and frozen ART cycles following transferring double embryos.

References: 1. Zander-Fox DL, Tremellen K, Lane M. Single blastocyst embryo transfer maintains comparable pregnancy rates to double cleavage-stage embryo transfer but results in healthier pregnancy outcomes. The Australian & New Zealand journal of obstetrics & gynaecology. 2011;51(5):406-10.

2. Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. Human reproduction update. 2012;18(5):485-503.

3. Templeton A, Morris JK. Reducing the risk of multiple births by transfer of two embryos after in vitro fertilization. The New England journal of medicine. 1998;339(9):573-7.

4. Pinborg A. IVF/ICSI twin pregnancies: risks and prevention. Human reproduction update. 2005;11(6):575-93.

5. Bechoua S, Astruc K, Thouvenot S, Girod S, Chiron A, Jimenez C, et al. How to demonstrate that eSET does not compromise the likelihood of having a baby? Human reproduction (Oxford, England). 2009;24(12):3073-81.

6. Cutting R, Morroll D, Roberts SA, Pickering S, Rutherford A. Elective single embryo transfer: guidelines for practice British Fertility Society and Association of Clinical Embryologists. Human fertility (Cambridge, England). 2008;11(3):131-46.

À Á 7. À Gianaroli L, Racowsky C, Geraedts J, Cedars M, Makrigiannakis A, Lobo R. Best practices of ASRM and ESHRE: a journey through reproductive medicine. Human reproduction (Oxford, England). 2012;27(12):3365-79.

**SUPPORT:** Not applicable.

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## REPRODUCTIVE AND PERINATAL OUTCOMES USING CRYOPRESERVED OOCYTES: AN ANALYSIS OF NATIONAL DATABASE SPANNING OVER A DECADE USING THREE CLINICAL MODELS.

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**OBJECTIVE:** With rising trend of freezing oocytes for donor egg bank and social fertility preservation indications, do assisted conception cycles using frozen oocytes affect live birth (LB) and perinatal outcomes compared to frozen embryos and fresh donor oocyte cycles?

**DESIGN:** Retrospective cohort study (2000-2016) using anonymised national database in the United Kingdom provided by the Human Fertilisation and Embryology Authority.

**MATERIALS AND METHODS:** Of 988 015 IVF cycles over a period of >15 years, 26 586 cycles were suitable for analysis. Three clinical models were used to assess the impact of oocyte cryopreservation on live birth and perinatal outcomes: 1. Cryopreserved donor oocytes (n=922) vs fresh donor oocytes (n=24 706). 2. Cryopreserved autologous oocytes (n=632) vs cryopreserved donor oocytes (n=922) 3. First cycle of cryopreserved donor oocytes (n=917) vs first cycle of cryopreserved embryos using autologous oocytes (n=326). Singleton birth data was used for calculating perinatal outcomes. Preterm birth (PTB) was defined as live birth before 37 weeks and low birth weight (LBW) was defined as birth weight <2500 gm. 100, 245 and 6537 singleton births were reported following cycles using autologous cryopreserved oocytes, cryopreserved donor oocytes, and fresh donor oocytes respectively.

**RESULTS:** The LB rate was lower in women having IVF cycles using cryopreserved donor oocytes than for fresh donor oocytes {30.7% vs 34.7%, OR 0.835 (95% CI 0.724 to 0.962,  $p=0.013$ ). LB rate was lower in women using autologous cryopreserved oocytes than those using cryopreserved donor oocytes {18.0% vs 30.7%, OR 0.497 (95% CI 0.388 to 0.636,  $p<0.001$ )}.

The PTB and LBW rates were not significantly different between cycles using autologous cryopreserved oocytes and cryopreserved donor oocytes or between cryopreserved donor oocytes and fresh donor oocytes.

The live birth rate per embryo transfer was significantly lower following the first cycle of cryopreserved donor oocytes as compared to first cycle of cryopreserved embryos using autologous oocytes (19.3% (177/917) vs 30.1% (98/326), OR 0.556 (95% CI 0.417 to 0.742),  $p<0.001$ ). Whilst the PTB rate was not significantly different in this model, women using frozen donor oocytes were more likely to have babies with LBW compared to those using cryopreserved embryos from autologous oocytes (17.5% (14/80) vs 5.9% (9/152), OR 0.297 (95% CI 0.122 to 0.720)  $p=0.005$ ).

Limitations: Confounders such as BMI, smoking status not known. The age of women were given as a range rather than as continuous data. LB outcomes possible only per cycle rather than per woman except for first cycle outcomes.

CONCLUSIONS: Women having cycles using cryopreserved oocytes have significantly lower live birth rate compared to cycles with fresh donor oocytes or cryopreserved embryos. This suggests the need for proper counselling of women considering elective oocyte freezing or using eggs from frozen donor egg banks.

SUPPORT: No financial support was sought from any funding agency.

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**DOES BODY MASS INDEX INFLUENCE THE ODDS OF A GOOD PERINATAL OUTCOME FOLLOWING FRESH AUTOLOGOUS IN VITRO FERTILIZATION CYCLES AMONG PATIENTS WITH POLYCYSTIC OVARY SYNDROME? A NATIONAL STUDY.**

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OBJECTIVE: To examine the association between body mass index (BMI) and the odds of a term, normal-weight, singleton live birth among women with polycystic ovary syndrome (PCOS) undergoing in vitro fertilization (IVF).

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: We utilized the 2012-2015 Society for Assisted Reproductive Technologies Clinical Outcomes Reporting System (SART CORS) to identify fresh, autologous IVF cycles among women aged < 41 with ovulatory dysfunction. We included only women with anti-Mullerian hormone (AMH) > 4.5 ng/mL to more accurately identify patients with PCOS.<sup>1</sup> Patients were assigned to BMI categories based on the World Health Organization guidelines. The primary outcome was a good perinatal outcome, defined as singleton live birth at ≥ 37 weeks gestation with birth weight ≥ 2500g and ≤ 4000g. A multivariable GEE model was used to assess the association between BMI and a good perinatal outcome while accounting for the correlation between repeated IVF cycles and adjusting for age, race, parity, diagnosis, and smoking.

RESULTS: The analysis included 9,611 cycles from 8,431 women. Baseline characteristics were similar among groups. With increasing BMI, patients had fewer oocytes retrieved and embryos cryopreserved despite higher gonadotropin doses (Table). Pregnancy and live birth rates decreased with increasing BMI, while miscarriage rates increased. After adjusting for covariates, women with class III or super obesity were half as likely to have a good perinatal outcome as normal weight women (OR 0.50, 95% CI 0.37-0.68, P<.0001).

CONCLUSIONS: Among PCOS patients undergoing IVF, the odds of a good perinatal outcome decrease as BMI increases. Obese women with PCOS should be counseled that weight loss may improve their odds of a term, normal-weight singleton, the ultimate goal of IVF.

Reference: 1. Wiweko B, Maidarti M, Priangga MD, Shafira N, Fernando D, Sumapraja K, Natadisastra M, Hestiantoro A. Anti-mullerian hormone as

a diagnostic and prognostic tool for PCOS patients. *Journal of assisted reproduction and genetics.* 2014;31(10):1311-1316.

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**EFFECT OF IN VITRO FERTILIZATION AND FRESH TRANSFER CONCEPTION ON BIRTHWEIGHT FOR GESTATIONAL AGE: A 2013-2016 FRENCH OBSERVATIONAL COHORT OF 49 224**

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OBJECTIVE: The purpose of this study is to establish whether babies born after In Vitro Fertilization (IVF) and fresh transfer are at higher risk of low birthweight (LBW) for gestational age (SGA).

DESIGN: This is an observational, exposed-unexposed national cohort study comparing pregnancies, births and neonatal data, focused on birth weight by gestational age, in births following IVF standard or using Intra Cytoplasmic injection (ICSI) and fresh transfers *versus* (vs) non-IVF controls data. The study included all 2,922,718 births from 2,832,578 deliveries registered between 2013 and 2016 in France, among which 1.7% (49,224) from IVF conception and immediate fresh transfer.

MATERIALS AND METHODS: Neonate's data from births 2013-2016 in France were analyzed by extracting the Information Systems Medicalization Program (PMSI) French database. Premature birth < 37 gestational weeks (WG), SGA and malformations were the main neonatal data investigated for the 49,224 IVF and 2,873,474 non-IVF neonates. SGA is defined as birth weight less than the 10th percentile of gestational age (GA), its frequency related to maternal characteristics, fetal sex, and single / multiple births.

RESULTS: Mean maternal age was 33.2 +/- 4.3 and 29.9 +/- 5.3 years in the IVF and non-IVF groups (p <0.0001). The frequency of multiple deliveries was 1.68% (48,425), including 13% from IVF. The frequency of premature deliveries was higher in IVF vs non-IVF group, 19.3% vs 6.9% (p <0.0001), as it was for single deliveries (9.0% vs 5.7%, p <0.001). The SGA rate was increased in IVF compared to non-IVF group, in all neonates, 21.6% vs 12.1%, (p <0.0001); in singletons, 14.9% vs 11.4% (OR = 1.37 [1.33-1.14], p <0.001), as in born >37 WG singletons, 13.6% vs 10.6% (OR = 1.33 [1.29-1.38], p <0.001). Univariate analysis indicated that the risk was identical according to sex, higher in multiple births (OR = 4.8) and premature births (OR = 2.9), if maternal smoking (OR = 2.2), and other maternal morbidity (MM) events except diabetes, and congenital malformation (OR = 1.7). In multivariate analysis, the added risk of SGA in IVF group was 2.1 [2.07-2.016] after adjustment for age, smoking, maternal obesity; 1.37 [1.34-1.40] if adjusted in addition to multiple births; 1.34 [1.30-1.37] if adjusted in addition to MM; 1.33 [1.30-1.36] if further adjusted for prematurity.

CONCLUSIONS: Large observational studies identified that IVF pregnancies are associated with a significant risk of concerns for babies. SGA babies are known to be at increased risks of perinatal morbidity and mortality. The results of this large cohort, whose strength is the completeness of IVF

Cycle Characteristics and Outcomes

	Underweight (N=233)	Normal (N=4181)	Overweight (N=2263)	Class I obesity (N=1576)	Class II obesity (N=947)	Class III obesity or Super obesity (N=411)	P
Total FSH dose	1792.3 ± 835.6	1910.1 ± 910.3	2057.7 ± 925.0	2307.4 ± 1009.7	2639.9 ± 1197.7	2900.4 ± 1264.5	< .0001
# Oocytes retrieved	18 (13, 25)	19 (13, 26)	18 (12, 25)	18 (12, 25)	16 (10, 23)	15 (9, 22)	< .0001
# Embryos frozen	4 (1, 7)	3 (0, 7)	3 (0, 7)	2 (0, 6)	2 (0, 5)	2 (0, 5)	< .0001
Clinical pregnancy	45.1%	44.2%	42.5%	42.2%	38.8%	33.8%	.0006
Miscarriage	10.5%	11.4%	15.4%	17.0%	18.5%	21.6%	< .0001
Live birth	39.9%	38.4%	35.4%	33.9%	31.4%	26.3%	< .0001
Multiple birth	24.7%	25.8%	30.0%	23.8%	27.6%	35.2%	.05
Preterm birth	23.7%	25.4%	30.4%	28.6%	33.0%	33.3%	.02
Low birth weight	22.8%	24.5%	24.2%	23.8%	27.6%	28.0%	.79
Good perinatal outcome	24.9%	22.7%	18.9%	18.5%	14.9%	12.4%	< .0001

Values represent mean ± standard deviation, median (interquartile range), or number (%).

and controls neonates data, provide evidence that the proportion of SGA birth post-IVF, including singletons, is increased compared to general population in multivariate analysis, after adjustment for age, smoking, maternal obesity, multiple births, maternal morbidity and prematurity. This is important to inform without worrying candidates for IVF, and understand possible concerns in IVF-children development. Further studies should allow to define more or less at-risk subgroups.

SUPPORT: None.

**P-467** Wednesday, October 16, 2019 6:30 AM

**OBSTETRIC, NEONATAL AND LONG-TERM OUTCOMES OF CHILDREN CONCEIVED FROM IN VITRO MATURED OOCYTES.**



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**OBJECTIVE:** To investigate the obstetric, neonatal, and long-term outcomes of in vitro maturation (IVM) compared to conventional IVF in women with polycystic ovarian syndrome (PCOS).

**DESIGN:** Matched retrospective case-control study.

**MATERIALS AND METHODS:** One hundred eighty-four patients undergoing IVM were compared with 366 patients undergoing IVF. All had PCOS and matched for patients' age, gestational age at birth, and the number of fetuses. Only women who had been conceived after fresh embryo transfer in the cycle of oocyte retrieval between January 1999 and December 2015 were included. Pregnancies using preimplantation genetic tests, testicular sperm extraction, or donor gametes were excluded. A questionnaire including pregnancy/neonatal outcomes and childhood medical problems/development was developed and distributed by reproductive specialists and administered via phone interview.

**RESULTS:** Women's mean age at oocytes retrieval was 32.6±2.9 years. Children's mean age was 7.5±2.3 years. There were no differences in the frequency of obstetric and neonatal outcomes between the two groups. No difference was found in birthweights between the two groups. The incidence of congenital anomalies was comparable between the groups (4.3% in IVM vs. 4.1% in IVF groups, p=0.65). No significant difference was observed be-

tween the two groups in the frequency and duration of hospitalization during childhood. Growth developmental status of both groups was within normal range.

**CONCLUSIONS:** In a matched setting between IVM and IVF babies born from women with PCOS, IVM is not associated with any additional risk compared to IVF after a mean follow-up of 7.5 years.

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**COMPARISON OF BIRTHWEIGHT AND GESTATIONAL AGE AT DELIVERY IN SINGLE FROZEN EMBRYO TRANSFERS (FET) WITH AND WITHOUT PGT-A.**



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**OBJECTIVE:** To compare perinatal outcomes and early hormonal trends between elective single blastocyst FET cycles with PGT-A and without PGT-A.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All patients undergoing an FET cycle of a single blastocyst between January 2015 and December 2017 were included. Cycles were divided into those with PGT-A and without. For PGT-A cycles, only euploid embryos were included. Inclusion criteria: delivery of a live singleton. Exclusion criteria: use of donor oocytes or multiple gestations. Primary outcomes were incidence of term or preterm delivery, low birth weight (LBW) and very low birth weight (VLBW). Secondary outcomes were early BhCG levels and trends. Groups were stratified by FET protocol, natural cycle or medicated.

**RESULTS:** 876 cycles met inclusion criteria, 502 with PGT-A and 374 without. Main results summarized in table. There was no difference in mean gestational age (GA), birth weight, or incidence of preterm delivery, LBW, or VLBW between the groups. This equivalence persisted after controlling for maternal age and FET protocol type. In FET cycles with PGT-A, median initial and second BhCG levels were significantly lower in cycles resulting in a LBW or VLBW infant compared to NBW. This difference did not persist in cycles without PGT-A.

**CONCLUSIONS:** Reassuringly, there was no difference in mean GA, birth weight, or incidence of preterm delivery, LBW, or VLBW infants between FETs with PGT-A and without. Initial BhCG levels were significantly lower in pregnancies resulting in LBW or VLBW infants as compared to NBW

Demographics	PGT-A (n= 502)		P	Non PGT (n=374)		P
	Mean ± SEM / N (%)			Mean ± SEM / N (%)		
Age	36.4± 0.2			33.5± 0.2		<b>&lt; 0.01</b>
BMI	23.4 ± 0.2			23.0 ± 0.2		
AMH	3.8 ± 0.2			4.3 ± 0.3		
Nulliparous	128 (25)			125 (33)		<b>0.01</b>
Multiparous	374 (75)			249 (67)		
<b>Perinatal Outcome</b>						
GA (Weeks)	39.2 ± 0.1			39.1 ± 0.1		
Term	442 (93)			331 (92)		
Preterm	37 (7)			28 (8)		
Birth Weight (g)	3384 ± 23			3356 ± 30		
NBW	455 (95)			335 (93.3)		
LBW <sup>a</sup>	24 (5)			22 (6.1)		
VLBW <sup>b</sup>	0 (0)			2 (0.6)		
<b>BhCG level (mIU/ML), Median (IQR)</b>						
	PGT-A (n=264)			Non PGT-A (n=200)		
	NBW (n=252)	LBW/VLBW (n=12)	P	NBW (n=189)	LBW/ VLBW (n=11)	P
BhCG 1	280 (181 - 424)	180 (73 - 314)	<b>&lt; 0.01</b>	257 (156 - 383)	218 (112 - 424)	ns
BhCG 2	783 (505 - 1996)	526 (316 - 1036)	<b>0.01</b>	727 (417 - 1201)	733 (396 - 1219)	ns
BhCG 2 day % increase	242 (216 - 276)	280 (255 - 294)	<b>0.01</b>	235 (207 - 259)	245 (239 - 263)	ns

Student T-test for continuous,  $\chi^2$  for categorical, Wilcoxon rank sum for nonparametric variables.

a: Low Birth Weight, <2500 g at delivery

b: Very Low Birth Weight, <1500 g at delivery

infants. This difference did not persist in cycles without PGT-A. Therefore, in pregnancies achieved by FET with PGT-A, early BhCG trends may be a useful prognostic indicator for neonatal birth weight. Further studies will need to elucidate the mechanism behind this difference.

**P-469** Wednesday, October 16, 2019 6:30 AM

**ASSOCIATION BETWEEN EMBRYO QUALITY AND BIRTH WEIGHT AMONG SINGLETONS AND TWINS CONCEIVED THROUGH AUTOLOGOUS FRESH IVF CYCLES.**



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**OBJECTIVE:** To determine if embryo quality is associated with birth weight for infants conceived via autologous fresh IVF.

**DESIGN:** Retrospective analysis of fresh autologous IVF cycles reported to SART CORS from 2008-2013.

**MATERIALS AND METHODS:** All autologous fresh IVF cycles resulting in livebirth with outcome confirmed by review of medical record were eligible for inclusion in the analysis. Cycles were excluded if more than 2 embryos or embryos of different quality were transferred.

The primary predictor was embryo quality (poor, fair, good). This grading system in SART CORS has been validated by *Vernon et al* (2011)<sup>1</sup>. Outcomes included continuous (in gram) and dichotomized birth weights (SGA: z-score  $\leq$  -1.28; LGA z-score  $\geq$  1.28). We adjusted for covariates (maternal age, BMI, race, smoking history, miscarriage, parity, infertility, gestational age, infant sex). Separate analyses were performed for singletons and twins, as well as for cleaved and blastocyst transfer. Depending on outcomes, multiple linear or logistic regression and Generalized Estimation Equation modeling were conducted.

**RESULTS:** There were 5262 (67.86%) singleton births (cleaved: 2089, blastocyst: 3173) and 2492 twin births (cleaved: 950, blastocyst: 1542) included in the analysis.

Among singletons conceived via cleaved embryo transfer, embryo quality was not predictive of birth weight. The difference in birth weight between fair vs. good quality was 33.6g (95% CI: -5.6, 72.8); poor vs. good: 123.7g (-30.1, 277.5). For singletons conceived via blastocyst transfer, fair quality was associated with decreased birth weight comparing with good quality (-38.0g (-74.1, -1.9)). No difference was seen for poor vs. good blastocysts (79.4g (-53.9, 212.7)). Among twins, quality for both cleaved embryos and blastocysts was not predictive of birth weight.

Among singletons, embryo quality was not predictive of SGA (Table 1). Among twins, quality of cleaved embryos was not predictive of SGA. However, for blastocysts, fair quality was associated with reduced odds of SGA. Embryo quality was not predictive of LGA in any comparison.

**CONCLUSIONS:** Embryo quality is generally not predictive of birth weight in infants conceived via autologous fresh IVF.

Reference: 1. Vernon M, Stern JE, Ball GD, Winger D, Mayer J, Racowsky C. Utility of the national embryo morphology data collection by the Society for Assisted Reproductive Technologies (SART): correlation between day-3 morphology grade and live-birth outcome. *Fertil Steril*. 2011;95(8):2761-2763.

**SUPPORT:** None.

TABLE 1.

	Singleton (N=5262)		Twin (N=2492)	
	Cleaved Embryo (N=2089)	Blastocyst (N=3173)	Cleaved Embryo (N=950)	Blastocyst (N=1542)
<b>SGA</b>	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Good ( <i>ref</i> )	1	1	1	1
Fair	0.81 (0.60, 1.10)	1.19 (0.92, 1.52)	1.10 (0.77, 1.56)	0.72 (0.52, 0.99)
Poor	0.48 (0.14, 1.62)	0.70 (0.25, 1.93)	0.40 (0.06, 2.76)	1.78 (0.36, 8.89)
<b>LGA</b>				
Good ( <i>ref</i> )	1	1	1	1
Fair	1.12 (0.78, 1.61)	0.83 (0.60, 1.15)	0.87 (0.55, 1.36)	1.13 (0.75, 1.70)
Poor	2.25 (0.94, 5.38)	1.41 (0.52, 3.79)	1.09 (0.27, 4.39)	0.99 (0.14, 7.01)

**P-470** Wednesday, October 16, 2019 6:30 AM

**IMPACT OF MODE OF CONCEPTION ON EARLY PREGNANCY HUMAN CHORIONIC GONADOTROPIN RISE AND BIRTHWEIGHT.**



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**OBJECTIVE:** Altered hCG kinetics have been observed in conceptions after fresh vs. frozen/thawed embryo transfers and following blastomere biopsies in cleavage stage embryos. While preimplantation genetic testing has improved pregnancy rates in some populations, the impact of trophoctoderm biopsy on hCG kinetics and subsequent birthweight is unknown. The aim of this study was to determine differences in first trimester hCG kinetics by mode of conception and subsequent risk of small and large for gestational age infants (SGA and LGA). Groups examined include unassisted natural conceptions, pregnancies after fresh embryo transfer (ET), frozen ET, and trophoctoderm preimplantation genetic testing for aneuploidy (PGT-A).

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** Serial serum hCG measurements were assessed for 598 singleton pregnancies between 10 and 28 days post-conception. All PGT-A subjects were also frozen embryo transfers. Chi-squared tests were used to test differences in the incidence of SGA and LGA by mode of conception. A joint random effects and logistic model was used to evaluate the effect of mode of conception on hCG slope (per day increase in log-transformed hCG) and incidence of SGA/LGA. Models were adjusted for maternal age, body mass index, and parity as appropriate. Odds ratios illustrate the change in risk associated with a one standard deviation increase in hCG slope.

**RESULTS:** Fresh ET had the highest incidence of SGA (12%) and frozen ET had the highest incidence of LGA (16%). PGT-A had the lowest incidence of each event among the groups observed (4% SGA and 8% LGA). Estimated hCG rise per day by group was as follows: Unassisted (0.41), fresh ET (0.39), frozen ET (0.43), PGT-A (0.45). Significant differences in hCG slope were found for all five pairwise group comparisons tested: PGT-A/unassisted (p < 0.01), PGT-A/fresh ET (p < 0.01), PGT-A/frozen ET (p = 0.02), fresh ET/frozen ET (p < 0.01), fresh ET/unassisted (p = 0.03). Slower hCG rise is associated with SGA (OR = 0.64, p < 0.01) but not with LGA (OR = 1.16, p = 0.33).

**CONCLUSIONS:** Slower hCG rise is associated with a higher risk of SGA, yet hCG rise does not impact LGA risk. There are differences in expected rate of hCG rise by mode of conception such that PGT-A has the fastest hCG rise, followed by frozen ET, unassisted, and fresh ET. Notably, PGT-A is not associated with abnormal fetal growth phenotypes, supporting the safety of this technology. These findings suggest the super-ovulated environment in fresh ET may predispose to abnormal trophoblast differentiation and early placentation resulting in altered hCG kinetics and fetal growth; yet the mechanisms of LGA in frozen embryo transfer may be mediated by other mechanisms beyond trophoblast function.

**SUPPORT:** 2P50HD068157-06A1.

### FIRST TRIMESTER VAGINAL BLEEDING DOES NOT PREDICT SMALL FOR GESTATIONAL AGE NEWBORNS FOLLOWING SINGLE EUPLOID FROZEN EMBRYO TRANSFER.



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**OBJECTIVE:** Newborns that are small for gestational age (SGA) have birth weights below the 10<sup>th</sup> percentile. Uterine/placental factors associated with SGA neonates include decreased blood flow to the uterus and placenta, placental abruption, placenta previa, and uterine infection. A secondary analysis of data from the NICHD Fetal Growth Studies suggests that more than one day of vaginal bleeding (VB) in the first trimester is associated with lower infant birth weight.<sup>1</sup> However, it is unclear whether this holds true in pregnancies achieved with the use of assisted reproductive technology treatment. The objective of this study was to determine in an infertile population undergoing in vitro fertilization (IVF) whether first-trimester VB is associated with the likelihood of having an SGA infant.

**DESIGN:** Retrospective, cohort study.

**MATERIALS AND METHODS:** The study included patients at an academic ART center who underwent a single euploid FET and experienced a live birth from 2012 to 2019. Natural language processing was performed to identify pregnancies complicated by VB prior to 10 weeks of gestation. Blind review of the database was conducted by two independent reviewers to verify data quality. Incidents of 'spotting' or 'staining' were excluded from the analysis. Primary outcome was the presence of a SGA infant, which was determined using the sex-specific weights for the 10<sup>th</sup> percentile of neonatal birth weight.<sup>2</sup> Data were evaluated using T-tests, chi-square tests, and multivariate logistic regression models.

**RESULTS:** A total of 1611 FET cycles with a live birth outcome from 1528 patients were included in the study. The overall incidence of VB was 17.69% (n=285). Pregnancies were divided into two groups: (1) pregnancies with VB prior to the 10<sup>th</sup> week of gestation and (2) pregnancies with no VB. Univariate analysis demonstrated significant differences in BMI, gravidity, and route of progesterone administration between groups. There was no difference in aspirin use, average birth weight, or gestational age at delivery between groups. There were a total of 18 (6.32%) SGA infants in the VB group, and 115 (8.67%) SGA infants in the no VB group. Controlling for BMI, gravidity, and route of progesterone administration, multivariate regression analysis did not demonstrate any significant association between VB and the incidence of SGA newborns (OR 0.53 [95% CI 0.24-1.20], p=1.23).

**CONCLUSIONS:** In contrast to the study published by Bever et al.,<sup>1</sup> patients who experienced first trimester VB did not demonstrate a higher incidence of SGA newborns. Use of natural language processing of electronic medical records enabled us to re-construct first trimester incidents not otherwise easily obtainable, limiting potential recall bias as a confounding variable. A limitation of our study design was the lack of a quantitative method to track quantity and duration of VB. Nevertheless, patients undergoing single euploid FET can be reassured that first trimester VB is not associated with a higher incidence of SGA infants.

References: 1. Bever AM, Pugh SJ, Kim S, et al. Fetal Growth Patterns in Pregnancies With First-Trimester Bleeding. *Obstet Gynecol.* 2018; 131(6):1021-1030.

2. Duryea EL, Hawkins JS, McIntire DD, et al. A Revised Birth Weight Reference for the United States. *Obstet Gynecol.* 2014; 124:16-22).

**SUPPORT:** None.

### DELAYED BLASTULATION HAS NO IMPACT ON NEONATAL OUTCOMES IN FROZEN-THAWED SINGLE BLASTOCYST TRANSFER CYCLES.



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**OBJECTIVE:** Blastocysts formed on day 6 (D6BL) are available in ART treatment although they are considered to be suboptimal for transfers due to delayed blastulation. As demonstrated in several reports, D6BLs have more abnormalities in mitotic apparatus, resulting poor clinical outcomes in transfers as compared with blastocysts formed on day 5 (D5BL). However, im-

pacts of delayed blastulation on prenatal outcomes after blastocyst transfers have not been fully investigated so far. The present study was designed to compare neonatal outcomes between singletons born after transfers of a frozen-thawed single blastocyst formed on Day 5 and Day 6.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** A total of 1137 neonates born after transfers of a frozen-thawed single D5BL and 134 neonates after D6BL transfers performed between 2008 and 2016 were analyzed. Blastocysts that reached grade 3 by the Gardner's score on day 5 or 6 were defined as D5BL and D6BL respectively. The following parameters were statistically analyzed using student's t-test or chi-square test between singletons born after D5BLs and D6BLs transfers: birth weight, birth height, gestational age at birth, sex ratio and occurrence of congenital abnormalities. Multiple linear regression analysis was performed to investigate the influential parameters on fetal growth among gender, gestational age and day of blastulation.

**RESULTS:** Birth weight (g), birth height (cm), gestational age (weeks), sex ratio (m/f) and congenital abnormality rates (%) of babies born after transfers of D5BLs vs D6BLs were 3057.3 ± 477.7 vs 3041.2 ± 447.8 (ns), 48.7 ± 2.6 vs 48.7 ± 2.9 (ns), 38.7 ± 2.0 vs 38.5 ± 1.8 (ns), 1.04 vs 1.23 and 3.5 vs 3.0 (ns), respectively. Multiple linear regression identified gender (p<0.01) and gestational age (p<0.01) as associated parameters with fetal growth. Delayed blastulation, on the other hand, was not related with either birth weight or height.

**CONCLUSIONS:** Our study showed that neonatal outcomes were not statistically different between babies born after transfers of D5BLs and D6BLs. Based on the results of both univariate and multivariate analyses, delayed blastulation has no influence on the neonatal outcomes, therefore transfer of D6BL is an optimal alternative for patients who miss blastocysts on day 5.

Reference: None.

**SUPPORT:** None.

### IS LOW BIRTH WEIGHT RELATED TO HIGH OOCYTE YIELD DURING FRESH TRANSFER ART CYCLES? RETROSPECTIVE ANALYSIS FROM HOMOLOGOUS CYCLES.



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**OBJECTIVE:** To determine if the number of retrieved oocytes correlates with live birth rate (LBR) and incidence of low birth weight (LBW).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All cycles of fresh embryo transfer with the use of homologous oocytes (n = 2216) between 2006-2017 performed at a private fertility center were reviewed and included. Groups were established in relationship to the number of retrieved oocytes (group 1: ≤10, group 2: 11-15, group 3: ≥16) and women age (≤35 and ≥36). Non adjusted comparisons between groups were calculated using t-test and chi-squared distribution. Furthermore, one-way analysis of variance (ANOVA) and Tukey posthoc test, were used to assess mean comparisons among the three groups. Pregnancy rates (positive serum hCG) and live birth rates were calculated. Finally, after excluding multiple pregnancy newborns, using the Intergrowth® matrix, each newborn was classified according to its weight (percentiles and z-score) to examine its relationship with the number of retrieved oocytes.

**RESULTS:** The younger group (≤35yo, n = 1176, 53%) had a pregnancy rate of 41.5% and LBR of 29.7% per cycle. The other group (≥36yo, n=1040, 47%) had a pregnancy rate of 28.2% and LBR of 16.7%. According to the number of retrieved oocytes, the group 2 and 3 had a statistically significant greater pregnancy rate (41.2% and 42%) than group 1 (29.8%) (p<0.001). However, there was no significant difference in the LBR between groups.

Comparative analysis between the number of retrieved oocytes, live birth rate and incidence of low birth weight (LBW) showed the following weight percentile means: group 1 - 46.84±23.79, group 2 - 43.78±27.38 and group 3 - 52.76±27.29, with no statistical differences found among the groups (p=0.077). No correlation was found after performing a linear regression for weight percentile or z-scores and number of retrieved oocytes for all patients and in the younger patients.

**CONCLUSIONS:** In homologous fresh transfer cycles there was no association between high number of retrieved oocytes and the incidence of live birth rate and LBW. Further studies are warranted to determine if a subgroup of women may be particularly vulnerable to certain maternal and fetal complications.

**INCIDENCE OF PRE-TERM BIRTH (PTB), LOW BIRTH WEIGHT (LBW), VERY LOW BIRTH WEIGHT (VLBW), AND MACROSOMIA IN GESTATIONAL CARRIER (GC) PREGNANCIES.**



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**OBJECTIVE:** Assisted reproduction (ART) has been associated with adverse perinatal outcomes, including extremes of birth weight (BW) and pre-term birth (PTB). We sought to explore the incidence of PTB, low birth weight (LBW), very low birth weight (VLBW), and macrosomia (MS) in GC pregnancies.

**DESIGN:** Retrospective analysis of all GC deliveries from a single agency from 2008-2019.

**MATERIALS AND METHODS:** Data from a large surrogate agency that consisted of matched GCs and intended parent (IP) couples for an index GC pregnancy were reviewed. The following was collected for each GC pregnancy: BW, number delivered, and gestational age (GA) at delivery. For each GC, history of PTB and history of multiple gestation were also collected. Definitions of LBW, VLBW, and MS were as defined by World

taken into account when identifying GC candidates and deciding on the number of embryos to transfer.

Reference: None.

SUPPORT: None.

**P-475 Wednesday, October 16, 2019 6:30 AM**

**PERINATAL OUTCOMES AFTER FRESH VERSUS FROZEN-THAWED EMBRYO TRANSFERS.**



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**OBJECTIVE:** This study intends to evaluate neonatal outcomes, regarding birth percentile, gestational age and birth weight stratifications, after frozen embryo transfer (FET) compared with fresh embryo transfer cycles.

TABLE 1.

			Gestational age at current GC delivery				
			Preterm birth in GC pregnancy	Full term birth in GC pregnancy	All deliveries	Missing	Total
Preterm birth history	History of preterm birth	Current singleton gestations	33 (76.7%)	10 (23.3%)	43 (100%)	29	82
		Current multiple gestations	3 (30.0%)	7 (70.0%)	10 (100%)		
		All gestations	36 (67.9%)	17 (32.1%)	53 (100%)		
No history of preterm birth	No history of preterm birth	Current singleton gestations	1 (0.7%)	146 (99.3%)	147 (100%)	388	570
		Current multiple gestations	1 (2.9%)	34 (97.1%)	35 (100%)		
		All gestations	2 (1.1%)	180 (98.9%)	182 (100%)		
Missing	Missing	All gestations	1 (4.2%)	23 (95.8%)	24 (100%)	160	184
Total	Total	Total	39 (15.1%)	220 (84.9%)	259 (100%)	577	836

Health Organization criteria. PTB was defined as < 37 weeks. Chi-squared was used for dichotomous variables and student's t-test for continuous variables.

**RESULTS:** Of 836 GC pregnancies reviewed, BW data was available for 536 deliveries. Average BW of GC index pregnancies was 7.27 lbs (SD 1.53, minimum 1.62 lbs, maximum 15.8 lbs). Incidence BW extremes were: 58 (10.9%) LBW; 10 (1.9%) VLBW; 70 (13.1%) MS. GA data was available for 259 index GC pregnancies (Table 1). Overall PTB rate (15.1%) was higher than national averages. Most PTB in index GC pregnancies was in those with a history of PTB, and more likely singletons rather than multiple gestation (76.7% in singletons vs 30% in multiples in patients with history of PTB, P<0.001). Those with no history of PTB and who carried multiples had a low rate of PTB; in fact, in this group, only 1 out of 35 patients had a PTB with multiples.

**CONCLUSIONS:** Incidence of LBW and VLBW were similar to national averages. PTB rate was higher and the majority were singleton gestations in women with a history of PTB. In women with prior full term deliveries, carrying multiples did not impart a greater risk of PTB. A GC's prior obstetric history appears to have the greatest impact on the GA at delivery in the GC pregnancy. These factors should be

**DESIGN:** Retrospective case-control study performed at an assisted reproduction clinic in Brazil. A total of 1929 embryos were analyzed. The data refers to a period from 2010 to 2019 and were collected from electronic records.

**MATERIALS AND METHODS:** A number of 1443 of embryo-transfers were included. Samples were divided into two groups: 1: Group 1 - frozen embryo (n=486) and Group 2 - fresh embryo transfer (n=1443). Categorical variables were expressed as percentage and were compared with Chi-square test, considering p<0.05.

**RESULTS:** Data show that births from frozen embryos have higher percentile when compared to fresh births. As well as 90% of these births presents weight greater than 2500g. We also found a statistical difference that related prematurity with births from fresh transfer. The main results are presented in Table 1.

**CONCLUSIONS:** Births from singleton pregnancies following FET have been associated with high birth weights, decrease incidence of preterm birth and rates of small for gestational age when compared to fresh transfer cycles. Our data corroborates with literature, demonstrating that prenatal outcomes from FET decrease the chances of prematurity and low birth weight.

TABLE 1. Differences between frozen embryo and fresh embryo births regarding birth percentile, gestational age and birth weight stratifications.

	Percentile*			Gestational age*			Birthweight*		
	<10	10_90	>90	<34 wks	34-37wks	>37 wks	<1500	1500-2499	>=2500
Fresh embryo (n=1443)(%)	219 (15,2)	1117 (77,4)	107 (7,4)	112 (7,8)	263 (18,2)	1067 (74,0)	44 (3,0)	290 (20,1)	1109 (76,9)
Frozen embryo (n=486)(%)	36 (7,4)	376 (77,4)	74 (15,2)	15 (3,1)	51 (10,5)	420 (86,4)	4 (0,8)	34 (7,0)	448 (92,2)
Total (n=1929)	255	1493	181	127	314	1487	48	324	1557

\*p<0.001.

**RISK OF ADVERSE PERINATAL OUTCOMES AFTER OOCYTE DONATION: A SYSTEMATIC REVIEW AND META-ANALYSIS.** Jose Antonio Moreno, MD,<sup>a</sup> Checa Angel Miguel, MD, PhD,<sup>b</sup> <sup>a</sup>Clinica de la Mujer - Medicina Reproductiva, vina del mar, Chile; <sup>b</sup>FERTTY, Barcelona, Spain.



**OBJECTIVE:** To assess if in women with singleton pregnancies conceived after assisted reproductive technologies, do the in vitro fertilization with oocyte donation (IVF-OD) affects the perinatal and maternal outcomes compared to autologous in vitro fertilization (IVF-AO)?

**DESIGN:** Systematic review and meta-analysis of studies comparing perinatal and maternal outcomes in singleton pregnancies resulting from IVF-OD versus IVF-AO.

**MATERIALS AND METHODS:** An electronic literature search in Pubmed, MEDLINE and Cochrane database was performed. The main outcome measures were preterm birth, early preterm birth, low birth weight, very low birth weight, hypertensive disorders in pregnancy, pregnancy induced hypertension, preeclampsia and severe preeclampsia.

**RESULTS:** 12 studies were included. IVF-OD is associated with a higher risk of preterm birth (RR 1.44; 1.20-1.74), early preterm birth (RR 1.68; 1.09-2.60), low birth weight (RR 1.26, 1.11-1.43), very low birth weight (RR 1.35, 1.21-1.50), hypertensive disorders in pregnancy (RR 2.66, 1.97-3.60), pregnancy induced hypertension (RR 1.69; 1.30-2.20), preeclampsia (RR 3.09; 2.60-3.68) and severe preeclampsia (RR 3.43; 2.34-5.04). There was no significant difference in the risk of small for gestational age.

**CONCLUSIONS:** IVF-OD patients must be considered an independent risk factor for some adverse perinatal outcomes, mainly hypertensive disorders in pregnancy, preeclampsia and severe preeclampsia, but also preterm birth and low birth weight. Immunological aspects may be involved in this results and further research focusing in the etiopathogenesis of these pathologies are needed.

**ART PREGNANCY RISKS**

**OVERVIEW OF 2016 U.S. ASSISTED REPRODUCTIVE TECHNOLOGY (ART) TREATMENT OUTCOMES AND CONTRIBUTION OF ART TO MULTIPLE-BIRTH AND PRETERM INFANTS IN THE UNITED STATES.**



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**OBJECTIVE:** To assess national and state-specific ART utilization and outcomes and the contribution of ART to multiple births and prematurity.

**DESIGN:** Population-based cross-sectional analysis.

**MATERIALS AND METHODS:** Data for ART procedures and birth outcomes in 2016 were obtained from CDC's National ART Surveillance System (years 2015 and 2016). Data for all infants born in the U.S. were obtained from 2016 National Vital Statistics System birth data. The number of ART procedures performed per million women 15-44 years of age (ART use), rates of elective single embryo transfers (eSET) among women <35 years, rates of ART preterm and multiple-birth infants, and proportions of ART-conceived infants among all infants, and all multiple-birth and preterm infants were calculated for each reporting area (50 States, District of Columbia, and Puerto Rico), by mother's state of residence. The proportion of infants who were small for gestational age (SGA) (i.e., born at <10th percentile of birthweight for gestational age) was calculated for singleton births that occurred at <37 weeks (preterm), 37-41 weeks (term), and 22-44 weeks (overall births) for ART and all infants.

**RESULTS:** Among 3,974,132 infants born in the U.S., 1.8% (70,600) were conceived with ART (range: 0.3% in Puerto Rico to 4.7% in Massachusetts). ART use ranged from 385 (Puerto Rico) to 7,371 (District of Columbia) procedures per million women aged 15-44 years. The national eSET rate among women <35 years was 42.7% (range: 8.3% in North Dakota to 83.9% in Delaware). The rates of multiple-birth and preterm infants were 31.5% and 29.9% among ART infants versus 3.4% and 9.9% among all infants, respectively. Nationally, the proportion of ART-conceived infants among multiple-birth and preterm infants was 16.4% and 5.3%, respectively. The percentage

of ART-conceived singletons that were SGA was 8.7% for preterm infants, 8.0% for term infants, and 8.1% overall; the corresponding percentages among all singletons were 9.3%, 10.5%, and 9.9%.

**CONCLUSIONS:** A higher proportion of ART infants are multiple and preterm in the U.S compared to all births. Wide variations were observed among reporting areas in the rates of ART utilization and eSET. Greater utilization of eSET, where appropriate, could reduce the contribution of ART to multiple-birth and preterm infants. Rates of SGA for singletons born preterm, term, and for all gestational ages were lower among ART-conceived infants compared with all infants, possibly indicating better health behaviors and care among ART patients.

**SUPPORT:** NONE.

**IN VITRO FERTILIZATION AND GESTATIONAL HYPERTENSION/PREECLAMPSIA RISK: EFFECT OF DIAGNOSIS VERSUS TREATMENT PARAMETERS.**



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**OBJECTIVE:** To evaluate the risks of gestational hypertension by maternal fertility status and infertility diagnosis.

**DESIGN:** Women in 8 States (CA, CO, FL, MI, NY, OH, PA, TX) who underwent in vitro fertilization (IVF) cycles resulting in a live birth during 2004-2013 were linked to their infant's birth certificates; a 10:1 sample of births from non-IVF deliveries were selected for comparison; those with an indication of infertility treatment on the birth certificate were categorized as subfertile, all others were categorized as fertile. The IVF pregnancies were additionally categorized by oocyte source (autologous vs donor) and embryo state (fresh vs thawed).

**MATERIALS AND METHODS:** Analyses within the IVF group were additionally categorized by infertility diagnosis. Gestational hypertension or preeclampsia (GH or PE) were identified from the birth certificate. GH and PE were considered as a composite primary outcome variable. GH/PE was modeled using logistic regression, and reported as adjusted odds ratios (AOR) and 95% confidence intervals (CI). For analyses of singleton fertile, subfertile, and IVF pregnancies, the reference group were fertile women. For analyses by oocyte source-embryo state within IVF pregnancies, the reference group was autologous-fresh for singletons.

**RESULTS:** The study population included 1,518,175 pregnancies (1,382,149 singleton/fertile, 7,815 singleton/subfertile, 86,907 singleton/IVF, and 41,304 twin/triplet/IVF). Compared to fertile women, subfertile women had increased risks for GH/PE [AOR 1.63, 95% CI 1.49, 1.79], but IVF women with autologous-fresh cycles did not [AOR 1.02, 95% CI 0.97, 1.08]. Among IVF singleton births, the risk of GH/PE was increased for all non-autologous-fresh groups (autologous-thawed, 1.32 [1.22, 1.43]; donor-fresh, 1.97 [1.76, 2.22]; donor-thawed, 1.79 [1.53, 2.09]; and donor-thawed or fresh, 1.92 [1.72, 2.14]) relative to the autologous-fresh group. The results were similar in multiple births. In analyses by infertility diagnoses, with the autologous-fresh group as the reference, autologous-thawed cycles had significantly elevated risks for GH/PE in 7 of 10 infertility diagnoses, AORs of 1.29 to 1.54; donor-fresh cycles had elevated risks in 8 of 10 diagnoses, AORs of 1.66 to 4.51; donor-thawed cycles had elevated risks for 4 of 9 diagnoses, AORs of 1.67 to 2.03.

**CONCLUSIONS:** The risk of gestational hypertension/preeclampsia is increased in pregnancies for subfertile women, in pregnancies from oocyte donation and from frozen embryo transfer, but not with pregnancies from fresh autologous IVF cycles.

**SUPPORT:** NIH Grant R01 HD84377.

**OBSERVATIONAL 4-YEARS STUDY OF OBSTETRIC COMPLICATIONS AFTER IN VITRO FERTILIZATION (IVF) AND FRESH EMBRYO TRANSFER IN A FRENCH NATIONAL COHORT OF 43,084 DELIVERIES.**



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**OBJECTIVE:** The objective of this large cohort study is to identify by univariate and multivariate analysis whether there is an excess of maternal morbidity (MM) in ongoing pregnancies and deliveries after IVF and fresh transfer techniques, when compared to spontaneous conceptions (SC).

**DESIGN:** This is an observational, exposed-unexposed cohort study comparing pregnancies, deliveries and births following IVF, standard or using Intra Cytoplasmic Injection (ICSI), and fresh transfers to non-IVF controls. The study included all 2,832,578 national deliveries registered between 2013 and 2016 in France, among which 1.5% (43,084) resulted from IVF and immediate fresh transfer.

**MATERIALS AND METHODS:** Pregnancies and deliveries were analyzed by extracting the Information Systems Medicalization Program (PMSI) French database. The main identified maternal morbidity indicators for the 43084 IVF and 2 789 494 non-IVF pregnancies were: venous and arterial thrombosis (VT, AT), gestational diabetes mellitus (GDM), pre-eclampsia (PE), Placenta Previa (PP), placenta abruption (PA) hemorrhage at delivery (HD). The risks of MM in IVF were estimated in multivariate analysis after adjustment for maternal age, smoking and obesity, and multiple deliveries.

**RESULTS:** The mean maternal age was 33.2 and 29.9 years in the IVF and control groups ( $p < 0.0001$ ). The rate of multiple deliveries was 1.68%, of which 13% if IVF conception. Diabetes and hypertensive disorders during pregnancy were more common in the IVF vs non-IVF group: 1.01% vs 0.9% ( $p = 0.01$ ) and 1.04% vs 0.9% ( $p < 0.001$ ). Tobacco dependence and obesity were less common in the IVF vs non-IVF group (2.2% vs 4.5%, and 3.9% vs 4.3%,  $p < 0.001$ ). The frequency of premature deliveries was higher in IVF vs non-IVF: 19.3% vs 6.9% ( $p < 0.0001$ ), persistent for single births (9.0% vs 5.7%,  $p < 0.001$ ). The risk of MM (VT, GDM, PE, PP, PA, HD) was higher in IVF vs non-IVF (20.9% vs 14.3%,  $p < 0.0001$ ), even if single pregnancies (19.6% vs 14.1%,  $p < 0.0001$ ) except arterial thrombosis. The risk of MM increased significantly with age for all events except for PE. In multivariate analysis, IVF is a significant risk factor for all MM events except arterial thrombosis. The adjusted risk of the occurrence of at least one concern after IVF is 1.29 [1.26-1.32] at all and 1.32 [1.28-1.35] in single deliveries. This risk is stable over the four years.

**CONCLUSIONS:** Large observational studies identified that IVF pregnancies are associated with a significant risk of complications, initially attributed to multiple pregnancies, as compared with pregnancies after SC. The strength of this large national exposed-unexposed cohort study lies in the number and completeness of subjects studied. Our data provide in turn evidence for increased adjusted risk of premature delivery and maternal morbidity (VT, GDM, PE, PP, PA, and HD) after IVF, including in single pregnancies. The knowledge of the excess risk is an essential tool for informing without worrying couples candidate for IVF, and analyzing neonatal health of IVF-children. Future developments should allow to refine the knowledge of more or less at-risk subgroups.

**SUPPORT:** None.

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#### **MODE OF DELIVERY IN GESTATIONAL CARRIER (GC) PREGNANCIES IN WOMEN WITH AND WITHOUT A HISTORY OF CESAREAN SECTION**

(CS). Meggie B. Smith, MD,<sup>a</sup> Rachel S. Mandelbaum, MD,<sup>a</sup> Jacqueline Ho, MD MS,<sup>a</sup> Kristin Bendikson, M.D.,<sup>b</sup> Richard J. Paulson, MD, MS,<sup>a</sup> <sup>a</sup>University of Southern California, Los Angeles, CA; <sup>b</sup>USC Fertility, Pacific palisades, CA.

**OBJECTIVE:** Pregnancies that result from in-vitro fertilization (IVF) have previously been shown to be associated with a higher rate of cesarean section (CS) compared to non-IVF pregnancies<sup>1</sup>. Gestational carriers (GC) are chosen based on a favorable obstetric history. Thus, GCs represent a unique population in which to study how assisted reproduction may affect obstetric outcomes. This study sought to report on mode of delivery from GC pregnancies and GC characteristics associated with CS.

**DESIGN:** Retrospective analysis of all GC singleton deliveries from a single agency between 2008-2019.

**MATERIALS AND METHODS:** Data from a large agency that consisted of matched GCs and intended parent (IP) couples for an index GC pregnancy were reviewed. GCs were excluded if route of prior deliveries and index GC pregnancy were not available. For each GC index pregnancy, the GC's prior parity, prior modes of delivery, year of delivery, age, BMI, and inter-preg-

nancy interval as well as the number of embryos transferred (ET), number delivered, and mode of delivery for the index GC pregnancy were collected. All variables were analyzed and compared using binary logistic regression and chi squared analyses where appropriate.

**RESULTS:** 836 GCs were included, of whom 319 (38.2%) delivered via CS and 517 (61.8%) delivered vaginally in the index GC pregnancy. 60 (18.8%) of the CS deliveries were due to multiple gestation. Primary CS rate in singleton GC pregnancies was 35.3%. In women without a history of CS, neither age, BMI, interpregnancy interval, prior parity, nor year of delivery impacted primary singleton CS rate (all,  $P > 0.05$ ). 350 (41.9%) GCs had a history of one or more prior CS, of which 85 (24.3%) were for multiple gestations. Of GCs with a history of a prior CS, 218 (62.3%) had a vaginal delivery after CS (VBAC) and 132 (37.7%) had a repeat CS. Women who had a VBAC were significantly younger than those who had repeat CS (mean 33.7 vs. 35.2 years,  $P = .003$ ). BMI was lower in patients who had a VBAC compared to those that had a repeat CS, but this did not reach statistical significance (mean BMI 24.6 vs. 25.5,  $P = 0.074$ ). In GCs with a history of CS, interpregnancy interval, year of delivery, prior parity, and multiple gestation in the index GC pregnancy did not impact mode of delivery. VBAC rates did not change over the study period ( $P = 0.757$ ).

**CONCLUSIONS:** Primary CS rates in singleton GC pregnancies are higher than national averages (21.9%)<sup>2</sup> and are independent of age, BMI, and interpregnancy interval. GCs with only prior vaginal deliveries should be duly counseled on their increased risk of primary CS and its affect on her own future pregnancies, should she plan on more children, or require other abdominal surgeries. In GCs with a history of a prior CS, VBAC rates well exceed national averages (12.8%)<sup>2</sup> and are higher in younger GCs with a lower BMI. In GCs with prior CS, factors related to overall success of VBAC rates should be discussed at screening to optimize any modifiable risk factors.

**References:** 1. Kozinsky Z et al. Obstetric and neonatal risk of pregnancies after assisted reproductive technology: a matched case control study. *Acta Obstet Gynecol Scand.* 2003 Sept; 82(9): 850-6.

2. Centers for Disease Control and Prevention. National Vital Statistics Reports – Births: Final Data for 2017. [https://www.cdc.gov/nchs/data/nvsr/nvsr67/nvsr67\\_08-508.pdf](https://www.cdc.gov/nchs/data/nvsr/nvsr67/nvsr67_08-508.pdf). Assessed 4/29/19.

**SUPPORT:** None.

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#### **THE IMPACT OF IVF ON NEONATAL BIRTHWEIGHT FROM 2000 TO 2017: A DRAMATIC REDUCTION OF LOW BIRTHWEIGHT INFANTS.**

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**OBJECTIVE:** IVF is a known risk factor for low birthweights among newborn infants, both multiples and singletons alike. In past decades, new approaches to care such as extended culture, cryo-all cycles, and near comprehensive single embryo transfer (SET) have become increasingly prevalent and have the potential to reduce adverse outcomes and neonatal health risks. This study seeks to determine the change in birthweights over time as these new approaches have been integrated into clinical practice.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Birthweights and demographics of 19,886 IVF pregnancies at a single center from 2000 to 2017 were collected. This cohort included all pregnancies without use of a gestational carrier. These data were compared to annual US statistics reported by the Centers for Disease Control, which reported 72,940,619 pregnancies between 2000 through 2017.

**RESULTS:** In our IVF population, incidence of low birthweight (LBW) decreased from 36.5% to 10.7%, only slightly higher than the general population risk of 8.3% the CDC reported for 2017. The drop in very low birthweight (VLBW) was even more dramatic and declined 8% in 2000 to 2.1% in 2017, almost equal to the national risk of 1.4%. Declines in LBW and VLBW births directly correlated with reduced transfer order: the 2017 national rate of twins in similar age groups was 2.4%, on par with our practice's IVF twin rate of 2.5% the same year. Currently, more than 97% of transfers are eSETs, independent of PGT-A use (~70% of cycles) and includes all age groups/all prior treatment histories.

**CONCLUSIONS:** Advances in clinical ART have resulted in marked improvements in sustained implantation rates (SIR), providing increased confidence in providers and patients while empowering effective use of eSET. Our prevalence of LBW and VLBW deliveries from IVF pregnancies now

Year	VLBW <1500g %	LBW 1500-2499g %	Normal BW ≥2500g %	SIR %	Total Births
US 2017	1.4	6.9	91.7		3,852,498
2000	8.0	28.5	63.5		99
2001	5.3	26.7	68.0		643
2002	6.2	26.7	67.1		469
2003	7.0	26.3	66.7		1188
2004	6.4	26.3	67.3		1066
2005	7.1	26.1	66.8	30.2	924
2006	6.6	24.8	68.6	32.1	864
2007	5.8	25.8	68.4	37.0	917
2008	6.9	26.8	66.3	40.9	956
2009	6.3	26.4	67.3	42.3	1086
2010	5.6	25.3	69.1	46.3	1071
2011	6.6	23.0	70.4	58.1	1094
2012	5.3	21.1	73.6	63.9	1232
2013	4.2	18.8	77.0	65.4	1251
2014	4.4	16.5	79.1	65.3	1614
2015	3.7	13.8	82.5	65.8	1615
2016	3.1	12.0	84.9	66.8	1894
2017	2.1	8.6	89.3	66.9	1903

approach that of the general population. This dramatic improvement was accomplished while simultaneously improving SIR such that delivery rates per transfer actually increased. It is now possible to perform SET in all patients without compromising delivery rates and drastically reducing the neonatal risks associated with LBW/VLBW endured by infertile couples and their progeny.

SUPPORT: None.

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**TWIN PREGNANCY OUTCOMES OF WOMEN WITH A DIDELPHUS UTERUS AFTER IN VITRO FERTILIZATION-EMBRYO TRANSFER.**

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**OBJECTIVE:** To investigate the twin pregnancy outcomes of women with a congenital didelphus uterus after in vitro fertilization embryo-transfer (IVF-ET).

**DESIGN:** A retrospective matched case-control study.

**MATERIALS AND METHODS:** A retrospective 1:4 matched case-control study was conducted of 16 cases of twin pregnancy in women with a congenital didelphus uterus after IVF-ET from January 2004 to December 2017. For each case in the study group, 4 consecutive control twin pregnancies in women with a normal uterus were included. Women in both groups were matched for maternal age (MA), body mass index (BMI), cause of infertility, infertility type and insemination methods. Patients with the monochorionic twins and twins with spontaneous or selective reduction were excluded. The pregnancy and obstetric outcomes between these two groups were compared.

**RESULTS:** The didelphus group and the control group were statistically similar with respect to MA, BMI, cause of infertility, infertility type and insemination methods ( $P > 0.05$ ).

Compared with the control group, the didelphus group had significantly higher rates of preterm delivery (75.0 vs. 42.2%;  $P = 0.019$ ), very preterm birth (42.9 vs. 8.5%;  $P = 0.001$ ), low birth weight (89.5 vs. 46.4%;  $P = 0.001$ ) and perinatal mortality (32.1 vs. 5.1%;  $P < 0.001$ ), and a significantly lower live birth rate (62.5 vs. 87.5%;  $P = 0.019$ ); the gestational age at delivery ( $31.3 \pm 5.7$  vs.  $35.6 \pm 3.8$  weeks;  $P = 0.017$ ) and the live birth weight ( $1944 \pm 387$  vs.  $2455 \pm 475$  g;  $P < 0.001$ ) were significantly lower in the didelphus group than those in the control group. Additionally, the miscarriage rate (12.5 vs. 7.8%;  $P > 0.05$ ) was higher in the didelphus group, but this difference was not significant.

**CONCLUSIONS:** Twin pregnancy was associated with increased rates of preterm delivery, low birth weight and perinatal mortality in women with a didelphus uterus after IVF-ET.

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**PRECONCEPTION AND VERY EARLY PREGNANCY BLOOD PRESSURE AND DEVELOPMENT OF PRE-TERM PREECLAMPSIA, TERM PREECLAMPSIA AND GESTATIONAL HYPERTENSION.**

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TABLE. The twin pregnancy outcomes between the didelphus group and the control group

Pregnancy outcomes	The didelphus group(n=16)	The control group (n=64)	P-value	OR (95% CI)
Miscarriage %,(n)	12.5 (2/16)	7.8 (5/64)	NS	1.67 (0.30-9.61)
Preterm delivery %,(n)	75.0 (12/16)	42.2 (27/64)	0.019	4.11 (1.20-14.14)
Perinatal mortality %,(n)	32.1 (9/28)	5.1 (6/118)	<0.001	8.84 (2.82-27.70)
Live birth rate %,(n)	62.5 (10/16)	87.5 (56/64)	0.019	0.240 (0.07-0.84)
The gestational age at delivery (weeks)	$31.3 \pm 5.7$	$35.6 \pm 3.8$	0.017	
< 32 gestational weeks %,(n)	42.9 (6/14)	8.5 (5/59)	0.001	8.10 (1.10-32.85)
The live birth weight (g)	1944 ± 387	2455 ± 475	<0.001	
<2500 g %,(n)	89.5 (17/19)	46.4 (52/112)	0.001	9.81 (2.16-44.46)

**OBJECTIVE:** Although elevations in early- to mid-pregnancy blood pressure are related to risk of developing a hypertensive disorder of pregnancy, little is known regarding when this differentiation in blood pressure begins. We evaluated the relationship of preconception and very early pregnancy blood pressure with risk of developing preterm preeclampsia (PE), term PE and gestational hypertension (GHTN).

**DESIGN:** Prospective cohort study set in the EAGeR trial, which enrolled 1228 couples attempting pregnancy who had a history of pregnancy loss. Women were randomized to receive 81mg aspirin or placebo for up to 6 menstrual cycles attempting pregnancy and, if they became pregnant, up to 36 weeks' gestation.

**MATERIALS AND METHODS:** Systolic and diastolic blood pressure were measured by trained staff at enrollment prior to conception and at gestational weeks 4, 8, 12, 16 and 20, and were used to derive mean arterial pressure. Hypertensive disorders of pregnancy, including preterm PE, term PE and gestational hypertension, were classified retrospectively from medical record abstraction. We excluded 9 participants with chronic hypertension (blood pressure over 140/90 mmHg and/or anti-hypertensive treatment during preconception or in early pregnancy). Log-binomial models assessed the relationship of blood pressure at preconception and in early- to mid-pregnancy with risk of developing a hypertensive disorder of pregnancy (preterm PE, term PE and GHTN), adjusting for maternal age, pre-pregnancy BMI, parity, and treatment assignment (low-dose aspirin or placebo). We additionally evaluated the interaction of blood pressure with assignment to aspirin due to its efficacy in preventing preeclampsia among high-risk women.

**RESULTS:** Of 588 women who had a live birth and no chronic hypertension, 10 developed preterm PE, 18 term PE and 24 GHTN. During preconception, systolic blood pressure levels were elevated to similar degrees for women who developed preterm PE, term PE and GHTN compared to women who did not develop hypertension in pregnancy. However, by 4 weeks gestation, those who developed preterm PE had relatively elevated blood pressure (124.8±3.6) compared to those with term PE (117.8±2.7) or GHTN (115.8±2.4), a trend that continued up to 20 weeks' gestation. By as early as 4 weeks gestation, higher mean arterial pressure was associated with higher risk of preterm PE (relative risk [RR] 2.28, 95% confidence interval [CI] 1.01, 5.16 per 10 mmHg) and term PE (RR 1.57, 95% CI 1.02, 2.41 per 10 mmHg). No differences were observed by assignment to low-dose aspirin.

**CONCLUSIONS:** Although preconception blood pressure levels were similarly elevated for women who developed preterm PE, term PE and GHTN as compared to women who did not develop a hypertensive disorder of pregnancy, a differentiation in blood pressure for each condition was observed as early as 4 weeks gestation. This suggests that some of the physiologic changes associated with preterm PE may occur prior to 4 weeks' gestation.

**SUPPORT:** Intramural Research Program, Division of Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development.

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#### PERCEPTIONS OF MULTIFETAL GESTATION AMONGST PATIENTS BEING TREATED FOR INFERTILITY.

Anne Hutchinson, M.D. Seth J. Barishansky, MS, Rafael Confino, BS, Angela K. Lawson, Ph.D., Mary Ellen Pavone, MD MSCI, Northwestern University, Chicago, IL.



**OBJECTIVE:** To assess the desire for multifetal gestation in our patient population and understand patient perceptions regarding maternal and fetal risks inherent in these pregnancies.

**DESIGN:** Cross-Sectional Study.

**MATERIALS AND METHODS:** We designed a 40-question digital survey based on a previously validated survey and approved by our IRB (Ryan et al. 2004). Between the months of February and April 2019, patients presenting with infertility were approached. After receiving verbal consent, patients were provided with a tablet, preloaded with our survey, which collected de-identified patient demographic information as well as treatment outcomes ranked in order of preference, specifically "no child", "singleton pregnancy", "twin pregnancy", "triplet pregnancy". Using a series of true/false questions we also assessed knowledge of the complications of multiple births with questions regarding risks to the mother's health during pregnancy and delivery, risks of cerebral palsy and long-term health problems in the infant and risk of death to the infant as well as knowledge of the financial and psychological risks of multifetal pregnancy.

These questions were then analyzed using chi-squared analysis to compare understanding of maternal and fetal risks of multifetal gestation between

groups who identified singleton pregnancy as desired outcome to those who desired twin or triplet pregnancy.

**RESULTS:** 71 patients completed our survey. 68% reported singleton pregnancy as ideal treatment outcome, 30% reported twin pregnancy as ideal treatment outcome, 2% reported triplet pregnancy as ideal treatment outcome.

A chi-squared analysis was used to compare the responses of patients desiring singleton pregnancy to those desiring twin or triplet pregnancy. Both groups showed similar understanding of increased risk of preterm birth in twin (88% vs. 100%) and triplet pregnancies (100% vs 92%). Similarly, both groups showed similar understanding of the increased risk of triplet pregnancies on maternal health (85% vs. 83%). Patients desiring twin and triplet pregnancies, however, showed less understanding of increased maternal risk in twin pregnancy (77% vs 52%, p<0.05), and increased risk of neonatal morbidity in twin pregnancy (17% vs 44%, p<0.05) and triplet pregnancy (26% vs 54%, p<0.05).

Both groups showed similar understanding of the increased risk of neonatal mortality in twin pregnancies (23% vs 22%) and triplet pregnancies (38% vs 30%).

**CONCLUSIONS:** A significant number of patients undergoing fertility treatment desire twin and triplet gestation. This desire seems to be associated with an incomplete understanding of maternal and neonatal risks associated with multifetal gestation. We believe that targeted patient education regarding these risks may decrease patient desire for multifetal gestation and help to bring patient and provider goals into better alignment.

**Reference:** Ryan GL, Zhang SH, Dokras A, Syrop CH, Van Voorhis BJ. The desire of infertile patients for multiple births. *Fertil Steril* 2004;81:500-4.

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#### RISK OF ECTOPIC PREGNANCY AFTER DIFFERENT OVARIAN STIMULATION PROTOCOLS IN FRESH SINGLE EMBRYO TRANSFER: ANALYSIS OF 71,831 CYCLES FROM THE JAPANESE ART REGISTRY.

Seung Chik Jwa, M.D., Ph.D., M.P.H.<sup>a</sup> Sachie Seto, M.D., Ph.D.,<sup>a</sup> Masashi Takamura, M.D., Ph.D.<sup>a</sup> Akira Kuwahara, M.D., Ph.D.<sup>b</sup> Takeshi Kajihara, M.D., Ph.D.<sup>a</sup> Osamu Ishihara, M.D., Ph.D.<sup>a</sup> <sup>a</sup>Saitama Medical University, Saitama, Japan; <sup>b</sup>Tokushima University, Tokushima, Japan.



**OBJECTIVE:** To investigate the risk of ectopic pregnancy following different ovarian stimulation protocols in fresh cycles.

**DESIGN:** Registry-based retrospective cohort study.

**MATERIALS AND METHODS:** This study included all autologous cycles that resulted in a clinical pregnancy after described ovarian stimulation protocols (natural, clomiphene (CC), CC+gonadotropin, GnRH agonist and GnRH antagonist) in fresh single embryo transfers between 2007 and 2015 in Japan. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using generalized estimating equations adjusted for potential maternal and treatment characteristics.

**RESULTS:** Among 71,831 clinical pregnancies, 1,049 (1.46%) ectopic pregnancies were reported. Ectopic pregnancy was more frequent for early cleavage stage embryo transfers than blastocyst transfers (1.54% vs. 1.29%, p = 0.008), and assisted hatching (AH) (1.75% in AH group vs. 1.36% in non-AH group, p = 0.003). The highest rate of ectopic pregnancy occurred with CC+gonadotropin (2.06%, 221/10, 711), followed by CC alone (1.77%, 160/9, 025), GnRH antagonist (1.49%, 216/14, 490) and GnRH agonist protocols (1.40%, 415/29, 585). The natural cycle had the lowest ectopic pregnancy rate of all ovarian stimulation protocols (0.46%, 37/8, 020). Compared with the natural cycle, all other ovarian stimulation protocols were associated with a significantly increased risk of ectopic pregnancy. Ovarian stimulation using CC+gonadotropin had the highest increased risk for ectopic pregnancy (adjusted OR, 4.39, 95% CI, 2.55 to 7.54). In each stimulation protocol, there was no association between the risk of ectopic pregnancy and the number of oocytes retrieved, except with ovarian stimulation using CC.

**CONCLUSIONS:** Ovarian stimulation protocols were associated with a significantly increased risk for ectopic pregnancy in fresh cycles. These results suggest that ovarian stimulation agents may affect the tubal and intra-uterine environment during fresh cycles.

**SUPPORT:** This study was supported by Health and Labour Sciences Research Grants.

**OBSTETRIC AND NEONATAL RISKS IN TERM-SINGLETON ASSISTED REPRODUCTIVE TECHNOLOGY (ART) PREGNANCIES: A SINGLE-CENTER REPORT IN A PERIOD OF 9 YEARS.**

Satoshi Furuya, MD, Kiyoshi Kubonoya, MD, Ken Kubonoya, MD, Kubonoya Ob/Gyn Clinic, Kashiwa City Chiba Prefecture, Japan.



**OBJECTIVE:** It is well documented that a singleton pregnancy is safer and healthier than a multiple pregnancy and term (defined as a period from 37 to 41 weeks of gestation) is the optimal timing to give birth for humans. As the number of infertile couples requiring ART is increasing today, pregnancies obtained by ART have often been reported to be associated with a higher risk of poor pregnancy outcomes. This can be accounted for in part by the higher frequency of ART-conceived preterm or multiple births included, and study-design heterogeneity among researchers. The aim of our study is to determine whether there is any increase in adverse obstetric and neonatal outcomes in ART pregnancies, compared with naturally conceived pregnancies, even when only term-singleton cases are selected as a base cohort.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** We reviewed 14297 consecutive term-singleton labor and delivery cases managed in our facility from January 2010 to March 2019. All information was collected from medical records, including maternal age, parity, details of infertility treatment, pregnancy course and mode of delivery, complications during labor and puerperium, status of the infant at birth, etc. Infertile cases conceived by other than ART (n=676) were excluded from our study subjects in order to assess the effect of ART more precisely. The remaining study population (n=13621) was divided into two categories as follows: cases conceived through ART procedures (Group A: n=750), and cases conceived naturally (Group B: n=12871). We used multivariate logistic regression analysis (shown as odds ratio (OR), 95% CI, and P value) to evaluate the impact of term-singleton ART pregnancy on obstetric and neonatal outcomes, while controlling for maternal age, parity, gestational weeks at birth, and neonatal sex.

**RESULTS:** Average maternal age in Group A and B was  $35.8 \pm 3.6$  (y  $\pm$  SD) and  $31.3 \pm 4.5$ , respectively. Group A constituted about 5.5% (750/13621) of all term-singleton births during the study period. Significant increased incidence of maternal medical complications (OR:1.42(1.10 - 1.81), P<0.01), HDP (hypertensive disorders of pregnancy; OR:1.64(1.18 - 2.25), P<0.01), forced delivery (i.e., emergency C-section or instrumental delivery; OR:1.52(1.28 - 1.80), P<0.001), abnormal postpartum hemorrhage (OR:2.29(1.77 - 2.94), P<0.001), placenta adhaerens/accrete (OR:3.33(1.81 - 5.82), P<0.001), velamentous umbilical cord insertion (OR:7.83(5.03 - 12.11), P<0.001), and heavy-for-date newborn infant (OR:1.71(1.03 - 2.71), P=0.03) was observed in Group A. Difference in the incidence of placental abruption and other neonatal outcomes (Apgar scores, umbilical artery pH value, NICU admission, congenital anomaly) between Group A and B could not be confirmed.

**CONCLUSIONS:** Among term-singleton pregnancies, cases achieved by ART carry an increased risk for several adverse maternal and neonatal outcomes, compared with those conceived naturally. In order to secure the safety, obstetricians should recognize term-singleton ART pregnancies as high-risk ones and manage them more cautiously than ever before.

**SUPPORT:** None.

**DOES OFFERING A FINANCIAL INCENTIVE FOR ELECTIVE SINGLE EMBRYO TRANSFER DECREASE RATE OF MULTIPLE GESTATION?**

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**OBJECTIVE:** The purpose of the study is to assess the impact of offering a financial incentive on the rate of elective single embryo transfer (eSET) at a single IVF center.

**DESIGN:** Prospective cohort study using historical controls.

**MATERIALS AND METHODS:** Patients who met the recent ASRM guidelines for eSET who were <38 years old with >1 embryo for transfer who did not have insurance coverage for frozen embryo transfer (FET) were candidates for a financial incentive program offered by a foundation associated with our IVF center to fund a second FET if they did not get pregnant from their initial FET of a single embryo. eSET program was initiated in July 2018. FETs performed in 2017 were used as historical controls. Outcomes included the percent of eSET, multiple gestation and adherence to ASRM guidelines.

**RESULTS:** There were a total number of 255 FETs performed in 2017 and 193 performed between 7/2018 through 3/2019. Rate of single FET in women of all ages prior to eSET program was 62.5% versus 85.0% after the initiation of the eSET program (p <0.001). Rate of double FET was 34.9% compared to 13.9% (p <0.001). In 2017, there were 132 self-pay cycles, 72 of which would have been eligible for eSET program, compared to 69 eligible cycles after the initiation of eSET program of which 63 participated (54.5 vs. 91.3%, p<0.001). Number of self-pay patients that did not follow ASRM guidelines prior to start of program was significantly higher than after the initiation of eSET program (23.5% vs. 2.9%, p<0.001) Rate of multiple gestation pregnancies was similar before and after initiation of eSET program, (5.4% vs 7.2%, p=0.58).

**CONCLUSIONS:** There are a significant percentage of patients in our population that do not insurance coverage for IVF. Our eSET incentive program, which provides a financial incentive for patients to transfer a single embryo without the financial burden of paying for an additional transfer if they do not get pregnant demonstrated significantly increased adherence to ASRM guidelines regarding eSET.

**AMONG WOMEN WITH CONGENITAL UTERINE ANOMALIES, IS A SHORT CERVIX PREDICTIVE OF PRETERM BIRTH.**

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**OBJECTIVE:** Cervical length measurements are used to assess risk for preterm birth in women with normal uterine architecture. The utility of this measurement in women with congenital uterine anomalies is not known. The objective of this study was to describe pregnancy outcomes among women with known congenital uterine anomalies based on cervical length.

**DESIGN:** A retrospective cohort of pregnant women with a congenital uterine anomaly (CUA) that delivered at a single tertiary academic center and its affiliated community hospital between June 1, 2013 and August 1, 2018 who underwent cervical length screening.

**MATERIALS AND METHODS:** Women were identified using ICD9 codes for both congenital uterine anomalies as well as for delivery. Prenatal records were reviewed to obtain demographic variables, obstetrics and gynecology history, ultrasound measurement of cervical length (CL) and delivery and neonatal outcomes. Short cervix was defined as a transvaginal cervical length of <25mm. The primary outcome was gestational age at delivery. Secondary outcomes included delivery at <37 weeks, delivery at <34 weeks, mode of delivery and neonatal death. Women with a short cervix were compared to those with CL  $\geq$  25mm using Wilcoxon rank sum, Fisher's exact and chi squared tests.

**RESULTS:** 95 women with congenital uterine anomalies delivered between June 1, 2013 and August 1, 2018 and were included in analysis. 10 women had a short cervix. Median cervical length in the short cervix group was 22mm (IQR 12,14) as compared to 39mm (IQR 35, 42) in the long CL cohort (p < 0.001). No significant difference in parity, prior preterm birth, history of abnormal pap smears, prior cervical surgery, tobacco use or infertility treatment was seen between groups. Women with a short cervix delivered at a median of 28.6 weeks (IQR 21.4, 34.1) as compared to 38.1 weeks (IQR 35.9, 39.4) in the long cervix cohort (p < 0.001). Delivery at less than 37 weeks (90% vs 38%, p = 0.002) and less than 34 weeks (70% vs 14%, p < 0.001) were also more common among women with a short cervix. Though mode of delivery did not differ, neonatal demise was ten times more common in the short cervix group (40% vs 4.7%, p = 0.004).

**CONCLUSIONS:** Among women with a CUA, short cervix may portend a more ominous outcome than among women with normal uterine architecture. Larger studies are needed to corroborate this data.

**THE BETTER TRANSFER STRATEGY IN WOMEN WITH A UNICORNUATE UTERUS: SINGLE BLASTOCYST-EMBRYO TRANSFER VERSUS DOUBLE CLEAVAGE-EMBRYO TRANSFER.**

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**OBJECTIVE:** To investigate the better transfer strategy between single blastocyst-embryo transfer (SBET) and double cleavage-embryo transfer (DCET) in women with a unicornuate uterus.

TABLE. Reproductive outcomes of SBET and DCET in women with a unicornuate uterus

Reproductive outcomes	DCET	SBET	P	aOR (95% CI)
Patients (n)	383	156		
Embryos transferred (n)	766	156		
Implantation, %(n)	47.9 ( 367/766 )	57.7 ( 90/156 )	0.011	0.628 ( 0.439-0.899 )
Clinical pregnancy, %(n)	68.1 ( 261/383 )	57.7 ( 90/156 )	0.039	1.522 ( 1.022-2.267 )
Live birth %(n)	52.0 ( 199/383 )	47.4 (74/156)	0.451	
Multiple pregnancy, %(n)	41.4 ( 108/261 )	3.3 ( 3/90 )	<0.001	20.046 ( 6.132-65.535 )
Pregnancy (n)	200	86		
Miscarriage, %(n)	19.5 (39/200)	15.1 (13/86)	0.378	
Preterm delivery, %(n)	24.5 (49/200)	12.8 (11/86)	0.028	2.213 ( 1.088-4.501 )
Term delivery, %(n)	53.0 (106/200)	72.1 (62/86)	0.003	0.437 ( 0.253-0.754 )
Babies born (n)	199	73		
Live births (n)	180	72		
Perinatal mortality, %(n)	9.5 (19/199)	1.4 (1/73)	0.027	9.900 ( 1.294-75.734 )
Low birth weight, %(n)	31.7 (57/180)	11.1 (8/72)	0.005	3.163 ( 1.416-7.062 )
Live birth weight (g)	2750 ± 650	3050 ± 500	<0.001	
Gestational age at delivery (weeks)	36.8±3.9	37.8±2.8	0.029	

DESIGN: A retrospective cohort study.

MATERIALS AND METHODS: 539 infertile patients with a unicornuate uterus who underwent SBET or DCET from January 2012 to December 2017 were enrolled. SBET and DCET were performed in 156 and 383 patients, respectively. Only the first transfer cycle was considered. The reproductive outcomes were compared between these two groups.

RESULTS: The two groups were statistically similar regarding age, body mass index and cause of infertility ( $p > 0.05$ ), however, the infertility duration, infertility type and insemination methods were significantly different ( $p < 0.05$ ).

Multivariate regression analysis showed a significantly lower implantation rate (47.9% vs. 57.7%), but markedly higher rates of clinical pregnancy (68.1% vs. 57.7%) and multiple pregnancy (41.4% vs. 3.3%) in the DCET group compared to the SBET group ( $p < 0.05$ ). While the live birth rate was similar. (52.0% vs. 47.4%,  $P = 0.451$ ).

The DCET group was associated with statistically higher risks of preterm delivery (24.5% vs. 12.8%), low birth weight (31.7% vs. 11.1%), perinatal mortality (9.5% vs. 1.4%) and lower live birth weight (2750 ± 650 vs. 3050 ± 500 g) and gestational age at delivery (36.8 ± 3.9 vs. 37.8 ± 2.8 weeks) compared to the SBET group ( $p < 0.05$ ). While no significant difference was found in the miscarriage rate (19.5% vs. 15.1%,  $p = 0.378$ ).

CONCLUSIONS: SBET could increase the implantation rate and decrease the risks of multiple pregnancy, preterm delivery and perinatal mortality, but with the same live birth rate as DCET. So SBET was recommended for women with a unicornuate uterus.

their subsequent frozen-thawed cycle. Monozygotic twinning was defined as 2 or more heart beats at 5-6 weeks ultrasound.

RESULTS: We analyzed 521 clinical pregnancies resulting from 1708 single embryo transfers in cleavage-stage (N=674) or blastocyst stage (N=1034). The overall MZT rate 2.87% (15/521), accounting for 0.87 % of cleavage stage derived pregnancies (1/115) and 3.45% of blastocyst stage derived pregnancies (14/406,  $p=0.01$ ).

The incidence of MZT was higher with ICSI (14/368) compared to conventional IVF (1/153), although not statistically significant ( $P=0.08$ ). In the blastocyst transfer group the incidence of MZT was not increased by Assisted Hatching (AH), Preimplantation Genetic Testing (PGT) nor was it affected by type of transfer, (either fresh or frozen) or quality/type of catheter used in the transfer.

Fifteen patients with MZT had 10 term deliveries with no neonatal complications, four of which had vanishing embryos, (one of them triple with a double vanishing embryo). One case had placenta accreta and underwent cesarean hysterectomy. Four patients miscarried, 2 in the first trimester and two in the second trimester (one due to cervical incompetence, and one voluntary interruption, due to a thoracopagus Siamese Twin pregnancy) Finally, one patient delivered at 30 weeks, with twin neonatal demise.

CONCLUSIONS: Although MZT is a rare event associated with ART it cannot be disregarded due to its potentially serious obstetric consequences, especially in a sET blastocyst transfer program. Monozygotic twinning is increased in blastocyst transfers, and special care should be taken to properly inform prospective parents when blastocysts are transferred.

## CONTRACEPTION/FAMILY PLANNING

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### PREVALENCE, RISK FACTORS AND OBSTETRIC OUTCOMES OF ZYGOTIC SPLITTING AFTER SINGLE EMBRYO TRANSFER CYCLES.

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OBJECTIVE: To describe the prevalence, main determining factors and obstetric outcomes of multiple pregnancy due to zygotic splitting after single embryo transfer (sET).

DESIGN: We performed a retrospective observational study in 521 clinical pregnancies resulting from cleavage-stage or blastocyst single embryo transfer (sET) following IVF or ICSI cycles with autologous or donated eggs. Fresh and frozen-warmed sET from January 2015 to June 2017 were included, and analyzed for the occurrence of assisted hatching, embryo biopsy for PGT, or insemination type (IVF vs. ICSI). We also evaluated embryo grading, blastocyst expansion grade, and quality of embryo transfer.

MATERIALS AND METHODS: We retrospectively analysed all pregnancies achieved through single embryo transfers at our center. The population included IVF or ICSI cycles with autologous or donated eggs, and/or

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### EFFECT OF SELF-ADMINISTERED LIDOCAINE IN-SITU GEL ON INTRAUTERINE DEVICE INSERTION PAIN: A RANDOMIZED CONTROLLED TRIAL.

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OBJECTIVE: Intrauterine contraceptive device (IUD) is a safe long-acting reversible contraceptive method. However, insertion-related pain presents a barrier to its widespread use in family planning. Our objective is to examine the analgesic effect of a novel self-administered lidocaine vaginal *in-situ* gel in alleviating pain during IUD insertion compared to placebo among parous women.

DESIGN: Randomized, double-blind, placebo-controlled trial (Clinicaltrials.gov: NCT02943135).

MATERIALS AND METHODS: Reproductive-aged parous women requesting Copper-T 380 A IUD insertion for birth control were counseled to participate. Eligible women based on WHO guidelines were recruited and randomized (1:1) to lidocaine *in-situ* gel vs. placebo using a permuted

block schedule. Ten minutes before IUD insertion, each participant self-inserted the prefilled syringe with 5 ml lidocaine or placebo in-situ gel vaginally. Neither cervical ripening agents nor analgesics were used before the insertion. The main study outcomes were the participant's self-rated pain perception utilizing a 10-cm Visual Analogue Scale (VAS) during cervical tenaculum placement, uterine sound and IUD insertion, then 15 minutes post-procedure. A 2 cm difference in VAS score between both arms was considered a clinically significant difference. The secondary outcomes included ease of insertion score, duration of insertion and need for additional analgesia. Mann Whitney and Fisher's exact tests were utilized for analysis of the outcomes.

**RESULTS:** One hundred twenty women were enrolled and randomized to lidocaine in-situ gel arm (n=60) or placebo (n=58). Both arms were homogeneous regarding age, parity, BMI, and the prior mode of delivery. Lidocaine group reported significantly lower pain scores during tenaculum (median[IQR]: 2[1-2] vs 4[3-4],  $p < 0.001$ ), uterine sound insertion (median [IQR]: 3[2-3] vs 5[4-6],  $p < 0.001$ ), IUD insertion (median[IQR]: 3[2-3.75] vs 6[5.5-7],  $p < 0.001$ ) and 15 minutes post-insertion (median[IQR]: 1[1-1.75] vs 2.5[2-3.75],  $p < 0.001$ ). The ease score of IUD insertion was significantly higher in the lidocaine group (median[IQR]: 8.5[8-9] vs. 7.5[6.25-8],  $p = 0.005$ ). Additionally, the IUD insertion in the lidocaine group was associated with less time incomparable to the placebo group (mean $\pm$ SD: 7.3 $\pm$ 1.19 vs. 8.75 $\pm$ 1.11 minutes,  $p = 0.048$ ). No difference regarding the need for additional analgesia.

**CONCLUSIONS:** self-administration of lidocaine in-situ gel 10 minutes before IUD insertion significantly reduces the induced pain with subsequent easier insertions.

References: - Ellah NH, Abouelmagd SA, Abbas AM, Shaaban OM, Hasanein KM. Dual-responsive lidocaine in situ gel reduces pain of intrauterine device insertion. *Int J Pharm.* 2018;538(1-2):279-86.

-Å Samy A, Abbas AM, Mahmoud M, Taher A, Awad MH, Hussein M, et al. Evaluating different pain lowering medications during intrauterine device insertion: a systematic review and network meta-analysis. *Fertil Steril.* 2019;111(3):553-61.

**SUPPORT:** A fund No. (2016-11) received from The Institutional Grants' office.

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#### **TREATMENT OF UNFAVORABLE BLEEDING PATTERNS IN CONTRACEPTIVE IMPLANT USERS.**

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**OBJECTIVE:** Some users of the etonogestrel (ENG) subdermal contraceptive implant experience unfavorable vaginal bleeding patterns. We evaluated whether a 7-day treatment with oral tamoxifen could reduce the number of bleeding/spotting days in women using an ENG implant who have documented frequent and/or prolonged vaginal bleeding.

**DESIGN:** Randomized, placebo-controlled, double blind treatment study.

**MATERIALS AND METHODS:** Subjects started treatment if they experienced  $\geq 3$  days of consecutive bleeding/spotting (B/S) and could repeat treatment every 30 days if needed during the 90-day study interval. We collected a daily record of B/S using an interactive text messaging service. The primary outcome was the total number of B/S free days in the 30 days following first tamoxifen treatment; secondary outcomes included time to B/S cessation and restart with treatment and number of B/S free days over 90 days.

**RESULTS:** From January 2017 to November 2018, 112 women enrolled in the study, 107 completed at least 30 days, and 89 completed 90 days. The average subject was 23 years old, white, and had some college education. Women randomized to tamoxifen had more B/S free days in both the first 30 days [20 (SD 7) vs. 15 (SD 7),  $p = 0.0001$ ] and 90 days [57 (SD 17) vs. 49 (SD 15),  $p = 0.026$ ]. The tamoxifen group also had faster cessation of B/S with first treatment [6 (SD 4) vs. 8 (SD 5),  $p = 0.037$ ] and longer time before bleeding restarted [19 (SD 19) vs. 8 (8),  $p = 0.0001$ ]. Study medications were well tolerated.

**CONCLUSIONS:** Most women using the ENG implant with documented frequent and/or prolonged bleeding will have cessation of bleeding within 6 days of starting a short-course of tamoxifen and a longer window of relief from bleeding after one treatment as compared to placebo.

**SUPPORT:** Grant support for this research was from Merck Women's Health Investigator Initiated Studies Program and the Oregon Clinical and Translational Research Institute (1 UL1 35RR024140 01) for access and use of REDCap electronic data capture system.

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#### **A NOVEL IN VITRO FLUORESCENT REPORTER PLATFORM FOR IDENTIFYING MALE CONTRACEPTIVES.**

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**OBJECTIVE:** Due to the challenges surrounding the development of male contraceptives, this study aimed to generate a high throughput testing platform to screen and identify potential male contraceptives.

**DESIGN:** To date, male forms of oral contraception have largely been ineffective. The current failures in developing male contraceptives stem from the lack of a robust, rapid, and unbiased human spermatogenesis platform. We previously developed a novel *in vitro*, human pluripotent stem cell model that mimics several aspects of human spermatogenesis.

**MATERIALS AND METHODS:** In order to address the challenges associated with male contraceptive development, we recently developed a novel *in vitro* fluorescent reporter platform. Testibow 1.0, that is coupled with our *in vitro* human spermatogenesis model. Testibow 1.0 is comprised of promoters for spermatogonia driving cyan fluorescent protein (eCFP) expression, promoters for primary spermatocytes driving green fluorescent protein (GFP) expression, and promoters for spermatids driving tdTomato expression. Since Testibow 1.0 utilizes fluorescence-based imaging, our model allows for the rapid identification of potential male contraceptives that successfully blocks spermatogenesis, but permits full restoration following treatment cessation.

**RESULTS:** Testibow 1.0 provides a unique, high content/ high throughput imaging platform that can rapidly and efficiently identify novel compounds that could be used as male contraceptives regardless of genetic background. Currently, we are developing a polycistronic version of our fluorescent reporter system, Testibow 2.0, that will express all three of our fluorescent reporters simultaneously in order to begin identifying and characterizing chemical compounds that block spermatogonia differentiation or meiotic entry. Furthermore, our novel fluorescent reporter platform can be used to begin addressing the safety and efficacy challenges that are hindering male contraceptive development.

**CONCLUSIONS:** In conclusion, our fluorescent reporter system represents a suitable platform for evaluating the safety and effectiveness of potential male contraceptives prior to clinical trials.

**SUPPORT:** National Institutes of Health: K22ES025418 (Easley, Charles) and Å Bill and Melinda Gates Grand Challenges Exploration Grant (Easley, Charles).

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#### **POLIDOCANOL/DOXYCYCLINE FOAM FOR NONSURGICAL PERMANENT FEMALE CONTRACEPTION: 6 MONTH DATA BABOON CONTRACEPTION STUDY.**

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**OBJECTIVE:** Our goal is the development of a safe and low cost nonsurgical approach to permanent contraception for women with high efficacy following a single treatment. We previously reported that the addition of doxycycline to polidocanol foam increases the rate of tubal occlusion. Here, we sought to determine if a single transcervical administration of polidocanol/doxycycline foam (PDF) would prevent pregnancy in female baboons.

**DESIGN:** Controlled nonhuman primate cohort study.

**MATERIALS AND METHODS:** Healthy regularly cycling female baboons underwent laparoscopy with chromopertubation, for evaluation of

baseline tubal patency and pelvic adhesions, followed by transcervical infusion of either 20 mL of 5% PDF (each 5 mL of foam contains 25 mg doxycycline; n=12, 8 nulliparous, 4 parous), 20 mL of 1% control methylcellulose foam (MC; n = 6, 5 nulliparous, 1 parous), or no additional treatment (Control; n=6, all nulliparous). All of the females received an intramuscular injection of depomedroxyprogesterone acetate (DMPA, 2 mg/kg) after the treatment. After recovery, females were socially-housed with males (n=4) of proven fertility, and observed for resumption of menstrual cyclicity and evidence of mating. The primary outcomes was pregnancy within 6 months of resumption of menses. We plan to follow pregnancy and safety outcomes thorough 18 months in the PDF-treated animals, and evaluate histologic features of tubal occlusion.

**RESULTS:** The baseline laparoscopy demonstrated bilateral tubal patency in all of animals selected for the study. All females resumed normal menstrual cycles and mating activity within 3 months of treatment. After 6 months of regular cycles, 11/12 (92%) of control females became pregnant (6/6 MC control, 5/6 untreated control). Significantly fewer (2/12, 16%) pregnancies occurred in PDF-treated females (p < .001, Fisher's exact test). All of the pregnancies were intrauterine. Both pregnancies in PDF-treated females occurred in nulliparous females - a group considered high-risk for failure. One progressed normally to term and one underwent spontaneous abortion.

**CONCLUSIONS:** A single transcervical treatment with PDF prevented pregnancy in most baboons. Pregnancy occurred in PDF-treated females considered at high risk of failure due to nulliparity.

**SUPPORT:** Bill and Melinda Gates Foundation OPP1025233, OPP1191953.

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**PRE-REMOVAL PLASMA LEVONORGESTREL LEVEL AND RETURN OF FERTILITY AFTER LEVONORGESTREL 52 MG INTRAUTERINE SYSTEM DISCONTINUATION.**

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**OBJECTIVE:** Evaluate return of fertility after levonorgestrel (LNG) 52 mg intrauterine system (IUS) discontinuation according to pre-removal serum levonorgestrel levels.

**DESIGN:** Prospective clinical trial.

**MATERIALS AND METHODS:** Nulliparous and parous women 16-45 years old received the Liletta® LNG 52 mg IUS in an IRB-approved multicenter trial to evaluate efficacy and safety for up to 10 years. Participants in a pharmacokinetics sub-study had frequent plasma LNG evaluations over the first 3 years of the trial. All study subjects, beginning at 3 years of LNG IUS use, had plasma LNG evaluations every 6 months and at IUS removal (if no level had been obtained in the prior 3 months). Women who desired pregnancy were followed for up to 12 months for pregnancy occurrence. This analysis compares LNG concentrations at IUS discontinuation between women who did and did not conceive and evaluates time to conception, using Fisher's exact and Mann Whitney U tests as indicated. We evaluated outcomes in women aged 16-35 years at study entry who had LNG levels within 90 days prior to or the day of IUS removal.

**RESULTS:** The analysis cohort includes 76 women who conceived and 19 women who did not conceive within 12 months of IUS removal. The majority of plasma LNG levels had been obtained on the IUS removal day in both groups (60/76 [79%] vs. 16/19 [84%], respectively, p=0.76). The ages of the women who conceived and did not conceive were 25.5 ± 3.1 and 25.2 ± 3.6 years. The proportion of nulliparous women in the two groups was similar (60/76 [79%] vs. 12/19 [63%], respectively, p=0.23). Fewer women who conceived had a body mass index (BMI) ≥ 30 kg/m<sup>2</sup> (9/76 [12%] vs. 7/

Median LNG levels (pg/mL)

	conceived	did not conceive	p-value
overall	109.5 (n=76)	78.2 (n=19)	<0.01
non-obese	123 (n=67)	99.4 (n=12)	0.09
obese	86.8 (n=9)	59.7 (n=7)	0.06
Obese = BMI ≥ 30 kg/m <sup>2</sup>			

19 [37%], respectively, p=0.02) although the median BMI among women who conceived and did not conceive was similar (23.7 vs. 24.9 kg/m<sup>2</sup>, respectively, p=0.11). Median duration of use was 4.3 (range 2.0-7.6) and 4.0 (range 2.0-6.4) years, respectively. Median LNG levels were higher among women who conceived than among those who did not (Table). Eight women conceived within one month and another 34 women by the end of 3 months. Median LNG levels among the 42 women who conceived within 3 months (124.5 pg/mL) after IUS discontinuation were similar to the 34 women who conceived at 4-12 months (103.5 pg/mL), p=0.23.

**CONCLUSIONS:** Plasma LNG levels were higher among women who conceived after LNG 52 mg IUS discontinuation compared to women who did not conceive. We found no evidence that higher LNG levels impact the ability to conceive or time to conceive following LNG 52 mg IUS removal.

**SUPPORT:** Medicines360.

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**SELF-ADMINISTERED VAGINAL LIDOCAINE IN-SITU GEL PRIOR TO INTRAUTERINE DEVICE INSERTION IS AN EFFECTIVE ANALGESIC IN WOMEN WITH NO PREVIOUS VAGINAL DELIVERY.**

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**OBJECTIVE:** Long-acting reversible contraception methods are highly effective for reduction of the unplanned pregnancy rate. The intrauterine device (IUD) can provide reliable, effective and long term contraception for many women. However, the insertion procedure can be associated with a troublesome degree of pain that prevent some women from choosing its use. Our objective is to assess the analgesic effect of self-administered vaginal lidocaine *in-situ* gel in pain relief during IUD insertion in women with no previous vaginal delivery.

**DESIGN:** Randomized, double-blind, placebo-controlled trial (Clinicaltrials.gov: NCT03166111).

**MATERIALS AND METHODS:** Reproductive-aged women who previously delivered only by cesarean section (CS) requesting Multiload-375 Copper IUD insertion were counseled to participate. Eligible women category 1 or 2 based on WHO guidelines were recruited and randomized (1:1) to lidocaine in-situ gel vs. placebo using a permuted block schedule. Each woman was supplied by a syringe filled with 5 ml lidocaine or placebo in-situ gel to be self-administered vaginally 10 minutes prior to insertion. The primary outcome was the difference in pain scores during IUD insertion using a 10-cm Visual Analogue Scale (VAS). A 2 cm difference in VAS score between both arms was considered a clinically significant difference. The secondary outcomes included the difference in pain scores during cervical tenaculum placement, uterine sound insertion and 15 minutes post-procedure, ease of insertion score and need for additional analgesia. Mann Whitney and Fisher's exact tests were used for the analysis of the outcomes.

**RESULTS:** The final analysis included 105 women randomized to lidocaine in-situ gel group (n=54) or placebo (n=51). Both arms were similar regarding age, parity, BMI, and a number of previous CS. Lidocaine in-situ gel group reported significantly lower pain scores during uterine sound insertion (median[IQR]: 3.5 [2-5] vs 6[5-8], p<0.001), IUD insertion (median[IQR]: 3.5[2-4.25] vs 5.5[4.75-8], p=0.002) and 15 minutes post-insertion (median[IQR]: 1.5[1-1.75] vs 4[2-4], p=0.03). No difference between scores during tenaculum placement (median[IQR]: 2.5[1.5-3] vs 3[2-4], p=0.07). The ease score of IUD insertion was significantly higher in the lidocaine group (median[IQR]: 8[7-9] vs. 6[5.25-8], p=0.001). No difference regarding the need for additional analgesia.

**CONCLUSIONS:** Self-administered vaginal lidocaine in-situ gel 10 minutes prior to copper IUD insertion is effective in pain reduction in women with no previous vaginal delivery.

**References:** - Ellah NH, Abouelmagd SA, Abbas AM, Shaaban OM, Hossain KM. Dual-responsive lidocaine in situ gel reduces pain of intrauterine device insertion. *Int J Pharm.* 2018;538(1-2):279-86.

-À Samy A, Abbas AM, Mahmoud M, Taher A, Awad MH, Hussein M, et al. Evaluating different pain lowering medications during intrauterine device insertion: a systematic review and network meta-analysis. *Fertil Steril.* 2019;111(3):553-61.

**SUPPORT:** A fund No. (2016-1) received from The Institutional Grants' office.

**WOMEN'S SATISFACTION WITH THE MULTIPURPOSE VAGINAL pH-REGULATOR (MVP-R; AMPHORA): RESULTS FROM THE PHASE 3 AMPOWER TRIAL.**

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**OBJECTIVE:** As a multipurpose vaginal pH-regulator, Amphora<sup>®</sup> is a novel, non-hormonal, woman-controlled, on-demand, contraceptive vaginal gel being investigated for prevention of pregnancy and sexually transmitted diseases. To better understand the treatment experience from the woman's perspective, the Satisfaction Questionnaire was administered in the phase 3 AMPOWER trial (NCT03243305).

**DESIGN:** The phase 3 AMPOWER trial is a single-arm, open-label study designed to evaluate the efficacy and safety of and women's satisfaction with Amphora over 7 cycles in sexually active women aged 18-35 years across 112 US sites. The primary efficacy endpoint was the cumulative 7-cycle pregnancy rate. Women's satisfaction with Amphora was an exploratory endpoint.

**MATERIALS AND METHODS:** The Satisfaction Questionnaire was given at baseline and the 3 subsequent study visits to assess women's satisfaction in 4 categories: 1) satisfaction with most recent/study birth control method; and likelihood of 2) recommending this method to others considering a vaginal contraceptive gel, 3) recommending this method to others considering another birth control option, and 4) continuing this method after study termination.

**RESULTS:** 1330 women were included in the Satisfaction Questionnaire. At Visits 3 (Cycle 2) and 4 (Cycle 5 or 6), more women reported being "very satisfied" or "satisfied" with the study method (85.3% [954/1118] and 89.5% [734/820], respectively), compared with their previous birth control method before enrollment (46.5% [616/1325]). At Visits 3 and 4, 86.6% (968/1118) and 89.8% (736/820) of women, respectively, were "very likely" or "likely" to recommend the study drug as a contraceptive vaginal gel, and as an alternative birth control option (85.7% [958/1118] and 88.2% [723/820], respectively) to others. 82.1% (918/1118) and 81.0% (664/820) of women surveyed at Visits 3 and 4, respectively, were "very likely" or "likely" to continue with Amphora if it were to be available, compared with 2.2% (25/1118) and 3.2% (26/820) of women who were "unlikely" to continue.

**CONCLUSIONS:** Data from the phase 3 AMPOWER trial indicate a very high level of satisfaction in women on Amphora compared with their previous birth control method;  $\geq 85\%$  of women on Amphora would recommend the study drug to others, and  $\geq 80\%$  of women were in favor of continuing with Amphora after study termination. Amphora has the potential of fulfilling an unmet need in women's sexual and reproductive health as a non-hormonal, woman-controlled, on-demand contraceptive option that offers a high level of satisfaction.

**SUPPORT:** Evoform Inc.

**REPRODUCTIVE AGE WOMEN ARE INTERESTED IN SELF-ADMINISTERED VAGINAL CONTRACEPTIVES THAT PREVENT SEXUALLY TRANSMITTED INFECTIONS.**

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**OBJECTIVE:** To develop a better understanding of women's knowledge of and desire for pregnancy and sexually transmitted infection (STI) prevention. Women's perspectives may help guide future development of new innovative products.

**DESIGN:** Questionnaire-based observational study.

**MATERIALS AND METHODS:** An IRB approved electronic survey investigating women's opinions on contraceptive choice and STI prevention was distributed at women's health clinics at the University of Cincinnati. Participation was voluntary and responses remained anonymous. The descriptive data were analyzed using percentages and medians.

**RESULTS:** One hundred and five surveys were completed. Participants ranged from 18-45 years of age (median 29, IQR 26, 33). The majority of participants were non-Hispanic white (82.9%) and sexually active (88.6%).

Approximately 83.7% were sexually attracted to men, while 7.7% were attracted to females and 8.7% were attracted to both. A history of an unintended pregnancy was reported by 26.7% of all participants and 20.0% had previously been diagnosed with an STI. In participants who were sexually attracted to men or both genders, 94.8% had used some form of contraception, including a hormonal pill (83.5%), barrier method (68.1%) or an intrauterine device (30.8%). Approximately 35.1% reported consistently using a method to prevent STIs, of which, 100% used male condoms. When asked if one felt empowered to choose her desired contraceptive method, 8.2% said no and 7.2% said they were unsure. Approximately 13.4% of participants reported that they feel pressured by their partner to not use contraception and 21.6% feel pressured by their partner to not use protection against STIs. When participants were asked if they were interested in using a self-administered, discrete product that could both prevent pregnancy and STIs, 38.5% responded yes and 31.3% were unsure.

**CONCLUSIONS:** In women sexually attracted to men, nearly two-thirds do not routinely use STI prevention and some feel pressured by their partner not to use contraception/condoms. This makes women more vulnerable for unwanted infections and pregnancy. Because of this, there is a role for discretely self-administered female contraceptive products that also prevent STIs.

**SUPPORT:** University of Cincinnati Office of Research Strategic Collaborative Grants Program.

**THE EFFECT OF DIFFERENT PROGESTOGEN ONLY CONTRACEPTIVE METHODS ON FEMALE SEXUAL FUNCTION IN THE FIRST-TIME USERS: A CROSS SECTIONAL STUDY.**

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**OBJECTIVE:** The progestin-only contraceptive (POC) methods are used frequently by women in the childbearing period. However; these methods are associated with female sexual dysfunction (FSD) especially injectables. There are potential predictors associated with FSD among POC users which should be put in the consideration during counseling for POC use. Our objective is to assess the female sexual function (FSF) in three POC methods among first-time users.

**DESIGN:** Cross-sectional study (Clinicaltrials.gov:NCT02579590).

**MATERIALS AND METHODS:** We included married women between 20-40 years with a heterosexually active relationship lasting for longer than four weeks. They were using one of the POC methods for at least six months for contraception only. Those women were first-time users with regular menstrual pattern, amenorrhea or even with minimal vaginal spotting not affecting the sexual life. The enrolled women were classified into four groups; non contraceptive users (group I), Depot Medroxyprogesterone Acetate 150 mg (DMPA) injection (group II), etonogestrel 68 mg subdermal implant (group III) and desogestrel 75  $\mu$ g oral pills (group IV) users for the first time. All participants were asked to complete the Arabic form of the female sexual function index (ArFSFI). A total score of less than or equal to 28.1 points was determined as FSD. The main outcome of the study was to identify the prevalence of FSD among those users. The predictors associated with FSD among POC users were also explored. The data were analyzed using ANOVA, Chi-square test and the logistic regression model.

**RESULTS:** Four hundred forty-four women consented to participate and divided into two groups; 222 women were non contraceptive users, and 222 women were POC users (88 women were DMPA users, 87 women were etonogestrel implant users and 47 women were desogestrel containing pills users). All groups (non contraceptive users and POC users) were homogeneous in the baseline data. The mean ArFSFI score was significantly lower in POC users than non-contraceptive users (26.92 $\pm$ 1.88 Vs 27.42 $\pm$ 2.02, p=0.006; respectively). The mean ArFSFI score was significantly lower in DMPA users in comparison to etonogestrel implant and desogestrel pills users (26.46 $\pm$ 1.75, 27.13 $\pm$ 1.89, 27.37 $\pm$ 1.93, p=0.010; respectively). Furthermore; the number of women with FSD was significantly higher in DMPA users in comparison to other users (68 women; 77.2%, 44 women; 50.5%, 16 women; 34.0%, p=0.000; respectively). The baseline characteristics that were revealed from the regression model and significantly associated

with a higher likelihood of FSD with POC were circumcision ( $p=0.001$ ), parity  $>3$  times ( $p=0.015$ ) and duration of use  $>12$  months ( $p=0.022$ ). A ROC curve analysis in the predictive model demonstrated that circumcision yielded the highest sensitivity (82.84%) while the parity  $>3$  times had the lowest one (59.76%) and the duration of use  $>12$  months had a sensitivity of 60.36%.

**CONCLUSIONS:** There is a high prevalence of FSD in POC users especially DMPA users. The circumcision, parity  $>3$  times and  $>12$  months of use are potential significant predictors of FSD in POC users.

**SUPPORT:** None.

**P-500** Wednesday, October 16, 2019 6:30 AM

**CHINA FEMALE CONDOM(FCC) FUNCTIONALITY STUDY AGAINST AN EQUIVALENT MARKETED FEMALE CONDOM(FC2).** Yimin Cheng Sr., M.D., National Research Institute for Family Planning, Beijing, China.



**OBJECTIVE:** To compare the differences of the rates of total clinical failure and four types of failures (Invagination, Misdirection, Slippage and Breakage) between two kinds of female condoms(FC) [China made FC (FCc) and USA made FC (FC2)] as well as to assess whether every failure of four rates is accord with the standard of WHO.

**DESIGN:** Prospective, double-blind randomized controlled.

**MATERIALS AND METHODS:** 300 participants were recruited. A computer-generated randomization sequence was used to assign the 300 participants to one of two groups (1:1). Group A used 5 FCc first, followed by 5 FC2s. Group B used 5 FC2s first, followed by 5 FCc. The FC2 is made from synthetic nitrile material and is manufactured by the Female Health Company (Chicago, IL, USA). The FCc is made of polyurethane and has a dumbbell shape. It is manufactured by Tianjin CondomBao Medical Polyurethane Tech. Co. (Tianjin, China).

**RESULTS:** The rate of loss to follow-up was 4.2% for FCc and 2.8% for FC2. The total clinical failure rate of FCc was 0.9% (95% confidence interval 0.5–1.3%) compared to 1.1% (95% confidence interval 0.7–1.5%) for FC2. The upper bound of the one-sided 95% confidence interval for FCc total clinical failure rate, minus the FC2 total clinical failure rate is equal to 0.2% ( $1.5\%-1.3\%=0.2\%$ ). The difference of the total clinical failure rates (1.1% vs. 0.9%) between FC2 and FCc was statistically no significant ( $P>0.05$ ). No breakage was found both in FCc users and in FC2 users. The failure rates of invagination, misdirection and slippage of FCc were 1.3%, 1.3% and 1.1% respectively. The failure rates of invagination, misdirection and slippage of FC2 were 1.8%, 0.1% and 2.5% respectively. The difference of slippage rates (2.5% vs. 1.1%) was statistically no significant ( $P>0.05$ ) between FC2 and FCc as well as the slippage rate of FCc was lower than the standard of WHO although the slippage rate of FC2 was slightly higher than that of FCc and slightly higher than the standard of WHO. The difference of invagination rates (1.8% vs. 1.3%) was also statistically no significant ( $P>0.05$ ) between FC2 and FCc. Although the rate of misdirection for FCc was higher than that for FC2 (1.3% vs. 0.1%) and although the difference of the misdirection rates between two groups was statistically significant, but the rate of misdirection for FCc (1.3%) is lower than that of WHO standard (1.5%).

**CONCLUSIONS:** (1) The results indicated that the total clinical failure rate of FCc is non-inferior to the total clinical failure rate of FC2; (2) The rates of four types of failure (Invagination, Misdirection, Slippage and Breakage) for FCc was that every failure rate is lower than the standard of WHO. (3) The upper bound of the one-sided 95% confidence interval for FCc total clinical failure rate, minus the FC2 total clinical failure rate is less than 3% ( $1.5\%-1.3\%=0.2\%$ ).

**SUPPORT:** National Research Institute for Family Planning provided financial support for this research.

**P-501** Wednesday, October 16, 2019 6:30 AM

**RISK FACTORS FOR NON-COMPLIANCE IN POST VASECTOMY FOLLOW UP.** Johnathan Doolittle, MD, Peter N. Dietrich, MD, Pranav Dadhich, MD, Sarah M. Brink, BS, Daniel Roadman, BS, Kayvon Kiani, BA, G. Luke Machen, MD, Jay I. Sandlow, MD. Medical College of Wisconsin, Milwaukee, WI.



**OBJECTIVE:** Vasectomy is regarded as the most effective method of birth control, with over half a million performed annually in the United States.

Poor compliance with providing a post vasectomy semen analysis (PVSA) has previously been reported in both the Family Medicine and Urologic literature, with rates ranging from 34–46%. Reasons for poor compliance with PVSA are not well described. Only one prior study was identified that examined socioeconomic factors predictive of non-compliance. We sought to further characterize this population by examining the pre operative characteristics of patients of a large volume vasectomy surgeon that were predictive of failure to provide a PVSA.

**DESIGN:** A retrospective, single institution chart review

**MATERIALS AND METHODS:** Records were reviewed from April 2015 to April 2018, which identified 1137 patients who underwent vasectomy by a single surgeon. Patients who underwent vasectomy for non-fertility related reasons were excluded. Other exclusion criteria included requiring in vitro fertilization to conceive prior to the procedure. Patient characteristics analyzed include age, race, marital status, insurance type, and number of children. Univariate and multivariate logistic regression were performed to compare our two cohorts and to assess for factors predictive of post vasectomy compliance.

**RESULTS:** 1,137 patients underwent vasectomy. The average age was 37.5 years. 89.5% and 88.7% of the patients were White/Caucasian and married, respectively. 27.5% of patients did not follow up for PVSA at any interval. Age was similar between patients who did and did not submit a PVSA (37.8 vs 37.3 years). However race, marital status, and insurance did differ, as patients in the no PVSA cohort were more likely to be African American (8.3% vs 3.7%), single (15.3% vs 9.7%) and have Title 19/Medicaid (2.9% vs 1.2%) insurance coverage (all  $p$  values  $<0.05$ ). On multivariate analysis, single relationship status was independently predictive of failing to present for post vasectomy semen analysis (RR 1.86,  $p=0.02$ ). Age (RR 1.02,  $p=0.08$ ) and increasing number of children (RR 1.11,  $p=0.09$ ) approached significance.

**CONCLUSIONS:** A significant percentage of patients do not provide a PVSA confirming sterility, with single relationship status being most predictive of noncompliance when controlling for all other preoperative variables. As with all vasectomy patients, counseling these patients that they are not sterile until proven with a PVSA is paramount.

**References:** 1. Å Barone MA, Hutchinson PL, Johnson CH et al: Vasectomy in the United States, 2002. *J Urol* 2006; **176**: 232.

2. Å Postvasectomy semen analysis: why patients don't follow-up. D. R. Smucker, H. E. Mayhew, D. J. Nordlund, W. K. Hahn, Jr, K. E. Palmer. *J Am Board Fam Pract.* 1991 Jan-Feb; 4(1): 5–9.

3. Å Postvasectomy semen analysis: are men following up? Ronald E. Christensen, Dalton C. Maples, Jr. *J Am Board Fam Pract.* 2005 Jan-Feb; 18(1): 44–47.

**SUPPORT:** N/A.

**P-502** Wednesday, October 16, 2019 6:30 AM

**RETURN TO FERTILITY AFTER 1-YEAR USE OF A SEGESTERONE ACETATE/ETHINYL ESTRADIOL CONTRACEPTIVE VAGINAL SYSTEM**



**USE.** Ginger Constantine, MD,<sup>a</sup> Kurt T. Barnhart, MD, MSCE,<sup>b</sup> Anne E. Burke, MD, MPH,<sup>c</sup> Ruth B. Merkatz, PhD,<sup>d</sup> Shelli Graham, PhD,<sup>e</sup> Brian Bernick, MD,<sup>e</sup> Sebastian Mirkin, MD,<sup>e</sup> Endo-Rheum Consultants, LLC, Malvern, PA; <sup>b</sup>University of Pennsylvania, Perelman School Of Medicine, Philadelphia, PA; <sup>c</sup>Johns Hopkins School of Medicine, Baltimore, MD; <sup>d</sup>Population Council, New York, NY; <sup>e</sup>TherapeuticsMD, Boca Raton, FL.

**OBJECTIVE:** To assess the return to menses and/or fertility in a subset of women who used a contraceptive vaginal system (CVS; approved by the FDA in August 2018) releasing a daily mean of segesterone acetate (SA) 0.15 mg and ethinyl estradiol (EE) 0.013 mg for up to 13 cycles.

**DESIGN:** Two multicenter, single-arm, open-label, pivotal, phase 3 studies of the SA/EE CVS; one US-only study (15 US sites) and one international study (5 in the US; 3 in Europe, 3 in Latin America, 1 in Australia).

**MATERIALS AND METHODS:** Women used the same SA/EE CVS on a 21/7-day in/out regimen for up to 13 cycles. Those who wished to become pregnant or use non-hormonal contraceptives after completing the 13 cycles could participate in a 6-month follow up for return to fertility. Women were instructed to perform a urine pregnancy test within 2–3 weeks following their last visit and then monthly if they experienced pregnancy symptoms and/or did not have a bleeding episode. Women were contacted every 2 months for pregnancy, menses, and contraceptive use information. Women who were pregnant returned to the clinic for pregnancy confirmation and a prenatal care referral. Bleeding  $<18$  days after last CVS use was considered

withdrawal bleeding, not menses. We report proportion of subjects who had return to fertility, defined as pregnancy within 6 months after final CVS use or menses occurring >18 days after last CVS use.

**RESULTS:** Of 212 women in the return-to-fertility population of the US study, 163 (76.9%) were able to be contacted and 16 women were excluded for hormonal contraceptive use leaving 147 women (69.3%) for analysis. All 147 (100%) women reported menses occurring >18 days after last CVS use (135 women; 91.8%) or pregnancy (12 women; 8.2%). Women reported 6 pregnancies at 2 months, 3 at 4 months, and 3 at ≥6 months.

In the international study, of 158 women in the return-to-fertility follow up, 154 (97.5%) were able to be contacted and 11 women were excluded for hormonal contraceptive use leaving 143 women (90.5%) for analysis. All 143 (100%) women reported menses as defined above (131 women; 91.6%) or pregnancy (12 women; 8.4%). Seven pregnancies were reported at 2 months, 5 at 4 months, and 1 at 6 months.

**CONCLUSIONS:** All women who desired pregnancy or used non-hormonal contraceptives became pregnant or had a return of menses in the 6 months after the last use of the SA/EE CVS, suggesting that the CVS does not delay or adversely affect return to fertility.

**SUPPORT:** The Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health (NICHD; Contract Number HHSN27500403372) funded and conducted the US study; the US Agency for International Development (USAID; Grant Number GPO-A-00-04-00019-00) funded the international study, which was conducted by the Population Council; the World Health Organization (WHO) Reproductive Health Research Department funded two international study sites.

**P-503** Wednesday, October 16, 2019 6:30 AM

**DECLINE IN FERTILITY WITH AGE: AN OVERLOOKED AND UNDER ADDRESSED TOPIC IN WOMEN'S HEALTH VISITS.**

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**OBJECTIVE:** To determine whether women discuss age-related fertility decline with healthcare providers, and women's response to an educational intervention on the impact of age on fertility.

**DESIGN:** We conducted a cross-sectional survey of women aged 20-45 visiting public markets in a metropolitan area.

**MATERIALS AND METHODS:** Participants completed a survey regarding demographics, reproductive life goals, and knowledge and thoughts about age and fertility at baseline and after an educational intervention. Our intervention was a hand out on the impact of age on fertility. Our primary outcome was the proportion of women who previously discussed age-based fertility with a provider. We used descriptive statistics to characterize our study population (age, parity, marital, employment and insurance status) and assess responses. We used a multivariable logistic regression model to determine the association between age and learning from the intervention.

TABLE 1. Survey responses regarding age-based fertility prior to and following an educational intervention.

	Desires future children, % respondents	Childbearing complete, % respondents	Undecided, % respondents
<b>Baseline data</b>			
<b>Age-based fertility:</b>			
Have discussed this with a healthcare provider	16% (17/104)	15% (9/59)	17% (8/46)
Would like to discuss this*	41% (41/101)	5% (3/59)	32% (15/47)
Would like more information*	52% (54/103)	7% (4/59)	26% (12/46)
<b>Following intervention</b>			
Learned something new	71% (72/102)	65% (37/57)	66% (29/44)
Plan to discuss with partner/family*	46% (47/103)	7% (4/54)	27% (12/44)
Considering changing reproductive plans*	13% (13/102)	2% (1/55)	23% (10/44)
Feel it would be helpful to discuss at routine visits*	79% (81/102)	45% (25/56)	73% (32/44)

\* = p<0.01

**RESULTS:** Our study population consisted of 212 women (mean age 30.6). The majority of women were employed (89%), insured (94%), college educated (72%), and had no living children (79%). Only 16% of participants reported prior discussion with a provider about age-based fertility, and 44% reported this topic made them feel anxious. Following an educational intervention, 68% of all participants, and 79% of participants who desire more children, reported it would be helpful to discuss age-based fertility in office visits (Table 1). Women were significantly more likely to report learning from the intervention if they were <35 years old (OR 2.41; 95% CI 1.16, 5.00) or had public insurance (OR 2.29; 95% CI 1.04, 5.07).

**CONCLUSIONS:** A minority of women are discussing the role of age on fertility with providers. This study suggests a potential role for counseling in well-women visits to assist women with reproductive planning. Women under age 35 or enrolled in public insurance are most likely to benefit from education on age-based fertility.

**EARLY PREGNANCY**

**P-504** Wednesday, October 16, 2019 6:30 AM

**ART CONCEPTIONS ARE NOT A HIGH-RISK POPULATION FOR CELL-FREE FETAL DNA TESTING.**

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**OBJECTIVE:** It has been suggested that Non-invasive Prenatal Testing (NIPT) based on the analysis of cell-free placental DNA (cf-DNA) is less accurate in patients conceiving via assisted reproduction technologies (ART) than in those with natural conception (NC). In this study, we are investigating in a larger clinical sample whether this assumption is correct.

**DESIGN:** Cohort study.

**MATERIALS AND METHODS:** A total of 17,735 patients were included in this retrospective study. Patients were divided in two groups, natural conception (NC) (n=13,108) and ART (n=4,627). The ART group was divided in those using their own oocytes (OO)(n=3,107) or using oocyte donation (OD)(n=1,520). NIPT by massively parallel sequencing to assess chromosome aneuploidy for 13, 18, 21 and sex chromosomes was offered to the patients past 10 weeks of gestation, in addition to conventional prenatal screening. NIPT was performed by using the Illumina's technology platform. Abnormal results rates, false positive rates, mean fetal fraction (FF) and rates of samples with FF<4% as a parameter indicating the percentage of samples with non-informative results were compared among all groups. For quantitative data, pairwise comparison using t-test with non-pooled SD (P value adjustment method: Bonferroni) was applied. In order to compare qualitative data Chi-square test (with Yates correction) was performed.

**RESULTS:** We observed that samples at risk of aneuploidy in the ART group were significantly lower than in the group of NC (1.73% vs. 2.76%;

$p < 0.0001$ ). These differences were due to the age of the OD group, which showed a percentage of samples at risk of aneuploidy of 0.72%. Comparable age groups of OO and NC did not show significant differences for the percentage of samples at risk of aneuploidy (2.22% vs. 2.76% respectively). No significant differences were observed in the false positive rates among any of the groups investigated. Unlike previously reported, we observed a significant higher FF in the ART group than in NC (9.8% vs. 9.5% respectively;  $p < 0.01$ ). No significant differences were observed in the percentage of samples with FF < 4 (3.5% in the NC, 3.3% for OO and 4% for the OD).

**CONCLUSIONS:** It has been suggested that woman who conceive by ART have a higher probability of false positives and non-call results in the NIPT due to low FF than those who conceive naturally. Our results in more than 17,000 patients showed similar false positive rates in both populations as well as FF rate in all groups analysed. We conclude that ART conceptions are comparable to those conceiving naturally in terms of NIPT test performance.

**SUPPORT:** None.

**P-505** Wednesday, October 16, 2019 6:30 AM

### IDENTIFICATION OF EARLY PLACENTAL HORMONE PRODUCTION IN PROGRAMMED EMBRYO TRANSFER CYCLES.

Robert Setton, MD,<sup>a</sup> Kelly McCarter, MD,<sup>b</sup> Lilli D. Zimmerman, MD,<sup>a</sup> Zev Rosenwaks, M.D.,<sup>a</sup> Steven Spandorfer, M.D.<sup>a</sup> <sup>a</sup>The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY; <sup>b</sup>Weill Cornell Medicine, New York, NY.



**OBJECTIVE:** There is a dearth of literature describing the onset of early placental steroidogenesis and the initiation of the luteal-placental shift. Patients undergoing programmed frozen-thawed embryo transfer (FET) or donor-egg recipient (DER) cycles offer a unique model to study this phenomenon, as these patients lack a corpus luteum. In this study we sought to identify the initiation of placental hormonal production as defined by the production of endogenous estradiol (E2) and progesterone (P4) in a cohort of patients undergoing programmed cycles with single embryo transfers resulting in liveborn singletons.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Patients undergoing programmed FET with autologous oocytes or DER cycles were screened for inclusion. Only patients who underwent a single embryo transfer, had a single gestational sac, and a resultant liveborn singleton were included. All patients were treated with E2 patches changed every other day and intramuscular progesterone injections daily. Main outcome measures were serial E2 and P4, with median values calculated for cycle days 28 (baseline) through 60. The baseline cycle day-28 median value was compared to each daily median cycle day value using the Wilcoxon Signed Rank test.  $P < 0.05$  was deemed statistically significant.

**RESULTS:** A total of 696 patients, 569 using autologous oocytes in programmed FET cycles and 127 using donor oocytes, from 4/2013 to 4/2019 who had a single embryo transfer with a resultant single sac and singleton livebirth met inclusion criteria. Serum E2 and P4 levels stayed consistent initially and then began to increase daily. Compared to baseline cycle day-28 E2 (415 pg/mL), the serum E2 was significantly elevated at 542 pg/mL ( $P < 0.001$ ) beginning on cycle day 36. With respect to baseline cycle day P4 (28.1 ng/mL), beginning on cycle day 48, the serum P4 was significantly elevated at 31.6 ng/mL ( $P < 0.001$ ).

**CONCLUSIONS:** These results demonstrate that endogenous placental estradiol and progesterone production occur by cycle day 36 and cycle day 48 respectively, earlier than traditionally thought. These findings also suggest that modifications to luteal support paradigms can be considered given that the placentas in most programmed FET cycles are independently steroidogenic before the seventh week of gestation.

**Reference:** None.

**SUPPORT:** None.

**P-506** Wednesday, October 16, 2019 6:30 AM

### DOES THE HYPERESTROGENIC MILIEU IN FRESH IN VITRO FERTILIZATION CYCLES IMPACT EARLY EMBRYONIC GROWTH?

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O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY.

**OBJECTIVE:** Recent studies have suggested that the hyperestrogenic milieu generated during ovarian stimulation may create a suboptimal peri-implantation environment, leading to adverse perinatal outcomes. In this study, we investigate whether supraphysiologic estradiol (E<sub>2</sub>) impacts early embryonic growth in vitro fertilization (IVF)-embryo transfer (ET) cycles.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Normal responder patients, <40 years old, undergoing fresh IVF-ET cycles resulting in live singleton births were included. Patients with PCOS, multiple births, vanishing twins, or unknown perinatal outcomes were excluded. The primary outcome of interest was crown-rump length (CRL) at 7 to 8 weeks of gestational age. Secondary outcomes recorded were low birth weight (LBW, <2500 grams), pre-term birth (PTB, <37 weeks of gestation) and birth weight. Primary and secondary outcomes were assessed according to peak E<sub>2</sub> quartiles. Receiver-operator-characteristic (ROC) curves were constructed for outcomes showing statistical significance.

**RESULTS:** A total of 4,071 patients with live singleton births were included. The median age, body mass index (BMI), E<sub>2</sub> level and birth weight for the study cohort was 36 (33-39) years, 22.3 (20.4-25.0) kg/m<sup>2</sup>, 1,554 (1,112.7-2,179) pg/mL, and 3,289 (2,920-3,628) grams, respectively. Singletons in the 4<sup>th</sup> E<sub>2</sub> quartile (8.56 mm) had a smaller CRL compared to all other E<sub>2</sub> quartiles. The rate of LBW rose from 6.4% (E<sub>2</sub> 2,001-2,500 pg/ml) to 20.7% (E<sub>2</sub> 3,501-4,000 pg/mL), without a corresponding rise in the rate of PTB. The odds of term LBW with E<sub>2</sub> >2,500 pg/mL were 6.1-7.9 times higher compared to the median E<sub>2</sub>. Peak E<sub>2</sub> level was a weak predictor of CRL (AUC=0.64), but a strong predictor of LBW (AUC=0.86).

**CONCLUSIONS:** The results of the current study suggest that the hyperestrogenic milieu of ovarian stimulation can adversely impact early embryonic growth and ultimately perinatal outcomes. Our results emphasize the importance of minimizing the supraphysiologic elevations of E<sub>2</sub> levels in fresh IVF-ET cycles to optimize the early peri-implantation environment and mitigate adverse perinatal outcomes.

**SUPPORT:** None.

**P-507** Wednesday, October 16, 2019 6:30 AM

### CHALLENGING CURRENT VIEWS: A PROSPECTIVE SERIES OF ANGULAR PREGNANCIES MANAGED EXPECTANTLY.

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**OBJECTIVE:** To describe the natural history and outcomes of the largest cohort of expectantly managed angular pregnancies diagnosed by specific ultrasound criteria.

**DESIGN:** Prospective case series.

**MATERIALS AND METHODS:** This was a prospective case series of women with prenatally diagnosed angular pregnancy at a single, academic, tertiary care center from March 2017 to February 2019. Participants were identified at a first-trimester ultrasound scan using specific

	Angular Pregnancy Outcomes n (% Proportion)	Pregnancy Outcomes in the General Population % Incidence
Total Deliveries	21 (51.2)	-
Spontaneous Vaginal Deliveries	15 (71.4)	68.1
Low Transverse Cesarean Section	6 (28.6)	31.9
Term Delivery	17 (81.0)	88
Preterm Delivery	4 (19)	12
Miscarriage	7 (17.1)	10
Continuing Gestations	13 (31.7)	-

diagnostic criteria of an angular pregnancy and followed expectantly. Diagnostic criteria included 1) Nonanomalous uterus; 2) Implantation of the embryo in the lateral angle of the uterine cavity; 3)  $\leq 1$  cm of myometrial thickness surrounding the gestational sac; 4) Presence of completely circumferential endometrium surrounding the gestation; and 5) Lack of an "interstitial line sign." Maternal and fetal data were gathered from the medical record.

**RESULTS:** Forty-two cases of angular pregnancy were identified at first-trimester ultrasound. At presentation, 33 patients (78.6%) were asymptomatic, eight (19.0%) had vaginal bleeding, and two (4.8%) had pain. The mean gestational age at diagnosis was  $7.4 \pm 1.0$  weeks, and the mean myometrial thickness was  $5.1 \pm 1.6$  mm (95% CI 4.6-5.6). At initial follow up, 23 cases (54.8%) had resolved, 13 cases (31.0%) persisted as angular pregnancies, and six cases (14.3%) resulted in miscarriage. Three cases (7.1% of total) that persisted had decreased myometrial thickness. At final follow up, 21 (51.2%) deliveries resulted in a live birth, seven (17.1%) in miscarriage, and 13 (31.7%) were continuing gestations. In cases of live birth, 15 (71.4%) were vaginal deliveries, six (28.6%) cesarean sections, 17 (81.0%) term deliveries, and four (19.0%) preterm deliveries. There were no cases of uterine rupture, maternal death, abnormal placentation, or hysterectomy.

**CONCLUSIONS:** In 42 cases of angular pregnancy diagnosed by first-trimester ultrasound, all but eight resolved with continued follow up. Outcomes were largely positive with a 51.2% live birth rate, 17.1% miscarriage rate, and 31.7% continuing pregnancy rate. Angular pregnancy may represent a clinical entity that more closely resembles a normal, non-eccentric intrauterine pregnancy rather than an ectopic pregnancy. Therefore, most cases can be safely observed, and efforts should be made to expectantly manage gestations while awaiting viability.

**P-508** Wednesday, October 16, 2019 6:30 AM

**INFLUENCE OF ASSISTED REPRODUCTIVE TECHNOLOGY (ART) ON EARLY FETAL GROWTH KINETICS.**

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**OBJECTIVE:** The aim of this study was to evaluate variations in embryonic size early in pregnancy and to investigate if *in vitro* fertilization (IVF) is associated with significant differences in crown rump length (CRL) prior to 7 weeks gestation. This work may lay the foundation for understanding the way in which assisted reproductive technology (ART) might impact embryonic and fetal growth dynamics, as well as the implications for adverse pregnancy outcomes.

**DESIGN:** Retrospective cohort study of data from an academic IVF practice between January and December 2017.

**MATERIALS AND METHODS:** Our study population included 88 patients undergoing a transvaginal ultrasound at 6-7 weeks gestation following fertility treatment with intrauterine insemination, timed intercourse with use of a trigger injection, or IVF (only when exact date of conception was known). Only women with singleton, intrauterine pregnancies with cardiac activity present were included. Patients with multiple gestations, spontaneous conception, or with first trimester miscarriage were excluded. At the time of the ultrasound, CRL, fetal heart rate, and average ultrasound age (AUA) were calculated. Fetal size was measured as CRL, and the difference between AUA and gestational age was calculated. The distribution of embryonic size was evaluated assuming a normal distribution of CRL. Differences in fetal size were evaluated using ANOVA to account for differences in age at scan. Deviations in AUA from expected gestational age were evaluated using student's t-test.

**RESULTS:** Approximately half (58%) of women in our cohort were non-Hispanic White and 27% were African American. The majority of women became pregnant through IVF (66%) with the rest resulting from IUI (27%) or timed intercourse (7%). Even within this relatively limited age range, there was variation in CRL. Among 20 ultrasounds performed at 47 days, the mean  $\pm$ standard deviation (SD) CRL was 0.73 (0.13) cm but the

smallest embryo was 0.37 cm and the largest was 0.96 cm. After accounting for gestational age at ultrasound, pregnancies conceived through IVF were slightly smaller, on average, than other pregnancies (mean  $\pm$ SD 0.56  $\pm$ 0.22 versus 0.62  $\pm$ 0.24), but this difference was not statistically significant ( $p=0.27$ ). IVF pregnancies achieved using fresh embryos were slightly larger (0.08  $\pm$ 0.05 cm) than those using frozen embryos, but this too was not statistically significant ( $p=0.13$ ).

**CONCLUSIONS:** Early ultrasound is the gold standard for dating pregnancies despite known variation in these measures, even in early pregnancy. The degree to which this variation reflects variation in embryonic size, measurement error, and/or error in estimating date of conception is unknown. In this study, we confirmed that even after accounting for timing of conception, substantial variation in fetal size remains, even between 6-7 weeks gestation. Furthermore, the method of conception may be one factor associated with variation in early embryo growth. Further research is needed to determine if this variation in early embryo size is associated with later pregnancy outcomes such as preeclampsia and/or fetal growth.

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**EARLY PLACENTAL GENE EXPRESSION DISCRIMINATES BETWEEN NON-VISUALIZED INTRAUTERINE PREGNANCIES VERSUS TUBAL ECTOPIC PREGNANCY IN UTERINE**



**ASPIRATES.** Maureen Baldwin, MD MPH,<sup>a</sup> Jon D. Hennebold, PhD,<sup>b</sup> <sup>a</sup>Oregon Health and Science University, Portland, OR; <sup>b</sup>Oregon National Primate Research Center, Division of Reproductive & Developmental Sciences, Beaverton, OR.

**OBJECTIVE:** All early pregnancies have the potential to be a life-threatening ectopic pregnancy, but there is no reliable diagnostic test to localize a pregnancy until almost two weeks after implantation, when it can be visualized using sonography. We aimed to demonstrate that early placental biomarkers expressed in endometrial samples can discriminate between non-visualized presumed intrauterine pregnancy (IUP) and confirmed ectopic pregnancy (EP).

**DESIGN:** Case-control.

**MATERIALS AND METHODS:** We collected uterine aspirates from asymptomatic patients undergoing induced abortion of pregnancy of unknown location (PUL) and those undergoing surgical management of tubal EP. PUL were assumed to be non-visualized IUP if estimated gestation  $\geq 42$  days, initial serum human chorionic gonadotropin (hCG)  $\geq 3,000$ , and hCG declined rapidly after uterine aspiration alone (typically defined as  $>50\%$  in 48 hours). All samples were stored in RNAlater<sup>®</sup> solution and homogenized in TRIzol<sup>®</sup> (Invitrogen). We performed RNA purification and extraction of 1 mL of homogenate with PureLink<sup>®</sup> RNA Mini Kit (Ambion<sup>™</sup>), including on-column DNase treatment with RQ1<sup>®</sup> RNase-free DNase (Promega<sup>™</sup>). We performed qRT-PCR (ABI Prism<sup>®</sup> 7900HT Sequence Detection System; Applied Biosystems) using an RNA-to-C<sub>T</sub><sup>™</sup> 1-Step Kit, gene-specific primers, and TaqMan<sup>®</sup> probes (ThermoFisher) to assess trophoblast-specific chorionic gonadotropin subunit beta (CGβ) and RPL10 (endogenous control). Relative expression of CBG and RPL10 expression in non-visualized IUP and EP endometrium was compared to non-pregnant endometrium. Sample size  $>3$  in each group had 80% power to demonstrate a statistical difference ( $\alpha=0.05$ ) in mean fold-change expression.

TABLE. Serial serum hCG trend among non-visualized pregnancies.

Estimated gestation (days)	Initial hCG (mIU/mL)	Serial hCG (mIU/mL)
26	268	34
28	336	46
33	852	137
37	1621	4*
38	266	38

All serial hCG trend over 2 days except 12 days for \*.

**RESULTS:** Using quantitative RT-PCR, the mean fold increase in expression of CGB in endometrial samples of non-visualized IUP (n=5) compared to non-pregnant endometrium, was 20.2 ( $\pm 2.0$ ), which was significantly greater (n=5;  $p < 0.01$  by t-test) than the 8.0 ( $\pm 2.8$ ) fold-increase observed in EP.

**CONCLUSIONS:** The early trophoblast biomarker CGB is detectable in uterine aspirates of non-visualized presumed IUP at a large threshold difference compared to endometrium from EP. This is consistent with localized CGB expression by the invasive syncytiotrophoblast. This preliminary data suggests that molecular targets to specific early placental markers could be utilized for diagnosis of pregnancy location after direct endometrial sampling.

**SUPPORT:** This work has been supported by the Society of Family Planning Research Fund SFPFR11-J11 and by the NIH Women's Reproductive Health Research Program (K12HD085809).

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### **PREDICTION OF PREGNANCY OUTCOME IN WOMEN WITH FIRST TRIMESTER BLEEDING BY THE DETECTION OF ALPHA-FETOPROTEIN (AFP) IN VAGINAL BLOOD.**



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**OBJECTIVE:** We previously published that high concentration of alpha-fetoprotein (AFP) in the vaginal blood confirms the presence of microscopic embryonic/fetal tissue. Here we attempted to detect the presence of AFP in dried blood collected on pads from women with first trimester bleeding.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** A sample of patients presenting to a fertility center or emergency department with positive pregnancy tests and vaginal bleeding were invited to participate in the study. After informed consent, a hygienic pad was collected from each participant. 1x1 cm<sup>2</sup> pad patches with dried vaginal blood were placed into 1 ml saline. The dissolved AFP that originated from the vaginal blood (AFPvb) was quantified by an automatic chemiluminescence assay. Two outcomes were evaluated: 1) clinical and/or histopathologic evidence of passage of intrauterine embryonic/fetal tissue (a failed intrauterine pregnancy); 2) a threatened miscarriage with subsequent ongoing clinical pregnancy (heartbeat documented on ultrasound on the day of pad collection or at least once within the subsequent 5 weeks).

**RESULTS:** To date, 15 women with first trimester bleeding were enrolled. For these women, the median age, gravidity, and parity (with ranges in parenthesis) were 32 (20-51) years, 3 (1-8), and 0 (0-3), respectively. Each woman provided a single pad with dried vaginal blood. Four women passed embryonic/fetal tissue and 11 had an ongoing pregnancy. AFPvb was detected in 5 specimens: 4 from the 4 women passing embryonic/fetal tissue and 1 from a woman with a threatened miscarriage. AFPvb was not detected in the other 10 specimens, all from women with a threatened miscarriage and ongoing pregnancies. The detection of AFP in the vaginal blood was significantly associated with pregnancy loss while its absence was seen exclusively in successful pregnancies ( $P = 0.004$  by Fisher exact test).

**CONCLUSIONS:** AFP can be extracted and detected in dried blood on pads collected from women with first trimester bleeding. When AFPvb is detected the likelihood of a failed intrauterine pregnancy is 80% whereas this likelihood drops dramatically when AFPvb is undetectable. Measurement of vaginal AFP may help to predict the fate of intrauterine pregnancy in the setting of first trimester bleeding.

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### **EARLY MULTIFETAL PREGNANCY REDUCTION OUTCOMES: NON-CHEMICAL-BASED METHOD YIELD IMPROVED PREGNANCY RATES AND MINIMIZE RISKS.**



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**OBJECTIVE:** Multifetal pregnancies increase maternal, and perinatal mortality, the presence of each additional fetus increases this risk; moreover, spontaneous loss of the entire pregnancy is 25% for quadruplets, 15% for triplets, and 8% for twins. Several studies showed that the reduction to a lower-order pregnancy (triplet or quadruplet to twin) reduces the risk of medical complications associated with maintaining multiple pregnancies. Multifetal pregnancy reduction is usually scheduled between 11 and 14 weeks of gestation, using chemical substances as adjuvants to help in the embryo reduction success rate. However, these chemical substances present alternative concerns and have been suggested to affect live birth rates. Therefore, we assessed a novel non-chemical-based procedure for fetal reduction performed during early gestation of high order pregnancies.

**DESIGN:** Single-arm prospective study conducted between December 2013 and September 2018.

**MATERIALS AND METHODS:** Multifetal pregnancy reduction was carried out between 6 and eight weeks of gestation. The patient was placed in a lithotomy position under general anesthesia. Using the same equipment used for transvaginal ultrasound-guided oocyte recovery, the smallest embryo, located in a position with the easiest access route and preferably the one nearest cervix, was selected for embryo reduction. An echo tipped needle (17 Cook medical ovum aspiration needle) was inserted through the posterior fornix and the posterior uterine wall to the intended gestational sac. Then the needle is inserted in the embryos cardiac area until the absence of fetal heartbeat was seen and confirmed by color and power Doppler. The needle is then extracted, and hemostasis is verified. We avoid aspiration and the use of any chemical substances. We verify the vitality of remaining embryos with color and power Doppler. Patients were followed until delivery, and the baby's weight was a record as well as any complications.

**RESULTS:** For the proof of principle, only patient with three gestational sacs were analyzed (n=296). None of the women presented or indicated of any complication due to the surgery. Embryo reduction typically took place during the 7<sup>th</sup> week (range: 5-10.5 week). After the procedure, 3 patients lost their pregnancy (1.0%); however, 89.9% maintained the remaining 2 gestational sacs and 9.1% for 1 gestational sac. The live birth rates were 94.4% for the 2 gestational sacs (birth weight: 2111 $\pm$ 625 grams) and 96.3% for 1 gestational sac (birth weight: 2546 $\pm$ 793 grams). There was no difference in the low birth weight rate (2 sacs: 14.5% v 1 sac: 11.5%). The most common for the 2 sacs group was requiring NICU intervention (4.5%), whereas, for the 1 sac group, was Restriction of Intrauterine growth (14.8%).

**CONCLUSIONS:** Here, we demonstrate that a non-chemical method can successfully reduce the number of embryos.

**SUPPORT:** ConacytA 250768.

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### **LUTEINIZING HORMONE (LH) SURGE SHOULD REPLACE LAST MENSTRUAL PERIOD (LMP) FOR IMPROVED ACCURACY OF PREGNANCY DATING.**



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**OBJECTIVE:** To compare the accuracy of pregnancy dating by Luteinizing Hormone (LH) surge versus last menstrual period (LMP).

**DESIGN:** Observational cohort study of prospectively collected data in two academic RPL Programs from 2005-2018. Inclusion criteria included: women with a history of recurrent early pregnancy loss (REPL), defined as  $\geq 2$  pregnancy losses  $< 10$  weeks size;  $\geq 1$  subsequent pregnancy with a known LMP, documented LH surge, natural conception, and transvaginal ultrasound (TVUS) prior to 8 weeks from LMP, which resulted in a live birth.

**MATERIALS AND METHODS:** We compared the gestational age (GA) by LH surge (GA<sub>LH</sub>) and LMP (GA<sub>LMP</sub>) to the sonographic gestational age (GA<sub>CRL</sub>), based on the first crown rump length (CRL) of  $\geq 5$  mm. Secondary analysis compared the accuracy of pregnancy dating with a measurable CRL  $< 5$  mm to the conventional CRL  $\geq 5$  mm. Scatter diagrams were created for the difference between GA<sub>CRL</sub>-GA<sub>LH</sub>, and GA<sub>CRL</sub>-GA<sub>LMP</sub>; paired T-tests were used for analysis. In addition, scatter diagrams were created for CRL vs. GA<sub>LH</sub> and CRL vs. GA<sub>LMP</sub>; correlation coefficients for each were compared using Fisher's z-test. SAS 9.4 was used for statistical analysis, with significance  $P < 0.05$ . Descriptive statistics were reported as mean, standard deviation and range.

**RESULTS:** A total of 115 women with a history of RPL, with 118 subsequent live births, met inclusion criteria. Subjects were 96% Caucasian and 6% Hispanic. Mean age at delivery was 35.6 years (3.4;26-43). Mean number of prior pregnancy losses <10 weeks was 3.6 (1.8;2-12) and mean number of prior live births was 0.78 (1.41;0-4).

Scatter diagrams of  $GA_{CRL}-GA_{LH}$  revealed tighter fit around zero vs.  $GA_{CRL}-GA_{LMP}$ . Paired T-test revealed a lower mean absolute difference between  $GA_{CRL}-GA_{LH}$  vs.  $GA_{CRL}-GA_{LMP}$  2.04 vs. 3.08 days,  $P<0.0001$ . Fisher's z-test revealed a greater correlation between  $GA_{CRL}-GA_{LH}$  compared to  $GA_{CRL}-GA_{LMP}$   $r=0.77$  vs.  $r=0.62$ ,  $P=0.0018$ . This indicates a greater accuracy when using LH surge.

57 subjects had at least one TVUS with a CRL of <5 mm. Scatter diagrams of  $GA_{CRL<5mm}-GA_{LH}$  revealed trend towards a tighter fit around zero vs.  $GA_{CRL\geq 5mm}-GA_{LH}$ . Paired T-test revealed a trend towards a lower mean absolute difference between  $GA_{CRL<5mm}-GA_{LH}$  vs.  $GA_{CRL\geq 5mm}-GA_{LH}$ , 1.86 vs. 2.25 days, although this did not reach statistical significance,  $P=0.33$ .

**CONCLUSIONS:** A highly accurate estimated date of delivery (EDD) improves antenatal surveillance and reduces iatrogenic prematurity. Pregnancy is optimally dated in the first trimester and becomes increasingly inaccurate with advancing GA. Numerous publications have concluded that LMP is unreliable for pregnancy dating. Based on the results in this study, LH surge should replace LMP for improved accuracy of dating of pregnancy. There was a trend towards improved accuracy with a CRL < 5mm.

The average GA at time of first ultrasound by all three methods (CRL, LH surge and LMP) was 7 weeks +/- 5 days. Therefore, we propose a new threshold for very early pregnancy dating that supports redating the pregnancy by sonographic CRL if there is a discrepancy of more than 2 days from the LH surge or 3 days from LMP for gestations  $\leq 7/6/7$  weeks.

References: Antenatal Care: Routine Care for the healthy pregnant woman. National Collaborating Centre for Women's and Children's Health. Royal College of Obstetricians and Gynaecologists. 2<sup>nd</sup> Edition. March 2008  
Morin I. Determinants and consequences of discrepancies in menstrual and ultrasonographic gestational age estimates. *Brit J Obstet Gynaecol* 2005;112(2):145-52.

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#### **SUBCHORIONIC HEMATOMA IN THE INFERTILE POPULATION: PREVALENCE AND OUTCOMES.**

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**OBJECTIVE:** Subchorionic hematoma (SCH) is common, however, is of unknown significance among the infertile population as most studies evaluate women without a history of infertility and with naturally conceived pregnancies. Additionally, 1 small study suggests that SCH is higher among patients undergoing IVF and specifically after frozen embryo transfer (FET). The objective of this study was to identify the prevalence of SCH in the infertile population, as well as risk factors for first trimester miscarriage in affected pregnancies.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** CPT codes were used to identify all obstetric (OB) scans performed at a single infertility clinic from 1/2015-3/2018. All viable intrauterine pregnancies on initial OB scan were included for analysis (n=1254). Chart review was performed to identify the presence of SCH. Data on patient demographics, fertility treatments and pregnancy outcomes were collected. Differences in rate of SCH among fertility treatment cycle were compared using Chi-square test. Bivariate analysis was performed to compare pregnancies with SCH that resulted in first trimester miscarriage (< 14 weeks) to those that did not.

**RESULTS:** SCH prevalence was 11.9% (n=149). SCH rates did not vary significantly when comparing fertility treatment type, specifically by the following groupings: 1) all infertility treatment cycle (orals, injectables, hybrid, IVF fresh or frozen, donor eggs) v. natural cycle (12.8% v. 9.1%,  $p = 0.08$ ), 2) oral cycle (clomiphene and letrozole) v. IVF (fresh and frozen) v. natural cycle (10.9% v 13.7% v 9.1%,  $p = 0.12$ ), and 3) IVF Fresh v. IVF Frozen (13.5% v. 13.7%,  $p = 0.96$ ). Among pregnancies with SCH, 18.1% (n=27) ended with first trimester miscarriage. Symptoms of vaginal bleeding or cramping were significantly associated with miscarriage

( $p < 0.008$  and  $p < 0.001$  respectively). Age, BMI, infertility diagnosis, medical co-morbidities, and SCH size were not significantly different between these groups. Aspirin use in this population was common at 49.7%, however was not significantly associated with first trimester miscarriage.

**CONCLUSIONS:** We found similar rates of SCH and subsequent first trimester miscarriage (0.5-22%, 3-29.5% respectively) in our study population to rates reported in the fertile population. Rate of SCH did not vary significantly amongst fertility treatment cycles, including IVF fresh v. frozen cycles, contrary to a prior study. Similar to the fertile population, symptoms are significantly associated with miscarriage. Symptomatic patients should be counseled on increased risk of miscarriage compared to patients with incidentally noted SCH. While prior studies have shown increased rates of SCH in infertile patients taking aspirin, this study is the first to evaluate outcomes and suggest that there is no increased risk of miscarriage with aspirin use in pregnancies affected by early SCH. Future research should further evaluate the effect of aspirin on SCH prevalence and the impact of continuing aspirin in affected pregnancies.

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#### **CHARACTERIZING TISSUE PROTEOME CHANGES IN THE DECIDUA AND TROPHOBLAST ASSOCIATED WITH VIABILITY AND LOCATION OF PREGNANCY.**



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**OBJECTIVE:** To compare changes in protein profiles of decidualized endometria and trophoblasts among women with different pregnancy outcomes, specifically a viable intrauterine pregnancy (IUP), ectopic pregnancy (EP), or fetal demise (FD), that identify potential biomarkers and provide insights into cellular pathways affected by fetus viability and location.

**DESIGN:** An exploratory proteomic profiling study.

**MATERIALS AND METHODS:** Trophoblast and endometrial tissue was collected from consenting, gestational-age matched women having a viable IUP (n=4), FD (n=4), or EP (n=2). Frozen tissue samples were homogenized, digested with trypsin and analyzed by nanocapillary LC-MS/MS using a Thermo Q-Exactive HF mass spectrometer. Data for each tissue type were processed using label-free quantitation with MaxQuant software. We performed pairwise comparisons of each pregnancy outcome (EP vs IUP, FD vs IUP, and EP vs FD), and protein changes having a fold change  $\geq 3$  and a Student's t-test p-value < 0.05 were considered significant.

**RESULTS:** A total of 4792 and 4757 high confidence proteins were identified in the decidua and trophoblast proteomes, respectively. The overall protein compositions from all three outcomes were similar with greater than 90% overlap in both tissue types. In the decidua, 125 protein quantities (2.6% of the proteome) were significantly different between EP and IUP, whereas FD and IUP decidua were more similar with only 68 (1.4%) differences. Non-viable pregnancies in different locations (EP vs FD) showed 191 differences (3.9%). A similar depth of analysis and degree of significantly changing proteins was observed in the trophoblast analyses. There are 66 (1.4%) differences between SAB and IUP and 177 (3.7%) differences when EP was compared to FD. However, the largest group of 355 differences (7.2%) was observed between EP and IUP trophoblasts. In both tissue types, proteins associated with ECM remodeling, cell adhesion and metabolic pathways showed decreases in EP specimens compared with IUP and FD. In trophoblasts, EP showed elevation of inflammatory and immune response pathways.

**CONCLUSIONS:** The differences between an EP and viable IUP are greater than the changes observed when comparing viable (IUP) and nonviable intrauterine pregnancies (FD) in both decidua and trophoblast proteomes. Furthermore, differences between EP and IUP were much higher in the trophoblast than in the decidua. This observation is true for the total number of protein changes as well as the extent of changes in upstream regulators and related pathways. This suggests that biomarkers of trophoblast function maybe the best predictors of early pregnancy location and viability.

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**ISOLATION AND PROFILING OF EXTRACELLULAR VESICLES IN UTERINE FLUID TO DETERMINE NOVEL MARKERS OF ENDOMETRIAL RECEPTIVITY.**

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**OBJECTIVE:** Uterine fluid contains endometrium-derived extracellular vesicles (EVs), which are membrane-bound cell-cell mediators containing lipids, proteins and nucleic acids. MicroRNAs (miRNA), the small non-coding RNA molecules directing post-transcriptional gene silencing, are a prominent cargo of EVs. Analysis of the miRNAs in EVs may offer insights into the endometrial secretome and endometrial-embryonic cross-talk around the time of implantation. This study is designed to characterize differentially expressed miRNAs from endometrium-derived EVs during the receptive phase versus pre-receptive phase in both natural and stimulated in-vitro fertilization (IVF) cycles, with the goal to identify novel markers of endometrial receptivity.

**DESIGN:** Healthy fertile women (Group 1, N=22) with regular menstrual cycles, and women under 40 years of age (Group 2, N=36) undergoing their first or second stimulated IVF cycles were recruited with informed consent. In Group 1, uterine fluid aspiration (UFA) sampling was performed on the day of LH+2 (pre-receptive) and LH+7 (receptive) in natural cycles. In Group 2, UFA sampling was performed on the day of hCG+2 (pre-receptive, day of egg retrieval) and hCG+7 (receptive, day of blastocyst transfer) in IVF cycles.

**MATERIALS AND METHODS:** Cellular pellet was removed from the aspirated uterine fluid by centrifugation. EVs in the supernatant were then isolated by two steps of ultracentrifugation at 100,000g for 70 minutes. Isolated EVs were characterized by Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA) and flow cytometry. RNAs were extracted from the isolated EVs and profiling of miRNAs was carried out by next-generation sequencing. Resulting sequencing counts were mapped to miRNAs and bioinformatic analysis was performed on all paired samples to determine differentially expressed miRNAs between conditions (adjusted p value < 0.05).

**RESULTS:** We confirmed the presence of EVs in human uterine fluid, which were characterized as 30-200 nm vesicles with bilayer membranes by TEM. NTA confirmed the high yield of EVs isolated by ultracentrifugation and demonstrated that the majority of these EVs were between 100-200 nm in size. Flow cytometry validated the successful isolation of EVs by their biomarkers CD9 and CD63. Through profiling of the miRNAs in EVs, over 100 miRNAs were found to be differentially expressed ( $\geq 2$ -fold change) between receptive phase and pre-receptive in either natural cycles or IVF cycles. Cross-referencing the differentially expressed miRNAs in both cycles, we identified three overlapping miRNAs (hsa-mir-331, hsa-mir-382, hsa-mir-505), all of which were up-regulated during the receptive phase, as potentially important miRNAs related to the establishment of endometrial receptivity.

**CONCLUSIONS:** This study validates that endometrium-derived EVs can be isolated and characterized from human uterine fluid by ultracentrifugation. It is the first study to comprehensively profile the miRNAs in these EVs and it has identified a small cohort of candidate miRNAs that may be the novel biomarkers of endometrial receptivity.

**SUPPORT:** The study is supported by the American Society for Reproductive Medicine (ASRM) research grant awarded to Dr. Crystal Chan in 2018.

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**EMBRYO ATP PRODUCTION CAN BE MODULATED BY MATERNAL MITOCHONDRIAL DNA SECRETED FROM THE HUMAN ENDOMETRIUM IN EXTRACELLULAR VESICLES.**

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**OBJECTIVE:** To identify the DNA cargo of extracellular vesicles (EVs) obtained from maternal endometrial fluid and determine whether cargo is incorporated into and thereby affects the embryo energetics regulation in terms of ATP modulation.

**DESIGN:** DNA cargo identification was performed by sequencing EVs [apoptotic bodies (ABs), microvesicles (MVs), and exosomes (EXOs)] isolated from human endometrial fluid (EF) samples from fertile donors (n=10). EVs populations originating from the same EF sample were evaluated in a paired design. The potential for EVs to transfer DNA to the embryo and to modify embryo energetics through ATP modulation was also investigated.

**MATERIALS AND METHODS:** EVs from human EF were treated with DNase to remove external DNA. Nextera XT DNA libraries were created and paired-end 300 cycles sequenced. EVs were labelled with 5-ethynyl-2'-deoxyuridine (specific DNA label) and incubated with hatching murine embryos (n=600) to investigate EVs DNA transfer into the embryo. Finally, hatching embryos (n=250) were cocultured with EVs, and embryonic ATP levels were quantified (FLASC kit, Sigma) and compared among embryos exposed to the different EV populations. Statistical comparisons were performed using ANOVA.

**RESULTS:** MVs were the only EV type in which specific DNA cargo was identified. NGS analysis revealed enrichment in mitochondrial DNA comprising the 13 coding genes (11.12  $\pm$  0.53-fold increase). Interestingly, transcription factor binding sites (TFBSs) were also enriched in this EVs population compared to ABs and EXOs (6.9  $\pm$  1.5 and 11  $\pm$  2.1-fold change, respectively), most of them mapping throughout the mitochondrial genome. Some of the associated transcription factors (SRF, GABP, E2F4, TR4, FOXA2, FOXA1, CTCF, GATA2, PAX5) are implicated in embryo development, gametogenesis, and cell-matrix adhesion. Further, DNA-tagged EV populations were taken up by murine embryos and exhibited different patterns of DNA integration into the cytoplasm and nuclei of the trophoblast. Interestingly, when embryos were cocultured in the presence of ABs, MVs, or EXOs, those in the presence of MVs maintained their ATP production when compared to EXOs (p < 0.001).

**CONCLUSIONS:** Our results suggest that EF-derived EVs may act as modulators of embryo energetics. Specifically, MVs convey DNA cargo enriched in coding and modulatory mitochondrial DNA and support maintenance of embryonic ATP production. Finally, the ability of EVs to transfer DNA to the embryo suggests that this mode of maternal-embryonic communication may have implications on embryo energetics regulation.

CS & FV contributed equally.

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**ENDOMETRIAL EPITHELIAL FOXO1 DIRECTLY MODULATES SIGNALING PATHWAYS NECESSARY FOR UTERINE RECEPTIVITY.**

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**OBJECTIVE:** We have previously shown that endometrial FOXO1 transcription factor protein expression is indispensable for murine embryo implantation. We also demonstrated that FOXO1 protein expression is concentrated in the uterine epithelial nuclei during the window of receptivity<sup>1</sup>. The objective of this study is to better understand the relevance of FOXO1 in human endometrial receptivity.

**DESIGN:** Differentially expressed genes (DEGs) from proliferative (non-receptive) versus mid-secretory (receptive) human endometrial epithelium were compared with DEGs of uterine-specific FOXO1<sup>del/d</sup> versus FOXO1<sup>if/</sup> mice to determine which FOXO1-regulated murine genes are also regulated during the transition to receptive human endometrium. Ingenuity Pathway Analysis (IPA) identified regulated pathway and FOXO1 ChIP-seq identified genes directly bound by FOXO1.

**MATERIALS AND METHODS:** Mouse uterine epithelium was isolated by laser capture on day 4.5 of natural pregnancy. The transcriptome of FOXO1 knockout epithelium was compared to that of wildtype mice using RNA sequencing, identifying DEGs defined by at least a 2-fold change. RNA sequencing was also used to generate DEGs from enzymatically separated human endometrial epithelium between non-receptive and receptive by at least a 1.5-fold change<sup>2</sup>. IPA of the FOXO1 altered murine genes conserved in human phase-related DEGs was used to identify altered pathways with known roles in endometrial receptivity. Subsequent comparison of the components of each pathway with FOXO1 ChIP-seq identified the direct role of FOXO1 on the altered pathway.

**RESULTS:** 1301 RNA species were common to both the mouse and human DEGs. 318 of these genes were directly bound by FOXO1. Pathway analysis identified Wnt/B-catenin signaling (-log(p-value) 4.14, Z score -0.626), estrogen mediated proliferation (-log(p-value) 10, Z score -3.05), and IL-6 signaling (-log(p-value) 4.73, Z score 0.943) as significantly altered by both human cycle phase and murine FOXO1 deletion, strongly suggesting a role of FOXO1 in these critical pathways for normal endometrial function. ChIP-Seq demonstrated direct FOXO1 binding to multiple regulated genes involved in estrogen-mediated proliferation, IL-6 signaling, and Wnt/b-catenin signaling, supporting a direct action of FOXO1 on these essential signaling pathways. FOXO1 was also found to directly bind several upstream regulators critical to endometrial receptivity, including CEBPB and CCND1.

**CONCLUSIONS:** Epithelial FOXO1 directly regulates key pathways necessary for human uterine receptivity.

References: 1. Vasquez YM, Wang X, Wetendorf M, et al. FOXO1 regulates uterine epithelial integrity and progesterone receptor expression critical for embryo implantation. PLOS Genetics. 2018;14(11). <https://doi.org/10.1371/journal.pgen.1007787>.

2. Arnold JT, Kaufman DG, Seppala M, Lessey BA. Endometrial stromal cells regulate epithelial cell growth in vitro: a new co-culture model. Hum Reprod. 2001;16(5):836-845.

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**PRESENCE OF p16-POSITIVE SENESENT CELLS IN HUMAN ENDOMETRIUM DURING THE MID-LUTEAL PHASE OF THE MENSTRUAL CYCLE.**



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**OBJECTIVE:** Biomarkers for cellular senescence such as p16<sup>ink4a</sup> are commonly measured in order to explore the level of senescence in reproductive tissues. It is known that p16<sup>ink4a</sup>-positive senescent cells in the human endometrium are involved in its receptivity and participate in the acute cellular remodeling at the time of embryo implantation. The objective of the present study was to evaluate and compare the percentage of p16-positive cells in the stromal, glandular and luminal epithelial compartments of the human endometrium.

**DESIGN:** We measured the percentage of p16<sup>ink4a</sup>-positive cells by immunohistochemistry in endometrial biopsy samples of 124 women.

**MATERIALS AND METHODS:** This is a prospective observational study of 124 fertile women who had an endometrial biopsy during the mid-luteal phase (7 days after LH surge) of the natural cycle. Patients older than 40 years, with BMI < 18 kg/m<sup>2</sup> or BMI ≥ 30 kg/m<sup>2</sup>, endometriosis, polycystic ovary syndrome (PCOS), endometrial polyps, abnormal uterine development and hydrosalpinx were excluded from the study. Endometrial biopsies were obtained by pipelle suction and they were immediately fixed in 10% formalin. The endometrial tissue was submitted to paraffin embedding for histological determination and subsequent analysis. Immunohistochemistry (IHC) was performed on the paraffin-embedded sections by Novolink Polymer Detection System (Leica Biosystems, Wetzlar, Hesse, Germany). We used polyclonal antibody against p16<sup>ink4a</sup> (Master Diagnostica, Granada, Spain) to identify senescent endometrial stromal as well as epithelial cells. The percentage of p16+ cells in each tissue compartment was calculated after enumeration by two independent in-

vestigators in multiple endometrial sections. Values were expressed as mean ± SD. Paired t-test and Spearman correlation coefficient were used as appropriate. P<0.05 was considered statistically significant.

**RESULTS:** The percentage of p16-positive cells in the endometrial stroma during the mid-luteal phase of the cycle ranged between 0.03% and 8.38%, while it varied between 0.06% and 51.02% in the glands, and between 1.69% and 90.88% in the luminal epithelium. The presence of p16+ senescent cells was significantly higher in the endometrial luminal epithelium compared to glandular and stromal compartments (28.71%±23.73% vs. 6.72%±7.73% vs. 0.82%±1.29%, p<0.01, respectively). We also observed a significant correlation between the percentage of p16+ cells in glands and those in the luminal epithelium (R=0.61; p<0.01). In contrast, the stromal p16-positive cells were not significantly correlated neither with the glandular senescent cells, nor with the luminal epithelial p16+ cells (p>0.05).

**CONCLUSIONS:** The endometrial luminal epithelium during the mid-luteal phase of the cycle has the highest percentage of p16-positive senescent cells, followed by the glands and the stroma. Moreover, the p16+ cells rate in the luminal epithelium is strongly positively associated with the proportion of senescent cells in the glands.

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**THE ENDOMETRIAL MICROBIOME OF CLINICAL MISCARRIAGE, ECTOPIC PREGNANCY AND DURING EARLY PREGNANCY IN A SUCCESSFUL LIVE-BIRTH.**



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**OBJECTIVE:** Characterize taxonomically and functionally the endometrial microbiome in clinical miscarriage, ectopic pregnancy and successful live-birth.

**DESIGN:** The endometrial microbiome was analyzed in patients undergoing ART. We describe the results of endometrial fluid samples analyzed prior to embryo transfer with euploid embryos resulting in 2 clinical miscarriages and 1 ectopic pregnancy (Patient 1), and a clinical miscarriage and a 4-week spontaneous successful pregnancy resulting in live birth (Patient 2).

**MATERIALS AND METHODS:** For taxonomic classification, 16S rRNA profiles were obtained using the Ion 16S metagenomics kit and sequenced on the Ion S5 XL system (ThermoFisher Scientific). Functional composition was assessed by Whole Metagenome Sequencing using the Nextera DNA Flex Library Preparation kit and sequenced on the NextSeq 500 system (Illumina).

**RESULTS:** The 16S rRNA sequencing of the endometrial fluid collected prior to clinical miscarriages and ectopic pregnancy showed the existence of a pathologic microbiota profile, whereas at 4-weeks of pregnancy had reversed to a normal *Lactobacillus*-dominated profile.

The functional metagenomics revealed different *Lactobacillus* species and associated functions between the clinical miscarriage and successful pregnancy. In clinical miscarriage, *L. crispatus* was detected with the indicated pathogens showing an unstable functional pattern with transposases and insertion elements. Whereas, in the same patient *L. iners* was the only bacteria present in the uterine cavity at the 4-week in the successful pregnancy, associated with defense mechanisms, energy production and cell division.

	Patient 1		Patient 2	
	Ectopic (%)	Miscarriage (%)	Miscarriage (%)	Live birth (%)
<i>Lactobacillus</i>	12.1	0.8	48	93.5
<i>Gardnerella</i>	32.8	28.8	32.8	0.1
<i>Pseudoalteromonas</i>	14.2	16.8	0.0	1.4
<i>Bifidobacterium</i>	8.8	5.0	6.2	0.0
<i>Rhodanobacter</i>	5.6	13.0	0.0	1.7
<i>Atopobium</i>	0.4	13.4	5.4	0.0
<i>Streptococcus</i>	1.0	0.2	0.0	0.1
<i>Pseudomonas</i>	3.2	2.7	0.1	0.5
Enterobacteriaceae	1.5	0.0	0.0	0.2
<i>Staphylococcus</i>	0.5	0.0	0.0	0.0

Finally, since bacteria produce G protein-coupled receptors ligands to modulate the host's physiology, we searched for sequences associated with N-acyl synthase protein family PFAM13444 detecting 44 sequences in the miscarriage versus 0 in the early successful pregnancy.

**CONCLUSIONS:** This is the first demonstration that the uterine microbiome in an early successful pregnancy is completely different from clinical miscarriages and ectopic pregnancy, revealing the different uterine microbial environment encountered by the embryo at implantation in these clinical outcomes. The potential of these new findings remains to be proven in larger series.

## ENDOMETRIOSIS

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### CHANGES IN ANTI-MÜLLERIAN HORMONE AND AMOUNT OF ETHANOL USED DURING ULTRASOUND GUIDED ASPIRATION OF OVARIAN CYST.

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**OBJECTIVE:** To evaluate the effect of transvaginal ultrasound-guided aspiration and ethanol sclerotherapy on ovarian reserve and anti-müllerian hormone (AMH) in patients with ovarian endometriomas. Setting: Teaching hospital affiliated with Chang Gung University, Taipei.

**DESIGN:** We retrospectively reviewed 124 patients with ovarian endometriomas who underwent trans-vaginal aspiration and sclerotherapy of endometrioma(s) in our hospital. Patients were grouped into minimal amount of ethanol retention, group 1, n = 80, ≤ 5 mL of retention of ethanol, and group 2, n = 44, > 5ml of retention.

**MATERIALS AND METHODS:** In all of 124 patients, preoperative evaluation included AMH, mid-cycle serum CA-125 level, and color Doppler ultrasonography to exclude possibility of malignancies. Patients underwent ultrasonographic guided transvaginal aspiration and sclerotherapy with 95% ethanol irrigation of the cystic cavity. Patients were grouped into group 1, n = 80, ≤ 5 mL of retention of ethanol, and group 2, n = 44, > 5ml of retention. Ultrasonography was performed at 3, 6, 9, and 12 months to determine persistence and size of cysts and AMH level was checked at 6 months after aspiration. Pain scores were evaluated pre- and post-operatively. Patients were followed up at 1 year for recurrent cysts. Those who were infertile prior to therapy were followed up for subsequent pregnancies (either by assisted reproductive technologies, or by natural conception). All statistics were two-sided and analyses were performed using SPSS software, version 25 (SPSS Inc., Chicago, IL).

**RESULTS:** The patients age, mean cyst size, bi/unilaterality in both groups were without significant differences. The mean pre-operative AMH levels for group 1 (≤ 5 mL of ethanol retention) and group 2 (>5ml of ethanol retention) were 3.80 and 3.06 respectively (p>0.05). The AMH at 6-month follow up for group 2 patients was significantly lower than for group 1 patients, with mean decrease of 0.72 (23.6%) and 0.10 (2.7%) respectively (p<0.05). No significant change in CA-125, recurrence rate or pain score was found within 1 year of aspiration.

**CONCLUSIONS:** Ultrasound-guided sclerotherapy with 95% ethanol is an effective therapy for ovarian endometriomas. The greater the amount of ethanol left in situ during sclerotherapy, the more AMH decreases post-operatively.

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### DOES ENDOMETRIOSIS IMPACT ON THE EMBRYONIC ANEUPLOIDY RISK?

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**OBJECTIVE:** Endometriosis, a highly frequent gynecological disease, is often associated with female infertility and poor in vitro fertilization (IVF) outcomes. Many factors have been suggested to cause the fertility problems in these patients, including poor oocyte quality and alterations in meiotic spindle that could affect embryo aneuploidy rates. Our main objective is to ascertain if endometriosis increases embryonic aneuploidy risk.

**DESIGN:** Multicenter retrospective assay of cohorts to compare the embryonic aneuploidy rate between patients with endometriosis (experimental group) and those without the disease (control group).

**MATERIALS AND METHODS:** Our study involved patients aged 18-42 years old undergoing IVF with preimplantation genetic screening (PGS) in IVIRMA Clinics between 2012 and 2017. To discard the impact of non-endometriosis disorders on embryo abnormalities, severe male factors and patients with altered karyotype or with chromosomopathy in previous embryos or pregnancies were excluded. The following PGS indications were included: Implantation failure (IF), Recurrent miscarriage (RM) and advanced maternal age (AMA). Both blastocyst and developing embryos analyzed for the complete chromosome set by comparative genomic hybridization (CGH) arrays or next generation sequencing (NGS) were only considered. Presence of endometriosis was evidenced at the time of abdominal surgery or after pelvic ultrasound or NMR findings. For the statistical analysis, chi-square test for categorical variables or Student's t-test for quantitative data were applied to compare baseline characteristics between groups.  $\chi^2$  test and Poisson regression model were used for comparing the proportion of aneuploid embryos in the different groups.

**RESULTS:** A total of 1622 embryos from 350 patients were biopsied in the endometriosis group while 17914 biopsied embryos from 4000 patients were included in the control group. Among these embryos, 1577 and 17566 were informative after PGS in the experimental and control group, respectively. One thousand seventy-two embryos from the endometriosis patients and 11997 from control patients were abnormal. No significant differences in the aneuploidy rate were observed when compared embryos from the experimental and the control group (68,0% versus 68,3%, respectively; p=0.794). Poisson regression analysis was performed, adjusting for baseline patient characteristics (age and body mass index), but the proportion of aneuploid embryos were still not significant.

**CONCLUSIONS:** Despite increased oocyte meiotic errors and chromosomal instabilities have been proposed as a potential cause of a lower IVF success in women with endometriosis, it does not seem to impact on their embryo aneuploidy risk. Further research is needed to determine if the embryo arrest before blastocyst PGS analysis could mask non-analyzed chromosomal anomalies. Anyway, disturbances during oocyte nuclear maturation, with subsequent less useful oocytes, could also explain the difficulties of these patients in becoming pregnant.

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### PREOPERATIVE SERUM ANTI-MULLERIAN HORMONE LEVELS IN WOMEN WITH OVARIAN ENDOMETRIOSIS COMPARED TO WOMEN WITH PERITONEAL ENDOMETRIOSIS.

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**OBJECTIVE:** Anti-Mullerian hormone (AMH) is an important serum marker to gauge ovarian reserve and predicted response to number of oocytes retrieved after ovarian stimulation. Patients who have ovarian involvement of endometriosis have clinically demonstrated lower baseline AMH levels. Whether or not patients with peritoneal endometriosis only have lower baseline AMH levels has not been established. Our aim is to investigate preoperative baseline AMH levels in women who have ovarian endometriosis versus women who have peritoneal endometriosis without ovarian involvement.

**DESIGN:** Retrospective cross-sectional analysis.

**MATERIALS AND METHODS:** Pre-operative AMH levels were evaluated for 111 women aged 19-42 who underwent laparoscopic surgery from January 2017 and July 2018 for suspected endometriosis. Patients were identified by those who desired future fertility and had preoperative AMH levels drawn. Patients with a diagnosis of polycystic ovaries or history of prior endometriosis excision surgery and/or oophorectomy were excluded. AMH levels were analyzed according to where endometriosis was anatomically located and confirmed by pathology, comparing women who had peritoneal endometriosis (n=71) without any ovarian involvement versus women with ovarian involvement (n=40). Subanalysis of AMH values was also performed within three different age groups.

**RESULTS:** Preoperative serum AMH level was not significantly different in the ovarian endometriosis group compared to the peritoneal endometriosis group (3.22 ± 3.07 ng/mL vs 3.94 ± 2.90 ng/mL, P=0.113). Subgroup analysis by age demonstrated significantly lower AMH levels for women with ovarian endometriosis aged 27-35 (2.62 ± 2.28 ng/mL vs 3.85 ± 2.98 ng/dL, P=0.045). Patients with ovarian endometriosis were significantly more likely to have more endometriotic lesions confirmed on pathology (16.95 ± 8.75 lesions vs 8.97 ± 6.85 lesions, P < 0.0001).

AMH Levels by Age Group Subanalysis (Mean ± 1 Standard Deviation)

Age Group (years)	AMH level (ng/dl)		P value
	in Ovarian Endometriosis	(ng/dl) in Peritoneal Endometriosis	
18-26	5.45 ± 4.2 (n = 10)	4.62 ± 2.81 (n = 28)	0.294
27-35	2.62 ± 2.28 (n = 23)	3.85 ± 2.98 (n = 31)	0.045
36-42	1.99 ± 2.12 (n = 7)	2.78 ± 2.64 (n = 11)	0.249
All years	3.22 ± 3.07 (n = 40)	3.94 ± 2.90 (n = 71)	0.113

**CONCLUSIONS:** Patients with ovarian endometriosis demonstrate lower serum AMH levels than baseline age-matched populations [1], thus reflecting ovarian function and potential success with oocyte retrieval. These findings indicate that some women with peritoneal endometriosis may be at a similar disadvantage in regards to ovarian reserve compared to women with endometriomas and could benefit from earlier intervention regarding active management of fertility preservation.

Reference: Muzii L, Di Tucci C, Di Feliciano M, Galati G, Di Donato V, Musella A, Palaia I, Panici PB. Antimüllerian hormone is reduced in the presence of ovarian endometriomas: a systematic review and meta-analysis. *Fertil Steril.* 2018 Oct;110(5):932-940.e1. <https://doi.org/10.1016/j.fertnstert.2018.06.025>.

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**DIENOGEST FOR PAIN AND INTESTINAL SYMPTOMS CAUSED BY RECTOSIGMOID ENDOMETRIOSIS: PROSPECTIVE COHORT STUDY.**

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**OBJECTIVE:** The aim of this study was to evaluate the efficacy of dienogest (DNG) for treating pain and intestinal symptoms in patients with rectosigmoid endometriosis.

**DESIGN:** 24-month open-label prospective cohort study.  
**MATERIALS AND METHODS:** This study included symptomatic women of reproductive age with rectosigmoid endometriosis. The diagnosis of rectosigmoid endometriosis was performed by transvaginal ultrasonography and confirmed by magnetic resonance imaging. Exclusion criteria for the study were: use of hormonal therapies for endometriosis in the 3 months before study entry (6 months for gonadotropin releasing hormone analogues), previous treatment with DNG, unwillingness to tolerate menstrual changes, undiagnosed vaginal bleeding, obstructive uropathy, complex adnexal cysts at imaging, estimated rectosigmoid stenosis >60%. Eligible women underwent hormonal treatment with DNG (2 mg/day) continuously for 24 months. Consultations were performed every 6 months. The primary endpoint of the study was patient satisfaction. Secondary endpoints were: changes in pain (assessed on a VAS scale) and intestinal symptoms (assessed by a 10-point symptom analogue scale and by the Gastrointestinal Quality of Life Index, GIQLI), changes in quality of life (assessed by the Endometriosis Health Profile 30, EHP-30), changes in sexual function (assessed by the Female Sexual Function Index, FSFI), tolerability of the therapy, changes in the volume of the rectosigmoid nodules (estimated by using the virtual organ computer-aided analysis, VOCAL).

**RESULTS:** 132 women were enrolled in the study and 114 (86.4%) completed the 24-months treatment. The mean (±SD) age of the study population was 34.8 (±4.1 years). 102 patients (77.3%) had already received previous hormonal treatment for treating endometriosis. 56 patients (42.4%) had previously undergone surgery for pelvic endometriosis. All pain symptoms (dysmenorrhea, non-menstrual pelvic pain, deep dyspareunia and painful defecation) significantly improved at 1-year of treatment compared with baseline. The severity of diarrhea, intestinal cramping and passage of mucus significantly improved at 6-, 12- and 24-month assessment compared with baseline. Abdominal bloating improved at 24-month assessment compared with baseline. The GIQLI, the EHP-30 and the FSFI were significantly improved at 24-month follow-up compared with baseline. There was a signif-

icant reduction in the volume of the bowel endometriotic nodules between baseline (4.3±0.8 cm<sup>3</sup>) and 12-month assessment (3.4±1.0 cm<sup>3</sup>; p<0.001) and between baseline and 24-month assessment (3.1±0.6 cm<sup>3</sup>, p<0.001). The volume of the nodules did not significantly change between the 12-month and the 24-month assessment. DNG was generally well tolerated, with no reported serious adverse events; the most common adverse effect was headache (8,3%).

**CONCLUSIONS:** A 2 year-therapy with DNG improves the symptoms caused by rectosigmoid endometriosis with a good safety-profile, proving also a slight reduction of the size of the bowel endometriotic nodules.

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**FIBER AND GLUTEN INTAKE AND RISK OF LAPAROSCOPICALLY-CONFIRMED ENDOMETRIOSIS.**

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**OBJECTIVE:** We examined the association between intake of fiber (total fiber, legume, vegetable, cruciferous vegetable, fruit, and cereal fibers) and gluten and diagnosis of laparoscopically-confirmed endometriosis.

**DESIGN:** A prospective cohort study using data collected from 81,789 premenopausal women from 1991-2013 as part of the Nurses' Health Study II (NHSII) cohort.

**MATERIALS AND METHODS:** Diet was assessed with a validated food frequency questionnaire every four years. Multivariable Cox proportional hazards models adjusted for race/ethnicity, menstrual cycle length, parity, age at menarche, body mass index, recent gynecologic/breast exam, and total calories, were used to calculate rate ratios (RR) and 95% confidence intervals (CI).

**RESULTS:** During 22 years of follow-up, 3793 incident cases of laparoscopically-confirmed endometriosis were reported. Higher intake of fruit fiber was associated with a lower risk of endometriosis diagnosis (RR for 5<sup>th</sup> quintile vs 1<sup>st</sup> quintile=0.89; 95% CI=0.80-0.98). A similar association was observed for cereal fiber (RR for 5<sup>th</sup> quintile vs 1<sup>st</sup> quintile=0.90; 95% CI=0.81- 1.00). In contrast, vegetable fiber intake was associated with a higher risk of endometriosis diagnosis (RR for 5<sup>th</sup> quintile vs 1<sup>st</sup> quintile=1.12; 95% CI=1.02-01.24). This association appear to be driven by the association with cruciferous vegetable fiber intake (RR for 5<sup>th</sup> quintile vs 1<sup>st</sup> quintile=1.17; 95% CI=1.06-1.30). No significant associations were observed with total fiber or legume fiber. Intake of gluten was associated with a lower risk of endometriosis diagnosis (RR for 5<sup>th</sup> quintile vs 1<sup>st</sup> quintile=0.82; 95% CI=0.72-0.93). This association was modified by fertility status. Specifically, the inverse association between gluten intake and endometriosis diagnosis was only apparent among women who had not reported infertility (RR for 5<sup>th</sup> quintile vs 1<sup>st</sup> quintile=0.82; 95% CI=0.71-0.95). The corresponding RR for those reporting infertility was 0.94 (95% CI=0.68-1.31).

**CONCLUSIONS:** Our findings suggest that different types of fiber intake are differentially associated with risk of endometriosis diagnosis. Further analyses are needed to identify whether these are associations are driven by consumption of the foods that contribute to fiber intake or due to the fiber content itself. Our finding that gluten intake was associated with a lower risk of endometriosis diagnosis among women who had not reported infertility, and thus were more likely to present with pain symptoms, suggests that gluten intake is unlikely to contribute to heightened endometriosis risk among the general population or exacerbation of pain symptoms among women with endometriosis. The inverse association observed deserves further study in well-designed observational and intervention studies.

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**IN UTERO AND EARLY LIFE EXPOSURES IN RELATION TO ODDS OF ENDOMETRIOSIS IN ADOLESCENTS AND YOUNG ADULTS.**

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**OBJECTIVE:** To investigate the relation between *in utero* / early life exposures and endometriosis diagnosis during adolescence and young adulthood.

**DESIGN:** We conducted a nested case-control study among participants of The Women's Health Study: From Adolescence to Adulthood (A2A), a longitudinal cohort of adolescents and young women enrolled from 2012-2018.

**MATERIALS AND METHODS:** Participants (n=604; 295 laparoscopically-confirmed endometriosis cases, 309 population-based controls) in the A2A study (age < 25 yrs at enrollment) completed a modified WERF EPHEct questionnaire at baseline. Information on *in utero* and early life factors were collected, including their mother's age at delivery, birthweight, gestation length, parents' smoking status during their pregnancy and/or during infancy and childhood, and if the participant was breastfed. We calculated odds ratios (OR) and 95% confidence intervals (CI) using logistic regression models, *a priori* adjusted for age at enrollment, race/ethnicity, maternal endometriosis diagnosis, and age at menarche. Analyses of birthweight were restricted to full term births.

**RESULTS:** Median age at enrollment was 22 y (range 7-24) in controls and 17 y (range 12-24) in cases, with 68% and 83% non-Hispanic white, respectively. Median age at menarche was 12 y (range 8-15) for both groups. Among cases, 50% had mothers with endometriosis while only 9% of the controls did. The almost all cases (95%) were rASRM stage I or II at diagnostic surgery. Participants who were breastfed had lower odds of endometriosis diagnosis < age 25 compared to those not breastfed (OR: 0.40, 95% CI: 0.21-0.74). Young women whose mothers smoked during pregnancy (n=13) were four times more likely to be diagnosed with endometriosis < age 25 (OR: 3.93, 95% CI: 0.80-19.43), while those with mothers who smoked during infancy to childhood were 2.5 times more likely to be diagnosed (OR: 2.64, 95% CI: 1.10-6.32). Low birthweight (OR: 0.64, 95% CI: 0.08-4.87) and preterm birth (OR: 1.30, 95% CI: 0.30-5.66) were not associated with endometriosis diagnosis < age 25.

**CONCLUSIONS:** Among adolescents and young adults, exposure to breastfeeding in early life was associated with lower odds of surgically diagnosed endometriosis. Exposure to maternal smoking during pregnancy and infancy/childhood was associated with greater odds of endometriosis, although the number exposed was small. Further exploration and replication are necessary to draw conclusions regarding risk among those diagnosed during adolescence compared to those diagnosed during adulthood. As these exposures are potentially modifiable, solidifying these associations will form the basis of informative public health messages to prevent endometriosis.

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### CUMULATIVE CLINICAL PREGNANCY AFTER SURGICAL TREATMENT OF INFERTILE WOMEN WITH ENDOMETRIOSIS.

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**OBJECTIVE:** Justification for reproductive surgical treatment of endometriosis in infertile women has been questioned given the current success rates with IVF. Although several studies support the use of laparoscopy for surgical treatment, particularly in cases of stage I or II disease, other studies propose either empirical treatment with COH/IUI or IVF. This study evaluated pregnancy outcomes in infertile women with endometriosis after surgical treatment.

**DESIGN:** IRB approved retrospective cohort study of women who presented with a complaint of infertility and were found to have endometriosis at the time of laparoscopic evaluation during 2012-2018.

**MATERIALS AND METHODS:** This study included 374 women, ages 18-44 years, who were found to have normal ovulation, normal male factor and patent fallopian tubes who chose to undergo laparoscopic evaluation for tubal/peritoneal factors as a potential cause of their infertility versus proceeding with IVF. All patients age 38 or older were encouraged to proceed directly to IVF. The primary reasons given for choosing laparoscopy over IVF involved financial issues and moral/ethical concerns about the IVF process. All surgeries were performed at the Ambulatory Surgery Center by a single endoscopic surgeon using a KTP laser for complete excision/vaporization of all identified endometriotic lesions and adhesions. 252 women were

found to have endometriosis at the time of the surgery and their pregnancy data is reported. After surgery, patients were allowed to attempt pregnancy on spontaneous cycles for 3 cycles for age equal to or greater than 35; 6 cycles for age less than 35 yr. If not pregnant, they were allowed a maximum of 3 cycles of COH/IUI prior to proceeding with IVF. Clinical pregnancy rates were determined for both spontaneous and COH/IUI cycles for each stage of endometriosis found.

**RESULTS:** Patients with stage I endometriosis had a spontaneous cycle pregnancy rate of 43.9% and an additional pregnancy rate of 36.4% on follow-up COH/IUI cycles. Cumulative pregnancy rate was 80.3%. Patient's with stage II endometriosis had a spontaneous cycle rate of 28.4% and an additional 15.7% on COH/IUI given a cumulative rate of 44.1%. Stage III patients had a spontaneous cycle rate of 18.5% and COH/IUI rate of 7.4% giving a cumulative pregnancy rate of 25.9%. Stage IV patients had a spontaneous cycle rate of 19.3% and COH/IUI rate of 15.8% giving a cumulative pregnancy rate of 35.0%.

**CONCLUSIONS:** Laparoscopic surgical treatment of endometriosis in infertile women, particularly in combination with COH/IUI, significantly enhances fertility rates. The cumulative pregnancy rate for all patients, 49.6%, compares favorably with prior studies as well as IVF data. Barri, et al<sup>8</sup> reported a 54.2% pregnancy rate surgical treatment of endometriosis. SART 2016 IVF pregnancy data for endometriosis patient showed a pregnancy rate of 46.6% for patients <35 yr and 41.1% for patient's age 35-37. We believe laparoscopic treatment of endometriosis is an excellent option for these patients, particularly those for whom IVF is not an acceptable treatment choice.

Reference: <sup>8</sup>Barri P.N., Coroleu B., Tur R., Barri-Soldevila P.N., Rodriguez I. A Endometriosis-associated infertility: Surgery and IVF, a comprehensive therapeutic approach. *Reproductive BioMedicine Online*, 21 A (2) ,A pp.Å 179-185 (2010). Å DOI: <https://doi.org/10.1016/j.rbmo.2010.04.026>

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### SERUM METABOLOMIC PROFILE AS A NON-INVASIVE ADJUNCT TOOL FOR THE DIAGNOSIS OF ENDOMETRIOSIS-RELATED INFERTILITY.

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**OBJECTIVE:** Nonsurgical methods for the diagnosis of endometriosis could avoid unnecessary laparoscopies and improve quality of life. We aimed to develop an adjuvant tool for the diagnosis of endometriosis, based on mass spectrometry (MS)-metabolomics.

**DESIGN:** Case-control study.

**MATERIALS AND METHODS:** Serum samples from 100 patients undergoing intracytoplasmic sperm injection (ICSI), from January 2017 to December 2017, in a private university-affiliated in vitro fertilization center were collected. Samples were split into two groups according to the cause of infertility: the Endometriosis Group (n = 50), consisting of samples derived from patients with grade III and IV endometriosis, classified according with the American Society for Reproductive Medicine (ASRM), and the Control Group (n = 50), comprising samples derived from patients with isolated male factor infertility. Clinical diagnosis and classification of subjects in the endometriosis group were performed through laparoscopic surgery followed by histology to confirm the presence of endometriotic lesions. The metabolomic profile of each sample was obtained by mass spectrometry. Partial least square discriminant analysis (PLS-DA) was applied to the dataset in order to determine the discriminatory components based upon the combination of variable influence on projection (VIP) values. These values were used to build a single receiver operating characteristic (ROC) curve. To validate the model, 30 samples from infertile women without any evidence of endometriosis were tested.

**RESULTS:** Except for the pregnancy rate, which was decreased in the Endometriosis-Group (32.0% vs 72.0%, for Endometriosis and Control groups respectively, p=0.007), the patient and cycle characteristics were similar between groups. A total of 429 and 484 ions for the positive and negative ionization modes were analysed, respectively. Considering components one, two and three, the PLS-DA was able to clearly distinguish the Endometriosis-Group from the Control-Group for both positive and negative ionization modes. Ten potential biomarkers were selected based on their importance for model prediction, five in the positive and five in the negative ionization modes. These ions were used to build the ROC curve, which presented an area under the curve (AUC) of 0.904 (CI 95%: 0.796-0.985), indicating the accuracy of the biomarkers for sample classification in the Control

or Endometriosis groups. Considering these ions as possible biomarkers, the model was able to correctly classify 84% of the patients and, when the validation set was tested, the model was able to correctly classify 86.6% of the samples. Two metabolites were identified by the database. Triacylglycerols and alpha-amino acids were more abundant in serum of positive endometriosis patients, while the other ions were not identified by the currently available database.

**CONCLUSIONS:** Our evidence suggests that serum metabolomics may be a valuable approach to the diagnosis of endometriosis and may be used as an adjunct tool for the selection of patients who must undergo laparoscopy to obtain a definitive diagnosis.

Reference: NA.

SUPPORT: None.

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**SYSTEMATIC REVIEW AND CRITICAL APPRAISAL OF CURRENT ENDOMETRIOSIS GUIDELINES INCLUDING 2018 SPANISH ENDOMETRIOSIS GUIDELINE WITH FOUR EVALUATION**



**METHODS.** María Carrera, MD,<sup>a</sup> Jose Antonio Dominguez, MD, PHD,<sup>b</sup> Enrique Perez de la Blanca, MD,<sup>c</sup> Roberto Matorras, Professor,<sup>d</sup> José María Gris, MD, PhD,<sup>e</sup> Gorka Barrenetxea, Professor,<sup>f</sup> Carmen María Segura, MD,<sup>g</sup> Miguel Caballero, MD, PhD,<sup>h</sup> Joaquin Llacer, PhD.<sup>i</sup> <sup>a</sup>Medical Doctor, Gynaecologist, Specialist in Reproductive Medicine, Madrid, Spain; <sup>b</sup>Reproducción specialist, Badajoz, Spain; <sup>c</sup>Hospital Quironsalud. Assisted Reproduction Unit., Malaga, Spain; <sup>d</sup>Basque Country University, Bilbao, Spain; <sup>e</sup>Hospital Vall d'Hebron, Barcelona, Spain; <sup>f</sup>MEDICAL DIRECTOR OF REPRODUCCION BILBAO, BILBAO, Spain; <sup>g</sup>Medical doctor, Madrid, Spain; <sup>h</sup>Gynecologist, Madrid, Spain; <sup>i</sup>Instituto Bernabeu, Alicante, Spain.

**OBJECTIVE:** To appraise methodological quality of main endometriosis guidelines, including the 2018 Spanish Fertility Society Endometriosis Guideline, with four different evaluation methods: Agree II Instrument, the Right statement, the Australian ICAHE Checklist and the German MiCheck, to explore methodological quality differences between them.

**DESIGN:** Appraisal of main Endometriosis Guidelines with different methodological quality assessment tools to establish differences among them and correlation between evaluation methods.

**MATERIALS AND METHODS:** Two reviewers (JAD, MCR) performed a systematic research at PubMed, EMBASE, and Web of Science from 2008 to 2019 for endometriosis guidelines, and consensus documents. Inclusion criteria: National or International Guidelines published in English, French or Spanish. The guidelines assessment was performed with the four appraisal tools described above.

Nine reviewers (GB, MCR, MCC, JAD, JMG, JLL, RM, EPB, CS) assessed the methodological quality of the guidelines to ensure each guideline was evaluated by at least four different reviewers with each of the four appraisal tools.

Guidelines scoring system was calculated for each method and standardising the result of the assessment for comparison.

Guidelines were categorised as high quality when they score between 67-100%, moderate quality between 34-66% and low quality between 0-33% of the total score for each appraisal tool.

**RESULTS:** Ten guidelines along with the Spanish Fertility Society Endometriosis Clinical Guideline (SEF 2018) were included in the review (in chronological order): the Korean Society of Endometriosis Guideline (2018), French Guideline (CNGOF 2018), the National Institute for Health

and Care Excellence (NICE 2017), the German Guideline (S2k 2014), the European Society of Human Reproduction and Embryology Endometriosis Guideline (ESHRE 2013), the World Endometriosis Society Montpellier Consensus (WES 2013), the Spanish Health Ministry Guideline (2013), the Australasian Guideline (ACCEPT, 2012), American Society of Reproductive Medicine Endometriosis Committee Opinion (ASRM 2012), and Society of Obstetricians and Gynaecologists of Canada Endometriosis Guideline (SOGC 2010). All of them were assessed by all four methods. Considerable methodological variability was found.

NICE 2017 was the best rated, followed by SEF, 2018 and CNGOF, 2018. According to guideline classification in tertiles, with the Agree II instrument four guidelines reached the upper tertile (high quality) (>67%) NICE 2017, SEF 2018, CNGOF 2018 and ESHRE 2013. All the rest scored as moderate quality (34-66%). With the Right checklist, the classification remained the same. With iCAHE and MiChe appraisal, 4 guidelines moved into the high quality tertile: Canada 2010, ACCEPT 2012, WES 2013, and S2K 2014.

**CONCLUSIONS:** Endometriosis Guidelines have a high degree of methodological variability when appraised by different evaluation tools. Due to this fact, appraisal seems necessary prior to apply recommendations. Guidelines assessment has to be quick and easy so clinicians can perform it themselves in daily practice.

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**PRESENCE OF HUMAN HERPESVIRUS 6 (HHV-6) ANTIGENS IN PAIRED SURGICAL SPECIMENS FROM WOMEN EVALUATED FOR ENDOMETRIOSIS: A SURVEY OF NINE PATIENTS.**



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**OBJECTIVE:** Endometriosis is a common disease afflicting more than 10% of American women of childbearing age, contributing to infertility in 30-50% of those affected.<sup>1</sup> A cause or trigger for the disease has not been identified. Human herpesvirus 6A and 6B (HHV-6) are members of the beta sub-family of herpesviridae that includes cytomegalovirus (CMV) and human herpesvirus 7 (HHV-7). Recent reports have identified the presence of HHV-6 infection within the endometrium in 43% of women with unexplained infertility, but not within the endometrial tissue of fertile women.<sup>2</sup> We examined biopsies from endometrial tissue and endometriosis implants for evidence of active HHV-6 infection to identify a potential association between HHV-6 and endometriosis-associated infertility.

**DESIGN:** Nine women with known infertility and suspected endometriosis underwent hysteroscopy and laparoscopy. With one exception, infertility patients without visually identified endometriosis did not have HHV-6 IHC testing. Endometriosis biopsies were obtained by laser excision and endometrial biopsies were obtained by curettage. Routine histologic evaluation was performed on all collected specimen. Endometriosis was confirmed visually and histologically in eight of the nine patients presented here. One patient had an endometrial polyp without visual evidence of endometriosis.

**MATERIALS AND METHODS:** Slides from archived formalin fixed paraffin embedded biopsy tissues were assessed by immunohistochemistry with a validated diagnostic assay for late viral proteins of HHV-6.

**RESULTS:** Of the eight patients with endometriosis, HHV-6 IHC was positive in two, with identification of HHV6 antigens in both endometrium and peritoneal implants. One patient without visually identified endometriosis was found to have HHV-6 IHC in a benign polyp as well as a paired curettage

Patient	Age(yrs)	Duration of infertility	Endometrial biopsy	Peritoneal biopsy(s)	Endometrial polyp
1	35	18 mo	Positive	Positive	N/A
2	33	4 yrs	Negative	Negative	N/A
3	36	10 mo	Positive	Positive	N/A
4	27	4 yrs	Negative	Negative	N/A
5	29	2 yrs	Negative	Negative	N/A
6	25	20 mo	Negative	Negative	N/A
7	31	3 yrs	Positive	N/A	Positive
8	34	6 yrs	Negative	Negative	N/A
9	34	2 yrs	Negative	Negative	N/A

specimen. HHV-6 late antigens were located in endometrial epithelial cells and were not seen in stromal or hematopoietic derived cells.

**CONCLUSIONS:** The presence of reactivated HHV-6 in endometrial epithelial cells and the ability of the virus to up-regulate the expression of cytokines (IL-8, TNF $\alpha$ ) and growth factors (VEGF) associated with proliferation, adhesion, and neoangiogenesis of endometrial cells, supports further study for a role of HHV-6 in the pathogenesis of endometriosis and associated infertility.

References: <sup>1</sup>Macer M. Endometriosis and Infertility: A review of the Pathogenesis and Treatment of Endometriosis-Associated Infertility. *Obstet Gynecol Clin North Am.* (2012);39:535-549.

<sup>2</sup>Marci R, Gentili V, et al. Presence of HHV-6A in Endometrial Epithelial Cells from Women with Primary Unexplained Infertility. *PLoS ONE.* 11(2016).

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**EFFECTS OF DIENOGEST ON BREAST : MCF CELL LINE DATA.** Hyun Jin Kim, M.D., Sung Hoon KIM, M.D., Ph.D., Young Sang Oh, M.S., DoYoung Kim, M.D., Sa Ra Lee, M.D. Ph.D., Hee Dong Chae, M.D., Ph.D., Byung Moon Kang, M.D. Ph.D. University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea, Republic of (South).



**OBJECTIVE:** Dienogest (DNG) is a widely used progestin which is safe and effective for long-term management of endometriosis. However, its association to breast cells remains to be elucidated. We perform this study to investigate whether in vitro treatment of DNG can cause any biologic changes on MCF cell line (human estrogen receptor (ER)-positive breast cancer cell line) experiments.

**DESIGN:** A laboratory study.

**MATERIALS AND METHODS:** Following in vitro culture of MCF cells, we treated those cells and compared cell viability and the expression of several markers have shown to be increased in breast cancer cells between with estradiol alone and estradiol with DNG. Cell viability was measured utilizing MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and the expression of PCNA (proliferating cell nuclear antigen) and PAK4 (p21 activated kinase 4) was measured by western blot analyses. VEGF (vascular endothelial growth factor) and IL (interleukin)-32 were analyzed by ELISA, and MMP2 (matrix metalloproteinase 2) activity was assayed by zymography.

**RESULTS:** In vitro treatment of MCF7 cells led to an increased cell viability by estradiol alone and decreased by both estradiol and DNG after 24 and 48-hour culture. The expression of PCNA after 48 hours showed the same result. VEGF and IL-32 were also significantly increased with estradiol and decreased following DNG treatment. However, there was no significant changes in MMP2 activity and PAK4 expression.

**CONCLUSIONS:** These findings suggest that DNG may have inhibitory effects on carcinogenesis of breast cells by suppressing specific biologic changes treated by estradiol. However, further study is necessary using normal human breast cells.

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**MAGNETIC RESONANCE WITH GEL ENEMA (MR-E) VERSUS COMPUTED TOMOGRAPHIC COLONOGRAPHY (CTC) FOR DIAGNOSING RECTOSIGMOID ENDOMETRIOSIS.** Simone Ferrero, MD, PhD,<sup>a</sup> Fabio Barra, MD,<sup>a</sup> Carolina Scala, MD,<sup>b</sup> Valerio Gaetano Vellone, MD, PhD,<sup>c</sup> Ennio Biscaldi, MD.<sup>d</sup> <sup>a</sup>DINOEMI, University of Genova, Genova, Italy; <sup>b</sup>Istituto G. Gaslini, Genova, Italy; <sup>c</sup>DISC, University of Genova, Genova, Italy; <sup>d</sup>Department of Radiology, Galliera Hospital, Genova, Italy.



**OBJECTIVE:** An accurate diagnosis of the presence, location and extent of rectosigmoid endometriotic nodules is critical for the clinicians to perform the correct counseling on the potential surgical or medical treatments. This study aims to compare the accuracy of magnetic resonance with gel enema (MR-e) and computed tomography-based virtual colonoscopy (CTC) for diagnosing rectosigmoid endometriosis.

**DESIGN:** Retrospective analysis of a prospectively collected database.

**MATERIALS AND METHODS:** This study included patients with pain and/or intestinal symptoms lasting at least 6 months and clinical suspicion of rectosigmoid endometriosis. Exclusion criteria for the study were previous

intestinal surgery (with the exception of appendectomy) or previous laparoscopic diagnosis of rectosigmoid endometriosis. Patients underwent both MR-e and CTC. Subsequently they underwent laparoscopy; rectosigmoid nodules were excised by segmental colorectal resection, nodulectomy or shaving. The surgical specimens were sent to the pathologist in order to be evaluated by standardized criteria.

**RESULTS:** Out of 90 women included in the study, 44 (48.9; 95% CI, 38.2%-59.7%) had rectosigmoid nodules. Seven patients underwent shaving of the colorectal nodules; nine underwent nodulectomy; 28 patients underwent segmental colorectal resection, in these patients the mean (+ SD) length of the resected bowel specimen was 12.0 + 2.1 cm. At histology, endometriosis infiltrated only the muscularis propria in 33 patients, the submucosa in 8 patients and the mucosa in 3 patients. There was no significant difference in the accuracy of both radiologic exams for diagnosing the presence of rectosigmoid endometriosis (p=0.344): in particular, for MR-e, sensitivity was 93.2% (95% CI, 81.3-98.6%), specificity 97.8% (95% CI, 88.5%-99.9%), positive predictive value (PPV) 97.6% (95% CI, 85.5%-99.7%) and negative predictive value (NPV) 93.8% (95% CI, 83.4%-97.9%). For CTC, sensitivity was 88.64% (95% CI, 75.44%-96.21%), specificity 93.48% (95% CI, 82.10%-98.63%), PPV 92.9% (95% CI, 81.2%-97.5%) and NPV 89.6% (95% CI, 80.0%-95.2%). The mean ( $\pm$  SD) largest diameter of the main endometriotic nodule at histology was 26.8 ( $\pm$  9.7) mm. The nodule was pre-operatively identified by both MR-e and CTC in 37 patients. MR-e was more accurate than CTC in estimating the largest diameter of the main rectosigmoid nodule (p < 0.001). The mean difference in the estimated length of the nodule was 3.1 mm (95% C.I., 2.4 to 3.7; limits of agreement, -0.7 to 6.8 mm) at CTC and 1.6 at MR-e (95% C.I., -1.0 to 2.1; limits of agreement, -1.8 to 4.9 mm) when compared with histology. MR-e was more precise than CTC in identifying multifocal disease. Patients complained more discomfort during CTC than during MR-e (p<0.001).

**CONCLUSIONS:** This study showed that MR-e and CTC have similar diagnostic accuracy in diagnosing rectosigmoid endometriosis. However, MR-e is more accurate in estimating the largest diameter of the main rectosigmoid nodule, in diagnosing multifocal disease and it is better tolerated than CTC. Moreover, MR-e does not require to administer ionizing radiations.

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**MARKERS OF LOCAL AND SYSTEMIC ESTROGEN METABOLISM IN ENDOMETRIOSIS.** Velja Mijatovic, M.D., PhD,<sup>a</sup> Essam R. Othman, MD,<sup>b</sup> Maha Y. Khashbhab, MSc,<sup>c</sup> Ibraheem I. Abdelaal, MD,<sup>d</sup> Ahmad Abo Markeb, PhD,<sup>e</sup> Ahmed N. Fetih, MD,<sup>d</sup> C. B. Lambalk, MD PhD.<sup>f</sup> <sup>a</sup>Amsterdam University Medical Center, location VU, Amsterdam, Netherlands; <sup>b</sup>Associate professor, OB-GYN department, Assiut, Egypt; <sup>c</sup>Women's Health Hospital, Assiut, Egypt; <sup>d</sup>Associate professor, Assiut, Egypt; <sup>e</sup>Lecturer, Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt; <sup>f</sup>Amsterdam University Medical Center, reproductive medicine department, Amsterdam, Netherlands.



**OBJECTIVE:** Endometriosis is an estrogen dependent disease. Estrogen metabolites can work independently of their parent hormones. Therefore, we hypothesize that in endometriosis patients estrogen is metabolized along hormonally active pathways to keep a highly estrogenic milieu.

**DESIGN:** Cross sectional study in which paired urine, eutopic and ectopic endometrial samples were taken from patients with endometriosis and control women and analyzed for estrogen metabolites.

**MATERIALS AND METHODS:** We recruited 62 Endometriosis cases (disease proven laparoscopically and histologically) and 52 control women (in whom laparoscopy was normal) among patients undergoing laparoscopy for pelvic pain and/or infertility during proliferative phase of cycle. Urine samples were collected preoperatively. At surgery we collected eutopic endometrial samples from endometriosis cases and control women and biopsies from ovarian endometriotic cysts (ectopic endometrium). Estrogen metabolites in urine and endometrial tissue were extracted and determined using Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS). These included: 2-hydroxyestrone (2OHE1), 16- $\alpha$  hydroxyestrone (16- $\alpha$ OHE1), 4-hydroxyestrone (4OHE1), 2-hydroxyestradiol (2OHE2), and 4-hydroxyestradiol (4OHE2). Non parametric statistics were used.

**RESULTS:** Endometriosis cases and control women had similar baseline characteristics. *Endometrial estrogen metabolites:* there was no significant difference among control endometrium, eutopic endometrium of

endometriosis patients or ectopic endometrium in levels of 16- $\alpha$  OHE1, 2OHE1, and 2OHE1/16- $\alpha$  OHE1 ratio. Eutopic endometrium of endometriosis patients, compared to control endometrium, had significantly higher 4OHE1 [30 (30-260) versus 30 (30-30) ng/ g tissue, respectively,  $p=0.017$ ], 2OHE2 [241 (100-960) versus 100 (100-100) ng/ g tissue respectively,  $p=0.0001$ ], and 4OHE2 [225 (200-1290.7) versus 200 (200-200) ng/g tissue respectively,  $P=0.0001$ ]. Levels of 4OHE2 were significantly elevated in eutopic endometrium of endometriosis patients than in ectopic endometrium [225 (200-1290.7) versus 200 (200-200) ng/g tissue respectively,  $P=0.0001$ ]. *Urinary estrogen metabolites:* Endometriosis patients; compared to control women, had significantly higher urinary levels of 16- $\alpha$ OHE1 [14.6 (3.4-34.6) versus 4.9 (3- 12.8) ng/mg creatinine respectively,  $P=0.024$ ] and 2OHE1 [10.7 (3.9-15.5) versus 4.8 (1.4- 13.7) ng/mg creatinine, respectively,  $P=0.018$ ]. All other metabolites did not differ significantly between cases and controls.

**CONCLUSIONS:** Eutopic endometrium of endometriosis patients metabolizes estrogen preferentially to estrogenically active (2OHE2), and potentially genotoxic (4OHE1, 4OHE2) metabolites. This adds explanation on endometriosis etiology, provides a link between endometriosis and cancer, and may help in identifying potential endometrial biomarker of the disease. In urine, a similar pattern could not be identified as ratio of antiproliferative 2OHE1 to proliferative 16- $\alpha$ OHE1 are similar between cases and controls.

**SUPPORT:** Science and Technology Development Fund (STDF) grant# 5525 to E.R.O.

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### **IMPACT OF GONADOTROPIN-RELEASING HORMONE AGONIST POST-OPERATIVE TREATMENT ON OVARIAN RESERVE CHANGES AFTER LAPAROSCOPIC SURGERY OF OVARIAN**



**ENDOMETRIOMA.** Yoo jin Shim, MD., Jung Ryeol Lee, MD. PhD., Seoul National University Bundang Hospital, Seongnam, Korea, Republic of (South).

**OBJECTIVE:** Hormonal treatment including gonadotropin-releasing hormone agonist, dienogest and oral contraceptive (OC) has been found to be effective in post-operative recurrence prevention. However, evidence is very limited regarding the change of ovarian reserve following these hormonal treatments. The objective of this study was to compare the impact of dienogest or OC alone versus gonadotropin-releasing hormone agonist (GnRHa) plus dienogest or OC on ovarian reserve.

**DESIGN:** Retrospective study at university hospital.

**MATERIALS AND METHODS:** A total of 81 patients undergoing laparoscopic ovarian cystectomy for ovarian endometriosis and subsequent treatment of either at least 2 times of GnRHa plus dienogest/OC (group A,  $n=46$ ) or dienogest/OC alone (Group B,  $n=35$ ) between October 2012 and April 2018 were retrospectively analyzed. Main outcome measures included AMH reduction ratio (preoperative - postoperative AMH / preoperative AMH x 100 ), AMH value at 3,6, and 12 months after operation, CA 125 reduction ratio (preoperative - postoperative CA-125 / preoperative CA-125 x 100) and 12 month recurrence of the 2 groups.

**RESULTS:** Prior to operation, there were no significant differences between the group A and B in terms of age ( $33.4 \pm 0.6$  vs  $32.1 \pm 5.6$ ,  $P=0.377$ ), body mass index ( $21.5 \pm 3.3$  kg/m<sup>2</sup> vs  $20.9 \pm 2.8$  kg/m<sup>2</sup>,  $P=0.365$ ), ASRM score ( $63.6 \pm 36.5$  vs  $69.4 \pm 43.2$ ,  $P=0.520$ ), bilaterality of endometrioma (54.3 % in both groups,  $P=0.996$ ), and pre-operative CA-125 levels ( $94.6 \pm 72.3$  U/mL and  $91.0 \pm 43.1$  U/mL ,  $P=0.163$ ).

Pre-operative AMH levels were not different in the two groups ( $3.9 \pm 3.3$  ng/mL vs  $3.6 \pm 1.6$  ng/mL, respectively,  $P=0.820$ ). At 3 and 6 months of treatment, AMH level was more reduced in Group A than Group B, but this difference was not statistically significant. ( $1.1 \pm 0.9$  ng/mL vs  $1.9 \pm 1.8$  ng/mL,  $P=0.311$  at 3 months,  $1.5 \pm 1.8$  ng/mL vs  $1.8 \pm 1.7$  ng/mL,  $P=0.610$  at 6 months) The AMH reduction ratio was non-significantly higher in Group A ( $64 \pm 23$  % vs  $51 \pm 25$  %,  $P=0.330$  at 3 months,  $63 \pm 20$  % vs  $55 \pm 30$  %,  $P=0.358$  at 6 months). At 12-month follow up, these trends were reversed and the AMH level was higher in group A, but this difference was also statistically not significant. ( $1.95$  ng/mL vs  $1.64$  ng/mL,  $p=0.615$ ). CA 125 level and reduction ratio at 12 months were not statistically different between the 2 groups. There was no recurrence at 12 months in both groups.

**CONCLUSIONS:** These results show that, use of GnRHa reduces immediate post-op AMH level more at 3 and 6 months. However, after 12 months this effect is reversed. Long-term effects of GnRHa treatment in ovarian reserve and recurrence could be elucidated with further study with longer follow-up.

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### **A LONGITUDINAL ASSESSMENT OF THE IMPACT OF ENDOMETRIOSIS ON PATIENTS' SALARIES.**



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**OBJECTIVE:** To evaluate the longitudinal indirect burden of endometriosis (EM) by assessing the impact of disease on salary and salary growth over a 5-year period.

**DESIGN:** A retrospective cohort study using data from the OptumHealth Reporting and Insights claims database.

**MATERIALS AND METHODS:** Women aged 18-49 years with  $\geq 1$  EM diagnosis (International Classification of Diseases code 617.x or N80.x) were matched 1:1 to women without EM (controls) by birth year, index year, employer industry, and geographic region. For EM patients, index dates were their first EM diagnosis date; for controls, index dates were random dates during the period where patients had continuous eligibility. Continuous eligibility in a health plan for  $\geq 1$  year pre- and post-index and active employment during the 1-year baseline period were required. Women with menopause or cancer diagnosis during baseline were excluded. Baseline characteristics were compared between EM and control cohorts with descriptive analyses. Average annual salaries for EM patients and controls were compared at each of the 5 post-index years using generalized estimating equations that accounted for matching. A multivariate longitudinal model was also used to estimate and compare the 5-year salary changes from baseline between the two cohorts. The model adjusted for baseline characteristics and correlations between observations. Salaries were inflated to 2018 USD using the Consumer Price Index.

**RESULTS:** Among the 6,851 matched pairs, the mean age at index date was 38.7 years. During baseline, EM patients, compared to matched controls, had significantly higher modified Charlson Comorbidity Index (CCI: 0.16 vs. 0.12,  $p<0.01$ ) and lower average annual salary (\$60,080 vs. \$64,081,  $p<0.01$ ). While oral contraceptive use was comparable between the two cohorts (20.1% vs. 20.2%,  $p=0.86$ ), more EM patients used NSAIDs (32.4% vs. 20.3%,  $p<0.01$ ) and opioids (44.6% vs. 26.0%,  $p<0.01$ ) during baseline than controls. In the first year after the index date, EM patients had an observed average annual salary of \$61,322 compared to \$64,720 for controls ( $p<0.01$ ). In each of the subsequent four years, the observed average salary for EM patients was less than that of controls by \$3,697, \$5,099, \$6,286, and \$6,600 (all  $p<0.01$ ) among matched pairs that both had observed data. In comparing the salary changes from baseline over the 5-year study period, EM patients consistently had smaller salary growth than controls ( $p=0.02$  for the difference in rate of salary growth between cohorts). In the first year after index date, EM patients had an estimated salary increase of \$438, while it was \$1,058 for controls. The salary changes from baseline for EM patients vs. controls were \$1,555 vs. \$2,562, \$2,672 vs. \$4,066, \$3,789 vs. \$5,570, and \$4,906 vs. \$7,074 for each of the subsequent four years, respectively.

**CONCLUSIONS:** Patients with EM had a lower salary at baseline and a smaller increase in salary over time compared to their matched controls.

**SUPPORT:** Financial support for conducting the study was provided by AbbVie.

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### **ELEVATED SERUM INTERLEUKIN-32 LEVELS IN PATIENTS WITH ENDOMETRIOSIS: A PROSPECTIVE CASE-CONTROL STUDY.**



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**OBJECTIVE:** Recently, interleukin (IL)-32 has been suggested to be involved in the pathogenesis of endometriosis. The aim of this study is to investigate whether serum IL-32 level might be used as a biomarker for diagnosis of endometriosis.

**DESIGN:** Prospective case-control study.

**MATERIALS AND METHODS:** We recruited the serum samples of 50 patients with histologically confirmed endometriosis and 35 controls. Enzyme-linked immunosorbent assay was used to analyze the serum IL-

32, IL-6, IL-10, tumor necrosis factor (TNF)- $\alpha$ , IL-1 $\beta$ , and CA-125 levels in patients with and without the disease and the diagnostic potentials of the cytokines were assessed using the area under the ROC curve (AUC).

**RESULTS:** Among evaluated cytokines, only serum IL-32 levels showed significant differences between patients with and without endometriosis ( $1111.24 \pm 149.59$  vs.  $631.10 \pm 120.23$ ,  $P=0.018$ , respectively). When the diagnostic power of serum IL-32 was evaluated, the area under the curve (AUC) was 0.638 (95% confidence interval (CI) 0.521-0.766,  $P=0.031$ ). When serum IL-32 levels were combined with serum CA-125 levels, the AUC was increased to 0.749 (95% CI 0.640-0.858,  $P<0.001$ ) with sensitivity and specificity of 60.0% and 82.9% at cut-off value of 0.640, which led to detect 25 more cases of endometriosis than the use of serum CA 125 with the cut-off value of 35 IU/ml (36/50 vs. 11/50,  $P<0.001$ ) without sacrificing the specificity of the marker.

**CONCLUSIONS:** Serum IL-32 levels are elevated in patients with endometriosis and with combination of serum CA-125 levels, it may serve as a potential biomarker for endometriosis.

**SUPPORT:** This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI16C1682).

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#### **ULTRALONG-TERM CYCLIC USE OF LOW-DOSE MONOPHASIC COMBINED ORAL CONTRACEPTIVE PILLS FOR THE MANAGEMENT OF RECURRENT SEVERE ENDOMETRIOSIS AFTER SECOND-LINE SURGERY.**

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**OBJECTIVE:** We performed this study to evaluate the efficacy of ultralong-term cyclic administration of low-dose monophasic combined oral contraceptive pills (OCP) for more than 60 months in the resolution of pain and regression of recurrent endometrioma and pseudocyst after second-line surgery for recurrent severe endometriosis.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Twenty-two patients who were prescribed low-dose monophasic combined OCP to be taken with follow-up ultrasonogram (USG) for more than 60 months after January 2001 for the treatment of recurrent severe pain and endometrioma and pseudocyst after second-line surgery were included. All patients included in the present study received cyclic therapy (daily 21 to 35 days followed by 7 day interval) with low-dose monophasic combined OCP (ethinyl estradiol 0.02 mg and desogestrel 0.15 mg daily or ethinyl estradiol 0.02 mg and drospirenone 3 mg daily). Pain and endometrioma and pseudocyst on ultrasonogram (USG) were evaluated. For the evaluation of pain improvement, visual analogue scale (VAS) was used.

**RESULTS:** In 22 patients included in this study, 6 patients had a unilateral endometrioma while 16 patients had bilateral endometriomas. Sixteen patients had pseudocyst while 6 patients had no visible pseudocyst. Duration of treatment ranged from 64 months to 150 months. Nine patients completed the treatment after complete resolution of dysmenorrhea and complete regression of endometriomas and pseudocysts but 13 patients are currently getting treatment. Pain score by visual analogue scale (VAS) was significantly lower from 12<sup>th</sup> month of treatment compared with baseline assessment ( $P < 0.001$ ) and all patients reported complete resolution of dysmenorrhea at 48<sup>th</sup> month of treatment. Endometrioma size measured at 12<sup>th</sup> month of treatment significantly decreased compared with baseline size ( $P < 0.001$ ) and consistently decreased and endometriomas assessed by USG were completely regressed in 20 patients (90.9%) at 60<sup>th</sup> month of treatment. Pseudocyst size measured by USG was significantly smaller from 12<sup>th</sup> month of treatment ( $P = 0.003$ ) and pseudocysts were completely regressed in all patients at 36<sup>th</sup> month of treatment. Eight patients (36.4%) of 22 patients reported breakthrough vaginal bleeding but bleeding was small and transient and did not cause discontinuations. Except vaginal bleeding, no patients reported any other adverse effects attributed to the ultralong-term use of low-dose monophasic combined OCP.

**CONCLUSIONS:** Ultralong-term treatment with low-dose monophasic combined OCP is effective without any serious adverse effect in eliminating pain and regressing recurrent endometriomas and pseudocysts in patients with recurrent endometriosis after second-line surgery. Therefore, ultralong-term treatment using low-dose monophasic combined OCP can be anef-

fective strategy in patients with pseudocysts as well as severe pain and recurrent endometriomas despite the second-line surgery.

**SUPPORT:** None.

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#### **THE USE OF DEXTROAMPHETAMINE SULFATE TO ALLEVIATE PELVIC PAIN DOES NOT LOWER LIVE DELIVERED PREGNANCY RATES FOLLOWING IN VITRO FERTILIZATION-EMBRYO TRANSFER (IVF-ET) IN YOUNGER WOMEN.**

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**OBJECTIVE:** Standard medical therapy with oral contraceptives, progestins, gonadotropin releasing hormone (GnRH) agonists or antagonists for pelvic pain syndromes preclude pregnancy while taking the medication, and there is no evidence that such treatment improves subsequent fecundity. Surgical treatment is frequently not effective in relieving pain and can sometimes cause oocyte depletion. Furthermore, it is not clear if surgical removal of endometriosis improves subsequent fecundity, or may have a negative effect related to diminishing oocyte reserve. One of the most effective treatments for pelvic pain is dextroamphetamine which would allow the patient to try to conceive while gaining pain relief. The objective of the present pilot study was to determine if the use of this sympathomimetic amine has any negative effects on pregnancy rates or adverse fetal consequences.

**DESIGN:** Prospective patient option controlled comparison study.

**MATERIALS AND METHODS:** Women with moderate to severe dysmenorrhea, who also wanted or needed IVF to become pregnant, were treated with dextroamphetamine sulfate. They were advised that in pharmacologic dosages the drug does not appear to be a teratogen, but its effect on pregnancy rates is not known. Patients were required to be aged  $\leq 35$  with normal oocyte reserve (serum anti-mullerian hormone (AMH) level over 1.06 ng/mL). If all embryos were frozen, or no day 3 embryos were created, the cycle was not counted. The dosage of dextroamphetamine sulfate varied from 9.4mg to 37.6mg. The clinical and live delivered pregnancy rates were compared to historical controls who did not necessarily have pelvic pain.

**RESULTS:** There were 23 women treated with dextroamphetamine sulfate who had day 3 embryo transfers. All stated their pelvic pain was moderately to markedly improved. There were 197 historical controls having day 3 transfers. The clinical pregnancy rate was 56.5% (13/23) vs. 47.2% (93/197) the live delivered pregnancy rate was 43.5% (10/23) vs. 37.6% (74/197) ( $p=NS$ , Chi-square analysis). The implantation rates were 39.5% vs. 32.5%. The average number of embryos transferred was 1.9 for both groups. All babies in the amphetamine treated group were normal.

**CONCLUSIONS:** Though the study group was small, there does not seem to be any negative effect of using dextroamphetamine sulfate for pelvic pain on pregnancy rates following IVF-ET. Since some believe that endometriosis may have a negative effect on IVF outcome, if there was a bias, it would be against the study group. If anything, there may have been a trend for higher pregnancy rates in the amphetamine treated group. Based on these data a randomized prospective study is planned comparing pregnancy outcome in women with pelvic pain taking amphetamine vs. no amphetamine who are undergoing IVF-ET to achieve a pregnancy.

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#### **ANALYSIS OF METABOLIC PATHWAYS AS A NOVEL CLINICAL BIOMARKERS OF ENDOMETRIOSIS.**

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**OBJECTIVE:** Endometriosis is a common gynecological disease and causes infertility. The discovery of biomarkers has been demonstrated to play an important role in medicine and diagnosing the patient. Metabolomics and metabolic profiling have become popular in many research projects and discovery novel biomarkers. Till now, little is known about the association of metabolic pathways and endometriosis. We investigate the seven metabolic pathways in endometriosis, including alpha-1 antitrypsin (AAT), alpha-1-acid glycoprotein (AGP-1), Hemoexin, Retinol-binding protein 4 (RBP4), Transferrin, Transthyretin and Vitamin D-binding protein (VDBP).

**DESIGN:** A case-control study in University-affiliated infertility center. A total number of 81 women were enrolled in this study. Women who were either undergoing laparoscopic confirmation of endometriosis (n =23) or were controls (n = 21). Women with endometriosis treated with Gonadotropin-releasing hormone agonist (GnRHa) (n=31) and 6 follow up women (n=6).

**MATERIALS AND METHODS:** Their plasma were collected and detected the metabolic pathways using Bioplex assay, seven metabolic pathways kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions.

**RESULTS:** We found that AAT, AGP-1 and RBP4 were increased in endometriosis patients. AAT is a protease inhibitor produced by the liver and belongs to the serpin superfamily of proteins. AAT protects tissue from enzymes secreted by inflammatory cells. AGP1, also known as orosomucoid, is an acute phase protein produced in the liver. AGP1 acts as a carrier of lipophilic and basic drug, steroids, and protease inhibitors. RBP4 belongs to the lipocalin family and is the specific carrier protein for retinol (Vitamin A), delivering retinol from liver stores to peripheral tissues. To further observe whether AAT, AGP-1 and RBP-4 could be a biomarker of endometriosis, we analyzed the ROC curve. RBP-4 showed high area under the curve (AUC) values of 0.8 (95% confidence interval, 0.6696-0.9276). The AUC of AAT and AGP-1 are 0.71 and 0.68, respectively. With a plasma RBP-4 level of 15.38µg/mL as the optimal cutoff value and defined the maximum sensitivity and specificity. RBP-4 showed 79.17 % sensitivity and 71.43% specificity. Moreover, AAT and RBP4 levels were significantly decreased in the GnRHa treatment group. Follow up studies also showed that GnRHa treatment decreased plasma AAT and RBP-4 levels in the same endometriosis patient.

**CONCLUSIONS:** These results found that RBP-4 may be a potential biomarker of endometriosis. AAT and RBP4 levels were significantly decreased in the GnRHa treatment group.

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**LONG TERM TREATMENT OF ENDOMETRIOSIS ASSOCIATED PAIN (EAP) WITH LINZAGOLIX: EFFICACY AND SAFETY AFTER 12 MONTHS OF TREATMENT.**

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**OBJECTIVE:** To assess safety and maintenance of efficacy of linzagolix after 52 weeks (w) of treatment.

TABLE 1

	PBO <sup>‡</sup>	50 mg	75 mg	100 mg	200/100 mg <sup>†</sup>
N	12 w 24 w 52 w	53 49 40 30	56 48 36	51 39 22	56 44 30
% responders OPP	12 w 24 w 52 w	34.5 49.4 52.5 66.7	61.5* 70.8 69.2	56.4* 66.7 53.8	56.3* 77.3 82.4
% responders DYS	12 w 24 w 52 w	28.5 43.3 47.5 50.0	68.2* 58.3 69.2	68.6* 82.1 69.2	78.9* 84.1 64.7
% responders NMPP	12 w 24 w 52 w	37.1 46.2 50.0 66.7	58.5* 72.9 69.2	61.5* 64.1 53.8	47.7 72.7 76.5
Dyspareunia Mean (SD) CFB	12 w 24 w 52 w	-0.4 (0.9) -0.6 (0.7) -0.7 (0.8) -0.5 (1.1)	-0.7 (0.8) -0.7 (0.8) -0.9 (0.8)	-0.7 (0.9) -0.6 (0.8) -0.7 (1.1)	-0.8 (1.1)* -1.0 (1.0) -0.9 (0.9)
Dyschezia Mean (SD) CFB	12 w 24 w 52 w	-0.7 (1.7) -1.4 (1.7) -1.5 (2.1) -2.3 (1.8)	-2.1 (2.5)* -2.2 (2.5) -2.1 (2.9)	-2.0 (1.8)* -2.0 (2.3) -2.9 (3.1)	-1.7 (2.3)* -2.5 (2.6) -2.6 (2.7)
BMD spine Mean (95% CI) % CFB	24 w 52 w	0.14 (-0.83, 1.11) 0.14 (-1.04, 1.31)	-0.80 (-1.57, -0.03) -1.14 (-2.21, -0.07)	-1.37 (-2.14, -0.59) -1.40 (-3.35, 0.55)	-2.60 (-3.56, -1.65) -2.19 (-3.59, -0.78)

<sup>‡</sup>PBO only to 12 w; <sup>†</sup>Subjects randomized to 200 mg received 100 mg from 24 to 52 w; \*p < 0.05 compared to PBO.

**DESIGN:** Linzagolix is an oral GnRH receptor antagonist that induces a dose-dependent reduction of estradiol and is being developed to treat EAP. EDELWEISS was a Phase 2b, double-blind, placebo (PBO)-controlled trial evaluating once daily linzagolix doses of 50, 75, 100 and 200 mg. At 24 w, subjects could extend active treatment up to 52 w.

**MATERIALS AND METHODS:** Participants were women with surgically confirmed endometriosis and moderate to severe EAP. Efficacy was assessed using a daily eDiary as the % of responders (≥ 30% reduction in mean 28-day scores) in overall pelvic pain (OPP), dysmenorrhea (DYS) and non-menstrual pelvic pain (NMPP). Dyspareunia and dyschezia scores were also assessed. Bone mineral density (BMD) of the femur, hip and spine were assessed by dual-energy X-ray absorptiometry (DXA).

**RESULTS:** At 12 w, there was a significant increase in the % of responders for OPP, DYS and NMPP for doses of 75 mg and above compared to PBO. These effects were generally maintained or increased at 24 and 52 w. At 12 w, there were significant improvements in dyspareunia (200 mg only) and dyschezia scores which were maintained or increased at 24 and 52 weeks. Mean BMD losses (spine) at 24 weeks were <1% at doses of 50 and 75 mg and increased with increasing dose up to 2.6% for 200 mg. A similar pattern was observed at 52 w. BMD changes in femur and hip were similar but generally smaller.

**CONCLUSIONS:** Linzagolix at daily doses of 75 mg and above significantly improved EAP symptoms at 12 w and these effects were maintained or increased at 24 and 52 w. These data support Phase 3 trials in women with EAP using linzagolix 75 mg once daily alone and 200 mg once daily with low-dose add-back hormonal therapy.

**SUPPORT:** The study was funded by ObsEva SA.

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**DECREASED CLINICAL PREGNANCY AND LIVE BIRTH RATES IN WOMEN WITH ENDOMETRIOSIS, IN THE "EIVF" DATABASE.**

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**OBJECTIVE:** To determine whether surgically-confirmed endometriosis is associated with decreased implantation, pregnancy, or live birth rates compared with male factor infertility in women undergoing in vitro fertilization (IVF).

**DESIGN:** Retrospective multivariable analysis of 34,278 fresh IVF cycles in Massachusetts between 2003- 2006, from the "eIVF" database.

**MATERIALS AND METHODS:** IVF cycles in women with surgically-confirmed endometriosis were compared with those in couples with male

	Surgical Endometriosis (n = 350)	Male Factor Infertility (n = 2824)	p-value
Age (mean)	35.4	36.3	<b>0.0011</b>
BMI (mean)	25.2	26.5	<b>0.0001</b>
AMH (median)	1.58	1.40	0.0913
Max FSH (median)	8.03	5.9	0.1340
Max E2	1966	2076	<b>0.0028</b>
Implantation rate 1 (GS/ET) >= 50%	22.0%	26.9%	<b>0.0492</b>
Implantation rate 2 (HB/ET) >= 50%	21.4%	26.4%	<b>0.0459</b>
Pregnancy rate	36.9%	41.9%	0.0747
Live birth rate	22.3%	25.5%	0.1916

factor infertility; only fresh IVF cycles that resulted in an embryo transfer were analyzed. Couples with both endometriosis and male factor infertility, and women with "suspected endometriosis" (not surgically confirmed), were excluded from analysis. Implantation rates were calculated in two ways: (1) gestational sacs (GS) per embryos transferred (ET), and (2) heartbeats (HB) per ET. Clinical pregnancy and live birth rates per cycle were also calculated. Means were compared using two-sample t-tests; medians were compared with Wilcoxon t-tests; pregnancy and live birth rates were compared using Chi-square tests; and multivariable analyses were performed using logistic regression. SAS version 9.4 (SAS Institute, Cary, NC) was used for all analyses.

**RESULTS:** 350 fresh IVF cycles in women with surgically-confirmed endometriosis were compared with 2,824 cycles in couples with male factor infertility only. Women with endometriosis were significantly younger and leaner, with lower max E2 levels. On univariable analysis, fewer women with endometriosis had implantation rates >= 50%, while no differences were found in pregnancy or live birth rates (Table). When adjusting for age and BMI in multivariable analysis, the odds of a clinical pregnancy was 35.5% higher in male factor infertility (OR 1.36, 95% CI [1.07-1.71]), and the odds of a live birth was 33.9% higher in male factor infertility (OR 1.34, 95% CI [1.02-1.76]), compared to women with surgically-confirmed endometriosis.

**CONCLUSIONS:** Compared with male factor infertility, surgically-confirmed endometriosis is associated with lower odds of implantation, clinical pregnancy and live birth in couples undergoing IVF.

**P-541** Wednesday, October 16, 2019 6:30 AM

#### **CIRCULATING PLACENTAL GROWTH FACTOR (PLGF) CONCENTRATION IN PREGNANT WOMEN WITH ENDOMETRIOSIS: A CASE-CONTROL STUDY.**

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**OBJECTIVE:** It is widely accepted that angiogenesis is pivotal to the establishment of endometriotic lesions and it is fundamental in the regulation of placental development starting from the early stages of pregnancy. Circulating vascular endothelial growth factor and placental growth factor (PLGF) levels have been reported to be increased in women with endometriosis when compared with controls, conversely decreased levels of PLGF have been reported in pregnant women developing preeclampsia. For this reason, the presence of endometriosis might be a protective factor for the development of early onset preeclampsia during pregnancy, since the increased level of PLGF in these patients might promote placental development. The objective of this study was to assess the first trimester serum concentrations of circulating PLGF in pregnant women with endometriosis compared to those without endometriosis.

**DESIGN:** Case-control study based on the retrospective analysis of a prospectively collected database.

**MATERIALS AND METHODS:** This study included 40 pregnant women who had histological diagnosis of endometriosis (E) and 40 pregnant women without endometriosis (C). Women included in the control group had no evidence of endometriosis at laparoscopy. Exclusion criteria were previous uterine surgery or uterine malformations, major fetal structural abnormalities, alcohol and/or drugs abuse, chronic hypertension disease, known autoimmune diseases, fetal aneuploidy or multiple gestations.

**RESULTS:** Compared to C women, those with E had no statistically significant difference regarding maternal demographic characteristics (age, BMI, parity, smoking, mode of conception). No statistically significant difference was observed in the first trimester PAPP-A levels, first trimester and mid-pregnancy mean UtA Doppler PI, neonatal birth weight (BW) centile, SGA fetuses and early onset preeclampsia (<37 weeks of gestation) prevalence. However, women with E had statistically significant higher first trimester concentration of PLGF compared to those without endometriosis (C) (group E: PLGF MoM 1.40; group C: PLGF MoM 1.19; p<0.05).

**CONCLUSIONS:** First trimester serum concentrations of PLGF are significantly higher in pregnant women with endometriosis compared to those without the disease. The major limitations of this study were that it was retrospective and it had a relatively small sample size. The small number of pregnant women with endometriosis did not allow performing a further subanalysis according to the different forms of endometriosis (peritoneal endometriosis, deep endometriosis, ovarian endometriosis).

**P-542** Wednesday, October 16, 2019 6:30 AM

#### **PELVIC FLOOR MUSCLE SPASM, COMORBID PAIN AND MENTAL HEALTH CONDITIONS IN WOMEN WITH ENDOMETRIOSIS-ASSOCIATED CHRONIC PELVIC PAIN.**

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**OBJECTIVE:** Describe pelvic pain pattern, and pain and mental health comorbidities in women with endometriosis-associated chronic pelvic pain (endo-CPP).

**DESIGN:** Baseline, cross-sectional data from a prospective, double-masked, placebo-controlled study of botulinum toxin injection for persistent endo-CPP despite optimal pain, surgical and hormonal treatment.

**MATERIALS AND METHODS:** Subjects described headache (including migraine) history and completed standardized questionnaires: Pelvic Pain Questionnaire; Patient-Reported Outcomes Measurement Information System (PROMIS) scales for anxiety, depression, fatigue, and sleep disturbances; Rome Criteria for irritable bowel syndrome (IBS); painful bladder syndrome (PBS); Oswestry Disability Index. Patients underwent pelvic exam to confirm pelvic floor spasm and determine pelvic pain pattern. Allodynia and hyperalgesia were assessed paraspinally to determine the extent of pelvic (T9-S2) and widespread (C2-S2) spinal segmental sensitization. Ordinal data were analyzed for trends using the Jonckheere-Terpstra Test. Unordered dichotomous variables were compared using Fisher's exact test.

**RESULTS:** Women (n=30, age 18-50yr) with endo-CPP (median duration 10.5yr; range 2-20) were evaluated. 22/23 women using hormonal methods (progestin IUD/combined hormonal contraception/depot medroxyprogesterone) had menses suppression; 7 others avoided hormones due to side effects. At pelvic exam, 30/30 had pelvic floor spasm that each identified as a primary focus of endo-CPP. Non-menstrual pelvic pain was reported by 29, dysmenorrhea at their last menses by 27, and dyspareunia by 14/15 who had sex in the last month; 7 others avoided sex because of pain. 17 women had widespread and 18 had pelvic spinal segmental sensitization. Most women reported anxiety (18), depression (14), fatigue (23) and sleep disturbances (18). 16 women met criteria for IBS, 22 for PBS, and 17 reported migraine. Moderate disability was reported by 14 women and severe disability by 3. Having either IBS or PBS was associated with depression (p=0.031), anxiety (p=0.003), and fatigue (p=0.029) but not sleep disturbances or disability. Disability was associated with pelvic and widespread sensitization (p=0.025 and p=0.009, respectively), but not PROMIS outcomes.

**CONCLUSIONS:** Not surprisingly, women with endometriosis-associated chronic pelvic pain persisting despite treatment report non-menstrual pain, dysmenorrhea and dyspareunia. Importantly, they experience significant

comorbid pain and mental health conditions. Pelvic muscle spasm and associated sensitization may be a key manifestation of their endometriosis-associated chronic pelvic pain. Comorbid pain conditions and mental health may factor into endo-CPP. These women merit comprehensive assessment and management of their pain patterns.

Clinicaltrials.gov: NCT01553201

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#### EFFECT OF ENDOMETRIOSIS ACTIVITY ON PREGNANCY OUTCOME IN PATIENTS WITH REPEATED IMPLANTATION FAILURE.

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**OBJECTIVE:** Patients face a problem in IVF/ICSI treatment, repeated implantation failure. In order to explore whether endometriosis affects the implantation of embryo when IVF/ICSI, this study compared the pregnancy outcomes of patients with endometriosis who failed to implant repeatedly. So as to explore the appropriate treatment plan for such patients.

**DESIGN:** Endometriosis patients with repeated implantation failure were grouped according to the timing of treatment and whether down-regulated or not.

**MATERIALS AND METHODS:** patients who asked for IVF/ICSI treatments in our Reproductive Center. A retrospective cohort study was performed on endometriosis patients with repeated implantation failure. The differences in pregnancy outcomes were compared. The comparison between quantitative data was tested by analysis of variance. The differences between the classification data were analyzed by chi-square test.

**RESULTS:** 1) According to treatment timings, the cumulative delivery rate (according to the numbers of people) was significantly lower than that of the early treatment group (28.17% vs 43.30%,  $P < 0.05$ ) and the late treatment group (28.17% vs 47.06%,  $P < 0.01$ ). The available embryo rate (93.56%) and high quality embryo rate (81.84%) in the early treatment group were significantly higher than those in the untreated group (85.20% and 62.93%) and the late treatment group (88.20% and 69.61%),  $P < 0.01$ . And the high quality embryo rate in the late treatment group (69.61%) was also significantly higher than that in the untreated group (62.93%),  $P < 0.05$ . 2) Kaplan-Meier curve analysis showed that the time required to get a pregnancy (live birth) for late treatment group was the shortest ( $12.21 \pm 1.05$ ,  $13.46 \pm 1.08$ ), and the time in the untreated group was longest ( $21.54 \pm 0.49$ ,  $22.27 \pm 0.45$ ),  $P < 0.001$ . 3) Down-regulation increased the clinical pregnancy rate of the untreated group and the late treatment group (41.18% vs 26.32% vs 50.00% vs 24.62%,  $P < 0.05$ ), and the live birth rate of the treatment group (38.78% vs 17.97% and 41.67% vs 17.69%,  $P < 0.01$ ). Down-regulation can increase the overall cumulative live birth rate (cycle) (27.78% vs 13.51%, 45.24% vs 12.99% and 46.30% vs 13.69%,  $P < 0.05$ ). Multivariate logistic regression analysis adjusted the basic line, the live birth rate of the down-regulated group was 2.249 times higher than that of the non-down-regulated group ( $P < 0.05$ ). 4) According to the EMs activity, the clinical pregnancy rate (38.84%) and live birth rate (29.75%) of the EMs-controlled patients were increased compared to the uncontrol group (26.25% and 18.13%,  $P < 0.05$ ).

**CONCLUSIONS:** 1) The treatment of endometriosis will improve the cumulative pregnancy rate and live birth rate of repeated implantation failure; 2) The down-regulation cycle can also improve the pregnancy outcome of endometriosis patients with repeated implantation failure; 3) The control of endometriosis activity can lead to better clinical outcomes in patients with repeated implant failures; 4) It is necessary to control the activity of endometriosis in patients with repeated implantation failures in IVF/ICSI treatment.

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#### SYMPATHOMIMETIC AMINE THERAPY MAY IMPROVE LIVE DELIVERED PREGNANCY RATES (PRS) FOLLOWING IN VITRO FERTILIZATION-EMBRYO TRANSFER (IVF-ET) IN WOMEN OF ADVANCED REPRODUCTIVE AGE – A PILOT STUDY.

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**OBJECTIVE:** A very effective medical therapies for various types of pelvic pain is the use of dextroamphetamine sulfate. The probable mechanism for pain relief seems to be by releasing more dopamine from sympathetic nerve fibers which diminishes cellular permeability. Increased cellular permeability may be the cause of pelvic pain related to increased absorption or irritating chemicals into pelvic tissues leading to excessive inflammation. Increased inflammatory cells, especially natural killer cells, could be a cause of infertility or miscarriage. There have been anecdotal reports of successful pregnancies in patients with repeated failures to successfully conceive despite multiple embryo transfers with this treatment. The objective of the present study was to perform a pilot study to determine if treatment with dextroamphetamine sulfate could improve the chance of a successful pregnancy following IVF-ET, in a poor prognosis group, i.e., women of advanced reproductive age.

**DESIGN:** Prospective patient option controlled study.

**MATERIALS AND METHODS:** Women age 40-42 with normal oocyte reserve as evidenced by a day 3 serum FSH  $\leq 11$  mIU/mL and a serum anti-mullerian hormone (AMH) level  $> 1.06$  ng/mL with a history of moderate to severe dysmenorrhea, dyspareunia, mittelschmerz, or chronic pelvic pain who required or requested IVF-ET were given the option of being treated with dextroamphetamine sulfate during the IVF cycle and the first trimester of pregnancy. They were advised of the theoretical benefit, but the lack of hard data, just anecdotal reports. The IVF would not be started until the dosage that best corrected the pain with acceptable side effects was achieved. The starting dosage was 9.4mg extended release capsules. The maximum dosage was 37.6 mg. All embryo transfers were performed on day 3.

**RESULTS:** There were 12 couples recruited (grp A) and 11 made it to ET. These results were compared to 77 historical controls (grp B). The historical control group did not have to have a history of pelvic pain. The average number of embryos transferred were 2 vs. 2.1 for grp A and B. The clinical PR per transfer was 27.3% (3/11) vs. 18.2 (17/77). The live delivered PR was 27.3% vs. 11.7% (9/77). The implantation rates were 18.2% and 11.8%. All 11 grp A women had marked improvement of their pelvic pain. The 3 babies born in grp A were full-term and healthy. The small pilot study group precluded meaningful statistical evaluation.

**CONCLUSIONS:** This pilot study showed sufficient benefit to improving fecundity in this poor prognosis group that we plan to submit a proposal to the IRB for a larger randomized control trial. Many of these grp A women may have had endometriosis. The advantage of dextroamphetamine sulfate over other medical therapies for pelvic pain is that it allows the patient to conceive while receiving pain relief.

#### ENDOMETRIOSIS - BASIC

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#### OVEREXPRESSION OF CD44v6 IS INVOLVED IN THE DEVELOPMENT OF THE EARLY ENDOMETRIOTIC LESION IN A XENOGRAFT MODEL.

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**OBJECTIVE:** We previously showed decreased development of endometriotic lesions in CD44 knockout mice compared to control.<sup>1</sup> CD44 has 10 different variants and a standard form. Menstrual endometrial cells (MECs) from women with endometriosis have increased adhesion and also express higher levels of CD44 variant 6 (v6), but not v3, compared to MECs from women without endometriosis.<sup>2</sup> Here, we assessed the effects of CD44 standard (CD44s), CD44v3 and CD44v6 overexpression (OE) on immortalized human endometrial epithelial (iEECs) and stroma cells (hESCs) *in vivo* attachment in a nude mouse xenograft model.

**DESIGN:** In vivo xenograft model.

**MATERIALS AND METHODS:** OE of CD44s, CD44v3 and CD44v6 was carried out using lipofectamine and their expression verified with qRT-PCR in iEEC and hESCs. Nude mice, 8-10 week old, were injected with estrogen 1 week prior to injection of iEECs and hESCs (n=7 per group). The cells were counted after transfection and at least 300,000 iEECs and 300,000 hESCs were injected per mouse. Transfected cells were tagged with cell tracker red (iEECs) and green (hESCs). Mice were sacrificed 48 hours after injection into the xenograft. Cells were counted using fluorescent stereo microscopy (FSM). The number of cells visualized by FSM divided by the number of transfected cells injected determined percent attachment. Unpaired student t-test was used to analyze differences in the percent attachment of the cells.

**RESULTS:** Expression of mRNA and protein confirmed appropriate OE of CD44s, CD44v3 and CD44v6 in the different cell types. CD44s OE did slightly induce CD44v6 expression. At necropsy, the majority of cells attached to the peritoneum. CD44v6 OE increased attachment of hESCs compared to control ( $p=0.03$ ). CD44v6 OE did not change attachment of iEECs. There was no difference in attachment in iEECs or hESCs with OE of CD44s or CD44v3.

**CONCLUSIONS:** Overexpression of CD44v6 increases attachment of ESCs to PMCs in an *in vivo* xenograft model. Menstrual endometrial cell type and CD44 variants play a complex role in the development of the early endometriotic lesion.

**References:** 1. Knudson JF, Tekmal RR, Santos MT, et al. Impaired Development of Early Endometriotic Lesions in CD44 Knockout Mice. *Reproductive sciences (Thousand Oaks, Calif.)*. 2016;23(1):87-91.

2. A Griffith JS, Liu YG, Tekmal RR, Binkley PA, Holden AE, Schenken RS. Menstrual endometrial cells from women with endometriosis demonstrate increased adherence to peritoneal cells and increased expression of CD44 splice variants. *Fertility and sterility*. 2010;93(6):1745-1749

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**P-546** Wednesday, October 16, 2019 6:30 AM

#### **ABERRANT EXPRESSIONS OF CHLORIDE CO-TRANSPORTERS IN ENDOMETRIOSIS.**

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**OBJECTIVE:** Recent studies have shown that cell membrane ion channels play an important role in cell migration, also shown in the context of cancer development and metastasis. Although endometriosis is a benign gynecological disease, endometriosis shows a behavior similar to cancer in terms of migration and invasion of nearby tissues and organs. However, there are only few studies on cell membrane ion channels and their association with endometriosis. The aim of this study was to investigate the effect of these ion channels on endometriosis.

**DESIGN:** Experimental study using human endometrial tissue and human endometrial stromal cell.

**MATERIALS AND METHODS:** In the endometriosis group ( $n=21$ ), eutopic endometrial tissue and ectopic endometrial tissue were obtained from the patients who had undergone laparoscopic ovarian cyst enucleation due to endometriosis. In the control group ( $n=18$ ), eutopic endometrium was obtained from patients who had undergone laparoscopic cyst enucleation for benign ovarian causes other than endometriosis. Quantitative real time PCR (qRT-PCR) and western blot were performed to quantify ion channel-related NKCC1, NKCC2 and CLC3 mRNA expressions and protein concentrations in endometrial tissue. Furthermore, to test the influence of ion channels on the migration ability of endometrial stromal cells, siRNA transfection and migration assay of eutopic endometrial cell of endometriosis patients.

**RESULTS:** mRNA expression of NKCC1, NKCC2 and CLC3 in ectopic endometrial tissue from endometriosis patients was significantly higher than in eutopic endometrium for both endometriosis and control group ( $p<0.05$ ). The mRNA expression of eutopic endometrium from endometriosis patients was higher than the control group, but the difference was not statistically significant. Western blot showed an increased expression of NKCC1, NKCC2 and CLC3 in both the eutopic and ectopic endometrium of endometriosis group, compared to the expression in eutopic endometrium of control group ( $p<0.05$ ). After siRNA transfection, qRT-PCR showed a decreased expression of MMP2 and MMP9. Migration assay further suggested a decreased migratory potential of the eutopic endometrial cells. Additional analysis showed that the magnitude of expression of NKCC1, CLC3 and the size of endometriotic ovarian cyst were positively correlated.

**CONCLUSIONS:** The expression of NKCC1, NKCC2 and CLC3 associated with plasma membrane ion channels is increased in endometriosis patients, which may be implicated in the increased cell migration potential in endometriosis.

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#### **PROPRANOLOL INHIBITS CATECHOLESTROGEN-INDUCED HUMAN ENDOMETRIAL STROMAL CELL SURVIVAL MEDIATED BY p38 MAPK SIGNALING: POTENTIAL THERAPY FOR ENDOMETRIOSIS.**

Rachel Grimes Sprague, MD, Joung Woul Kim,



PhD, Asli Ozmen, PhD, Xiaofang Guo, MD, Anthony N. Imudia, MD, Charles J. Lockwood, MD, MHCM, Ronald R. Magness, PhD, Umit A. Kayisli, PhD. Department of Obstetrics and Gynecology, Morsani College of Medicine, University of South Florida, Tampa, FL.

**OBJECTIVE:** Catecholestrogens (CCEs), 2-Hydroxyestradiol (OHE<sub>2</sub>) and 4-OHE<sub>2</sub>, are biologically active metabolites of 17 $\beta$ -estradiol (E<sub>2</sub>). Studies indicate that local increases in E<sub>2</sub> production as well as aberrant expression of E<sub>2</sub> metabolizing enzymes enhance local generation of CCEs in women with endometriosis. CCEs have low binding affinity to estrogen receptors whereas CCEs display high binding affinity to  $\beta$ 2-adrenergic receptor (AR), which induce uterine endothelial cell proliferation during gestation. Our recent data demonstrated  $\beta$ 2-AR expression in eutopic and ectopic endometrial tissue. In addition, binding of CCEs to  $\beta$ -AR enhances human endometrial stromal cell (HESC) viability, suggesting contribution of CCEs to the pathogenesis of endometriosis. Thus, we tested the hypothesis that the mechanism of CCE-enhanced HESC viability involves alterations in either proliferation or apoptosis mediated by  $\beta$ -AR-induced common intracellular signaling pathways, *i.e.* AKT, MAPK and/or NF $\kappa$ B.

**DESIGN:** BrdU, Apoptotic Cell Detection ELISA, q-PCR, Western blot and XTT analyses were performed on cultured HESCs derived from endometrial biopsies.

**MATERIALS AND METHODS:** Cultured HESCs treated with 10<sup>-8</sup> M E<sub>2</sub> or 2-OHE<sub>2</sub> or 4-OHE<sub>2</sub> were measured by BrdU for proliferation and ELISA for apoptosis ( $n=3$  with quadruplicate). Total RNA from cultured HESCs was isolated and pro-apoptotic, anti-apoptotic, and proliferation markers were evaluated by q-PCR ( $n=5$  with duplicate). Total and phosphorylated AKT, p38 and ERK1/2 MAPKs, and NF $\kappa$ B levels were detected in lysates of cultured HESCs ( $n=3$  with triplicate) treated for 10 min with vehicle (control) or 10<sup>-8</sup> M E<sub>2</sub> or 2-OHE<sub>2</sub> or 4-OHE<sub>2</sub>  $\pm$  2 $\times$ 10<sup>-5</sup> M non-specific  $\beta$ -antagonist (propranolol). Subsequently, XTT assays were conducted with p38 MAPK inhibitor to assess the role of p38 MAPK on CCE-induced HESC viability ( $n=4$  with triplicate). Results were analyzed by One-way ANOVA and *post hoc* Tukey test.

**RESULTS:** An increased HESC proliferation index by E<sub>2</sub> and 4-OHE<sub>2</sub> ( $P<0.05$  and  $P<0.05$ , respectively) and decreased apoptosis were detected in HESCs treated with 2-OHE<sub>2</sub> and 4-OHE<sub>2</sub> vs. control ( $P<0.01$  and  $P<0.01$ , respectively). Analysis of apoptotic markers by q-PCR revealed a significant decrease in Bax mRNA expression in response to 2-OHE<sub>2</sub> treatment vs. control ( $P<0.01$ ). Among the several intracellular signaling cascades analyzed, only phosphorylation levels of p38 MAPK were increased by either treatment with 2-OHE<sub>2</sub> or 4-OHE<sub>2</sub> ( $P<0.05$  and  $P<0.05$  vs. control, respectively), but not with E<sub>2</sub>.  $\beta$ -AR antagonism with propranolol mitigated this increased phosphorylation in p38 MAPK levels ( $P<0.05$  and  $P<0.01$ , respectively) and inhibition of p38 MAPK by SB203580 blocked CCE-induced HESC survival ( $P<0.05$  and  $P<0.001$ , respectively).

**CONCLUSIONS:** These data indicate that induction of endometrial stromal cell viability by CCE- $\beta$ -AR interactions results from an imbalance in both proliferation and anti-apoptotic mechanisms and is specifically mediated by the activation of p38 MAPK signaling. These data also suggest that inhibition of  $\beta$ 2-ARs with propranolol may be a novel treatment option in endometriosis.

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#### **ENDOMETRIOSIS INCREASED ATHEROSCLEROSIS IN A MURINE MODEL.**

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**OBJECTIVE:** Epidemiologic studies have identified an association between endometriosis and subsequent development of cardiovascular disease. Here we used an animal model to determine if endometriosis caused atherosclerosis. Further, identifying the molecular mechanisms responsible for atherosclerosis in women with endometriosis is necessary to develop targeted treatment strategies to reduce cardiovascular risk in women with endometriosis. This study aims to determine if endometriosis increases aortic plaque formation in a murine model and explores the conditions that are mechanistically responsible for the observed changes.

**DESIGN:** Experimental endometriosis was induced in mice to identify changes related to atherosclerosis and cardiovascular disease. Oil Red O (ORO) staining, biochemical assays and qRT-PCR were performed to measure the degree of atherosclerotic plaque development, lipid levels, and the differential gene expression of inflammation mediators.

**MATERIALS AND METHODS:** Endometriosis was induced in 9-week-old female ApoE<sup>-/-</sup> C57BL/6 mice by suturing donor uterine tissue to the walls of the peritoneal cavity. A sham control group was also created using no uterine tissue. After 23 weeks post-surgery, mice were euthanized and serum was collected from the blood. Biochemical assays were carried out for lipid profile at the Yale Core Center. Total RNA was extracted from the serum using Trizol reagent and used for qRT-PCR to analyze the gene expression of inflammatory mediators. Whole aortas were dissected and stored in DPBS at 4°C until being subjected to ORO staining. The degree of staining was quantified using ImageJ software. The total area of each longitudinally-opened aorta was measured and the percent of red stain was then calculated using the same red threshold for all samples.

**RESULTS:** The mice in the endometriosis group showed noticeable bilateral atherosclerotic lesions, while no lesions were found in the corresponding location in the control mice. ORO staining of the aorta indicated minimal plaque formation in the control mice and a significant increase in plaque development in the endometriosis group. The difference in average percent stain between the groups was 4.75% indicating that the endometriosis group showed significantly more staining than the control group: control, 3.13 ± 0.95, n=5; endometriosis, 7.89 ± 1.56, n=5, (mean ± SEM; P=0.03, unpaired t test). Biochemical assays from serum showed no significant difference between the control and the endometriosis groups with respect to total cholesterol, HDL, LDL, TG, and glucose levels. However, serum inflammation markers associated with cardiovascular disease such as TNF-α, C-Reactive Protein, and IL-6 were altered by endometriosis in the mouse model.

**CONCLUSIONS:** Endometriosis increased atherosclerotic plaque formation in mice unrelated to blood lipid levels. We expect that these changes to the cardiovascular system in mice with endometriosis occur through inflammatory mechanisms. Targeted treatment of endometriosis and endometriosis-associated inflammation may reduce the risk of cardiovascular disease.

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#### PROSTAGLANDIN E2 ACTIVATES COMPLEMENT PROTEIN CD55 TO ENHANCE CELL ADHESION IN ENDOMETRIOSIS.

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**OBJECTIVE:** Endometriosis is a leading cause of pelvic pain and infertility. Prostaglandin E2 (PGE2) is widely regarded to be central to its pathogenesis.<sup>1</sup> A promising novel target of PGE2 signaling is decay accelerating factor (CD55) which has increased RNA expression in endometriotic stromal cells.<sup>2</sup> CD55 has a well described role in the complement pathway, but emerging evidence indicates other functions in cell proliferation and activation of cellular signaling. The objective of this work was to determine whether CD55 protein is expressed in ectopic endometrium, whether PGE2 is a regulator of CD55 expression, and whether CD55 is necessary and sufficient for endometriosis pathogenesis via a non-canonical pathway.

**DESIGN:** Laboratory investigation.

**MATERIALS AND METHODS:** To study the principal cellular compartments of endometriotic lesions *in vitro*, immortalized endometrial stromal (T-HESC) and endometriotic epithelial (12Z) cell lines were studied. Immunohistochemistry was performed to localize the presence of CD55 in a random sample of eight human pathology specimens of eutopic and ectopic endometrium. CD55 expression analysis was performed on cell lines (RNA expression by quantitative real-time PCR; protein expression by immunoblotting and flow cytometry) treated first with 0, 1, 3, 10, 30, and 100 uM of PGE2 then with 10uM PGE2 for 0, 1, 2, 6, and 24 hours. Overexpression of CD55 in 12Z cells was achieved using lentiviral transduction with an empty vector control. Cell adhesion was determined by plating cells on cell culture plates coated with fibronectin, collagen I, or bovine serum albumin at different time points, washing the plates, then quantifying remaining adherent cells using a luminescent ATP detection assay. Intra- and inter-experimental variation was addressed by multiple wells per condition and ≥ 2 replicates per experiment.

**RESULTS:** Both 12Z and T-HESC lines demonstrated CD55 expression at baseline, as did histologic specimens of eutopic and ectopic endometrium. Treatment with PGE2 of 12Z and T-HESC lines resulted in increased CD55 expression in a dose dependent fashion until maximal treatment effect (3-fold) was achieved at 10 uM. In time course experiments, CD55 RNA expression was maximal at 6 hours and protein expression at 18 hours. 12Z cells overexpressing CD55 demonstrated more than 2-fold number of adherent cells as compared to wildtype and empty vector controls.

**CONCLUSIONS:** These preliminary cellular studies indicate that PGE2 induction of CD55 can lead to promotion of adhesion of endometriotic cells, a necessary step in the pathogenesis of endometriosis. Future *in vivo* studies using CD55<sup>-/-</sup> mice will investigate CD55 function in adhesion in mouse models of endometriosis. Understanding this downstream pathway of PGE2 signaling may identify points of fragility for future translational therapies.

References: 1. Wu et al. Prostaglandin E2: the master of endometriosis. *Exp Biol Med* (Maywood). 2010 Jun;235(6):668-77.

2. Rekker et al. High-throughput mRNA sequencing of stromal cells from endometriomas and endometrium. 2017 Jul;154(1):93-100.

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#### α1D-ADRENERGIC RECEPTOR EXPRESSION IN HUMAN ENDOMETRIAL STROMAL CELLS CONTRIBUTES TO CATECHOLESTRADIOL-INDUCED CELL SURVIVAL: IMPLICATIONS FOR ENDOMETRIOSIS.



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**OBJECTIVE:** Catecholestrogens (CCEs), 2-Hydroxyestradiol (OHE<sub>2</sub>) and 4-OHE<sub>2</sub>, are biologically active metabolites of 17β-estradiol (E<sub>2</sub>). Local increases in E<sub>2</sub> production as well as aberrant expression of E<sub>2</sub> metabolizing enzymes enhance generation of CCEs in women with endometriosis. During gestation, CCEs bind adrenergic receptors (ARs) to mediate uterine endothelial cell proliferation. Our recent data demonstrated that compared to the basalis layer of the endometrium, the functionalis layer displayed increased β2-AR expression in women with endometriosis. Moreover, binding of CCEs to β-ARs enhanced human endometrial stromal cell (HESC) viability in culture, suggesting a modulatory role for CCE-β-AR binding in retrograde menstruation that contributes to endometriosis development. It is unknown if, in addition to β-ARs, α-ARs also have a role in this process. We, therefore, tested the hypothesis that ectopic endometrial tissue expresses α1D-AR and that 2-OHE<sub>2</sub> and 4-OHE<sub>2</sub> will potentiate HESC viability via α-ARs.

**DESIGN:** Immunohistochemistry was performed on paired eutopic/ectopic endometriotic tissue and XTT analysis was conducted on cultured CCE-treated HESCs derived from endometrial biopsies.

**MATERIALS AND METHODS:** Paired eutopic/ectopic endometrial sections from women with endometriosis in the proliferative (n=5) or secretory (n=4) phases were immunostained using α1D-AR antibody and evaluated semi-quantitatively by HSCORE. Confluent HESCs derived from endometrial biopsies at time of surgery for benign reasons were cultured in 96-well plates (5x10<sup>3</sup> cells/well) and treated with vehicle (control) or with 10<sup>-8</sup> M E<sub>2</sub> or 2-OHE<sub>2</sub> or 4-OHE<sub>2</sub> ± nonspecific α-AR inhibitor (phentolamine) for 48h. XTT assays measured cell viability. Experiments (n=4) were performed in duplicate and results were analyzed by One-way ANOVA and *post hoc* Tukey test.

**RESULTS:** Immunohistochemistry analysis revealed overall weak to moderate α1D-AR staining in endometrial epithelial cells and weak staining in endometrial stromal cells with no significant difference between eutopic and ectopic endometrial tissues in either phase. Compared to the basalis layer, both stromal and epithelial cells in the functionalis layer of eutopic endometrium displayed stronger α1D-AR staining. *In vitro* XTT analyses revealed phentolamine partially inhibited (P<0.05 and P<0.001, respectively) 2-OHE<sub>2</sub> and 4-OHE<sub>2</sub>-enhanced HESC survival versus control (P<0.05 and P<0.05, respectively). This inhibitory effect of phentolamine was specific to CCEs since E<sub>2</sub>-enhanced HESC survival was not inhibited by phentolamine.

**CONCLUSIONS:** Immunostaining with α1D-AR showed similar expression patterns as β2-AR in that there was increased staining to the functionalis layer of the endometrium; albeit, α1D-AR staining was overall weaker in both ectopic and eutopic tissue. Our *in vivo* and *in vitro* results indicate that induction of HESC viability by CCE-α-AR binding may contribute to the propagation of endometriosis. Future studies into different α-AR isoform expressions in endometriotic lesions are warranted.

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#### NFκB2 EXPRESSION IN ENDOMETRIOSIS IS REGULATED BY miRNA Let-7b.

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**OBJECTIVE:** Endometriosis is a debilitating gynecologic disease characterized by aberrant inflammation. We have previously demonstrated differential expression of several microRNAs (miRNAs) in endometriosis, and dysregulated expression of several inflammatory cytokines. Altered miRNA expression may modulate the inflammatory response, ultimately increasing severity of disease. Nuclear factor-kappaB (NFkB) is a transcription factor involved in the immune response. It is activated initially by inflammatory cytokines and chronically activated in endometriosis, thus capable of promoting a chronic inflammatory state and altered progesterone response. We hypothesize that miRNAs (Let-7b, 3613-5p, and 125b) alter expression of *NFkB1*, *NFkB2*, and progesterone receptor gene (*PgR*) in women with endometriosis.

**DESIGN:** *in vitro* human primary cell culture.

**MATERIALS AND METHODS:** Primary eutopic endometrial cells from 6 subjects were cultured in six-well plates (1x10<sup>5</sup> cells). Once cells reached 70% confluence they were transfected with miRNA Let-7b, 3613-5p, or 125b miRNA mimic or each respective miRNA inhibitor. Each transfection was carried out with respective controls and in duplicate. Total RNA was extracted 48 hours post-transfection. Quantitative RT-PCR was performed for genes of interest (*NFkB1*, *NFkB2* and *PgR*). Relative expression was calculated using the 2<sup>-ΔΔC<sub>T</sub></sup> method. Student's t-test was used for statistical analysis.

**RESULTS:** Cells transfected with miRNA Let-7b Mimic demonstrated a 2.25-fold decrease in *NFkB2* expression (p=0.04); there was no significant change in *NFkB2* expression in cells treated with Let-7b inhibitor. There was no difference in *NFkB2* expression in cells transfected with miRNAs 3613-5p or 125-5p mimic or inhibitor. There was no significant effect on expression of *NFkB1* or *PgR* when cells were transfected with miRNAs Let-7b, 3613-5p, or 125-5p (mimic or inhibitor).

**CONCLUSIONS:** Endometriosis sequela are in large part due to the inflammatory nature of the disease. As *NFkB2* is a key mediator in the inflammatory response, aberrant miRNA levels can mediate inflammation and aggressiveness of disease through regulation of *NFkB2* expression. Here we demonstrate that low Let-7b levels that we have previously reported in endometriosis lead to increased *NFkB2* expression and increased inflammation. Let-7b regulation in endometriosis is a key endogenous regulator of inflammation. MiRNA Let-7b and *NFkB2* represent novel, non-hormonal targets for treating endometriosis.

R01 HD076422

SUPPORT: NIH U54 HD052668.

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**A REPLICATION STUDY OF PLASMA MICRORNA EXPRESSION IN ADOLESCENTS WITH ENDOMETRIOSIS.**

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Boston, MA; <sup>c</sup>Michigan State University College of Human Medicine, Grand Rapids, MI; <sup>d</sup>Michigan State Univ and Harvard T.H. Chan SPH and Boston Ctr for Endometriosis, Grand Rapids, MI.

**OBJECTIVE:** Micro-ribonucleic acids (miRNAs), stable noncoding RNAs involved in gene regulation, have diagnostic potential. Our purpose was to validate 59 plasma miRNAs identified within a discovery study of adolescents with endometriosis vs. controls.

**DESIGN:** Nested case-control study.

**MATERIALS AND METHODS:** Adolescents (<26 y) with surgically-visualized endometriosis (n=54, 96% rASRM Stage I-II) and controls (n=10, no endometriosis history), matched on age (±2 yrs, mean 21 y) and hormone use (y/n), were included. WERF EPHect protocols were used. miRNAs included 48 from a discovery study of plasma of adolescent cases vs. controls (n=10 each, via qt-PCR) plus 11 previously published miRNAs. For this replication study, plasma miRNAs were quantified on a bead-based platform (Firefly™, Abcam). Wilcoxon signed-rank tests compared miRNA expression between cases/controls. Stepwise logistic regression models were built using miRNAs with p-values <0.10 (p=0.20 for entry and stay).

**RESULTS:** Overall, 9 miRNAs were chosen: 4 among hormone users, 2 among non-users, and 4 in all participants. AUCs were similar for the three models: 0.74, 0.77 and 0.74, respectively (Table). In models restricted to pathology-confirmed cases (26 hormone users, 7 non-users) AUCs improved slightly (0.75, 0.86, 0.83). These miRNAs are associated with cell adhesion and proliferation, tumor metastasis, and embryonic neuro- and myogenesis.

**CONCLUSIONS:** Several miRNAs were significantly dysregulated in plasma from adolescents with rASRM stage I/II endometriosis vs. controls, and together suggest fair to good (AUC 0.74-0.86) discrimination. The small number of miRNAs that replicated among the groups reinforces the need for multi-phased discovery and targeted replication. Hormonal medications appear to impact miRNA expression differently in cases v. controls. Future studies of miRNA variation by stage, hormonal and non-hormonal treatments and among disease phenotypes are needed.

SUPPORT: Marriott Daughters Foundation support of the Boston Center for Endometriosis.

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**INCREASED EXPRESSION OF YAP (YES-ASSOCIATED PROTEIN) IS ASSOCIATED WITH THE DECREASED CELL AUTOPHAGY IN THE EUTOPIC ENDOMETRIAL STROMAL CELLS OF WOMEN WITH ENDOMETRIOSIS.**



Wei Huang, Ph.D, M.D.,<sup>a</sup> Tianjiao Pei, M.D.,<sup>a</sup> Xin Huang, M. M. Candidate,<sup>b</sup> Yujing Li, M.D.<sup>c</sup> <sup>a</sup>West China Second University Hospital of Sichuan University, Chengdu, China; <sup>b</sup>Department of Obstetrics and Gynecology, West China Second University Hospital of Sichuan University, Chengdu, China; <sup>c</sup>Affiliation not provided.

**OBJECTIVE:** To explore the role of Yes-associated protein (YAP) in the regulation of cell autophagy in the eutopic endometrial stromal cells (ESCs) from a subset of women with endometriosis.

		Validation Study					
		Hormone users		Not on Hormones		All (adjusted for hormone use)	
		(42 cases, 84 controls)		(12 cases, 24 controls)		(54 cases, 108 controls)	
		OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
miRNAs selected	Discovery Study	AUC = 0.74		AUC = 0.77		AUC = 0.74	
slnhsa_mir_376b_3p	Hormone Users	0.38 (0.18, 0.81)	0.01				
slnhsa_mir_1908_5p	Adult Literature	0.26 (0.08, 0.86)	0.03			0.41 (0.13, 1.25)	0.12
slnhsa_mir_542_3p	Hormone Users	0.67 (0.37, 1.19)	0.17				
slnhsa_let_7g_3p	Adult Literature	2.09 (1.01, 4.34)	0.05				
slnhsa_mir_296_3p	Not on Hormones					1.72 (1.08, 2.75)	0.02
slnhsa_mir_154_5p	Hormone Users			15.8 (1.10, 227.6)	0.04		
slnhsa_mir_219a_5p	Both Horm Users			2.65 (0.76, 9.20)	0.12		
slnhsa_mir_124_3p	Hormone Users					1.55 (0.84, 2.83)	0.16
slnhsa_mir_23b_5p	Hormone Users					0.61 (0.35, 1.07)	0.08

**DESIGN:** Experimental study using primary cell culture, quantitative real-time PCR (qRT-PCR), Western blotting, drug interference, and transfection in isolated ESCs.

**MATERIALS AND METHODS:** Endometrial samples were collected during hysteroscopy, including eight patients diagnosed with endometriosis by laparoscopy and six women laparoscopically diagnosed endometriosis-free as controls. The expressions of YAP pathway and cell autophagy markers (mTOR, LC-3) in ESCs of women with or without endometriosis were validated by qRT-PCR and Western blotting. The protein levels of autophagy markers were detected in the eutopic ESCs after verteporfin and rapamycin treatments and the transfection with YAP-knockdown vector in ESCs, respectively. Student's t test was used for comparisons between two groups after assessing the normality and homogeneity of variance.

**RESULTS:** The mRNA levels of YAP, TEAD, mTOR were all increased in the eutopic ESCs of women with endometriosis compared with controls, but no statistically difference ( $P > 0.05$ ). The protein levels of YAP ( $P < 0.05$ ) and mTOR ( $P < 0.05$ ) were significantly increased in the eutopic ESCs of women with endometriosis compared with controls, whereas the ratio of the autophagy marker protein LC3-II/LC3-I ( $P < 0.05$ ) was significantly decreased in the eutopic ESCs of women with endometriosis compared with controls. Moreover, verteporfin treatment interfered the YAP function and led to an increase trend of cell autophagy level, but it had no effect on mTOR expression; rapamycin treatment and YAP knockdown in the eutopic ESCs both inhibited the expression of YAP and increased the level of cell autophagy significantly with an increased ratio of LC3-II/LC3-I ( $P < 0.05$ ).

**CONCLUSIONS:** Our study demonstrates that the decreased cell autophagy level is associated with the increased expression of YAP and YAP may participate in the mTOR-autophagy pathway in the eutopic ESCs of endometriosis.

**SUPPORT:** This research was supported by the Grant from the Science and Technology Bureau of Sichuan (2018SZ0124) (to Wei Huang).

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**CD4 AND CD8 BUT NOT DN MUCOSA-ASSOCIATED INVARIANT T CELLS FOSTER THE DEVELOPMENT OF ENDOMETRIOSIS: A PILOT STUDY.** Huanhuan Jiang, Doctor, Kaihuan Bi, Bachelor's, Zhimin Lu, Bachelor's, Caihua Li, Doctor, Peipei Guo, Graduate, Yunxia Cao, Doctor, The First Affiliated Hospital of Anhui Medical University, Hefei, China.



**OBJECTIVE:** Our study aims to demonstrate the relationship between Mucosa-associated invariant T (MAIT) cells and endometriosis.

**DESIGN:** Case-control study.

**MATERIALS AND METHODS:** The study group comprised 32 patients with a diagnosis of endometriosis. 18 women with only ovarian benign cysts or uterine leiomyoma who underwent laparoscopy were recruited as control group. Peritoneal fluid (PF) was collected during laparoscopy. Peripheral blood (PB) was obtained shortly before the surgery. We investigated MAIT cells and their different subpopulations in PB and PF from endometriosis (EMS) and control group (CG). MAIT cells were characterized as CD3<sup>+</sup>CD161<sup>+</sup>Vα7.2<sup>+</sup> cells by flow cytometry. Next based on CD4 and CD8, the cells were divided into three subsets: CD8 MAIT cells, CD4/CD8<sup>-/-</sup> (double negative, DN) MAIT cells and CD4 MAIT cells. And IL-8, 12, 18, 17, MMP-9, INF-g from the peritoneal fluid and plasma were analyzed by ELISA kit.

**RESULTS:** Our results revealed that there were enrichments of MAIT cells, especially CD4 and CD8 MAIT subset. Moreover, CD8 MAIT cells had a greater activation in EMS group as compared to the results from CG patients. In line with this data, EMS patients produced higher level of IL-8/12/17 as compared to these from controls. On the contrary, control patients exhibited a dramatic upregulation of DN MAIT cells, however, these DN MAIT cells from controls showed a higher expression of PD-1. At the end we performed the relevance analysis, and we discovered that the accumulation of PB MAIT cells correlated with elevated level of serum CA125 production.

**CONCLUSIONS:** Our study suggests functional diversities of MAIT cells subsets in the development of endometriosis. CD4 and CD8 MAIT cells could be a promoter in endometriosis, whereas DN MAIT cells might be a protector for the host.

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**ESTROGEN PROMOTES THE CHEMOTACTIC MIGRATION AND DIFFERENTIATION OF BONE MARROW MESENCHYMAL STEM CELLS IN ENDOMETRIOSIS.** Wenbi Zhang, doctor,<sup>a</sup> Lu Li, profes-



sor,<sup>b</sup> Xiaoxi Sun, professor<sup>c</sup> <sup>a</sup>Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China; <sup>b</sup>Affiliation not provided; <sup>c</sup>Obs/Gyn hospital of fudan university, shanghai, China.

**OBJECTIVE:** To confirm the theory of endometriosis (EMT) stem cell origin and to investigate the role of estrogen on the process of bone marrow mesenchymal stem cell (BMSC) chemotactic migration and differentiation.

**DESIGN:** To illustrate this hypothesis, we employed 17β estradiol for the co-culture of BMSC and endometrial stromal cells (ESC) *in vitro* and established EMT mice model *in vivo*.

**MATERIALS AND METHODS:** Primary cultured BMSCs and ESCs were identified by flow cytometry and immunohistochemical analysis. BMSCs and ESCs were co-cultured and divided into four groups: BMSC group, BMSC+17β estradiol treatment group, BMSC+ESC group, BMSC+ESC+17β estradiol treatment group. After 5 days of culture, the chemotaxis of 17β-estradiol was observed through Transwell experiments, and the chip technique was used to analyze the expression of chemokines in the culture medium. Mouse EMT model was established and HE staining was performed *in vivo*. BMSCs were injected through the tail vein into the EMT mice. The mice were divided into two groups according to BMSC 17β-estradiol pretreated or not. The immunofluorescence was used to detect the expression of protein B-cell lymphoma-2 (BCL-2), proliferating Cell Nuclear Antigen (PCNA), Matrix metalloproteinase (MMP-1) in ESC after one month.

**RESULTS:** We showed that the migration of BMSC promoted by ESC, and the migration ability of BMSC was enhanced after the treatment of 17β-estradiol. Through gene chip detection, 17β-estradiol may accelerate the secretion of chemokines by ESC, which can promote the expression of 25 chemokines, especially for stromal cell derived factor-1a as the main target. Furthermore, the immunofluorescence results in animal experiments showed that the expression of BCL-2, PCNA, MMP-1 in 17β-estradiol pretreated group was higher than control group. It confirmed that 17β-estradiol might promote the differentiation, proliferation and apoptosis of ESC in ectopic lesions through the migration, differentiation and proliferation of BMSCs, thereby increasing the degree of lesions in ectopic lesions.

**CONCLUSIONS:** Estrogen promoted the chemotactic migration of BMSC to a appropriate microenvironment and differentiation into endometrial cells forming endometriosis.

**SUPPORT:** the National Natural Science Foundation of China (Grant number: 81501234).

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**ENDOMETRIOSIS RISK ALLELE IN WNT4 MAY INTERACT WITH RARE MUTATIONS IN HDAC2 GENE.** Kenneth Ward, MD, Rakesh Chettier, MS, Hans M. Albertsen, PhD. Juneau Biosciences, LLC, Salt Lake City, UT.



**OBJECTIVE:** To discover genes that may interact with the endometriosis risk allele in the WNT Family Member 4 (*WNT4*) gene.

**DESIGN:** Endometriosis is a common gynecological condition with complex etiology defined by the presence of endometrial glands and stroma in ectopic locations outside of the uterus. Twin and family studies have shown increased relative risk in families. Multiple genome-wide association studies (GWAS) show that several polymorphisms in the region harboring *WNT4* and Cell Division Cycle 42 (*CDC42*) are associated with endometriosis across multiple ethnicities. In this study, we explored whole exome sequencing (WES) data in women carrying the risk allele T (rs2235529) in the *WNT4* gene to see if the risk allele interacts with rare protein altering variants in other genes.

**MATERIALS AND METHODS:** WES was conducted on 1731 women with a confirmed diagnosis of endometriosis and 774 population controls of Northern European Ancestry. Whole exome sequencing (WES) was performed using Ion Proton Instrument with the AmpliSeq Exome Capture Kit. All missense and truncating mutations including stop gain, stop loss, splicing, and frameshifts were considered for downstream analysis. Population frequency of these variants are provided if present in the gnomAD database (n=65,000).

**RESULTS:** The risk allele T in *WNT4* (rs2235529) is present in either homozygous or heterozygous form in 787 subjects (554 endometriosis cases and 233 controls). Eight endometriosis patients and none of the controls had histone deacetylase 2 (*HDAC2*) protein altering mutations identified. The T risk allele was associated with *HDAC2* altering mutation burden [ $p=1.7E-03$ , OR=15.4 (95% confidence limits 1.9-125.5)].

**CONCLUSIONS:** In this study, we found that women with mutation in *HDAC2* gene in the background of *WNT4* risk allele T are more likely to

be susceptible to endometriosis. It has been reported that the levels of *HDAC1* and *HDAC2* are deregulated in endometriotic stromal cells. *HDAC1* and *HDAC2* are key regulators of *WNT* and *p53* pathways. During nucleosome remodeling, the deacetylase complex physically interacts with the *WNT4* chromatin in an *HDAC*-dependent manner, leading to suppression of the *WNT4* gene and *WNT4* dependent morphogenesis. Analyses of the ten other human *HDAC* genes are underway.

SUPPORT: Juneau Biosciences, LLC.

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WITHDRAWN

## ENDOMETRIUM

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### CELL-SPECIFIC EFFECTS OF CLOMID AND E2 ON ENDOMETRIUM: INSIGHTS INTO WNT SIGNALING AND STROMAL-EPITHELIAL INTERACTIONS.

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**OBJECTIVE:** Hormonal effects on epithelial cells of endometrium are often mediated through stromal cell receptors. Endometrium from women undergoing IVF using minimal stimulation (MS-IVF) with clomiphene citrate (CC) is characterized by marked atrophy of endometrial glands accompanied by relative increases in stromal cells, despite supraphysiologic levels of E2. Previously, we discovered dramatic stromal cell-specific upregulation of Wnt antagonists (secreted frizzled related proteins 1,4, SFRP) in endometrium from MS-IVF. Although sFRPs inhibit Wnt7a signaling during endometrial decidualization and gland formation in endometrial cancer, physiologic regulation of Wnt signaling in endometrium is not well understood. Our objective was to test the hypothesis that sFRPs are secreted constitutively by stroma cells but regulated in vivo by secretions from endometrial epithelial cells.

**DESIGN:** Epithelial cells (Epi, Ishikawa) and primary human endometrial stromal cells were used alone or in co-culture with transwell cell culture inserts. Stromal cells were pretreated for 48 h in serum free media prior to treatment with vehicle or estradiol (E2, 3.6 nM) ± CC (20nM).

**MATERIALS AND METHODS:** Gene expression was quantified by qPCR and normalized to two housekeeping genes, *GAPDH* and *h36B4*. Endometrial tissue explants from spontaneously ovulating women (24 h after LH surge, n = 4) were studied. ANOVA and Student's T test were used for statistical analyses as appropriate.

**RESULTS:** E2 treatment of stromal cells increased *PR-B* (from 1 ± 0.1 to 6.7 ± 0.4 RU, p < 0.01) and total *PR* (from 1.0 ± 0.11 to 5.4 ± 0.23 RU, p < 0.01). CC was not an ER antagonist in stromal cells also increasing *PR* gene expression. In contrast to in vivo results, CC did not alter expression of *sFRP1* or *4* in stromal cell cultures. In co-culture, Epi did not alter E2-induced upregulation of *PRs* in stroma. However, co-culture with Epi downregulated stromal cell *sFRP4* (83 ± 3%). The magnitude of *sFRP4* suppression was dose-dependent with increasing number of Epi cells (from 10<sup>4</sup>–10<sup>5</sup>/cm<sup>2</sup>). Epi co-culture also decreased stromal *sFRP1* to 34 ± 11% and expression of stromal growth factors (*FGF-9* and *TGF-α*) significantly. To investigate physiologic relevance, treatment of tissue explants from ovulatory women with E2 for 72 h (to induce epi growth) resulted in suppression of *sFRP4* (from 1.44 ± 0.3 to 0.55 ± 0.08 mRNA, p < 0.02).

**CONCLUSIONS:** Although CC did not have direct effects on *sFRP* in stroma, secretions from glandular epithelial cells suppressed Wnt antagonists *sFRP1* and *4* in stroma. These results support the hypothesis that CC-induced inhibition of epithelial cell growth results in immature, atrophic glands that are insufficient to suppress Wnt inhibitors in stroma thereby accentuating loss of epithelial differentiation and growth. In the absence of CC, E2 induces epithelial cell growth and suppression of stromal sFRPs culminating in full maturation of the endometrium. Although it is believed that CC exhibits its effects simply as an ER antagonist, these studies indicate that the effects of CC are more complex and involve inhibition of Wnt signaling in cell-specific compartments.

Reference: Carmon, K. S., and D. S. Loose. "Secreted Frizzled-Related Protein 4 Regulates Two Wnt7a Signaling Pathways and Inhibits Proliferation in Endometrial Cancer Cells." *A Molecular Cancer Research*, vol. 6, no. 6, Jan. 2008, pp. 1017–1028., <https://doi.org/10.1158/1541-7786.mcr-08-0039>.

SUPPORT: This work was supported by the tissue core laboratory of NIH HD087150.

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### DOES UNIVERSAL SCREENING FOR CHRONIC ENDOMETRITIS IMPROVE PREGNANCY RATES?

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**OBJECTIVE:** A growing body of evidence suggests a link between chronic endometritis (CE) and infertility. We investigated whether the implementation of universal screening for CE at the time of oocyte retrieval was correlated with a change in pregnancy rates after initial single thawed euploid embryo transfer (STEET).

**DESIGN:** Retrospective cohort analysis at a high volume private fertility center.

**MATERIALS AND METHODS:** The analysis included the initial STEET of all patients undergoing autologous IVF/PGT-A with endometrial biopsy screening (EMB) on day of oocyte retrieval from January 2017 to December 2018 and historic controls without universal EMB from September 2015 to August 2016. Cycles performed in the six-month window surrounding the policy onboarding were excluded. The pathologic diagnosis of CE was established via CD 138 staining of endometrial specimens. Patients found to have CE after biopsy were treated with doxycycline and metronidazole and re-biopsied. If persistent CE was demonstrated, a protocol designated second and third-line treatment algorithms prior to embryo transfer. Patients using a gestational carrier, oocyte or embryo donation, and those who had previous embryo transfer were excluded from the analysis. The CE rate, implantation rates (IR) and ongoing pregnancy rates (OPR) were calculated. Statistical analysis was done with T test, Mann-Whitney U, Chi squared tests, and logistic regression where appropriate.

**RESULTS:** A total of 375 initial STEETs were analyzed. The average age of the EMB screened and non-screened population differed (35.7 ± 3.9 vs 36.7 ± 4.1, p=0.0157). In analyses controlled for age, there was no difference in IR (AOR 1.12, 95% CI 0.77-1.80) or OPR (AOR 1.06, 95% CI 0.69 - 1.63) between screened and non-screened cohorts. The rate of CE found on day of oocyte retrieval was 14.0% (n=31). The median number of CD138 cells per 10 high power fields was 11 (IQR 8-15) in the CE positive group and 0 (IQR 0-1) in the CE negative group (p < 0.0001). No difference was seen in the IP (76.3% vs 67.7%, p=0.31) or OPR (60.5% vs 58.1%, p=0.80) between the treated, CE positive and CE negative groups in their first embryo transfer. CE cure rate was 81% (n=21). Nineteen percent of CE positive patients (n=6) were not cured after 3 cycles of antibiotics. The non-cured group had an 83% OPR.

**CONCLUSIONS:** A universal screening strategy detected a lower baseline CE rate than has been previously reported. No association was demonstrated between universal CE screening and IR or OPR. A larger sample size is needed to confirm these findings.

P-560 Wednesday, October 16, 2019 6:30 AM

### GATA BINDING PROTEIN 2 EXPRESSION AT IMPLANTATION WINDOW DIMINISHES IN WOMEN WITH ADENOMYOSIS: IMPLICATIONS FOR IMPAIRED ENDOMETRIAL RECEPTIVITY.

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**OBJECTIVE:** Adenomyosis in reproductive age women has negative impacts on embryo implantation. Impaired progesterone (P4) responsiveness causes female infertility associated with endometrial receptivity. Mice deficient in the transcription factor, GATA binding protein 2 (GATA2), are infertile due to embryo implantation failures associated with defective decidualization and endometrial receptivity. Uterine tissues of GATA2 deficient mice display decreased progesterone receptor (PR) levels and diminished P4 responsiveness. We hypothesize that reduced endometrial GATA2 expression during the window of implantation may contribute to implantation failure seen in women with adenomyosis.

**DESIGN:** Thus, we evaluated the endometrial expression of GATA2 in patients with adenomyosis.

**MATERIALS AND METHODS:** Uterine specimens was obtained during the window of implantation (cycle days 18-23) from patients with adenomyosis (n=5, age <45 years) who underwent hysterectomy and from age-matched controls who had no endometriosis or adenomyosis (n=5). The GATA2 expressions were detected by immunohistochemistry, quantified by a histologic scoring system (HSCORE) and statistically compared using a *t*-test.

**RESULTS:** During the window of implantation, both endometrial and myometrial cells displayed predominantly nuclear immunoreactivity for GATA2 expression. Compared to those in the control group, patients with adenomyosis showed significantly reduced GATA2 expression in endometrial luminal epithelium (Mean  $\pm$  SEM 103.9  $\pm$  15.5 vs. 55.4  $\pm$  14.1, *p* = 0.049), stromal cells (128.6  $\pm$  20.7 vs. 53.3  $\pm$  10.6; *p* = 0.012) and myometrial cells (133.7  $\pm$  12.3 vs. 72.6  $\pm$  15.9; *p* = 0.016). On the other hand, glandular epithelial cells were weakly GATA2 immunoreactive with no difference between women with or without adenomyosis (85.0  $\pm$  9.9 vs. 55.6  $\pm$  26.3; *p* = 0.325).

**CONCLUSIONS:** The significant reduction in human uterine GATA2 expression may impair endometrial receptivity by diminishing P4 responsiveness in patients with adenomyosis and, thereby, contributing to possible mechanism(s) behind the reduced embryo implantation reported in women with adenomyosis.

**P-561** Wednesday, October 16, 2019 6:30 AM

#### DEFINING CHRONIC ENDOMETRITIS: ARE PLASMA CELLS SUFFICIENT?

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**OBJECTIVE:** There is considerable variability in the diagnostic criteria used for chronic endometritis: including number of plasma cells, immunohistochemistry and inclusion of stromal changes. The objective of this study was to compare the prevalence of chronic endometritis in women with unexplained recurrent pregnancy loss (RPL) using different diagnostic criteria.

**DESIGN:** Cohort Study.

**MATERIALS AND METHODS:** IRB approval was obtained. The cohort included women with two or more pregnancy losses, endometrial biopsy (EMB) between 1/2016 and 12/2018, TSH values < 4 mU/L, negative antiphospholipid antibodies and normal uterine anatomy. H&E and CD138 immunohistochemical staining were performed. A single pathologist blinded to patient history recorded the number of plasma cells per 10 HPF and the presence or absence of endometrial stromal changes (spindling, edema, foci of breakdown, inflammatory cells, and pigment deposition).

**RESULTS:** 50 women were included, with a mean age of 35.2 (SD 4.1) years, BMI of 27.1 (SD 6.3) kg/m<sup>2</sup> and 3.1 (SD 0.9) prior pregnancy losses. The preceding pregnancy was a mean of 117 (SD 146) days prior to EMB and had a gestational age of 6 weeks (SD 3.2). When chronic endometritis was defined by the presence of plasma cells on H&E alone, 24% (12/50) of the cohort met criteria if  $\geq$  1 plasma cell was used, 16% (8/50) with  $\geq$  2 plasma cells and 4% (2/50) with  $\geq$  5 plasma cells. When the presence of both endometrial stromal changes and plasma cells were required, the prevalence decreased to 14% (7/50) with  $\geq$  1 plasma cells, 12% (6/50) with  $\geq$  2 plasma cells and 4% (2/50) with  $\geq$  5 plasma cells. When CD 138 staining was used to identify plasma cells, the prevalence of chronic endometritis increased to 56% (28/50) with  $\geq$  1 plasma cell, 44% (22/

#### Defining Chronic Endometritis (n=50)

	Stromal Changes Not Required	Stromal Changes Required
H&E: 1 or more plasma cells/10 HPF	24% (12/50)	14% (7/50)
H&E: 2 or more plasma cells/10 HPF	16% (8/50)	12% (6/50)
H&E: 5 or more plasma cells/10 HPF	4% (2/50)	4% (2/50)
CD 138: 1 or more plasma cells/10 HPF	56% (28/50)	30% (15/50)
CD 138: 2 or more plasma cells/10 HPF	44% (22/50)	28% (14/50)
CD 138: 5 or more plasma cells/10 HPF	26% (13/50)	16% (8/50)

50) with  $\geq$  2 plasma cells and 26% (13/50) with  $\geq$  5 plasma cells. When stromal changes and plasma cells by CD138 were required, the prevalence was 30% (15/50) with  $\geq$  1 plasma cells, 28% (14/50) with  $\geq$  2 plasma cells and 16% (8/50) with  $\geq$  5 plasma cells.

**CONCLUSIONS:** Establishing specific diagnostic criteria for chronic endometritis is necessary for both research and evidence based treatment guidelines. The definition of chronic endometritis significantly alters its prevalence. Recruitment of a control cohort is currently ongoing to establish the most appropriate diagnostic criteria for chronic endometritis.

**SUPPORT:** Friends of Prentice Grant.

**P-562** Wednesday, October 16, 2019 6:30 AM

#### IMPROVEMENT OF ENDOMETRIAL RECEPTIVITY THROUGH THE USE OF AUTOLOGOUS PLATELET-DERIVED MICROPARTICLES.

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**OBJECTIVE:**

The goal of the present trial is to evaluate the effectiveness of the application of platelet-rich plasma (PRP) derived from the patient's autologous plasma in the basal layer of the endometrium for the treatment of patients with suboptimal endometrium.

**DESIGN:** This is a single arm trial carried out at the Fertility Institute of Madrid. The trial included eighteen patients which presented a suboptimal endometrium, refractory to estrogen therapy between June 2014 and November 2017.

**MATERIALS AND METHODS:** The trial included thirteen patients that presented a suboptimal endometrium (<7 mm) and five presented implantation failures (in more than three embryo transfers with high quality embryos).

Blood was extracted the same day of the application. The blood was centrifuged at 600G for ten minutes to separate red blood cells from plasma. The

Patient	Age	IVF/OVO	Endometrium before/after	Pregnancy
1	41	OVO	7/8,3mm	yes
2	36	OVO	6/7,5mm	yes
3	45	OVO	7/8,1mm	yes
4	50	OVO	8/10,6mm	yes
5	35	FIV	6,2/8mm	yes (miscarriage)
6	48	OVO	5/6mm	No
7	42	FIV	5/7,2mm	No
8	43	OVO	7/8,3mm	yes
9	39	OVO	6,6/7,6mm	yes (biochemical pregnancy)
10	41	OVO	6/7,1mm	No
11	41	OVO	7,2/8mm	No
12	46	OVO	8/10mm	yes
13	35	FIV	6,8/8,2mm	No
14	46	OVO	6,6/7mm	No
15	48	OVO	10/12mm	yes
16	38	FIV	10/14mm	No
17	38	OVO	6,4/7,6mm	No
18	40	OVO	6,6/7,2mm	yes

supernatant was separated into three fractions, from low to rich factor concentration. Calcium Chloride were added in order to activate the platelets. A hysteroscopy was carried out using saline solution as a distension medium and a puncture needle of 17G and 300mm was introduced through the hysteroscope work guide during menstrual period. Several subendometrial injections were administered using the needle, in the uterine lining, until the entire plasma (15 ml) was injected. After the hysteroscopy, the patients started the replacement treatment with oestradiol valerate with a dosage of 6mg per day. Once the endometrium had a thickness of more than 7mm, 200 mg of progesterone was applied 3 times a day, and the embryo transfer was scheduled.

**RESULTS:** The thickness of the endometrium increased in all 18 patients. All except for one achieved the minimum 7mm required for the transfer. On average, the endometrium thickness increased by 1,52mm. Ten patients showed positive BHCG thirteen days after the transfer (55%). Two of these ten patients miscarried on the first trimester (20%). The rest of the pregnancies concluded with the birth of a healthy child. No adverse effects have been reported.

**CONCLUSIONS:** PRP can stimulate the proliferation and regeneration of tissues with a great amount of growth factors and cytokines. This is the first study where PRP are applied via a hysteroscopy and subendometrial injections instead of through intrauterine perfusions with a canula. It is suggested that PRP increase the chances of pregnancy in patients with suboptimal endometrium and recurrent implantation failures. It is necessary to carry out randomised and controlled clinical trials to confirm these results.

**P-563** Wednesday, October 16, 2019 6:30 AM

**REDUNDANT ENDOMETRIUM AND ENDOMETRIAL POLYPS: IS THERE A LINK?**

Irene Peregrin Alvarez, MD,<sup>a</sup> Robert Roman, MD,<sup>a</sup> Mary Emily Christiansen, MD,<sup>a</sup> Ghassan Saed, MD,<sup>b</sup> Laura Detti, MD.<sup>a</sup> <sup>a</sup>University of Tennessee Health Science Center, Memphis, TN; <sup>b</sup>Wayne State University School of Medicine, Detroit, MI.



**OBJECTIVE:** Endometrial polyps (EP) and redundant endometrium (RE) are often detected incidentally during routine transvaginal ultrasonography. Several studies on the expression of hormone receptors, oncogenes and anti-mitotic proteins have been conducted to elucidate the molecular mechanisms underlying EP, however, no studies have been reported on the biology of RE. We explored whether the expression of different endometrial markers could vary in patients with EP and RE and what is their role the etiology and pathogenesis of endometrial pathology.

**DESIGN:** Pilot experimental study.

**MATERIALS AND METHODS:** We examined the expression of estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), insulin-like growth factor receptor 1 (IGFR-1), B-cell lymphoma 2 (bcl-2), Ki67, HOXA10 and thyroid receptor beta 1 (TR beta 1) in EP and RE. We obtained endometrial specimens from 16 patients aged 20-45 years, that presented to our center between September 2017 and May 2018 who were undergoing hysteroscopy for benign gynecologic pathology (EP, RE or submucosal fibroids). Fragments of the endometrial samples were processed for real-time RT-PCR analyses for the expression of the above-mentioned markers. The main outcome measure was tissue expression of these markers and comparison between EP and RE. We performed ANOVA for analysis among the 3 groups. Our results were summarized as median and quartiles (Q1, Q3) and we used SPSS v25 for Windows (SPSS, Chicago, IL); a p<0.05 defined significance.

**RESULTS:** 8 patients had RE, 5 had EP, 1 RE plus EP, 2 had normal endometrium. Compared to EP, RE showed increased bcl2 and Insulin-R but similar Ki67, IGF-R1 and HOXA10 expression. Compared to normal endometrium, RE showed increased bcl2, IGF-R1 and Insulin-R expression, while Ki67 was decreased and HOXA10 unchanged.

**CONCLUSIONS:** RE showed biochemical characteristics similar to endometrial polyps, both stemming from environmental factors. Cell differentiation seemed more enhanced than replication. Similarly to EP, RE could be detrimental for embryo implantation, especially when extensive.

This should be of consideration in women undergoing fertility treatments.

**References:** (1) Peregrin-Alvarez I, Roman RA, Christiansen ME, Detti L. Endometrial abnormalities: Correlation between different diagnostic modalities. Presented at the American Institute of Ultrasound in Medicine (AIUM) Annual Convention, April 6-9, 2019, Orlando, FL.

(2) Pinheiro A et al. Expression of hormone receptors, Bcl-2, Cox-2 and Ki67 in benign endometrial polyps and their association with obesity. *Mol Med Rep.* 2014 Jun;9(6):2335-41. <https://doi.org/10.3892/mmr.2014.2125>. Epub 2014 Apr 9.

(3) Taylor LJ et al. The differential expression of estrogen receptors, progesterone receptors, Bcl-2 and Ki67 in endometrial polyps. *BJOG.* 2003 Sep;110(9):794-8.

**P-564** Wednesday, October 16, 2019 6:30 AM

**PREVALENCE OF CHRONIC ENDOMETRITIS IN PATIENTS WITH ENDOMETRIAL POLYPS AND UNEXPLAINED INFERTILITY.**

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**OBJECTIVE:** To assess the prevalence of chronic endometritis (CE) in patients with endometrial polyps and unexplained infertility compared to patients without history of infertility.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** We evaluated patients underwent hysteroscopic polypectomy in the period of 2015 to 2018. The inclusion criteria were age 25-42 and histologically confirmed endometrial polyps. Patients with cycle day 3-5 FSH > 10 mIU/mL, with intrauterine devices, history of repeated implantation failure and recurrent pregnancy loss, autoimmune diseases, suspected placental residua, endometrial cancer, atypical hyperplasia, previous diagnosis of CE, and received any antibiotic treatment in the period of 3 months before hysteroscopy were excluded. Study group included patients with unexplained infertility. The control group included those with no previous history of infertility, not taking hormone treatment in the past 3 months before hysteroscopy or having spontaneous pregnancy in the previous 3 years before the procedure. The diagnosis of CE was established after hematoxylin and eosin and CD 138 staining and was based on the presence of one or more plasma cells per 10 high-power fields. The primary outcome was the prevalence of CE compared between infertile and fertile patients. The secondary outcomes included clinical pregnancy rate (CPR), live birth rate (LBR) and miscarriage rate (MR) of infertile patients after CE treatment (Doxycycline 100 mg twice daily for 14 days) compared to infertile patients without CE. To determine factors significantly associated with CE we used multivariate logistical regression. A sample size of 100 in each group has 80% power of showing a 15% difference in primary outcome with an alpha of 5%.

**RESULTS:** A total of 237 patients were included in the analysis. Demography, hysteroscopy cycle day, polyp location and diameter were similar between the groups. The prevalence of CE in group of patients with unexplained infertility (n=137) was significantly higher compared to the control group (n=140) [22.6% vs. 8.6%; P = 0.001]. Cumulative CPR, LBR and MR were similar between women with treated CE (n=31) and patients without CE (n=106). Multivariate logistical model showed that infertility diagnosis was significantly associated with the diagnosis of CE (OR 3.16; 95% CI 1.53 – 6.49).

**CONCLUSIONS:** In women with endometrial polyps the prevalence of CE is higher in patients with unexplained infertility compared to patients without infertility history. The pregnancy outcome of infertile patients with CE treated with one course of Doxycycline was similar to those without CE.

Variable	Redundant Endometrium Median (Q1, Q3)	Polyp Median (Q1, Q3)	Normal Median (Q1, Q3)	p-value RE vs Polyp	p-value RE vs normal
BCL2 (fg/ug RNA)	0.263 (0.16, 0.32)	0.082 (0.07, 0.09)	0.068 (0.06, 0.07)	0.0271	0.0184
HOXA-10 (fg/ug RNA)	35.05 (28.8, 43.7)	35.33 (29.2, 48.9)	40.56 (40.4, 41.7)	ns	ns
Ki67 (fg/ug RNA)	1.45 (1.1, 2.3)	2.51 (2.5, 7.3)	5.72 (4.5, 5.9)	ns	0.0009
IGF1-R (fg/ug RNA)	6.25 (4.5, 7.0)	4.42 (3.9, 5.8)	2.61 (2.6, 2.8)	ns	0.0184
Insulin-R (fg/ug RNA)	53.33 (47.6, 58.6)	39.69 (26.8, 44.4)	15.6 (15.4, 16.0)	0.0318	0.0002

## WHAT IS THE MOST EFFECTIVE TREATMENT FOR ENDOMETRITIS IN WOMEN UNDERGOING ASSISTED REPRODUCTIVE TECHNOLOGY?



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**OBJECTIVE:** Treatment of chronic endometritis (CE) improves implantation rates in patients undergoing assisted reproductive technology (ART), but causative organisms are difficult to identify and the most effective treatment regimen remains undefined<sup>1</sup>. Our objective was to identify the optimal duration and choice of antimicrobial agent(s) on clearance of CE.

**DESIGN:** Retrospective cohort study of patients between 1/2017 and 12/2018 at a single academic center with an endometrial biopsy (EMB) showing CE.

**MATERIALS AND METHODS:** All patients diagnosed with CE (defined as >1 plasma cell/HPF, stained for CD138) on EMB followed by test of cure biopsy (TOC) were included. Antimicrobial agents prescribed and length of course were recorded. Regimens were classified as 14 days or less versus 15 days or more (up to 21 days), and by spectra of coverage: Gram positive, Gram negative, Anaerobe, Atypical and Anti-fungal. Primary outcome was presence or absence of CE on TOC. If a patient remained positive on TOC, subsequent treatment(s) were included as separate course(s) for analysis. Statistical analysis included chi square test of independence and a stepwise multiple logistic regression, with  $p < 0.05$  significant.

**RESULTS:** 144 women with an initial EMB positive for CE received a total of 225 treatment courses. 11 TOC results were unavailable, leaving 214 courses of treatment with known TOC outcomes. The most common indication for EMB was failed frozen embryo transfer(s) (FET) (mean  $0.98 \pm 1.00$ , range 0-7), euploid pregnancy loss or recurrent pregnancy loss. The mean age of women in the cohort was  $36.90 \pm 3.93$  years (range 27-47). Mean number of courses required for clearance was  $1.55 \pm 0.88$  (range 0-6). All courses included antimicrobials providing gram positive and negative coverage. 62.6% (134/214) included anaerobic coverage and 66.3% (142/214) included atypical agent(s). 2 courses included anti-fungals. Including anaerobic coverage did not affect outcome (58.2% with vs 61.3% without,  $p = 0.67$ ), nor did use of an atypical agent (59.2% with vs 59.67% without,  $p = 1.00$ ). Antibiotic regimens lasting 14 days or less ( $n = 155$ ) had lower rates of CE clearance when compared to those lasting 15 days or more (54.8% vs 71.2%,  $p < 0.03$ ). Stepwise multiple logistic regression showed that only length of antimicrobial course retained a significant impact on TOC ( $B = -0.762$ ,  $p < 0.027$ , Omnibus test for variance  $p < 0.024$ , Hosmer-Lemeshow test for fit  $p = 0.99$ ).

**CONCLUSIONS:** CE is a treatable but poorly defined inflammatory process, which may affect ART success. Ideally, CE is cleared with the first course of antibiotics. Our results show that longer courses (15-21 days) are more effective, regardless of antimicrobial choice. This suggests that patients do not need to take less tolerable agents to achieve high clearance rates, and highlights the need for further, prospective analyses.

References: <sup>1</sup>: Cicinelli E, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. *Human Reproduction* 2015;30(2):323-330.

**SUPPORT:** None.

## P-566 Wednesday, October 16, 2019 6:30 AM

### NEW RELATION BETWEEN DYSBIOSIS OF THE VAGINAL AND ENDOMETRIAL MICROBIOTA AND RIF FOUND.



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**OBJECTIVE:** Repeated implantation failure (RIF) is estimated to occur in 15%–20% of infertile women undergoing *in vitro* fertilization-embryo transfer (IVF-ET). Molecular identification recently confirmed that the uterine micro-

biota may have implications for reproductive and obstetrical outcomes. We evaluated dysbiosis of the vaginal and endometrial microbiota in patients with RIF to comprehensively analyze their microbiota using 16S rRNA gene sequencing and compared the microbiota profiles in the RIF patients and healthy women.

**DESIGN:** This study was conducted from October 2017 to June 2018. It was performed retrospectively for 166 women who consented to participate. It was approved by the Saint Mother Clinic's ethical committee.

**MATERIALS AND METHODS:** 145 women who had been diagnosed with RIF were enrolled in the study. 21 healthy women were also enrolled as controls. We investigated their vaginal and endometrial microbiotas using 16S rRNA gene sequencing and compared the microbiota profiles in the RIF patients and controls.

**RESULTS:** The endometrial microbiotas had a higher alpha diversity than did the vaginal microbiotas (controls  $p = 2.41 \times 10^{-7}$ ; RIF patients  $p < 2.2 \times 10^{-16}$ ). To compare the compositional dissimilarity between the endometrial and vaginal microbiotas, the beta diversity was analyzed. By the principal coordinates analyses (PCoA) based on weighted UniFrac distance, significant associations were observed between microbiotas ( $p = 0.001$ ).

Assessing the alpha diversity revealed no significant differences between the control and RIF groups in either the uterus or vagina. Beta diversity of the endometrial microbiota showed no significant associations between the controls and RIF patients ( $p = 0.301$ ). Beta diversity of the vaginal microbiota did not differ significantly between the controls and RIF patients ( $p = 0.052$ ), but a weak difference in bacterial composition was noted.

In the endometrial microbiotas, 20 bacterial genera (*Delftia*, *Schlegella*, *Burkholderia*, *Gardnerella*, *Sphingobacterium*, *Prevotella*, *Megasphaera*, *Cloacibacterium*, *Dietzia*, *Rothia*, *Enterococcus*, *Atopobium*, *Micrococcus*, *Staphylococcus*, *Ralstonia*, *Exiguobacterium*, *Hydrogenophaga*, *Sediminibacterium*, *Limnochlamydomonas*, and *Vagococcus*) exhibited significantly different levels between the controls and RIF patients (all  $p < 0.05$ ). In the vaginal microbiota, 7 bacterial genera (*Corynebacterium*, *Atopobium*, *Megasphaera*, *Varibaculum*, *Gardnerella*, *Peptoniphilus*, and *Prevotella*) showed significantly higher levels in the RIF patients ( $p < 0.05$ ). In contrast to previous reports, we discovered no significant differences in the endometrial *Lactobacillus*, with average levels of  $51.6 \pm 38.33\%$  in the controls and  $51.15 \pm 37.48\%$  in the RIF patients ( $p = 0.961$ ). However, the average vaginal *Lactobacillus* levels differed significantly at  $91.8 \pm 22.73\%$  in the controls and  $76.38 \pm 38.85\%$  in the RIF group ( $p = 0.015$ ).

**CONCLUSIONS:** Analysis of the vaginal and endometrial microbiota using 16S rRNA gene sequencing may be a new biomarker of RIF and may help treat RIF and consequentially help raise the implantation success rate for RIF patients.

References: 1. Margalioth EJ, Ben-Chetrit A, Gal M, Eldar-Geva T. Investigation and treatment of repeated implantation failure following IVF-ET. *Hum Reprod*. 2006;21(12):3036-43. doi:10.1093/humrep/del157

2. Coughlan C, Ledger W, Wang Q, Liu F, Demiroglu A, Gurgan T, Cutting R, Ong K, Sallam H, Li TC. Recurrent implantation failure: definition and management. *Reprod Biomed Online*. 2014;28:14-38.

3. Salim R, Ben-Shlomo I, Colodner R, Keness Y, Shalev E. Bacterial colonization of the uterine cervix and success rate in assisted reproduction: results of a prospective survey. *Hum Reprod*. 2002;17(2):337-40. doi:10.1093/humrep/17.2.337

4. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Nikita L, Galuppi M, Lamont RF, Chaemsaihong P, Miranda J, Chaiworapongsa T, Ravel J. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome*. 2014;2(1):4. doi:10.1007/s12275-014-0004-2

5. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L, Forney LJ. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA*. 2011;108(Suppl):4680-7.

6. Moreno I, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, Jimenez-Almazán J, Alonso R, Alamá P, Remohí J, Pellicer A, Ramon D, Simon C. Evidence that the endometrial microbiota has an effect on implantation success or failure. *Am J Obstet Gynecol*. 2016;215(6):684-703.

7. Baker JM, Chase DM, Herbst-Kralovetz MM. Uterine Microbiota: Residents, Tourists, or Invaders? *Front Immunol*. 2018;9:208.

8. Kyono K, Hashimoto T, Nagai Y, Sakuraba Y. Analysis of endometrial microbiota by 16S ribosomal RNA gene sequencing among infertile patients: a single-center pilot study. *Reprod Med Biol*. 2018;17(3):297-306.

9. Aagaard K, Riehle K, Ma J, Segata N, Mistretta TA, Coarfa C, Raza S, Rosenbaum S, Van den Veyver I, Milosavljevic A, Gevers D, Huttenhower C, Petrosino J, Versalovic J. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PLoS One*. 2012;7(6):e36466.

**COMPARISON OF THE ENDOMETRIAL RECEPTIVITY ARRAY TO ENDOMETRIAL THICKNESS, ESTRADIOL AND PROGESTERONE LEVELS AS A MARKER FOR ENDOMETRIAL RECEPTIVITY PRIOR TO FROZEN EMBRYO TRANSFER.** Shannon T. Alexa, DO. Inspira Health Network, Vineland, NJ.



**OBJECTIVE:** Endometrial thickness (ET), estradiol (E2) and progesterone (P4) levels have been traditionally used as a marker for endometrial receptivity when preparing for frozen embryo transfer (FET) with patients undergoing in vitro fertilization (IVF). We propose that using these known receptivity markers in conjunction with Endometrial Receptivity Array (ERA) results will increase the sensitivity of detecting optimal endometrial receptivity for embryo transfer and implantation.

**DESIGN:** Retrospective Chart Review.

**MATERIALS AND METHODS:** A retrospective chart review of 143 patients who had undergone testing for the ERA at the Reproductive Science Center of New Jersey, Eatontown NJ, between 2016-2019 was done. All of the 143 patients underwent a mock cycle with endometrial biopsy and a subsequent ERA modified cycle with FET. These patients then underwent medical management and evaluation with laboratory testing of their E2 and P4 levels and ultrasounds to evaluate ET and uterine artery blood flow. Data on the patient's age, body mass index (BMI), ERA Results, ET, E2 levels, P4 levels, Pulsatility Index (PI), Resistance index (RI) and modified cycle pregnancy outcome were collected. Exclusion criteria included chronic endometritis, congenital uterine abnormalities, endometriosis, and patients who underwent biopsy but whose cycle was not managed by the primary site leaving 91 total patients.

**RESULTS:** Utilizing SAS, pairwise comparisons were made for the 4 ERA results and a T-test was done using a pooled standard error. The 4 ERA results were compared to E2, P4, number of hours of P4 given prior to biopsy/transfer, ET and uterine artery PI and RI. Of these variables only the hours of P4 given during a modified ERA cycle was significant ( $p < 0.0001$ ). In addition, E2 and number of hours of P4 during the modified cycle were highest for patients with a pre-receptive result (mean 457.1 and 140.3) and BMI was highest in patients with post-receptive results. There were minimal differences in means independent of the ERA result for P4 levels, hours of P4 given prior to biopsy, ET, PI and RI. Despite identification of higher results for some of the variables none of the differences were statistically significant. In addition there was no significant difference in pregnancy outcome of modified cycles for previously receptive vs. non receptive ERA results

**CONCLUSIONS:** In conclusion only the number of hours of P4 given during a patients modified cycle had a significant effect on the ERA result. Given this, it would appear that no linear relationship between these variables including the ERA result exists and that there is no significant relationship between any ERA result and pregnancy outcome exists suggesting a more multifaceted relationship that warrants further exploration. Further understanding of a cumulative effect allows for higher rates of implantation reducing the total number of transfers needed, total cost and emotional burden to the patient.

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**PROSPECTIVE EVALUATION OF HUMAN HERPESVIRUS 6 (HHV-6) IN ENDOMETRIAL TISSUES OF WOMEN WITH REPEAT IMPLANTATION FAILURE.**

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**OBJECTIVE:** Recent reports have demonstrated active human herpesvirus 6 (HHV-6) infection in the endometrium of >43% of women with unexplained infertility and in 0% of fertile women.<sup>1</sup> The studies demonstrated HHV-6 associated alterations in eNK cells and multiple cytokines, and suggested that HHV-6 may perturb the uterine micro-environment and disadvantage embryo implantation and placentation. To assess whether HHV-6 could play a role in embryo implantation we initiated a prospective evaluation of active HHV-6 infection in endometrial biopsies (EB) of women with Repeat Implantation Failure (RIF) following transfer of morphologic high quality or PGT-A tested euploid embryos.

**DESIGN:** EB taken at LH post 5-9 days or progestin + 5days were obtained from 24 patients with a history of two or more failed ET/FET. Pipelle segments were placed in 10% buffered formalin and submitted for formalin fixed paraffin embedding (FFPE) and immunohistochemical (IHC) evaluation.

**MATERIALS AND METHODS:** IHC analysis for an HHV-6 late protein was performed on the EB. The HHV-6 IHC assay is a validated diagnostic

test utilizing a monoclonal (Mab) antibody directed at a late structural protein of the virus paired with an isotypic Mab to control for background staining.<sup>2</sup> Fisher's Exact Test and paired *t* Test were used to analyze difference between HHV6+ and HHV-6 patients.

**RESULTS:** Of the 24 patients demonstrating 2 or more failed embryo transfers, 12 (50%) were positive for HHV-6 late viral proteins in endometrial epithelial cells and 12 (50%) were negative. There were no significant differences in age or diagnosis of endometriosis or PCOS between groups. The HHV-6 positive group had undergone significantly more failed transfers than the HHV6-negative group ( $p = 0.022$ ).

**CONCLUSIONS:** The ability of human herpesvirus 6 (HHV-6A/B) to infect and cause disruption or failure of specific organ systems has precedence in hematopoietic stem cell (HSC) transplantation. The presence of HHV-6A/B and disruption of HSC graft function and failure of marrow engraftment has been confirmed.<sup>3</sup> The HHV-6 induced factors that cause HSC engraftment interference may be operative in preventing the natural or assisted implantation of human embryos. The data presented here strongly suggests that further studies of HHV-6 in infertility and RIF are warranted.

**References:** <sup>1</sup> Marci R, Gentili V, et al. Presence of HHV-6A in Endometrial Epithelial Cells from Women with Primary Unexplained Infertility. PLoS ONE. 11(2016).

<sup>2</sup> Charnot-Katsikas A, Baewer D, et al. Fulminant hepatic failure attributed to infection with human herpesvirus 6 (HHV-6) in an immunocompetent woman. J Clin Virol.75(2016): 27-32.

<sup>3</sup> Carrigan D, Knox K. HHV-6 Associated Bone Marrow Suppression in Bone Marrow Transplant Patients. Blood. 84(1994): 3307-3310.

P-569 Wednesday, October 16, 2019 6:30 AM

**IDENTIFICATION OF NEW BIOMARKERS OF HUMAN ENDOMETRIAL RECEPTIVITY AND MATERNAL-FETAL DIALOGUE.**

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**OBJECTIVE:** The endometrial receptivity is a key process for the success in assisted reproductive technology. Despite careful embryo selection, two of every three in vitro fertilization (IVF) cycles fail to result in pregnancy, making reproduction in humans an inefficient process. The key to successful implantation is synchronization. The embryo must not only evolve to the blastocyst stage, but the endometrium must also achieve a specific receptive status and cross-talk between the embryo and endometrium must occur during the window of implantation (WOI). Therefore, it appears essential to identify inadequate endometrial receptivity to offer personalized care management. Molecular diagnostic tools currently available to characterize this process are very limited. In this study, we describe the development of a new personalized molecular test based on endometrial receptivity and maternal-fetal dialogue.

**DESIGN:** As a result of a single site study at ovo clinic from December 2016 to March 2019, the development and clinical validation of a new test, Adhesio RT, allowed us to analyze 215 biopsies of which 50 endometrial biopsy samples and 35 autologous endometrial co-culture samples were analyzed by using microarray technology and 130 biopsies from IVF-patients with a known pregnancy outcome were used for clinical validation.

**MATERIALS AND METHODS:** Microarray data from 50 endometrial biopsies obtained during the optimal theoretical implantation window LH+7 to LH+11 in natural cycle (35 with successful clinical pregnancy 15 with implantation failure). Similarly, a total of microarray data obtained from 29 co-culture biopsies were performed on autologous- endometrial co-culture (14 endometrial cells cultured in absence of embryo, 5 in presence of good-quality embryo successfully transferred, 10 with good quality embryo but with implantation failures). Microarray data were analyzed and selected biomarkers were assessed using RT-qPCR.

**RESULTS:** 10 genes have been identified for the first time by using a new approach that incorporates two specific transcriptomic signatures obtained by different bioinformatics and statistical technologies applied to microarray analyses:

A first specific transcriptomic signature of 1717 genes specifically modulated associated to biopsies from patients with successful clinical pregnancy *versus* biopsies from patients with implantation failure. Gene ontology analyzes revealed that cell division, cellular proliferation, cell adhesion and mitotic cycle are the most over-represented biological terms in this group of genes.

A second specific signature of 60 genes associated to endometrial co-culture successfully transferred was obtained using class prediction approach.

Gene expression was validated by RT-qPCR. Clinical validation was performed on 130 biopsies from IVF-patients with a known pregnancy outcome.

**CONCLUSIONS:** Evaluation of receptivity and embryo implantation with this new molecular signature can predict IVF success and may help in the management of endometrial preparation for embryo transfer and optimizes chances of successful pregnancy for many couples.

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**PROGESTERONE AND ESTRADIOL CONCENTRATIONS IN HUMAN ENDOMETRIUM DURING THE MID-LUTEAL PHASE OF THE MENSTRUAL CYCLE.**

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**OBJECTIVE:** The aim of this study was to estimate the local progesterone and estradiol concentrations in the endometrial tissue, to compare them with their serum concentrations and to investigate possible associations between them.

**DESIGN:** Observational study.

**MATERIALS AND METHODS:** The concentration of (P4) and estradiol (E2) were investigated in serum and endometrial biopsy samples from 58 women aged between 26 and 41 years during mid-luteal phase phase (7 days after LH surge). The endometrial samples were weighed and homogenized in a glass homogenizer with a Teflon pestle in a PBS buffer followed by centrifugation at 12,000 g for 10 minutes at 4°C. The obtained supernatant was used for P4 and E2 measurement by electrochemiluminescence immunoassay (ECLIA) on the Cobas e411 analyser (Roche Diagnostics, Mannheim, Deutschland). Statistical analysis was performed using SPSS v.21 (IBM Corp., Armonk, NY, USA). Descriptive parameters and patient characteristics were reported as mean ± SD and median. P<0.05 was considered statistically significant.

**RESULTS:** The observed endometrial P4 levels ranged from 0.001 to 270.54 ng/mg tissue, with a mean of 23.41 ± 57.63 ng/mg tissue and a median of 0.86 ng/mg tissue. The endometrial E2 concentrations ranged between 0.01 and 0.29 pg/mg tissue with a mean of 0.1 ± 0.06 pg/mg tissue and a median of 0.08 pg/mg tissue. The determined mean P4 concentration in the endometrial tissue was 17.9 times higher than the P4 found in the serum samples, while the mean tissue E2 concentration was 1510 times lower in comparison with the E2 serum levels. As a result, the mean P4/E2 ratio (P4 [ng/mg] /E2 [ng/mg]) in the tissue (18538 ± 16638), was 136 times higher than the corresponding P4/E2 ratio in the serum (136.15 ± 85.30). A significant but relatively low Spearman correlation was found when comparing the endometrial and serum P4 concentrations (R=0.34; p=0.01). A similar relation was observed between the E2 levels in the endometrial tissue and in the serum (P=0.35; p=0.02). Again, modest but significant relation was present between the P4 and E2 tissue levels (R=0.35; p=0.02).

**CONCLUSIONS:** We conclude that the mid-luteal endometrium contains relatively high levels of P4 and significantly low levels of E2 compared to their serum levels. Endometrial P4 and E2 concentrations are positively but slightly associated between each other and with their respective levels in serum.

**P-571** Wednesday, October 16, 2019 6:30 AM

**LIPID PROFILING OF PERI-IMPLANTATION ENDOMETRIUM IN PATIENTS WITH PREMATURE PROGESTERONE RISE IN LATE FOLLICULAR PHASE.**

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**OBJECTIVE:** To investigate alterations of lipid profile at the window of implantation in patients with premature progesterone rise.

**DESIGN:** Lipidomics variation of endometrium was evaluated by ultra-high performance liquid chromatography coupled with electrospray ionization high-resolution mass spectrometry (UHPLC-ESI-HRMS).

**MATERIALS AND METHODS:** 43 patients undergoing IVF/ICSI by the reason of tubal factor or male factor were included in this study. The patients were divided into high progesterone group (P ≥ 1.5ng/ml, 15 patients) and control group (P < 1.5ng/ml, 28 patients) on the day of hCG administration. The endometrium tissues were obtained by pipelle biopsy 7 days after hCG trigger.

**RESULTS:** A total of 1026 ions were identified and 25 lipids were showed significantly up-regulated. The endometrium lipid profile was characterized

by significant increase in concentration of phosphatidylcholine (PC), phosphatidylethanolamine (PE), lysophosphatidylcholine (LPC), diacylglycerol (DG), ceramide (Cer), phosphatidylinositol (PI), phosphatidylserine (PS) in patients with premature progesterone rise at the end of the follicular phase. The correlation analysis between progesterone level with the lipids showed stronger negative correlation between PE and PS with progesterone level.

**CONCLUSIONS:** Premature progesterone elevation disturbs lipid homeostasis of endometrium in the peri-implantation period. The altered lipids may impair endometrial receptivity and early embryo implantation.

**SUPPORT:** This study was financially supported by the National Natural Science Foundation of China (No. 81601347, 81503156.), Natural Science Foundation of Guangdong Province (No. 2014A030310096) and Public Welfare Research and Capacity Building Fund of Guangdong (No. 2016A020218006).

**P-572** Wednesday, October 16, 2019 6:30 AM

**EFFECT OF ESTRACE ON PREGNANCY RATES FOR WOMEN WITH THIN ENDOMETRIAL LINING UNDERGOING INTRAUTERINE INSEMINATION.**

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**OBJECTIVE:** To evaluate the effect of exogenous estradiol on pregnancy rates for women with thin endometrial lining undergoing intrauterine insemination (IUI) as compared to women who did not receive estradiol for endometrial support; we hypothesize that there was no difference in pregnancy rates between the two groups.

**DESIGN:** Retrospective chart review.

**MATERIALS AND METHODS:** All IUI cycles completed at Stanford University Clinic for Reproductive Medicine from March-December 2017 were reviewed. All monitored IUI cycles were included. Cycles with the addition of exogenous estradiol given vaginally or orally were compared to those without exogenous estradiol. Differences in endometrial parameters, pregnancy rates, miscarriage rates and live birth rates were compared between both groups.

**RESULTS:** A total of 885 IUI cycles were included. In 85 cycles, exogenous estradiol was initiated for thin endometrium. Baseline characteristics including maternal age, body mass index, ethnicity, number of IUI cycles per patient, type of IUI cycle, and total motile sperm count were similar between the two groups. Mean baseline endometrial lining was thicker in the non-estradiol group, and the non-estradiol group was more likely to have a diagnosis of unexplained infertility whereas the estradiol group was more likely to have a diagnosis of diminished ovarian reserve. Despite initiation of estradiol, the mean endometrial thickness at trigger scan remained significantly thinner in estradiol group as compared to the non-estradiol group (6.4 ± 1.3 cm vs. 8.4 ± 1.9 cm, respectively, p < 0.001), although the change in thickness in the estradiol group from baseline to trigger scan did increase on average by 2.2 cm. Pregnancy, miscarriage and live birth rates were similar between the estradiol and non-estradiol groups (see Table 1).

**CONCLUSIONS:** Although there is limited data supporting the use of exogenous estradiol to improve outcomes during IUI cycles, this low risk intervention is often employed in the setting of a thin endometrial lining in the late follicular phase. In women undergoing IUI with exogenous estradiol supplementation due to thin endometrial lining, pregnancy, miscarriage and live birth rates were similar to women undergoing IUI without exogenous estradiol use.

TABLE 1.

Outcomes	Estradiol (n=85)	No Estradiol (n=800)	P-value
Endometrial Lining thickness (cm), mean ± standard error			
Baseline	4.2 ± 1.3	4.9 ± 1.7	<0.001
Estradiol initiation	4.9 ± 0.8	6.4 ± 1.7	<0.001
Trigger	6.4 ± 1.3	8.4 ± 1.9	<0.001
<b>Pregnancy Rate, n (%)</b>	14 (20%)	81 (10%)	0.12
<b>Spontaneous Abortions, n (%)</b>	8 (10%)	30 (4%)	0.08
<b>Live births to date, n (%)</b>	5 (10%)	42 (10%)	0.78

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### ACUTE EXPOSURE TO UNHEALTHY AIR QUALITY DURING THE 2018 CAMP FIRE WAS NOT ASSOCIATED WITH ADVERSE LABORATORY OUTCOMES IN PATIENTS WHO UNDERWENT ASSISTED REPRODUCTION TREATMENT.



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**OBJECTIVE:** The Camp Fire, California's most destructive wildfire, began on November 8<sup>th</sup> 2018 and the Bay Area's air quality index (AQI) rose from Healthy (AQI < 50) to Unhealthy (AQI 150-200), enduring for two weeks. Though devastating, this event presents a unique opportunity to assess the short-term effects of exposure to poor quality air on a fertility population. This study investigates the effects of unhealthy air quality on in vitro fertilization (IVF) outcomes of patients undergoing treatment at the University of California, San Francisco (UCSF) during this period.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Data regarding AQI were obtained directly from the Environmental Protection Agency. A LifeAire System is used for air purification in UCSF's IVF laboratory. During the fires, there were <10ppb volatile organic compounds and <1 ug/m<sup>3</sup> of particles (0.1-10 μm) measurable in the laboratory. Clinical outcome data were collected from exposed patients with oocyte retrievals within a month following the Camp Fire (from November 8<sup>th</sup> to December 8<sup>th</sup>, 2018). Data on fertilization, blastocyst, and euploid rate were compared to a control population of patients who had oocyte retrievals at UCSF within the year prior to the Camp Fire. Student's t-test was used to analyze differences in mean rates for each clinical outcome. Chi squared test was used to compare cycle cancellation rate between groups. Regression analyses with cluster analysis for pairwise comparison were performed on exposed patients with prior unexposed cycles, to assess for differences in clinical outcomes within a patient.

**RESULTS:** Median AQI in the year prior to the Camp Fire was 37 (IQR 31-52), in comparison to a median AQI of 164 (IQR 151-173) during the two weeks of the Camp Fire (p=0.001). One hundred and twelve patients were exposed during the fire, and 45% of them completed preimplantation genetic screening for aneuploidy (PGT-A). There were 969 patients in the control population, with 45% completing PGT-A. No significant differences were noted in age, body mass index, race, infertility diagnosis, or stimulation protocol between the groups. When comparing control group to fire exposed group, there were no significant differences between the fertilization (79% vs 77%, p=0.44), blastocyst (51% vs 57%, p=0.16), or euploid (40% vs 47%, p=0.14) rates. There were also no differences in cycle cancellation rate. Sixty-six exposed patients had non-exposed cycles with the same or different stimulation protocol. When comparing all non-exposed to exposed cycles within a patient, no differences were noted in fertilization (73% vs 76%, p=0.67), blastocyst (48% vs 41%, p=0.22), or euploid (31% vs 41%, p=0.24) rate.

**CONCLUSIONS:** Unhealthy AQI during the 2018 Camp Fire was not associated with statistically significant differences in clinical outcomes of patients undergoing IVF treatment in a code complaint laboratory. These findings suggest that acute exposure to unhealthy air does not impact egg or sperm function, however, further studies are needed to assess for impact of long-term exposure on outcomes.

**SUPPORT:** None.

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### EFFECT OF GENISTEIN AND DAIDZEIN LEVELS ON OVARIAN RESPONSE AND EMBRYO QUALITY IN PGT-A CYCLES.



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**OBJECTIVE:** To evaluate if genistein and daidzein (soy-derived phytoestrogens) levels in urine and follicular fluid (FF) could have an impact on the number of antral follicles, MII oocytes, fertilization, embryo quality on day 3, and aneuploidy rates.

**DESIGN:** Prospective, observational study including preimplantation genetic testing for PGT-A (PGT-A) cycles in which urine and FF was collected the day of ovum pick-up (December 2013 - July 2018).

**MATERIALS AND METHODS:** A total of 36 PGT-A cycles in women <38 years were analyzed by Next Generation Sequencing (NGS). Indications for PGT-A were recurrent miscarriage or repetitive implantation failure. Genistein and daidzein were measured using Ultra-Performance Liquid Chromatography/Electrospray Mass Spectrometry (UPLC/ESI-MS) and normalized according to creatinine levels. In urine samples genistein and daidzein levels were classified in three categories: <10ng; 10-50ng and >50ng. The number of informative urine samples with levels above the limit of detection was 26 for genistein and 36 for daidzein. In FF, lower levels were detected and were classified as follows: <2ng; 2-5ng and >5ng. The number of informative FF samples with levels above the limit of detection was 13 for genistein and 9 for daidzein. The statistical comparisons among groups were carried out using the GraphpadInstat v. 2.05a package (Graphpad Software, San Diego, CA, USA).

**RESULTS:** For genistein levels in urine, a significant increase in the mean number of antral follicles was observed in the group with higher concentration (14.9±5.3; 18.2±5.3 and 24.5±9.6; p<0.05). A similar trend was observed for the mean number of MII oocytes and 2PN, but without significant differences. Day 3 embryos showed a significant increase in mean blastomere number (6.6±2.0; 5.9±1.8 and 7.2±1.0; p<0.05), and a decrease in fragmentation degree (8.1±9.6; 8.6±8.5 and 5.0±4.1; p<0.05) in the group with higher urine genistein concentration. Genistein levels in FF were correlated with the levels in urine and significant differences were found for the same variables: mean number of antral follicles (11.2±3.7; 19.5±3.5 and 22.5±14.8; p<0.05); mean blastomere number (6.7±3; 6.0±1.9 and 7.5±0.7; p<0.05) and fragmentation degree (6.7±5.8; 11.0±8.2 and 2.5±3.5; p<0.05). Aneuploidy rates were significantly decreased in urine genistein levels >50ng (60.8% vs. 42.3%; p<0.05), and a similar trend was observed for FF >5ng, but without reaching statistical significance (69.7% vs. 47.1%). For daidzein levels in urine and FF, no clear correlation was observed with ovarian response and embryo quality.

**CONCLUSIONS:** In PGT-A couples, genistein, a soy-derived phytoestrogen, could enhance ovarian response, embryo quality and euploidy rates. A protective effect of a soy-diet has been previously described in a mouse model (Muhlhauser et al., 2009). In ART patients, soy isoflavones intake has been positively related to livebirths (Vanegas et al., 2015). Therefore, a soy-enriched diet could be beneficial for women undergoing ART, and more specifically in PGT-A cycles.

References: Muhlhauser A, Susiarjo M, Rubio C, Griswold J, Gorence G, Hassold T, Hunt PA. Bisphenol A effects on the growing mouse oocyte are influenced by diet. *Biol Reprod.* 2009 May;80(5):1066-71.

Vanegas JC, Afeiche MC, Gaskins AJ, Minguez-Alarcón L, Williams PL, Wright DL, Toth TL, Hauser R, Chavarro JE. Soy food intake and treatment outcomes of women undergoing assisted reproductive technology. *Fertil Steril.* 2015 Mar;103(3):749-55.e2.

**SUPPORT:** Merck research grant 2015-2018.

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### THE ASSOCIATION BETWEEN SEASON AT CYCLE START AND CLINICAL PREGNANCY FOLLOWING FRESH EMBRYO TRANSFER.



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**OBJECTIVE:** Improvements in laboratory techniques and clinical protocols have led to increasing livebirth rates from in vitro fertilization (IVF). Despite this impact, the reproductive potential of patients remains a primary determinant of success. It is therefore of interest to investigate any environmental factors that may influence this potential. As delivery rates from spontaneous conception vary according to season, outcomes from IVF may also be season-dependent. The present study was designed to test the hypothesis that there is an association between season at IVF cycle start and clinical pregnancy.

**DESIGN:** Retrospective cohort of 5,878 fresh embryo transfers.

**MATERIALS AND METHODS:** Start dates for all autologous cycles resulting in fresh cleavage or blastocyst stage transfers performed in our IVF program between January 2012-December 2017 were categorized by season

(Spring: March, April, May; Summer: June, July, August; Fall: September, October, November; Winter: December, January, February). Dates were linked to local temperature (min, max, average) and day length measurements obtained from meteorological records ([www.wunderground.com](http://www.wunderground.com)). Average maximum temperature and day length were categorized into tertiles. Multivariable logistic regression, adjusted for age and quadratic age, was used to model odds of clinical pregnancy, defined as presence of fetal sac.

**RESULTS:** Patient characteristics were similar among seasons. As expected, temperature and day-length varied by season. When compared with cycles started during Winter, there was no difference in clinical pregnancy rate in Spring (OR: 1.08, 95% CI: 0.94-1.25), Summer (OR: 1.10, 0.95-1.27), or Fall (OR: 1.02, 0.88-1.19). Comparisons of clinical pregnancy by month of cycle start revealed that cycles started in June (45.6%) and July (45.1%) had 38% greater odds of clinical pregnancy compared to cycles that started in January (37.5%) (OR: 1.38, 1.08-1.77 for June; OR: 1.38, 1.08-1.76 for July). No other comparisons by month reached statistical or clinical significance. There was a positive linear trend between day-length and clinical pregnancy (P-value, test for linear trend=0.05) but no association with average temperature (P-value, test for linear trend=0.52).

**CONCLUSIONS:** Our results do not support the hypothesis that clinical pregnancy after fresh transfer is associated with season at cycle start. However, our more detailed analyses found greater odds of clinical pregnancy for cycles started in June and July compared with January and a positive linear trend between clinical pregnancy and day-length, but not temperature. Together, these findings suggest an association between maternal light exposure and outcomes from IVF. The exact mechanism underlying this association is not known but may implicate a role of melatonin and/or Vitamin D on improved oocyte quality and/or endometrial receptivity.

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#### REGIONAL DIFFERENCE OF METAL LEVELS IN FOLLICULAR FLUID AND SERUM AMH LEVEL BETWEEN INFERTILE WOMEN FROM EASTERN CHINA AND SOUTHERN CHINA. Jinqun Xu, MD,<sup>a</sup>

Yanyun Ying, B.S.Med,<sup>b</sup> Jianpeng Chen, MD,<sup>c</sup> Dan Li, MD,<sup>c</sup> Dan Zhang, MD, PhD.<sup>b</sup> <sup>a</sup>Key Laboratory of Reproductive Genetics (Ministry of Education) and Department of Reproductive Endocrinology, Hangzhou, Zhejiang, China; <sup>b</sup>Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China; <sup>c</sup>Key Laboratory of Reproductive Genetics (Ministry of Education) and Department of Reproductive Endocrinology, Hangzhou, China.

**OBJECTIVE:** The metal exposure can result in different bioaccumulation in the reproductive tissues as well as diverse disturbance of the reproductive outcomes in different regions, which requires additional researches on regional effects on environmental exposure and reproductive toxicity. Therefore, the study aims to assess the regional difference of metal levels in follicular fluids and serum Anti-muller test tube hormone (AMH) of infertile women from Eastern China and Southern China.

**DESIGN:** A cross sectional study was approved by the Institutional Review Board Committee, and was conducted between September 2017 and December 2017 in infertility ward in Women's Hospital, Zhejiang University School of Medicine. 648 female patients diagnosed with unexplained infertility from Eastern China and Southern China were included.

**MATERIALS AND METHODS:** After informed written consent signed by the included patients, FF samples during the oocyte retrieval were collected. Nineteen elements (vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, strontium, molybdenum, silver, cadmium, tin, antimony, barium, titanium, and mercury) were analyzed in FFs by inductively coupled plasma mass spectrometry (ICP-MS), and serum AMH levels are tested. The places of residence were collected from the electronic medical record of the hospital. The Associations of metal levels between AMH levels were adjusted by age, BMI and reproductive hormones (follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P), testosterone (T) and prolactin (PRL)).

**RESULTS:** We observed that iron (Fe) levels and Cobalt (Co) levels in FFs were both inversely related to serum AMH level ( $P < 0.05$ , respectively). Infertile women living in the Eastern China have a significant higher level of Fe and Co in FF ( $P < 0.05$ , respectively) with a significant lower AMH level ( $P < 0.05$ , respectively) compared to infertile women from Southern China.

**CONCLUSIONS:** Bioaccumulations of Fe and Co were quite different between infertile women living in the Eastern China and Southern China. Fe and Co might have significant inverse effects on AHM, which also showed significant regional differences between Eastern China and Southern China.

There might be some difference of Fe and Co exposure pathways between two areas, while additional prospective research is needed to corroborate these findings in the general population.

**SUPPORT:** This work was supported by the National Key Research and Development Program of China (2017YFC1001003), the National Natural Science Foundation of China (No. 81771535), the Natural Science Foundation of Zhejiang Province (No. LZ18H040001), and the Zhejiang Provincial Key Medical Technology Program (WKJ-ZJ-1826).

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#### CIGARETTE SMOKE-INDUCED OXIDATIVE STRESS ALTERS DNA METHYLATION PATTERNS IN SPERM AND NEUROLOGICAL GENE EXPRESSION PATTERNS IN OFFSPRING. Patrick J. Murphy, PhD,<sup>a</sup>

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**OBJECTIVE:** We investigated the impact of pre-conception paternal cigarette smoke exposure on sperm DNA methylation and gene expression in the offspring as well as the mechanism underlying smoke-induced changes. In addition, we evaluated the capacity for sperm DNA methylation patterns to correct following removal of smoke exposure for 1-5 spermatogenic cycles (28-171 days).

**DESIGN:** Mouse model-based intervention versus non-intervention study.

**MATERIALS AND METHODS:** Male mice were exposed to tobacco smoke at the body mass-adjusted equivalent of 10 to 20 cigarettes per day for 60 days. Following the exposure period, some exposed and unexposed mice were bred to unexposed female mice, while additional exposed and control mice (recovery group) were maintained for 28-171 days following removal of smoke exposure. Sperm were collected from the sires and recovery animals, and reduced representation bisulfite sequencing (RRBS) was performed to analyze genome-wide sperm DNA methylation patterns associated with cigarette smoke exposure and recovery. Offspring of exposed and control mice were euthanized at 14-17 weeks of age, and DNA methylation and gene expression patterns in the frontal cortex were evaluated to determine whether paternal smoking status impacted offspring neurological profiles. The Bismark RRBS pipeline and the USeq program DefinedRegionDifferentialSeq were utilized for measuring changes in DNA methylation and gene expression respectively. Statistical analysis was performed using R.

**RESULTS:** We identified significant differences in sperm DNA methylation patterns associated with smoking status. Remarkably, the changes in sperm DNA methylation were largely recapitulated in *Nrf1*<sup>-/-</sup> mice independent of smoke exposure. The assessment of heritable effects revealed changes in DNA methylation patterns as well as gene expression in the offspring of mice exposed to cigarette smoke, and strikingly the epigenetic and transcriptional changes identified in the offspring of smoke-exposed mice were also observed in *Nrf1*<sup>-/-</sup> offspring irrespective of paternal smoking status. Recovery experiments indicated that about half of differentially methylated regions returned to normal within 28 days of removal from smoke, however additional recovery following a longer recovery period was not observed, indicating potential long-term effects following smoking cessation.

**CONCLUSIONS:** The current study provides abundant evidence that cigarette smoke exposure induces epigenetic changes in sperm. Further, studies in offspring suggest that pre-conception paternal smoking status impacts neurological epigenetic and gene expression status in a consistent manner. Parallel studies performed in *Nrf1*<sup>-/-</sup> mice provide strong evidence for oxidative stress as the predominant underlying mechanism for smoke-induced epigenetic changes to sperm as well as the offspring of smoke-exposed sires. Lastly, recovery experiments indicate that while many epigenetic changes are corrected following removal from smoke exposure, aberrant methylation persists at a significant number of regions even after five spermatogenic cycles.

**SUPPORT:** This work was supported by a grant from the Eunice Kennedy Shriver National Institute of Child Health and Human Development, USA (R01HD082062).

**AMBIENT TEMPERATURE IN THE WEEK PRIOR TO DELIVERY AND RISK FOR STILLBIRTH.**



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**OBJECTIVE:** To assess the impact of higher ambient temperature on the risk of stillbirth during the warm season. Extreme ambient temperature events are becoming more prevalent and changes in ambient temperature represent an understudied but potentially modifiable stillbirth risk factor.

**DESIGN:** Retrospective cohort study based on hospital delivery admission electronic records from 19 hospitals for all deliveries 20 weeks gestation or later.

**MATERIALS AND METHODS:** We identified the first stillbirth case per mother (n=498) among singleton deliveries in the NICHD Consecutive Pregnancies Study (Utah, 2002-2010). Ambient temperature was derived from the Weather Research and Forecasting model and air pollution data were based on modified Community Multiscale Air Quality models. We conducted a case-crossover analysis to estimate the hazard ratio (HR) and 95% confidence interval (95% CI) for the risk of stillbirth for each increase of 1° Celsius during the warm season (May – September). Risk periods included day of delivery and each of the 7 days prior to delivery as well as the average temperature for the week prior to delivery. Two control periods were selected: two weeks prior to delivery and two weeks after delivery. Women serve as their own controls in this analysis and all non-time-varying factors are controlled by design. Models were adjusted for time-varying relative humidity, ozone, and particulate matter <2.5 microns.

**RESULTS:** During the week prior to delivery, daily risk of stillbirth significantly increased between 5-11% for each 1° Celsius increase in temperature beginning 2 days prior to delivery (HR=1.05; 95% CI: 1.00-1.09) with the highest risk observed at 7 days prior (HR=1.11; 95% CI: 1.06-1.17). Point estimates for the day of delivery, the day immediately preceding delivery and average temperature in the week prior to delivery were elevated but not significantly associated with stillbirth.

**CONCLUSIONS:** Our findings suggest temperature may be a modifiable risk factor for stillbirth. Notably, the risks we observe beginning 2 days prior to delivery appear consistent with the fact that most stillbirths occur 48-72 hours prior to delivery. High temperature can induce physiologic stress including increased heart rate and inflammatory processes, but the specific underlying biologic mechanisms related to stillbirth remain to be explored. Stillbirth risk associated with ambient temperature merits attention given anticipated increases in ambient temperature over time.

Time prior to delivery	Hazard Ratio	95% Confidence Interval
Day of delivery	1.02	0.98 -1.06
1 day	1.04	0.99 - 1.08
2 days	1.05	1.00 - 1.09
3 days	1.07	1.02 - 1.11
4 days	1.06	1.02 - 1.11
5 days	1.06	1.01 - 1.10
6 days	1.09	1.04 - 1.14
7 days	1.11	1.06 - 1.17
Average for the week prior	1.04	1.00 - 1.08

**SUPPORT:** This research was supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

**AMBIENT TEMPERATURE AND HUMIDITY DURING KEY PHASES OF THE MENSTRUAL CYCLE AND REPRODUCTIVE OUTCOMES: DIFFERENCES BY SEASON AND LOW-DOSE ASPIRIN.**



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**OBJECTIVE:** Exposure to temperature extremes is associated with a greater burden of inflammation and oxidative stress, which in turn may impact fecundity through disruption of key reproductive processes. We investigated the relationship of temperature and humidity during ovulation and implantation with pregnancy and pregnancy loss, and whether these relationships differ by season and randomization to low-dose aspirin.

**DESIGN:** Prospective cohort study of 1228 participants of a randomized clinical trial of preconception-initiated 81mg aspirin or placebo from 4 U.S. study centers (Salt Lake City, Utah; Denver, CO; Buffalo, NY; Scranton, PA).

**MATERIALS AND METHODS:** Women were followed for up to 6 menstrual cycles and, if they became pregnant, throughout pregnancy. Timing of ovulation was assessed using fertility monitors and pregnancy using end-of-cycle hCG tests. Pregnancy loss was documented on absence of ultrasound confirmation of hCG pregnancy or later observed loss. Daily temperature and humidity were abstracted from local weather stations and averaged for ovulation (3 days before to one day following ovulation) and implantation (6 to 10 days following ovulation). Discrete-time proportional hazards models evaluated menstrual-cycle probability of pregnancy, a pregnancy ending in a loss, and a pregnancy ending in a live birth adjusting for season, ozone, particulate matter <2.5 microns, serum vitamin D, age, body mass index, parity and study site. We evaluated interactions with season (warm [April-September] vs. cold [October-March]) and treatment assignment (low-dose aspirin vs. placebo).

**RESULTS:** Of 797 women who became pregnant, 188 had a pregnancy loss. Overall, there were no clear associations of temperature or humidity with pregnancy and pregnancy loss. However, we observed seasonal trends, with a 2% increase in humidity during implantation associated with a 6% (95% confidence interval [CI] 1.00, 1.12) greater odds of a pregnancy ending in a loss only during the warm season. We additionally observed key differences by treatment assignment, with a 2% increase in humidity during ovulation and implantation both associated with a 4% greater odds (95% CI 1.01 1.07 and 95% CI 1.01, 1.08, respectively) of pregnancy only in the placebo group. This increased odds of pregnancy in the placebo group appeared to be confined to pregnancies ending in a loss, with a 2 degree Celsius increase in temperature and 2% increase in humidity during ovulation independently associated with a 15% (95% CI 1.00, 1.32) and 7% (95% CI 1.01, 1.13) greater odds of a pregnancy ending in a loss. No clear associations of temperature and humidity with pregnancy or pregnancy loss were observed in the aspirin group.

**CONCLUSIONS:** Higher ambient temperature and humidity may increase the odds of a pregnancy ending in a loss, particularly during the warm season. Findings suggest that low-dose aspirin may mitigate some of these effects.

**SUPPORT:** Intramural Research Program, Division of Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development.

**PROXIMITY TO NEAREST MAJOR ROAD AND FECUNDABILITY IN AN HISTORICAL COHORT.**



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**OBJECTIVE:** To examine the relationship between distance to major roadway, a proxy for traffic-related air pollution, and fecundability.

**DESIGN:** Our analysis was conducted within the North Carolina Early Pregnancy Study (n=221) a prospective time-to-pregnancy cohort.

**MATERIALS AND METHODS:** Pregnancy attempt time, which allows estimation of the per cycle probability of conception, was calculated as the number of cycles from enrollment until conception, or until study end if no conception occurred. Our primary definition of conception included all detected conceptions, including early pregnancy loss, clinical pregnancy loss, and singleton or twin deliveries. In a secondary analysis, conception was defined as clinical pregnancy only. Residences were geolocated for each participant using ArcGIS and roadway information from the U.S. Census Bureau and a 1980 Official Highway Map from the North Carolina Department of Transportation. Residential proximity to nearest major road

TABLE. Fecundability Ratios for roadway proximity.

	Conception <sup>1</sup> yes, 170 cycles no, 438 cycles			Clinical Pregnancy <sup>2</sup> yes, 150 cycles no, 545 cycles		
	cycles	Adjusted <sup>3</sup>		cycles n	Adjusted <sup>3</sup>	
		FR	95%CI		FR	95%CI
Per 100m increase Roadway proximity (m)	608	0.99	(0.98, 1.01)	695	1.00	(0.99, 1.02)
<200	79	1.42	(0.94, 2.14)	97	1.07	(0.68, 1.68)
200 - <500	157	1.11	(0.77, 1.60)	188	0.80	(0.53, 1.21)
500 - <1000	137	1.18	(0.83, 1.67)	155	1.10	(0.76, 1.59)
>1000	235	1		255	1	

1. "Conception" includes: early pregnancy losses, singletons, twins, pregnancy losses.
2. "Clinical pregnancy" excludes early pregnancy losses.
3. Adjusted for: female age, male age, education, income, occupation.

was calculated for each participant. We used generalized linear regression models to estimate fecundability ratios (FR). We also used a logistic regression to estimate the odds ratios (OR) for early pregnancy loss.

**RESULTS:** In our primary analysis, fecundability was higher for couples living near a major road, but the confidence interval included the null (FR <200 meters vs >1000 meters, 1.42 (95% confidence interval (CI): 0.94, 2.14) (Table). For clinical pregnancies, proximity was not associated with fecundability (Table). Odds of early pregnancy loss was higher in women who lived < 200 meters from a major road (OR: 2.08, 95%CI: 0.85, 5.09) or who lived 200 - <500 meters away from a major road (OR: 1.82, 95%CI: 0.78, 4.24), but numbers were small (47 losses).

**CONCLUSIONS:** Living near a major roadway was not associated with reduced fecundability. Proximity to major roads may be associated with early pregnancy loss, but this should be investigated in a cohort with a larger number of early losses. Planned analyses of existing data in this cohort include implantation timing and characteristics of early loss in relation to proximity.

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#### WOMEN'S KNOWLEDGE ABOUT THE IMPACT OF FEMALE AND MALE AGE, WEIGHT, AND SMOKING ON FERTILITY: RESULTS FROM A NATIONAL SURVEY.

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**OBJECTIVE:** Women's misconceptions about the impact of female and male age, weight, and smoking on a couple's fertility likely lead to uninformed decisions regarding reproductive health and family planning; however, little research has examined women's fertility knowledge. The goal of this study was to provide a large-scale assessment of women's knowledge about the impact of risk factors on female and male fertility.

**DESIGN:** A national, cross-sectional survey.

**MATERIALS AND METHODS:** 327 women were recruited through an e-newsletter in March 2019; no incentive was provided. Eligible participants were aged 18 to 59, identified as women, lived in the USA and provided informed consent. Participants completed an online survey that assessed their knowledge about the impact of female and male age, weight, and smoking on fertility. The data were analyzed using descriptive statistics and dependent sample t-tests; the power was excellent (.99).

**RESULTS:** Participants ranged in age from 18 to 59 ( $M = 34.11$ ,  $SD = 6.64$ ) and the majority identified as heterosexual (95%) and had a partner (81%).

3 items assessed knowledge about the impact of age on female fertility, and 3 items assessed knowledge about the impact of age on male fertility (e.g., "female fertility significantly declines between the ages of 35 and 39" (T); "male fertility significantly declines between the ages of 45 and 49" (T)). Participant responses on all 6 items were coded as correct or incorrect. 21% answered all items about female age correctly; 13% answered all items about male age correctly. A dependent samples t-test revealed that women were less knowledgeable about the impact of male age on fertility ( $M = 0.95$ ,  $SD = 2.07$ ,  $range = 0 - 3$ ) than female age on fertility ( $M = 2.00$ ,  $SD = 0.69$ ,  $range = 0 - 3$ );  $t(326) = 15.33$ ,  $p < .001$ .

2 items assessed participants' knowledge about the impact of weight and smoking on female fertility, and 2 items assessed knowledge about these im-

pacts on male fertility (e.g., "a woman's weight affects her chances of conceiving" (T); "a man's weight does not impact his likelihood of impregnating his partner" (F)). Participant responses on all items were coded as correct or incorrect. 87% answered both items about female weight and smoking correctly; 49% answered both about males correctly. A dependent samples t-test revealed that women were less knowledgeable about the impact of male weight and smoking on fertility ( $M = 1.41$ ,  $SD = 0.64$ ,  $range = 0 - 2$ ) than female weight and smoking on fertility ( $M = 1.86$ ,  $SD = 0.38$ ,  $range = 0 - 2$ );  $t(326) = 13.13$ ,  $p < .001$ .

**CONCLUSIONS:** These results suggest that women are relatively informed about the impact of their own age, weight, and smoking on fertility but less informed about the impact of male age, weight, and smoking on fertility. These misconceptions may disproportionately assign responsibility for preconception health to women. Providers should be aware of these misconceptions in order to educate patients on the role of male fertility risk factors. Correcting these misconceptions may be a critical step towards decreasing infertility by changing unhealthy behaviors and alleviating the emotional load on opposite-sex coupled women.

**SUPPORT:** This research was funded by Modern Health, Inc.

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#### THE DEGRADATION OF VITAMIN D ACROSS TIME: AN ISSUE LEADING TO UNRELIABLE RESULTS IN REPRODUCTIVE RESEARCH.

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**OBJECTIVE:** Vitamin D deficiency is widely reported with significant impact on many health processes, including reproduction. However, many studies evaluate the impact of Vitamin D utilizing banked samples, and the stability of vitamin D after a prolonged storage is often not taken into account. We aimed to determine if 25-hydroxyvitamin D (25(OH)D<sub>3</sub>) and its main catabolite 24,25-dihydroxyvitamin D (24,25(OH)<sub>2</sub>D<sub>3</sub>) in serum and follicular fluid, are stable across time in frozen samples.

**DESIGN:** Prospective, non-interventional study.

**MATERIALS AND METHODS:** Controlled ovarian stimulation was performed in thirty-five egg donors using an antagonist protocol and standard doses of subcutaneous FSH. After 36 hours of a GnRH agonist bolus, the oocytes retrieval was performed. Serum samples and pooled follicular fluid from mature follicles were collected during pick-up for 24,25(OH)<sub>2</sub>D<sub>3</sub> and 25(OH)D<sub>3</sub> measurements via LC-MS/MS using a UPLC-TQ-S Xevo Waters system with a Waters Acquity BEH C18 (1,7µm 2,1x100 mm) column. A baseline Vitamin D analysis and a second one after seven months of storage at -80°C were performed. After testing the normal distribution of metabolites concentration with Shapiro-Wilcoxon, a t-test (when normal distribution) or a Wilcoxon test (for non-normal distribution) was performed in order to contrast mean differences before and after storage.

**RESULTS:** A significant decrease in 25(OH)D<sub>3</sub> concentrations after 7 months of storage was found in serum (from 91.56 ± 39.01 nM to 62.235 ± 24.09 nM,  $p$ -value=2.68e-11) and follicular fluid (from 58.13 ± 19.55 to

36.37 ± 12.82 nM, p-value=2.52e-11). In contrast, serum and follicular fluid concentrations of 24,25(OH)<sub>2</sub>D<sub>3</sub> remained more stable, (from 15.62 ± 11.00 to 16.94 ± 10.55, and from 11.27 ± 6.09 to 11.86 ± 6.58 respectively, p-value=NS).

**CONCLUSIONS:** 25(OH)D<sub>3</sub> degrades significantly in stored samples, thus limiting its usefulness in research and clinical practice. The more stable values of 24,25(OH)<sub>2</sub>D<sub>3</sub> suggest it is as a more accurate and reliable metabolite evaluating vitamin D status when utilizing stored specimens.

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**IMPACT OF AMBIENT TOTAL VOLATILE ORGANIC COMPOUND (TVOC) DURING IN VITRO FERTILIZATION (IVF) LABORATORY PROCEDURES UPON SUBSEQUENT EMBRYO IMPLANTATION: A RETROSPECTIVE STUDY.**

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**OBJECTIVE:** To investigate the associations between laboratory TVOC levels measured during *in vitro* manipulation of human oocytes/embryos and subsequent implantation.

**DESIGN:** Retrospective cohort study in a private IVF center.

**MATERIALS AND METHODS:** Consecutive IVF cycles (n=103; female age 35.9±4.5 yr) performed at Reproductive Medicine Center, Tianjin United Family Hospital, between August 2018 and April 2019 were included. Intracytoplasmic sperm injection (ICSI) cycles were excluded due to the confounding effect of extra exposure of oocytes during sperm injection. Ambient TVOC readings were continuously logged at 6-minute intervals by a specialized designed device (HuChuang, China) at a fixed position in the embryology laboratory. The readings were retrieved at the closest time point to 4 procedures where embryos were exposed to the ambient environment, namely egg collection, insemination, fertilization check, and D3 embryo check. Embryos were cultured in MINC incubators (Cook) at 37 °C perfused with clean cylinder gas (6% CO<sub>2</sub>, 5% O<sub>2</sub>, and balance N<sub>2</sub>) post in-line VOC removal. One or two embryos ranked the highest from the cohort were transferred on either D3 or D5, depending on the prognosis of individual patients. Implantation detected by rising hCG was evaluated via multiple variable logistic regression against cycle characteristics, embryology parameters and TVOC levels, expressed by odds ratio (OR) and 95% confidence interval (CI). Proportional embryology parameters were compared using the  $\chi^2$  analysis.

**RESULTS:** TVOC levels ranged from 0.15 to 1.98 ppm despite extensive filtration of laboratory air (*in-situ* HEPA filters, stand-alone IQAir and Coda Tower), reflecting the influence of outside atmospheric conditions. Multiple variable logistic regression showed statistically significant associations between implantation and female age (OR=0.817[0.723-0.924], P=0.001), D3 or D5 embryo transfer (OR=12.078[1.105-132.062], P=0.041), and the TVOC levels at egg collection (OR=0.171[0.035-0.835], P=0.029). No effect was seen on the number of previous attempts (1.240[0.725-2.120], P=0.432), stimulation protocol (1.269[0.486-3.316], P=0.627), number of eggs collected (0.954[0.828-1.098], P=0.509), number of embryos transferred (8.954[0.871-92.021, P=0.065]), TVOC levels at insemination (1.782 [0.162-19.664], P=0.637), at fertilization check (1.013 [0.277-3.704], P=0.984) and at D3 embryo check (1.401[0.563-3.489], P=0.468). Using a cut-off at the median TVOC reading (0.64 ppm) at egg collection, there were no significant differences in the fertilization rates (64.1% vs 65.0%, P=0.776), D3 good quality embryo rates (65.5% vs 65.1%, P=1) and embryo utilization rates (50.5% vs 50.6%, P=1). However, a significantly reduced implantation rate (57.7% vs 37.3%, P=0.038) was seen when comparing low to high TVOC groups.

**CONCLUSIONS:** High levels of TVOC at egg collection, rather than at insemination or fertilization check or D3 embryo check, were associated with a reduced chance of implantation following IVF. However, conventional embryology parameters were not adversely affected.

**P-584** Wednesday, October 16, 2019 6:30 AM

**SEASONAL ENVIRONMENTAL CONTAMINANTS APPEAR TO INFLUENCE GAMETE PRODUCTION AND PREGNANCY OUTCOME FOLLOWING ART.**

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**OBJECTIVE:** It is well known that environmental contaminants can affect many aspects of human health including fertility. While most research has focused on compounds like bisphenol A (BPA), there are other compounds, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and Paraquat, that could be of concern in areas with intensive agriculture. Due to their chemical structures, common herbicides and pesticides could potentially interrupt reproductive function. The objective of this study is to correlate environmental exposure differences between urban and rural populations with fertility treatment success and to better understand patient responsiveness to assisted reproductive technologies (ART) as a result of these environmental exposure differences.

**DESIGN:** Chart review study evaluating the relationship between patient environment and ART outcomes.

**MATERIALS AND METHODS:** Patients were assessed based off of ART procedure reports from 2014-2017 (N= 267) and were categorized into urban and rural populations. For male patients, sperm concentration, semen volume, and percent motility were evaluated pre- and post-wash. For female patients, the number of oocytes retrieved, normal versus abnormal fertilization, embryo development, and pregnancy outcome were analyzed. Further, pregnancy outcomes were evaluated based on month of retrieval to examine seasonal differences related to agricultural practices of the region. Statistical significance was determined using statistical package for social sciences (SPSS) to run two-way ANOVA, Tukey, and one-way statistics.

**RESULTS:** Men who lived in rural environments had significantly lower pre-wash sperm concentrations (p=0.05) than men who lived in urban environments; however there was no difference in pre-wash semen volume (p=0.06). Women who lived in rural environments had lower numbers of embryos retrieved (p<0.05), lower numbers of atretic embryos (p<0.05), and lower numbers of healthy embryos (p=0.05) compared to women from urban environments. However, fertilization rates, embryo development, and pregnancy outcome did not differ (p>0.45). While not statistically significant due to not reaching power, pregnancy outcomes based on season appear to correlate with decreased success in months with intensive agricultural activity with success rates ranging from 8.3%-34.8% in March, April, September, and October while in months during the growing season and post-harvest, ART success rates range from 41.7%-69.2%.

**CONCLUSIONS:** Semen parameters vary between urban and rural populations, as demonstrated in previous research. Pre-fertilization parameters are different between women from urban and rural environments. However, embryo development and pregnancy outcomes are not different. Pregnancy outcomes from assisted reproductive technologies may be affected by season and corresponding environmental factors. There appears to be a change in success of ART procedures during various times of year that could be caused by agricultural activity, but further research is needed to determine if and how environmental factors affect gamete production and ART outcomes.

**SUPPORT:** None.

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**THE USE OF PRESCRIPTION DRUGS AMONGST MEN AND WOMEN UNDERGOING ASSISTED REPRODUCTIVE TECHNOLOGY (ART) PROCEDURES.**

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**OBJECTIVE:** A pilot study aiming to determine the trends of prescription medication amongst men and women undergoing ART and the associated live birth rates.

**DESIGN:** This was a retrospective cohort study of heterosexual couples undergoing ART between October 2016 and November 2017 in a tertiary reproductive medicine unit. This pilot study was conducted as an undergraduate medical student project.

**MATERIALS AND METHODS:** A predefined proforma was used to extract data manually from each patient record for couples who had underwent ART in the defined time period. Information obtained included the drug history of both partners as well as smoking status and units of alcohol consumed each week. The outcome of the ART cycle was recorded. This data was then entered electronically into a spreadsheet and prevalence and

trends of prescription medication use were analysed. Since the sample size was insufficient for reliable statistical analysis, a descriptive report of the prescription drug utilisation patterns was created.

#### RESULTS: • MATERNAL MEDICATION

Out of 400, there were 90 (22.5%) women taking prescription medications and 44 (11%) on no medications. There were 266 (66.5%) women on folic acid and/or vitamin D alone. The live birth rate of the women on prescription medications was 32.2% (n=29). The live birth rate of the 44 women on no medications was 29.5% (n=13). The live birth rate of the women on folic acid and/or vitamin D was 33.5% (n=89).

There were a total of 60 different medications at an average of 1.4 per patient.

The most common medications were asthma medications (n=22), levothyroxine (n=12), selective serotonin re-uptake inhibitors (SSRIs) (n=10), ferrous sulphate (n=8), and diabetic medications (n=7).

Food and Drug Administration categories: 1 medication and 12 prescriptions at Category A. Category B: 20 medications and 29 prescriptions. Category C: 25 medications and 62 prescriptions. Category D: 6 medications and 6 prescriptions. Category X: 1 medications and 1 prescription.

#### • PATERNAL MEDICATION

Out of 400, 88 male partners were on prescription medication (22%). The live birth rate for those on medication was 30.7% (n=27) compared to those not on medication 33.0% (n=103).

#### • SMOKING

Female: out of 376, 19 smoked (5.1%) averaging 6.1 per day (Standard Deviation (SD) 4.47). Live birth rate in the smokers was 21.1% (n=4) compared to non-smokers, 30.5% (n=109).

Male: Out of 316 male patients, 44 smoked (13.9%) averaging 8.3 per day (SD 6.9). The live birth rate in smokers was 27.3% (n=12) compared to 32.0% (n=101).

#### • ALCOHOL

Female: out of 275, 178 drank alcohol (47.5%), averaging 5.8 units per week (SD 5.6). The live birth rate in drinkers was 28.1% (n=50) compared to 36.5% (n=72).

Male: out of 375, 240 drank alcohol (64%), averaging 9.1 units per week (SD 8.2). The live birth rate in drinkers was 33.8% (n=81) compared to 30.4% (n=41).

CONCLUSIONS: The study found that a large number of women are prescribed category C, D or X drugs when attempting ART. The effect these drugs have on the success of ART is unclear. More information is required in order to expand these results and help counsel couples on prescription drug use, smoking and alcohol consumption.

SUPPORT: None.

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#### DIETARY CADMIUM INTAKE AND FECUNDABILITY IN A NORTH AMERICAN PRECONCEPTION COHORT STUDY.

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OBJECTIVE: To evaluate the association between dietary cadmium intake (D-Cd) and fecundability. Diet is one of the main sources of cadmium, and D-Cd is often used as indicator of cadmium exposure, particularly in non-smoking populations. In a previous preconception cohort study of 501 couples,<sup>1</sup> high female cadmium concentrations measured in whole blood were associated with reduced fecundability.

DESIGN: Prospective cohort study (2013-2018).

MATERIALS AND METHODS: Pregnancy Online Study (PRESTO) is a North American prospective preconception cohort of pregnancy planners. At baseline, female participants aged 21-45 years completed a web-based questionnaire on demographic, lifestyle, medical and reproductive factors. Ten days after enrollment, participants completed the National Cancer Institute Dietary History Questionnaire II, a validated food frequency questionnaire (FFQ) of average intake during the previous year. D-Cd ( $\mu\text{g}/\text{day}$ ) was estimated by combining FFQ responses with US Food and Drug Administration data on food cadmium content. Participants were then followed for up to 12 months or until reported pregnancy, whichever came first. The analysis included 4,768 women attempting to conceive for  $\leq 6$  cycles at study entry

and not using fertility treatment. We used a proportional probabilities regression model to estimate fecundability ratios (FR) and 95% confidence intervals (CI), adjusted for age, body mass index (BMI), smoking history, parity, physical activity, last method of contraception, daily use of multivitamins, race/ethnicity, education, income, geographic region, and the 2010 healthy eating index score. We used the nutrient residual approach to adjust for energy intake.

RESULTS: Median D-Cd was 8.0  $\mu\text{g}/\text{day}$  (interquartile range: 7.0-9.1  $\mu\text{g}/\text{day}$ ). The top 5 contributors to D-Cd were nuts and seeds; fried potatoes; dark green lettuce; cooked greens; and white potatoes. Compared with an average D-Cd of  $< 6.8 \mu\text{g}/\text{day}$ , FRs for D-Cd quintiles of 6.8-7.6, 7.7-8.4, 8.5-9.5, and  $\geq 9.6 \mu\text{g}/\text{day}$  were 1.03 (CI: 0.92-1.14), 1.07 (CI: 0.96-1.18), 1.07 (CI: 0.96-1.19), and 1.08 (0.97-1.20), respectively. Results were not appreciably different among never smokers with no current passive smoke exposure, for whom cadmium exposure from other sources (e.g., cigarettes) would be lower (respective FRs: 1.02, 1.05, 1.06 and 1.02). Results did not differ materially by age ( $< 30$  vs.  $\geq 30$  years), BMI ( $< 30$  vs.  $\geq 30 \text{ kg}/\text{m}^2$ ), total fiber intake ( $< 25$  vs.  $\geq 25 \text{ g}/\text{day}$ ), geographic region of residence (West, Midwest, Northeast, South, Canada), or attempt time at study entry ( $< 3$  vs.  $\geq 3$  cycles).

CONCLUSIONS: Dietary intake of cadmium was not appreciably associated with fecundability, though exposure misclassification and confounding could explain the null results.

References: <sup>1</sup> Buck Louis GM, Sundaram R, Schisterman EF, Sweeney AM, Lynch CD, Gore-Langton RE, Chen Z, Kim S, Caldwell KL, Barr DB. Heavy metals and couple fecundity, the LIFE Study. *Chemosphere*. 2012 Jun;87(11):1201-7. <https://doi.org/10.1016/j.chemosphere.2012.01.017>. Epub 2012 Feb 4. PubMed PMID: 22309709; PubMed Central PMCID: PMC3327819.

SUPPORT: NIH/NICHD grant: R01HD086742.

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#### ACCURACY OF SELF-REPORTED MENSTRUAL CYCLE CHARACTERISTICS AND INFERTILITY IN A COHORT HIGHLY EXPOSED TO ENDOCRINE-DISRUPTING COMPOUNDS (EDCs).

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OBJECTIVE: To determine whether reproductive health outcomes are associated with changes in menstrual function among women within the Michigan Polybrominated Biphenyl (PBB) Registry.

DESIGN: Cross-sectional survey of women in the Michigan PBB Registry.

MATERIALS AND METHODS: In 1973, accidental contamination of livestock feed with PBB led to the Michigan Health Department establishing a registry of highly exposed individuals who have been followed for  $> 40$  years. Women who were not pregnant, breastfeeding, using hormonal medications, developmentally disabled, diagnosed with cancer, amenorrheic <sup>3</sup> three months, or with prior hysterectomy were recruited into a menstrual function study. 176 women completed a reproductive health survey, obtained daily morning urine samples throughout 4 menstrual cycles, and completed 6 months of daily menstrual cycle diaries. The morning urine samples were analyzed for Estrone-3-glucuronide (E13G), Pregnanediol-3-glucuronide (Pd3G), mid-cycle creatinine, and day of luteal transition (DLT). We used a nested mixed linear model to quantify the accuracy of menstrual cycle data and to test for association between endometriosis, infertility, and urinary hormone metabolites.

RESULTS: Women's self-reported cycle and bleed length correlated accurately with their actual cycle length ( $p=0.002$ ) and bleed length ( $p=2.31\text{E}-13$ ) from urinary metabolite data. Women with self-reported endometriosis were noted to have higher preovulatory E<sub>1</sub>3G ( $p=0.001$ ), mid-luteal E<sub>1</sub>3G ( $p=0.0006$ ), and overall luteal phase E<sub>1</sub>3G ( $p=0.033$ ) levels. Women with self-reported infertility were noted to have higher mid-luteal E<sub>1</sub>3G ( $p=0.019$ ) and overall luteal phase E<sub>1</sub>3G ( $p=0.008$ ) levels.

CONCLUSIONS: Women with self-reported diagnoses of endometriosis and infertility showed statistically significant changes in their menstrual function urinary metabolites. Additionally, this study found that the self-

reported cycle characteristics were positively associated with actual cycle characteristics. Further analyses are required to determine the clinical implications of these menstrual function metabolite changes within this population of women highly exposed to endocrine disrupting compounds.

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#### IMPACT OF NANOPARTICLES ON MOTILITY OF HUMAN SPERMATOZOA.

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**OBJECTIVE:** Nanomaterials, including a large array of nanoparticles are integral components of daily-used products including food, sunscreens, cosmetics and pharmaceuticals. Human and environmental exposure to nanomaterials is commonly occurring, and as the use of nano-enabled products become more widespread, so too will concerns around their safety and impact on human and environmental health. Nanoparticles are reported to be amongst major factors that influence development of various medical conditions including reproductive disorders. But until now, the impact of nanoparticles on sperm motility has not been established. Here we investigate for the first time how nanoparticles affect human sperm motility.

**DESIGN:** Experimental study with human normal sperm.

**MATERIALS AND METHODS:** We treated human normal sperm with titanium dioxide (E171) and nanodiamond. Then we performed human spermatozoa motility after incubation with titanium dioxide and nanodiamond particles. Human spermatozoa were incubated with 1 ng, 10 ng, 100 ng and 1000ng of each of the nanoparticle class. Sperm motility profiling was done using computer-aided sperm analysis (CASA) system every 30 min until overnight. We analysis whether nanoparticles were attracted to regions of spermatozoa by electrical microscope. Furthermore, we imaged the surface with electrical microscopy.

**RESULTS:** Both nanoparticles have shown significantly decreased in sperm motility. Titanium dioxide has been shown 20~30% decrease motility compares with control group. Nanodiamond also reduced 10 ~ 20% motility of sperm. In the CASA study, both nanoparticles showed significantly reduction in straight movement pattern compare with control sperm. However, both nanoparticles did not show any significant cytotoxicity to the human sperm up to 1000 ng/ml concentration.

**CONCLUSIONS:** In this study, we showed that nanogram concentration of nanoparticles decreases motility of human sperm. There is no literature evidence regarding the exposure of human testis and sperm to nanoparticles that are in the blood stream. But these studies suggest that both nanoparticles reduce motility, thus impact negatively on fertilization. Currently titanium dioxide (TiO<sub>2</sub>) nanoparticles (NPs) are widely used in food, agriculture products, personal care products, cosmetics, sun protection and toothpaste, electronics, and food packaging. Our future research will investigate the mechanism behind the observed effects, because such information would facilitate the production of nanoparticles with increased biosafety.

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#### THE EFFECT OF CURRENT AND PRIOR SMOKING, ALCOHOL CONSUMPTION, AND DRUG ABUSE ON IVF OUTCOMES.

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**OBJECTIVE:** To assess the effect of cigarette smoking, alcohol consumption, and drug use on semen parameters, number of oocytes retrieved, fertilization rate, embryo development, and pregnancy outcomes in infertile couples undergoing IVF.

**DESIGN:** Retrospective study in a University based fertility program

**MATERIALS AND METHODS:** All ART cycles (n = 935) performed from July, 31, 2010 to January 31, 2017 at DHMC IVF program were

analyzed. Semen parameters, embryology data and pregnancy outcomes were compared by the male and female's current or prior cigarette smoking, alcohol consumption, and drug use. The control group consisted of infertile couples without current or prior smoking, alcohol consumption and drug use.

**RESULTS:** There were 493 couples (mean female age 34.42 ± 4.63) with a total of 935 ART cycles. In males, being a former smoker, alcohol consumer and drug user significantly reduced semen volume (p = 0.004), sperm count (p = 0.04), and total motile sperm (p = 0.05). Male drug users who were current (p=0.05) or former (p = 0.02) smokers had significantly lower mean sperm count. Male alcohol consumers who were also former smokers had significantly lower mean sperm motility (p=0.01). Female former smokers, alcohol consumers, and drug users had significantly lower number of eggs retrieved as compared to the control group (p=0.02). However, for these females, mean embryo cleavage and 2PN were only trending toward significantly lower compared to the control group (p=0.06). Significantly higher mean abnormal fertilization (1PN and 3PN) were observed in current female smokers (p=0.04). The effects of smoking, alcohol, and drug use variables for females on a categorical pregnancy variable with levels of positive, negative, and Ectopic/SAB were tested, which showed significantly higher percentages of Ectopic/SAB than the other levels, for female current smokers (p = 0.04) and female alcohol consumers who are also current smokers (p = 0.01), compared to the control group.

**CONCLUSIONS:** Our study showed lower semen parameters in infertile males and lower embryology and pregnancy outcomes in infertile females who were either current or former smokers, alcohol consumers, or drug users.

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#### BISPHENOL A INDUCES INSULIN RESISTANCE IN SKELETAL MUSCLE BY DOWN-REGULATING THE EXPRESSION OF IRS1 THROUGH ESTROGEN RECEPTOR.

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**OBJECTIVE:** To investigate the effect of human relevant doses of bisphenol A (BPA), an endocrine disruptor, on insulin resistance and the underlying mechanisms.

**DESIGN:** Mice were administered with water containing BPA of human relevant doses. C2C12 myocytes were treated with BPA and selective estrogen/androgen receptor down-regulator. Bioinformatic analysis was applied to search for estrogen receptor response element (ERE) in Irs1.

**MATERIALS AND METHODS:** Mice were administered with water containing BPA of human relevant dose (2.5 µg/L) or high human relevant dose (25 µg/L) from the day they were born to 8-week-old. The serum levels of fasting glucose and insulin were measured. Differentiated C2C12 myocytes were treated with BPA, and, with or without ICI 182,780 or flutamide. ICI 182,780 and flutamide are selective estrogen and androgen receptor down-regulator. The expression levels of key players in insulin signaling pathway of skeletal muscle and C2C12 myocytes was measured by real-time PCR. The expression levels of key players in lipid metabolism and transportation of skeletal muscle were also measured. Bioinformatic analysis was applied to search for ERE in Irs1.

**RESULTS:** Mice of 25µg/L group exhibited increased fasting insulin levels and visceral adipose weight compared with control. The expression levels of Irs1 were down-regulated in skeletal muscle of mice from both BPA groups. In contrast, the expression levels of other key players in insulin signaling pathway, including Ir, Akt2, As160 and Glut4, showed no difference between BPA and control groups. Furthermore, key players in lipid metabolism and transportation of skeletal muscle, including Fatp1, Cd36, Atgl, Pnpla3, Dgat1 and Spt, didn't show significant difference in expression levels between groups, either.

In consistence with results in mice, C2C12 myocytes administered with BPA showed decreased expression of Irs1 compared with control. Treatment of both BPA and flutamide also resulted in decreased transcription of Irs1. In contrast, C2C12 myocytes treated with both BPA and ICI 182,780 showed no difference in the transcription level of Irs1 compared with control, indicating that BPA might down-regulate the expression of Irs1 through estrogen receptor. Furthermore, we found ERE of high score in the promoter of Irs1 through bioinformatic analysis.

**CONCLUSIONS:** Human relevant exposure of BPA induces insulin resistance in young mice. BPA may induce insulin resistance in skeletal muscle by down-regulating the expression of Irs1 through estrogen receptor.

## FIBROIDS

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### IMPROVEMENT IN HEALTH-RELATED QUALITY OF LIFE (HRQoL) IN WOMEN WITH UTERINE FIBROIDS (UF) TREATED WITH VILAPRISAN (VPR): A SUMMARY OF RESULTS FROM ASTEROID 1 AND 2.



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**OBJECTIVE:** To assess the impact of VPR, ulipristal acetate (UPA) and placebo on HRQoL in women with UFs.

**DESIGN:** Two multicenter, double-blind, randomized phase 2 studies examining the efficacy and safety of VPR compared with placebo (ASTEROID 1 and 2), and an active comparator (ASTEROID 2).

**MATERIALS AND METHODS:** 309 and 172 women with UFs and heavy menstrual bleeding (HMB) were enrolled in ASTEROID 1 and 2, respectively. In ASTEROID 1, women received oral placebo or VPR 0.5 mg, 1 mg, 2 mg, or 4 mg once daily (OD) for 12 weeks, with follow-up at 24 weeks. In ASTEROID 2, women received VPR 2 mg, UPA 5 mg, or placebo OD for one or two 12-week treatment periods, with 12 weeks' follow-up. HRQoL was assessed using the Uterine Fibroid Symptom and Quality of Life (UFS-QoL) questionnaire and the Short-Form 36 Health Survey Version 2 (SF-36v2) during baseline (BL), end of treatment (EoT) and end of follow-up (EoFUP) visits.

**RESULTS:** Across treatment groups in ASTEROID 1 at BL, the mean (SD) UFS-QoL total HRQoL scores were between 46.2 (24.1) and 49.9 (25.0). SF-36v2 physical component scores (PCS) were between 45.6 (7.3) and 47.5 (7.8), and SF-36v2 mental component scores (MCS) were between 43.3 (11.5) and 47.5 (8.3). All BL scores were in line with previously published data for women with UFs.<sup>1</sup> At EoT, the mean total and subscale UFS-QoL and SF-36v2 scores in all VPR-treated groups increased compared with BL. These increases were greater than those observed with placebo. The mean (SD) UFS-QoL total HRQoL scores increased to 83.2 (19.7) in the highest and 78.3 (24.0) in the lowest VPR dose group, versus 56.2 (24.8) in the placebo group. Mean (SD) SF-36v2 PCS and MCS increased to 51.7 (6.2) and 52.0 (8.9) in the lowest VPR dose group, and 53.7 (6.5) and 51.1 (8.9) in the highest VPR dose group, respectively. By comparison, the mean (SD) PCS and MCS at EoT in the placebo group were 48.4 (7.8) and 43.9 (12.2), respectively. The EoT UFS-QoL and SF-36v2 scores of women that received VPR were in the range of those previously published for healthy women without UFs.<sup>1</sup>

Similar results were observed in ASTEROID 2. Mean UFS-QoL HRQoL and SF-36v2 BL scores increased continuously during treatment with VPR for up to 24 weeks, whereas increased scores were observed for the first 12 weeks with UPA but did not continue to 24 weeks (not statistically tested). The mean (SD) UFS-QoL total HRQoL scores across VPR dosage groups increased from between 78.5 (21.0) and 81.9 (16.4) after 12 weeks, to between 82.4 (17.5) and 85.6 (13.2) after 24 weeks' treatment. In the UPA group, this score decreased from 75.0 (19.6) at 12 weeks to 73.4 (20.8) at 24 weeks.

In general, the mean UFS-QoL total HRQoL and SF-36v2 scores decreased only slightly between treatment cessation and EoFUP across active treatment groups in ASTEROID 1 (6 months) and 2 (3 months).

**CONCLUSIONS:** In ASTEROID 1 and 2, increases in mean UFS-QoL and SF-36v2 HRQoL scores were observed alongside reduced HMB in women with UFs during active treatment with VPR and UPA for up to 24 weeks, and were sustained to EoFUP. Furthermore, by EoT, HRQoL scores increased to levels previously published for healthy women without UFs.<sup>1</sup>

**References:** 1. Coyne KS, Margolis MK, Bradley LD, et al. Further validation of the uterine fibroid symptom and quality-of-life questionnaire. *Value Health*. 2012;15(1):135–142

**SUPPORT:** This study was funded by Bayer AG. Medical writing support provided by Huntsworth Health was funded by Bayer.

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### VALIDATION OF A MENSTRUAL PICTOGRAM AND A DAILY BLEEDING DIARY AS ALTERNATIVES TO THE ALKALINE HEMATIN (AH) METHOD FOR ASSESSMENT OF EFFICACY OF TREATMENTS FOR UTERINE FIBROIDS (UF) IN CLINICAL STUDIES.



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**OBJECTIVE:** To determine whether the menstrual pictogram super absorbent polymer containing version 3 (MP SAP-c v3) and uterine fibroid daily bleeding diary (UF-DBD) demonstrate reliability, validity, and sensitivity to change and can replace the Alkaline Hematin (AH) method for assessment of efficacy in UF clinical trials.

**DESIGN:** Post-hoc analysis of vilaprisan phase 2 (ASTEROID 1 and 2) clinical study data in terms of psychometric properties, missing data, and comparability of methods.

**MATERIALS AND METHODS:** ASTEROID 1 (N=623) study data collected by MP SAP-c v3, UF-DBD, and the AH method were used to assess psychometric properties of the MP SAP-c v3 and UF-DBD, and degree of comparability and extent of missing data with the AH method. Daily scores aggregated over 28 days (monthly) and during bleeding episodes at randomization (RND) and end of treatment (EoT) were analyzed. ASTEROID 2 (N=228) study data were used to confirm ASTEROID 1 findings as appropriate.

**RESULTS:** ASTEROID 1 data analysis showed that the response distributions of MP SAP-c v3 and UF-DBD appropriately reflected the natural cycle of menstrual bleeding and treatment-related changes. The full range of responses were used to assess bleeding severity. Based on bleeding severity defined by the AH method and overall patient global impression of severity, differences in MP SAP-c v3 and UF-DBD scores between low- and high-severity groups were large and significant ( $p < 0.001$ ). Strong Spearman's rank correlations were observed between MP SAP-c v3 monthly sum scores and those of both AH ( $r_s = 0.72$  and  $0.97$ ) and UF-DBD ( $r_s = 0.56$  and  $0.89$ ) at RND and EoT, respectively. Moderate to strong correlations were also observed between UF-DBD and AH monthly sum scores at RND ( $r_s = 0.44$ ) and EoT ( $r_s = 0.84$ ). Test-retest reliability and sensitivity to changes were also demonstrated. Analyses of ASTEROID 2 data largely confirmed findings from ASTEROID 1.

In ASTEROID 1, details of more sanitary protection items were provided using the MP SAP-c v3 than with the AH method. Fewer days with missing data were observed with the MP SAP-c v3 and UF-DBD than with AH; over the course of the study the mean absolute (relative) number of days with missing values per patient was 16.1 (11.8%) for MP SAP-c v3, 15.5 (11.6%) for UF-DBD and 18.1 (15.9%) for AH.

Both instruments showed good agreement with the AH method in assessments of study eligibility (MP SAP-c v3) and treatment response (MP SAP-c v3, UF-DBD). The positive predictive value (PPV) of MP SAP-c v3 to distinguish women with heavy menstrual bleeding at baseline vs. the AH method (reference standard) was 75.8%. The PPVs of MP SAP-c v3 and UF-DBD for reaching amenorrhea were 100.0% and 99.3%, respectively.

**CONCLUSIONS:** The MP SAP-c v3 and UF-DBD are valid, reliable, and sensitive measures of menstrual bleeding severity in the UF population. Use of both instruments is more convenient, less burdensome, and associated with greater compliance than the AH method. Coupled with positive results from cognitive interviews and Bland-Altman analyses, these results support the use of MP SAP-c v3 and UF-DBD in UF clinical studies instead of the AH method.

**SUPPORT:** This study was funded by Bayer AG. Medical writing support provided by Huntsworth Health was funded by Bayer.

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### PHENOME-WIDE ASSOCIATION STUDY OF UTERINE FIBROIDS USING A LARGE MULTI-RACIAL CLINICAL POPULATION OF WOMEN.



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**OBJECTIVE:** Uterine fibroids affect up to 70% of women by menopause. Prior studies have identified several clinical factors associated with fibroid risk by evaluating candidate risk factors from observational epidemiology. We identified novel clinical characteristics of women associated with fibroids, gaining insight on causal mechanisms by broadly evaluating the clinical phenome.

**DESIGN:** A phenome-wide association study (PheWAS) tests disease diagnoses across a patient's clinical record for association with a specific outcome. We conducted a PheWAS of uterine fibroids utilizing diagnoses from electronic health records (EHRs) of patients at Vanderbilt University Medical Center (VUMC) in Nashville, TN.

**MATERIALS AND METHODS:** Fibroid cases and controls were identified using a previously validated phenotyping algorithm. We conducted PheWAS analyses with logistic regression models adjusted for body mass index

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(BMI) in black (N=3,571 cases; 12,329 controls) and white (N= 7,348 cases; 57,149 controls) women to observe associations between patient disease diagnoses (>1700 diseases) with fibroid outcome. PheWAS outcomes were categorized into disease groups and a hypergeometric analysis was performed to test for enrichment of specific disease groups among significant ( $p < 5 \times 10^{-5}$ ) and nominally significant results ( $p < 0.005$ ) with Bonferroni correction for multiple testing.

**RESULTS:** Two hundred ninety-one and 486 diagnoses were significantly associated with fibroids in black and white women, respectively. Most associations (black = 95.1%; white 90.4%) had odd ratios >1, suggesting that the diseases are related to increased risk of fibroids. As expected, across racial groups the most significant associations were menstrual disorders including infertility (whites  $p < 1.38 \times 10^{-103}$ ; blacks  $p < 7.56 \times 10^{-30}$ ) and endometriosis (whites  $p < 8.41 \times 10^{-244}$ ; blacks  $3.20 \times 10^{-57}$ ). Novel associations were found for cervical cancer and other malignant neoplasms, malnutrition, diverticulosis, bullous dermatoses, and osteoarthritis. Across racial groups, when analyses were evaluated with disease groups, genitourinary and pregnancy complication diagnoses were overrepresented and circulatory, digestive, and injury (bone fractures) groups were underrepresented.

**CONCLUSIONS:** We observed novel associations of diagnoses with fibroids and confirmed prior observed associations. Both individual disease analyses and analyses by group support an increased risk of fibroids and other genitourinary diseases (e.g. endometriosis, cervical cancers). This PheWAS provides novel insights into fibroids and suggests associative disease complexes, but further research into the phenotype networks and causal mechanisms is needed.

**P-594** Wednesday, October 16, 2019 6:30 AM

#### **PATIENT-REPORTED OUTCOMES OF A PHASE 1 CLINICAL TRIAL OF INJECTABLE COLLAGENASE CLOSTRIDIUM HISTOLYTICUM (EN3835) FOR TREATMENT OF UTERINE FIBROIDS.**

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**OBJECTIVE:** Uterine fibroids may cause a significant reduction in quality of life for affected women. Since fibroids contain an excessive extracellular matrix, we injected fibroids with purified collagenase *Clostridium histolyticum* (EN3835) under transvaginal ultrasound guidance in women scheduled for fibroid removal. Here we report the impact of the treatment on the fibroid-related symptoms following injection with study drug and before fibroid removal.

**DESIGN:** Phase 1 clinical trial.

**MATERIALS AND METHODS:** Changes in subject's quality of life and fibroid-related symptoms were assessed at baseline and following injection of study drug. Standardized-validated questionnaires were used to assess the patient-reported outcomes of quality of life and fibroid-associated symptoms. The McGill Pain questionnaire, the Uterine Fibroid Symptom Health-Related Quality of Life (UFS QOL), and the Visual Analogue Scale (VAS) for pain were utilized. Study subjects were divided into 2 groups. Group 1 had injection followed by surgery at 2-3 days, and subjects in Group 2 had injection followed by surgical removal of fibroids 60-90 days later. Therefore, Group 1 subjects (n=3) completed the post intervention questionnaire at 24-48 hours post-study drug injection. Group 2 subjects (n=9) completed the post intervention questionnaires at 4-8 days and 60-90 days post study drug injection. To compare the changes in patient-reported outcomes, generalized linear mixed effects models with random intercepts for the person and paired t-tests were used;  $p < 0.05$  was considered significant.

**RESULTS:** No clinically significant adverse events related to the study drug were reported. Of note, all subjects reported a decrease in fibroid-related pain on the McGill Pain Questionnaire following study drug injection. Specifically, for Group 1 there was a trend ( $p = 0.195$ ) and for Group 2: 4-8 days post injection ( $p = 0.056$ ), and 60-90 days post injection ( $p = 0.079$ ). Similar trends were observed on the Visual Analogue Scale for pain. No Group 1 subject reported an increase in pain post study drug injection. In Group 2, no subject reported an increase in pain 4-8 days post study drug injection ( $p = 0.854$ ) and only 3 out of 9 reported a mild increase in pain 60-90 days post study injection ( $p = 0.6982$ ). UFS-

QOL Part-1: the symptom severity score for Group 1 showed a mild increase in symptoms in 2 out of 3 subjects, for Group 2, the general trend was a decrease in symptom severity both at 4-8 days and 60-90 days post injection. UFS QOL Part 2: all 3 subjects in Group 1 reported an improvement in health related quality of life, for Group 2, 7 out of 9 subjects reported an improvement in health-related quality of life 4-8 days post injection, and 5 of these subjects sustained the increasing trend at 60-90 days post study drug injection.

**CONCLUSIONS:** Injection of Collagenase *Clostridium histolyticum* into fibroids was well tolerated by all study participants. Interestingly, fibroid-related pain was reduced and there was a trend of decreasing fibroid-related symptoms and improving quality of life. (ClinicalTrials.gov number: NCT02889848).

**SUPPORT:** BioSpecifics Technologies Corporation.

**P-595** Wednesday, October 16, 2019 6:30 AM

#### **MEASURING PATIENT-REPORTED OUTCOMES IN WOMEN WITH HEAVY MENSTRUAL BLEEDING ASSOCIATED WITH UTERINE FIBROIDS: THE BLEEDING AND PELVIC DISCOMFORT SCALE.**

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**OBJECTIVE:** The Uterine Fibroid Symptom and Quality of Life (UFS-QoL) instrument consists of 6 scales and has been used frequently in clinical trials; the symptom severity scale has 8 items covering different types of symptoms and is multi-factorial. The objectives were to a) determine from the UFS-QoL symptom severity scale a factor consisting of items relevant to most women with heavy menstrual bleeding (HMB) associated with uterine fibroids (UF), b) assess the psychometric properties of the new subscale (factor), and c) determine a threshold based on which responders to treatment can be identified.

**DESIGN:** Analyses were based on pooled, blinded data from the first one-third of patients with HMB enrolled in 2 Phase 3 studies of relugolix in UF (N=254), who completed the Patient Global Assessment (PGA) of symptom severity and the UFS-QoL at Baseline and at Week 24.

**MATERIALS AND METHODS:** To assess the factor structure of the UFS-QoL symptom severity scale, factor analyses were performed. The subscale (factor) consisting of symptoms experienced by most patients was chosen for further analysis; psychometric properties were evaluated, including item performance (floor and ceiling effect, item-total correlation, item discrimination index), internal consistency reliability, known-groups validity, and ability to detect change. The meaningful change threshold, which is the smallest improvement on the new subscale that is considered meaningful by patients, was derived by applying anchor-based methods with change in PGA of symptom severity as an anchor. Within and between anchor group changes from Baseline to Week 24 on the subscale were evaluated using the pair t-test analysis of variance. Cumulative distribution function and probability density function curves of change from Baseline by anchor categories were used as supportive information in determining the threshold.

**RESULTS:** Factor analyses revealed that the UFS-QoL symptom severity scale consisted of 3 factors with 3, 2, and 2 items, respectively. Factor 1 had 3 items representing symptoms of HMB in UF that are associated with a high burden experienced by most patients: heavy bleeding during period, passing blood clots during period, and feeling tightness or pressure in pelvis. Hence, this factor, named the Bleeding and Pelvic Discomfort (BPD) scale, was further assessed psychometrically. The BPD scale had no ceiling effects, and all response options were used by patients. The items of the BPD scale were found to work cohesively to inform the total score and to adequately distinguish between patients of different severities. Descriptive statistics supported the construct validity and responsiveness of the BPD scale. A 20-point change on the BPD scale score (range from 0-100) was recommended as the minimum meaningful change threshold for defining a responder. This threshold estimation was based on the '1 category improvement' PGA group as the anchor.

**CONCLUSIONS:** The BPD scale is a short, patient-reported outcome measure relevant to patients with HMB due to UF that can be used to assess their symptom burden.

**SUPPORT:** Myovant Sciences, Inc. provided financial support for the MVT-601-3001 and MVT-601-3002 clinical trials that data are from in this abstract.

**TO STUDY THE EFFECT OF ULIPRISTAL ACETATE (UPA) TREATMENT IN INFERTILE PATIENTS WITH SINGLE TYPE 2-3 FIGO MYOMAS UNDERGOING IVF.**



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**OBJECTIVE:** To study the effect of ulipristal acetate (UPA) treatment in infertile patients with single type 2-3 FIGO myomas undergoing IVF.

**DESIGN:** Prospective study.

**MATERIALS AND METHODS:** This study included infertile women of reproductive age who had single type 2 or 3 FIGO myoma and had to undergo IVF. Ovarian stimulation and oocyte retrieval were performed before treating the uterine myomas. All patients underwent transvaginal ultrasonography and hysteroscopy before and after 3-month treatment with UPA (5 mg/day). The largest diameter and volume of uterine myomas (estimated by virtual organ computer-aided analysis, VOCAL) were recorded before and after UPA treatment. Hysteroscopy was performed after UPA treatment to assess if the myoma distorted the uterine cavity. Patients with myomas that were not distorting the uterine cavity underwent embryo transfer; the other patients underwent hysteroscopic or laparoscopic myomectomy. Pregnancy rate was defined as fetal heart beat observed by transvaginal ultrasonography.

**RESULTS:** 46 women were included in the study. The mean age (±SD) of the study population was 35.6 (±3.8) years. 25 patients had type 2 FIGO myomas and 21 had type 3 FIGO myomas. The mean (±3.8) diameter of the myomas was 3.1 (±1.7) cm. The 3-month UPA treatment was completed by 43 patients (93.5%; 95% C.I., 82.1%-98.6%). After UPA treatment, hysteroscopy showed that the percentage of myomas that were not distorting the uterine cavity was significantly higher in patients with type 3 myomas (n = 9; 42.9%; 95% C.I., 21.8%-66.0%) than in those with type 2 myomas (n = 3; 12%; 95% C.I., 2.5%-31.2%; p = 0.018). These patients underwent frozen-thawed embryo transfer. All patients with myomas distorting the uterine cavity after UPA treatment underwent myomectomy. There was no significant difference in the pregnancy rate per embryo transfer in patients who underwent myomectomy (12/34; 33.3%; 95% C.I., 9.9%-65.1%) and did not undergo surgery (4/12; 35.3%; 95% C.I., 19.7%-53.5%; p = 0.902). The patients underwent a median of two embryo transfer (range, 1-4). There was no significant difference in the pregnancy rate per patient in women who underwent myomectomy (18/34; 52.9%; 95% C.I., 35.1%-70.2%) and in those did not undergo surgery (7/12; 58.3%; 95% C.I., 27.7%-84.8%; p = 0.104).

**CONCLUSIONS:** In patients with FIGO type 3 myomas, 3-month treatment with UPA may allow to avoid myomectomy and to immediately perform embryo transfer.

**THE IMPACT OF ISOLATED NON-CAVITY DISTORTING INTRAMURAL FIBROIDS ON PREGNANCY OUTCOMES IN IN VITRO FERTILIZATION CYCLES: A SYSTEMATIC REVIEW AND META-ANALYSIS.**



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**OBJECTIVE:** To investigate the impact of non-cavity distorting intramural fibroids on pregnancy outcomes in women undergoing IVF.

**DESIGN:** An exhaustive literature search using EMBASE, MEDLINE, Google Scholar, Cochrane Library, and PUBMED was performed from inception to May 2018. Relevant search terms included: "intramural fibro\*," "intramural leiomyoma\*" or "intramural myoma\*" and "IVF" or "in vitro fertilization." We also searched Biomed Central, [ClinicalTrials.gov](http://ClinicalTrials.gov), WHO International Clinical Trials Registry Platform (ICTRP) and Thomson CenterWatch for unpublished works and ongoing clinical trials.

**MATERIALS AND METHODS:** We included studies with women undergoing IVF treatment who had at least one non-cavity distorting intramural fibroid. The studies had to report one of: Live birth rate (primary outcome), implantation rate, clinical pregnancy rate and miscarriage rate (secondary outcomes). We excluded studies where women also had submucosal fibroids

or had undergone myomectomy. Two authors independently selected studies and extracted data. Methodological quality was assessed using PRISMA guidelines.

**RESULTS:** Among all 15 observational studies that were included (10 retrospective and 5 prospective), patients with non-cavity distorting intramural fibroids had 32% lower odds of clinical pregnancy (estimated average OR = 0.68, 95%CI = 0.56 to 0.83, p = 0.0002) and 44% lower odds of live birth than patients without fibroids undergoing IVF (OR=0.56, 95%CI=0.46-0.69, p<0.0001). While there was a trend toward lower implantation rate (estimated average OR = 0.76, 95%CI = 0.58 to 1.12, p=0.06) and increased miscarriage rate (estimated average OR = 1.38, 95%CI = 0.98 to 1.95, p = 0.07) in patients with these fibroids, the results did not reach statistical significance.

Among studies that explicitly excluded patients with concurrent subserosal fibroids, patients with isolated intramural fibroids showed consistently lower odds of clinical pregnancy and live birth (OR = 0.70, 95%CI=0.50-0.97 and OR = 0.62, 95%CI=0.45-0.84, respectively). Similarly, this trend was consistent among studies that reported pregnancy rate per IVF cycle (0.70, 95%CI=0.53-0.92) as well as those that reported cumulative pregnancy rates (0.72,95%CI=0.55-0.94).

**CONCLUSIONS:** This meta-analysis demonstrates that non-cavity distorting intramural fibroids are associated with decreased live birth rates and clinical pregnancy rates in women undergoing IVF. However, RCTs and prospective studies that standardize their patient selection criteria and IVF methods are needed in order to better address this question.

**RISK FACTORS FOR COEXISTENT LEIOMYOMA AND ADENOMYOSIS COMPARED TO COEXISTENT LEIOMYOMA AND ENDOMETRIOSIS IN PATIENTS WITH SYMPTOMATIC LEIOMYOMA REQUIRING MYOMECTOMY.**



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**OBJECTIVE:** To investigate the risk factors for coexistent leiomyoma with endometriosis and leiomyoma with adenomyosis in a population of women who elect for myomectomy due to symptomatic leiomyoma.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** This was a single-center case control study to compare patients with leiomyoma only, to those that had either coexistent leiomyoma and endometriosis or leiomyoma and adenomyosis. Multinomial logistic regression models were used to compute relative-risk ratios (RRR) for the association between patients with endometriosis or adenomyosis in the presence of leiomyoma with patients with leiomyoma only. The analysis was first performed for each independent variable alone and subsequently, a best-subset selection method was used to construct a parsimonious model by selecting the model with lowest Akaike information criteria (AIC).

**RESULTS:** 961 patients underwent surgery for symptomatic leiomyoma only, and either coexistent leiomyoma and adenomyosis or endometriosis (leiomyoma only (L) – 799, leiomyoma and endometriosis (LE) – 98 and leiomyoma and adenomyosis (LA) – 64 patients respectively) between

TABLE 1. Relative-risk ratios (RRR) from most parsimonious\* multinomial logistic regression model

	RRR	95% C.I.	p-value
<b>Fibroids only</b>	(base outcome)		
<b>Fibroids + Endo</b>			
Age	0.960	0.912, 1.010	0.107
Parity	0.308	0.109, 0.875	0.027
Uterine length	0.931	0.864, 1.002	0.058
Constant	1.368	0.204, 9.163	0.747
<b>Fibroids + Adeno</b>			
Age	1.087	1.032, 1.146	0.002
Parity	1.389	0.993, 1.943	0.055
Uterine length	0.825	0.746, 0.911	<0.001
Constant	0.027	0.003, 0.256	0.002

Note: Constant estimates baseline relative risk for each outcome.

\* Based on model with lowest Akaike information criteria (AIC)

2013 and 2018 at a free-standing ambulatory surgical setting. The LE group was significantly younger (35.2 +5 years) (RR 0.95 95% C.I. 0.911-0.986) compared to the LA group (39.4 +5 .7 years) (RR 1.08 95% C. I. 1.035 -1.129) (  $p < 0.001$ ). LE group (RR 0.23 95% C.I 0.08 – 0.661) ( $p < 0.006$ ) had higher nulliparity rate compared to LA group (RR 1.6 95% C.I. 1.193-2.148) (  $p < 0.002$ ). The uterine length was significantly shorter in the LA group compared to the LE group. (L – 12.3 +3.7 cm, LE 11.7 +3.7, LA 10.4 +3.1 cm ( $p < 0.001$ )).

**CONCLUSIONS:** Age is an important predictor of developing adenomyosis but not endometriosis. The risk of developing adenomyosis in patients with leiomyoma increases by almost 9% for every year of age compared to the leiomyoma only group. Both nulliparity and uterine length are important predictors of both endometriosis and leiomyoma in patients compared to the leiomyoma control group.

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**IMPACT OF NON-CAVITY DISTORTING INTRAMURAL MYOMAS ON PREGNANCY OUTCOMES IN EUPLOID FROZEN EMBRYO TRANSFER CYCLES & DONOR EGG RECIPIENTS: A PROSPECTIVE STUDY.**



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**OBJECTIVE:** A recent ASRM guideline highlights that there is insufficient evidence to determine whether non-cavity distorting intramural myomas is associated with a decreased likelihood of achieving pregnancy in patients undergoing fertility treatment. We sought to determine if the presence of non-cavity distorting intramural myomas has an impact on pregnancy outcomes in an ideal study group of patients: patients undergoing either frozen-thawed embryo transfer (FET) of normal euploid embryos or elective single embryo transfer of donor oocytes.

**DESIGN:** Prospective cohort study, interval analysis.

**MATERIALS AND METHODS:** Patients who underwent cycles with either autologous FET after preimplantation genetic testing for aneuploidy (PGT-A) or donor egg recipient (DER) cycles were included in this prospective study which began enrollment in September 2018. Patients were stratified based on whether myomas were detected (group A) or no myomas were detected (group B) on pelvic ultrasonography at the time of study enrollment during the patient's treatment cycle. The FIGO classification system was used and the distance from the endometrial lining to the closest myoma was recorded. The primary outcome was positive pregnancy rate. The secondary outcomes were ongoing pregnancy rate and miscarriage rate. Statistical analysis included Mann-Whitney U test and chi-square test.  $P < 0.05$  was deemed statistically significant.

**RESULTS:** Currently, 53 patients enrolled in the study have completed their ART cycles. 15/53 (28.3%) had a non-cavity distorting intramural myoma. The patients who had myomas were older and had a higher BMI. The peak endometrial thickness was similar between the two groups. Of the patients who had myomas 11/15 conceived, and of the patients without myomas 30/38 conceived. There was no difference in the primary outcome of positive pregnancy rate. There was also no difference for the secondary outcomes of ongoing pregnancy rates and miscarriage rates between the two groups.

**CONCLUSIONS:** An interval analysis of our ongoing prospective study suggests that non-cavity distorting myomas do not affect positive pregnancy rates, ongoing pregnancy rates, or miscarriage rates in a good-prognosis population with patients undergoing either autologous FET with PGT-A or donor egg recipient cycles. However, this study is ongoing and will continue to evaluate this further.

Reference: Practice Committee of the American Society for Reproductive Medicine. Removal of myomas in asymptomatic patients to improve fertility and/or reduce miscarriage rate: a guideline. *Fertil Steril*, 2017.

SUPPORT: None.

**FIBROIDS - BASIC**

**P-600** Wednesday, October 16, 2019 6:30 AM

**EFFECT OF SIMVASTATIN ON INTEGRIN-β1 AND ITS DOWNSTREAM MEDIATORS IN HUMAN LEIOMYOMA CELLS.**



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**OBJECTIVE:** Integrins, extracellular matrix (ECM) receptors, are key mediators of out-in and in-out signaling between a cell and its ECM environment and neighboring cells. Leiomyoma cells were shown to overexpress integrin-β1, which on activation, induces FAK auto-phosphorylation and activates its downstream signaling including ERK, p38 MAPK, PI3K and cyclin D1. In addition, phosphorylated FAK leads to activation of AKAP13 and RhoA which further recruits ROCK and MLCK activation. Activation of this cascade promotes leiomyoma development by increasing proliferation, cell spreading and ECM deposition. Therefore, integrin-β1 signaling may serve as a therapeutic target for uterine leiomyoma. Current evidence from *in vitro*, *in vivo*, and *epidemiologic* studies suggests that simvastatin possesses anti-tumor effects on uterine leiomyoma. Our objectives in this study are to examine the effect of simvastatin on integrin-β1 expression and its downstream mediators in human leiomyoma cells.

**DESIGN:** *In vitro* laboratory study using human leiomyoma cells.

**MATERIALS AND METHODS:** Human leiomyoma (huLM) cells were treated with simvastatin (0.001 to 10 μM) for 24 to 72 hours. Anti-proliferative effect of simvastatin was determined by MTT assay. The effect of simvastatin on the expression of integrin-β1 and its downstream mediators p-FAK, AKAP13, ROCK1 and MLCK were examined using western blotting after 48-hour treatment. Furthermore, the expression of cyclin D1, a downstream marker of FAK signaling, was evaluated. Student's t-test was used to determine statistically significant differences ( $P < 0.05$ ).

**RESULTS:** Simvastatin exhibited significant anti-proliferative effects on leiomyoma cells in a dose- and time-dependent manner. At 48 hours, 85% and 51% proliferation inhibition were noted at 0.01 to 1 μM simvastatin, respectively. Simvastatin treatment at 1 μM for 48 hours was associated with 48% decrease in the expression of integrin-β1. The ratio of phosphorylated to total FAK (p-FAK/total FAK ratio) was reduced by 28% at 1 μM of simvastatin and there was a dose-dependent pattern. At 1 and 10 μM of simvastatin, the expression of AKAP13 was suppressed by 56% to 34% whereas the expression of ROCK1 and MLCK were decreased by 65% to 43% and 63% to 57%, respectively. Additionally, the expression of cyclin D1 demonstrated a 46% to 36% reduction at 1 and 10 μM simvastatin.

**CONCLUSIONS:** These encouraging results indicate that the simvastatin might have a significant therapeutic impact on uterine leiomyoma growth through modulating the ECM-integrin-β1 interaction in leiomyoma. Down-regulated p-FAK/FAK, AKAP13, ROCK1 and MLCK signaling molecules after simvastatin treatment may correct the mechanical signaling, already disordered in leiomyoma. Suppressing integrin-β1 and its downstream mediators after simvastatin treatment may serve as a promising approach for uterine leiomyoma treatment.

SUPPORT: Supported by NIH grant 1R01HD094380-01.

	Group A: Non-cavity distorting myoma (n=15)	Group B: No non-cavity distorting myoma (n=38)	p
Age (years)	41.7 ± 3.2	37.3 ± 5.1	<0.001*
BMI (kg/m <sup>2</sup> )	25.5 ± 7.9	22.2 ± 3.3	0.014*
Gravidity	1.5 ± 1.8	1.5 ± 1.3	0.66
Parity	0.33 ± 0.5	0.53 ± 0.8	0.53
Peak Endometrial Stripe (mm)	10.6 ± 2.4	9.4 ± 2.1	0.37
Positive Pregnancy Rate	0.73	0.79	0.72
Ongoing Pregnancy Rate	0.67	0.63	1.00
Miscarriage Rate	0	0.16	0.26

**SIMVASTATIN INHIBITS RhoA ACTIVATION, COLLAGEN EXPRESSION AND GEL CONTRACTION IN HUMAN LEIOMYOMA CELLS.** Sadia Afrin, PhD, Mostafa A. Borahay, MD, PhD Johns Hopkins University, School of Medicine, Baltimore, MD.



**OBJECTIVE:** There is a strong evidence that altered mechanical homeostasis and signaling play a key role in uterine leiomyoma development and growth. Mechanical stress from disordered extracellular matrix (ECM) can initiate the activation of RhoA and its downstream signaling pathways. In turn, activated RhoA contributes to the production of collagen type 1, alters the viscoelastic properties of tissue and contribute to increased ECM stiffness, a key feature of leiomyomas. Therefore, mechanical signaling pathway seems to be a conceivable pharmacologic target in leiomyoma. The objective of this study is to examine the effect of simvastatin on: i) RhoA activation; ii) collagen type 1 expression; iii) gel contraction; and iv) cell migration in human leiomyoma cells.

**DESIGN:** *In vitro* laboratory study using immortalized human leiomyoma cells.

**MATERIALS AND METHODS:** Human leiomyoma (huLM) cells were treated with simvastatin (0.001 to 10  $\mu$ M) for 48 hours. RhoA activation was measured using the Rhotekin RBD Agarose beads to selectively isolate and pull-down the active (GTP-bound) form of RhoA, to be quantified by western blot. Simvastatin effect on collagen gel contraction was measured by culturing the leiomyoma cells in three-dimensional (3D) condition. Photographs were taken at the end of treatment. Simvastatin effect on cells migration were observed by wound closure assay and the wound areas were analyzed by Image J software. Western blot analysis was performed for examining the effect of simvastatin on collagen type 1 expression. Student's t-test was used to determine statistically significant differences ( $P < 0.05$ ).

**RESULTS:** Simvastatin significantly decreased RhoA activation (active/total RhoA ratio) at 1  $\mu$ M by 33% compared to control. Furthermore, simvastatin suppressed collagen type 1 expression by 58% at 10  $\mu$ M. In addition, simvastatin inhibited 3D collagen gel contraction at as low concentrations as 0.001  $\mu$ M with the higher concentrations of simvastatin (1 and 10  $\mu$ M) inducing maximal gel relaxations, similar to collagen gel without cells. Finally, simvastatin decreases the migration ability of leiomyoma cells up to 43% compared to control cells.

**CONCLUSIONS:** Simvastatin has significant attenuating effects on RhoA activation, a key mediator of leiomyoma mechanical signaling that contributes to its development and growth. Also, simvastatin inhibits the expression of type 1 collagen. Additionally, the remarkable gel contraction noted in leiomyoma 3D culture was inhibited by simvastatin, which also reduced the migration ability of leiomyoma cells. These findings indicate the therapeutic efficacy of simvastatin for the treatment of uterine leiomyoma by targeting its disordered mechanical signaling.

**SUPPORT:** Supported by NIH grant 1R01HD094380-01.

**EXPRESSIONS OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND ANGIOPOIETIN-1 IN MED12 MUTATED AND WILD-TYPE UTERINE LEIOMYOMAS.**

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**OBJECTIVE:** To investigate whether the expressions of vascular endothelial growth factor (VEGF) and angiopoietin-1 (Ang1) in uterine leiomyomas are influenced by the presence *MED12* gene mutation. *MED12* gene mutation is the most frequent cause of uterine leiomyomas and *MED12* wild-type leiomyomas are larger, produce more erythropoietin (EPO), and have mature vessels compared to mutated-type. *MED12* mutated and wild-type leiomyomas may also have differences in the expressions of factors effecting tumor angiogenesis that support tumor growth.

**DESIGN:** Retrospective study of clinical data and gene mutation.

**MATERIALS AND METHODS:** This study was approved by the Ethical Committee of Yokohama City University and written informed consent was obtained from all subjects. The leiomyoma tissue samples and clinical data of

over a hundred of uterine leiomyoma patients who underwent surgery in our hospital were collected. The mutations in *MED12* exon 2, the mutation hot-spot of *MED12* gene, were analyzed by Sanger sequencing. The mRNA expression levels of VEGF and Ang1 of leiomyoma tissue were measured by real-time reverse transcription-polymerase chain reaction. The relationship between *MED12* gene mutation statuses, mRNA expression levels, and clinical backgrounds were analyzed. Mann-Whitney U test or  $\chi^2$  test for trend were performed for statistical analyses and *P*-values of less than 0.05 were considered statistically significant.

**RESULTS:** Fifty-two *MED12* mutated and 56 wild-type leiomyomas were included in this study. The *MED12* wild-type leiomyomas were confirmed to be significantly larger compared to *MED12* mutated leiomyomas. Larger leiomyomas had the trend to be *MED12* wild-type ( $P = 0.004$ ). VEGF mRNA was 1.3-fold higher in *MED12* mutated leiomyomas ( $P = 0.024$ ); however, Ang1 mRNA and other clinical backgrounds did not have difference between *MED12* mutated and wild-type leiomyomas.

**CONCLUSIONS:** Contrary to our expectation, *MED12* mutated leiomyomas expressed higher VEGF mRNA levels. We speculate that *MED12* wild-type and mutated leiomyomas grow in different mechanisms including angiogenesis and vessel maturation. The growth of *MED12* mutated leiomyomas may be supported by the angiogenetic effect of VEGF rather than EPO and *MED12* wild-type leiomyomas may have an advantage in increasing tumor size by reinforced vessel maturation due to EPO.

**MULTI-OMIC ANALYSIS OF UTERINE LEIOMYOMAS FROM HEREDITARY LEIOMYOMATOSIS AND RENAL CELL CANCER PATIENTS.**

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**OBJECTIVE:** Mutation in the fumarate hydratase (FH) gene causes hereditary leiomyomatosis and renal cell cancer (HLRCC). Impaired FH activity leads to accumulation of cellular fumarate resulting in widespread post-translation modifications, such as succinated cysteinyl residues. A comprehensive multi-omics analysis was conducted to identify proteogenomic determinants underlying risk of developing leiomyomas in HLRCC patients.

**DESIGN:** We performed multi-omic analyses of uterine leiomyomas (ULMs) from HLRCC patients to decipher novel mechanistic insights for improved management of ULMs in HLRCC patients.

**MATERIALS AND METHODS:** ULMs from HLRCC (n=17) and non-syndromic (n=12) patients were obtained under IRB consent from a single institution. Tissues were processed for genomic DNA and RNA to support whole genome sequencing (WGS) and total RNA-seq analysis (Illumina Hi-Seq X/3000), as well as for quantitative proteomics using a comprehensive mass spectrometry-based approach. Proteomic database searches included variable modifications for 2-succinyl-cysteine (2SC) residues. Differential expression and functional inference analyses were performed using commercial and in-house bioinformatic pipelines.

**RESULTS:** WGS analyses revealed 16 of 17 HLRCC patients harbored recurrent mutations, insertion, or deletion events fumarate hydratase (FH), whereas 8 of 12 non-syndromic ULM cases exhibited mutations in the mediator complex subunit 12 (*MED12*) gene. Differential analyses identified 504 proteins (LIMMA  $p < 0.05$ , LogFC  $\pm 0.5$ ) and 1,022 genes (edgeR  $p < 0.05$ ) as significantly altered between HLRCC and non-syndromic ULMs, 51 of which were co-significant and exhibited high quantitative correlation trends (Spearman=0.866). Pathway analysis suggested marked alteration of mitochondrial activity in HLRCC versus non-syndromic ULMs as reflected by increased expression of electron transport and ATP synthase proteins. Comparison with historic gene expression studies (Vanharanta S, 2006) validated 36 transcripts and 16 proteins as significantly altered between HLRCC and non-syndromic ULMs. Furthermore, 364 2SC-modified peptides corresponding to 239 protein targets were identified, 47 of which have been

previously described as being modified by 2SC in FH-mutated cancer cell lines (Ternette N, 2013 and Yang M, 2014). Pathway analysis of 2SC-modified proteins revealed altered regulation of cytoskeletal organization, cell death and cell migration signaling in HLRCC ULMs. Quantitative analyses revealed 63 unique 2SC-modified peptides were 2.45 ( $\pm$  0.03)-fold elevated in HLRCC versus non-syndromic patients. These candidates included a peptide modified on C106 of Parkin 7 (PARK7), a potent cellular deglycase and sensor of oxidative stress.

**CONCLUSIONS:** Multi-omic revealed protein alterations and post-translational modifications impacting mitochondrial and oxidative stress signaling in HLRCC versus non-syndromic ULMs. These findings define proteogenomic alterations that may better support the treatment of ULMs in HLRCC patients.

**P-604** Wednesday, October 16, 2019 6:30 AM

**REPROGRAMMING OF ESTROGEN SIGNALING BY MLL1 LINKS DEVELOPMENTAL EXPOSURE TO THE RISK OF UTERINE FIBROIDS.** Mohamed Ali B.Pharm, M.Sc.<sup>a</sup> Ayman Al-Hendy, MD PhD,<sup>b</sup> Qiwei Yang, PhD.<sup>c</sup>



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**OBJECTIVE:** Environmental exposure to endocrine disrupting chemicals (EDCs) reprograms developmental organs, which leads to their predisposition to tumorigenesis later in life. Uterine fibroids (UFs) are monoclonal tumors arising from aberrant stem cells (SCs) in the myometrium (MM). We have previously demonstrated that MMSCs are the targets for epigenome reprogramming, and the expression of estrogen responsive genes (ERGs) was altered in response to early life exposure to EDCs. However, the mechanism responsible for initiation of this persistent EDCs-induced epigenetic alteration is unknown.

**DESIGN:** Laboratory research studies using Eker rat fibroid model MM tissues as well as MMSCs.

**MATERIALS AND METHODS:** Female newborn rats were treated S.C. with vehicle (VEH) or 10  $\mu$ g/kg of diethylstilbesterol (DES-a tool compound of environmental EDCs) on postnatal days 10-12, a key period of uterine development. MMSCs were isolated from 5 month adult MM tissue (N=5 for each group) using Stro-1 and CD44 surface markers. To determine the role of MLL1 for changes in H3K4me3 in response to DES, knockdown (KD) of *Taspase 1* (*Tasp1*) was performed using 3 lentiviral particles. To identify targets of epigenomic reprogramming in MMSCs, whole genome RNA-sequencing and ChIP-sequencing (using H3K4me3 antibody) was performed in DES- and VEH-MMSCs. Protein and gene expressions have been measured using Western blot (WB), immunofluorescence (IF) and qRT-PCR. Prime-PCR array of estrogen receptor (ER) signaling has been used. Ingenuity Pathway Analysis (IPA) software was used.

**RESULTS:** Our previous findings showed that DES exposure increased the expression of ERGs via epigenetic active marker H3K4me3. In this study, IPA analysis of RNA-seq data demonstrated that  $\beta$ -estradiol and ESR1 upstream regulators were highly activated, which was tightly correlated to the diseases of endocrine and reproductive systems. Also, ER signaling involving 47 molecules was activated in DES-MMSCs. By WB and IF analyses, the expression levels of H3K4me3 and activated form of MLL1 were increased in DES- vs. VEH-MMSCs. To identify that MLL1 was the methyltransferase responsible for H3K4me3 mediated reprogramming, we inactivated MLL1 by KD of the *Tasp1* protease, which is required to generate the C- and N-terminal fragments that form the active MLL1 heterodimer. WB demonstrated KD of *Tasp1* by 90% vs. scramble particle (P<0.05). *Tasp1* KD abrogated the increase in expression of H3K4me3-reprogrammed genes *Esr1*, *Pgr*, *Cd9*, *Cxcl12*, *Ar*, and *Tgm2* in DES-MMSCs (P<0.05). To determine the additional estrogen pathway related molecules, which can be altered by MLL-1, Prime-PCR array of ER signaling was performed in *Tasp1* KD and scramble DES-MMSCs. The data showed that the c-terminal fragment from MLL1 cleaved by *Tasp1* is responsible for regulating ER signaling via direct and indirect epigenetic mechanism.

**CONCLUSIONS:** Our data demonstrate novel findings that MLL1 activation is required for H3K4me3 regulated ERG expression that are vulnerable to disruption by environmental exposures. *Tasp1* KD reverses the DES exposure-induced ERG reprogramming and modulates the ER signaling.

**References:** 1. Cook JD, Davis BJ, Cai SL, Barrett JC, Conti CJ, Walker CL. Interaction between genetic susceptibility and early-life environmental

exposure determines tumor-suppressor-gene penetrance. Proc Natl Acad Sci U S A. 2005 Jun 14;102(24):8644-9. Epub 2005 Jun 3. PMID:15937110.

2. Cook JD, Davis BJ, Goewey JA, Berry TD, Walker CL. Identification of a sensitive period for developmental programming that increases risk for uterine leiomyoma in Eker rats. Reprod Sci. 2007 Feb;14(2):121-36. PMID:17636224.

3. Mas A, Nair S, Laknaur A, Simón C, Diamond MP, Al-Hendy A. Stro-1/CD44 as putative human myometrial and fibroid stem cell markers. Fertil Steril. 2015 Jul;104(1):225-34.e3. <https://doi.org/10.1016/j.fertnstert.2015.04.021>. Epub 2015 May 16. PMID:25989979.

4. Yang Q, Mas A, Diamond MP, Al-Hendy A. The Mechanism and Function of Epigenetics in Uterine Leiomyoma Development. Reprod Sci. 2016 Feb;23(2):163-75. <https://doi.org/10.1177/1933719115584449>. Epub 2015 Apr 28. Review. PMID:25922306.

5. Mas A, Stone L, O'Connor PM, Yang Q, Kleven D, Simon C, Walker CL, Al-Hendy A. Developmental Exposure to Endocrine Disruptors Expands Murine Myometrial Stem Cell Compartment as a Prerequisite to Leiomyoma Tumorigenesis. Stem Cells. 2017 Mar;35(3):666-678. Epub 2016 Nov 11. PMID:27739139.

**SUPPORT:** NIH grants: RO1 ES028615, U54 MD007602.

**P-605** Wednesday, October 16, 2019 6:30 AM

**SINGLE CELL RNASEQ ANALYSES OF UTERINE FIBROIDS AND FIBROID-FREE MYOMETRIA REVEAL PREVIOUSLY UNIDENTIFIED CELL TYPE AND STATE.** Wanxin Wang, PhD, candidate,<sup>a</sup> Aymara Mas, PhD,<sup>b</sup> Javier Monleón, MD,<sup>c</sup> Stephen Quake, DPhil,<sup>d</sup> Carlos Simon, MD, PhD.<sup>e</sup>



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**OBJECTIVE:** Whole tissue studies of uterine fibroids provided information on transcriptomic and genomic signatures of the tumor, but were limited in providing mechanistic and therapeutic insights due to the undefined intra- and inter-tumor heterogeneity. We performed single-cell RNA-seq analyses on uterine fibroids (uF) and fibroid-free myometria (uM) at both expression and mutation level to better understand the molecular and cellular origin of the tumor and to identify targets for less invasive treatment.

**DESIGN:** Single cell RNAseq analyses on both expression and mutation level were performed on 5582 single cells from uF and matched uM from 6 patients with uF diagnosed, as well as uM from patients with no uF diagnosed.

**MATERIALS AND METHODS:** uF and uM were dissected after hysterectomy and dissociated separately into single cell suspension. Single cells were index-sorted into 384 well plates containing lysis buffer and ERCC. Full-length RNA was reverse transcribed and amplified (23 cycles) following an adapted SmartSeq2 protocol. Dual-indexed cDNA libraries were sequenced on a Novaseq to  $\sim$ 1e06 reads/cell. Mutations were called using an adapted GATK pipeline. Downstream analyses such as quality control, dimension reduction, and differential expression were performed using custom R scripts. Cell type and state validation was performed via RNA FISH.

**RESULTS:** uF and uM consist of cell types and states that are more complex than previously known. While both are composed of the primary hierarchy of smooth muscle cells, fibroblasts, blood vascular endothelia, and immune cells, in uF we identified previously unreported lymphatic vascular endothelia as well as further heterogeneity in fibroblasts and immune cells that does not exist in uM. For uM we report a previously uncharacterized ion-responding cell state. In addition, we observe an overall inflammatory state in uF, manifested as the transcriptomic signature of the immune cells, the presence of lymphatic system, and the interplay between the two via CCL21-CCR7 ligand-receptor pair. This state and the mutation load in the uF suggest a tumorigenic scheme that might differ between this benign tumor and cancer.

**CONCLUSIONS:** Our comprehensive single cell delineation of the cellular hierarchy of uF and uM revealed previously unidentified cell type and state for both. The difference in the cellular hierarchy between the two and the differentially expressed genes in cell types that are common to both provide cellular and molecular targets for less invasive treatment for the tumor.

**SUPPORT:** 1. Chan Zuckerberg Biohub, 2. Igenomix Foundation.

### VITAMIN D3 AND ITS ANALOGUE PARICALCITOL REVERSE DNA DAMAGE IN HUMAN UTERINE FIBROID STEM CELLS: MECHANISM FOR POTENTIAL PREVENTIVE THERAPY.

Mohamed Ali B.Pharm, M.Sc.<sup>a</sup> Lauren Prusinski Fernung, PhD,<sup>b</sup> Ayman Al-Hendy, MD PhD,<sup>a</sup> Qiwei Yang, PhD.<sup>a</sup> <sup>a</sup>University of Illinois at Chicago, Chicago, IL; <sup>b</sup>Medical College of Georgia at Augusta University, Augusta, GA.



**OBJECTIVE:** The prevailing model for Uterine Fibroids (UFs) pathogenesis invokes the genetic transformation of a single myometrial stem cell (MMSC) into a tumor-initiating cell (UFSC) that seeds and sustains clonal tumor growth. UFSCs are known to have higher prevalence in African American (AA) women, which is related in part to their vitamin D deficiency, yet its exact preventive mechanism of action has not been fully revealed yet. Growing body of evidence showed chemopreventive effect of vitamin D. We have recently demonstrated increased DNA repair defect in UFSCs compared to MMSCs. Collectively, we hypothesize that vitamin D3 or its potent analogues, through reparation of an impaired DNA damage response, will provide therapeutic benefits for UFSCs.

**DESIGN:** Laboratory research studies using Stro-1+/CD44+ MMSCs and UFSCs

**MATERIALS AND METHODS:** Surgically removed fresh human UF and adjacent MM tissues were collected from two AA patients, and subjected to MM and UFSC isolation using dual Stro-1 and CD44 surface markers. Human UFSC cells were treated with concentration ranges (10 nM-1000 nM) of 1, 25 dihydroxyvitamin D3 and its three analogues (Paricalcitol, Doxercalciferol and Elocalcitol). The growth inhibitory effect was assessed by MTT assay after 24, 48 and 72 hr. To determine the role of vitamin D and Paricalcitol on DNA damage repair system, UF SCs from 2 AA patients were treated with 100 nM of 1, 25 dihydroxyvitamin D3 or Paricalcitol for 3 days. Total RNA was extracted and DNA damage signaling pathway was examined using Prime-PCR array including 84 genes. The expression levels of 6 DNA double strand breaks repair genes including *BRCA1*, *CHECK1*, *RAD50*, *RAD51*, *NBS1* and *MRE11* were validated using RT-qPCR. In addition, protein lysates were extracted from treated and untreated cells and expression levels of DNA damage marker  $\gamma$ H2AX as well as RAD51, NBS1 and MRE11 were measured by Western Blot (WB). Unpaired Student t-test was used to measure statistical significance. ( $P < 0.05$ ) was considered significant.

**RESULTS:** Using MTT assay, Vitamin D3 and its analogues treatment showed a potent significant anti-proliferative effect on human UFSCs in a concentration and time dependent manner ( $P < 0.05$ ). Using Prime-PCR array of DNA damage signaling, Vitamin D induced upregulation of 67 DNA repair genes while seven were downregulated and one was unchanged. Paricalcitol showed similar results by inducing the expression of several DNA related genes. Using RT-qPCR, expression of *BRCA1*, *CHECK1*, *RAD50*, *RAD51*, *NBS1*, *MRE11* were validated in response to Vitamin D3 and paricalcitol treatment in favor of significant upregulation as compared to untreated control. WB analysis showed that both treatments significantly decreased protein expression of  $\gamma$ H2AX while increased the protein levels of key DNA damage repair members including RAD51, NBS1 and MRE11.

**CONCLUSIONS:** Our studies demonstrate a tight link between DNA damage and vitamin D in UFSCs. Vitamin D3 and its analogue(s) suppress the UF phenotype via targeting DNA damage repair pathway, therefore providing a novel mechanistic insight into clinical effectiveness of Vitamin D3 and analogues on UFSCs.

**SUPPORT:** National Institutes of Health grants R01 HD089553-01 and U54 MD007602.

### VERTEPORFIN INHIBITS FIBROSIS, INFLAMMATION AND ANGIOGENESIS RELATED GENES IN UTERINE FIBROID CELLS.

Md Soriful Islam, PhD, Jacqueline Yano Maher, MD, MA, Sadia Afrin, PhD, Szu-Chi Su, MS, James Segars, MD, Johns Hopkins University, School of Medicine, Baltimore, MD.



**OBJECTIVE:** Uterine fibroids are characterized by abnormal cell proliferation and apoptosis, leading to excessive growth and secretion of an altered extracellular matrix (ECM). A key signaling pathway controlling cell proliferation and apoptosis is the Salvador/Warts/Hippo pathway. Hippo signaling is mediated by the TEA domain family member (TEAD)/Yes-associated protein (YAP)/(transcriptional co-activator with PDZ-binding motif (TAZ) tran-

scription factors. Verteporfin, a benzoporphyrin derivative, is known to inhibit the interaction between TEAD and YAP/TAZ. We previously reported that Hippo signaling was altered in fibroid cells compared to myometrium and that verteporfin reduced expression of YAP responsive genes (*CTGF* and *CYR61*). Here we tested the hypothesis that inhibition of Hippo targets by verteporfin would alter expression of key genes involved in uterine fibroid pathogenesis.

**DESIGN:** Translational research study with Human uterine fibroid (P53F) cells.

**MATERIALS AND METHODS:** Human myometrial and fibroid tissues were used to assess the expression levels of YAP, and TAZ by immunohistochemistry (IHC). Next, we tested the effect of verteporfin on key ECM-related genes involved in fibroid formation and growth: collagen1A; versican; integrin  $\alpha 6$ ; matrix metalloproteinase (MMP)-14; the profibrotic growth factors transforming growth factor (TGF)- $\beta 1$ , and TGF- $\beta 3$ ; inflammation related gene, interleukin (IL)-8; and the angiogenesis related gene, endothelin1 in uterine fibroid cells. Cells were treated with vehicle or verteporfin at 0.5 or 1  $\mu$ M for 24 hr. Viability curves showed  $> 90\%$  viability at 1  $\mu$ M concentration. RNA was extracted using an RNeasy Plus Mini Kit and converted to cDNA using iScript cDNA Synthesis Kit. Real-time qPCR was performed on LightCycler 96 System, using FastStart Essential DNA Green Master Kit and gene-specific primers. Prior experiments confirmed activation of YAP phosphorylation by verteporfin in P53 myometrial cells.  $P < 0.05$  was considered as significant.

**RESULTS:** YAP and TAZ were differentially expressed in human myometrial and fibroid tissues as measured by IHC. Treatment of fibroid cells with verteporfin significantly reduced steady-state mRNA levels of versican and MMP-14 in a dose-dependent manner in uterine fibroid cells, compared to control. Integrin  $\alpha 6$  transcript levels were not affected, but IL-8 levels were reduced. Verteporfin also decreased profibrotic growth factors, TGF- $\beta 1$ , and TGF- $\beta 3$  mRNA expression levels in fibroid cells, compared to untreated controls. Furthermore, we found that endothelin1 mRNA levels were reduced in response to verteporfin treatment in fibroid cells, compared to controls.

**CONCLUSIONS:** Inhibition of Hippo signaling by inactivation of nuclear YAP with verteporfin led to a reduction in mRNA expression levels of key genes involved in fibroid pathogenesis. These data support the possibility that altered Hippo signaling may contribute to the altered fibrosis, inflammation and angiogenesis of fibroids.

### NAV2: A NOVEL NEURAL PROTEIN AND REGULATOR OF THE CYTOSKELETON IS UPREGULATED IN LEIOMYOMA AND MYOMETRIUM.

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**OBJECTIVE:** Uterine leiomyoma are common reproductive-age benign tumors that contribute to severe morbidity and infertility, and account for 40% of hysterectomies in the US. Developing potential drug targets for medical treatment is of clinical importance. We have identified several neural genes present in leiomyoma tissue, including members of the neuron navigator (NAV) family. NAV2 plays a role in migration, cellular outgrowth and F-actin tethering to the myoskeleton in other tissues. However, its function is unknown in leiomyoma. The objective of this study is to assess NAV2 expression and its role in human uterine leiomyoma, and to identify potential oral medications that affect its expression.

**DESIGN:** Laboratory Study.

**MATERIALS AND METHODS:** RNA sequence (RNAseq) analysis was performed on placebo-treated patient matched leiomyoma and normal myometrium samples from a prospective, randomized, placebo-controlled clinical trial. These results were confirmed with qRT-PCR, western blotting, and immunohistochemistry (IHC).

**RESULTS:** RNAseq analysis of placebo-treated fibroids compared to myometrium demonstrated the presence of transcripts encoding for several neuronal proteins. For NAV2, RNA sequence analysis demonstrated increased expression in leiomyoma as compared to myometrium (2.78 fold  $\pm 0.32$ ,  $p < 0.0001$ ). Confirmatory qPCR results on leiomyoma and myometrial patient samples demonstrated an increase in expression of NAV2 in fibroids (3.86 fold  $\pm 0.98$ ,  $p = 0.005$ ). Additionally, qRT-PCR on immortalized leiomyoma and myometrial cell lines similarly demonstrated an increase in expression of NAV2 in leiomyoma (3.06 fold  $\pm 1.02$ ,

p=0.034. Western blot analysis on patient matched leiomyoma and myometrium supported these findings. IHC demonstrated a qualitative increase of NAV2 protein in fibroids as compared to myometrium by visual examination in morphologic appearance and staining intensity in 75% of patients examined.

**CONCLUSIONS:** NAV2, a member of the neuron navigator family, is identified in leiomyoma and myometrial tissue. NAV2 RNA and protein is elevated 2-3 fold in leiomyoma compared to myometrium. NAV2 is associated with F-actin and actin tethering, providing a novel target for compounds that regulate the cytoskeleton.

**SUPPORT:** This research was supported by Uniformed Services University of the Health Sciences, Department of Obstetrics and Gynecology and a research grant from Allergan.

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of Defense, or the United States Government.

**P-609** Wednesday, October 16, 2019 6:30 AM

### **IL6 AND STAT-3 PATHWAY HIGHLIGHT THE DIFFERENCES IN MOLECULAR RESPONSES IN MYOMETRIUM AND UTERINE FIBROIDS.**

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**OBJECTIVE:** Human fibroids are highly prevalent and symptomatic uterine tumors. Phenotypically, they are different from normal myometrium because of massive production of extracellular matrix (ECM), which is a hallmark of these benign tumors resulting in symptoms including abnormal bleeding and pain. Dysregulated production of inflammatory cytokine, IL6 and its regulated JAK/STAT-3 pathway are known to contribute to the fibrotic process. Our objective was to understand the effect of IL6 on fibroid and myometrium cells and the role of JAK/STAT pathway in production of matrix proteins that play a major role in fibroid pathogenesis.

**DESIGN:** Laboratory study.

**MATERIALS AND METHODS:** Leiomyoma and patient-matched myometrium cell lines exposed to different concentrations of IL6 and JAK/STAT-3 modulators for various exposure time periods, in a 2D culture model system. Changes in expression of ECM proteins and regulating pathways were assessed using western blot.

**RESULTS:** In leiomyoma cells, IL6 activation of STAT-3 peaked (2.32±/0.21 fold) as early as 1.5hr of continuous exposure, and was 1.4±/0.04 fold increased at end of 3hr of exposure. The maximum increase (1.59±/0.07 fold) in the feedback loop protein, Suppressor of Cytokine Signaling (SOCS3) required a short exposure to IL6 (30min) followed by collection after 3hr. We also observed an increase (1.9±/0.11 fold) in the ECM structural collagen-1 after continuous exposure to IL6 (3hr) in leiomyoma cells. No significant effect of IL6 was observed in myometrium cells. Collagen-1 protein was also affected significantly in the leiomyoma cells on use of JAK/STAT-3 modulators. An increase of 2.21±/0.052 fold was observed on direct activation of the pathway in leiomyoma cells. Increase were also observed in ECM protein fibronectin in response to the JAK/STAT-3 modulators. Response of transforming growth factor beta3 (TGFβ3) protein, involved in fibrotic specific pathway, on use of JAK/STAT-3 modulators indicated that both pathways may interact to regulate the production of the ECM proteins in leiomyomas.

**CONCLUSIONS:** Direct effect of inflammatory cytokine IL6, on leiomyoma and not myometrium cells indicate that this differential response to IL6 and JAK/STAT3 pathway may be the key to increased ECM production and growth in uterine leiomyomas.

**SUPPORT:** USUHS Military Women's Health Award OBG6422-309325.

## **GENETIC COUNSELING**

**P-610** Wednesday, October 16, 2019 6:30 AM

### **OFFERING UNIVERSAL CARRIER SCREENING TO WOMEN OF REPRODUCTIVE AGE SEEKING ROUTINE GYNECOLOGIC CARE.**

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**OBJECTIVE:** To investigate differences in demographic and clinical characteristics of reproductive age women who express interest in carrier

screening consultation with genetic counseling offered as part of routine gynecologic care and those who do not.

**DESIGN:** Cross-sectional implementation study at a tertiary care gynecology practice.

**MATERIALS AND METHODS:** Women ages 18-40 years presenting for routine gynecologic care were eligible for participation. Women were given a packet with information about the benefits of genetic evaluation and assessment prior to conception and offered referral for genetic counseling consultation with the possibility of opting for carrier screening. Interested women were scheduled for comprehensive genetic counseling appointments which included evaluation, assessment, and discussion of available testing options. Demographic and clinical characteristics were obtained by review of the electronic medical record and a survey completed by women at the time of gynecologic visit. The electronic medical record was also reviewed to obtain information on the genetic counseling appointment, type of testing ordered, and test results. Statistical analysis was performed as appropriate with  $p < 0.05$  to compare relevant characteristics.

**RESULTS:** From October 2018 to March 2019, 131 women were screened for participation. 105 women consented to participate, of which 4 were excluded due to participant or partner having undergone permanent surgical sterilization. Of the 101 women included in this study, 41 expressed interest in genetic counseling referral. Women most likely to express interest were those presenting for infertility evaluation (75.0%) and those presenting for preconception counseling (66.7%). Women presenting for other visit types were less likely to express interest (33.3% for annual exams, 28.6% for problem visits, and 26.7% for contraceptive counseling,  $p < 0.05$ .) Nulliparous women were more likely than multiparous women to express interest (49.3% vs 16.3%,  $p < 0.05$ .) Women of higher level of education were also more likely to express interest with least likely groups being those with high school degree or equivalent (30.7%) or associate's degree (14.3%) and most likely groups being those with a master's degree (88.9%) or professional degree/doctorate (60.0%) ( $p < 0.05$ ). No significant differences were seen between differing age, race, ethnicity, employment status, marital status, insurance type, or past history of carrier screening. Of women who expressed interest, 13 (31.7%) attended their scheduled genetic counseling visit and 7 (53.8%) opted to undergo carrier screening.

**CONCLUSIONS:** After a brief introduction to genetic counseling services during routine gynecologic care in a single tertiary care clinic, nearly half of reproductive age women expressed interest in referral with possibility of carrier screening prior to conception. Nulliparous women, women of higher level of education, and women presenting for infertility evaluation or preconception counseling may be more likely to express interest in these services.

**SUPPORT:** None.

**P-611** Wednesday, October 16, 2019 6:30 AM

### **PATERNAL ADVANCED AGE AND MALE FACTOR ARE INDICATORS FOR PRE-IMPLANTATION GENETIC TESTING IN EGG DONATION CYCLES.**

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**OBJECTIVE:** Oocyte donation in fertility programs has become the most effective alternative to achieve pregnancy, mainly in women with low or no ovarian reserve as well as other complications. It has been shown that the use of young donors in oocyte donation cycles tends to increase success in pregnancy and live birth rates. Although there is a wide variety of studies that demonstrate the influence of the oocyte source's age on the success of assisted reproduction treatments, the effect of paternal age has been studied to a lesser extent. Therefore, our goal was to assess semen quality between the spouse and donors and in vitro fertilization outcomes.

**DESIGN:** Retrospective.

**MATERIALS AND METHODS:** 394 IVF cycles in 5 different Mexican IVF labs where egg donation (age range: 18-35 years) was performed were considered. All donors underwent similar IVF stimulation protocols. The oocytes were aspirated and fertilized using ICSI technique. Biopsies were performed on Day 5 or Day 6, and chromosome integrity was determined by Next-Generation Sequencing. Insemination was performed with both spousal semen (n=332) and donor semen (n=62). Semen characteristics were evaluated by seminogram, and seminal quality was assessed by measuring the total amount of normal progressive motile sperm (TNPM). Associations were determined using logistic regression.

**RESULTS:** A total of 1449 embryos were biopsied, 995 for Day 5 and 454 for Day 6. The aneuploidy rates for Day 5 and six biopsies were not significantly different (22.2% and 22.7%, respectively). Independent of the sperm source, there was an observable trend between embryo aneuploidy rates and the sperm source's age; however, only for spousal sperm was the trend significant (odds ratio=1.022, 95%CI: 1.003-1.040, p=0.022). Interestingly, higher TMPN levels were associated with lower aneuploidy rates, but there was no significant association. When the cohort was separated by sperm characteristics, normospermia presented with similar aneuploidy rates (22%) as terato- (22%), oligo- (27%), crypto- (22%), and a-zoospermia (15%); however, asthenozoospermia samples presented with a higher aneuploidy rate (56%). Lastly, sperm quality how no effect on fertilization rates, blastocyst formation, or implantation rates.

**CONCLUSIONS:** Here, the poor semen quality did not affect the IVF outcomes. However, we show that the spousal's age is associated with the aneuploidy rate, whereas donor sperm does not. Therefore, it would be prudent to perform PGT when the sperm donor is of advanced age.

**SUPPORT:** ConacytA 231793.

**P-612** Wednesday, October 16, 2019 6:30 AM

### EXPANDED CARRIER SCREENING FOR RECESSIVELY INHERITED GENETIC DISORDERS: FACTORS IN DECISION-MAKING WHEN ONE INDIVIDUAL IN A COUPLE IS IDENTIFIED AS A CARRIER.

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**OBJECTIVE:** Expanded carrier screening (ECS) is a method of identifying individuals that are carriers for recessively inherited genetic disorders with the goal of reducing the risk of having a child affected by a genetic disease. While some couples are screened in tandem, others may be screened sequentially to reduce need for a second individual's test if a partner is found not to carry any mutations. However, it is unknown how often partners of individuals found to be carriers complete the recommended testing with a sequential approach. The goal of this study was to determine the frequency with which the partner of an individual identified as a carrier chooses to undergo testing and what factors may influence that decision.

**DESIGN:** Retrospective chart review.

**MATERIALS AND METHODS:** All individuals at a university-affiliated reproductive endocrinology and infertility practice identified to be carriers of a recessively inherited mutation using the Counsyl/Foresight ECS between 9/1/2013 and 4/1/2019 were included. Conditions were categorized by severity (profound, severe and moderate) according to the classification system previously described by Lazarin et al.<sup>1</sup> If an individual screened positive for more than one condition the category corresponding to the more severe condition was used.

**RESULTS:** A total of 2,061 patients were screened. 760 (36.9%) screened positive as carriers of one or more recessively-inherited disorders. Of these, 577 (75.9%) had reproductive partners listed in the medical record. One-hundred and fifty six (27%) of positively-screened individuals with reproductive partners did not have their partner undergo screening. When compared to those who had a profound mutation, those with a moderate mutation had a trend towards a reduced odds for having their partner screened (OR 0.36 95%CI 0.12-1.05, p=0.06). However, those with a severe mutation did not demonstrate

a reduction in odds for having their partner screened when compared with those who had a profound mutation (OR 0.60, 95% CI 0.21-1.74 p=0.35). Number of conditions a patient screened positive for was not predictive of subsequent partner screening (OR 0.95, 95% CI 0.72-1.25 p=0.72).

**CONCLUSIONS:** Though the greatest utility of ECS is when the carrier status of both reproductive partners is known, not all patients that carry recessively-inherited genetic disorders choose to have their reproductive partner screened. Patients found to be carrier of more debilitating genetic disorders may be more likely to screen their reproductive partners. The emphasizes the importance of the role of the provider in counseling patients prior to performing ECS, as well as genetic counseling after results are received.

**Reference:** <sup>1</sup>Lazarin GA, Hawthorne F, Collins NS, Platt EA, Evans EA, Haque IS. Systematic classification of disease severity for evaluation of expanded carrier screening panels. PLoS One. 2014;9:e114391.

**P-613** Wednesday, October 16, 2019 6:30 AM

### AGE BASED COUNSELING FOR WOMEN PLANNING TO UNDERGO IVF WITH PGT-A.

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**OBJECTIVE:** Aging leads to both a decrease in embryo yield and euploid embryo frequency. With increasing utilization of PGT-A technologies, the ability to counsel patients as to the number of expected cycles required to produce a euploid embryo is an important tool. This study aims to provide such a counseling tool utilizing retrospective outcomes data.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** A total of 614 IVF cycles with intent for PGT-A from January 1, 2015 to March 15, 2019 at a single institution were reviewed. All cycles proceeding to oocyte retrieval were included, even if no embryos were available to biopsy. Patients who had an oocyte thaw with subsequent embryo biopsy were excluded. Cycles were analyzed to determine number of embryos biopsied, number of euploid embryos, and number of cycles with at least one euploid embryo. These data were utilized to obtain number of euploid embryos per cycle and calculate the estimated number of cycles required to attain at least one euploid blastocyst.

**RESULTS:** A total of 544 cycles proceeded to biopsy with 2573 embryo biopsies. Of those, 368 (67.6%) cycles had at least one euploid embryo per cycle. Four patients had a transfer and biopsied remaining embryos within the same cycle. The percentage of cycles with euploid embryos decreased by age group from 86.1% of cycles in women less than 35 years old to 23.4% of cycles in women over age 42. Extrapolating data from the number of euploid embryos per cycle by age group, the estimated number of cycles to achieve at least one euploid blast increased from one cycle for women less than age 35 to five cycles for those greater than 42 years of age.

**CONCLUSIONS:** Managing patient expectations during IVF treatment is critical. Clinicians and patients alike frequently underestimate the likelihood of not having embryos available for biopsy as well as the chance of having a euploid embryo with each cycle. With increasing utilization of PGT-A technology, a tool for estimating the number of cycles anticipated to achieve at least one euploid blastocyst can prove useful to set expectations with preparation for the possibility of multiple cycles.

TABLE 1. Cycle characteristics and biopsy results by patient age

Age Group	<35	35-37	38-40	41-42	>42	All Groups
Total Number of Cycles (%)	115 (19)	111 (18)	194 (32)	117 (19)	77 (13)	614 (100)
Cycles with No Biopsy (%)	8 (7)	10 (9)	17 (9)	21 (18)	14 (18)	70 (11)
Number Embryos Biopsied	702	553	736	404	178	2573
Number Euploid Embryos (%)	299 (43)	180 (33)	198 (27)	85 (21)	22 (12)	784 (30)
Cycles with at Least One Euploid per Cycle (%)	99 (86)	79 (71)	117 (60)	57 (49)	18 (23)	370 (60)
Number of Embryos Biopsied per Cycle	6.10	4.98	3.79	3.45	2.31	4.19
Number Euploid Embryos per Cycle	2.60	1.62	1.02	0.73	0.29	1.28
Number of Cycles to Euploid	1.16	1.41	1.66	2.05	4.28	1.66

**IMPORTANCE OF EXPANDED CARRIER SCREENING IN THE ASHKENAZI JEWISH POPULATION.**



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**OBJECTIVE:** Compared to the general population, patients of Ashkenazi Jewish descent have an increased risk of being genetic carriers for certain diseases, with an overall carrier rate ranging from 1 in 4 to 1 in 5<sup>1</sup>. Therefore, the American College of Obstetricians and Gynecologists (ACOG) strongly recommends this population be offered carrier screening for four conditions: Tay Sachs, Cystic Fibrosis, Familial Dysautonomia, and Canavan Disease<sup>2</sup>. Some experts have advocated for a more comprehensive screening panel, and subsequently, ACOG recognized that screening for the following Jewish Genetic Diseases can be offered to patients: Bloom syndrome, Familial hyperinsulinism, Fanconi anemia, Gaucher disease, Glycogen storage disease type I, Joubert syndrome, Maple syrup urine disease, Mucopolysaccharidosis type IV, Niemann-Pick disease, and Usher syndrome<sup>2</sup>. Given the genetic risks inherent in this population, carrier screening programs have been created to test for these founder mutations and have been successful in significantly decreasing the incidence of certain autosomal recessive conditions. Recently, however, with the advent of pan-ethnic, expanded carrier screening, we have the means to identify carriers for a broader array of conditions beyond the fourteen aforementioned<sup>3</sup>. The objective of this study is to assess whether the current screening recommendations are sufficient in diagnosing carrier status in the Ashkenazi Jewish population.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This was a retrospective chart review. Students at a single institution underwent genetic testing with expanded carrier screening through an outreach program at Rutgers University Hillel in October 2015. All the students were of Jewish descent. The genetic conditions tested in the expanded carrier screening were grouped into the following three categories based on ACOG's 2017 committee opinion regarding carrier screening: the four strongly recommended genetic conditions, the fourteen genetic conditions that can be offered, and the genetic diseases that are not specifically mentioned for screening in this population. The results were then divided according to this categorization.

**RESULTS:** A total of 81 patients were screened. Of these, 36 (44.4%) were found to be carriers of at least one disease. Out of the 36 patients, 28 were found to be a carrier for one disease, 7 for two diseases, and 1 for three diseases, representing 45 total identified mutations. The carrier rate was 7/45 (15.6%) for the four recommended Jewish Genetic Diseases, 20/45 (44.4%) for the fourteen offered conditions, and 25/45 (55.6%) for genetic diseases that were not recommended in this population.

**CONCLUSIONS:** If carrier screening for the Ashkenazi Jewish population was limited to only founder Jewish mutations in fourteen disorders, 44.4% of carriers would not have been identified. Our data supports that individuals of Ashkenazi descent should be offered pan-ethnic, expanded carrier screening.

**References:** 1. American College of Obstetricians and Gynecologists. A Carrier Screening for Genetic Conditions. ACOG Committee Opinion No. 691. *Obstet Gynecol* 2017; 129: e41-55.

2. Baskovich, B., Hiraki, S., Upadhyay, K., Meyer, P., et al. Expanded genetic screening panel for the Ashkenazi Jewish population. *Genetics in medicine: official journal of the American College of Medical Genetics*. 2015; 18(5), 522-528.

3. Gross, S. J., Pletcher, B. A., Monaghan, K. G., & Professional Practice and Guidelines Committee. Carrier screening in individuals of Ashkenazi Jewish descent. *Genetics in medicine: official journal of the American College of Medical Genetics*. 2008;10(1), 54-56.

**SUPPORT:** None.

**REPRODUCTIVE ENDOCRINOLOGISTS' UTILIZATION OF GENETIC COUNSELORS AND THEIR SERVICES: IS THERE AN UNMET NEED?**



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**OBJECTIVE:** Use of genetic testing options is now routine for patients utilizing assisted reproductive technologies (ART) under the care of reproductive endocrinologists (REIs). Carrier screening, and often expanded

carrier screening, and preimplantation genetic screening including preimplantation genetic diagnosis (PGD) and preimplantation testing for aneuploidy (PGT-A) have become popular options within this patient population. Research suggests that patients utilizing these technologies have found genetic counseling regarding these tests and their results to be beneficial, but limited research has been conducted to examine current practices for genetic counseling in the assisted reproduction setting. The objective of this study was to assess current practices for providing genetic counseling in the ART setting and assess whether there is a need for additional genetic counselor involvement in this medical subspecialty.

**DESIGN:** Research study conducted via web-based survey.

**MATERIALS AND METHODS:** REIs practicing in the United States were identified using the American Society of Reproductive Medicine (ASRM) member directory and invited via e-mail to participate in a survey about their current practices for providing genetic counseling to their patients, including who is currently responsible for providing this service and for what indications patients receive genetic counseling. Participants were also asked whether they currently employ a genetic counselor, their reasons for doing so or not, and about any future plans for adding a genetic counselor to their staff. Demographic information was collected to determine how representative the sample was of the U.S. REI population and to assess any associations between participant characteristics and attitudes regarding the role of genetic counselors in ART. The survey was designed and administered using REDCap, a web-based tool. SAS version 9.4 was used for statistical analysis.

**RESULTS:** Of the 135 responses included in the data analysis, over a quarter (27.4%, n=37) of participants said they currently have at least one genetic counselor on their staff. Of those who do have a genetic counselor on staff, over a quarter (27.0%, n=10) reported that they see a need for additional genetic counselors at their practice. Of those who do not currently have a genetic counselor on their staff, just under half (41.8%, n=41) reported that they see a need for one. Current individuals providing genetic counseling in ART practices include physicians, nurses and advanced practice nurses, on-staff genetic counselors, outside genetic counselors and in one case, medical assistants. Genetic counseling is currently provided for a variety of indications in these practices, including ordering and discussing genetic testing, obtaining informed consent, eliciting a comprehensive family history, and interpreting genetic testing results and risk.

**CONCLUSIONS:** This study provides insight into current practices for genetic counseling in ART and suggests increasing involvement of genetic counselors, as well as a need for more genetic counselors, in this medical subspecialty.

**INFORMED CONSENT FOR GENETIC TESTING ON GAMETE DONORS.** Pamela Callum, M.S., Lauren Isley, M.S., L.C.G.C., California Cryobank, Los Angeles, CA.



**OBJECTIVE:** To illustrate the need for informed consent for genetic testing on gamete donors.

**DESIGN:** Reviewed and summarized the indications and potential implications of additional genetic testing needs on qualified gamete donors during 2018.

**MATERIALS AND METHODS:** Not applicable.

**RESULTS:** In 2018, 231 genetic tests were performed on qualified sperm donors based on requests from clients or to investigate health issues in donor-conceived individuals. Carrier screening was requested in 196 cases to evaluate the risk to future offspring when a prospective recipient was known to be a carrier. Screening for 21-hydroxylase deficient congenital adrenal hyperplasia was requested 17 times. Biotinidase deficiency carrier screening was performed in 4 cases and galactosemia in 6 cases.

Offspring health issues were investigated in 8 cases. 13 tests were performed for immune compatibility needs or parentage studies. Ten cases involved PGT-M design. While PGT-M analyses may involve flanking marker analysis, set-up in other cases requires that the donor's DNA is evaluated for the presence of the pathogenic mutation known in the recipient.

**CONCLUSIONS:** Donors are informed of their results from all tests, regardless of whether the results are positive or negative. Gamete donors will test positive as carriers for autosomal recessive conditions at general population carrier frequencies. The large volume of requests for common conditions leads to numerous donors testing positive on these evaluations. The likelihood that a donor will have a positive result is substantially more likely when testing is performed following a diagnosis in a donor-conceived offspring. While a new carrier result will typically have low implications to the donor's own health, the actual impact of this information on the donor and his family may be significant depending upon his current personal circumstances and reproductive plans at the time the information becomes known.

PGT-M analysis and some offspring investigations often involve autosomal dominant conditions. Results of these investigations may have substantial implications for the donors' own health and can more greatly disrupt the donor's life, particularly if unexpected.

By engaging donors in the testing and informed consent process, gamete providers allow donors to be informed about risks, possible results, likelihood of positive results, and the potential implications to them and their own families, as with any other patient. Recipients and their providers are increasingly sending semen specimens for genetic testing without consent from donors or authorization from the gamete providers. Through this approach, well-intended clinicians and laboratory personnel place gamete providers in the difficult position of contacting donors regarding positive results for which they did not provide consent, and for which they may be greatly unprepared. For these reasons, and out of respect for autonomy of gamete donors, all testing on donors should be performed with consent and facilitated by the gamete provider.

SUPPORT: California Cryobank.

## HEALTH DISPARITIES

P-617 Wednesday, October 16, 2019 6:30 AM

**DETERMINANTS OF DISPARITIES IN MINIMALLY INVASIVE HYSTERECTOMY.** Alicia Y. Christy, MD Veterans Administration, kensington, MD.



**OBJECTIVE:** Addressing racial disparities in access to minimally invasive surgery among women will require understanding factors that impact use of minimally invasive techniques for hysterectomy, the second most common surgery in women. Our objective was to identify trends in utilization of minimally invasive hysterectomy (MIH) among Black and White women Veterans over time in the Veterans Health Administration (VHA) and whether racial disparities in MIH varied by geographic region or by whether they were performed at VA facilities or paid for by VA but performed at non-VA facilities.

**DESIGN:** Cross-sectional study.

**MATERIALS AND METHODS:** Using VA clinical and administrative data, we identified all women Veterans undergoing hysterectomy in fiscal years 2012–2014 with a diagnosis of fibroids ( $n=1714$ ). We determined hysterectomy route (laparoscopic with/without robot assist, vaginal, abdominal) by International Classification of Diseases-9th edition codes. We employed multivariable logistic regression to estimate trends in racial disparities in MIH over time and to test whether racial disparities in MIH differed by geographic region or by whether procedures were performed by VA or paid for by VA. Models adjusted for socio-demographic and health-related risk factors.

**RESULTS:** Disparities in the proportion of MIH decreased over time ( $p$ -value for interaction  $<0.001$ ) primarily due to increases in MIH among Black women (Black: 26% to 39%; White: 50% to 46%). Disparities in the proportion of MIH procedures in Black versus White women did not differ by whether the procedure was performed by VA or paid for by VA, although the overall proportion of MIH was slightly higher within VA. The proportion of MIH procedures was highest in the Northeast (Black 45%, White 62%), but the disparity in MIH was also the greatest in this region (difference=16%). The proportion of MIH was lowest in the South (Black 34%, White 43%) but the disparity was smallest in this region (difference=9%).

**CONCLUSIONS:** In the enhanced access to care environment of the VHA, disparities in MIH appear to be narrowing over time. Variations existed in MIH proportion by geographic region, with observed Black-White disparities greatest in regions where MIH was more common overall. Efforts are needed to increase access to MIH in VHA in the Southern US in particular; however, any efforts to increase MIH must also address equity to avoid worsening racial disparities. Further studies, including qualitative research, are needed to determine optimal strategies for decreasing persistent racial disparities in access to new medical technologies, such as MIH.

Reference: None.

SUPPORT: None.

P-618 Wednesday, October 16, 2019 6:30 AM

**ASSOCIATION OF PREGNANCY OUTCOMES WITH AREA DEPRIVATION INDEX.** Vinita Alexander, MD,<sup>a</sup> Jean-Claire "Mandi" Powe Dillon, MD,<sup>b</sup> Emily S. Jungheim, MD, MSCI.<sup>b</sup> <sup>a</sup>Washington University in St. Louis, St. Louis, MO; <sup>b</sup>Washington University School of Medicine, St. Louis, MO.



**OBJECTIVE:** Living in a socioeconomically deprived neighborhood has been associated with an increased risk of adverse birth outcomes. However, variation in the effect of socioeconomic deprivation has not been studied in the U.S. population undergoing in vitro fertilization (IVF). In this study, it was of interest to explore the relationship between socioeconomic deprivation and clinical pregnancy rate, live birth outcomes, and preterm birth rates.

**DESIGN:** A retrospective cohort study of 516 women undergoing their first cycle of IVF at a single academic fertility center in St. Louis, MO from January 2015 to December 2018 was conducted.

**MATERIALS AND METHODS:** The Area Deprivation Index (ADI) has been validated to the neighborhood-level by Dr. Amy Kind at the University of Wisconsin-Madison. It facilitates the rankings of neighborhoods by socioeconomic status disadvantage. Using published ADI maps, neighborhood-level deprivation index was obtained per individual patient (from the 2013 American Community Survey). To construct a model with relevant factors, independent samples t-test were conducted for continuous variables of interest and chi-square analysis carried out for binary variables of interest. Logistic regression analysis was carried out to determine the relationship between clinical pregnancy (CP), live birth (LB), and preterm birth. Covariates included in the original model were: age, BMI, number of oocytes retrieved, intracytoplasmic injection (ICSI), number of 2PN embryos transferred, and anti-mullerian hormone (AMH) level.

**RESULTS:** Overall, there was no significant difference between the CP rate in the highest national quintile deprivation index group (most deprived) and those in the lowest (least deprived) group. Compared to the least deprived quintile, the OR for CP in second least deprived quintile was: 1.344 (95% CI: 0.735-2.456,  $p=0.337$ , and in the most deprived group was 0.605 (95% CI: 0.212-1.723,  $p=0.347$ ). Factors significantly associated with CP in the studied cohort were: AMH, ICSI, and age at start of treatment. Overall, there was also no significant relationship between ADI and LB rate, with the most deprived group (compared to the least deprived quintile) having an OR of LB of 1.021 (95% CI 0.343-3.040,  $p=0.970$ ). Interestingly, the hazard ratio of preterm birth at  $<37$  weeks was elevated in the second and third quintiles of deprivation compared to the areas with the lowest deprivation index: 1.626 (95% CI: 1.26-2.10) and 1.66 (95% CI: 1.25-2.2). Also interestingly, there was a nonsignificant trend in increasing odds ratio of multiple births in the most deprived quintiles compared to the least deprived quintile (OR 1.935, 95% CI: 0.440 to 8.509,  $p=0.382$ ).

**CONCLUSIONS:** We found no significant association between neighborhood deprivation index and probability of CP or LB after IVF. Given that the academic center is in St. Louis, MO and attracts many patients coming from Illinois, a state that mandates fertility coverage, it may be interesting to further investigate whether those in the most deprived ADI groups (and possibly fertility insurance coverage) are more likely to have multiples.

P-619 Wednesday, October 16, 2019 6:30 AM

**EXPLORING THE INTERSECTION OF RACE, RELIGION, AND GENDER IN BLACK WOMEN WITH INFERTILITY.** Nicolas A. Johnson, B.S., David A. Grainger, M.D., University of Kansas School of Medicine-Wichita, Wichita, KS.



**OBJECTIVE:** Investigating the cultural and psychosocial factors that affect Black women's access to fertility treatment; including intraracial differences between infertile women who have accessed fertility treatment compared to women with infertility that have not accessed care.

**DESIGN:** Qualitative study in a community-based setting.

**MATERIALS AND METHODS:** The first author of the study consented participants via phone and conducted semi structured interviews with Black women with an ICD9 or ICD10 diagnosis of female factor or unexplained infertility. Interviews were audio recorded, transcribed, and analyzed in NVIVO using phenomenological methods.

**RESULTS:** Each of the 12 participants were married or partnered, and the mean age of the women was 39 years. Most women were college educated from working middle class households. All participants were insured. Thematic analysis revealed each woman's journey to motherhood, challenges navigating the healthcare system, and the value of their religion throughout their experience. *Journey to Motherhood:* Participants expressed their experience with pregnancy loss, delayed diagnosis, the anxiety around inheriting trauma from an adopted child, and belief in the motherhood mandate. *Healthcare System:* Challenges included: feelings of discrimination, high cost of fertility treatment, and lack of accessibility and knowledge about treatment. *Religion:* Emerging themes around religion included the belief in accepting God's plan, using it as a form of solace. This belief did not impact their overall perception of fertility medication or the use of assisted reproductive technologies to build their families.

**CONCLUSIONS:** Black women face unique intersectional challenges in their experience living with infertility. The results of this study may serve as a tool for improving physician-patient interactions and foster a better understanding of modern reproductive health disparities and minority health outcomes.

**P-620** Wednesday, October 16, 2019 6:30 AM

**PROLONGED TIME TO DIAGNOSIS OF PRIMARY OVARIAN INSUFFICIENCY (POI) IN AN URBAN REPRODUCTIVE ENDOCRINOLOGY (RE) CLINIC.**



Shweta J. Bhatt, MD,<sup>a</sup> Valerie S. O'Besso, BA,<sup>b</sup> Natak C. Douglas, MD, PhD,<sup>c</sup> Peter McGovern, MD,<sup>d</sup> Jacquelyn Loughlin, MD,<sup>e</sup> Sara S. Morelli, MD, PhD.<sup>b</sup> <sup>a</sup>Rutgers New Jersey Medical School, New York, NY; <sup>b</sup>Rutgers New Jersey Medical School, Newark, NJ; <sup>c</sup>Associate Professor, Newark, NJ; <sup>d</sup>University Reproductive Associates, NJ; <sup>e</sup>Rutgers New Jersey Medical School, Newark, New Jersey, NJ.

**OBJECTIVE:** Prompt recognition of symptoms and subsequent diagnosis of POI are critical given its consequences on quality of life and long term health. Poor access to care in low-income populations may contribute to delayed diagnosis. We previously demonstrated a dearth of board-certified RE physicians providing care for Medicaid patients in New Jersey (1). Given the adverse effects of prolonged hypoestrogenism, we aimed to evaluate length of time to diagnosis of POI in a low-resource/low-income population presenting to an urban university-based RE clinic.

**DESIGN:** Case series.

**MATERIALS AND METHODS:** All new patients seen at the RE clinic at University Hospital in Newark, NJ from June 2014 through June 2018 were included. POI was diagnosed in women with oligo/amenorrhea and menopausal levels of follicle stimulating hormone. The primary outcome was time to diagnosis from onset of symptoms.

**RESULTS:** Of 524 new patients seen, 19 (3.6%) were diagnosed with POI (Table 1). Mean time to diagnosis of POI from onset of symptoms was 6 years. 17/19 (89.5%) women were Hispanic and/or Black. 13/19 (68.4%) reported hypoestrogenic symptoms at time of referral. 21.1% were diagnosed with Turner mosaicism. 14 patients completed DEXA scan, of which 35.7% were diagnosed with low bone mass or osteoporosis. Of those diagnosed prior to referral to RE (9/19, 47.4%), only 4 had initiated hormone therapy.

**CONCLUSIONS:** Prolonged time to diagnosis of POI has adverse effects, as reflected by hypoestrogenic symptoms and decreased bone mineral den-

TABLE 1. Patient Characteristics (n=19)

Follicle Stimulating Hormone (M±SD)	82.0±31.5 mIU/mL
Estradiol (M±SD)	12.3±17.5 pg/mL
Anti-Mullerian Hormone (M)	<0.015 ng/mL
Age at Symptoms Onset in Years (M±SD)	25.1±10.0
Time to Diagnosis from Symptoms Onset in Months (M±SD)	72.4±61.3
Ethnicity	
Hispanic, n (%)	6 (31.6)
Not Hispanic, n (%)	13 (68.4)
Race	
White, n (%)	2 (10.5)
Black, n (%)	11 (57.9)
Other, n (%)	6 (31.6)
History of smoking, n (%)	4 (21.1)
BMI (M±SD)	25.6±5.4
Nulliparous, n (%)	14 (73.6)
Symptoms on Presentation to Reproductive Endocrinology	
Vasomotor, n (%)	12 (63.2)
Vaginal, n (%)	6 (31.6)
Hormone Therapy Initiated Prior to Referral, n (%)	4 (21.1)
Etiology Identified, n (%)	8 (42.1)
Chemotherapy, n (%)	2 (10.5)
Turner Mosaic, n (%)	4 (21.1)
Fragile X premutation, n (%)	2 (10.5)
DEXA Completed, n (%)	14 (73.7)
Low Bone Mass or Osteoporosis, n (%)	5 (26.3)
Hormone Therapy	
Combined oral contraceptive pills, n (%)	6 (31.6)
Cyclic estrogen and progestin, n (%)	12 (63.2)

sity. Our study demonstrates a need for more aggressive evaluation of oligo/amenorrhea in underrepresented minority women. Delayed diagnosis and management of POI may be related to health care disparities facing these women, and warrants action to improve access to care.

Reference: <sup>1</sup>Holden EC, Kashani BN, Bhatt SJ, Cho M, McGovern PG. NJ Medicaid Patients Have Limited Access to Providers for Reproductive Endocrine Care. *Fert Ster.* 2017; 108 (3):e110.

SUPPORT: None.

**P-621** Wednesday, October 16, 2019 6:30 AM

**IMPACT OF MATERNAL ETHNICITY ON PREGNANCY OUTCOME IN INFERTILE WOMEN WITH POLYCYSTIC OVARIAN SYNDROME.**



Fabiola D'Ambrosio, MD, Humberto Scoccia, MD. University of Illinois at Chicago, Chicago, IL.

**OBJECTIVE:** Polycystic ovarian syndrome (PCOS) is the most prevalent endocrine pathology seen among women of reproductive age. The objective of the study is to investigate the impact that ethnic background has on pregnancy outcomes, including live birth rates in infertile women with PCOS going through IVF compared to women without PCOS.

**DESIGN:** Retrospective cohort study in an academic IVF center.

**MATERIALS AND METHODS:** This study used a coded REDCap data set of 486 women, 18-45 years of age who underwent IVF and embryo transfer between 1/1/2010 and 12/31/2015 at an academic IVF Center after IRB approval (IRB 2015-0623). Patients underwent their 1<sup>st</sup> IVF cycle following progesterone withdrawal (norethindrone acetate) using GnRH agonist or antagonist protocols with mixed gonadotropins and demographic data, including race and ethnicity was obtained. Data collected during the IVF process included peak estradiol, number of mature oocytes, fertilization rate, number of top quality embryos on Day 3 of development, number of embryos transferred, implantation rate, clinical pregnancy rate, live birth rate, clinical miscarriage rate, and ectopic pregnancy rate. In women, whose pregnancy resulted in a live birth, additional maternal and neonatal variables were collected, including estimated gestational age (EGA) at delivery, mode of delivery, and weight at birth. Using R analytics software, data was analyzed using logistic regression and linear models. For logistic regression, the estimated coefficient was the log-odds-ratio. A significant *p* value was considered <0.05.

**RESULTS:** Of the 486 initial women, 360 women were included in the final analysis. Only women with known/reported ethnicity were included. Not Hispanic is the referent cohort for ethnicity and non-PCOS is the referent group when comparing PCOS status. There was no significant difference found for implantation rate (*p*=0.53) and pregnancy rate (*p*=0.99) when comparing women without PCOs with the women with PCOS. When taking into account the ethnicity factor in PCOS and the women who conceived and had a live birth, there was no significant difference between being Hispanic with PCOs and the referent non-Hispanic group. However, when evaluating women who started the IVF cycle, women with PCOS are less likely to have a pregnancy that leads to a live birth compared to women without PCOS (*p*=0.046). Being Hispanic by itself does not seem to affect live birth (*p*=0.81). Hispanic women with PCOS have the same probability of having a vaginal delivery compared to the referent group (*p*=0.935). Women with PCOS are more likely to deliver ~2 weeks earlier than non-PCOS patients (*p*=0.038). Being Hispanic and having PCOS did not affect the EGA at delivery (*p*=0.83) or affected fetal weight compared to the referent group (*p*=0.58).

**CONCLUSIONS:** This pilot study did not find a significant difference in most of the variables studied comparing Hispanic PCOS women with non-Hispanic women without PCOS. However, further studies with a larger number of subjects are needed to assess the impact of ethnicity and PCOS on IVF pregnancy outcomes.

**IMAGING**

**P-622** Wednesday, October 16, 2019 6:30 AM

**ORAL DICLOFENAC POTASSIUM IS AN EFFECTIVE ANALGESIC DURING HYSTEROSALPINGOGRAPHY.**



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**OBJECTIVE:** Tubal abnormalities are found in 30-40% of cases of infertility and evaluation of tubal patency is so crucial in their diagnostic workup. Hysterosalpingography (HSG) is a reliable, simple and cost-effective method for evaluation of tubal patency. Our objective is to investigate the analgesic effect of oral diclofenac potassium in pain alleviation during hysterosalpingography (HSG).

**DESIGN:** A randomized double-blinded controlled trial.

**MATERIALS AND METHODS:** Reproductive-aged infertile women scheduled for HSG were considered for enrollment. Eligible women were recruited and randomized (1:1) to oral diclofenac or Placebo group. All women received oral 50 mg diclofenac potassium or placebo tablets one hour before HSG. The study outcomes were the participant's self-rated pain perception using a 10-cm Visual Analogue Scale (VAS) during speculum application, cervical tenaculum application, injection of the dye, 5 minutes and 30 minutes post-procedure. A 2 cm difference in VAS score between both groups was considered a clinically significant difference. Other outcomes included the number of women who need additional analgesics and the adverse effects of the study medications. Mann Whitney test and Fisher's exact test were used for the analysis of the outcomes.

**RESULTS:** Two hundred women were enrolled and randomized to diclofenac arm (n=100) or placebo (n=100). No difference between both groups in age, parity, BMI, type, duration of infertility and the prior mode of delivery. Women in the diclofenac group reported lower VAS scores during injection of the dye, 5 minutes and 30 minutes post procedure (median: 3 vs. 5.5, p=0.001; 2 vs. 4, p=0.001; 1.5 vs. 3, p=0.003, respectively). No significant differences in VAS score during speculum or tenaculum application. Additionally, twenty-five women asked for additional analgesics in the placebo group versus nineteen women in the diclofenac group (p=0.062). No difference in the rate of adverse effects.

**CONCLUSIONS:** oral diclofenac potassium one hour before HSG significantly alleviates the induced pain during and 30 min after the HSG procedure.

**SUPPORT:** None.

**P-623** Wednesday, October 16, 2019 6:30 AM

#### **UTERINE PERISTALSIS DURING IMPLANTATION PERIOD; EXPERIENCE OF 3,672 PATIENTS WITH 3 OR MORE FAIRULE OF EMBRYO TRANSFERS.**



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**OBJECTIVE:** Uterine peristalsis caused by uterine contraction is thought to be one of the risk factor for implantation failure, because the uterus is quiescent at the time of implantation period. Previous studies suggested more than 2 or 3 waves/min may be a threshold for implantation failure. Although those reports focused on frequency and direction of the uterine contraction, as far as we know, there were no reports regarding intensity and location of the uterine contraction. Therefore, we investigated intensity and location as well as frequency and direction of the uterine contraction in the largest number of patients with recurrent failure of embryo transfers.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Transvaginal ultrasonography scans of uterine peristalsis were performed at the mid luteal phase (7 days after progesterone administration = P+7 on hormone replacement cycle or 9 days after HCG administration = HCG+9 on natural cycle). The transvaginal probe (Logiq V5 Expert, 6 to 10 MHz, GE Healthcare) was introduced into the vagina as gently as possible to avoid stimulating the uterine cervix. After scanning mid-sagittal plane of the uterus, the probe was fixed as steady as possible while 3 min, video was recorded simultaneously. The video images were analyzed at 10 time the normal speed using Quick Time Player (Ver. 10.4) by a single observer. Frequency, intensity, location and direction of the uterine contractile activity were recorded and evaluated. Intensity was divided into 3 categories; movement with the whole endometrium (strong), with the middle and the surface of the endometrium (medium), and just the surface of the endometrium (weak). Direction was complicated with many patterns (e.g., lower → upper → lower).

**RESULTS:** Of 3671 patients (average age, 37.5), 1936 (52.7%) did not show any uterine peristalsis, 1735 (47.3%) had uterine peristalsis. In the peristalsis group, frequency was 55.2% for 1 to 3 (times/3 min), 30.2% for 4 to 6, 10.8% for 7 to 9, and 3.8% for 10 or more. Intensity was almost equal among 3 categories (strong 34.1%, medium 37.4%, weak 28.5%). Most uterine peristalsis was observed in the whole uterine cavity (80.7%), whereas those in the upper, middle and lower part of the uterus were 9.7%, 1.6% and 8.1%, respec-

tively. In terms of direction, about half (48.1%) of uterine peristalsis was observed as "lower → upper → lower", followed by "upper → lower → upper" (16.9%), "lower → upper" (14.5%), "upper → lower" (14.3%), and unfocused (5.9%). Pregnancy outcome of patients (N=24) who had strong uterine peristalsis with 10 or more times/3 min was retrospectively evaluated after taking piperidolate hydrochloride (150mg/day). Patients with live birth or ongoing pregnancy with 22 weeks or more were 11 (45.8%), those with biochemical pregnancy or miscarriage were 6 (25.0%), and those without pregnancy were 7 (29.2%).

**CONCLUSIONS:** These data suggest that uterine peristalsis was frequently observed in patients with recurrent implantation failure. However, we have to determine the cutoff line that should be treated. Further studies will be required.

**Reference:** None.

**SUPPORT:** None.

**P-624** Wednesday, October 16, 2019 6:30 AM

#### **TRENDS IN EMERGENCY DEPARTMENT UTILIZATION IN WOMEN AGED 18-50 WITH OVARIAN CYSTS (2006-2014).**

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**OBJECTIVE:** Ovarian cysts may be functional or non-functional with management ranging from expectant, to medical, or surgical. Clinical concerns regarding ovarian cysts include risk of torsion, rupture, hemorrhage, or underlying malignancy. Pain is often an associated feature prompting women to seek evaluation in the Emergency Department (ED), whereas other times ovarian cysts are identified incidentally. The aim of this study was to examine the frequency, trends, and associated features of women presenting to the ED with a diagnosis of an ovarian cyst.

**DESIGN:** Retrospective cross-sectional study.

**MATERIALS AND METHODS:** Data from the Nationwide ED Sample (NEDS) database of Health Cost and Utilization Project (HCUP; Rockville, MD), were queried for all ED visits of women aged 18-50 years old with a primary or secondary diagnosis (ICD-9) of ovarian cysts, between 2006-2014. Variables assessed included age, hospital type, medical insurance, household income quartile and disposition.

**RESULTS:** Between 2006 and 2014 the estimated number of ED visits for ovarian cysts increased (410,435 in 2006 to 628,425 in 2014). However, the percentage of patients admitted to the hospital for this condition decreased during the same time period (12.1% in 2006 to 7.3% in 2014). This decrease far outpaced the trend of decreased admission rates in age matched women who presented to the ED for all other diagnoses (8.2% in 2006 to 7.4% in 2014). Across the years analyzed, the 20-24 age category more frequently sought ED care for ovarian cysts while the older 45-50 age category was admitted at a higher rate. Overall, women that visited the ED for ovarian cysts were more likely to have private insurance or Medicaid, to live in zip codes of the bottom two income quartiles, to visit metropolitan EDs in areas with population >1M, and to live in the southern states. The most frequently associated secondary diagnoses, when ovarian cyst was the principal diagnosis for that ED visit, included tobacco disorder, abdominal pain and female genital symptoms.

**CONCLUSIONS:** While the total ED visits of women with a primary or secondary diagnosis of an ovarian cyst increased from 2006 to 2014, the proportion of women admitted during the same time period decreased. This decrease in admission rate may be attributed to a shift away from acute surgical management of ovarian cysts and/or an increased in the number of low-acuity cases of ovarian cysts presenting to the ED. A disproportionate number of women evaluated in the ED for ovarian cysts were in the lowest two income quartiles highlighting a potential disparity in healthcare delivery and utilization.

**P-625** Wednesday, October 16, 2019 6:30 AM

#### **PREVENTING UNNECESSARY PITUITARY MAGNETIC RESONANCE IMAGING: PROLACTIN TO TESTOSTERONE RATIO PREDICTS PITUITARY ADENOMAS IN MALE PATIENTS WITH MILD HYPERPROLACTINEMIA.**

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**OBJECTIVE:** Serum prolactin (PRL) levels are routinely obtained in men presenting with clinical hypogonadism or infertility with mild hyperprolactinemia, often prompting pituitary magnetic resonance imaging (pitMRI) to assess for adenoma. The utility of obtaining pitMRI in this population has not been adequately studied, and no society guidelines exist to inform this decision. We hypothesize that a combination of laboratory findings predicts positive pitMRI findings in patients with mild hyperprolactinemia and, given the high rate of negative pitMRIs among young men with mild hyperprolactinemia, sought to identify patients in whom pitMRI can safely be avoided.

**DESIGN:** Retrospective, case-control chart review.

**MATERIALS AND METHODS:** Male patients under the age of 50 with mild hyperprolactinemia (15-55 ng/ml) who presented with erectile dysfunction, low libido, hypogonadism, or infertility who had undergone pitMRI were included. Those with a prior diagnosis of prolactinoma, hormonal or dopaminergic therapy, or incomplete clinical data were excluded. Presenting symptoms, age, PRL, body mass index (BMI), testosterone (T), luteinizing hormone (LH), follicle-stimulating hormone (FSH), creatinine (Scr), all medications, and MRI findings were collected. Means of continuous variables were compared with the Wilcoxon-Rank Sum test, and categorical variables were compared with Fisher Exact or Chi-squared tests. Fitted binomial distributions were used to generate Receiver Operating Characteristics (ROCs) and Area Under the Curve (AUC) calculations.

**RESULTS:** 62 men met inclusion criteria. Pituitary adenomas were identified in 18 patients (29%) with a mean adenoma size of  $5.4 \pm 5$  mm. Mean PRL differed in men with and without adenomas (37.8 ng/ml vs 24.9 ng/ml,  $p < 0.001$ ), as did mean T (198 ng/dl vs 301 ng/dl,  $p < 0.01$ ) with considerable overlap. Age, BMI, LH, FSH, and Scr were not associated with presence of adenoma ( $p > 0.05$ ).

A novel ratio of PRL (ng/mL) to T (ng/dL) (PRL/T) was superior to PRL or T alone in predicting positive pitMRI findings. PRL/T outperformed PRL or T when  $PRL < 30$  ng/ml (AUC 0.88 vs 0.76, 0.83 respectively) and when  $T < 300$  ng/dl (AUC 0.83 vs 0.80, 0.73).

A PRL/T ratio  $> 0.1$  identified adenomas ( $p < 0.001$ ) with high sensitivity (89%, 16/18 adenomas identified). 43% of pitMRIs could have been prevented if this metric were applied. No patients had pituitary abnormalities when  $PRL/T < 0.1$  and  $PRL < 30$  ng/ml. A more conservative approach of ordering pitMRI when  $PRL/T$  ratio  $> 0.1$  and/or  $PRL \geq 30$  retains 100% sensitivity for identifying adenomas (18/18;  $p < 0.01$ ). This more conservative guideline would have prevented 32% of pitMRIs when applied to the study cohort.

**CONCLUSIONS:** The PRL/T ratio is a superior metric to PRL or T alone in identifying young male hypogonadal patients with mild hyperprolactinemia who have imaging-confirmed pituitary abnormalities. A conservative clinical heuristic of ordering pitMRI in patients with hypogonadism with  $PRL/T > 0.1$  and/or  $PRL \geq 30$  ng/ml detects adenomas with 100% sensitivity and prevents 32% of pitMRIs without changing clinical management, thereby reducing healthcare costs.

**P-626** Wednesday, October 16, 2019 6:30 AM

### THE UTILITY OF PELVIC ULTRASOUNDS IN ADOLESCENTS PRESENTING TO THE EMERGENCY DEPARTMENT WITH HEAVY MENSTRUAL BLEEDING.

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**OBJECTIVE:** Unlike in adults, the utility of pelvic ultrasounds (PUS) for heavy menstrual bleeding (HMB) in adolescents who seek care in the Emergency Department (ED) is not well known; therefore, this study was conducted to analyze both decision-making and data utilization around performing PUS in this population.

**DESIGN:** Retrospective chart review.

**MATERIALS AND METHODS:** Patients between the ages of 11 and 19 years who presented to the ED at a tertiary care hospital from 2006-2018 were identified by ICD-9 and ICD-10 codes for HMB. Patients who had PUS were divided into three groups based on endometrial stripe measurement (EMS): EMS  $\leq 5$ mm (group 1), EMS 6-9mm (group 2), and EMS  $\geq 10$ mm (group 3). Patients were further divided into those admitted to the hospital versus those discharged from the ED. Outcome of treatment was evaluated in admitted patients by progress notes indicating when bleeding resolved. Statistical analysis was performed across all groups with cross tab and Chi-Square test, and logistic and linear regression analysis. Approval of this study was granted by the Institutional Review Board.

**RESULTS:** Two-hundred fifty-eight adolescent females presented to the ED with HMB during this timeframe, of which 113 (43.8%) had PUS. PUS were more likely to be performed if a patient was seen by gynecology, as opposed to hematology or both specialties together ( $p < 0.001$ ). Additionally, the lower the hemoglobin value, the more likely PUS were to be performed ( $p < 0.003$ ). The decision of whether or not to order PUS did not differ based on age ( $p > 0.1$ ) or duration of bleeding ( $p > 0.1$ ). There were no structural abnormalities noted on PUS. Forty-nine patients (43.4%) had an EMS that was  $\leq 5$ mm (group 1), 32 (28.3%) had an EMS between 6-9mm (group 2), and 32 (28.3%) had an EMS  $\geq 10$ mm (group 3). There was no difference between thickness of the EMS and duration of bleeding prior to presentation ( $p < 0.91$ ). Among those who had PUS, 67 (59%) patients were treated with hormonal suppression and 46 (41%) were not. There were no significant differences in treatment choices across all EMS groups: 22, 13, and 16 patients were treated with oral contraceptive pills (OCP); 1, 1, and 4 patients used progesterone only pills (POP); and 3, 0, and 7 patients received IV estrogen in groups 1, 2, and 3 respectively ( $p < 0.061$ ). To compare treatment outcomes, we analyzed the 44 patients who were admitted to the hospital, of which 34 (77.3%) had PUS. The distribution of treatments was evenly spread throughout the three EMS groups ( $p < 0.34$ ). There were no significant differences with respect to the amount of time it took bleeding to either significantly taper down or stop completely after initiating treatment ( $p < 0.227$ ,  $p < 0.211$ ,  $p < 0.229$ , respectively for OCP, POP, and IV estrogen).

**CONCLUSIONS:** In adolescents with HMB in the ED, performing a PUS did not affect treatment decisions or outcomes. Providers may want to reconsider ordering PUS in adolescents who present for this purpose, as unlike in adults, structural abnormalities are rare and there does not appear to be utility in treating based on the EMS.

**P-627** Wednesday, October 16, 2019 6:30 AM

### ANALYSIS THE CHARACTERISTIC OF CLINICAL MANIFESTATIONS, PATHOLOGY AND IMAGING IN 39 PATIENTS WITH DISORDER OF SEX DEVELOPMENT.

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**OBJECTIVE:** To investigate the relationship between clinical manifestations, pathology and imaging findings of subtypes of disorder of sex development (DSD) in order to improve the understanding of the disease.

**DESIGN:** A total of 39 patients with DSD diagnosed in the first affiliated Hospital of Zhengzhou University from September 2008 to December 2018 were collected and analyzed retrospectively. The clinical manifestations, imaging findings and pathological results of 39 cases of different types of DSD comprehensively analyzed in order to improve the understanding of the disease.

**MATERIALS AND METHODS:** We retrospectively analyzed thirty-nine patients with DSD (16 cases of 46, XY, 14 cases of 46, XX, 9 cases of Sex chromosome DSD). Clinical manifestations, karyotypes, sex hormonal levels, surgical pathology and MRI/US/CT imaging were analyzed.

**RESULTS:** All of these 39 patients received sex hormones examination, MRI and ultrasonography examination. Social genders of the patients included 11 males and 28 females. Clinical presentations included: toward female external genitalia in 25 cases, male external genitalia in 9 cases and ambiguous external genitalia in 5 cases. 12 patients had primary amenorrhea and 3 patients had secondary amenorrhea. Orificium vaginae was seen in 11 cases, hypospadias was seen in 7 cases and 2 cases accompanied by common urogenital sinus opening. Short phallus was seen in 11 cases. 12 patients showed clitoral hypertrophy. Short stature was seen in 7 cases. 30 patients had abnormal sex hormone levels. Hypertension and hypokalemia in 3 cases. 23 patients underwent laparoscopic gonad exploration. In 16 cases of 46, XY DSD, laparoscopic gonad exploration surgery discovered 29 gonads and ultrasound/MRI examination showed 21 gonads (21/29, 72%) including 3 ovaries, 17 cryptorchidism and 1 ovariectomia. 8 gonads were discovered in laparoscopic gonad exploration surgery while not showed in ultrasound/MRI examination and all located in pelvic cavity including 6 streak ovary gonads and 2 dysplastic testes. 3 gonads were neither found in both examinations. In 14 cases of 46, XX DSD, ultrasound/MRI examination showed 24 gonads (24/28, 85%) including 20 ovaries, 1 teste and 3 ovariectomia, 4 gonads were not showed. Ultrasound/MRI examination showed 6 gonads (6/18, 33%) in 9 cases of sex chromosome DSD. There were 19 cases of infantile uterus, 8 cases of primordium uterus and 3 cases of normal uterus showed in imaging results in 39 cases of DSD patients and the imaging results did not show any uterus in 9 patients. We found 12 cases of normal vagina and 14 cases of vaginal stenosis/atresia including 2 cases accompanied by

common urogenital sinus opening showed in imaging results. In 11 cases of DSD no vagina was displayed.

**CONCLUSIONS:** MRI and ultrasonography examination is effective at detection of dysplasia gonads, uterus and vagina, in combination with clinical manifestations, pathology, it is valuable in improving the diagnosis and treatment of patients with disorder of sex development.

**P-628** Wednesday, October 16, 2019 6:30 AM

### THE UTILITY OF REPEAT SALINE INFUSION SONOHYSTEROGRAM (SIS) IN THE INFERTILITY WORKUP.

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**OBJECTIVE:** The purpose of this study is to evaluate the utility of repeat SIS prior to fertility treatment cycles (FTC) and to identify risk factors for uterine abnormality recurrence or the development of new abnormalities after initially normal imaging.

**DESIGN:** Retrospective cohort study of women undergoing initial infertility workup and treatment at a single institution who had at least two imaging studies performed 1/1/2007-12/31/2017.

**MATERIALS AND METHODS:** Initial imaging included hysterosalpingography and/or SIS, while repeat imaging  $\geq 9$  months later included only SIS. Patient characteristics, imaging results, and FTC data were abstracted from patient charts and a clinical IVF database. Analysis was stratified by initial imaging result: normal or abnormal. In each stratum, result of repeat SIS was compared to patient characteristics using Chi-square test, t-test, or Wilcoxon Rank Sum test.

**RESULTS:** Of 1163 patients identified, 436 were eligible for study inclusion. Of these, 318 (72.9%) had normal initial imaging and 118 (27.1%) had abnormal initial imaging. Among the former, 22% had an abnormal repeat SIS; among the latter, 54% had an abnormal repeat SIS [ $p < 0.0001$ , RR 2.39 (95% CI 1.83-3.12)]. On average, 22.6 $\pm$ 13.9 months passed between imaging studies. In both groups, women with abnormal repeat SIS were older than those with normal repeat SIS ( $p < 0.01$ ). Women with normal initial imaging were more likely to have had a live birth in the interim if their repeat imaging was normal (29.9 vs 6.7%,  $p < 0.001$ ), an association that did not hold for women with abnormal initial imaging (26.1 vs. 18.0%,  $p = 0.338$ ). Regardless of initial imaging outcome, there was no association found between repeat imaging outcomes and total number of FTCs [IVF, FET, ovulation induction, or natural cycle (timed intercourse or IUI)] performed, max total gonadotropins used, or maximum peak estradiol level between imaging studies. Finally, there was no difference in the live birth rate among cycles started within one year after repeat SIS across groups.

**CONCLUSIONS:** Uterine cavity evaluation is a critical component of the infertility workup. Normal initial imaging does not preclude the identification of new abnormalities as patients progress through fertility treatments or return after a break from treatment or a successful pregnancy, as 22% of women with a normal initial scan will have abnormal repeat imaging. Detection and correction of uterine abnormalities helps equalize the live birth rate of women with and without uterine abnormalities. Therefore, repeat uterine cavity evaluation should continue to be performed as the standard of care.

**P-629** Wednesday, October 16, 2019 6:30 AM

### THE UTERINE MICRO-PERISTALSIS PHENOMENON RELATING TO CLINICAL OUTCOME OF IN VITRO FERTILIZATION AND EMBRYO TRANSFER.

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**OBJECTIVE:** Uterine peristalsis has been reported to influence pregnancy chances in both natural and artificial cycles, but it is not convenient to observe clinically. Moreover, there is no tool for evaluating endometrial receptivity by invisible peristalsis of the uterus. We aim to identify the existence of a new phenomenon which is defined as uterine micro-peristalsis (UMP) by ultrasound video features, and to investigate the association between UMP and outcome of *in vitro* fertilization and embryo transfer (IVF-ET).

**DESIGN:** A prospective research study.

**MATERIALS AND METHODS:** Fifty-One infertility women were recruited, including 22 non-pregnant and 29 pregnant women. Transvaginal ultrasound videos, demographic characteristics and clinical pregnancy outcomes of IVF-ET were collected. First of all, invisible UMP was magnified by video magnification[1]. Then the UMP was characterized by a new index named histogram entropy of peristalsis (HEP), which was the result of the video processing consisted of frame difference method and volume local phase quantization[2]. Subsequently, logistic regression analysis was applied to assess the effect of HEP and other independent variables relating to clinical pregnancy outcomes of IVF-ET. Besides, a comparison test of HEP between pregnant and non-pregnant patients was conducted.

**RESULTS:** There was visible UMP in the video after video magnification, which was invisible with naked eyes in the original ultrasound video. The logistic regression result reveals that HEP ( $p < 0.01$ ) and luteinizing hormone ( $p < 0.05$ ) have significant effects on the clinical pregnancy outcome of IVF-ET, while other independent variables are not significantly associated with the clinical pregnancy outcome. Further, there exists a significant difference ( $p < 0.01$ ) in HEP between pregnant patients and non-pregnant patients after video magnification.

**CONCLUSIONS:** The existence of uterine micro-peristalsis is identified by the proposed micro-motion magnification and characterization strategy. As a promising phenomenon, uterine micro-peristalsis provides a new insight for evaluating the endometrial receptivity of IVF-ET patients.

**References:** [1] Wadhwa, N., et al., *Phase-based video motion processing*. ACM Transactions on Graphics (TOG), 2013. 32(4): p. 80.

[2] Päiväranta, J., E. Rahtu, and J. Heikkilä. *Volume local phase quantization for blur-insensitive dynamic texture classification*. in *Scandinavian Conference on Image Analysis*. 2011. Springer.

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## INTRAUTERINE INSEMINATION

**P-630** Wednesday, October 16, 2019 6:30 AM

### ASSESSMENT OF SPERM RETENTION AFTER INTRAUTERINE INSEMINATION (IUI) WITH USE OF CERVICAL DEVICE.

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**OBJECTIVE:** Retention and colonization of sperm in cervical/uterine mucus is a purported mechanism of action for intrauterine insemination (IUI) efficacy. This study was designed to assess whether a use of a silicone device to plug/cap the cervix reduces reflux and loss of sperm after intrauterine insemination.

**DESIGN:** Prospective, assessor-blinded, randomized controlled trial (RCT) for the application of device with an intra-cervical stem and soft external cap, designed to be placed and maintained during insemination.

**MATERIALS AND METHODS:** IRB approval was obtained prior to recruiting subjects presenting for IUI as a planned part of treatment in a single office setting over a period of 17 months. With consent, subjects were assigned to routine IUI or IUI with use of the device by randomized number generation. Semen preparation for IUI was a standard dual wash to prepare 0.5 ml sample. A 5 mL vaginal lavage was collected from each subject before and after IUI and processed by lab technicians blinded to assigned study group. The two vaginal washes were processed by centrifugation and re-suspension to 0.5mL. A Makler chamber was used for total sperm count (motile and non-motile). Primary outcome was proportion of sperm retained after IUI. Secondary outcomes were ease of use, evaluation of device design, patient comfort/satisfaction, incidence of conception and ongoing pregnancy rates. Statistical analysis was performed using Student t-Test analysis (or Wilcoxon Rank Sum).

**RESULTS:** Sperm retention was evaluated between 50 patients relegated to randomly assigned Group A (n=26) in which IUI was performed with device in place versus Group B (n=24) in which IUI without device was performed. Data analysis with the Student t-Test was applied. Results failed to show a difference in our primary outcome of retention of sperm between the Group A & Group B demonstrating an insignificant ( $p = 0.3023$ ). An evaluation of the relationship between time interval of device and proportion of retention within the cases using a correlation analysis, did show the data was significant. A correlation analysis of case subjects with relation to device interval verified ( $r = -0.35$ , with  $p = 0.0126$ ). In further evaluation of absolute value of sperm number in inseminate of both Case and Control subjects and sperm retention percentage, no demonstrable difference was seen in either group ( $r = 0.033$ , and  $p = 0.81$ ).

**CONCLUSIONS:** Despite overall low fertility rates, IUI remains a common first step in the management of infertility given ease of treatment and low cost. Our device and study design failed to show a significant increase in sperm retention above conventional IUI technique. Notable, and not a surprising finding, is a frequent and measurable sperm reflux (sperm loss) after routine IUI, suggesting further research is warranted to improve IUI efficiency. Guiding future efforts, the finding of a significant correlation of sperm retention with extended device placement suggests a need to place and retain a therapeutic device for a prolonged period. Further, this study design suggests viability of using pre- and post-IUI vaginal washing technique for future studies.

**References:** Duran, H.E., Morshedi M., Kruger T., Ochninger, S. (2002) Intrauterine insemination: a systematic review on determinants of success. *Human Reproduction Update*, Vol.8, No 4. 373-384.

Cantineau, A.E., Cohlen, B.J., Heineman, M.J., Farquhar, C. (2013). Intrauterine insemination versus fallopian tube sperm perfusion for non-tubal infertility. *Cochrane Database Syst Rev*. 2012 (10):CD001502. <https://doi.org/10.1002/14651858.CD001502.pub4>.

Mamas, L. (2006) Comparison of fallopian tube sperm perfusion and intrauterine tuboperitoneal insemination: a prospective randomized study. *Fertil Steril*. 85, 735-40.

Hendin, B.N., Falcone, T., Hallak, J., Nelson, D.R., Vemullapalli, S., Goldberg, J., Thomas, A.J. and Agarwal, A. (2000) The effect of patient and semen characteristics on live birth rates following intrauterine insemination: a retrospective study. *J. Assisted Reprod. Genet.* 17, 245-252.

**SUPPORT:** NA.

**P-631** Wednesday, October 16, 2019 6:30 AM

#### **A MULTI-CENTRIC, PROSPECTIVE TEST OF CAP-SCORE'S™ ABILITY TO PREDICT A MAN'S PROBABILITY OF GENERATING PREGNANCY.**

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**OBJECTIVE:** Semen analysis lacks an evaluation of fertilizing ability, and fails to diagnose many cases of male factor infertility. Previously, Cap-Score™, the percentage of sperm that can capacitate, showed strong correlations with male fertility (retrospective and cohort comparison studies), and prospectively identified low versus normal fertility using a simple cut-off. However, male fertility is a continuum; logistic regression based on clinical pregnancy outcomes revealed how Cap-Score relates to the probability of generating a pregnancy (PGP) in 3 cycles (Schinfeld et al, 2018; n=124; 5 clinics). Here, we prospectively tested the relationship between the predicted PGP and actual intrauterine insemination (IUI) outcomes.

**DESIGN:** A multicentric prospective test of the PGP model's ability to predict pregnancy. IUI was used as the experimental model to ensure collection of outcomes and provide control over number and timing of inseminations relative to ovulation. For inclusion, men had to have ≥ 3 million cells post-wash, and female partners could not have factors precluding IUI, e.g., tubal occlusion, hydrosalpinges.

**MATERIALS AND METHODS:** Studies approved by Weill Cornell's IRB (1210013187) or WIRB (20152233). Cap-Score and outcomes were obtained from 6 clinics (n=292). A total of 128 finished treatment (pregnant or ≥ 3 IUIs). The PGP model was tested in two ways. First, the new outcomes were added to the prior 124 and the model was recalculated to determine change. Second, the 128 new outcomes were divided into rank-ordered groups of roughly equal size. When split into 5 groups, each had 25-26 observations; when split into 6 groups, each contained 21-22 observations. The proportion of individuals successfully generating pregnancy within a group was compared to the average predicted PGP within a group (linear regression).

**RESULTS:** Only a slight change (average 2.6%) from the original model ( $PGP = 1/[1 + \exp\{-2.86 + 0.08 * \text{Cap-Score}\}]$ ; n=124; p<0.01) was noted when new data were added ( $PGP = 1/[1 + \exp\{-2.26 + 0.06 * \text{Cap-Score}\}]$ ; n=252; p<0.001), and fit improved. When predicted PGPs were compared to observed pregnancies, significant linear relationships were seen for n=5 ( $y = 0.81x + 0.10$ ;  $R^2 = 0.84$ ; p=0.03) and n=6 ( $y = 0.69x + 0.14$ ;  $R^2 = 0.86$ ; p<0.01). The slopes were not different from 1 and intercepts were not different from 0 (p>0.05; t-tests).

**CONCLUSIONS:** Despite the potential for introducing noise when using cases from diverse settings, there was no significant change upon doubling the data set. A 1:1 relationship was detected between predicted PGPs and the observed proportion of men generating pregnancy. These results further demonstrate the strong association between Cap-Score, sperm function/fertilizing ability, and the ability to generate pregnancy.

**References:** Schinfeld et al. A Cap-Score™ Prospectively Predicts Probability of Pregnancy. *Mol Reprod & Devel.* 2018;85 (8-9), 654-664

**SUPPORT:** Androvia LifeSciences performed the Cap-Score Male Fertility Assay.

## LGBTQ REPRODUCTIVE ISSUES

**P-632** Wednesday, October 16, 2019 6:30 AM

#### **QUALITY OF LIFE AFTER FERTILITY PRESERVATION AMONG TRANSGENDER PEOPLE.**

Amanda Adeleye, MD,<sup>a</sup> Garrett Michael Reid, BS,<sup>b</sup> Yiu Ho Au, B.S.,<sup>b</sup> J. A. M. E. S. F. SMITH, M.D.,<sup>c</sup> Evelyn Mok-Lin, MD<sup>b</sup> <sup>a</sup>UCSF REI fellow, San Francisco, CA; <sup>b</sup>REI UCSF, Center for Reproductive Health, San Francisco, CA; <sup>c</sup>University of California, San Francisco, SAN FRANCISCO, CA.

**OBJECTIVE:** There are limited data on the quality of life among transgender people who sought fertility preservation or family building. This pilot study sought to describe the quality of life among transgender people who sought fertility services through the Gender Expansive Attitudes about Reproductive Health (GEAR) study.

**DESIGN:** Cross sectional survey.

**MATERIALS AND METHODS:** This survey queried transgender people who underwent ovarian stimulation or semen cryopreservation at an academic medical center between January 1, 2015 and March 31<sup>st</sup>, 2019. Enrollment is ongoing. Primary outcomes included the number of healthy days and depressed/anxious days as measured by the CDC health related quality of life survey and whether or not ovarian stimulation or semen cryopreservation was emotionally challenging. Primary outcomes were compared by gender identity and ease of gamete collection using a Fisher's Exact or Wilcoxon Rank-Sum test where appropriate.

**RESULTS:** Among 40 transgender people who presented for care, 18 initiated the survey and 16 completed the survey (n=12 transfeminine people, n=4 transmasculine people).

The median number of healthy days for the entire cohort was 21 (IQR 15.5-25.5). Transmasculine people experienced more healthy days than transfeminine participants (p=0.01). There were no associations between gender identity and the number of depressed or anxious days (p=0.09 and 0.14 respectively.)

Fourteen participants completed the survey about the ease of gamete collection. The majority of people, 64.3% (n=8 transgender women, n=1 transgender man) found the process of ovarian stimulation or sperm cryopreservation "not at all difficult" or "neither difficult or easy." Five participants (n=4 transgender women, n=1 transgender man) found the process "somewhat difficult" or "very difficult." The ease or difficulty of fertility preservation was not associated with either gender identity (p=0.604) nor the number of healthy days, depressed days or anxious days (p=0.688, 0.528 and 1.00 respectively).

**CONCLUSIONS:** In this pilot study, transmasculine people experienced more healthy days compared to transfeminine people. Gender identity was not associated with the number of depressed or anxious days. Whether or not participants found the process of ovarian stimulation or sperm cryopreservation emotionally difficult, was not associated with quality of life metrics.

**SUPPORT:** None.

**P-633** Wednesday, October 16, 2019 6:30 AM

#### **IUD CHOICE IN TRANSGENDER AND GENDER DIVERSE INDIVIDUALS.**

Lauren Abern, MD,<sup>a</sup> Glen DeGuzman, MD,<sup>b</sup> Jake Cook, BA,<sup>c</sup> Kristen Kiely, WHNP-BC,<sup>a</sup> Karla Maguire, MD, MPH,<sup>d</sup> <sup>a</sup>Harvard Vanguard Medical Associates, Somerville, MA; <sup>b</sup>University of Nevada Las Vegas School of Medicine, Las Vegas, NV; <sup>c</sup>Philly FIGHT, Philadelphia, PA; <sup>d</sup>University of Miami, Miami, FL.

**OBJECTIVE:** Although use of the intrauterine device (IUD) is increasing, the appeal among transgender and gender diverse individuals is unknown. Our objective is to assess the reasons IUD users in this population are choosing one of the five FDA-approved devices available and if they are satisfied.

**DESIGN:** Cross-sectional, survey-based study.

**MATERIALS AND METHODS:** Transgender and gender diverse individuals assigned female at birth age 18 and older that currently have an IUD participated in an online survey about reproductive history, rationale for IUD choice, unwanted side effects, and satisfaction.

**RESULTS:** 85 surveys were completed. The mean age was 25.8 (SD 4.7). 14 (16%) identified as transgender, 70 (82%) as genderqueer or non-binary, and 1 (1%) as agender. The majority (71 (85%) was white and had minimum of a college education (47, 55%). 72 (85%) were sexually active, and 63 (88%) were at risk for pregnancy. 62 (73%) chose a 52mg-Levonorgestrel (LNG) IUD (Mirena®/ Liletta®), 5 (6%) the lower dose IUDs (Kyleena®/ Skyla®), and 17 (20%) the copper IUD (Paragard®).

Menstrual manipulation was the main reason for choosing a 52mg-LNG IUD (35, 56%). Other influential factors included how long the IUD lasted (39, 63%), provider recommendation (28, 45%), and to avoid side effects experienced from other methods of contraception (28, 45%). 24 (39%) experienced unwanted side effects including worsening cramping (8, 33%), pelvic pain (7, 29%), bloating (7, 29%) and weight gain (7, 29%). 6 (25%) reported these side effects within the first 0-6 months. 6 (25%) desired removal. Of those that desired removal, 2 (33%) would opt for another IUD.

The main reasons for choosing the lower dose IUDs were the size of IUD (2, 40%), and lower hormone dose (2, 40%). Other influential factors included insurance coverage (4, 80%), how long the IUD lasted (4, 80%), and menstrual manipulation (3, 60%). 2 (40%) experienced unwanted side effects including heavy bleeding (2, 100%), worsening cramping (2, 100%), and pelvic pain (2, 100%). 1 (50%) reported these side effects within the first 3-6 months. Neither (2, 100%) desired removal.

The majority of participants selecting the copper IUD did so to avoid hormones (12, 71%). Other influential factors included how long the IUD lasted (12, 80%), to avoid side effects experienced by other methods of contraception (9, 60%), and provider recommendation (6, 40%). 10 (67%) stated they were experiencing unwanted side effects including irregular bleeding (7, 70%) and worsening cramping (5, 50%). 4 (40%) reported these side effects in the first 0-6 months. However, only 2 (20%) desired removal, and both would opt for another type of IUD.

**CONCLUSIONS:** Of the IUD options available, the majority of transgender and gender diverse individuals surveyed opted for a 52mg-LNG IUD and chose this specific IUD type for menstrual manipulation. Although side effects were experienced with all options, many occurred within the first 6 months, and few desired removal. As a result, providers should counsel this population about the benefits of an IUD as well as expected side effects including those that should resolve over time.

**P-634** Wednesday, October 16, 2019 6:30 AM

**FERTILITY PRESERVATION KNOWLEDGE AMONG TRANSGENDER WOMEN: PRELIMINARY FINDINGS FROM THE GEAR STUDY.**

Amanda Adeleye, MD,<sup>a</sup> Garrett Michael Reid, BS,<sup>a</sup> Yiu Ho Au, B.S.,<sup>a</sup> J. A. M. E. S. F. SMITH, M.D.,<sup>b</sup> Evelyn Mok-Lin, MD.<sup>a</sup> <sup>a</sup>REI UCSF, Center for Reproductive Health, San Francisco, CA; <sup>b</sup>University of California, San Francisco, SAN FRANCISCO, CA.



**OBJECTIVE:** The American Society of Reproductive Medicine and the Endocrine society guidelines recommend a discussion about fertility prior to the commencement of gender affirming hormonal therapy (HT). There are limited data about how transgender people obtain this information and their understanding of HT on their fertility. This pilot study sought to describe how transgender patients acquire information about their fertility and the accuracy of their knowledge through the Gender Expansive Attitudes about Reproductive Health (GEAR) study.

**DESIGN:** Cross sectional survey.

**MATERIALS AND METHODS:** This survey queried transgender people who sought consultation for ovarian stimulation or semen cryopreservation at an academic medical center between January 1, 2015 and March 31<sup>st</sup>, 2019. Enrollment is ongoing. Participants were asked about their most helpful resource when learning about fertility or family building options. Transgender women were assessed on whether their knowledge was aligned with clinical practice. A Fisher's exact test was used to determine whether fertility preservation answers differed by the educational resource used.

**RESULTS:** Among 40 eligible patients, 12 transgender women completed the survey. Seventy-five percent (n=9) of transgender women cited the internet as their primary source of fertility education. A minority, (n=3) stated their medical team (n=2) or friends (n=1) as their primary source of information. Sixty-six percent of participants (n=8) thought that HT should be discontinued prior to sperm freezing. The source of knowledge (internet vs. medical team or friends) was not associated with responses to whether HT should be discontinued (p=0.24). A minority of participants, 16.7% (n=2)

stated that it was not possible to freeze sperm after starting HT. Both participants cited the internet as their most useful resource, however there was no difference in the preferred educational resource and the answer to this question (p=0.545). Although sperm can be retrieved through surgical means when necessary, the majority of participants 83.3% (n=10) believed that ejaculation was required for sperm cryopreservation. There were no differences in participant answers by their preferred educational resource.

**CONCLUSIONS:** In this pilot study, the majority of transgender women obtained their information about fertility preservation from the internet. A minority of transgender women had misconceptions about their fertility potential after starting HT. Future studies may consider targeting the fertility knowledgebase among transgender women.

**SUPPORT:** None.

**P-635** Wednesday, October 16, 2019 6:30 AM

**IN VITRO FERTILIZATION (IVF) OUTCOMES IN GAY AND SINGLE WOMEN USING DONOR SPERM.**

Sara E. Barton, M.D., Sue McCormick, BS, William B. Schoolcraft, MD, Mandy G. Katz-Jaffe, Ph.D., Colorado Center for Reproductive Medicine, Lone Tree, CO.



**OBJECTIVE:** To describe the IVF outcomes in gay and single women using donor sperm.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** The first IVF oocyte retrieval in women using donor sperm due to lack of a male partner from January 1, 2016 to December 31, 2018 were reviewed at a single IVF center. Women with ≥3 failed IVF attempts at a previous clinic or >1 failed IVF attempt at our center, oocyte donation, and transfers to a gestational carrier were excluded.

The primary outcome was ongoing pregnancy rate. Secondary outcomes are listed in Table 1. Additionally, we calculated the odds of having no embryo transfer [women with failed blastocyst development or no euploid embryos in cycles where preimplantation genetic testing for aneuploidy (PGT-A) was performed]. Results are expressed as odds (%) and in median (range) where applicable.

**RESULTS:** 90 women met study inclusion criteria (n=32 gay women with a female partner; n=58 single women). The median maternal age was 40.0 (25-47) years. 28 women (31.1%) did not have an embryo transfer. The ongoing pregnancy rate was 45.6% (41/90) per oocyte retrieval and 66.1% (41/62) per transfer. One patient experienced a pregnancy loss after fetal cardiac activity was detected (2.4%) (Table 1).

Of the 90 women included, 86 women had a freeze-all cycle (84 for PGT-A; 2 for other indications) and the remaining 4 women had a fresh transfer. In the freeze-all group for PGT-A, 21.4% (18/84) had no euploid embryos, and 11.9% (10/84) had no blastocyst development; therefore 32.6% (28/86) freeze all cycles did not have a transfer. The median maternal age of those with no blastocyst development was 43 (range 40-45) years and no euploid embryos 42.5 (35-47) years, significantly older than women who had an embryo transfer [39 (25-45) years, P<0.05].

**CONCLUSIONS:** It is notable that women using IVF for lack of a male partner presented at advanced maternal age. While the outcomes of this study suggest this population may have favorable livebirth rates compared to infertile women of the same age, female age remains a strong predictor of a failed cycle. Women presenting to infertility clinics at advanced reproductive ages should be counseled regarding the negative impact of age on fertility, regardless of previous fertility attempts. These data should be useful to guide counseling in gay and single women pursuing IVF treatment without prior infertility.

Reference: NA.

SUPPORT: None.

TABLE 1. Outcome variable

	N=90
# oocytes retrieved	16 (2-44)
# fertilized oocytes (2PN)	8 (1-28)
# usable blastocysts	2 (0-15)
	<b>N=62</b>
# embryos transferred	1 (1-2)
Positive HCG	82.3%
Implantation rate (FHT)	62.3%
Ongoing pregnancy rate per FET	66.1%
Ongoing pregnancy rate per retrieval	45.6%
Miscarriage rate	2.4%

\*results displayed as median (range) or odd (%)

P-636 Wednesday, October 16, 2019 6:30 AM

**DELTA-9 THC CAN BE DETECTED AND QUANTIFIED IN THE SEMINAL FLUID OF MEN WHO ARE CHRONIC USERS OF INHALED CANNABIS.**

Malinda S. Lee, MD, MBA, Andrea Lanes, PhD, Elizabeth S. Ginsburg, MD, Janis H. Fox, MD. Brigham and Women's Hospital, Boston, MA.

**OBJECTIVE:** To detect whether delta-9 tetrahydrocannabinol (THC) and THC metabolites can be identified and quantified in human seminal fluid.**DESIGN:** Proof-of-concept study in which serum, urine and semen testing was conducted in 12 male chronic users of inhaled cannabis.**MATERIALS AND METHODS:** Healthy men aged 18-45 years who identified as chronic and heavy users of inhaled cannabis (at least 4 times per week for at least one year) were eligible to participate. Eligibility screening took place via structured phone interviews and preceded a single study visit performed at Brigham and Women's Hospital, Boston, Massachusetts. Participants were asked to abstain from ejaculation for 48-72 hours prior to their study visit, and to use cannabis within 24 hours of their visit. After informed consent was obtained, participants provided urine, semen and serum samples on site.

Semen analyses were performed as standard practice, using a Hamilton-Thorn IVOS Semen Analyzer. The remaining ejaculate, as well as the urine and serum samples, were frozen and stored at -80 degrees C. Cannabinoid assay testing in all three fluid matrices was performed through high performance liquid chromatography/tandem mass spectrometry by NMS Labs (Willow Grove, PA). Serum and semen were tested for THC (the primary active component of cannabis), 11-hydroxy delta-9 THC (11-OH THC, the main psychoactive metabolite of THC), and delta-9 carboxy THC (THC-COOH, an inactive metabolite of THC). Urine was tested for THC-COOH, the main metabolite of THC in urine, as well as creatinine to provide a normalized ratio.

**RESULTS:** The median age and BMI of participants was 27.0 years and 24.7 kg/m<sup>2</sup>, respectively. Over half the participants were daily users of cannabis and had been using cannabis regularly for over five years. On average, participants used cannabis 10 hours prior to their study visit and abstained for 53 hours from their last ejaculation. The median sperm concentration, motility and morphology was 75.5 million/mL, 69.5% and 5.5%.

Urinary THC-COOH was detected in all 12 participants, whereas at least one serum THC metabolite was present in 10 of 12 participants. Two semen samples had insufficient volume to be analyzed. Delta-9 THC was above the reporting level of 0.50 ng/mL in the seminal fluid of two of the remaining ten participants. The major downstream THC metabolites were not detected in any of the semen samples. Seminal delta-9 THC was moderately correlated with serum levels of delta-9 THC (r=0.66), serum 11-OH THC (r=0.57), and serum THC-COOH (r=0.67). Seminal delta-9 THC was not correlated with urinary cannabinoid levels or semen analysis parameters.

**CONCLUSIONS:** This is the first study to report that delta-9 THC can be identified and quantified in human seminal fluid. Seminal delta-9 THC was found to be moderately correlated with serum THC and THC metabolites.**SUPPORT:** This study was founded by the Expanding the Boundaries Grant from the Dept. of Obstetrics, Gynecology & Reproductive Biology, Brigham and Women's Hospital.

P-637 Wednesday, October 16, 2019 6:30 AM

**COMPREHENSIVE GENE EXPRESSION ANALYSIS BY RNA SEQUENCING OF TESTICULAR TISSUE EXOSOMES IN AZOOSPERMIC MEN: PREDICTING THE PRESENCE OF SPERM.**Joshua Stewart, M.D.,<sup>a</sup> Philip Xie, B.S.,<sup>a</sup> Alessandra Parrella, M.Sc.,<sup>a</sup> David Lyden, M.D., Ph.D.,<sup>b</sup> Zev Rosenwaks, M.D.,<sup>a</sup> Gianpiero D. Palermo, M.D., Ph.D.<sup>a</sup> <sup>a</sup>The Ronald O. Perleman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY; <sup>b</sup>Weill Cornell Medical College, New York, NY.**OBJECTIVE:** To isolate and characterize exosomes<sup>1</sup>, extracellular vesicles containing functional biomolecules, from testicular tissue in azoospermic men and to perform gene expression analysis in order to classify exosomes within the testicular germinal epithelium to predict spermatogenic reserve.**DESIGN:** A case and control prospective study.**MATERIALS AND METHODS:** A total of 17 surgically retrieved testicular specimens from 17 subjects (13 non-obstructive azoospermia (NOA): 7 with sperm identified at testicular biopsy and 6 with no sperm identified; 4

obstructive azoospermia (OA), all with retained spermatogenesis) were obtained from consenting men from March 2018 to August 2018. Exosome isolation was performed by a standardized differential ultracentrifugation protocol. Nanoparticle tracking analysis was used for characterization of exosome size and concentration. Protein concentration was measured by BCA assay and mass spectrometry proteomics analysis was performed. Gene expression was determined by RNA sequencing. Sequences were queried against the Homo sapiens reference genome and filtered of contaminants. The Wald test and ANOVA were used to determine significance.

**RESULTS:** The total number of isolated exosomes was 71x10<sup>9</sup>/uL specimen volume with a mean size of 129 nm. Global transcriptional change in men with OA (retained spermatogenesis) was compared to men with NOA and no sperm identified at the time of testicular biopsy by analysis of 17,571 genes. A single gene (POS) was found to be significantly upregulated in the exosomes of men with retained spermatogenesis as compared to those without sperm identified (log<sub>2</sub> fold change: 5.89, p-value 0.049). Paradoxically, within the overall NOA cohort, the majority of genes were significantly upregulated in the testicular exosomes of men that had no sperm identified at the time of biopsy compared to those with sperm identified (1,005 genes significantly upregulated, 147 significantly downregulated, p-value <0.05), including retinoic acid signaling mediators and regulators of the self-renewal capacity of germline cells. Furthermore, both groups within the NOA cohort had unique protein expression profiles with 1,926 proteins specific to men with NOA and retained spermatogenesis as compared to men with no sperm identified at the time of biopsy.**CONCLUSIONS:** Investigators have identified many potential biomarkers in men with infertility, however, few are clinically applicable currently<sup>2,3</sup>. We show that the testicular germinal epithelium secretes exosomes, which carry unique gene expression profiles in azoospermic men with and without retained spermatogenesis. Specifically, we identify a single gene (POS) essential for germline specification which is significantly upregulated in the exosomes of men with retained spermatogenesis. These transcripts may serve as a biomarker for spermatogenesis, as well as the functional capacity of spermatozoa. Furthermore, the molecular expression of testicular tissue exosomes indicates that these extracellular vesicles may interact with the germinal epithelium in order to ordain new waves of spermatogenesis.References: 1. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol.* 2013 Feb 18;200(4):373-83. <https://doi.org/10.1083/jcb.201211138>.2. Barcelo M, Mata A, Bassas L, Larriba S. Exosomal microRNAs in seminal plasma are markers of the origin of azoospermia and can predict the presence of sperm in testicular tissue. *Hum Reprod.* 2018 Jun 1;33(6):1087-1098. <https://doi.org/10.1093/humrep/dey072>.3. Kovac JR, Lamb DJ. Male infertility biomarkers and genomic aberrations in azoospermia. *Fertility and Sterility.* May 2014, Volume 101, Issue 5, e31. <https://doi.org/10.1016/j.fertnstert.2014.02.029>.**SUPPORT:** None.

P-638 Wednesday, October 16, 2019 6:30 AM

**AN AGE-BASED NOMOGRAM BASED ON CUT OFF VALUES OF SEMEN ANALYSIS RESULTS, FROM 2010 WHO REFERENCE VALUES FOR SEMEN CHARACTERISTICS.**Guy Shrem, M.D.,<sup>a</sup> Michael H. Dahan, MD,<sup>a</sup> Jacques Balayla, M.D.,<sup>b</sup> Naama Steiner, M.D.,<sup>c</sup> Alexander Volodarsky-Perel, M.D.,<sup>b</sup> Weon-Young Son, Ph.D.,<sup>d</sup> Mali Salmon-Divon, Ph.D.<sup>e</sup> <sup>a</sup>McGill University, Montreal, QC, Canada; <sup>b</sup>Affiliation not provided; <sup>c</sup>McGill University Health Centre, Montreal, QC, Canada; <sup>d</sup>Division of Reproductive Endocrinology and Infertility, McGill University Health Care Centre, Montreal, QC, Canada; <sup>e</sup>Senior Lecturer, Ariel, Israel.**OBJECTIVE:** To create a nomogram for sperm parameters along with the male life.**DESIGN:** A retrospective evaluation of all records of Computer-Assisted Semen Analysis (CASA) (and human-verified) performed between January 2009 to December 2018 at a University Health Center.**MATERIALS AND METHODS:** We encountered 17,915 CASA at all ages. Samples that did not meet the WHO lower reference limit [1] (concentration  $\geq 15$  mil/ml, motility  $\geq 40\%$ , morphology  $\geq 4\%$ ) were excluded, leaving 8045 samples.**RESULTS:** For concentration, percentiles 25<sup>th</sup> to 75<sup>th</sup> of the population had a three-phasic pattern reflecting an increase in sperm concentration until around age 30 years, followed by a plateau in sperm concentration until age 45 years, and then a decrease in sperm concentration begins.

For sperm motility, 50-95 percentiles demonstrate a triphasic distribution with an increase until 30 years of age, a plateau until the age of 40 years and

then a decrease in motility. In the groups of two lowest percentiles (10<sup>th</sup> and 25<sup>th</sup>), a modest decrease begins at age 30 years, whereas a steeper slope is seen after the age of 40 years.

For sperm morphology, there are two different phasic trends. The 50<sup>th</sup> percentile and above exhibit a decrease in normal morphology throughout the twenties, subsequently values stabilize. Opposed to this trend, the groups of two lowest percentiles (10<sup>th</sup> and 25<sup>th</sup>) have stable low morphology values up to the 7<sup>th</sup> decade.

**CONCLUSIONS:** Males have the best semen parameters from age 30-40 years. This may be acting as a compensatory mechanism to obtain pregnancy with female fertility falling at this age.

Reference: [1] Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HWG, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update 2009;16:231-45. <https://doi.org/10.1093/humupd/dmp048>.

P-639 Wednesday, October 16, 2019 6:30 AM

**YO<sup>®</sup> HOME SPERM TEST'S MOTILE SPERM CONCENTRATION AND YO SCORE<sup>™</sup> CORRELATES WITH AUTOMATED SEMEN ANALYSIS<sup>™</sup>**



**RESULTS.** Stan Honig, MD,<sup>a</sup> Lev Rabinovitch, PhD,<sup>b</sup> Natan Bar-Chama, MD,<sup>c</sup> <sup>a</sup>Yale University, New Haven, CT; <sup>b</sup>Medical Electronic Systems, Caesarea Industrial Park, Israel; <sup>c</sup>Icahn School of Medicine at Mount Sinai, New York, NY.

**OBJECTIVE:** To evaluate the accuracy of the YO<sup>®</sup> Home Sperm Test ("YO") motile sperm concentration (MSC) and YO SCORE<sup>™</sup> results in the hands of TRAINED professional as compared to the SQA-V automated laboratory sperm analyzer (Medical Electronic Systems).

**DESIGN:** Multi center, Double-blind prospective study.

**MATERIALS AND METHODS:** 316 human semen samples were tested by TRAINED professionals at three sites utilizing the YO Home Sperm test kit. In parallel, the same samples were tested on the SQA-V automated semen analyzer (Medical Electronic Systems). Samples were collected, liquefied, split and run in a blinded fashion. TRAINED professionals ran the YO test using the YO device (mini-microscope) on either a Galaxy or iPhone Smartphone following the YO app. instructions. YO automatically reports (a) LOW MSC <6m/mL or MODERATE/NORMAL MSC ≥ 6m/mL, and (b) a YO SCORE displayed as a two-digit integer, from 10 to 90+ MSC centile levels derived from the 2010 WHO 5<sup>th</sup> edition Table A1-2 of semen parameter centile distribution for recent fathers. The YO MSC results from the TRAINED professional were analyzed statistically vs. the SQA-V based on negative and positive percent agreement (NPA and PPA). The TRAINED professional YO SCORE results were analyzed for accuracy vs. SQA-V semen quality groupings with an allowance of ± one YO SCORE deviation.

**RESULTS:** The YO device demonstrated high levels of PPA, NPA and accuracy when TRAINED professional MSC levels were compared vs. SQA-V results: 97.6%, 97.0% and 97.3% respectively with inter-site CV ≤ 2%: YO SCORE results obtained by TRAINED professionals demonstrated an overall accuracy of 94.3% for distinguishing between MSC semen quality groups which were established based on SQA-V MSC.

**CONCLUSIONS:** Using the YO<sup>®</sup> Home Sperm Test TRAINED professionals showed a high level of accuracy for motile sperm concentration when compared to the SQA-V automated laboratory sperm analyzer in 316 semen samples. This study also demonstrated that the YO SCORE is a reliable tool for defining different motile sperm concentration categories.

Reference: None.

SUPPORT: Medical Electronic Systems.

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**FERTILITY AND INFERTILITY TREATMENT KNOWLEDGE AMONG MEN AGED 18-50 IN THE U.S.**



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**OBJECTIVE:** To validate the Fertility and Infertility Treatment Knowledge Scale (FIT-KS) among men aged 18-50 in the United States, and to assess fertility knowledge among men in the general population, with comparison to the female population in the original validation study.

**DESIGN:** Cross-sectional web-based survey study.

**MATERIALS AND METHODS:** An online survey with format identical to that previously constructed for the original FIT-KS validation study was administered to English-fluent men aged 18-50 residing in the United States. STATA v15.1 was used to compute descriptive statistics, and conduct analyses, including the Student's t, Pearson's X<sup>2</sup>, Spearman's ρ, and Kruskal-Wallis tests, to assess for correlation to demographics and comparison between the male and female cohorts. The study received IRB exemption.

**RESULTS:** In preliminary analysis, 99 men completed the survey, with median age 30 [28, 37]; 50 (50.5%) were single, with 14 (14.1%) in a relationship, 33 (33.3%) married, 2 (2%) divorced. Most (65.7%) had no children, and identified as White (74.8%), with 9.1% Hispanic or Asian, and 7.1% Black. The majority (89.9%) reported an annual household income at or below \$100k, and 60.6% held a college or higher degree.

The mean FIT-KS score was 12.3 +/- 0.34 (out of 29, 42.4% correct). Increasing age was the only significant demographic predictor of higher FIT-KS score (p=0.002). In item analysis, notable findings include: though 74 (74.8%) knew at which ages women are most fertile, many (48.5%) overestimated age of maximal fertility decline, fecundability at age 30 (63.6%) or at age 40 (71.7%), and 74.7% underestimated the spontaneous miscarriage rate. Only 6.1% agreed that men can contribute to a couple's infertility, though 25.3% acknowledged male age could impact fertility. Only 17.2% knew how long sperm survive in the female reproductive tract. A majority were generally aware of lifestyle issues that impact fertility, though only 31.3% knew about lubricants. When asked about IVF, 19.2% overestimated success rates at female age 35 and 85.9% at age 44. The twin rate was underestimated by 70.7%, and 95% overestimated success rates for oocyte cryopreservation.

When compared to the original validation cohort, men in this sample scored lower than women on total FIT-KS score (12.3 +/- 0.34 vs. 16.2 +/- 0.32), as well as in natural fertility and infertility treatment sub-sections (all p<0.0001).

**CONCLUSIONS:** These preliminary results uphold the conclusion that fertility knowledge in the general population is low. Though the validation analysis for the FIT-KS in men is ongoing, these findings suggest that men also tend to overestimate natural fertility and infertility treatment success rates and underestimate risks and impact of lifestyle. Most surprising, the low rate of acknowledging the male role in infertility suggests a particular need for education in this area. Outreach efforts aimed at educating the public about fertility must target both men and women to sufficiently penetrate the general population and correct gaps in knowledge.

SUPPORT: None.

TABLE 1

Site Name, Location	N	YO MSC TEST		
		Results: TRAINED vs. SQA-V		
		PPA	NPA	Accuracy
Xytex Corporation, Augusta, GA	82	100.0%	98.2%	99.1%
Xytex Corporation, New Brunswick, NJ	136	97.0%	97.1%	97.1%
Medical Electronic Systems, Caesarea, IL	98	96.2%	95.8%	96.0%
OVERALL	316	97.6%	97.0%	97.3%
Inter-site CV		2.0%	1.2%	1.6%
<b>YO SCORE Agreement TRAINED vs. SQA-V</b>				
SQA-V Semen Quality Group	SQA-V MSC Range, 10 <sup>6</sup> /mL	YO SCORE	YO SCORE Accuracy vs. SQA-V	
LOW (n = 90)	0 - <6	0	96.7%	
LOW NORMAL (n = 55)	6 - 32	10 - 30	94.5%	
AVERAGE NORMAL (n = 78)	32 - 63	40 - 60	93.6%	
HIGH NORMAL (n = 93)	63 - >94	70 - >90	92.5%	
OVERALL (n = 316)			94.3%	

**EFFICACY OF ANTIOXIDANT SUPPLEMENTATION ON CONVENTIONAL AND ADVANCED SPERM FUNCTION TESTS IN PATIENTS WITH IDIOPATHIC MALE INFERTILITY.**

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**OBJECTIVE:** Antioxidants have long been used in the empirical treatment of infertile men. While a positive effect has been reported by a number of studies, others have failed to reproduce any benefit leading to controversy regarding their efficacy in the treatment of infertility. The aim of the present study was to evaluate the effects of antioxidant combination therapy on conventional semen parameters and advanced sperm function tests in men seeking fertility.

**DESIGN:** Prospective clinical trial.

**MATERIALS AND METHODS:** 148 patients presenting with male factor infertility to a tertiary medical center with at least one abnormal semen parameter over a period of 6 months were included. Patients with varicocele, leukocytospermia, history of genitourinary infections, any febrile illness and exposure to chemo-radiation were excluded.

All participants were treated with the antioxidant supplement FH-PRO (1000 mcg B12, 30mg Zinc, 140mcg Selenium, 350mg Arginine, 2000mg, 200mg Co-Q10, 120mg Vitamin C, 200IU Vitamins E) (Fairhaven Health, Bellingham, WA) for a period of 3 months. Semen analysis, sperm DNA fragmentation (SDF) (Halosperm kit, Halotech, Madrid, Spain), oxidation reduction potential (ORP) (MiOXSYS, Aytu BioScience, Englewood, CO) and hormones (estradiol, FSH, LH, prolactin, and testosterone) were performed on all participants initially and following treatment. Numbers (percentages) were used to report categorical values while mean ± SE to report numerical values. Results were compared using Wilcoxon Signed Ranks Test and a p value of <0.05 was considered statistically significant.

**RESULTS:** The mean age of study participants was 35.9 ± 0.5 years and body mass index 29.6 ± 0.4 Kg/m<sup>2</sup>. Compared to the pretreatment test results, there was statistically significant improvement in conventional semen parameters including sperm concentration, total and progressive motility and normal morphology after 3 months of treatment with FH-PRO. Furthermore, a significant improvement in advanced sperm function tests (SDF & ORP) was also observed following antioxidant supplementation.

**CONCLUSIONS:** Treatment of patients with idiopathic male infertility with FH-PRO antioxidant regimen for 3 months resulted in significant improvement in conventional semen parameters and advanced tests of sperm function. It may offer promise to the medical treatment of idiopathic male infertility.

TABLE

Parameters	Pre-treatment	Post-treatment
Semen volume (ml)	3.18 ± 0.12	3.12 ± 0.11
Sperm concentration (10 <sup>6</sup> /ml)	22.23 ± 2.01	30.57 ± 2.26*
Total motility (%)	34.59 ± 1.43	38.47 ± 1.54*
Progressive motility (%)	4.00 ± 0.61	8.06 ± 0.81*
Normal morphology (%)	2.86 ± 0.19	3.98 ± 0.26*
SDF (%)	38.63 ± 2.10	32.04 ± 1.82*
ORP (mV/10 <sup>6</sup> sperm/mL)	10.26 ± 1.29	6.21 ± 1.18*

\* P<0.05.

**THE FREQUENCY OF PARENTAL CONSANGUINEOUS MARRIAGE AND EFFECT ON CLINICAL PARAMETERS IN MEN WITH IDIOPATHIC NON-OBSTRUCTIVE AZOOSPERMIA.**

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**OBJECTIVE:** To determine the frequency of parental consanguineous marriage (PCM) in men with idiopathic non-obstructive azoospermia (INOA) and to search predictive clinical parameters on the success of sperm retrieval with micro-TESE operation.

**DESIGN:** A retrospective cohort study.

**MATERIALS AND METHODS:** 246 azoospermic men with INOA were analyzed retrospectively. Micro-TESE operation was performed in all pa-

tients. Patients were divided into two groups as group 1 who have PCM and group 2 who have not PCM. The clinical parameters and surgical sperm retrieval rates were compared between the groups.

**RESULTS:** A total of 81 out of 246 (33%) patients with INOA have parental consanguinity. In 72 out of 246 (29.3%) men, sperm recovery was successful with micro-TESE operation. There was no difference in sperm retrieval rate among men who had parental consanguinity (23.4%) compared to men who did not (32.1%) (p = 0.18). Men who had parental consanguinity had significantly lower FSH (13.7 vs 21.9 mIU/mL; p=0.0001), larger testes (14.1 vs 11.8 mL; p=0.0008) and higher total testosterone levels (3.8 vs 3.4; p=0.02) compared to men who did not. Men who had successful sperm retrieval had smaller testes compared to men who did not.

**CONCLUSIONS:** In our INOA cohort, we found that 33% of the men have PCM. The testis volumes were higher, serum FSH levels were lower and mostly pathological pattern was showed maturation arrest in INOA patients with parental consanguinity compare to patients who have not. Genetic research on consanguineous families may enable the emergence of new knowledge about male reproductive genetics, which will improve the management of infertile men.

**SUPPORT:** None.

**VARICOCELE AND TESTICULAR HYPERTHERMIA: INFRARED DIGITAL THERMOGRAPHIC MEASUREMENT OF SCROTAL AND INGUINAL TEMPERATURES AMONG VARICOCELE PATIENTS AND NORMAL CONTROLS.**

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**OBJECTIVE:** Testicular hyperthermia has been considered the primary pathophysiology leading to spermatogenic dysfunction in patients with varicocele. This study aims to compare scrotal and inguinal temperature measurements between varicocele patients and normal controls linking such measurement with semen parameter results.

**DESIGN:** Prospective comparative study.

**MATERIALS AND METHODS:** Patients presenting with left clinical varicocele to our male infertility unit over a period of 1 year were included. The exclusion criteria were history of genitourinary infections/surgery and prior infertility related treatment. Conventional semen analysis (WHO 2010) and oxidation reduction potential (ORP) measurement (MiOXSYS, Aytu Bioscience, Englewood, USA) were performed on all study participants. Controls (group I) with no varicocele and normal semen (sperm concentration ≥ 15x10<sup>6</sup>/ml + total motility ≥ 40% + normal morphology ≥ 4%) were recruited (n=32). Varicocele patients were classified into normal semen (group II; n=57); and abnormal semen (group III; n=22) (≥ 1 abnormal parameter). Bilateral scrotal and inguinal infrared digital thermographic imaging (FLIR E6, FLIR systems, Wilsonville, USA) was performed on patients and controls. Temperature measurements were compared between all study groups using ANOVA. Pearson's correlation was performed to examine the link between temperature findings and different variables.

**RESULTS:** The study population's mean age was 33 ± 4.5 years. Left scrotal, inguinal & right scrotal temperatures were significantly higher in group III compared with controls. ORP was significantly higher in group III than the other 2 groups.

	(I) Controls	(II) Varicocele + Normal Semen	(III) Varicocele + Abnormal Semen
Left Scrotal temp (°c)	33.1 ± 0.8*	33.2 ± 0.7	33.6 ± 0.8*
Right Scrotal temp (°c)	32.9 ± 0.9*	33.1 ± 0.7	33.6 ± 0.8*
Left Inguinal temp (°c)	34.3 ± 1.0*	34.5 ± 0.9	34.9 ± 1.2*
Right inguinal temp (°c)	33.9 ± 1.1	33.9 ± 0.8	34.4 ± 1.3
Left Testicular size (cm <sup>3</sup> )	16.5 ± 5.1*	15.1 ± 4.8 <sup>†</sup>	11.4 ± 5.0* <sup>†</sup>
Right Testicular size (cm <sup>3</sup> )	16.9 ± 5.7	19.3 ± 7.2	12.9 ± 4.2
sORP (mV/10 <sup>6</sup> sperm)	2.2 ± 1.5*	1.8 ± 1.2 <sup>†</sup>	22.5 ± 12.7* <sup>†</sup>

\*Significance between (III) & (I); <sup>†</sup> Significance between (III) & (II).

Left scrotal temperature was significantly negatively correlated with sperm concentration (-0.268,  $p=0.004$ ), total motility (-0.337,  $p<0.001$ ), normal morphology (-0.282,  $p=0.003$ ) & left testicular size (-0.292,  $p=0.002$ ). While it was significantly positively correlated with ORP (0.374,  $p=0.01$ ), left inguinal temperature (0.521,  $p<0.001$ ) & right scrotal & inguinal temperatures (0.843,  $p<0.001$ ; 0.521,  $p<0.001$ ).

**CONCLUSIONS:** Increased scrotal and inguinal temperatures are detected in patients with testicular dysfunction secondary to clinical varicocele.

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#### RE-EXAMINING THE INCIDENCE OF KARYOTYPIC ABNORMALITIES AND Y CHROMOSOME MICRODELETIONS IN MALES WITH AZOOSPERMIA OR SEVERE OLIGOSPERMIA.



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**OBJECTIVE:** Prior studies evaluating the prevalence of karyotypic abnormalities and Y chromosome microdeletions in males with azoospermia and severe oligospermia are limited by small sample sizes and may differ from those seen in the modern clinical infertility setting<sup>1-6</sup>; thus, we set out to evaluate the prevalence of these abnormalities in a large clinical cohort undergoing IVF.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** Couples treated with in vitro fertilization (IVF) from January 2014- April 2019, with 'male factor' assigned as an infertility diagnosis were screened against inclusion criteria: sperm concentration < 5 million sperm/mL and available results for karyotype and/or Y chromosome microdeletion panel. Patients were included in the analysis whether autologous sperm was available for IVF/ICSI (89.4%) or donor sperm needed to be used. The prevalence of karyotypic abnormalities and Y microdeletions were calculated within this population.

**RESULTS:** Over 7300 male partners were screened, and 1315 unique male patients met inclusion criteria. When normal variants were excluded, 9.0% of all patients had a karyotypic abnormality or Y microdeletion. Of these, 76 (5.9%) patients had abnormal karyotypes and 42 (4.4%) patients had Y microdeletions. The most common karyotypic abnormalities were Klinefelter Syndrome (38.2%), balanced translocations (17.1%), and Robertsonian translocations (15.8%). Chromosome 9 inversions, which are classified as normal variants, were found in 15 patients (1.2%). All Y microdeletions identified were in AZFc, which may be due to better prognosis for obtaining sperm for ICSI among men with AZFc deletions relative to AZFa and AZFb microdeletions.

**CONCLUSIONS:** In a large population of azoospermic and severely oligospermic men whose female partners underwent IVF, the incidence of karyotypic abnormalities was approximately 6%, which is similar to previous reports. Our study population was nearly four-times larger than prior studies. While non-existence of results positive for AZFa and AZFb microdeletions must be taken into account, the low incidence (4.4%) of AZFc microdeletions in our population indicates that Y chromosome microdeletions may be less common in men with azoospermia and severe oligospermia than previously reported.

**References:** 1. Reijo R, Alagappan RK, Patrizio P et al: Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet* 1996; 347:1290.

2. Pryor JL, Kent-First M, Muallem A, Van Bergen AH, Nolten WE, Meisner L, et al. Microdeletions in the Y chromosome of infertile men. *N Engl J Med* 1997; 336:534-9

3. Van Assche E, Bonduelle M, Tournaye H, et al. Cytogenetics of infertile men. *Human reproduction (Oxford, England)*. 1996;11 Suppl 4:1-24; discussion 25-26.

4. Chandley AC. Chromosome anomalies and Y chromosome microdeletions as causal factors in male infertility. *Human reproduction (Oxford, England)*. 1998;13 Suppl 1:45-50.

5. Ghorbel M, Gargouri Baklouti S, Ben Abdallah F, et al. Chromosomal defects in infertile men with poor semen quality. *Journal of assisted reproduction and genetics*. 2012;29(5):451-456.

6. Mascarenhas M, Thomas S, Kamath MS, et al. Prevalence of chromosomal abnormalities and Y chromosome microdeletion among men with severe semen abnormalities and its correlation with successful sperm retrieval. *Journal of human reproductive sciences*. 2016;9(3):187-193.

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#### IMPACT OF COMMERCIAL SPERM SUPPLEMENTATION ON SEMINAL PARAMETERS.



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**OBJECTIVE:** Various products on the market claim to improve seminal parameters, but the impact of such supplements has not been well studied in a clinical setting. We sought to evaluate the effects of a new commercially available supplement on patients with male factor infertility.

**DESIGN:** Prospective.

**MATERIALS AND METHODS:** An initial semen analysis (SA) was performed and, if abnormalities were noted, men were started on Androferti (Innovus Pharmaceuticals, San Diego, CA), a commercial supplement in powder form, taken twice daily. Androferti is a blend of nutrients including L-Carnitine, Vitamin C, Selenium, CoQ-10, Zinc, Vitamin E, Folate, and Vitamin B12. A second SA was then performed after a minimum of 30 days to evaluate the results of supplementation.

**RESULTS:** A total of 120 male patients with an abnormal initial SA and at least 30 days of taking Androferti (149.15  $\pm$  146.26 days on average) were prospectively evaluated. Of these patients, 94 had been on Androferti for 60 or more days. There were no differences in average semen volume (2.93 vs 2.80 cc,  $P=NS$ ) or concentration (46.2 vs 42.1  $\times 10^6/cc$ ,  $P=NS$ ) between the first and second SA, but there were significant increases in sperm motility and progression (36.6% vs 40.2%,  $P=0.023$ ; 2.19 vs 2.35,  $P=0.034$ , respectively). The average number of total motile sperm per ejaculate, however, did not differ between the first and second SA (39.6 vs 39.6 Millions,  $P=NS$ ). There was also a trend towards improved strict sperm morphology after 30 days on Androferti (1.93% vs 2.74%,  $P=0.061$ ).

**CONCLUSIONS:** Supplementation with Androferti significantly improved sperm motility and progression, with a trend to improve strict morphology, as early as 30 days after supplementation. Future studies will evaluate whether such supplementation will improve spontaneous conception or IUI success rates.

**Reference:** None.

**SUPPORT:** None.

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#### REPEAT SEMEN ANALYSIS – AN UNNECESSARY DELAY IN UROLOGIC EVALUATION?



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**OBJECTIVE:** Most conventionally accepted guidelines recommend obtaining a repeat semen analysis (SA) following an abnormal initial test. The objective of this study is to determine if repeating a SA when one or more abnormal values is identified may unnecessarily delay REI referral to a Urologist by determining the likelihood that a patient with an abnormal SA will have an entirely normal SA with subsequent tests.

**DESIGN:** Retrospective cohort study at a single academic medical center.

**MATERIALS AND METHODS:** All men who underwent two or more SA (one to six months apart) from January 2016 to December 2018 at one large academic fertility center were included. Semen samples were analyzed manually by trained technicians according to 2010 World Health Organization (WHO) criteria. Normal values included concentration  $\geq 15$  million/mL, motility  $\geq 40\%$ , and Kruger morphology  $\geq 4\%$ . Total motile sperm concentration (TMSC) was calculated by multiplying the concentration  $\times$  volume  $\times$  motility divided by 100 and considered normal at  $>20 \times 10^6$  per ejaculate. SA parameters were sequentially analyzed for differences between the first and any subsequent SA to determine how often an abnormal SA becomes entirely normal with additional tests. We assumed that abnormalities in any SA parameter would result in Urology referral and analyses were performed with and without consideration of morphology defects.

**RESULTS:** Five hundred fifty first and second SA from 275 men were analyzed, each of whom had at least one defect in the first SA (Table). The most common abnormality was morphology defects. Seventy-nine percent (N=217) of men had at least one abnormality on the second test as well, while the remaining 21% had SA that normalized entirely with a second

Initial defect	% Normal second SA	% Persistent defect	% Different defect
Any	21.1	78.9	–
Concentration	8.8	72.8	18.4
Motility	8.8	60.5	30.7
Morphology	19.8	71.1	9.1
TMSC	10.3	71.7	17.9

SA, including morphology defects. When morphology defects were excluded, approximately 3/4 (73.3%) of men with an initial abnormal SA had persistently abnormal results on a second test, while the remaining 26.7% had a normal second SA. Among patients with at least two initial defects, only 8.1% had a normal second SA; when morphology defects were excluded, this figure increased to 16.4%.

**CONCLUSIONS:** The majority of men with abnormal semen analyses on initial testing have persistent abnormalities on repeat testing that warrant referral to Urology. Less than 1 in 10 men with two or more defects on initial testing had a normal second SA. These results suggest that referral to a Urologist may be considered after a single abnormal SA to expedite male-factor infertility workup and treatment.

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#### LONG TERM SAFETY AND EFFICACY OF CLOMIPHENE CITRATE FOR THE TREATMENT OF MALE HYPOGONADISM.

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**OBJECTIVE:** The aim of our study was to assess the ability of clomiphene citrate (CC), a selective estrogen receptor modulator, to maintain eugonadal testosterone levels and improve the symptoms of hypogonadism in men being treated with CC for extended periods of time.

**DESIGN:** A retrospective chart review was performed to identify all patients treated with CC for hypogonadism at two institutions from 2010-2018. Duration of CC therapy, serum testosterone levels, improvement in hypogonadal symptoms, and side effects while on CC were assessed.

**MATERIALS AND METHODS:** Hypogonadism was defined as a baseline serum testosterone < 300ng/dL. Side effects while on CC were subjectively reported by patients. As the longest duration of CC treatment in the literature to date is 3 years, patients were divided into those on CC treatment

for ≤ 3 years, and those on treatment for > 3 years. Unpaired t-test was used to evaluate changes in testosterone and estradiol between groups. Fisher's exact test was used to compare side effects, symptom improvement, and requirement for anastrozole between groups.

**RESULTS:** 400 patients were treated with CC from 2010-2018. Mean patient age was 39 ± 11 years. Mean length of CC treatment was 25.5 ± 20.48 months with a range of 0-84 months. 280 patients were treated with CC for ≤ 3 years (mean CC duration 12.75 ± 9.52 months), and 120 patients were treated with CC for > 3 years (mean CC duration 51.93 ± 10.52 months). Following treatment with CC for > 3 years, 106 patients (88%) achieved eugonadal testosterone levels, 92 patients (77%) reported improvement in hypogonadal symptoms, and 10 patients (8%) reported side effects on CC. There was not a statistically significant difference in the results between patients treated > 3 years and patients treated ≤ 3 years. The most common side effects reported by patients treated > 3 years included changes in mood (N=5), blurred vision (N=3), and breast tenderness (N=2). There were no significant adverse events with long term sequelae in any patients treated with CC.

**CONCLUSIONS:** Testosterone replacement therapy (TRT) has traditionally been the primary treatment for hypogonadism in men. However, exogenous testosterone disrupts the hypothalamic-pituitary-gonadal (HGP) axis and suppresses intratesticular testosterone production and spermatogenesis. CC is commonly used to treat hypogonadism in men desiring to preserve spermatogenesis and fertility, and may be used as an off-label primary treatment for hypogonadism. There is a paucity of long-term data on the efficacy and safety of CC, with no published data with the use of CC in men for durations longer than three years. CC has not historically been offered as a primary treatment for hypogonadism in men who do not desire fertility preservation, perhaps in part due to the lack of data regarding long term safety and efficacy of CC. This data demonstrates that CC is safe and effective with few side effects when used as a long-term treatment for hypogonadism.

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#### PATERNAL AGE IS A PREDICTOR OF ELEVATED SPERM DNA FRAGMENTATION IN INFERTILE MEN.

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**OBJECTIVE:** Increased sperm DNA fragmentation (DF) has been associated with reduced embryo quality and pregnancy rates, and increased miscarriage rates. The underlying cause of increased SDF is unknown. Our objective is to examine clinical factors associated with abnormal DF in infertile men.

**DESIGN:** Cross sectional study.

TABLE 1. Characteristics of males by level of DF

	0<SCSA ≤ 30% (n=119)	SCSA > 30% (n=28)	P value
Sperm concentration (million/ml)	20.0 (11.0, 40.0), n=119	18.0 (12.0, 51.5), n=28	0.624
Normal morphology (%)	5.0 (3.0, 8.5), n=104	5.0 (2.0, 11.0), n=19	0.666
Total motility (%)	45.2 ± 15.6, n=119	38.2 ± 20.5, n=28	<b>0.048</b>
Age (yrs)	33.0 (30.0, 36.0), n=119	36.0 (32.5, 40.0), n=28	<b>0.009</b>
BMI (mg/kg <sup>2</sup> )	27.7 (24.2, 31.3), n=118	28.0 (24.3, 30.9), n=27	0.994
History of Smoking			0.748
Never	71/119 (59.7)	17/28 (60.7)	
Current	106/119 (89.1)	2/28 (7.1)	
Former	5/119 (4.2)	9/28 (32.1)	
History of Alcohol Use			0.476
Never	8/119 (6.7)	0/28 (0.0)	
Current	106/119 (89.1)	27/28 (96.4)	
Former	5/119 (4.2)	1/28 (3.6)	
Varicocele (self-reported)			1.000
Yes	11/119 (9.2)	2/28 (7.1)	
No	108/119 (90.8)	26/28 (92.9)	
Duration of Infertility (months)	24.0 (16.0, 36.0), n=116	24.0 (13.0, 36.0), n=26	0.951

Wilcoxon's rank-sum test, Chi-square, or Fisher's exact test were used where appropriate.

**MATERIALS AND METHODS:** A secondary analysis of 147 infertile males enrolled in the Male, Antioxidant, and Infertility (MOXI) Trial. MOXI participants, who were 18-40 years old with at least one abnormal semen parameter, provided a semen sample and completed questionnaires including baseline demographics, health and lifestyle factors. Semen samples underwent standard semen analysis and DF testing using sperm chromatin structure assay. Abnormal DF was defined as > 30%. Bivariate analysis and subsequent multivariable regression analysis were performed. Variables were introduced to the multivariable regression analysis in a step-wise fashion, using a p-value of <0.10 on the bivariate analysis to enter and a p-value of < 0.05 to remain.

**RESULTS:** Nineteen percent of subjects had DF >30%. Males with abnormal DF were older and had lower total sperm motility compared to controls (Table 1). No differences were seen in environmental or lifestyle exposures between groups (data not shown). Only male age remained a significant predictor of abnormal DNA fragmentation in the regression model (OR 1.16; 95% CI 1.03,1.32; p=0.02).

**CONCLUSIONS:** Older male age and lower sperm motility, but not smoking, obesity, or environmental or lifestyle exposures are associated with increased DF among infertile males. Longitudinal studies are needed to confirm causal inference. The role of abnormal DF during infertility treatment as well as optimizing treatment options in men with abnormal DF is worthy of further study.

**SUPPORT:** R25 HD 0757375; U10HD077844, U10HD077680, U10HD077841, U10HD027049; U10HD038992; U10HD039005; and U10HD055925.

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**ANTIOXIDANT COMBINATION THERAPY: A NEW HOPE FOR OLIGOASTHENOTERATOSPERMIC PATIENTS.**

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**OBJECTIVE:** Idiopathic oligoasthenoteratospermia (iOAT) is a challenging condition often seen in up to 40% of infertile men and has been linked with increased seminal oxidative stress. This study aims at evaluating the effect of antioxidant combination formula (FH PRO) on the semen parameters and advanced sperm function tests in patients with iOAT.

**DESIGN:** Prospective clinical trial.

**MATERIALS AND METHODS:** Patients presenting to the Male infertility clinic with semen parameters showing iOAT (sperm concentration > 1 and ≤ 15 million/ml, motility ≤ 40%, normal forms ≤ 4.0%) were included in the study. Patients with clinical varicocele, epididymo-orchitis, irradiation or chemotherapy, history of recent STDs infection, malignancy and recent antioxidant use were excluded. Study subjects received antioxidant formula FH Pro, Fairhaven Health (1000 mcg B12, 30mg Zinc, 140mcg Selenium, 350mg Arginine, 2000mg, 200mg Co-Q10, 120mg Vitamin C, 200IU Vitamins E) (Fairhaven Health, Bellingham, WA) daily for 3 months.

Semen samples were collected before and after treatment and analyzed according to WHO 5<sup>th</sup> edition guidelines and for oxidation reduction potential (ORP) (MiOXSYS analyzer, Aytu Bioscience, Englewood, USA) and sperm DNA fragmentation (Halosperm kit, Halotech, Madrid, Spain). Numbers (percentages) were used to report categorical values while mean ± SE was used to report numerical values. Results were compared using Kruskal Wallis Test and a p value of <0.05 was considered statistically significant.

**RESULTS:** 52 infertile patients completed the study with a mean age 35.7±6.6 years and a mean infertility duration 5.9±4.2 years. There was a significant improvement in semen parameters including sperm count (p0.001), progressive motility (p0.002) and normal morphology (p0.001)

Parameters	Pre-treatment	Post-treatment
Vol (ml)	3.26±0.28	3.09±0.23
Concentration (millions/ml)	6.21±0.66	11.46±1.82*
Motility (%)	18.56±1.62	27.2±2.73*
Progressive Motility (%)	0.23±0.16	3.37±0.91*
TMSC (millions/ejaculate)	4.288±0.83	11.86±2.65*
Normal Morph (%)	1.4±0.13	2.4±0.28*
DNA Fragmentation (%)	49.58±5.34	35.68±4.68*
ORP (mV/106 sperm/mL)	18.79±3.09	10.34±1.67*

\*p<0.05.

compared to pre-treatment results. Significant decrease in seminal oxidation reduction potential was observed (p 0.001), as well as significant decrease in sperm DNA fragmentation (p0.007).

**CONCLUSIONS:** Medical treatment of infertile men with idiopathic OAT by Fairhaven Pro resulted in a significant improvement in semen parameters, reduction in seminal oxidative stress and sperm DNA fragmentation. We conclude that these changes should lead to improvement in men's fertility and better outcome in natural conception as well as in assisted reproduction.

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**THE VALUE AND USAGE OF DNA BANKING ON SEMEN DONORS.** Lauren Isley, M.S., L.C.G.C., Kara Baldwin, M.S., Pamela Callum, M.S., California Cryobank, Los Angeles, CA.



**OBJECTIVE:** To illustrate the uses and benefits of banked extracted DNA on semen donors based on our experience with genetic evaluation needs after the donor's initial qualification.

**DESIGN:** Data was compiled for all additional genetic evaluation needs on California Cryobank (CCB) semen donors from 2017 to 2018 following the donor's initial qualification. Cases involving stored DNA as the utilized sample type were identified. The data was then evaluated based on the specific indication for the additional testing.

**MATERIALS AND METHODS:** Not applicable.

**RESULTS:** Banked extracted DNA was utilized for genetic evaluation purposes in 24 cases involving 19 donors. In the majority of cases (13/24), the additional testing was performed based on a recipient's request to evaluate the donor's carrier status for an autosomal recessive condition for which the recipient was a carrier. One case involved a request to perform HLA testing for compatibility purposes. In seven cases, extracted DNA was utilized for preimplantation genetic testing (PGT) assay creation based on recipient need. Three cases involved additional genetic testing on the donor prompted by the report of a genetic condition in a donor-conceived offspring. In 2 of these 3 cases, testing confirmed the donor's carrier status for an autosomal recessive condition for which he was not previously tested, resulting in restricted distribution of remaining vials and recipient notifications.

**CONCLUSIONS:** Banking extracted DNA on gamete donors is advantageous both for gamete donor facilities and recipients. Banked DNA is valuable when a donor is unavailable for sample collection and may serve multiple purposes, including additional evaluations to investigate reports of genetic diagnoses in donor-conceived offspring. Given the rapid evolution and availability of genetic testing, gamete donor facilities may consider a uniform approach to DNA banking on donors with careful attention to the initial consent process for DNA collection and re-contacting donors to discuss requests for specific uses of their DNA samples.

**SUPPORT:** California Cryobank.

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**COMPARISON OF SEMEN QUALITY IN NORTHERNTAIWAN BETWEEN 2017 AND 2001-2010.**

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**OBJECTIVE:** Semen quality is a crucial indicator of male reproductive ability. This study aimed to show the trend of men sperm quality in northern Taiwan in the year of 2017.

**DESIGN:** We recruited 1125 male samples in 2017 from Center of reproductive Medicine, Taipei Medical University Hospital. The semen data of 2017 were compared to the semen data from 2001 to 2010.

**MATERIALS AND METHODS:** Semen analysis was performed through standardized methods outlined in the World Health Organization laboratory manual. Furthermore, sperm sample of low quality rate was calculated.

**RESULTS:** The median of sperm volume, total sperm count, progressive sperm motility and rapid progressive sperm motility in 2017 was decreased by 0.5ml, 2×10<sup>6</sup>/ml, 8% and 3% respectively, compared to the data of 2001-2010. Low quality rate of sperm concentration, volume, total sperm count, progressive motility and rapid progressive motility in 2017 were increased significantly.

**CONCLUSIONS:** These finding shows the fact that man sperm parameter values

were significantly decreased in the year of 2017 in comparison with the data in 2001-2010. Moreover, it was estimated that total sperm count was decreased by 2.85×10<sup>6</sup>/ml annually.

### PERSISTENT GENDER GAP AND A TREND TOWARDS SUBSPECIALIZATION: CHARACTERISTICS OF SURGEONS PERFORMING VASECTOMY IN THE UNITED STATES.

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**OBJECTIVE:** We sought to characterize trends in the characteristics of urologic surgeons performing vasectomy over time.

**DESIGN:** Retrospective, cross-sectional study.

**MATERIALS AND METHODS:** We examined surgeon characteristics for case logs from American Board of Urology (ABU) certifying urologists between 2004 and 2013, which included information on surgeon age, gender, certification cycle, self-reported subspecialty, and practice area population. We used generalized estimating equations (GEE) with a log link and negative binomial distribution to determine whether the association between a surgeon characteristics, such as gender, and the count of vasectomies, changed over time. Analyses were conducted in R version 3.4.3 and SAS 9.4.

**RESULTS:** A total of 115,146 vasectomies were performed by 5,415 individual certifying urologists. Mean surgeon age was 43.9±8.3 years, which remained stable throughout the study. The majority of surgeons self-identified as general urologists (80.6%). A small proportion identified as andrology and infertility specialists (1.7%), pediatric urologists (1.4%), and other specialists (16.4%). Surgeons were equally distributed across the various certification cycles.

Median number of vasectomies performed per certifying surgeon during the study period was 12 (interquartile range [IQR] 5-23), ranging from 10 to 12.5 for each individual certifying year. Based on the distribution of vasectomies performed per cycle year, The majority of vasectomies were performed by high-volume surgeons (≥ 23 vasectomies) ranging from 51.5% - 66.0%, whereas the proportion performed by low-volume (≤ 5 vasectomies) surgeons ranged from 4.2% - 6.13%. The maximum number of vasectomies performed by a single certifying surgeon was 1,183. Female surgeons accounted for approximately 7.0% of all certifying urologists. Over time, the percent of female surgeons performing vasectomies increased from 2.9% in 2004 to 9.2% in 2013. Male surgeons performed vasectomies 2.33 times more frequently than female surgeons (CI: 2.05 to 2.65; p<.0001). There was no statistical evidence to suggest this gap has changed over time. In other words, the interaction between year and surgeon gender on the count of vasectomies was not found to be statistically significant.

**CONCLUSIONS:** While the majority of surgeons performing vasectomy identify as general urologists, there are clear trends towards subspecialization of vasectomy among a small number of high-volume surgeons. Furthermore, while the proportion of vasectomies performed by female surgeons has increased over time, a gender gap persists.

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### UTILIZATION OF EJACULATED SPECIMEN AFTER ICSI FAILURE WITH TESTICULAR SPERMATOZOA FROM MEN WITH HIGH DNA FRAGMENTATION.

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**OBJECTIVE:** To assess the benefit of using ejaculated spermatozoa from men with high DNA fragmentation in their ejaculate after intracytoplasmic sperm injection (ICSI) failure with testicular specimen.

**DESIGN:** Consenting couples with a history of assisted reproductive technology (ART) failure, and male partners with elevated DNA fragmentation in their ejaculate, were treated by testicular biopsy and underwent a subsequent ICSI cycle with ejaculated spermatozoa. Embryology and clinical outcome were compared between the two semen origins.

**MATERIALS AND METHODS:** A total of 43 couples had their ejaculate assessed for DNA fragmentation by terminal deoxynucleotidyl dUTP nick-end labeling (TUNEL). A threshold of <15% was considered normal, with at least 500 spermatozoa assessed per patient. ICSI and testicular biopsy were performed in the standard fashion.

**RESULTS:** A total of 43 couples (maternal age, 36.6±6; paternal age, 41.3±10) underwent 65 cycles with testicular biopsy due to a prior history of ART failure and high DNA fragmentation in the male partners' ejaculate.

The parameters for the testicular specimens were an average concentration of 1.8±4 million and 5.0±11% motility. ICSI with testicular spermatozoa (ICSI-TESE) had a fertilization rate of 53.0%, an implantation rate of 13.2%, and a clinical pregnancy rate (CPR) of 23.0%, which led to a delivery rate of 21.1%. The pregnancy loss rate was 1.9%. Subsequently, these couples attempted cycles (n=85) with ejaculated spermatozoa. These specimens were characterized by an average volume of 2.6±1 mL, a concentration of 12.6±23 million, and 16.9±20% motility. The resulting fertilization rate was 62.7% (P < 0.001), the embryo implantation rate was 17.0%, and the CPR was 31.9%, which resulted in a delivery rate of 29.0%. The pregnancy loss rate was 2.9%.

When we consider as a denominator only the couples that failed to achieve a pregnancy with testicular biopsy, the actual fertilization rate was 64.2% (P < 0.0001), with an embryo implantation rate of 19.4% and a CPR of 35.3%, which resulted in a delivery rate of 31.4%. The pregnancy loss rate was 3.9%.

**CONCLUSIONS:** It has been recently proposed that couples with recurrent ART failure and male partners with high DNA fragmentation in their ejaculate to attempt ICSI with testicular spermatozoa. In these couples, if the ICSI-TESE fails, it seems reasonable to make an additional ICSI attempt with ejaculated spermatozoa. This prevents the surgical risk of TESE and reduces the potential financial and emotional hardships related to the procedure.

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### PREDICTIVE BIOMARKER AS MICROBIOMES IN THE SEMINAL PLASMA ASSOCIATED WITH SPERMATOGENESIS STATUS.

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**OBJECTIVE:** Whether microbiota in seminal plasma has a specific co-relationship with spermatogenesis status on male fertility or not?

**DESIGN:** Andrology at the in vitro fertilization center.

**MATERIALS AND METHODS:** We investigated that whole microbiome screen in the seminal plasma of normal group (4 cases), abnormal group (4 cases; sertoli cell only syndrome (SCO) 2 cases, hypospermatogenesis 2 cases) using next generation sequencing (NGS). We harvested normal semen and azoospermia semen by SCO, Klinefelter's syndrome, and hypospermatogenesis cases. And we performed microbiome analysis used by NGS for identification of microbiome in the seminal plasma of normal and azoospermia patients. Therefore, we analyzed microbiome's taxonomic composition of each sample from phylum to genus level.

**RESULTS:** Based on the metagenomics-NGS, we found that total 638 microbiome genome counts in the seminal plasma. Therefore, non-obstructive azoospermia present 437 genomes count number and normal spermatozoa present 384 genomes count of microbiomes. We investigated specific microbiomes on the genus level in the azoospermia patient compared to normal group. Azoospermia group present a significantly higher population of *Prevotellaceae*, *Prevotella*, *Porphyromonas*, *Streptococcus*, and *Sutterellaceae* compared to normal spermatozoa group. In the case of hypospermatogenesis group showed a specifically more abundant *Prevotellaceae*, *Prevotella*, *Streptococcus* than *Porphyromonae*, *Sutterellaceae* in the seminal plasma. Normal group revealed more variable microbiota regarding family and genus bacteria compared to azoospermia patients and have no major dominant microbiota.

**CONCLUSIONS:** Specific microbiome profiling data may be valuable for prediction of normal spermatogenesis. The small sample size used in the present study may be insufficient to clarify the role of microbiota in this preliminary study. However, this data showed possibility for the external validation study of seminal plasma microbiota. Male infertility may be associated with the residential bacterial flora as microbiomes microenvironment.

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### SUB-FERTILITY AND ITS PSYCHOLOGICAL IMPACT ON MEN.

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**OBJECTIVE:** Infertility affects an estimated 15% of couples attempting to conceive. Of these couples, male factor etiology is thought to play a part in

50% of cases. While practitioners strive to provide comprehensive care to these men, the psychologic impact of subfertility on individuals has not traditionally been addressed, and there is a dearth of data on the subject, particularly in regards to the experience of men.

**DESIGN:** At our institution, men presenting for an infertility evaluation are routinely administered a survey assessing their psychological well-being and concerns at their initial visit. This study aims to assess the results of these surveys as a means to quantify the psychological impact of sub-fertility on men.

**MATERIALS AND METHODS:** This single-center prospective study utilized a questionnaire containing both narrative questions and a Likert survey to probe several psychological and emotional domains relevant to the their impact of sub-fertility on males. Specifically, the effects of sub-fertility on mood, marital relations and sexual experience were assessed. The Likert survey was utilized to better characterize patient's abilities to cope with sub-fertility as well as the desire for additional resources in regards to its impact. Data were analyzed using SPSS v24.

**RESULTS:** One hundred sixty-four men completed the questionnaire. Of those, 83 men (51.6%) reported a negative effect on mood, 40 (24.8%) reported a negative effect on their relationship and 40 (24.8%) described a negative effect on their sexual experience. Approximately one third of men (34.6%) doubted their ability to manage the emotional impact of this pathology. Lastly, around one-fourth of men (25.7%) requested additional resources to aid in coping with these psychological impacts.

**CONCLUSIONS:** Sub-fertility has a significant impact on the emotional and psychological well-being of men who presented to our infertility clinic. As indicated above, one in four men feel the need for additional resources or treatment to address the psychological impact of this pathology. When encountered in clinic, these particular individuals are provided pamphlets and/or appropriate referrals when indicated. While the medical management of infertility remains paramount, it is important to consider the emotional toll this pathology has on patients and possible need for further resources.

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### RELATIONSHIP BETWEEN SEMINAL PROSTAGLANDINS ON INTRAUTERINE INSEMINATION PREGNANCY OUTCOMES.

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**OBJECTIVE:** Mammalian semen consists of spermatozoa and accessory glands fluid secretions which promote sperm survival, motility and overall male fertility. A primary component of seminal fluid are prostaglandins (PG), which have a dual role: facilitation of sperm transport within the female reproductive tract, via inducing peristaltic contractions (PGF<sub>2α</sub>), and induction of sperm motility (PGE<sub>1</sub>; PGE<sub>2</sub>). A partial loss or an altered ratio of a prostaglandin(s) may contribute to male infertility. This pilot study compared prostaglandin PGF<sub>2α</sub> content and PGE<sub>1</sub>:PGE<sub>2</sub> ratios on pregnancy outcomes following intrauterine insemination (IUI).

**DESIGN:** Retrospective analysis of seminal fluid prostaglandin content and IUI pregnancy outcomes.

**MATERIALS AND METHODS:** After an initial recording of semen volume, liquefaction, degree of viscosity and pH an aliquot (~5 μL) of semen was loaded onto a disposable counting sperm chamber and evaluated for sperm concentration and motility. An aliquot (0.5-2.0 mL) of semen was saved, stored (-80°C) and diluted for PG analysis via a specific ELISA for each prostaglandin (PGF<sub>2α</sub>, PGE<sub>1</sub> and PGE<sub>2</sub>). Sperm were washed free of seminal fluid via centrifugation (300g) prior to IUI. Prostaglandin content and IUI pregnancy outcomes were compared by regression analysis and Students' *t*-test.

**RESULTS:** A total of 49 semen samples were evaluated as described. The overall IUI pregnancy rate was 24.5%. Patients who became pregnant had a lower PGE<sub>1</sub>:PGE<sub>2</sub> ratio (5.95 pg/mL vs 9.06 pg/mL) and lower PGF<sub>2α</sub> content (1770.12 pg/mL vs 2381.71 pg/mL) than those that did not become pregnant, respectively.

**CONCLUSIONS:** The preliminary findings suggest that the PGE<sub>1</sub>:PGE<sub>2</sub> ratio may be important for pregnancy potential but not sperm motility. PGF<sub>2α</sub> content may not influence sperm motility as it does with pregnancy outcomes. Additional studies are warranted to determine the optimal ratio and content of the prostaglandins to insure sufficient sperm motility, transport and subsequent fertilization for a successful pregnancy outcome.

**SUPPORT:** Faculty Research Development Award, Department of Surgery, Prisma Health Upstate, Greenville, SC.

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### THE OVERALL HEALTH STATUS OF INFERTILE MEN IN THE UNITED STATES IS SIMILAR TO THAT OF FERTILE MEN.

Jesse Benjamin Persily, BA, Bobby B. Najari, MD, MSc. New York University School of Medicine, New York, NY.



**OBJECTIVE:** Epidemiologic studies have found that a greater degree of comorbidity is associated with worse fertility potential. However, these findings are largely based on retrospective studies of men interacting with the health care system. Our objective was to evaluate the association of fertility and health status in men in the United States using a nationally representative survey.

**DESIGN:** We compared the demographics, healthcare utilization, and overall health status of fertile and infertile men in the National Survey for Family Growth (NSFG).

**MATERIALS AND METHODS:** We performed an analysis of the male 2011-2017 cycles of the NSFG, a nationally representative survey of family planning. Infertile men were defined as men who had ever used infertility services or men who self-reported as non-surgically sterile. Men who reported completed pregnancies were considered fertile.

**RESULTS:** Of the 13,861 men surveyed, 1,071 men were infertile, and 5,661 men were known to be fertile. Projecting to the national population, this translates to 5,205,771 infertile men and a 26,577,702 fertile men. Of the total population of sexually active men aged 15-49, roughly 8.5% (95%CI: 7.8-9.3) of men were infertile. Compared to known fertile men, infertile men had significant demographic and healthcare utilization differences (Table). Infertile men were wealthier, better educated, more likely to be white, more likely to be married, and more likely to have private insurance. Importantly, infertile men and fertile men had similar overall health status. On multivariate analysis, differences in income, marital status, and usual healthcare place remained significant.

TABLE. Demographics, Healthcare Utilization, and Overall Health Status of Fertile and Infertile Men

	Infertile		Fertile	
	Men	Men	Bivariate	Multivariate
<b>Age, years</b>	34.1	34	0.867	N/A
<b>Income, % of poverty level</b>	318.5	263.1	<0.001	<0.001
<b>Religion, %</b>			0.017	0.05
No Religion	25	25.9		
Catholic	23.5	22.4		
Protestant	40.2	44.6		
Other Religions	11.2	7.1		
<b>Race, %</b>			0.001	0.59
Hispanic	18.8	24.4		
White	61.9	55.1		
Black	12.2	14.8		
Other	7.2	5.6		
<b>Marital Status, %</b>			<0.001	0.001
Married	66.7	57.9		
Widowed	0.1	0.2		
Divorced/Separated	5.9	11.9		
Never Married	27.3	30.1		
<b>Education, %</b>			<0.001	0.267
Less than High School	12.7	17.5		
High School	64.7	67.7		
College	22.6	14.9		
<b>Health Insurance Status %</b>			<0.001	0.259
Private	69.8	59.2		
Medicaid	9.7	10.1		
Military	4.9	4.9		
Uninsured	15.6	25.7		
<b>Usual Place for Healthcare, %</b>	79.2	69.2	<0.001	<0.001
<b>Health Status, %</b>			0.378	0.472
Excellent	24.5	27.7		
Very Good	42.6	39.3		
Good	26.2	25.3		
Fair	5.9	6.5		
Poor	0.8	1.2		

**CONCLUSIONS:** While infertile men do have significant demographic and healthcare utilization differences compared to fertile men, the overall health status of both infertile and fertile men appear similar.

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**AN EVIDENCE-BASED ANALYSIS OF INGREDIENTS IN POPULAR MALE FERTILITY SUPPLEMENTS.** Manish Kuchakulla, B.S., Yash Soni, B.S., Premal Patel, MD, Ranjith Ramasamy, M.D University of Miami Miller School of Medicine, Miami, FL.



**OBJECTIVE:** To study the level of evidence available for ingredients of popular over-the-counter male fertility supplements.

**DESIGN:** Systematic review

**MATERIALS AND METHODS:** We performed a systematic search using the terms “male fertility supplement”, “male sperm supplement”, and “male reproductive supplement”. We identified the top male fertility supplements available from the most commonly used online retailers in the United States: A1 Supplements, Amazon, Vitamin Shoppe, and Walmart. The ingredients of each of these supplements were identified and a systematic review was performed to identify randomized controlled trials studying each ingredients impact on sperm parameters and/or live birth rates using search terms, “Xingredient and sperm,” “Xingredient and male fertility,” and “Xingredient and sperm parameters.” A score was assigned to each ingredient based on its available evidence using The American Heart Association Evidence-Based Scoring System. Subsequently, a composite level of evidence score was calculated for each supplement to assess its overall level of evidence.

**RESULTS:** Ninety unique ingredients were identified from the top 17 listed male fertility supplements. The most commonly used ingredients were Vitamin E, Folic Acid, Zinc, Vitamin C, Selenium, Vitamin B12, L-Carnitine, and Maca. Only 17% of ingredients had published data showing positive effect on semen parameters, of these, the most studied ingredients are L-Carnitine, Vitamin E, Vitamin C, CoQ10, and Zinc. None of the supplements had any published evidence of their use in a randomized controlled trial. Our scoring system gave an average composite rating of 1.66 (on a scale to 5) for the evidence level of the popular supplements. Evolution 60 and Conception XR had the highest composite scores with 3.6 and 3.5, respectively. Mitamen and Standard Process scored the lowest with 0 and -.33, respectively.

**CONCLUSIONS:** Many fertility supplements claim to improve fertility; however, their promises are rarely backed by evidence. Very few ingredients used in popular fertility supplements had positive evidence demonstrated in randomized clinical trials. These findings can help providers counsel men attempting conception about the use of over the counter supplements.

**P-659** Wednesday, October 16, 2019 6:30 AM

**ELEVATED BLOOD SUGAR PARAMETERS IN YOUNG INDIAN MEN ATTENDING OUR FERTILITY CLINIC.** Madhavi M. Panpalia, MS,<sup>a</sup> Sujatha Reddy, MD,<sup>a</sup> Chitra Ishwar, MD,<sup>a</sup> Meenal Khandeparkar, MS,<sup>a</sup> Dattatray Naik, MSc,<sup>a</sup> Suresh Dhumal, MSc,<sup>a</sup> Prashant Makwana, MSc,<sup>a</sup> Firuza Rajesh Parikh, MD DNB PhD.<sup>b</sup> <sup>a</sup>Jaslok Hospital and Research Centre, Mumbai, India; <sup>b</sup>Genexplore Diagnostics and Research Centre Pvt. Ltd., AHMEDABAD, India.



**OBJECTIVE:** India is considered the diabetic capital of the world (1). This study aims to review the levels of high blood sugar parameters for the incidence of Type 2 Diabetes in young male partners of Indian couples seeking fertility treatment since there is a paucity of studies documenting blood sugar levels in young Indian men.

**DESIGN:** Retrospective observational study in the young Indian male visiting our Fertility Centre.

**MATERIALS AND METHODS:** Over a 6 month period, 727 male partners of Indian ethnicity (median age 35 years, range 24 - 45 years) of couples visiting our fertility clinic for the first time were investigated for blood sugar levels with one or more of the following parameters:

- Fasting plasma glucose (FPG) with no caloric intake for at least 8 hours.
- 2 hours plasma glucose (2 hr PG) during oral glucose tolerance test (OGTT) using a glucose load containing 75 gm glucose.
- Glycosylated Haemoglobin (HbA1C).

Criteria by the American Diabetes Association (ADA) for the diagnosis of Diabetes

	Fasting plasma glucose (FPG)	2 hrs plasma glucose in OGTT	HbA1C
Diabetic	≥ 126 mg/dl	≥ 200 mg/dl	≥ 6.5 %
Prediabetic	≥ 100 - 125 mg/dl	≥ 140 - 199 mg/dl	≥ 5.7 - 6.4 %
Normal	< 100 mg/dl	< 140 mg/dl	< 5.7 %

**RESULTS:** Of the 727 young male partners, while 62 (8.5 %) were diabetic, 279 (38.4 %) were prediabetic. The remaining 386 (53.1 %) were normal.

**CONCLUSIONS:** Our study found that Indian men showed deranged blood sugar parameters indicating the prevalence of diabetes in young Indian men. Furthermore it highlights the importance of screening male partners prior to fertility treatment. In India, a trend indicates that there is a rapid increase in the number of individuals becoming diabetic and a decline in the mean age of onset of Type 2 Diabetes. This is a disturbing trend as Type 2 Diabetes is seen in Indian males a decade earlier than in Caucasian males (2).

**References:** (1) Singh U. Prevalence of diabetes and other health related problems across India and worldwide: An overview. *Journal of Applied and Natural Science.* 2016;8(1):500-505.

(2) Ramachandran A et al. Current scenario of diabetes in India. *Journal of Diabetes.* 2009;1:18-28.

**P-660** Wednesday, October 16, 2019 6:30 AM

**HIV/OTHER STD INFECTIONS AMONG 338,432 INFERTILE POPULATIONS SHOULD RECEIVE MORE ATTENTION IN HUNAN, CHINA, 2012-2018: A CROSS-SECTIONAL STUDY.** Gang Liu, PhD,<sup>a</sup> Weina Li, PhD,<sup>b</sup> <sup>a</sup>Institute of Reproduction and Stem Cell Engineering, Central South University, Changsha, China; <sup>b</sup>Reproductive and Genetic Hospital of CITIC-Xiangya, Changsha, China.



**OBJECTIVE:** Although infertile populations were not at high risk for HIV compared with sex workers and MSM groups, they do not adjust their high risk practices in natural pregnancies for reproduction. However, data regarding HIV/STD testing, infections and coinfections among infertile couples are limited. This study aimed to assess HIV/other STD prevalence among infertile populations in China.

**DESIGN:** This study was performed as a retrospective survey of 338,432 infertile populations in Hunan, China, from 2012-2018 in our hospital.

**MATERIALS AND METHODS:** A cross-sectional hospital-based study was conducted to evaluate the prevalence of HIV/other STDs (HBV, HCV, syphilis, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* (MG)) among 338,432 infertile populations. We calculated linear trends in prevalence using bivariate linear regression.

**RESULTS:** The overall prevalence rates of HIV, chlamydia, gonorrhea, MG, syphilis, HBV and HCV antibody positivity in this study were 0.04%, 1.73%, 0.05%, 2.60%, 2.15%, 12.01% and 0.56%, respectively. The predominant infection was HBV, followed by MG, syphilis, and chlamydia. Of those participating, 16.65% (56336/338432) had at least one positive test; 0.59% (1999/338432) had more than one positive test. Only 1.13% of participants (382/338432) reported STD signs and symptoms suggesting genital tract infection. However, the variation in HIV prevalence was not significant ( $\beta=0.000$ ,  $P_{TREND}=0.907$ ) during this period. From 2012-2018, the characteristics of the HIV-infected infertile population had not shifted dramatically: women composed 32.56% of HIV cases in China, and the incidence rate for men was 2 times the rate in women. Concordant infections were found in 4.65% of infertile couples (6/129).

The highest incidence of 54.26% (70/129) was found at 30–39 years of age. Overall, 87.60% of the HIV-infected population had a relatively low education. All HIV-positive women discontinued treatment, but 45.98% (40/87) of HIV-positive men continued their assisted reproductive therapy with donor semen.

**CONCLUSIONS:** Therefore, screening for STDs should be emphasized regardless of symptoms in the clinical setting, and targeted interventions

TABLE. Comparison of variables among different BMI groups

	Underweight	Normal	Overweight	Obese	P value
Number	53	554	849	775	
Age	32.2 ± 2.3	34.9 ± 0.4	36.4 ± 0.4	36.6 ± 0.3	< 0.001
Volume	2.57 ± 0.34	3.12 ± 0.08	3.05 ± 0.05	3.09 ± 0.05	
Count	22.4 ± 5.7	30.6 ± 1.5	27.0 ± 1.2	22.4 ± 1.1	0.001
Total motility	34.64 ± 5.06	42.71 ± 1.08	39.32 ± 0.87	38.02 ± 0.92	
Progressive Motility	12.7 ± 3.4	17.2 ± 0.7	14.7 ± 0.6	13.5 ± 0.5	0.002
Normal Morphology	6.2±1.3	7.3±0.3	6.4±0.2	5.8±0.3	0.007
SDF	26.22 ± 5.16	26.19 ± 1.36	28.8 ± 1.24	26.71 ± 1.17	
E2	97.6 ± 8.1	98.2 ± 5.2	104.7 ± 3.3	123.4 ± 4.7	0.001
LH	3.43 ± 0.37	4.65 ± 0.21	4.49 ± 0.13	4.24 ± 0.13	
FSH	4.39 ± 0.85	5.32 ± 0.32	5.26 ± 0.23	5.54 ± 0.28	
Prolactin	242.38 ± 22.97	260.43 ± 9.22	262.87 ± 9.6	262.91 ± 7.72	
Testosterone	19.7 ± 2.02	19.1 ± 0.7	17.8 ± 0.5	15.4 ± 0.47	< 0.001

should focus especially on infertile populations with low income and less education.

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**IMPACT OF BODY WEIGHT ON SEMEN PARAMETERS AND REPRODUCTIVE HORMONES OF MEN WITH IDIOPATHIC INFERTILITY.**



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**OBJECTIVE:** Obesity is known to have a detrimental impact on human health including reproductive potential. We aim to evaluate the effect of body weight on semen parameters & reproductive hormones of men with idiopathic infertility

**DESIGN:** Retrospective Chart review.

**MATERIALS AND METHODS:** Charts of 6,483 patients who presented to our infertility clinic between 2012 - 2016 were screened. Patients with idiopathic male infertility were included while those with genetic abnormalities, varicocele, genitourinary infections, surgery and history of radiotherapy or chemotherapy were excluded. Demographic & clinical data (semen parameters, hormones & BMI) of the initial visit (before any treatment) was collected.

Patients were grouped according to the WHO BMI classification into underweight, normal, overweight and obese. Kruskal-Wallis one-way analysis of variance test was used to compare the data between them. The different variables were correlated with Spearman correlation.

**RESULTS:** A total of 2231 patients were included in the study. Their mean age was 36.11 ± 0.17 years & their mean BMI was 28.71 ± 0.13 kg/m<sup>2</sup>. While the means of age differed significantly between the study groups, they belonged to the same age generation.

Sperm concentration, progressive motility & normal morphology were highest in patients with normal body weight compared with the other patient groups. A significant increase in estradiol & a decrease in testosterone were noted as BMI increased.

A significant (p<0.001) but weak negative correlation was observed between BMI & sperm concentration (-0.135), progressive motility (-0.116), normal morphology (-0.110) & serum testosterone levels (-0.226). While a significantly positive correlation was noted with age & serum estradiol levels (0.109, 0.215 respectively, p<0.001).

**CONCLUSIONS:** Increasing body weight can influence semen parameters & reproductive hormones providing an explanation to the reduced fertility potential of men with idiopathic infertility.

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**SIGNIFICANT DELAYS IN EVALUATION OF MALE PARTNER AMONGST INFERTILE COUPLES.**



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<sup>b</sup>University Hospitals Urology Institute/Case Western Reserve University, Cleveland, OH; <sup>c</sup>University of Utah School of Medicine, Salt Lake City, UT.

**OBJECTIVE:** To characterize the prior evaluation and intervention that infertile couples received prior to male evaluation.

**DESIGN:** Retrospective review of couples presenting for male infertility evaluation by fellowship trained male reproductive urologists.

**MATERIALS AND METHODS:** Couples presenting for infertility to a male infertility specialist were identified and charts were reviewed for duration of attempting pregnancy, prior reproductive workup, and prior use of assisted reproductive technology (ART). Variables were compared between couples presenting for primary versus secondary infertility and between couples who had undertaken ART and those who had not. Physical exam findings at evaluation, and subsequent therapeutic interventions were recorded.

**RESULTS:** A total of 806 patients were included for analysis. The mean age at presentation was 36.2 (range 20–73) years for men, and 32.42 (range 19-53) years for women (p<0.001). 39% (312/799) of couples were first evaluated by a gynecologist only, 25% (200/799) a reproductive endocrinologist (REI) only, 18% (147/799) presented without a female workup, and 18% (140/799) of couples saw both a gynecologist and REI prior to presentation at our clinic. In total, 14% previously attempted ART, 6% (46/776) underwent intrauterine insemination (IUI) (range 1-8 cycles); 6% (43/776) underwent invitro fertilization (IVF) (range 1 to 8 cycles); 3% (20/776) underwent both IUI and IVF. Couples who had undertaken ART were attempting pregnancy for 39 months versus 22 months for those who had not undergone ART (p<0.001). The majority (63%) of females had no abnormality in their workup. 72% (78/109) of men undergoing ART had at least one abnormality diagnosed at examination. Varicocele was the most common abnormality diagnosed amongst these men (Table 1). Varicocele repair (VR) (41%, 45/109) and testicular sperm extraction (11%, 12/109) were the most common interventions pursued following evaluation.

**CONCLUSIONS:** Our findings highlight that a male workup for infertile couples often lags behind a female workup and sometimes even ART. We

	Exam Findings	
	Overall (N=806)	ART Cohort (N=109)
High Riding Testicle	2%	3%
Varicocele (Vx)	54%	57%
Absence of Vas	1%	1%
Epididymal Cyst/Granuloma	3%	3%
Hypospadias	1%	1%
	Interventions Pursued	ART Cohort (N=109)
	Overall (N=806)	
Vx Repair	28%	41%
Testicular Sperm Extraction	16%	11%
Transurethral Resection of Ejaculatory Ducts	1%	3%
Microepididymal Sperm Aspiration	4%	2%

identified that undergoing a simple, inexpensive male workup composed of scrotal ultrasound, semen analysis and hormone levels identifies several correctable forms of male infertility and prompt surgical interventions such as VR that can potentially improve outcomes.

**SUPPORT:** A.W.P. is a National Institutes of Health (NIH) K08 Scholar supported by a Mentored Career Development Award (K08DK115835-01) from the National Institute of Diabetes and Digestive and Kidney Diseases. This work is also supported in part through a Urology Care Foundation Rising Stars in Urology Award (to A.W.P.) and NIH grant K12 DK0083014, the Multidisciplinary K12 Urologic Research (KURe) Career Development Program awarded to DJL (NT is a K12 Scholar) from the National Institute of Kidney and Digestive Diseases to Dolores J Lamb. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

**P-663** Wednesday, October 16, 2019 6:30 AM

#### MALE MULTIVITAMIN USE AND SEMEN QUALITY.

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**OBJECTIVE:** To prospectively evaluate the association between male multivitamin use and semen quality. Male factors contribute up to 50% of couple infertility, but few modifiable factors have been identified. Several studies have examined the influence of male multivitamin use on semen quality, but findings have been inconsistent.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Pregnancy Study Online is a web-based preconception cohort of North American pregnancy planners. At baseline, female participants completed a questionnaire on demographics, lifestyle, and reproductive history, including multivitamin use. Multivitamin use was ascertained by asking "Which of the following vitamins and/or minerals did you take on a regular basis (daily or almost every day)?" with "multivitamins" as a response option. Female participants invited their male partners to complete a similar baseline questionnaire. During October 2015 through April 2019, a subset of male participants from the U.S. whose partners reported regular menstrual cycles were invited to use Trak, an FDA-approved device that measures sperm concentration and semen volume at home. Men were instructed to provide up to two semen tests, with at least 3 days of abstinence time, and upload their results online via self-report and smartphone photo images. We used generalized estimating equations, accounting for within-person correlation, to estimate risk ratios (RR) and 95% confidence intervals (CI) for the association between male multivitamin use and low semen volume ( $\leq 2$  vs  $>2$  ml), low sperm concentration ( $\leq 20$  vs  $>20$  million/ml), and low total sperm count (TSC,  $\leq 50$  vs  $>50$  million). The analysis included 223 men who provided a total of 375 samples. The median and interquartile range of attempt time for men at study enrollment was 1 (1-3 cycles). We adjusted for abstinence time, age, body mass index (BMI), and lifestyle and socio-demographic factors.

**RESULTS:** At baseline, 34% of male participants reported taking multivitamins on a regular basis. Nearly 14% of samples had semen volume  $\leq 2$  ml, 18% had sperm concentration  $\leq 20$  million/ml, and 14% had TSC  $\leq 50$  million. Compared with men not taking multivitamins regularly, RRs for men reporting regular multivitamin use were 0.98 (CI: 0.54-1.78) for low semen volume, 0.66 (CI: 0.40-1.09) for low sperm concentration, and 0.74 (CI: 0.42-1.31) for low TSC.

**CONCLUSIONS:** In a geographically heterogeneous cohort of U.S. men, we observed slight inverse associations between regular multivitamin use and low sperm concentration and low TSC; little association was observed for semen volume.

**SUPPORT:** This research was supported by R01HD086742, R21HD094322, and R21HD072326.

**P-664** Wednesday, October 16, 2019 6:30 AM

#### WHAT DO MBA STUDENTS KNOW ABOUT MALE FERTILITY?

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**OBJECTIVE:** To assess knowledge of male fertility amongst Master of Business Administration (MBA) students.

Male age	Estimated fecundability $\leq 15\%$
<35	19.2%
35-40	32.4%
41-45	47.7%
>46	56.2%

Male age	Estimated IVF success rates $\leq 15\%$
<35	21.5%
35-40	19.2%
41-42	25.4%
42-44	40.8%
45-57	46.8%

**DESIGN:** Cross sectional study.

**MATERIALS AND METHODS:** A Research Electronic Data Capture survey that included 31 questions on male fertility was distributed via email to all students enrolled in the MBA program at the Kellogg School of Management at Northwestern University. Answers were compiled and descriptive statistics were analyzed.

**RESULTS:** One hundred and thirty-three students (30 males and 103 females) with a mean age of 28.9 years completed the survey. Most participants in the sample were partnered with 35.4% married and 40.0% in a committed relationship. The remaining 24.6% were single. Four and a half percent reported having children, 3.8% had used medical assistance to achieve a pregnancy in the past, and 96.0% reported a desire to have children in the future.

More than one fifth of males and almost one third of females stated that a man's age never impacts reproductive outcomes, although approximately 10% of all participants believed that men stopped producing sperm somewhere between 55 and 75 years of age. When the impact of age on semen analysis parameters was assessed, over 10% of males were not aware that sperm concentration and quality decrease, while more than 30% of females did not know that these parameters decline as men grow older. Only approximately 55% of males and 40% of females reported that the likelihood of miscarriage increases as men age. Participants were also asked to estimate whether male age alters fecundability and success rates with IVF in women under 35 years old. The percentage of participants who estimated a likelihood of pregnancy of 15% or lower, despite female age less than 35, are shown in the tables.

**CONCLUSIONS:** Even in a highly-educated cohort of MBA students, knowledge of the relationship between male age and reproduction is limited. One third of female participants were not aware that there are decreases in quality and quantity of sperm as men age. When knowledge of both spontaneous and IVF pregnancy rates were assessed, participants believed that the likelihood of success was dependent on paternal age, and low success rates were estimated in the oldest male patients despite young female age. As delayed childbearing becomes more common, particularly amongst those with high educational attainment, attention should be focused on ensuring that all individuals have a comprehensive understanding of the relationship between male age and reproductive outcomes.

**P-665** Wednesday, October 16, 2019 6:30 AM

#### EFFECT OF MEDICAL COMORBIDITIES OVER SEMINAL PARAMETERS AND SPERM DNA FRAGMENTATION.

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**OBJECTIVE:** Several publications have been shown that it could be a relationship between male infertility and general health status. The aims of this study were to investigate the prevalence and effect of some medical comorbidities over sperm parameters and DNA fragmentation in an Argentinian population.

**DESIGN:** Retrospective controlled cohort study.

**MATERIALS AND METHODS:** Under the approval of the institutional ethics committee, a retrospective study was performed for 1,092 men who were examined due to infertility between August 2017 and April 2019. The initial evaluations were comprised of a complete medical history, a physical examination, endocrine assessment, and at least two semen analyses. Sperm parameters and DNA fragmentation were compared between men with and without medical comorbidities.

**RESULTS:** Significant medical comorbidities were found in 112 of 1092 (10.3%) men, including 3.6% with hypertension, 2.3% with hypothyroidism, 2% with mental, 2% with diabetes/dyslipemia and 0.6% with respiratory disease.

Semen volume, sperm count and progressive motility were significantly lower in men with comorbidities than in men without comorbidities ( $p=0.045$ ,  $p=0.036$  and  $p=0.025$ , respectively). Regarding sperm DNA fragmentation, it was higher in patients with comorbidities ( $p=0.018$ ). Sperm vitality and strict morphology were not significantly different. Within patients with comorbidities, patients with diabetes/dyslipemia and anxiety disorders presented significantly higher levels of DNA fragmentation ( $p=0.001$ ).

**CONCLUSIONS:** After this preliminary study, we can conclude that medical comorbidities are associated with the impairment of sperm production and function. It has been published that obesity and metabolic disorders could be associated with impair sperm function by altering physical and molecular structure of germ cells. A complete male infertility evaluation, including an exhaustive anamnesis, could offer the possibility of specific therapy in order to improve some semen parameters. We didn't assess this theoretical benefit, however it would be very interesting to evaluate if that therapy, despite the improvement of the general health status, could improve the spermatogenesis.

## MALE REPRODUCTION AND UROLOGY - BASIC

**P-666** Wednesday, October 16, 2019 6:30 AM

**ROLE OF LEPTIN AS A PARACRINE FACTOR CRITICAL FOR HUMAN LEYDIG STEM CELL FUNCTION AND DIFFERENTIATION.** Himanshu Arora, PhD, Ranjith Ramasamy, M.D. University of Miami Miller School of Medicine, Miami, FL.



**OBJECTIVE:** Impaired testosterone production as a result of Leydig cell loss or dysfunction can occur in men with testicular failure. Although several testosterone formulations are available, none are capable of replicating the physiological pattern of testosterone secretion. We have shown in our recent study conducted in murine models that, Leydig stem cell transplantation along with peritubular myoid cells and Sertoli cells could be used to physiologically increase serum testosterone thereby potentially minimizing the adverse effects. However, in order to optimize the function of Leydig stem cells, we need to understand the paracrine factors released by myoid and Sertoli cells. In the present study we evaluated the significance of paracrine factors secreted by human peritubular myoid cells and Sertoli cells on Leydig stem cell function.

**DESIGN:** A total of 8 men with testicular failure underwent testis biopsies for sperm retrieval. Using an IRB approved protocol, about 10mg of testicular tissue from each of these men were processed for Leydig stem cell isolation, culture and characterized.

**MATERIALS AND METHODS:** The presence of Leydig stem cells (LSCs), Sertoli cells (SCs) and peritubular myoid cells (PMCs) in the harvested cellular pool was validated by immunofluorescence and quantitative real time PCR (qPCR) using PDGFR- $\alpha$ , 3 $\beta$ HSD and Sox-9, PZLF, respectively. After stimulation by Luteinizing hormone (LH), the levels of 3 $\beta$ HSD mRNAs were increased. Additionally, the CD146 (+) cells representing LSCs were sorted using MACS kit and maintained along with unsorted cells in charcoal stripped medium. Condition media was collected from both the cell types and screened for secreted protein using RayBio Human Antibody Array for a total of 80 molecules.

**RESULTS:** We successfully isolated and cultured LSCs from all 8 testis biopsies. We were able to culture up to 3 million cells / biopsy. Of the cells cultured, up to 70% of the cells were Leydig stem cells and 10% of them were Sertoli-cell in origin on day 14. IF and qPCR data showed as the majority of cell population was undifferentiated (PDGFR- $\alpha$ ). Upon stimulation by LH, the expression of 3 $\beta$ HSD (mature Leydig cells) was increased and that of PDGFR- $\alpha$  was decreased. Importantly, human antibody protein array demonstrated increased expression of only one cytokine - LEPTIN in the media of LSC's that were co-cultured with Sertoli cells and myoid cells compared to the media from purified LSCs cultures (CD146 positive). Further results confirmed that Leptin is upstream of desert hedgehog signaling in regulation of LSC differentiation.

**CONCLUSIONS:** Our results indicate that LSCs can be isolated and cultured from men with testicular failure. Leptin is a specific paracrine factor which is released by adjacent Sertoli and myoid cells which could be critical for LSC differentiation and testosterone production. Further studies are ongoing to validate the implications of Leptin in terms of their role in LSCs function, differentiation and survival.

**SUPPORT:** Supported by the American Urological Association Research Scholar Award and Stanley Glaser Award to RR. J.M.H. is supported by NIH grants 1R01 HL137355, 1R01 HL107110, 1R01 HL134558, 5R01 CA136387, 5UM1 HL113460 and Soffer Family Foundation.

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## IDENTIFICATION OF TWO NOVEL SEMINAL PEPTIDES, WHICH ACT AS NEUTRAL ENDOPEPTIDASE INHIBITORS AND MODULATE SPERM MOTILITY.

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**OBJECTIVE:** Poor sperm motility is highly predictive of male factor infertility. Semenogelins, and their peptide products, are recognized as important determinants of sperm motility; most research suggests they act as inhibitors of sperm motility. Peptidomic analysis of semen identified several semenogelin-derived peptides. Based on sequence analysis, these peptides may act as substrates, and thereby inhibitors, of neutral endopeptidase (NEP). Since inhibition of NEP activity has been associated with increased sperm motility, this raises the intriguing possibility that certain semenogelin-derived peptides may activate sperm motility. The present study determined if two novel seminal semenogelin-derived peptides, (RSIY-15 and SSIY-15), were indeed NEP inhibitors and if they had a positive effect on sperm motility.

**DESIGN:** A colorimetric assay was performed using recombinant human NEP enzyme at 0.1  $\mu$ g/ml and fluorogenic NEP peptide substrate. RSIY-15 and SSIY-15 were synthesized and the colorimetric assay was performed to evaluate their inhibitory nature and Ki. Semen analysis was undertaken in order to determine the effects of RSIY-15 and SSIY-15 on sperm motility.

**MATERIALS AND METHODS:** 50 $\mu$ L of substrate, at a range of concentrations, was added to 50 $\mu$ L of NEP enzyme followed by 50 $\mu$ L of a range concentrations of RSIY-15 and SSIY-15. Dixon and Lineweaver Burk plots were generated to evaluate the inhibitory nature of RSIY-15 and SSIY-15. Semen samples from patients presenting for routine semen analysis were collected; semen from each patient was divided into aliquots and motility analyzed following addition of 1 $\mu$ L of 75 $\mu$ M RSIY-15 or SSIY-15. Addition of 1 $\mu$ L 75 $\mu$ M RSIY-11 or 200  $\mu$ M Opiorphin (peptides previously identified as NEP inhibitors) were utilized as positive controls, and vehicle (PBS) was utilized as negative control. Additionally, 1 aliquot was set aside without addition. 2 $\mu$ L of semen was then placed into a 4-chamber microcell disposable counting chamber slide. Progressive and non-progressive motility was assessed at 0, 30, and 60 minutes after the addition of peptide. Wilcoxon Rank-Sum Tests were used to evaluate differences in sperm motility between groups.

**RESULTS:** Colorimetric assays indicate that RSIY-15 and SSIY-15 both act as competitive inhibitors of NEP. Both peptides appear to have Ki similar to RSIY-11 (12.58  $\pm$  3.75  $\mu$ M and 13.69  $\pm$  5.44  $\mu$ M, respectively). Semen analysis for 30 patients was undertaken. Compared to PBS controls, the addition of RSIY-15 and SSIY-15 lead to improved progressive and total sperm motility ( $p < 0.05$ ).

**CONCLUSIONS:** The novel seminal semenogelin-derived peptides RSIY-15 and SSIY-15 act as competitive inhibitors of NEP. Both peptides increased progressive and total sperm motility when added to semen samples. Contrary to the prevailing viewpoint that semenogelin is primarily an inhibitor of sperm motility, our observations demonstrate that semenogelin and its peptide products both activate and inhibit sperm motility. Further studies are underway to investigate the breakdown products of these peptides, and to compare the peptidomic profiles of fertile and infertile men.

**SUPPORT:** NIH/NIDDK (DK107807 awarded to KD).

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## SUBTLE SIGNATURES OF AGING PERSIST OVER TWO GENERATIONS THROUGH THE PATERNAL GERM LINE.

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**OBJECTIVE:** Determine if epigenetic signatures of aging in sperm persist over multiple generations.

**DESIGN:** DNA methylation analysis of stored sperm samples.

**MATERIALS AND METHODS:** 32 sperm samples were assessed for DNA methylation signatures. All samples were collected by men of a similar age (31 to 33) and whose father's age at conception was also held constant (26 to 29 years of age). 16 men had paternal grandfathers younger than 24 years of age at the time of conception (young grandfathers; YG) and 16 men had paternal grandfathers who were over 40 years of age at the time of conception (aged grandfathers; AG). We performed sperm DNA methylation analysis using the Illumina 850K (EPIC) array. Samples were assessed for differential methylation signatures between the AG and YG groups with standard approaches. Germ line age calculation was also performed. Additionally, novel analyses were applied to identify subtle remnants of aging signals known to be present in aged men (assessment of sites known to change with age). Fisher's exact tests were performed to assess significance and permutations were performed to validate identified patterns.

**RESULTS:** Differential methylation analysis revealed no significant difference between the YG and AG groups. Additionally, germ line age calculations successfully predicted the men's age with a typical amount of variability, but showed no sign of age acceleration in either the YG group of the AG group. We performed an additional analysis to assess 140 regions known to show decreases in methylation with age and found that ~80% of these regions were lower in the YG group when compared to the AG group. Outside of these regions, we have previously shown a slight whole genome methylation increase in aged sperm. We performed 10 random screens of the genome that each randomly selected 10,000 CpGs and assessed differences between YG and AG. In each of these 10 screens between 70 and 75% of the CpGs observed had slight increases in methylation in the AG compared to the YG. Permutations were performed and used to generate statistics using a Fisher's exact test. In both cases (regional assessment and random whole genome screening), the findings were significant with a  $p < 0.001$ .

**CONCLUSIONS:** Previous assessments of this data set from our lab have failed to show significant results due to the fact that only conventional approaches were employed. Due to the extremely small changes identified and lack of consistency between samples, the findings reported herein were never previously identified. When thresholds for magnitude were removed and averages generated across each group we began to see subtle but clear signals commonly observed in "aged" sperm. It is clear that the aging signal is nearly all removed through the epigenetic reprogramming process in the embryo, but the fact that some very subtle signals remain suggest that this process is incomplete or susceptible to some degree of error. Perhaps some of this subtle epigenetic transmission over multiple generations plays a small role in previously identified patterns of increased incidence of neuropsychiatric disease in the grand offspring of older grand fathers.

Reference: None.

SUPPORT: None.

**P-669** Wednesday, October 16, 2019 6:30 AM

**TO STUDY OF APOPTOTIC GENE EXPRESSION THROUGH HYPOTHALAMUS-PITUITARY-GONADAL AXIS IN.** Kamla Shukla Shukla, PhD. All India Institute of Medical Sciences, Jodhpur, India.



**OBJECTIVE:** To investigate the apoptotic gene expression of Bcl-2, Cytochrome C, Caspase and procaspase with male hormone profile in infertile subjects for their relationship to sperm quality and cell death parameters.

**DESIGN:** We undertook gene expression on a total of 400 individuals, including 100 fertile donors as controls and three subgroups of infertile men, normozoospermic (idiopathic unexplained;  $n=100$ ), oligozoospermic ( $n=100$ ) and asthenozoospermic ( $n=100$ ). These participants were selected from Departments of Urology, King George's Medical University, Lucknow, India.

**MATERIALS AND METHODS:** We used ELISA, quantitative real time PCR (qPCR) with lightCycler Fast Start DNA PLUS SybrGreen kit for IL-6 and TNF alpha, Bcl-2, Cytochrome C, Caspase and procaspase mRNA and their relation to male fertility.

**RESULTS:** We found decreased sperm motion kinetics and altered male hormone profile was associated with decreased Bcl-2 and procaspase expression and increased of cytochrome c expression was significantly increased in the oligozoospermic and asthenozoospermic infertile subjects compared to healthy fertile subjects.

**CONCLUSIONS:** Male hormones, Bcl-2, Cytochrome C and Caspase gene expression were altered in men with impaired fertility possibly via their associations with sperm count, motility and morphology.

**P-670** Wednesday, October 16, 2019 6:30 AM

**FAM9B WAS ASSOCIATED WITH HUMAN SPERMATOGENESIS AND MALE-SPECIFIC STERILITY.** Xin-jie Zhuang Dr, Ping Liu, prof., Reproductive Medicine Center, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China.



**OBJECTIVE:** Spermatogenesis is the process of gamete formation, which includes mitosis in spermatogonia, meiosis in spermatocytes, and spermiogenesis. A key event is the formation of the synaptonemal complex (SC). Mutations of genes encoding SC components (such as SYCP3, synaptonemal complex protein 3) lead to infertility or subfertility due to germ cell death. Fam9B mapped on the human chromosome X (Xp22.3) was more similar to SYCP3 in their amino acid sequences and expressed in human testis. However, the expression and precise underlying mechanisms of Fam9B have not been clearly in testes during the spermatogenesis and infertility. And Fam9B mutation associated with sterility?

**DESIGN:** This study was an analysis of azoospermia, sertoli cell only syndrome (SCOS) and proven fertile patients, including 162 patients diagnosed with azoospermia, 65 patients diagnosed with SCOS and 10 proven fertile patients at the Reproductive Medical Center of Peking University Third Hospital between January 2015 and January 2019. Fam9B was cloned, expressed and identified. Genome DNA sequencing analysis, RT-PCR, Western-blot, Immunohistochemistry and immunocytochemistry were performed.

**MATERIALS AND METHODS:** Fam9B was analyzed with blood and testicular biopsy samples from azoospermia patients by genome DNA sequencing analysis. These likely mutations were further screened in SCOS patients and in men proven to be fertile. And Fam9B was cloned, expressed and identified with testicular samples using RT-PCR, western blot, Immunohistochemistry and immunocytochemistry. The concentration of testosterone in azoospermia and SCOS patients were determined, and their relativity was studied by statistic methods.

**RESULTS:** Fam9B mRNAs and protein were detected in human testis, and Fam9B was expressed at different level in patients of azoospermia, SCOS and normal groups. Immunohistochemical staining showed that Fam9B was expressed in the nucleus of primary spermatocyte in fertile persons. Moreover, Fam9B was more similar to SYCP3 expression pattern and located on the SC during the leptotene and diplotene spermatocytes. Furthermore, genome DNA sequencing analysis revealed three fam9b deletion patients and five patients of point mutation in fam9b gene. Spermatogenesis of these three patients was arrested at spermatocytes and accompanied by germ cells lost. Interesting, testosterone concentrations levels of these three patients were decreased compared with controls ( $P < 0.01$ ).

**CONCLUSIONS:** Fam9B may be a SC related protein participated in human normal spermatogenesis. And mutations of fam9b may be risk of spermatocytes arrest and male-specific sterility.

References: 1. Roumelioti FM, Louizou E, Karras S, et al. Unbalanced X;9 translocation in an infertile male with de novo duplication Xp22.31p22.33. *J Assist Reprod Genet.* 2019 Jan 24. <https://doi.org/10.1007/s10815-019-01405-0>.

2. Niu Y, Zhou C, Xu H, et al. Novel interstitial deletion in Xp22.3 in a typical X-linked recessive family with Kallmann syndrome. *Andrologia.* 2018 Feb 14. <https://doi.org/10.1111/and.12961>.

3. Hinton RB, Opoka AM, Ojarikre OA, et al. Preliminary Evidence for Aortopathy and an X-Linked Parent-of-Origin Effect on Aortic Valve Malformation in a Mouse Model of Turner Syndrome. *J Cardiovasc Dev Div.* 2015 Jul 10;2(3):190-199. <https://doi.org/10.3390/jcdd2030190>.

**SUPPORT:** This study was supported by Beijing Natural Science Foundation (NO.7172236) and National Natural Science Foundation of China (NO.81671513 and 81200466).

**P-671** Wednesday, October 16, 2019 6:30 AM

**PSMA5 AND RARRES1, SEMINAL PLASMA PROTEIN MARKERS OF SPERM DNA FRAGMENTATION, ARE NOT ALTERED BY SMOKING.** Tamashiro, BSc.,<sup>a</sup> Paula Intasqui, PhD,<sup>a</sup> Ricardo P. Bertolla, DVM, PhD,<sup>b</sup>



Mariana Pereira Antoniassi, PhD<sup>a</sup> Sao Paulo Federal University, São Paulo, Brazil; <sup>b</sup>Head of Research UNIFESP, SAO PAULO, Brazil.

**OBJECTIVE:** Sperm DNA fragmentation is one of the major cellular mechanisms of male infertility and can be observed in 20% to 25% of infertile men. An earlier study has found that RARRES1 and PSMA5 proteins are altered in seminal plasma in men with high DNA fragmentation. Another study has shown that smokers present high sperm DNA fragmentation. Therefore, in this study, we wanted to evaluate whether these proteins are also altered in other causes of infertility, such as smoking.

**DESIGN:** Cross-sectional study.

**MATERIALS AND METHODS:** For this study, men aged between 20 and 50 years referred to our andrology laboratory were included. For controls, 29 normozoospermic, non-smoker men were included, and for the smoking group, 26 men who smoke only cigarettes were included. Samples were collected by masturbation, with an ejaculatory abstinence of 2 to 5 days. After liquefaction, an aliquot was used for semen analysis according to the 2010 WHO manual and another was used for sperm DNA fragmentation analysis using an alkaline comet assay. The Comet Distributed Moment variable were recorded using software analysis. The remaining volume was centrifuged and seminal plasma was used for protein quantification. A volume corresponding to 50  $\mu$ g protein was used for quantification of RARRES1 and PSMA5 proteins by Western blotting. Bands were then normalized by total protein by Ponceau S. Protein contents were shown as normalized values as well as PSMA5/mL and PSMA5/ejaculate and RARRES1/mL and RARRES1/ejaculate. For statistical analysis, data normality of was assessed by a Kolmogorov-Smirnov test and groups were compared by an unpaired Student's t test or Mann-Whitney test, with an alpha of 5%.

**RESULTS:** Control and smokers groups did not differ regarding DNA fragmentation (mean; standard deviation of 40.5; 17.45 and 47.3; 15.97 respectively). On the other hand, sperm concentration, count, and morphology were lower in the smoking group ( $p = 0.005$ ,  $p = 0.002$ ,  $p = 0.019$ ), respectively. There was no difference between groups for PSMA5/mL (median;interquartile range of 0.23;0.18 and 0.17;0.21) and PSMA5/ejaculate (0.65;0.62 and 0.65;0.65). There was no difference between groups for RARRES1/mL (1.15;0.87 and 1.32;1.00) and RARRES1/ejaculate (4.02;3.64 and 4.57;4.79).

**CONCLUSIONS:** Minimal levels of PSMA5 and RARRES1 are not altered due to smoking. It is an important finding because it corroborates our hypothesis that these proteins are exclusively associated with sperm DNA fragmentation, but not with other infertility conditions, such as smoking.

P-672 Wednesday, October 16, 2019 6:30 AM

#### SINGLE-CELL RNA SEQUENCING REVEALS NOVEL MARKERS OF STEM/PROGENITOR SPERMATOGONIA IN HIGHER PRIMATES.

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**OBJECTIVE:** Determining the unique biological features of higher primate spermatogonial stem cells (SSCs) to facilitate the translation of SSC based therapies to the human fertility clinic.

**DESIGN:** We performed high throughput, unbiased, single-cell RNA-sequencing (scRNA-seq) of healthy adult primate (human and rhesus macaque) testicular tissue.

**MATERIALS AND METHODS:** Human and monkey single cell suspensions were generated by a two-step enzymatic digestion protocol, using 1 mg/ml collagenase type IV and 0.25% Trypsin-EDTA plus 1mg/mL DNase I. Live cells were selected by either DAPI exclusion by FACS or using a MACS dead cell removal kit. The single cell suspensions were diluted to 280 cells/ul and processed using the Drop-seq platform and sequencing was performed on a HiSeq-2500 (Illumina). Data analysis was performed with the Seurat R package (version 1.4).

**RESULTS:** We have generated ~33,800 human and monkey single cell transcriptomes. Dimensionality reduction and unsupervised clustering partitioned the single cells into transcriptionally distinct populations representing the major cell types found in the primate testes. Differential expression analysis has identified genes GPC4, GPC3, FMR1, TSPAN33, MAGEB2, DNAJB6, MORC1, TCF3, LITD1 and GPX1 as potential markers of stem/progenitor spermatogonia. These genes are implicated in WNT, Hedge-

hog, FGF, BMP, MAP2K/AKT signaling as well as metabolism that may be important in regulating primate SSC growth dynamics in vivo and/or in vitro.

**CONCLUSIONS:** Our single cell data may reveal novel mechanisms regulating higher primate SSCs that can be exploited for sorting, enhancing survival and expansion in culture or other applications that improve the fundamental knowledge about SSCs in higher primates, and may enable applications in the male infertility clinic.

P-673 Wednesday, October 16, 2019 6:30 AM

#### IMPACT OF INTERACTION BETWEEN OXIDATIVE STRESS ADDUCTS (OSA) LEVELS AND ACCESSORY CELLS ON SPERM DNA INTEGRITY AND COMPLEMENT REGULATORY PROTEIN.

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**OBJECTIVE:** Factors present in the semen such as Zinc (Zn), white blood cells (WBC), Round (RC) and epithelial cells (EC) may increase the levels of oxidative stress (OS) and consequently, the formation of oxidative stress adducts (OSA) which may interact with sperm key biomolecules such as complement regulatory proteins required (CRP) for acrosome reaction and DNA producing sperm defective function. The aims of this study were (i) to determine if the levels of OSA are associated with the concentrations of Zn, WBC, RC, and EC and (ii) to correlate if those OSA levels induce both the increased of DNA fragmentation and loss of expression of (CRP).

**DESIGN:** Retrospective.

**MATERIALS AND METHODS:** Frozen semen samples from 186 men were evaluated for sperm, WBC, RC and EC concentrations by microscopy. Oxidative Stress (OSA) and Zinc ( $\mu$ M) were determined by spectrophotometry. The DFI (%), HDS (%), WBC (CD45) X 10<sup>6</sup>/ml and Expression of CD46 (CRP) were measured by flow cytometry. For the data analysis, the samples were classified into 3 OSA categories: Normal  $3.8 \geq$  (n=31), BL 3.81-4.4 (n=53) and Abnormal  $>4.4$  (n=102). The statistical analysis was performed by ANOVA sigma stat ( $p < 0.01$ ).

**RESULTS:** The sperm DFI scores were increased both BL and Abnormal categories when were compared to Normal OSA (Normal  $12.75 \pm 3.39$  BL  $24.45 \pm 2.19$  Abnormal  $38.59 \pm 8.16$   $p < 0.001$ ). The concentrations of leukocytes were different for BL  $0.8 \pm 0.2$  and Abnormal  $6.8 \pm 2.3$  when were compared to Normal  $0.4 \pm 0.1$  ( $p < 0.01$ ). The values of CRP measured as expression of CD46 indicated that BL  $12.73 \pm 3.07$  and Abnormal  $11.32 \pm 4.07$  are significant different when compared to Normal  $15.48 \pm 5.18$  ( $p < 0.05$ ). Although no differences were found an increasing tendency are observed when the OSA levels are increasing for Zn (Normal  $241.1 \pm 17$   $\mu$ M, BL  $256.4 \pm 19$   $\mu$ M and Abnormal  $261.4 \pm 18.4$   $\mu$ M), RC (Normal  $3.06 \pm 1.55$ , BL  $4.55 \pm 3.67$  and Abnormal  $5.26 \pm 5.74 \times 10^6$  /ml), HDS (Normal  $5.06 \pm 2.12$ , BL  $8.72 \pm 3.27$  and Abnormal  $9.45 \pm 3.60$ ) and no differences were found among the 3 OSA groups for sperm and EC concentrations.

**CONCLUSIONS:** The levels of oxidative stress measured as (OSA) are associated with DNA fragmentation (DFI) and the loss of the expression of complement protein (CD46). These results also suggest a possible role for leukocytes in generating oxidative stress, and demonstrate the application of CD45 staining and flow cytometry to identify leukocytes.

References: 1. A Kitamura M1, Matsumiya K, Yamanaka M, Takahara S, Hara T, Matsumoto M, Namiki M, Okuyama A, Seya T. Possible association of infertility with sperm-specific abnormality of CD46. J Reprod Immunol. 1997 Apr; 33(1):83-8.

2. A Tavalae M1, Parivar K1, Shahverdi AH2, Ghaedi K3,4, Nasr-Esfahani MH5,6. Status of sperm-born oocyte activating factors (PAWP, PLC $\alpha$ ) and sperm chromatin in uncapacitated, capacitated and acrosome-reacted conditions. Hum Fertil (Camb). 2017 Jan 12:1-8.

3. McLaughlin PJ1, Holland SJ, Taylor CT, Olah KS, Lewis-Jones DI, Hara T, Seya T, Johnson PM. Soluble CD46 (membrane cofactor protein, MCP) in human reproductive tract fluids. J Reprod Immunol. Oct;31(3):209-19. 1996.

4. A Seya T, Hara T, Matsumoto M, Kiyohara H, Nakanishi I, Kinouchi T, Okabe M, Shimizu A, Akedo H. Membrane cofactor protein (MCP, CD46) in seminal plasma and on spermatozoa in normal and "sterile" subjects. Eur J Immunol. 1993 Jun;23(6):1322-7.

5. Carver-Ward JA1, Hollanders JM, Jaroudi KA, Einspinner M, Al-Sedairy ST, Sheth KV. Progesterone does not potentiate the acrosome reaction in human spermatozoa: flow cytometric analysis using CD46 antibody. Hum Reprod. 1996 Jan;11(1):121-

6. Â Kawamoto A1, Ohashi K, Kishikawa H, Zhu LQ, Azuma C, Murata Y. Two-color fluorescence staining of lectin and anti-CD46 antibody to assess acrosomal status. *Fertil Steril*. 1999 Mar;71(3):497-501.

SUPPORT: ReproSource-Quest Diagnostics.

P-674 Wednesday, October 16, 2019 6:30 AM

**DOES THE gp130 ADENINE (A)/THYMINE (T) (rs1900173) GENE POLYMORPHISM AFFECT SEMEN QUALITY OR SPERM DNA DAMAGE?**



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**OBJECTIVE:** To Investigate a possible correlation between the glycoprotein subunit 130 (gp130) gene adenine (A)>thymine (T) (rs1900173) polymorphism and semen quality or sperm DNA damage.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** A prospective cohort study enrolled 364 men seeking fertility care. DNA was extracted from peripheral blood, and the gp130 gene A>T (rs1900173) polymorphism was genotyped using real-time PCR with the Taqman Universal PCR Master Mix and Taqman SNP genotyping assays. Patients were genotyped for the gp130 polymorphism and were categorized as follow: A/A (n=286); A/T(n=75); or T/T(n=3). Semen analyses were compared between genotype groups.

A portion of each semen samples was used for analysis according to the WHO guidelines/morphological analysis by motile sperm organelle morphology examination(MSOME). The remainder of the semen samples were tested for sperm DNA fragmentation using TUNEL assay; sperm apoptosis was analyzed using the annexin V assay; sperm chromatin packing/protamination was assessed using chromomycin A3(CMA3) staining; and sperm mitochondrial membrane potential(MMP) was analysed using MitoTracker Green. At least 200 spermatozoa were examined in each evaluation.

**RESULTS:** No correlation was observed between gp130 gene genotypes and potential confounders (age, abstinence time, smoking, drinking alcohol, and varicocele). No association was observed between gp130 gene A/T genotypes and general semen parameters or sperm DNA damage. Table 1 shows the data.

**CONCLUSIONS:** There appears to be no association between gp130 gene A>T single nucleotide polymorphism (SNP) and semen quality or sperm DNA damage. However, more studies stratified for different ethnic background should be performed in the future to clarify the possible roles of gp130 gene SNPs in the pathogenesis of male infertility.

SUPPORT: Merck Grant for Fertility Innovation (GFI-2014).

P-675 Wednesday, October 16, 2019 6:30 AM

**KARYOTYPING SINGLE SPERM: TARGETED NEXT GENERATION SEQUENCING ALLOWS FOR ACCURATE CHROMOSOME COPY NUMBER ANALYSIS OF HAPLOID GENOMES.**



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**OBJECTIVE:** Available NGS-based PGT-A platforms have been validated and clinically implemented for karyotyping of trophoctoderm biopsies, where at least 5 cells are available for DNA amplification. However, their application on single cells remains challenging, especially when dealing with haploid genomes. Given the current urge to investigate genetics at a single cell level and the promising advances of in vitro generated gametes, this study aimed to evaluate the performance of a targeted NGS-based PGT-A platform in single spermatozoa, an application that could be extended to any haploid genome.

**DESIGN:** Experimental and methodology validation study.

**MATERIALS AND METHODS:** Surplus washed sperm samples from two subjects undergoing an IVF-ICSI cycle were used in this study. For sperm isolation, single spermatozoa with progressive motility were immobilized with an ICSI pipette under a microscope and placed on a 2 µL microdrop of loading buffer, then the microdrop was loaded into a PCR tube. Finally, all samples were subjected to a targeted 2-step PCR protocol for DNA amplification before undergoing NGS. The targeted nature of DNA amplification of this platform results in higher sequencing depth, which allows for accurate single nucleotide polymorphism (SNP) genotyping across all chromosomes. Therefore, if complete loss of heterozygosity was evidenced by more than 95% of SNPs across the whole genome being homozygous, the sample was called haploid. In addition, SNP allele frequency patterns provided further evidence to detect chromosomal abnormalities. For instance, the presence of heterozygous SNPs demonstrates an imbalance of two chromosomes in single sperm, whereas a total absence of SNP data would indicate a chromosome nullisomy.

**RESULTS:** 52 single spermatozoa (20 from subject 1 and 32 from subject 2) were isolated and subjected to DNA amplification and sequencing. One sample from each subject did not show DNA amplification. In total, 39 samples (Subject 1 = 15, subject 2 = 24) retrieved a haploid genome. Of these, 21 (53.8%) samples presented the X chromosome and 18 (46%) the Y. Based on SNP data and heterozygosity plots, the remaining 11 samples were not categorized as pure haploid, which could be indicative of sample contamination, presence of more than one spermatozoon or suboptimal DNA amplification. Furthermore, 1 in 15 sperm karyotypes from subject 1 and 1 in 24 from subject 2 presented both a nullisomy of chromosome 15, which indicates a sperm aneuploidy rate of 6.67% and 4.17% respectively for each subject.

**CONCLUSIONS:** This NGS-based PGT-A platform is coupled with targeted DNA amplification, thus resulting in sufficient sequencing depth to allow genotyping that can be used to evaluate abnormality rates in sperm

TABLE 1. gp130 gene A>T (rs1900173) genotypes vs. population and semen parameters

Semen parameter	gp130 gene A/T (rs1900173) polymorphism genotypes			P
	A/A	A/T	T/T	
pH	8.1±0.2	8.1±0.2	8.0±0.2	0.59
Volume (ml)	2.8±1.5	2.8±1.2	1.4±0.8	0.12
Concentration(mlx10 <sup>6</sup> )	62.4±50.4	64.2±56.4	90±78.6	0.82
Progressive motility(%)	53.0±16.5	53.6±16.0	68.3±8.1	0.16
Total motility (%)	60.1±16.4	60.9±16.0	74.7±8.5	0.17
Leukocytes (x10/ml)	0.4±0.8	0.3±0.3	0.2±0.2	0.19
Vitality (%)	61.6±15.9	64.3±14.4	73.0±7.1	0.26
Normal spermatozoa (%)	0.6±0.8	0.7±0.5	1.2±0.3	0.23
DNA fragmentation (%)	13.7±7.7	13.5±8.0	8.5±0.7	0.59
Apoptosis (%)	19.5±8.5	19.9±6.1	21.5±2.1	0.54
CMA3 positivity (%)	55.1±17.1	58.1±16.0	56±26.9	0.56
Abnormal MMP (%)	25.8±17.4	26.4±16.0	22.5±14.8	0.95

with higher precision. This application also highlights its ability to be used in other haploid samples such as second polar bodies or in vitro-generated gametes. In addition, our data supports the fact that aneuploidy rates in human sperm are low.

**P-676** Wednesday, October 16, 2019 6:30 AM

#### **EFFECT OF OXIDATION-REDUCTION POTENTIAL ON MITOCHONDRIAL MEMBRANE POTENTIAL AND VITALITY OF PHYSIOLOGICALLY NORMAL HUMAN SPERMATOZOA.**



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**OBJECTIVE:** Physiological levels of reactive oxygen species (ROS) are necessary for optimal sperm functions such as total and progressive motility. In our previous study, we have demonstrated that higher levels of seminal oxidation-reduction potential (ORP) negatively affects total and progressive motility. Furthermore, motility is directly related to sperm vitality and mitochondrial membrane integrity. The objective of the present study was to investigate the effect of ORP on vitality and mitochondrial membrane potential (MMP) of physiologically normal spermatozoa.

**DESIGN:** Physiologically normal sperm from donor semen samples (n=8) were exposed to different titrated levels of oxidative stress (ORP: 1.48 and 2.75 mV/10<sup>6</sup> sperm/mL) in sperm wash medium (SWM). MMP and sperm vitality were measured at different time intervals (0, 60 and 120 minutes). The sample size for this study was calculated with an 80% power and a significance of P<0.05.

**MATERIALS AND METHODS:** ORP of SWM was taken as base line (control) and the different ORP levels (1.48 and 2.75 mV/10<sup>6</sup> sperm/mL) were generated by titrating SMW with defined concentrations of the oxidative stress inducer, cumene hydroperoxide. Equal concentrations (~20 x 10<sup>6</sup>/mL) of double-density gradient centrifugation-selected sperm (motility >90%) were incubated in SMW with different ORP levels for up to 120 minutes. Eosin-nigrosin staining was performed to evaluate the vitality; whereas, JC-1 dye was used to stain the sperm cells (~1 x 10<sup>6</sup>) to evaluate the depolarization of mitochondrial membrane. MMP was analyzed using flow cytometry after 60 and 120 minutes. Pairwise comparison analysis was carried out to determine the statistical significance.

**RESULTS:** MMP remained unchanged after sperm exposure for 60 minutes. MMP decreased to 2.5% (P=0.0014) and 61.1% (P<0.0001) at 120 minutes when sperm was exposed to ORP values of 1.48 mV/10<sup>6</sup> sperm/ml and 2.75 mV/10<sup>6</sup> sperm/ml, respectively. Vitality decreased to 21.2% (P=0.0001) at 60 minutes and 41.1% (P<0.0001) at 120 minutes when sperm were exposed to ORP values of 2.75 mV/10<sup>6</sup> sperm/ml.

**CONCLUSIONS:** The current findings demonstrate that spermatozoal MMP and vitality were affected at ORP levels of ≥ 1.48 mV/10<sup>6</sup> and ≥ 2.75 mV/10<sup>6</sup> sperm/ml, respectively. Hence, high seminal ORP may have a negative effect on sperm functionality and therefore on the fertilizing ability of spermatozoa.

Reference: None.

SUPPORT: None.

**P-677** Wednesday, October 16, 2019 6:30 AM

#### **MAPPING EVOLUTION OF MAMMALIAN SPERMATOGENESIS VIA HIGH RESOLUTION TRANSCRIPTOMICS.**



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**OBJECTIVE:** Sperm are unique, highly specialized cells that carry genetic information from father to offspring and provide a continuous link between the past, present, and future of a species. In all mammals, the foundational unit of fertility is the spermatogonial stem cell (SSC), which must balance self-renewal with differentiation to ensure continuous sperm production. This is reliant on coordinated intrinsic (germ-cell mediated) and extrinsic (soma mediated) regulation to guide differentiation, commit-

ment to **meiosis**, and morphological maturation. While decades of research in mice have provided a critical foundation of data, studies in primates have been limited to targeted subtypes based on *a priori* knowledge applied from rodents. However, fundamental differences exist between lineages, limiting the utility of mouse models. As a result, these processes are not well understood in humans and efforts to restore impaired spermatogenic function have had limited success. Here, we aim to identify key differences among species in these processes by conducting unbiased global evolutionary comparisons of expression between rodents and primates in the germline and soma throughout the course of spermatogenesis.

**DESIGN:** Single cell RNA sequencing was performed on adult human and nonhuman primate testis, and datasets were analyzed both individually and also globally compared with our previously published mouse single cell atlas.

**MATERIALS AND METHODS:** Cryopreserved testes samples from 4 human and 5 macaque individuals were dissociated to single cell suspensions and isolated via microfluidics using the Drop-seq platform to conduct single-cell sequencing on multiple technical replicates.

**RESULTS:** The data revealed a continuous developmental progression from spermatogonia to spermatids in adult humans (n=4, ~14,000 cells) and rhesus macaques (n=5, ~22,000 cells), thus capturing the complete germ cell differentiation process and analogous somatic cell types across all three species. Comparing pseudotime alignments of germ cell trajectories across species identified areas of similarity and dis-synchrony of germ cell maturation program, including differences in starting and ending states and a variable "clock rate" within the trajectories. Targeted analysis of spermatogonia computationally aligned discrete molecular states between species, revealing a unique undifferentiated population in primates, potentially containing SSCs. Characterization of underlying transcriptional programs and somatic cell inputs identified additional features of divergence.

**CONCLUSIONS:** Our datasets provide new insights into differences in the intrinsic germ cell program and extrinsic signals required to promote germ cell differentiation in human, nonhuman primate, and rodent testes.

**P-678**

**WITHDRAWN**

**P-679** Wednesday, October 16, 2019 6:30 AM

#### **COMPARATIVE PROTEOMIC ANALYSIS REVEALS DIFFERENTIAL REGULATION OF REDOX HOMEOSTASIS AND PERTURBED OXIDATIVE PHOSPHORYLATION PATHWAY IN UNILATERAL COMPARED TO BILATERAL VARICOCELE CONDITION.**



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**OBJECTIVE:** Oxidative stress is pronounced in varicocele patients and differs between unilateral and bilateral conditions. At subcellular level, excess of oxidative stress induces damage to the cell organelles and plasma membrane. The main objective was to have a proteomic insight into seminal plasma for delineating the possible pathways involved in the etiology of sperm dysfunction in unilateral and bilateral varicocele condition.

**DESIGN:** Proteomic profiling of seminal plasma (unilateral varicocele, bilateral varicocele and fertile healthy men) was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Bioinformatic analysis was conducted using ingenuity pathway analysis (IPA) software.

**MATERIALS AND METHODS:** Pooled seminal plasma samples from unilateral (n=5), bilateral (n=5) varicocele patients and fertile healthy men (n=5) were subjected to quantitative proteomic analysis. Proteins identified by LC-MS/MS in both varicocele groups were compared separately and also as combined varicocele group with the fertile group. Differentially expressed proteins (DEPs) obtained from three different analysis were subjected to comparison analysis using IPA software.

TABLE 1. Proteins involved in oxidative phosphorylation pathway

DEPs	Unilateral varicocele (Z-score)	Biilateral varicocele (Z-score)	Combined varicocele (Z-score)
SDHA	2.27	0	0
COX4I1	-2.33	0	0
ATP5F1B	-3.25	0	0
UQCRC2	-1.96	0	-1.63
CYC1	-4.71	0	0
NDUFS3	-7.09	0	0
COX5B	-4.89	0	-2.28
NDUFV2	-7.74	0	0
ATP5PO	-10.15	0	0
NDUFS1	-5.77	-2.78	-3.76

\*Considered significant when Z score is >2 or <-2.

**RESULTS:** Seminal plasma proteomic analysis revealed the presence of cellular proteins particularly of mitochondrial origin in seminal plasma. Proteins involved in the oxidative phosphorylation pathway of spermatozoa were present (Z score = -3.5) in unilateral varicocele patients. Whereas, the Z-score was not available for combined varicocele and bilateral varicocele groups (Table 1). In addition, proteins regulating the cellular antioxidant mechanism such as SOD1 (Z score = 3.94) and SOD2 (Z score = 8.08) were detected in unilateral varicocele patients. Whereas, IL-8 signaling pathway was activated in bilateral varicocele group (Z score = 2.236) compared to unilateral varicocele group (Z score = 1.342).

**CONCLUSIONS:** Our proteomic result implies release of spermatozoal proteins into seminal plasma of unilateral varicocele patients may be due to oxidative damage of sperm membrane or inflammation originating from mitochondrial dysfunction. On the other hand, in case of bilateral varicocele it may be due to apoptosis which might have been phagocytized thereby, no cellular content is released into seminal plasma.

Reference: None.

SUPPORT: None.

**P-680** Wednesday, October 16, 2019 6:30 AM

**S100A9, AN INFLAMMATORY AND IMMUNE PROTEIN, IS INCREASED IN SEMEN OF SMOKERS.**

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**OBJECTIVE:** Cigarette smoking is one of the most important lifestyle-associated factors involved in human diseases, and both men and women who smoke present increased rates of infertility. We have previously shown that smoking leads to an altered seminal plasma proteome, with increased inflammatory mechanisms and immune function. We hypothesize that this inflammatory milieu is dependent on at least one inflammatory protein – S100A9 – by a Calcium and Zinc binding-dependent mechanism. Thus, this study aimed to compare seminal plasma S100A9 levels in smokers and non-smokers.

**DESIGN:** cross-sectional study.

**MATERIALS AND METHODS:** 48 adult men who reported smoking cigarettes, referred to our andrology laboratory were included. 39 adult men without semen alterations were included as controls. Men who were exposed to other drugs, or with systemic or urogenital alterations that affect testicular function were excluded. Semen was collected and analyzed as per 2010 WHO guidelines. The remaining volume was centrifuged and seminal plasma was collected for western blot analysis. 50 µg of protein (quantified by a modified Lowry assay) was used for S100A9

quantification and bands were normalized to total protein by Ponceau S. Protein contents were calculated as S100A9/mL and S100A9/ejaculate. Due to a non-normal data distribution, groups were compared by a Mann-Whitney test (p <0.05).

**RESULTS:** Smokers presented a 28% lower sperm concentration and 30% lower total sperm count (p=0.047 and 0.013, respectively). S100A9 was higher in seminal plasma of smokers, with a fold-change of 1.9 for concentration (p=0.038) and 2.2 for total S100A9 in the ejaculate (p=0.023). Seminal concentrations of S100A9 (per mL) were weakly correlated to sperm concentration, but this was lost after correction for ejaculate volume, and no linear model could be constructed, indicating variables were independent.

**CONCLUSIONS:** Our results confirm our hypothesis that S100A9 is increased in seminal plasma of smokers, which serves as a suggestive inflammatory pathway in these men. This is an important finding because it could lead to targeted therapeutics based on this inflammatory aspect. However, it should be noted that seminal plasma is produced by fluid of various sources, so that identifying its tissue of origin could assist in understanding how smoking affects male fertility potential. Also, because there is an important exchange of information between the spermatozoa and the epididymis cells, it is of interest to demonstrate how inflammation disturbs this exchange, and what the consequences are.

**SUPPORT:** CNPq (140995/2016-1).

**P-681** Wednesday, October 16, 2019 6:30 AM

**EXOME SEQUENCING IDENTIFYING ADDITIONAL QRICH2 MUTATIONS IN OLIGO-ASTHENOTERATOZOOSPERMIA AND ASTHENOSPERMIA PATIENTS.** Wenming Xu, Ph.D, Xiao Liang Li, Ms., American Society of Human Genetics, Chengdu, China.



**OBJECTIVE:** Our recent study has shown that loss-of -function of QRICH2, a testis specific expressed gene, is associated with male infertility with multiple morphological abnormalities of the flagella (MMAF), the current study aim to determine whether QRICH2 mutations were associated with other more common forms of male infertility, such as oligo-asthenotatozoospermia and asthenospermia

**DESIGN:** Experimental study recruited from male infertility clinic and human samples of case and control were collected.

**MATERIALS AND METHODS:** 84 cases of male infertility patients were recruited. WES was performed for all subjects. All identified variants were confirmed by Sanger sequencing. Immunostaining result was used to determine the specific localization of QRICH2 in human sperm. Western blot were used to detect the expression of QRICH2 in oligo-asthenotatozoospermia. Co-Immunoprecipitation (Co-IP) with QRICH2 antibody in human testis and proteomics analysis were conducted to identify the binding partner. IVF/ICSI outcome were followed to determine whether the mutation of QRICH2 have effect on the normal development of offspring.

**RESULTS:** We identified five unrelated patients (5/84, 5.9%) with homozygous and compound heterozygous mutations in the QRICH2 gene, which is specifically expressed in human and mouse testis. Three of the samples harbor a recurrent deletion, (g.17:74288566\_74288568del,c.1742\_1744del,p.581\_582del). None of these mutations were reported in control sequence databases. 4 of mutation is located in the SNC-N domain, while one mutation is located in the Glutamine rich domain. Co-IP result indicated that mitochondrial proteins, such as VDAC1 is associated with QRICH2. Western blot result shows that QRICH2 expression is -down-regulated in patients. And IVF/ICSI outcome analysis indicates that normal offspring development could be observed in the patients.

**CONCLUSIONS:** Compared with other reported genes associated with male infertility, high frequency of QRICH2 mutations were detected with WES. QRICH2 is important for sperm motility. The mutation of QRICH2 gene, especially high frequency mutations of SMC\_N domain are likely responsible for the phenotypes of both oligo-asthenotatozoospermia and asthenospermia.

P-682 Wednesday, October 16, 2019 6:30 AM

**ASSOCIATION OF MENTAL HEALTH DIAGNOSES AND UTERINE ENDOMETRIAL THICKNESS IN WOMEN UNDERGOING IN-VITRO FERTILIZATION.**



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**OBJECTIVE:** To assess the association between mental health diagnoses and uterine receptivity, operationalized as endometrial thickness.

**DESIGN:** Due to the lack of data regarding the impact of mental health diagnoses on intermediate outcomes of in vitro fertilization (IVF), we performed an exploratory retrospective cohort study of women undergoing IVF at an academic medical center from 2018 to 2019.

**MATERIALS AND METHODS:** A total of 101 patients undergoing IVF were recruited and underwent controlled ovarian hyperstimulation with an antagonist protocol. Women on clomiphene or letrozole, those seeking fertility preservation and those with uterine factor were excluded. Mental health diagnoses and medications were abstracted from chart review. Endometrial thickness was assessed via transvaginal ultrasound on the final day of stimulation. We used linear regressions to assess the associations between 1) anxiety 2) depression 3) any psychiatric diagnosis 4) current treatment with antidepressant or anxiolytic medications.

**RESULTS:** Of the 101 women, 12 (11.9%) had previously been diagnosed with anxiety, 10 (9.9%) had been diagnosed with depression, of these women 15 (14.9%) were currently being treated with antidepressants or anxiolytic medications. Women had a mean of 35.1±4.0 years of age, a mean BMI of 26.3±5.6 kg/m<sup>2</sup>, and a mean endometrial thickness of 10.3±2.7 mm. Women self-reported as Caucasian (52.5%), Asian (24.8%), or African American (15.8%). A mental health diagnosis (p=.01) and use of antidepressants or anxiolytic medications (p=.002) were negatively associated with endometrial thickness. All associations remained significant after controlling for BMI. Endometrial thickness was not associated with cycle characteristics (peak estradiol level, total gonadotropin dose, or number of oocytes retrieved).

**CONCLUSIONS:** Mental health diagnoses and current antidepressant or anxiolytic treatment are associated with a thinner endometrial thickness, a marker of uterine receptivity. Prior studies have indicated women with history of anxiety and depression may have lower live birth rates; this is perhaps due to the influence of stress on endometrial thickness and interaction with the reproductive hormonal axis. These findings are important given the strong association of mental health diagnoses and infertility, with anxiety and depression often exacerbated by the stress of fertility treatment. Future studies will examine biochemical markers of stress to further explore the mechanism behind this finding.

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**PATH TO PREGNANCY; A MULTINATIONAL SURVEY OF WOMEN'S EXPERIENCES AND EXPECTATIONS WHEN PLANNING PREGNANCY.**



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**OBJECTIVE:** When deciding to start a family, there are many suggested actions a woman could take to increase chances of a healthy pregnancy. However, it is not clear where women find relevant information, their mindset and their actions. This research aimed to understand more about women's path to pregnancy in five countries; USA, UK, Germany, Italy and China.

**DESIGN:** On behalf of SPD, Ipsos Suisse SA surveyed a sample of a minimum of 1000 women per country regarding their attitudes and behaviours regarding starting a family. Interviews were conducted online (computer assisted web interview) between 27<sup>th</sup> of July to 7<sup>th</sup> of August 2018.

**MATERIALS AND METHODS:** Women were aged 20-45 years old who were able to have children and who chose to take part in our survey from Ipsos Panel.

**RESULTS:** The majority of participants considered as conceivers (tried to get pregnant in the past 12 months; currently trying or who will try in the next

12 months), were in the state of mind regarding getting pregnant: "I want to maximise my chance of getting pregnant as soon as possible" (71% USA, China 61%, 55% UK, 54% Germany, 54% Italy) as opposed to "prefer to let nature take its course and wait".

Partner involvement in planning pregnancy differed between countries; in China, only 1% of women reported their partner was not very involved, USA had similarly high involvement (only 3% not involved), but lack of partner involvement was much higher for UK (9%), Italy (10%) and Germany (11%).

Recalling their last, planned pregnancy participants, many women stated it took longer to conceive than they expected (43% USA, 39% UK, 31% Germany, 35% Italy, 42% China). Women in China (53%) started to become worried that they may have problems getting pregnant less than 3 months after they started trying for a baby. A lower proportion were worried after 3 months in other countries (27% USA, 15% UK, 12% Germany, 23% Italy).

Among women who were wishing to have children now or in the future, sources of fertility information included; gynaecologist/doctor (top source for all countries), internet (highest UK 55%, lowest China/Italy 38%), mobile phone App, parenting magazines, friends, mother and books.

Common behaviours when trying to get pregnant were; adopting a healthier diet, taking folic acid supplements, seeking information on the internet, tracking their cycle and increasing intercourse frequency.

**CONCLUSIONS:** This survey found that attitudes towards pregnancy differed between countries, with Chinese women, in particular, wanting to manage their pregnancy plans and achieve success quickly. Most women are prepared to change behaviours to help conception and healthy pregnancy, with doctors being the most important source of information.

**SUPPORT:** Study funded by SPD Swiss Precision Diagnostics GmbH.

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**PREIMPLANTATION GENETIC TESTING: PSYCHOMETRIC PROPERTIES OF QUESTIONNAIRES DESIGNED TO ASSESS PATIENT DECISIONAL DISTRESS AND DECISIONAL UNCERTAINTY.**



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**OBJECTIVE:** Limited scientifically rigorous research exists on decisional distress and uncertainty experienced by individuals considering preimplantation genetic testing (PGT), and no questionnaire (Q) exists to measure these constructs. This study assessed the psychometric properties of the new Pre-implantation Genetic Testing (PGT) Decisional Distress Q and the PGT Decisional Uncertainty Q.

**DESIGN:** Prospective online Q's and associated psychometric evaluation of the Q's.

**MATERIALS AND METHODS:** The Q's were developed by the investigative team based on a careful review of the relevant literature and our prior experience with questionnaire design for aspects of reproductive health. A semi-structured interview was conducted to assess face validity. Eligibility required women had used or considered using PGT in the previous 6 months. The new Distress Q has 22 items. The new Decisional Uncertainty Q has 13 items and assesses (through three subscales): clarity of the perceived benefits/drawbacks of testing (5 items), degree of input by respondent in the decision (1 item), and decision certainty (7 items). Means, variances (SD), and ranges were calculated, followed by Cronbach's alpha, and correlation between the Q's. Estimated sample size of n=100 was based on alpha=0.05 and 80% power to detect correlations of r≥0.28 between the contributors and the Q scores.

**RESULTS:** N=106 females (mean age 36.5±4.8 years, range 26-45 years; 15% non-Caucasian; 9% Hispanic) completed the online questionnaire, and 17 states were represented. All scales had excellent internal consistency (Cronbach's α's 0.92-0.94). Distress levels were low (mean 1.00 [SD=0.75] on a 0-4 scale, higher score=greater distress). Decision clarity and decision certainty were good (mean 3.26 and 3.06 [SD=0.79 and 0.89] respectively on a 0-4 scale, higher score=greater clarity/certainty). Distress was inversely correlated with decision clarity (r= -0.34, p<0.01) and decision certainty (r= -0.65, p<0.01). Decision clarity was positively correlated with decision certainty (r= +0.73, p<0.01). For degree of input in the testing decision, more than half (62%) indicated that they and their partner would have equal input, and 32% said that they would have more input than their partner.

**CONCLUSIONS:** Infertility patients must cull through many clinical and personal factors prior to making the decision to utilize PGT, and modern

genetic testing options can raise moral, ethical, and personal issues that result in distress and uncertainty. We document initial reliability and validity of new instruments to measure these constructs in female patients who are considering PGT, either for single gene disorders or for chromosomal disorders. The novel Q's can be used in research and clinical settings (e.g., genetic and repro-psychologic counseling). Educational materials can be developed to address information gaps, and counseling can ease psychological dilemmas. Data collection is ongoing for future analysis on whether decisional distress and uncertainty vary by patient demographics, PGT-M vs PGT-A, etc.

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### IS A DIAGNOSIS OF UTERINE FIBROIDS WORSE THAN A DIAGNOSIS OF CONGESTIVE HEART FAILURE OR CHRONIC LUNG DISEASE?

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<sup>a</sup>Johns Hopkins School of Medicine, Baltimore, MD; <sup>b</sup>University of Mississippi Medical Center, Jackson, MS; <sup>c</sup>Johns Hopkins University, School of Medicine, Baltimore, MD.



**OBJECTIVE:** More than 80% of African American women and 70% of white women have detectable uterine fibroids by age 50. Despite the high prevalence of disease, the psychosocial impact of fibroid disease has not been quantitatively compared to other chronic conditions. Here we rigorously analyzed available evidence pertaining to the psychosocial burden of uterine fibroids compared to chronic diseases using quality of life indicators.

**DESIGN:** Systematic literature review using PRISMA guideline of three databases (PubMed, PsycINFO, Cochrane library) for English language publications between January 1990 and September 2018.

**MATERIALS AND METHODS:** Search phrases included: depression AND fibroids; stress AND fibroids; sexuality AND fibroids; psychological AND fibroids; and fear AND fibroids; in addition to: uterine fibroids; quality of life; leiomyoma; and myofibroma. We considered studies eligible if they published standardized, validated questionnaires of the effect of fibroids at baseline and after treatment. A total of 13 different validated questionnaires were included, such as the SF-36, UFS-QoL and SAQ. To be included, the study must have referenced women with fibroids specifically. Study quality was assessed by established PRISMA guidelines. Internal and external validity were evaluated.

**RESULTS:** Of the 2,422 articles identified, 21 studies met inclusion/exclusion criteria, representing a total of 2,361 patients. Of note, the data showed that for 7 out of 8 categories on the SF-36(Short Form (36) Health Survey) questionnaire, a diagnosis of uterine fibroids was accompanied by a disability score that exceeded (i.e., was a greater psychosocial stressor) than the diagnosis of congestive heart failure (CHF), diabetes mellitus (DM), or chronic lung disease (CLD). At baseline, quality of life scores were considerably lower in all instruments measuring these variables in women with uterine fibroids, indicating significantly impaired psychosocial functioning. Uterine fibroids were associated with significant patient-reported health disabilities related to bodily pain, emotional and mental health, social functioning, and satisfaction with sex life. Women with symptomatic fibroids regarded an improvement in quality of life as a major driver of decision making, more so than clinical indices, such as increase in hemoglobin levels or decrease in fibroid size.

**CONCLUSIONS:** The level of disability associated with uterine fibroids exceeded that of chronic diseases such as CHF, DM and CLD. Attention to the impact of uterine fibroids on the quality of life in women affected by fibroids will lead to increased patient satisfaction.

**SUPPORT:** This effort is supported in part by the Howard and Georgeanna Jones Research Endowment.

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### A COMPREHENSIVE EXAMINATION OF INFERTILITY STIGMA AMONG FERTILE AND INFERTILE WOMEN IN THE UNITED STATES.

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**OBJECTIVE:** Infertility impacts 1 in 6 couples; however, having children is a social norm, potentially stigmatizing infertile individuals. This research expands previous qualitative and small-scale studies with a large-scale survey of both fertile and infertile women's societal perceptions of female

and male infertility stigma as well as infertile women's internalized experiences of stigma.

**DESIGN:** A national, cross-sectional survey.

**MATERIALS AND METHODS:** 327 women were recruited through an e-newsletter in March 2019; no incentive was provided. Eligible participants were ages 18 to 59, identified as women, and lived in the USA. After providing informed consent, participants completed an online survey to assess societal perceptions of female and male infertility stigma. The survey also assessed infertile women's internalized experiences of stigma and emotions. The data were analyzed using one-sample and independent sample t-tests and bivariate correlations; the power for these analyses was excellent (.99).

**RESULTS:** Participants ranged in age from 18 to 59 ( $M = 34.11$ ,  $SD = 6.64$ ). The majority identified as heterosexual (95%) and had a partner (81%). Infertility was defined as a diagnosis of infertility or 12 months of unprotected sex without becoming pregnant; 33% of the participants were infertile.

An examination of societal perceptions of infertility stigma revealed that both fertile ( $M = 2.90$ ,  $SD = 0.76$ ) and infertile women ( $M = 2.76$ ,  $SD = 0.81$ ) felt that female infertility was stigmatized (the means were statistically higher than the midpoint of the 5-point scale; fertile women,  $t(217) = 4.89^*$ ; infertile women,  $t(108) = 5.48^*$ ). Fertile ( $M = 2.56$ ,  $SD = 0.73$ ) and infertile women ( $M = 2.49$ ,  $SD = 0.71$ ) did not believe male infertility was stigmatized (the means were not statistically higher than the midpoint; fertile women,  $t(217) = -0.15$ , *ns*; infertile women,  $t(108) = 0.95$ , *ns*). A comparison of societal perceptions of female and male infertility stigma revealed that both fertile and infertile women felt that female infertility stigma was significantly higher than male infertility stigma (fertile women:  $t(217) = 5.96^*$ ; infertile women:  $t(108) = 4.80^*$ ). Infertile women indicated feeling internalized stigma, as the mean ( $M = 4.18$ ,  $SD = 0.84$ ) was significantly higher than the midpoint of the 5-point scale,  $t(108) = 3.04^*$ . Internalized stigma was associated with feeling afraid ( $r = .25^*$ ), uncertain ( $r = .22^*$ ), anxious ( $r = .24^*$ ), stressed ( $r = .36^*$ ), ashamed ( $r = .46^*$ ), and guilty ( $r = .52^*$ ).

**CONCLUSIONS:** Despite the increased awareness of infertility and emergence of new technologies increasing treatment success, infertility stigma persists, particularly for women. The results suggest that women believe infertile women are stigmatized, and there is greater stigma for infertile women than men. Further, infertile women report feeling stigmatized, which is related to negative emotions. Infertility stigma puts strain on relationships, may lead individuals to hide their diagnoses from friends or family and delay or avoid treatment. In turn, this could lead to worse prognoses for these patients.

Note  $*p < .05$ ; *ns* = not significant.

**SUPPORT:** This research was funded by Modern Health, Inc.

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### THE PSYCHOLOGICAL IMPACT OF SURROGACY ON THE FAMILIES OF GESTATIONAL CARRIERS: IMPLICATIONS FOR CLINICAL PRACTICE.

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**OBJECTIVE:** ASRM has issued ethics and practice committee guidelines for gestational carriers (GCs) that include recommendations for the psychological consideration of a GC's own family. At present, there are no studies on the impact of surrogacy within GC family systems that can offer guidance to mental health professionals (MHPs) who counsel potential GCs. This study seeks to explore the psychological impact of surrogacy on families in order to guide MHPs in educating GCs on how this experience might affect their families.

**DESIGN:** IRB approved, cross-sectional survey study.

**MATERIALS AND METHODS:** Participants ( $n = 53$ ) were recruited via an ad posted on surrogacy websites and forums. Research packets were mailed to GC families with designated questionnaires for each family member to fill out and return. All family members filled out a detailed questionnaire on the experience of surrogacy along with the Family Assessment Measure, Version III (FAM-III). Children were asked to fill out the Piers-Harris Children's Self-Concept Scale, 2<sup>nd</sup> Edition (Piers-Harris 2). Data was entered and analyzed by SPSS software.

**RESULTS:** Children of GCs ( $n = 23$ ) endorsed excitement, curiosity, surprise, and pride at the highest rate amongst emotions experienced from surrogacy. 74% of children reported the experience as having a positive impact on their life. Children scored within normal limits on all domain scales on the Piers Harris 2, including behavioral adjustment, freedom from anxiety,

popularity, and overall happiness. On the FAM-III, parents' overall rating scales were significantly more defensive than their children's ( $p < .05$ ). The overall ratings given by the children reflected more dysfunction, although scores were still within normal limits. Relative strengths reported by all family members were *Involvement* (reflecting nurturing and supportive involvement between family members) and *Values and Norms* (reflecting a congruence of the family values system). Relative weaknesses endorsed included Role Performance (reflecting a lack of agreement regarding roles within the family system) and Task Accomplishment (reflecting problems with task identification and problem-solving strategies). Children's advice to other kids whose mothers are considering surrogacy included: "Have fun...it's a rollercoaster", "Don't be scared or angry...your parents will not get rid of you or your siblings"; "Be proud. Your mom is creating LIFE, People!"

**CONCLUSIONS:** Families in which the mother has been a GC appear to be functioning well across a number of psychosocial domains and feel that surrogacy has been a positive experience within their family system. These families appear to foster nurturing relationships and place importance on family values and consistency in interpersonal interactions within their families. Parents' perceptions of the experience of surrogacy did not differ from their children's endorsed emotions and children express pride in their mothers' desire to help others. Research into the impact of surrogacy on the families of GCs is critical for MHPs who work with this unique population to ensure adequate psychological preparation.

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**THE IMPACT OF THE FERTISTRONG APP ON ANXIETY AND DEPRESSION IN MEN.** Alice D. Domar, Ph.D.,<sup>a</sup> Lauren Jasulaitis, CRC,<sup>b</sup> Sue Jasulaitis, M.S.N., R.N.,<sup>c</sup> Elizabeth A. Grill, PsyD,<sup>d</sup> Meike L. Uhler, M.D.<sup>e</sup> <sup>a</sup>Boston IVF, Waltham, MA; <sup>b</sup>Affiliation not provided; <sup>c</sup>Fertility Centers of Illinois, Glenview, IL; <sup>d</sup>Weill Cornell Medical Center, Rye Brook, NY; <sup>e</sup>Fertility Centers of Illinois, Chicago, IL.



**OBJECTIVE:** The impact of infertility on distress levels in men is poorly explored; however initial research does indicate that men commonly report symptoms of anxiety and depression. Despite the publication of dozens of studies on psychological interventions with women, there is a paucity of such research on men. The goal of the present pilot study was to determine if a recently developed cognitive-behavioral and relaxation mobile app, targeted at men experiencing infertility, could lead to decreases in psychological distress.

**DESIGN:** This pilot project utilized a randomized controlled design.

**MATERIALS AND METHODS:** Thirty nine men participated in a randomized study of the FertiStrong app May/June of 2018. There was only a brief period of time allowed for recruitment as the app was launched nationwide less than two months after it was developed. Participants completed a demographic form, the Hospital Anxiety and Depression Scale (HADS) and Fertility Problem Inventory (FPI) at baseline and follow-up. Participants randomized to the intervention group were introduced to the FertiStrong app, instructed to download it on their phone, and to use it when needed. Control participants received routine infertility care. The follow-up testing was approximately one month after recruitment. The HADS and FPI were converted to numeric values and compared baseline to follow up with a paired t test. The Shapiro Wilk test was used to test for normality of distributions.

**RESULTS:** One participant was excluded, resulting in 38 participants, 19 in each group. There were no differences between the two groups on any of the demographic characteristics ( $P > 0.31$ ). For the HADS anxiety domain, the control group had a small increase between baseline and follow up while the intervention group had a small decrease, but no statistical significance. For the HADS depression domain, there was a slight increase in the control group and no change in the intervention group but there was no significant difference. For the FPI, the control group had a two point increase, changing from moderately stressed at baseline to extremely high while the intervention group had a five point decrease, changing from extremely high to moderately high, but not significant. Each of the FPI five domain-specific scores in the intervention group decreased at follow up and one, Rejection of Childfree lifestyle, was significant ( $P = 0.03$ ). Several statements increased in both groups at follow-up but the increase in stress level was significantly greater in the control group than the intervention group ( $P > 0.02$ ).

**CONCLUSIONS:** Recruitment was challenging for this study due to the short recruitment phase and the sample size was thus smaller than planned. The small size could have contributed to the lack of significant differences in the HADS. However, despite the small sample size, there were several sig-

nificant improvements noted in the intervention group and on all testing, the intervention group trended to less distress. More research is needed on convenient interventions for men experiencing infertility.

**SUPPORT:** This study was supported by an unrestricted educational grant from Ferring Pharmaceuticals.

**P-689** Wednesday, October 16, 2019 6:30 AM

### MOTIVATING FACTORS AND QUALITY OF LIFE FOR MALE PARTNERS OF INFERTILE COUPLES.

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**OBJECTIVE:** Motivating factors for pursuing fertility treatment may be difficult to ascertain, as there may be fear of a "wrong" answer. Given that infertility is a couples' condition, it is important to understand the male's perspective. We sought to assess the male motivations and quality of life (QoL) during fertility treatment.

**DESIGN:** Cross-sectional survey study.

**MATERIALS AND METHODS:** 2 anonymous paper surveys were given to 70 male partners after providing a semen sample for assisted reproductive technology at a fertility clinic. Men were alone during this time. 1<sup>st</sup>, a questionnaire assessed demographics, motivating factors, and fertility history. 2<sup>nd</sup>, the fertility quality of life (FertiQoL) survey assessed the impact of infertility in diverse life areas. The FertiQoL contained 2 QoL domains: Core (with Mind-Body, Emotional, Relational, and Social subdomains) and Treatment (with Tolerability and Environment subdomains). Responses were scaled 0-100. A higher score indicated higher QoL. Eligible respondents included men receiving treatment as part of an infertile couple. Responses were analyzed via descriptive statistics, chi-square analysis, and multivariable regression analysis.

**RESULTS:** Out of 70 anonymous surveys, 61 (87.1%) and 52 (74.3%) completed the 1<sup>st</sup> and 2<sup>nd</sup> surveys entirely. 62 (88.6%) men were married, 51 (75.0%) did not have prior children, and 19 (27.9%) reported prior in vitro fertilization (IVF). 23 (33.8%) men had been trying to conceive for <12 months, 20 (29.4%) for 12-24 months, and 25 (36.8%) for >24 months.

When asked, "Why are you pursuing a fertility evaluation?" 89.6% (60/67) said it was "because both my partner and I want a child." When asked, "Do you want children?" 91.0% (61/67) said "yes," but 9.0% (6/67) said "no." Of these men, 4/5 were planning to undergo IVF. In contrast, 66.7% (40/60) of men who did desire children were planning to undergo IVF. Duration of infertility, age, income, and marital status were not related to male desire for a child ( $p > 0.05$ ). Not having a prior child was related to male desire for wanting children ( $p = 0.003$ ).

Mean FertiQoL scores were: Overall 78.9 +/- 9.9, Core 79.0 +/- 9.6, and Treatment 78.5 +/- 14.4. Men who did not want children scored lower in the Core interpersonal subgroups (Relational and Social) than those who did want children, but this was not significant ( $p > 0.05$ ).

**CONCLUSIONS:** 9% of males self-reported that they did not want a future child, yet 4/5 were planning to undergo IVF. Not having a prior child was related to desire for future children. Understanding these motivations provides an opportunity to better care for male partners.

**P-690** Wednesday, October 16, 2019 6:30 AM

### KNOWLEDGE, ATTITUDES AND CONCERNS TOWARDS ELECTIVE SINGLE EMBRYO TRANSFER (ESET) IN COUPLES UNDERGOING FRESH/FROZEN EMBRYO TRANSFER CYCLES IN ASIAN POPULATION.

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**OBJECTIVE:** With increasing trend towards elective single embryo transfer (eSET), it is essential to explore crucial questions like how patients perceive, understand and decide for these options. In countries where the number of embryos transferred is still unregulated and left for clinician's discretion, there is a need to reduce couples desire for multiple embryo transfer. Particularly, in a patient population with poor health literacy and mostly

taking self-funded treatment, understanding how patient balances the risks and benefits of single embryo transfer is vital. The purpose of this study is to assess the patients' and spouses' knowledge, attitude and concerns regarding single embryo transfer.

**DESIGN:** Prospective questionnaire study at a tertiary-care, university-affiliated teaching hospital.

**MATERIALS AND METHODS:** 240 couples participated in this 25 item questionnaire survey at their routine counselling visit before embryo transfer. The common practice in the centre during the study period was double embryo transfer (DET). The treatment cycles were self-funded and the patients received no reimbursements. All couples received a psychological counselling session, written information as brochure and consultation with ART clinician, explaining DET, SET and Multiple pregnancy, before responding to questionnaire. Descriptive statistics were computed, chi square tests were performed to compare the frequencies according to population demographics and response characteristics.

**RESULTS:** 240 women and 232 men answered the questionnaire for analysis. 62% preferred singleton conception in their next embryo transfer cycle. Yet, 92% of men and 93% of women indicated that they would happily accept if conception resulted in twins in the current IVF attempt. Cancelled cycle (82%) was perceived as unacceptable risk followed by failed cycle (67%) and multiple pregnancy (45%). Twin conception risks are perceived as important by the couples but still prefer two embryo transfer stating 'Have a positive attitude and wouldn't happen to all' (76%). 87% of men and 89% of women would prefer eSET if results unchanged and comparable to DET.

The top concerning factor for choosing DET over eSET was 'understand the benefits but feel it will prolong the time to conceive' (69% men and 74% women). Compared to women, men were more likely to choose eSET over the factor 'less risks to mother' in singleton conception (63% Vs 35%,  $P=0.04$ ). About 74% choose DET over eSET in the next cycle even after feeling well informed and understood benefits.

**CONCLUSIONS:** Twin conception risks were perceived as important by the couples but still prefer double embryo transfer. Couples believe accepting eSET would prolong the time to conceive. These results could help in counselling patients addressing their concerns and specific information provision about risks. Couples would prefer eSET programs if it may provide comparable success rates and time to conception which would require careful patient selection. Thus, strategies to maintain existing rates of successful conception per oocyte recovery may reduce couples desire to choose multiple embryo transfer.

**P-691** Wednesday, October 16, 2019 6:30 AM

#### **LIFESTYLE RELATED FACTORS ASSOCIATED WITH PREGNANCY OUTCOME AFTER IN VITRO FERTILISATION-EMBRYO TRANSFER CYCLES.**



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**OBJECTIVE:** Lifestyle factors have a dramatic impact on the reproductive performance of infertile population undergoing Assisted Reproductive Technology. The present study focussed on IVF population and explored association between lifestyle factors primarily during the implantation window and implantation success. This study aims at determining the independent contribution of female lifestyle related factors following embryo transfer leading to ART success. Also, the secondary aim is to compare the differences between pregnant and non-pregnant cycles and to draw strategies to improve ART outcome.

**DESIGN:** Cross sectional questionnaire based study.

**MATERIALS AND METHODS:** This study was undertaken in our university affiliated and tertiary referral private hospital. We recruited 130 women who underwent frozen/fresh embryo transfer (IVF/ICSI cycles) over a period of 12 months. We categorized lifestyle factors into diet and nutrition related, physical activity related and emotional support related behaviours. A structured questionnaire with 15 questions was framed. The survey was conducted using the computer assisted telephone interviewing system. The women completed the questionnaire based on their lifestyle factors from the time of embryo transfer to serum pregnancy testing. The primary outcomes were the result of Serum beta hCG ( $>25\text{mIU/mL}$  considered to be positive) on day 14 after embryo transfer.

**RESULTS:** Among the 130 women receiving ET, 50/130(38%) resulted in implantation. The mean age of the study population was  $31.23\pm 3.21$  years with a mean BMI of  $25.2\pm 3.2\text{kg/sq.m}$ . Age, duration of infertility, previous IVF attempts all showed a correlation with negative outcome. A BMI consistent with being overweight (BMI 25-29.9 kg/m<sup>2</sup>) and obese (BMI  $>30$  kg/m<sup>2</sup>) was associated with a lower pregnancy rate compared with women of a BMI of 19 - 24.9(Implantation rate- 23%). A comparison of the physical activity variables among the pregnant and non-pregnant groups yielded no significant differences among them in logistic regression analysis. There was a significant association between plant based diet and inclusion of fresh fruits to successful outcome ( $P=0.043$ ). All women responded that they had received adequate emotional/psychological support and there was no statistical differences between two groups ( $P=0.521$ ).

**CONCLUSIONS:** Women had a tendency to limit physical activity levels post embryo transfer and bed rest has no correlation with ART success and there is a clinical need to emphasize that prolonged bed rest following ET is not necessary. Women maintained a plant based diet showed an association to positive pregnancy outcome. A structured counselling to facilitate lifestyle changes may optimise reproductive performance and improve their chance of success.

**P-692** Wednesday, October 16, 2019 6:30 AM

#### **FAMILY-BUILDING AFTER CANCER: UNDERSTANDING PATIENT SUPPORT NEEDS, PREFERENCES FOR SUPPORT, AND RECOMMENDATIONS FOR CARE.**



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**OBJECTIVE:** Fertility is one of the most distressing issues for adolescent and young adult female (AYA-F) cancer survivors. Family-building often requires reproductive medicine, with associated challenges (e.g., high cost). This study examined AYA-F survivors' fertility experiences and perceptions of care related to family-building after cancer treatment.

**DESIGN:** Semi-structured interviews (45-60 minutes) were conducted with AYA-F cancer survivors (N=25) exploring themes related to fertility and family-building.

**MATERIALS AND METHODS:** Coding categories were derived based on the Ottawa Decision Support Framework and augmented by grounded theory. The coding team (n=3) completed an iterative process of coding and review, resulting in adequate inter-rater reliability ( $\alpha>.7$ ).

**RESULTS:** Participants averaged 29 years old (SD=6.2; range, 15-39) and were primarily White and well educated; 32% had undergone fertility preservation. Three main themes were identified: Unmet Needs, Preferences for Support Delivery, and Recommendations for Providing Support. Multiple areas of unmet needs were discussed, including lack of information about fertility and family-building options (36%), psychological support (16%), and logistical help navigating access to care and resources (32%). AYA-Fs believed the best way to learn about resources was through online platforms (72%) or doctor-initiated discussions during clinic visits (40%). Their preferred format for receiving in-depth information and counseling was through face-to-face interactions (80%). Thus, a combined approach was preferred such that information (via web-based communication) should be provided first, with follow-up in-person visits and referral to fertility specialists available when needed. AYA-Fs wanted providers to communicate with more empathy, spend more time discussing fertility and family-building, and initiate honest, open dialogues that could continue throughout care (40%). They also wanted to be referred to trusted resources tailored to their age group for informational and emotional support (36%). Specific recommendations to address family-building costs and financial planning were identified (16%).

**CONCLUSIONS:** Informational and psychological support services are needed to better educate patients about gonadotoxic effects and options to have children after cancer and address the emotional burden. Future work should evaluate how multidisciplinary care between cancer and reproductive medicine may inform the development of interactive web-based patient resources, coupled with in-person supportive interventions, and referrals.

**SUPPORT:** This research was supported by a grant from the National Cancer Institute (NCI; R03CA212924-02, PI: Benedict).

**INSIGHTS FROM WOMEN WITH FERTILITY CONCERNS ABOUT THEIR CHOICES WHEN ATTEMPTING TO IMPROVE THEIR ABILITY TO CONCEIVE.**

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**OBJECTIVE:** The goal of this survey-based research was to gather additional information from women who have been actively trying to conceive, on barriers to access of fertility-related treatment and perception of value of such services.

**DESIGN:** Three online surveys of women who were trying to conceive and voluntarily responded to a request for participation.

**MATERIALS AND METHODS:** 330 women ages 18-44 completed the first questionnaire on their overall feelings towards fertility. 132 unique women completed the second questionnaire on emotional state. 93 unique respondents answered questions regarding their interest in various fertility related services and sources of information.

**RESULTS:** 65% (214/330) had been trying less than seven months, 17% (55/330) 7-12 months, and 18% (61/330) more than a year. 54% (127/236) had not yet seen a physician in relation to fertility concerns. The two most common reasons for not seeing a physician were 'feeling they could get pregnant on their own' (42%; 96/230) and 'wanting to try a more natural approach' (23%; 53/230). 80% (180/224) believed that their emotions could have an impact on their fertility. When asked about most helpful fertility-related services, which could be made available to them, access to certified reproductive experts (39%; 36/92) was followed by nutrition-related services (23%; 21/93).

**CONCLUSIONS:** Most of the research available to fertility specialists is conducted on women already seeking consultation. A significant number of women not yet under fertility treatment prefer to seek out natural means of conception and believe in the importance of their emotional state in improving their chances of conception. Educating women about real options to increase chances of conception should be a priority.

**SUPPORT:** This research was sponsored by Nestle Healthcare Nutrition.

**ASSOCIATIONS BETWEEN PSYCHOLOGICAL STATUS AND SEMEN QUALITY PARAMETERS AMONG MALE PARTNERS OF COUPLES ATTEMPTING FERTILITY.**

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**OBJECTIVE:** To study associations of semen quality parameters with psychological status including depression, stress and anxiety.

**DESIGN:** A cross-sectional single-center study.

**MATERIALS AND METHODS:** A total of 412 men attending fertility center from 2017 to 2018 were investigated. Participants completed a questionnaire on lifestyle factors, self-rating depression scale, self-rating anxiety scale and perceived stress scale. Semen samples were collected to test semen volume, sperm concentration, progressive motility rate, vitality, normal forms rate.

**RESULTS:** Men with depression symptoms were detected to have lower sperm vitality( $p=0.026$ ) and progressive motility rate( $p=0.01$ ). Higher anxiety scores were accompanied with decreased sperm vitality( $p=0.03$ ). While no significant associations between self-reported stress and semen parameters were found.

**CONCLUSIONS:** Depression and anxiety are associated with lower levels of semen quality, which may lead to infertility of men.

**EFFECT OF PHYSICIANS' PERSONAL REPRODUCTIVE EXPERIENCES ON COUNSELING INFERTILITY PATIENTS.**

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**OBJECTIVE:** Physicians' personal medical experiences have been shown to affect patient counseling and management. Our objective was to assess

whether reproductive endocrinologists' personal experience with infertility and multiple gestation correlate with clinical counseling and treatment recommendations.

**DESIGN:** A web-based cross-sectional survey.

**MATERIALS AND METHODS:** An anonymous survey was emailed to Society for Reproductive Endocrinology and Infertility members, regarding personal and close contacts' experience with infertility and multiple gestation, and factors influencing embryo transfer (ET) number and twin risk counseling. Responses were compared using Fisher exact and Mann-Whitney U tests as appropriate, with significance at  $p < 0.05$ .

**RESULTS:** Responses were provided by 109 physicians, who were 51% female, 85% white and 56% age 50 years or older. Most (91%) reported being parents, and 28% had a personal history of infertility. Among respondents, 12% reported they or their partners had conceived multiples (83% using assisted reproduction), and half had family or close friends with multiples. Physicians with a history of infertility regularly shared their experiences with multiples more often than those without infertility (50% vs. 16%,  $p=0.01$ ). Twins were considered an adverse outcome by 86%, regardless of their reproductive experiences. When counseling about multiples, physicians rated their concern for preterm birth and neonatal morbidity highest (mean 4.9 on a scale of 1 [not at all] to 5 [to a large extent]); familial stress and maternal mental health were rated lowest (3.6). Incidence of preterm birth in twins was underestimated by 34% of physicians, and 44% underestimated twin infant mortality, irrespective of personal or close contacts' multiple gestations (including preterm deliveries). Most (79%) "encourage SET whenever possible." In deciding ET number, avoidance of multiples and patients' obstetrical history were rated highest (mean 4.4) while self-pay status (2.5) and body mass index (BMI, 2.3) were rated lowest. These ratings did not vary by reproductive history, though physicians reporting strong influence of patient BMI on ET number had significantly lower BMI than those reporting little to no effect (22.8 vs. 25.0 kg/m<sup>2</sup>,  $p=0.05$ ).

**CONCLUSIONS:** There is a high incidence of infertility diagnoses (28%) and multiple gestation (12%) among reproductive endocrinologists. Physicians reported strongly advocating for SET to reduce risk of multiples, which were widely considered an adverse outcome, independent of personal experience with infertility or multiple gestation. This is despite at least one-third of respondents underestimating twin morbidity or mortality.

**AN ANALYSIS OF THE CORRELATION BETWEEN MARITAL ADJUSTMENT TEST AND SELF-ESTEEM AND DEPRESSION IN CHINESE INFERTILE WOMEN.**

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**OBJECTIVE:** To investigate the status of marital adjustment test and self-esteem and depression in the Chinese infertile women and to explore the mediating effect of self-esteem on the relationship between marital adjustment test and depression.

**DESIGN:** A cross-sectional descriptive study was conducted with 244 Chinese infertile women from February 2019 to April 2019 in our reproductive center. All included women had voluntarily participated in this self-evaluation and the informed consents were obtained from all participants.

**MATERIALS AND METHODS:** This study was conducted at the Reproductive and Genetic Hospital of CITIC-Xiangya (Changsha, China). The 244 Chinese infertile women who had completed 4 questionnaires were recruited by simple random sampling. The questionnaires included (a) General information (made by the author), (b) Locke-Wallace marital adjustment Test (MAT), (c) Self-Esteem Scale (SES) and (d) Center for Epidemiological Studies Depression Scale (CES-D). Pearson's correlation was used to investigate the relationship. Structural equation model (SEM) was constructed by AMOS 22.0.

**RESULTS:** The mean scores of these Chinese infertile women were  $101.42 \pm 20.76$ ,  $28.24 \pm 4.96$  and  $17.82 \pm 10.47$  based on MAT, SES and CES-D, respectively. The total mean score of CES-D was found to have a significant negative correlation with the score of SES ( $r=-0.609$ ,  $p < 0.01$ ) and MAT ( $r=-0.548$ ,  $p < 0.01$ ). While the total mean score of MAT was found to have a significant positive correlation with the score of SES ( $r=0.441$ ,  $p < 0.01$ ). SEM indicated that marital adjustment test had significant direct effects on self-esteem ( $\beta=0.44$ ,  $P < 0.01$ ) and depression ( $\beta=-0.35$ ,  $P < 0.01$ ); self-esteem had a significant direct effect on marital adjustment test ( $\beta=-0.46$ ,  $P < 0.01$ ). Furthermore, self-esteem partially mediated the relationship between marital adjustment test and depression.

**CONCLUSIONS:** The depression level of infertile women was higher, and was negatively correlated with marital adjustment test and self-esteem. The mediating role of self-esteem may provide a potential mechanism for exploring the relationship between marital adjustment test and depression. These results suggested that medical staff should pay attention to the patients' depression, especially to the infertile women with marital disorders and low level of self-esteem. And then, humanistic care which helps the patients to vent their inner depression in a variety of ways could be implemented during treatment. In addition, we should actively carry out health education for infertile women and their families, so that they could have an enhanced understanding of reproductive knowledge, and gradually improved psychosociality and coping ability to deal with various problems arising in the course of treatment.

**P-697** Wednesday, October 16, 2019 6:30 AM

#### **DOES DURATION OF INFERTILITY AFFECT THE MALE'S FERTILITY QUALITY OF LIFE?**

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**OBJECTIVE:** An infertility diagnosis can confer a significant amount of personal stress and strain on a couple. There is limited data on how infertility affects quality of Life (QoL) and the male's perspective on his relationship with his partner. The purpose of this study was to determine if duration of infertility affects males' QoL and partner intimacy.

**DESIGN:** Cross-sectional survey study.

**MATERIALS AND METHODS:** 2 anonymous paper surveys were given to 70 male partners after providing a semen sample for assisted reproductive technology at a fertility clinic. Men were alone during this time. 1<sup>st</sup>, a questionnaire assessed demographics, motivating factors, and fertility history. 2<sup>nd</sup>, the fertility quality of life (FertiQoL) survey assessed the impact of infertility in diverse life areas. The FertiQoL contained 2 QoL domains: Core and Treatment. The Core consisted of 4 subscales, each focusing on psychosocial QoL factors. Treatment consisted of 2 subscales: environment and tolerability (of treatment). Responses were scaled 0-100; a higher score indicated higher QoL. Eligible respondents included men receiving treatment as part of an infertile couple. Responses were analyzed via descriptive statistics, chi-square analysis, and multivariable regression analysis.

**RESULTS:** Out of 70 anonymous surveys, 61 (87.1%) and 52 (74.3%) completed the 1<sup>st</sup> and 2<sup>nd</sup> surveys in their entirety. 62 (88.6%) men were married, 51 (75.0%) did not have prior children, and 19 (27.9%) reported prior in vitro fertilization (IVF). 23 (33.8%) men had been trying to conceive for <12 months, 20 (29.4%) for 12-24 months, and 25 (36.8%) for >24 months.

Mean FertiQoL scores for all men were: Overall 78.9 +/- 9.9, Core 79.0 +/- 9.6, and Treatment 78.5 +/- 14.5. For infertility duration <12 months, scores were 81.8 +/- 6.5, 83.1 +/- 6.4, and 78.7 +/- 13.9, respectively. For infertility duration 12-24 months, scores were 79.7 +/- 11.0, 79.6 +/- 10.8, and 79.5 +/- 15.4, respectively. For infertility duration >24 months, scores were 76.1 +/- 10.8, 75.5 +/- 9.9, and 77.4 +/- 14.9, respectively. There were no significant differences between overall or domain scores and the duration of infertility groups (p>0.05). However, there was a downward trend in scores the longer the couple was trying to conceive, and duration of infertility >24 months was significantly related to one's Relational score (subscale of the Core domain, p=0.019).

**CONCLUSIONS:** For most FertiQoL domains, duration of infertility did not affect scores. This is reassuring that longer durations of infertility do not seem to impact male QoL. Infertility for at least a 2-year period did affect the Relational score, which may suggest that the longer durations of infertility >2 years may cause stress on a couple's intimate relationship. Awareness of how infertility affects the male partner's quality of life and relationship with his partner provides an opportunity to enhance our care of the infertile couple.

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#### **PREVALENCE OF INFERTILITY TREATMENT IN PATIENTS WITH VULVODYNIA.**

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**OBJECTIVE:** There is a paucity of data on the fertility desires of patients with vulvodynia, and if pain associated with vaginal intercourse limits these patients' reproductive goals. Thus, the objective of this study was to determine the prevalence of infertility treatment in patients with vulvodynia. We hypothesized that patients with vulvodynia would have higher rates of infertility treatment compared to the general public and that, of those seeking infertility treatment, uptake of intrauterine insemination (IUI) would be higher compared to uptake of oral or gonadotropin cycles requiring vaginal intercourse, given the potential barrier of painful intercourse.

**DESIGN:** Retrospective chart review.

**MATERIALS AND METHODS:** Self-administered questionnaires detailing symptom history, vulvar pain characteristics, pregnancy desires, and history of infertility treatment, were completed by patients seeking evaluation at an ambulatory vulvar disorders clinic from 1996 to 2018. Patients with diagnoses other than vulvodynia were excluded. Primary outcome was prevalence of infertility treatment, defined as use of oral induction agents, gonadotropins, IUI, in-vitro fertilization (IVF), or surgical procedures for tubal factor infertility. Secondary outcomes included desire for pregnancy and frequency of vaginal intercourse. Descriptive statistics were used to characterize these distributions. Approval of this study was granted by the Institutional Review Board.

**RESULTS:** 379 patients diagnosed with generalized or localized vulvodynia were included in this study. Mean age of patients was 40.3 years (SD 14.9). 30.3% (N=115) patients reported desiring pregnancy, and 81.7% (N=94) of their partners were in agreement with this desire. 7.65% (N=29) patients had sought or were seeking infertility treatment, compared to 12.5% reported in population prevalence studies. 35.6% (N=135) patients reported having vaginal intercourse at least once weekly, while 0.53% (N=20) patients reported never having vaginal intercourse. Of the patients who sought infertility treatment, 10 (32.0%) received oral induction agents, 3 (9.6%) utilized gonadotropins, 5 (16.0%) employed IUI, 4 (12.9%) underwent surgical procedures for tubal factor infertility, 3 (9.6%) received IVF, and 6 (19.3%) were unaware of treatment modality.

**CONCLUSIONS:** Prevalence of infertility treatment in patients with vulvodynia is similar to that of the general public. A large proportion of patients with vulvodynia desire pregnancy; these patients do not refrain from vaginal intercourse and do not have higher rates of IUI uptake compared to other treatment modalities.

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#### **SPOUSAL CONCORDANCE IN ASSISTED CONCEPTION: PROSPECTIVE COHORT STUDY OF COUPLES UNDERTAKING THEIR FIRST IVF CYCLE.**

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**OBJECTIVE:** Assortative mating and cohabitation concordance cause married/cohabiting couples to share similar traits, with spousal concordance known to contribute to cardiovascular disease and be associated with worse treatment outcomes in other medical specialties. Maternal and paternal characteristics/behaviors are known to affect both natural fertility and success of IVF treatment but the contribution of concordance is unknown. This study was designed to examine the extent to which heterosexual couples undergoing IVF are concordant with respect to baseline characteristics/behaviors and whether this impacts upon outcome?

**DESIGN:** Prospective cohort study of consecutive couples undertaking their first IVF cycle.

**MATERIALS AND METHODS:** Couples were assessed prior to undertaking NHS Scotland funded IVF treatment, with assessment of demographic, anthropometric, lifestyle and medical factors. Spousal concordance was assessed by spearman correlation for continuous variables, whilst kappa analysis was employed for categorical variables, with regression modelling for their association with outcomes.

**RESULTS:** There were 306 couples with complete baseline data, of which 264 underwent fresh embryo transfer, with 125 ongoing pregnancies (47.3%). Couples were strongly concordant for age (r=0.59 p<0.000), alcohol consumption (k=0.661), educational attainment (k=0.655) and smoking status (k=0.45) but not BMI (r=0.11, p=0.44). Only exercise concordance was significantly associated with outcome, with exercise discordance a predictor of biochemical pregnancy (OR: 1.86; 95% CI 1.18-2.92 p=0.008). Furthermore, females in discordant couples were significantly less physically active than females in concordant couples (mean difference = 0.4527 times/week, p=0.003).

**CONCLUSIONS:** Couples undertaking assisted conception are concordant for many baseline characteristics, with couples with discordant exercise habits having increased rates of biochemical pregnancy. Shared education and public health initiatives to attain spousal concordance of lifestyle factors may be beneficial for overall health outcomes if they converge towards healthy behaviors, but concordance per se had limited impact on clinical ART outcomes.

**P-700** Wednesday, October 16, 2019 6:30 AM

#### **ATTITUDES TOWARDS PREGNANCY IN PATIENTS WITH VULVODYNIA.**

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**OBJECTIVE:** Qualitative studies indicate that vulvodynia affects women's reproductive desires and timing [1]. However, little is known about the prevalence of these attitudes amongst patients with vulvodynia, or the relationship between pain severity and reproductive planning. We aimed to further characterize the effects of vulvodynia on women's reproductive wishes, hypothesizing that desire for pregnancy would decrease with increasing pain score and fear of pregnancy would increase with worsening pain score.

**DESIGN:** Retrospective chart review.

**MATERIALS AND METHODS:** We retrospectively analyzed patient intake questionnaires completed prior to evaluation at an ambulatory vulvar disorders clinic from 1996 to 2018. Questions addressed symptom history, vulvar pain characteristics, and pregnancy desires. Only those diagnosed with vulvodynia in their subsequent clinic visit were included in our sample. Patients with incomplete questionnaires were excluded. Our primary outcomes were pain severity and unpleasantness scores (0-100) compared between women reporting presence versus absence of desire for pregnancy, as well as between those noting fear of pregnancy or lack thereof. Descriptive statistics and Student's t-tests were used as appropriate. This study was approved by the University of Michigan Institutional Review Board.

**RESULTS:** 424 patients diagnosed with vulvodynia (generalized or localized) were eligible for analysis. Their mean age was 40.2 years (SD 15.1); 13.2% (N=56) of them had never had a pregnancy. Nearly one third of the sample (27.8%, N=118) reported a desire for pregnancy. Of those desiring pregnancy, 63.6% (N=75/118) were having at least weekly vaginal intercourse. Mean pain intensity score among those desiring pregnancy was not different between those having and not having at least weekly intercourse (67.1 vs 71.1, p=0.38). Similarly, mean pain unpleasantness was comparable between these groups (78.1 vs 79.0, p=0.81). Of the total 424 patients, 15.1% (N=64) fear pregnancy. This fear was not associated with increased pain intensity (p=0.99) or unpleasantness scores (p=0.28).

**CONCLUSIONS:** Although vulvodynia has far-reaching effects on women's quality of life, our study suggests that women with more intense or unpleasant pain do not avoid or fear pregnancy more than those with less pain.

**Reference:** 1. Johnson, N. S., Harwood, E. M., & Nguyen, R. H. (2015). "You have to go through it and have your children": reproductive experiences among women with vulvodynia. *BMC Pregnancy & Childbirth*, 15(1), 114.

**SUPPORT:** None.

#### **NUTRITION**

**P-701** Wednesday, October 16, 2019 6:30 AM

#### **GLYCEMIC LOAD, DIETARY FIBER, AND ADDED SUGAR AND SPONTANEOUS ABORTION.**

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**OBJECTIVE:** To prospectively evaluate the association between preconception dietary factors including glycemic load (GL), dietary fiber (DF), and added sugar, and spontaneous abortion (SAB). To the authors' knowledge, there have been no studies of the association between GL and SAB.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Pregnancy Study Online is a web-based preconception cohort study of pregnancy planners in North America. At baseline, female participants completed a questionnaire providing data on demographic, lifestyle, medical, and reproductive histories. Ten days after enrollment, participants completed the National Cancer Institute's Dietary History Questionnaire II, a validated food frequency questionnaire. Participants were followed with bi-monthly questionnaires for up to 12 months or until a reported conception. Data on SAB, first positive pregnancy test date, due date, and gestational weeks at loss were ascertained from follow-up questionnaires, an early pregnancy (<12 weeks' gestation) and a late pregnancy (~32 weeks' gestation) questionnaire. We calculated GL (glycemic index times portion size); DF, soluble fiber, insoluble fiber (grams (g)/day); and added sugar (teaspoons (tsp)/day), based on reported frequencies of individual foods, standard recipes for mixed foods, and average serving size. We used Cox proportional hazards regression to estimate hazard ratios (HR) and 95% confidence intervals (CI), using gestational weeks as the time scale. We adjusted for age, body mass index (BMI), healthy eating index score (HEI-2010), energy intake, and lifestyle and demographic factors.

**RESULTS:** Of the 3,565 female participants included in this analysis, 756 (21%) had a SAB over the course of follow-up. The median gestational week at loss was 6 weeks (interquartile range: 5-9 weeks). Compared with an average daily GL  $\leq 100$ , HRs for GL of 101-114, 115-125, 126-140, and  $\geq 141$  were 0.95 (CI: 0.77-1.18), 0.80 (CI: 0.64-1.01), 0.89 (CI: 0.71-1.12), and 1.07 (CI: 0.84-1.35), respectively. Compared with daily total DF intake of  $\leq 16$  g/day, HRs for 17-20, 21-24, and  $\geq 25$  g/day were 1.00 (CI: 0.85-1.30), 1.13 (CI: 0.90-1.43), 0.83 (CI: 0.64-1.07), respectively. Relative to soluble fiber intake of  $\leq 4$  g/day, HRs for 5-6, 7-8, and  $\geq 9$  g/day were 1.04 (CI: 0.85-1.27), 1.02 (CI: 0.81-1.27), and 0.86 (CI: 0.68-1.08), respectively. Relative to insoluble fiber intake of  $\leq 10$ , HRs for 11-13, 14-17, and  $\geq 18$  g/day were 1.19 (CI: 0.96-1.48), 1.03 (CI: 0.80-1.33), and 1.02 (CI: 0.77-1.33), respectively. Compared with added sugar intake of  $\leq 6$  tps/day, HRs for 7-9, 10-12, 13-17, and  $\geq 18$  tps/day were 0.98 (CI: 0.77-1.24), 0.96 (CI: 0.75-1.22), 1.00 (CI: 0.78-1.28), and 1.04 (CI: 0.80-1.36), respectively. Results were similar for early (<8 weeks' gestation) and late ( $\geq 8$  weeks' gestation) SAB.

**CONCLUSIONS:** GL, total DF, insoluble fiber, and added sugar intakes were not appreciably associated with SAB. A slight inverse association was seen for higher intake of soluble fiber and SAB risk. Chance remains a plausible explanation of these associations.

**SUPPORT:** This research was supported by NIH/NICHD grants R01HD086742 and R21HD072326.

**P-702** Wednesday, October 16, 2019 6:30 AM

#### **HIGH-FAT DIET LEADS TO INCREASED OVARIAN LIPID DEPOSITION EVEN IN THE ABSENCE OF OBESITY.**

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**OBJECTIVE:** We have previously shown that high-fat diet (HFD) feeding leads to depletion of the ovarian reserve, subfertility, and altered expression of key ovarian genes, irrespective of the development of obesity<sup>1,2</sup>. It has been proposed that HFD-induced ovarian dysfunction is due to increased lipid accumulation in the ovary<sup>3,4</sup>, however, it is currently unknown if there is increased HFD-induced lipid accumulation in the ovary in the absence of obesity. We hypothesized that HFD exposure would increase ovarian lipid deposition regardless of the induction of obesity.

**DESIGN:** Prospective laboratory animal study.

**MATERIALS AND METHODS:** 5-week-old C57BL/6J mice were fed either a 60% HFD or standard chow (N = 15) for 10 weeks. After 10 weeks body weights were determined. Lean and fat mass was measured by quantitative MRI. HFD mice were divided into HFD-lean (HFLn, N=10) and HFD-obese (HFOb, N=9) groups based on body weight: mice < 26 g were considered HFLn and mice  $\geq 30$  g were considered HFOb. Ovaries were collected, one for qRT-PCR analysis of the lipid droplet protein *plin2* and the other for immunohistochemical analysis of lipid droplets via anti-*plin2* staining. A one-way ANOVA (Tukey's post hoc) was used for statistical analysis.

**RESULTS:** After 10 weeks of diet HFOb mice weighed more and had a higher percentage of body fat ( $33.2 \pm 0.9$  g,  $37.2 \pm 1.2\%$ , respectively) than both HFLn mice ( $23.5 \pm 0.6$ g,  $17.7 \pm 1.5\%$ , respectively) and chow controls ( $21.6 \pm 0.3$ g,  $11.2 \pm 0.6\%$ , respectively) (p<0.0001). Ovarian expression of *plin2* was dramatically increased after HFD, with a 5-fold increase observed in HFLn mice and a 4.7-fold increase observed in HFOb mice (p

= 0.01) compared to chow-fed controls. Immunohistochemical analysis of *plin2* in the ovary confirmed our qRT-PCR data showing dramatically increased levels of *plin2* in the ovaries of both the HFLn and HFob mice. In particular, increased lipid deposition was observed in the corpora lutea, ovarian stroma, and outside of the theca cells of large follicles, and to a far lesser extent in the granulosa cells.

**CONCLUSIONS:** HFD exposure leads to increased ovarian lipid deposition regardless of the development of obesity in mice. This phenomenon may be responsible for the ovarian dysfunction and reproductive defects observed with HFD exposure, even in the absence of obesity. The presence of increased lipid droplets in the ovaries of lean mice fed a HFD provides further evidence that even if a lean phenotype is maintained, consumption of HFDs will still negatively impact the ovary. More research is needed to determine the exact mechanism by which excessive ovarian lipid accumulation is responsible for the HFD-induced reproductive dysfunction.

Reference: 1) Skaznik-Wikiel et al. *Biol Reprod* 2016; 94(5):108. 2) Hoshos et al. *Mol Cell Endocrinol*. 2018; 470:199. 3) Wu et al. *Endocrinology* 2010; 151(11):5438. 4) Reynolds et al. *Reprod Fertil Dev*. 2015; 27(4):716.

**SUPPORT:** American Society for Reproductive Medicine Research Grant to M.E.S.-W.

**P-703** Wednesday, October 16, 2019 6:30 AM

### **MATERNAL SERUM VITAMIN D LEVELS CORRELATE NEGATIVELY WITH THE LENGTH OF INTERBIRTH INTERVALS IN RHESUS MACAQUES.**



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**OBJECTIVE:** This study was to investigate the association between maternal vitamin D status and outcomes of spontaneous pregnancies in a nonhuman primate model.

**DESIGN:** Healthy rhesus macaques (*Macaca mulatta*) in the breeding groups, which had never participated in other research projects, were studied.

**MATERIALS AND METHODS:** Adult female macaques (n = 53; 5-13 years old; relative to 18-35 year old women) were housed in outdoor shelters at a national research facility. Diet consists of monkey chow containing 6.6 IU/g vitamin D3, fruit, vegetables and water ad libitum. Blood samples were collected, and body weight measured, during June-July (summer; non-breeding season) and September-October (fall; beginning of the breeding season) for all animals within the same year. Animals were not pregnant or nursing at the time of or between the two blood collections. Serum 25-hydroxyvitamin D3 (VD) concentrations were assessed by electrochemiluminescence assay. Two successive births resulted from spontaneous pregnancies, one prior to and one post blood sample collections, were recorded. The length of time between the two births was termed as interbirth interval (IBI). The offspring birth weight was measured. In a multivariable setting, partial correlation was used to analyze the relationship between serum VD concentrations (average of two measurements) and pregnancy outcomes which were normalized by the maternal age and body weight (average of two measurements).

**RESULTS:** Two distinct cohorts of animals were identified based on their IBIs. While the majority of animals (n = 44) had IBIs less than 16 months (13.3 ± 0.2 months), which was common for rhesus macaques, IBIs of 9 animals were greater than 20 months (24.1 ± 0.8 months; p < 0.001). After being normalized by animal age and body weight, serum VD concentrations of animals in the short IBI group (166 ± 6 ng/ml) were higher (p < 0.05) than those of the long IBI group (143 ± 8 ng/ml). The offspring birth weight values were comparable (p > 0.05) between animals in the short (0.51 ± 0.01 kg) and long IBI groups (0.46 ± 0.05 kg). For animals with short IBIs, the serum VD concentrations correlated negatively with the length of their IBIs when animal age and body weight were taken into account (r = -0.48; p < 0.01). There was no significant correlation between maternal serum VD concentrations and the offspring birth weight (r = -0.24; p > 0.05).

**CONCLUSIONS:** These data demonstrate a relationship between maternal vitamin D status and pregnancy outcomes. Macaques with relatively low serum vitamin D levels require more time to achieve spontaneous pregnancy, though the offspring birth weight does not appear to be affected by maternal serum vitamin D levels. The findings are consistent with previous observations of improved ovulation and menstrual cyclicity via vitamin D supplementation in patients with polycystic ovary syndrome.

**SUPPORT:** The study was supported by NIH OD P51OD011092.

**P-704** Wednesday, October 16, 2019 6:30 AM

### **HIGH-AGE DIET CAUSES ESTROUS CYCLE IRREGULARITIES AND OVARIAN FUNCTION CHANGES IN MICE.**

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**OBJECTIVE:** Advanced glycation end products (AGEs) are highly reactive pro-inflammatory molecules that are formed following the heating process of several diets. Following their ingestion, these AGEs get absorbed and could cause several organs' dysfunction, including the female reproductive system. Using human granulosa cell model, it was previously shown that AGEs *in vitro* induce changes in genes important in steroidogenesis and follicular development, potentially leading to ovarian dysfunction. This study aimed at studying the effects of dietary AGEs *in vivo* on female reproductive function.

**DESIGN:** *In vivo* experiments using mouse model.

**MATERIALS AND METHODS:** 6 week-old C57Bl/6 female mice were fed for 9-10 weeks either Low-AGE (AIN-93-G, 3.84 kcal/gram, 64.6% carbohydrate, 18.8% protein, 16.6% fat) (n=5) or High-AGE diet (n=5). The High-AGE diet was prepared by heating up the Low-AGE diet at 120° C for 30 min. Mice were weighed weekly and vaginal smears were performed for 2 weeks after 8 weeks of feeding (at approximately 14 weeks of age). Mice were then superovulated with exogenous gonadotropins and were sacrificed 16 h after hCG, then oviducts were harvested, and oocytes that were deposited into the oviducts were quantified. The oocytes present within the oviducts were counted by an individual blinded to diet status. The ovaries were harvested and subjected to RT-PCR for genes important in steroidogenesis and folliculogenesis. Data are presented as mean ± SEM. Mann-Whitney U test was performed for comparison. To confirm that the conditions used to prepare the High-AGE diet indeed increased the AGE content (in particular CML [N-carboxymethyl-lysine]), an ELISA kit for CML was used on the diet itself before and after heating.

**RESULTS:** CML levels in the High-AGE diet were 10 times higher compared to levels in Low-AGE diet (60.5 ug/mL vs. 6.2 ug/mL; respectively) indicating that the heating process increased AGEs' levels. Although there was no difference in weight or the number of oocytes deposited between both groups (19.2 ± 5.4 vs. 24.0 ± 5.1, respectively; p=0.5), mice on High-AGE diet spent significantly more time in the diestrus phase compared to mice on Low-AGE diet (4.3 ± 0.3 vs. 3.2 ± 0.4, respectively; p=0.04). RT-PCR data on ovarian tissue showed that *fshr* mRNA expression level was 100% higher (p=0.049) and *gdf-9* mRNA expression level was 40% higher (p=0.046) in the High-AGE diet group compared to the Low-AGE diet group.

**CONCLUSIONS:** These results indicate that heating diet at high temperatures increases AGEs' content in the food and could alter both estrous cyclicity and ovarian folliculogenesis/steroidogenesis. Future studies should adjust for macro- and micro-nutrients that could change due to the heating process of diet, and which represent a confounding variable for studies using High-AGE diet in female reproduction.

2) Grant from Society for Reproductive Investigation (SRI)

References: 1) Uribarri J, et al. (2005) Diet-derived advanced glycation end products are major contributors to the body's AGE pool and induce inflammation in healthy subjects. *Ann N Y Acad Sci*. 1043:461-466.

2) Kandaraki E, et al. (2018) Advanced glycation end products interfere in luteinizing hormone and follicle stimulating hormone signaling in human granulosa KGN cells. *Exp. Biol. Med*. 243, 29-33.

3) Merhi Z, et al. (2018) Advanced glycation end products alter steroidogenic gene expression by granulosa cells: an effect partially reversible by vitamin D. *Mol Hum Reprod*. 24:318-326.

**SUPPORT:** 1) Grant from American Society for Reproductive Medicine (ASRM).

### **OVARIAN RESERVE**

**P-705** Wednesday, October 16, 2019 6:30 AM

### **PREGNANCY OUTCOMES FOLLOWING INTRAUTERINE INSEMINATION (IUI) IN YOUNG WOMEN WITH DECREASED OVARIAN RESERVE.**

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Linnea R. Goodman, MD.<sup>c</sup> <sup>a</sup>Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA; <sup>b</sup>IVI-RMA New Jersey, Basking Ridge, NJ; <sup>c</sup>University of North Carolina, Raleigh, NC.

**OBJECTIVE:** To evaluate pregnancy outcomes following IUI in young women with low ovarian reserve compared to age-matched controls.

**DESIGN:** Retrospective cohort study

**MATERIALS AND METHODS:** Patients aged <35 years undergoing their first IUI cycle with a documented anti-mullerian hormone (AMH) level at a single large IVF center between 1999 and 2018 were included. All patients had evidence of patent fallopian tubes and severe male factor infertility (total motile sperm count < 10 million on IUI) was excluded. Patients with AMH <1.0 ng/mL were compared to those with levels >1.0 ng/mL. The primary outcome was positive pregnancy test. Secondary outcomes included live birth, biochemical loss, clinical miscarriage (loss after visualized gestational sac) and ectopic pregnancy. Student's *t*-tests and chi square testing were used where appropriate.

**RESULTS:** There were 3438 patients included: 428 with AMH <1.0 ng/mL and 3010 with AMH >1.0 ng/mL. Mean AMH values were 0.63 ± 0.26 vs 5.7 ± 5.6 ng/mL, respectively. Mean antral follicle count was 10.6 ± 5.2 vs 22.9 ± 12.7. There were no differences in age (31.6 ± 2.4 vs 30.6 ± 2.7 years), body mass index (26.0 ± 6.4 vs 26.3 ± 6.5 kg/m<sup>2</sup>) or infertility diagnosis (54% vs 51% with unexplained or ovulatory dysfunction excluding polycystic ovarian syndrome) between patients with decreased ovarian reserve compared to those without. Reproductive outcomes are depicted in Table 1.

TABLE 1. Intrauterine insemination (IUI) outcomes of 3438 patients under the age of 35 years stratified by anti-mullerian hormone (AMH) level of < 1.0 ng/mL (n=428) and > 1.0 ng/mL (n=3010).

Outcome % (n)	AMH < 1.0 ng/mL	AMH ≥ 1.0 ng/mL	<i>p</i>
Positive Pregnancy Test	32.0% (137)	37.8% (1139)	<b>0.02</b>
Biochemical Loss	10.3% (44)	15.1% (454)	<b>0.006</b>
Clinical Loss	4.2% (18)	5.6% (168)	0.24
Ectopic	0.5% (2)	0.7% (20)	0.69
Live birth	17.1% (73)	16.5% (497)	0.77

**CONCLUSIONS:** Young patients (<35 years) with decreased ovarian reserve conceived less often after IUI as compared with age-matched controls. However, once pregnant, such patients had fewer biochemical losses and similar live birth and clinical miscarriage rates as compared to controls. These data imply a quantitative, not qualitative, distinction between groups. Future prospective studies carefully controlling for infertility diagnosis are required to confirm these relationships.

**SUPPORT:** None.

**P-706** Wednesday, October 16, 2019 6:30 AM

### THE RATE OF ANTRAL FOLLICLE COUNT DECLINE DECREASES WITH OLDER AGE AND LOWER ANTRAL FOLLICLE COUNT.

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**OBJECTIVE:** There is a paucity of longitudinal data examining the rate of antral follicle count (AFC) decline. We aimed to assess the correlation between baseline age and baseline AFC, and the rate of AFC loss over repeated measures in healthy, reproductive-aged women.

**DESIGN:** Prospective Cohort Study.

**MATERIALS AND METHODS:** 256 women aged 25 to 45 with regular menstrual cycles lasting between 25-35 days enrolled in the Ovarian Aging (OVA) study between 2006 and 2010 were included. A baseline AFC was obtained during the initial study period and a follow-up AFC was obtained an average of 3.9 years later for each individual. Given the multilevel longitudinal data, mixed models with random intercepts and random slopes were used in order to account for the varying baseline ages and AFCs, and differing AFC decline rates between individuals. The models were adjusted for smoking status, oral contraceptive use and baseline age where appropriate.

**RESULTS:** The mean baseline AFC for the age groups 25-29, 30-34, 35-39 and 40-45 was 21.8 (SD 9.7), 17.8 (SD 9.5), 8.4 (SD 5.4) and 3.9 (SD 3.5), respectively. The rate of AFC decline was found to gradually accelerate with increasing baseline age until age 40 with the highest

TABLE. Yearly AFC Change by Baseline Age and AFC Groups

Baseline Age Group (Years)	N	Yearly AFC Change	SE	95% CI
25-29	53	-0.75	0.24	-1.22 to -0.29
30-34	74	-1.54	0.19	-1.92 to -1.16
35-39	72	-1.74	0.20	-2.12 to -1.35
40-45	66	-1.31	0.21	-1.72 to -0.90
Baseline AFC Group (Follicles)				
40+	5	-6.79	0.59	-7.95 to -5.63
30-39	18	-2.84	0.31	-3.51 to -2.28
20-29	41	-1.57	0.20	-1.97 to -1.17
10-19	113	-1.36	0.13	-1.62 to -1.10
0-9	88	-0.67	0.15	-0.97 to -0.38

rate of AFC loss seen in the 35-39 age group. After age 40, there was a deceleration in the AFC loss rate compared to the 35-39 age group (-1.3 vs. -1.7 follicles per year, *p*<0.001). The rate of AFC decline was also found to gradually decelerate in women with lower baseline AFC, with the lowest AFC group of 0-9 follicles experiencing the slowest rate of loss of -0.7 follicles per year compared to all other AFC groups including the 10-19 follicle group with a rate of loss of -1.4 follicles per year (*p*<0.001) (Table).

**CONCLUSIONS:** In healthy, non-infertile women with regular menstrual cycles, our longitudinal data suggest that older women start with fewer follicles at baseline and experience follicle loss at a slower rate after the age of 40. In addition, women with lower baseline follicle counts, irrespective of age, experience slower declines than women with higher baseline follicle counts. These findings perhaps indicate a compensatory mechanism to preserve ovarian function in women of older age, or lower ovarian reserve, toward the end of their reproductive lifespan.

**SUPPORT:** Grant Support: R01 HD044876; 1R01AG053332-01A1.

**P-707** Wednesday, October 16, 2019 6:30 AM

### EXOME SEQUENCING REVEALED SIGNIFICANT DELETERIOUS DNA VARIANTS ASSOCIATED WITH PREMATURE DIMINISHED OVARIAN RESERVE.

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**OBJECTIVE:** Diminished ovarian reserve (DOR) is a condition where the number or quality of oocytes is compromised, significantly impacting a woman's reproductive potential. While this is common as women age, about 10% of younger women will be impacted by premature DOR, resulting in poor fertility outcomes. With the causes of early-onset DOR being largely unknown, the objective of this study was to utilize exome sequencing to investigate the underlying molecular mechanisms associated with DOR in young women (≤ 31 years).

**DESIGN:** Research study.

**MATERIALS AND METHODS:** Whole peripheral blood was collected from IRB consented female patients and donated to research: young, fertile, oocyte donor controls (CONT ≤ 31 years; n=11), and age-matched young women presenting with diminished ovarian reserve (DOR ≤ 31 years; n=11). DNA was isolated using the QIAamp DNA Mini kit (Qiagen). Exome sequencing libraries were prepared using SureSelect<sup>XT</sup> (Agilent) and sequenced on the Illumina NovaSeq 6000. Sequences were processed using the GATK4 Best Practices exome analysis pipeline. Functional and rare variants found exclusively in DOR samples were evaluated for pathogenicity and corresponding genes were tested for pathway enrichment using Ingenuity Pathway Analysis (Qiagen). Sequencing validation was performed using qPCR with Taqman SNP Genotyping Assays (Applied Biosystems).

**RESULTS:** Exome sequencing revealed 730 significant DNA variants across the genome that were observed exclusively in the young DOR sample set (*P*<0.01). Bioinformatic analysis revealed the top significantly enriched signaling pathways associated with young DOR: Glucocorticoid receptor (GR) and Notch (*P*<0.01). The GR signaling pathway had 32 deleterious DNA variants within 16 different genes, all of which would significantly affect protein function (*P*<0.01).

Each young DOR patient had an average of 2.9 different deleterious DNA variants impacting the GR signaling pathway. Glucocorticoid receptors are crucial for the establishment and maintenance of reproductive function and stress response, influencing oocyte maturation and developmental potential. The Notch pathway had 5 missense DNA variants observed in young DOR patients ( $P < 0.01$ ), which could be responsible for abnormal folliculogenesis and affect meiotic spindle assembly. To date, DNA variant validation has been performed on 5 genes in the GR signaling pathway, including AGT which has been implicated in reduced ovulatory capacity, and KRT19, involved in proliferation of the surface epithelium during ovarian development.

**CONCLUSIONS:** Exome investigation of young DOR patients revealed significant deleterious DNA variants in genes crucial to ovarian function, folliculogenesis and oocyte maturation. The combination of these adverse hits across key signaling pathways would impact the reproductive stress response, growth and maturation of ovarian follicles, as well as downstream oocyte quality. Identifying the underlying molecular mechanisms responsible for premature DOR could lead to preventative treatments that slow the process of early ovarian aging.

**SUPPORT:** None.

**P-708** Wednesday, October 16, 2019 6:30 AM

### INTEREST OF THE USE AUTO-TRANSPLANTATION OF THE OVARIAN CORTEX AFTER DORMANT FOLLICLES IN VITRO ACTIVATION (IVA) IN PATIENTS WITH PREMATURE OVARIAN INSUFFICIENCY (POI).



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**OBJECTIVE:** In women with POI, spontaneous conception and response to ovarian stimulation are considerably limited; oocyte donation is therefore the only effective treatment. But in some social cultures, like ours, egg donation is forbidden. The aim of this study is to evaluate the efficacy of autotransplantation of the ovarian cortex after in vitro activation of dormant follicles in these patients in order to have their own genetic children.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** From September 2018 to February 2019, 19 patients with POI according to the Bologna criteria agreed to participate in this study on IVA treatment. The main characteristics of women are: age ( $35.4 \pm 2.9$  years), duration of amenorrhea: ( $2.6 \pm 1.9$  years), FSH ( $51.5 \pm 20.3$  IU/L), E2 ( $13.0 \pm 3.6$  pg/ml) and AMH: ( $0.04 \pm 0.03$  ng/ml). all women received pretreatment with an oral estrogen/progesterone (OEPP). on days 20 to 22 of this pretreatment, a first laparoscopy is performed to remove an entire ovary which will immediately be transferred to the IVF laboratory. The biologist separates the ovarian cortex from the medulla and cuts it into small cubes ( $0.5-1$  cm x  $1-2$  mm thickness). 10 to 20% of this ovarian cortex is used to histological analysis to determine the presence of residual primordial follicles (RPF), the rest is cultured for 48 hours with a PI3K stimulator and a PTEN inhibitor. A second laparoscopy is performed to auto-transplant 20 to 30 of the cultured cortex ovarian cubes, in each of the 2 peritoneal pockets created bilaterally. The OEPP is stopped and after the onset of withdrawal bleeding, we randomized 2 groups of patients: in Group1(9 women), we performed

weekly follow up in order to detect a spontaneous follicular development using ultrasound and measure of serum FSH and estradiol levels. In Group2 (10 women), ovarian stimulation is started by the daily combination of GnRH agonist and rFSH + rLH for in vitro fertilization.

**RESULTS:** On histological examination we found 13 of 19 patients, (68.4%) had RPF, 6 patients of 13 (46.15%) had follicular growth. Follicular growth was also seen in 3 of 6 patients (50%) who did not have RPF. A total 9 of 19 patients (47.4%) showed follicular development reaching the preovulatory stage, 3 in group1(33%) and 6 in group 2 (60%). In group 1, one became pregnant spontaneously (evolutive at 21 weeks). In group 2, we collected, after  $20.3 \pm 2.8$  days of stimulation, 17 oocytes (10 matures). 8 embryos were developed. Following fresh embryo transfer in 3 patients, one became pregnant (evolutive at 11 weeks). 4 embryos were frozen waiting to be transferred for the 3 other patients.

**CONCLUSIONS:** In our country oocyte donation is prohibited, it would be interesting to better develop this technique for not only women with premature ovarian insufficiency but also for those who have ovarian failure due to age in order to have legitimately their own genetic children. the presence, on histological examination of the ovaries, of residual primordial follicles, seems to be an important factor for the success; their prior quantification would help to identify the target patients.

**P-709** Wednesday, October 16, 2019 6:30 AM

### DECLINING TREND OF AMH LEVELS IN INDIAN WOMEN OF REPRODUCTIVE AGE GROUP : THE JASLOK EXPERIENCE.



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**OBJECTIVE:** To study the declining trend of AMH levels in young Indian women visiting our Fertility Center.

**DESIGN:** Retrospective case control study of AMH levels in Indian women visiting our Fertility Center.

**MATERIALS AND METHODS:** AMH (n=800) of these women was measured (ng/ml) using Electro-chemiluminescence Immuno Assay (Roche machine e601 ECLIA). Their age and AMH values were compared.

**RESULTS:** Of the 800 women (Table 1), 31 % of women in < 30 yrs of age group and 51 % of women in the 31 to 35 age group had AMH levels < 2.0 ng/ml as compared to 18 % and 35 % in the fertile control groups respectively ( $p < 0.05$ ). It is interesting to note that 13.9 % of women in < 30 years age group and 26 % of women in 31-35 year age group had AMH of < 1 ng/ml as compared to 2.6 % and 13.4 % respectively in the control group. This study indicates the troubling trends of low AMH in Indian women in the reproductive age group.

**CONCLUSIONS:** Young Indian women in their late 20's and early 30's visiting our center for infertility treatment showed a worrisome declining trend of AMH. Speculation can point towards the ubiquitous role of plastics and Endocrine Disrupting Chemicals (EDCs) that entered the Indian environment 30-35 years ago.

TABLE 1

Age (yrs)	AMH (ng/ml)												
	<=0.55	0.56-1.0	Total (<1)	1.01-1.55	1.56-2.0	Total (1-2)	Total (<2)	2.01-2.55	2.56 – 3.0	Total (2-3)	3.01-4.0	Total (2-4)	>4
<b>&lt; 30</b> n =215 (n=39)	6.0% (0%)	7.9% (2.6%)	13.9% (2.6%)	10.7% (10.3%)	6.5% (5.1%)	17.2% (15.4%)	31.1%* (18.0%)	9.8% (17.9%)	10.2% (10.2%)	20.0% (28.1%)	16.7 (17.9%)	36.7% (46.0%)	32.10% (35.90%)
<b>31-35</b> n =327 (n=52)	12.5% (1.9%)	13.5% (11.5%)	26.0% (13.4)	14.1% (15.4%)	10.7% (5.8%)	24.8% (21.2%)	50.8%* (34.6%)	11.3% (21.2%)	7% (19.2%)	18.3% (40.4%)	11.9% (13.5%)	30.2% (53.9%)	19% (11.50%)
<b>36-40</b> n =202 (n=9)	17.3% (0%)	18.3% (22.2%)	35.6% (22.2%)	18.3% (66.7%)	12.4% (0%)	30.7% (66.7%)	66.3% (88.9%)	8.4% (0%)	5.9% (0%)	14.3% (0%)	8.4% (0%)	22.7% (0%)	10.9% (11.10%)
<b>&gt; 40</b> n =56 (n=0)	44.6% (0%)	17.9% (0%)	62.5% (0%)	14.3% (0%)	12.5% (0%)	26.8% (0%)	89.3% (0%)	3.6% (0%)	1.8% (0%)	5.4% (0%)	1.8% (0%)	7.2% (0%)	3.60% (0%)
<b>Total</b> n=800 (n=100)													

(Within parenthesis is the value from fertile control group) \*p value (<0.05) from respective fertile control group

**THE EFFECT OF THE RELATIVE DEGREE OF HOW LOW IS THE SERUM ANTI-MULLERIAN HORMONE (AMH) LEVEL IN WOMEN AGED < 39 ON OUTCOME FOLLOWING IN VITRO FERTILIZATION-EMBRYO TRANSFER (IVF-ET).**



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**OBJECTIVE:** To determine the relative adverse effect of decreasing levels of AMH on IVF-ET in women aged ≤39 with diminished oocyte reserve.

**DESIGN:** Prospective observational study.

**MATERIALS AND METHODS:** Women with diminished oocyte reserve, as evidenced by a serum AMH <1 ng/mL, who were undergoing IVF-ET during a finite time period, were divided into 4 subcategories: grp 1 – AMH ≤0.39, grp 2 – AMH 0.40 to 0.59, grp 3 – AMH 0.60 to 0.79, and grp 4 – AMH 0.80 to 0.99 ng/mL. Only mild gonadotropin stimulation was used. All transfers were on day 3. A couple was included only one time. Power analysis suggested a study group size of 75 patients having oocyte retrieval with marked DOR (AMH <0.39) and 75 with AMH 0.4 to 0.99 considering that the very low AMH group would be less likely to have oocyte retrieval result in embryo transfer.

**RESULTS:** Pregnancy rates following IVF-ET (day 3 transfers) according to serum AMH levels in women aged ≤39 with diminished oocyte reserve are seen in the table below.

	Group 1	Group 2	Group 3	Group 4
AMH Levels (ng/mL)	<0.39	0.40-0.59	0.60-0.79	0.80-0.99
# Retrievals	73	27	24	20
# Transfers	36	18	17	14
Average age	36.0	38.4	35.9	36
% Clinical/pregnancies/transfer	11.1%	27.8%	47.1%	21.4%
% Delivered	5.6%	16.7%	17.6%	21.4%
Avg. no. embryos transferred	1.47	1.7	1.9	1.6
Implantation rate	6.7%	22.6%	27.3%	26.1%

Live deliveries are possible even in women aged ≤39 with the lowest serum AMH levels (≤0.39 ng/mL). Overall, the live delivered pregnancy rate following day 3 embryo transfer was 13%/transfer (11/85) in women with diminished oocyte reserve as evidenced by serum AMH <1 ng/mL. Overall, oocyte retrieval led to an embryo transfer 53% of the time. Even grp 1 (AMH <.39) had an embryo transfer 50% of the time and had 1.7 embryos transferred. Excluding grp 1, the live delivered pregnancy rates for groups 2-4 was 18.3% (9/49).

**CONCLUSIONS:** Knowledge of the likelihood of success based on the degree of oocyte deficiency can help a couple aged ≤39 with diminished oocyte reserve to decide to try IVF with their own oocytes or choose donor oocytes. Comparing a 5.6% live delivered pregnancy rate in grp 1 vs. 18.3% for groups 2-4, with the same average number of embryos transferred, it would seem that oocyte quality is markedly reduced with serum AMH extremely low, but otherwise oocyte quality is only mildly to moderately compromised with serum AMH levels of 0.4 to 0.99 ng/mL.

**SHOULD ANTI-MÜLLERIAN HORMONE BE TESTED FOR PATIENTS UNDERGOING INTRAUTERINE INSEMINATION?**



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**OBJECTIVE:** Anti-Müllerian hormone (AMH) has been proven to be a useful biomarker to predict the ovarian response to controlled ovarian stimulation (COS) for in vitro fertilization (IVF). However, there is a lack of evidence for the role of testing ovarian reserve markers in couples undergoing infertility treatment that does not include controlled ovarian stimulation. Despite this lack of evidence of utility, AMH is often tested and even pro-

moted as a potential marker of reproductive potential. The aim of this study was to determine if AMH is predictive of pregnancy following ovulation induction and intrauterine insemination for unexplained infertility.

**DESIGN:** A retrospective cohort study at our institution analyzed the association of AMH levels with pregnancy rates following intrauterine insemination (IUI) from November 2017 to November 2018.

**MATERIALS AND METHODS:** Data collected included female age, AMH, IUI attempt number, oral ovulation induction medication used, pre- and post-wash semen analysis parameters, and pregnancy rate. All unexplained infertility patients with known AMH values and pregnancy outcomes were included in the study. A survival analysis was used to determine association of AMH with pregnancy outcome. Cox proportional hazards regression right-censored models were performed for pregnancy event, treating non-pregnancy at the last cycle as censored. Furthermore, restricted cubic spline logistic regression models were analyzed to determine the predictive power of AMH on eventual successful outcome, measured as pregnancy.

**RESULTS:** Ultimately, 294 women were included in the analysis. Of these, 31 (10.5%) were aged 22 to 30 years, 118 (40.1%) were aged 30 to 35 years, 109 (37.1%) were aged 35 to 40 years, and 36 (12.2%) were aged greater than 40 years. AMH values were left skewed and had a median of 2.7 ng/mL (IQR, 1.3- 4.8). After log-transformation AMH values had a mean of 0.82 ng/mL (SD, 1.2). The majority of patients, 214 (72.8%), had one to three total attempted cycles. The overall cumulative pregnancy rate was 24% (71/294).

Univariate model of age revealed that older patients have a lower probability of conceiving in one year than their younger counterparts OR 0.92 (CI: 0.87-0.98, P= 0.010). Univariate model of log(AMH) indicates that the probability of conceiving in patients having one unit larger log(AMH) was higher compared to those with the smaller log(AMH) OR 1.30 (CI: 1.02-1.67, P=0.035). However, when using a multivariable model including age and log(AMH), these results were no longer significant: Cox multivariable Wald p-value for age was 0.067 and for log(AMH) is 0.240. As expected, age and log(AMH) had moderate correlation relationship (Pearson r=-0.414, P<.0001).

**CONCLUSIONS:** In women undergoing IUI in our study population there is no association between AMH and pregnancy when controlling for age of individual. Therefore, AMH does not add meaningful predictive power for success of IUI for fertility treatment. Given these findings, providers should be cautioned against ordering AMH solely for this purpose.

**References:** 1. Wu CH, Chen YC, Wu HH, Yang JG, Chang YJ, Tsai HD. Serum anti-Müllerian hormone predicts ovarian response and cycle outcome in IVF patients. *J Assist Reprod Genet.* 2009;26(7):383-389.

2. Steiner AZ, Pritchard D, Stanczyk FZ, et al. Association Between Biomarkers of Ovarian Reserve and Infertility Among Older Women of Reproductive Age. *JAMA.* 2017;318(14): 1367–1376.

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**PCOS/ANDROGEN EXCESS**

**BROWN ADIPOSE TISSUE IN WOMEN WITH POLYCYSTIC OVARY SYNDROME: RELATIONSHIP WITH BODY MEASURES, PLASMA IRISIN LEVELS AND THE USE OF METFORMIN.**



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**OBJECTIVE:** Brown adipose tissue (BAT) has been recently identified in adult humans through positron emission tomography-computed tomography (PET-CT). Irisin is a myokine that can induce BAT formation. Polycystic ovary syndrome (PCOS) is a chronic dysfunction associated with obesity and metabolic disorders. The aim of this study was to evaluate whether BAT activity in women with PCOS differs from controls, correlates with plasma irisin levels and can be rescued by metformin.

**DESIGN:** Prospective cross-sectional study and randomized controlled trial.

**MATERIALS AND METHODS:** In the cross-sectional study, we included women aged 18-45 years with PCOS (n=45) and a healthy control group (n=25) matched by age and body mass index (BMI). The 45 participants of the PCOS group were subsequently randomized into a metformin subgroup (1500 mg/day during 60 days, n=21) and a placebo subgroup

(n=24). BAT activity was measured using <sup>18</sup>F-FDG PET-CT, while plasma irisin levels were assayed using ELISA.

**RESULTS:** Total BAT activity was significantly reduced in women with PCOS (maximal standardized uptake value [SUV<sub>max</sub>]: median 7.4 g/ml, interquartile range 0.9 to 15.4) compared to controls (median 13.0 g/ml, interquartile range 4.7 to 18.4, p<0.05). However, this difference was no longer significant after adjustment for waist circumference, a surrogate marker of central adiposity. In the PCOS group, BAT activity correlated negatively with BMI (Spearman's r = -0.63, p<0.001) and waist circumference (r = -0.59, p<0.001) but not with plasma irisin levels. Neither metformin nor placebo resulted in significant changes in BAT activity or plasma irisin levels.

**CONCLUSIONS:** BAT activity was reduced in women with PCOS possibly due to increased central adiposity. In PCOS women, BAT activity did not correlate with plasma irisin levels and did not change after a brief treatment with metformin.

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#### **WOMEN WITH POLYCYSTIC OVARIAN SYNDROME AND ELEVATED LEVELS OF INSULIN RESISTANCE ARE MORE PRONE TO BENEFIT FROM DIETS TO IMPROVE INSULIN SENSITIVITY: A META-ANALYSIS.**

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**OBJECTIVE:** Women with polycystic ovary syndrome (PCOS) are associated with increased levels of insulin resistance (IR). Most likely, IR is exacerbated by obesity, which is common, but not exclusive, with these women. Other than treatment with insulin-sensitizing drugs, specialized diets have also been implemented concurrently with drug treatments, as to reduce the patient's weight. However, the capacity of certain diets, with respect to the level of IR, to reduce IR has not fully been explored. Therefore, we conducted a meta-analysis to determine in subjects with higher IR, if hypocaloric diets improve insulin sensitivity.

**DESIGN:** Systematic review with a meta-analysis.

**MATERIALS AND METHODS:** PubMed, SCOPUS, EBSCO, and LACS databases and retrieved studies' bibliographies were searched for prospective studies that investigated the association between diet and IR in PCOS until October 2018. Diet was defined as a modification of the patients' nutrition intake according to caloric restriction, change in protein intake, or by using a specialized diet. IR measures (HOMA1-IR), pre- and post-intervention were extracted. Using Comprehensive meta-analysis software, depending on the level heterogeneity, determined by the  $\psi^2$ -based Q-test and the I<sup>2</sup>-test, Fixed-Effects or Random-Effects models were used to calculate the pooled standard paired differences and standard error. No publication bias was detected by the Begg-Mazumdar's test and Egger's test.

**RESULTS:** From 2,880 retrieved records, 21 publications (27 studies) fulfilled the inclusion criteria. Due to the heterogeneity of the diets, the random effects model was used. In 52% of studies, the diets led to a decrease of IR, where 30% had no effect. In 3 studies, the diet increased IR. Overall, the diets decreased IR (-0.53 ± 0.21, p<0.01). Subjects with high IR (HOMA1-IR>9) had a marked improvement (-1.28 ± 0.44, p<0.01). This was also determined with elevated IR (HOMA1-IR: 3.0-9.0; -0.68 ± 0.19, p<0.01). However, subjects with low IR (HOMA1-IR<3.0), diets did not improve IR (-0.13 ± 0.32, p=0.68).

**CONCLUSIONS:** Here, we demonstrate that in subjects with higher IR, diets are more likely to improve IR in women with PCOS. Therefore, it is crucial to determine a subjects IR status before considering any intervention containing a diet.

**SUPPORT:** Conacyt 231793.

**P-714** Wednesday, October 16, 2019 6:30 AM

#### **PREVALENCE AND PREDICTORS OF ADEQUATE PHYSICAL ACTIVITY IN A MULTIETHNIC POLYCYSTIC OVARY SYNDROME PATIENT POPULATION.**

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**OBJECTIVE:** To 1) identify correlates of adequate physical activity and 2) describe exercise behaviors in a multiethnic polycystic ovary syndrome (PCOS) patient population, in order to identify high-risk group(s) that could benefit from targeted intervention.

**DESIGN:** Cross sectional cohort study.

**MATERIALS AND METHODS:** Participants were recruited from 2006-2019 from a PCOS multidisciplinary clinic at a single academic center. Exercise data were ascertained by the International Physical Activity Questionnaire, from which we calculated metabolic equivalents (METs) as the unit of energy expenditure. Adequate physical activity was defined by the US Department of Health and Human Services (DHHS) guidelines, either as 150 minutes/week of moderate-intensity, or 75 minutes/week of vigorous-intensity, or an equivalent combination of moderate- and vigorous-intensity aerobic activity. Exercise data were analyzed by self-reported ethnicity (White, Hispanic, East/Southeast Asian, South Asian, and African American), income level, education, parity, and place of birth (US-born vs. foreign-born). Primary outcome was adequate physical activity, coded as a binary variable. Logistic regression analysis was used to identify correlates of adequate physical activity after controlling for age (SAS v9.4). Further, we used the Kruskal-Wallis test to compare the distribution of METs from moderate-intensity, vigorous-intensity, and total (moderate- plus vigorous-intensity) exercise between ethnic groups.

**RESULTS:** Of the 466 women evaluated, 62% (n = 287) were White, 15% (n = 71) were Hispanic, 11% (n = 52) were East/Southeast Asian, 7% (n = 32) were South Asian (SA), and 5% (n = 23) were African American (AA). The cohort was notable for AA patients being older (p = 0.02), and Hispanic and AA patients having higher BMI (p < 0.01) and waist circumference (p < 0.01) compared to other remaining ethnic groups. Overall prevalence of adequate physical activity was 66% in our cohort. Logistic regression analysis, controlling for age, demonstrated ethnicity as a predictor for adequate physical activity (p = 0.01), with SA patients having the lowest frequency of meeting DHHS guidelines (47%, compared to 71% in White patients). Parous status and education level (specifically, not having a college degree) were also identified as predictors of lower frequency of adequate physical activity (p = 0.02 and <0.01, respectively). Lastly, we noted significant differences in distribution of METs from vigorous exercise (MET<sub>vig</sub>) and total exercise (MET<sub>total</sub>) between ethnic groups (p = 0.01 and 0.01, respectively), with South Asian patients having the lowest mean rank MET<sub>vig</sub> and MET<sub>total</sub>.

**CONCLUSIONS:** We observed significant differences in frequency of adequate physical activity by ethnicity, parity, and education level in our cohort. Providers of PCOS patients should consider focusing on South Asian patients and women who are parous and/or with lower educational attainment with targeted interventions to promote healthy exercise behaviors.

**P-715** Wednesday, October 16, 2019 6:30 AM

#### **DECREASED LIFETIME FECUNDITY IN WOMEN WITH PCOS: FINDINGS FROM A HIGH FECUNDITY POPULATION.**

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**OBJECTIVE:** European studies have shown that women with polycystic ovary syndrome have similar numbers of children to women without this diagnosis [1]. However, these studies have generally been conducted in populations in which the mean number of children born to a woman is less than 2. In Utah, the mean number of children per woman has ranged between 2.33 and 2.65 between 1990 and 2014 [2]. We sought to determine whether women with PCOS in a high fecundity population have similar numbers of children compared to women without PCOS, and whether there are differences in ages at first and last birth.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** The Utah Population Database (UPDB), which contains demographic and detailed genealogy data, as well as medical records from the University of Utah healthcare system from 1994 through 2014, was queried for this study. PCOS cases were identified by ICD-9 codes. PCOS cases were diagnosed between the ages of 10 and 54, had least 3 generations of genealogical data in the UPDB (to increase the likelihood of having birth certificate data available for their children), and had given birth to at least one child. Controls were

matched for sex, birth place (Utah or elsewhere), and 5-year birth cohort. In addition, controls were required to have had at least one child. Children born to PCOS cases and controls, and maternal age at each birth, were determined using birth certificate data. We report mean values, standard deviation (SD) and range using conventional methods. As the data were not normally distributed, the Mann-Whitney U test was used to compare age and number of children between the PCOS cases and matched controls.

**RESULTS:** A total of 1,022 PCOS cases who had given birth to at least child and 1,022 matched population controls and were used in this analysis.

TABLE 1. Fecundity patterns in women with and without PCOS

	PCOS cases: mean (SD), range	Controls: mean (SD), range	P-value
Age at censoring (yrs)	38.96 (7.27), range: 23-70	39.02 (7.46), range: 23-73	0.891
Number of children born	2.20 (1.19), range: 1-10	2.60 (1.36), range: 1-11	6.75e-13
Age at first birth (yrs)	26.66 (4.97), range: 15-44	23.97 (3.94), range: 14-41	2.2e-16
Age at last birth (yrs)	30.37 (5.17), range: 17-44	28.82 (4.59), range: 16-44	4.04e-12

**CONCLUSIONS:** The mean number of births for both PCOS cases and matched controls in this high fecundity population was greater than 2 for both groups. PCOS cases had significantly lower parity on average than matched controls. PCOS cases were an average of 2.7 years older at the birth of their first child and 1.5 years older at the birth of their last child. As only women with at least one live birth were included, the detrimental impact of PCOS on lifetime fecundity may be greater than estimated here.

References: 1. Hudecova M, Holte J, Olovsson M, Sundstrom Poromaa I. Long term follow-up of patients with polycystic ovary syndrome: reproductive outcome and ovarian reserve. *Hum Reprod* 2009; 24: 1176-83.

2. Perlich PS. Utah's fertility rate is at a historic low. *Kem C. Gardner Policy Institute, University of Utah*, 2016.

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**P-716** Wednesday, October 16, 2019 6:30 AM

### IS HIRsutISM A MARKER OF METABOLIC DYSFUNCTION?

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**OBJECTIVE:** To determine if hirsutism alone is a marker of metabolic dysfunction.

**DESIGN:** Prospective community-based cohort study.

**MATERIALS AND METHODS:** Women (age > 14) with a modified Ferriman-Galleway (mFG) score and markers of metabolic dysfunction were included. Hirsutism was defined by an mFG score  $\geq 4$  and oligomenorrhea as <8 cycles/year. Markers of metabolic dysfunction included body mass index (BMI), waist to hip ratio (WHR), high-density and low-density lipoprotein (HDL, LDL), insulin and glucose levels during a 2-hr oral glucose tolerance test (OGTT). Categorical variables were compared using  $\chi^2$  tests and continuous variables were compared using Kruskal-Wallis or ANOVA, as appropriate. Linear regression models were used to determine correlation of degree of hirsutism with severity of metabolic dysfunction.

**RESULTS:** 497 hirsute patients were identified, of which 236 were oligomenorrheic (H/O) and 261 were eumenorrheic (H/NO); 303 non-hirsute controls (CONTROLS) were included. The groups were similar in race and age, WHR, LDL and HDL. Hirsute groups had higher BMI values (Table 1). Fasting, 1- and 2-hr insulin values were significantly higher for hirsute groups vs controls. In H/O women, mFG scores negatively correlated with HDL ( $\beta = -0.94$  95% CI -1.82- -0.06) and positively correlated with 1- and 2-hr insulin levels (1-hr:  $\beta = 6.1$  95% CI 1.1-11.0; and 2-hr:  $\beta = 4.3$  95% CI 0.97-7.62). These relationships were not significant after adjusting for BMI. In H/NO

TABLE 1

	H/O	H/NO	CONTROLS	p-value
n	236	261	303	
Age (SD) years	29.1 (6.6)	29.1 (7.0)	30.9 (7.1)	0.45
Race				
White Non-Hispanic	117	130	158	0.09
Hispanic	59	63	52	
Black	35	35	40	
Asian/Other	23	32	52	
BMI (SD) kg/m2	31.5 (8.3)	30.9 (7.8)	28.6 (8.0)	<b>0.0001<sup>a</sup></b>
OGTT time 0 (SD) mg/dL	87.3 (11.7)	86.4 (13.4)	87.6 (25.9)	0.44
OGTT 2-hr (SD) mg/dL	119.2 (78.3)	105.7 (40.3)	102.2 (30.1)	0.06
Insulin time 0 (SD) uIU/mL	21.8 (65.5)	15.0 (16.4)	11.9 (13.6)	<b>0.01<sup>a</sup></b>
Insulin 1-hr (SD) uIU/mL	120.3 (122.2)	117.2 (107.3)	81.7 (77.8)	<b>0.003<sup>a</sup></b>
Insulin 2-hr (SD) uIU/mL	86.7(83.6)	91.3 (103.9)	59.9 (58.4)	<b>0.01<sup>a</sup></b>

Pairwise comparisons: <sup>a</sup>H/O vs CONTROL and H/NO vs CONTROL p<0.05.

women, mFG scores positively correlated with 1- and 2-hr insulin values (1-hr:  $\beta = 5.38$ , 95% CI 1.7-9.1; and 2-hr:  $\beta = 4.27$  95% CI 0.67-7.88), even after adjusting for BMI.

**CONCLUSIONS:** Hirsutism is associated with a higher BMI and markers of metabolic dysfunction. In hirsute women with oligo-ovulation, evidence of metabolic dysfunction appears to be primarily associated with BMI. However, in eumenorrheic hirsute women, increasing mFG scores correlate with markers of metabolic dysfunction, even after adjusting for BMI. Thus, both increasing BMI and hirsutism alone may be suggestive of metabolic derangement and should be used as a marker for metabolic screening.

**P-717** Wednesday, October 16, 2019 6:30 AM

### BONE MORPHOGENETIC PROTEIN SUPPRESSES THE ANDROGEN PRODUCTION IN PCOS THECA IN-VITRO CELL MODEL.

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**OBJECTIVE:** Polycystic Ovarian Syndrome (PCOS) is a metabolic disorder characterized by inflammation, infertility and excess ovarian androgen production by theca cells in the ovary. Though its etiology is not fully understood. Women with PCOS exhibit the increased expression of steroidogenic pathway genes (CYP17A1, and CYP11A1) involved in androgen production. Most available therapies aim to decrease ovarian androgen production to enhance fertility. The Bone morphogenetic proteins (BMPs) which are members of the transforming growth factor  $\beta$  (TGF $\beta$ ) family plays a significant role in controlling the enzymes of the steroidogenic pathway which suppresses the androgen production. Our previous study showed the effect of mesenchymal stem cells (MSCs) secretome on steroidogenic pathway genes. It is reported in the literature that MSCs also secrete BMPs in its secretome. In this study, we evaluated the utility of BMPs on Human H295R adrenocarcinoma cell line, an in-vitro model for the investigation of the steroidogenic pathway. The H295R cell line expresses genes that encode for the key enzymes for the steroidogenesis.

**DESIGN:** We hypothesize that bone morphogenetic proteins are able to inhibit the androgen biosynthesis in PCOS in-vitro cell model by affecting the steroidogenic pathway genes expression.

**MATERIALS AND METHODS:** Human adrenocarcinoma cell line (H295R), purchased from ATCC and cultured as per the protocol. Cells were seeded on six-well plates at a density of  $1.8 \times 10^5$  cells per well and cultured for 60 hours. Cells were treated with BMP 6 and 7 with different concentrations 25, 50, 100 ng per ml with respective control (without BMP) for 48 hours. After 48 hours the media was removed and cells were washed with PBS and serum free media were added for further 24 hours. After 24 hours incubation, cells and media were collected for analysis. The

expression of mRNA for CYP17A1, CYP11A1, and DENND1A genes was quantified by real-time PCR while testosterone level in media estimated by radioimmunoassay (RIA). Student t-test was used for statistical analysis.

**RESULTS:** Human H295R cells treated with BMP 6 and 7 with concentrations of 25, 50 and 100 ng/ml for 48 hours secreted significantly lower level of testosterone in a dose-dependent manner (25ng/ml: BMP-6, 35.80 ± 0.70 ng/dl, BMP-7, 43.45 ± 0.91 ng/dl), (50ng/ml: BMP-6, 25.40 ± 0.00 ng/dl, BMP-7, 26.30 ± 0.00 ng/dl), (100ng/ml: BMP-6, 16.75 ± 0.21 ng/dl, BMP-7, 22.70 ± 2.54 ng/dl) with compared to their respective control (without BMP treatment) (Control: BMP-6, 80.65 ± 0.35 ng/dl, BMP-7, 58.00 ± 2.96 ng/dl) respectively. The expression of CYP17A1 and DENND1A gene was decreased significantly ( $P < 0.05$ ) in a dose-dependent manner only in the case of BMP-6 in all the tested concentrations while the changes in CYP11A1 expression was not relevant.

**CONCLUSIONS:** The Bone morphogenetic protein (BMP-6) showed a significant change in steroidogenic pathway genes and decrease androgen production in H295R cells compared to the control. This results may offer a promising lead and explanation of MSCs secretome effect and offer a rational for its use in treatment of infertility associated with PCOS.

**SUPPORT:** Start-up Fund from The University of Illinois, Chicago, USA.

**P-718** Wednesday, October 16, 2019 6:30 AM

### DECREASE IN PERIPHERAL 8-HYDROXYDEOXYGUANOSINE LEVELS AND MITOCHONDRIAL DNA COPY NUMBER FOLLOWING METFORMIN TREATMENT IN WOMEN WITH POLYCYSTIC OVARY SYNDROME.

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**OBJECTIVE:** We examine the longitudinal changes in markers of oxidative stress in patients with PCOS who were given metformin for 12 months.

**DESIGN:** This is an observational, prospective cohort study.

**MATERIALS AND METHODS:** A cohort of 86 women with PCOS were enrolled from a reproductive endocrinology clinic in an university-affiliated medical center from 2009 to 2015. PCOS was diagnosed using the Rotterdam criteria, and metformin was given daily for 12 months. Evaluations were undertaken before and after 3, 6, and 12 months of treatment. The primary end points were the changes in mitochondrial DNA copy number (mtDNA) and the changes in serum 8-hydroxydeoxyguanosine (8-OHdG), an oxidative stress marker of DNA damage and repair, at the 4 aforementioned time points.

mtDNA copy number was assessed using quantitative PCR (SYBR green system, Applied Biosystems, Waltham, MA, USA) on DNA isolated from lysates of whole blood samples. This is reported as the ratio of mitochondrial to nuclear DNA. Serum levels of 8-OHdG were assessed using the HT 8-oxodG ELISA Kit II (Trevigen, Gaithersburg, MD, USA).

Treatment responses were stratified by BMI using a cut-off of 25 kg/m<sup>2</sup>. Comparisons between the BMI groups at baseline were undertaken with the Mann-Whitney U test. Correlations between 8-OHdG, mtDNA copy number and various characteristics at baseline were evaluated using the Spearman correlation analyses. Longitudinal analyses were done using the linear mixed models. All statistical tests employed a two-tailed test with an alpha of 0.05.

**RESULTS:** The higher BMI group was different in regards to anthropometric, metabolic and inflammatory markers, such as BMI, AC sugar, insulin, HOMA-IR, GPT, blood pressure, and CRP levels at baseline. Factor that did not differ between the BMI groups were age, testosterone levels, GOT, 8-OHdG and mtDNA.

At study baseline, the mtDNA copy number was not correlated with any of the baseline parameters, such as age, BMI, insuline, AC sugar, GOT, GPT and CRP. The serum 8-OHdG was negatively correlated with AC sugar, and GPT levels. The serum 8-OHdG and mtDNA copy number did not correlate at baseline.

The use of metformin was associated with a progressive decrease in BMI, testosterone, AC sugar, GOT, GPT, mean dBP, CRP, 8-OHdG and mtDNA copy number over time. No significant change was seen in insulin levels and mean sBP in response to metformin use. In particular, mtDNA copy number exhibited a progressive decrease from a median of 55.38 (26.14-100.3) to 34.78 (19.67-74.68), 33.42 (20.48-55.67) and 24.11 (17.16-66.13) at 3, 6 and 12 months, respectively ( $p = 0.003$ ). 8-OHdG exhibited a decrease from a

median of 34.62 (27.7-45.4) to 31.94 (23.18-42.10), 29.96 (21.21-39.09), and 18.57 (12.14-31.15) at 3, 6 and 12 months, respectively ( $p < 0.001$ ). A higher BMI was considered to be a significant factor affecting the changes in mtDNA copy number over time ( $p = 0.017$ ).

**CONCLUSIONS:** Metformin treatment is associated with decreased markers of oxidative stress in patients with PCOS. BMI appears to play a role in the change in MtDNA copy number, while changes in 8-OHdG appears to be unaffected.

**SUPPORT:** This study was supported by grant MOST 105-2628-B002-043-MY4 from the Ministry of Science and Technology of Taiwan.

**P-719** Wednesday, October 16, 2019 6:30 AM

### ANDROGEN ALTERS SENP3 EXPRESSION AND INDUCES AUTOPHAGY IN GRANULOSA CELLS: A NOVEL MECHANISM INVOLVED IN POLYCYSTIC OVARY SYNDROME.

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**OBJECTIVE:** Polycystic ovary syndrome(PCOS) is a common endocrine disorder in reproductive-aged women, which is mainly associated with androgen excess[1]. Autophagy is responsive to energy stress and is activated in the ovarian tissue of PCOS [2]. The role of autophagy in PCOS related metabolic disorders is increasingly recognized [3-4]. Small ubiquitin-like modifier (SUMO) modification is an important post-translational protein modification in various cell types and is associated with autophagy activation[5]. SENP3, one of Sentrin/SUMO-specific proteases (SENPs) family members, can remove SUMO2/3 from proteins and SENP3 expression is closely related to oxidative stress[6]. Therefore, this study was designed to research the relationship between testosterone, SENP3 and autophagy in PCOS granulosa cells.

**DESIGN:** We collected granulosa cells of patients treated by in vitro fertilization with the same controlled ovarian stimulation protocol and were divided into two groups between January 2017 to January 2018. Group A included 75 PCOS patients and group B included 75 tubal-factor infertile patients. Cultured human ovarian granulosa-like tumor cell line (KGN cells) were treated or not with testosterone.

**MATERIALS AND METHODS:** Granulosa cells were isolated by gradient centrifugation from the follicle fluid aspirated during oocyte retrieval. Western blotting, quantitative PCR analysis were used to detect the SENP3, LC3 and p62 levels in human granulosa cells and KGN cells.

**RESULTS:** In group A, LC3III/I expression ( $1.00 \pm 0.05$  vs  $0.5 \pm 0.04$ ,  $P < 0.01$ ) was significantly higher than group B, whereas p62 ( $0.69 \pm 0.02$  vs  $0.78 \pm 0.01$ ,  $P = 0.01$ ) expression significantly decreased. After granulosa cells were treated by testosterone for 24 hours, the LC3III/I expression ( $1.35 \pm 0.08$  vs  $1.0 \pm 0.05$ ,  $P = 0.02$ ) was significantly higher than control group, whereas the expression of P62 levels ( $1.35 \pm 0.08$  vs  $1.0 \pm 0.05$ ,  $P = 0.02$ ) were obviously lower, which showed that ovarian granulosa cell of polycystic ovary syndrome autophagy is activated, and androgen excess is involved in the autophagy activation of granulosa cells in polycystic ovary syndrome patients. In KGN cells, SENP3 expression declined as the dose of androgen increased ( $0.61 \pm 0.01$  vs  $0.93 \pm 0.04$ ,  $P < 0.01$ ); whereas LC3III/I expression significantly increased ( $2.62 \pm 0.04$  vs  $1.5 \pm 0.06$ ,  $P < 0.01$ ). LC3III/I expression and mRNA levels were decreased after SENP3 overexpression and were elevated after the transfection of siRNA ( $P < 0.05$ ). It suggested that SENP3 is involved in the process of hyperandrogenism induce autophagy in KGN. granular cell of two groups cultured and treated with 10  $\mu$ M testosterone, SENP3 expression ( $1.13 \pm 0.10$  vs  $1.54 \pm 0.03$ ,  $P = 0.02$ ) was lower in PCOS group treated with testosterone, which suggested that SENP3 plays an important role in the autophagy activation of granulosa cells in patients with hyperandrogenism polycystic ovary syndrome.

**CONCLUSIONS:** Our results show that androgen excess alters SENP3 and granulosa cell autophagy, which may play a role in PCOS. This work may shed some light on the pathogenesis of PCOS.

References: 1. Chan Jessica L, Kar Sujata, Vanky Eszter et al. Racial and ethnic differences in the prevalence of metabolic syndrome and its components of metabolic syndrome in women with polycystic ovary syndrome: a regional cross-sectional study. [J] .Am. J. Obstet. Gynecol., 2017, 217: 189.e1-189.e8.

2. Li Da, You Yue, Bi Fang-Fang et al. Autophagy is activated in the ovarian tissue of polycystic ovary syndrome. [J] .Reproduction, 2018, 155: 85-92.

3. Maixner Nitzan, Bechor Sapir, Vershini Zlata et al. Transcriptional Dysregulation of Adipose Tissue Autophagy in Obesity. [J] .Physiology (Bethesda), 2016, 31: 270-82.

4. Lee Hae-Youn, Kim Jinyoung, Quan Wenyong et al. Autophagy deficiency in myeloid cells increases susceptibility to obesity-induced diabetes and experimental colitis. [J]. *Autophagy*, 2016, 12: 1390-403.

5. Shamini Vijayakumaran, Dean L. Pountney. SUMOylation, aging and autophagy in neurodegeneration. [J]. *Neurotoxicology*, 2010.

6. Han Yan, Huang Chao, Sun Xuxu et al. SENP3-mediated de-conjugation of SUMO2/3 from promyelocytic leukemia is correlated with accelerated cell proliferation under mild oxidative stress. [J]. *J. Biol. Chem.*, 2010, 285: 12906-15.

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**P-720** Wednesday, October 16, 2019 6:30 AM

#### **ESTABLISHING AN ANTI-MÜLLERIAN HORMONE (AMH) CUT-OFF TO DETERMINE POLYCYSTIC OVARIAN MORPHOLOGY (PCOM) SUPPORTING DIAGNOSIS OF POLYCYSTIC OVARIAN SYNDROME (PCOS): THE APHRODITE STUDY.**

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**OBJECTIVE:** To derive and validate a cut-off for AMH to discriminate PCOM using the Elecsys<sup>®</sup> AMH Plus immunoassay.

**DESIGN:** APHRODITE is a case-control study of PCOS-positive (cases) and PCOS-negative (controls) women aged 25–45 years. Cases were defined using Rotterdam criteria, showing the full phenotype A (irregular cycles/ovulatory dysfunction, clinical or biochemical hyperandrogenism and PCOM); controls had an antral follicle count (AFC)  $\leq 20$ , based on the new international guideline for PCOS.

**MATERIALS AND METHODS:** The discovery cohort included 290 cases and 575 controls, whereas the validation cohort consisted of 455 cases and 500 controls. Serum levels of AMH were measured using the Elecsys<sup>®</sup> AMH Plus immunoassay; AFC was determined by transvaginal ultrasound. An AMH cut-off was optimised in the discovery cohort based on concordance analysis. Performance (sensitivity, specificity and area under the curve [AUC]) of the defined cut-off was evaluated in the validation cohort. Exploratory analyses in different sub-cohorts (including age groups) were also performed.

**RESULTS:** Compared with controls, PCOS cases were younger (median age 29.0 vs 34.0 years), with a higher body mass index (median 29.2 vs 23.8 kg/m<sup>2</sup>) and higher AMH level (median 6.23 vs 2.13 ng/mL). Good correlation was observed between AMH and AFC in the discovery and validation cohorts, with Spearman correlation coefficients of 0.83 and 0.84, respectively. A serum AMH cut-off of 3.5 ng/mL (25 pmol/L) was determined in the discovery cohort, which achieved 85.9% sensitivity and specificity. In the validation cohort, this cut-off achieved 82.4% (95% confidence interval [CI] 78.6–85.8) sensitivity and 89.8% (95% CI 86.8–92.3) specificity, with an AUC of 94.0% (95% CI 92.6–95.5). In women aged  $\leq 35$  years, the AMH cut-off of 3.5 ng/mL showed 84.2% (95% CI 81.3–86.9) sensitivity and 83.5% (95% CI 80.0–86.6) specificity; in women aged  $> 35$  years, specificity remained high (91.8% [95% CI 89.2–93.9]) but sensitivity was lower (77.4% [95% CI 63.8–87.7]).

**CONCLUSIONS:** The Elecsys<sup>®</sup> AMH Plus immunoassay provides a robust method for identifying PCOM as part of PCOS diagnosis with a cut-off of 3.5 ng/mL (25 pmol/L).

**SUPPORT:** These analyses were sponsored by Roche Diagnostics International Ltd.

**P-721** Wednesday, October 16, 2019 6:30 AM

#### **PROBIOTICS AND SYNBiotics FOR POLYCYSTIC OVARIAN SYNDROME: A SYSTEMATIC REVIEW AND META-ANALYSIS.**

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**OBJECTIVE:** To evaluate the effectiveness of probiotics and synbiotics on metabolic, hormonal and inflammatory parameters of PCOS, to identify the effect on potential fertility mediators. Probiotics and synbiotics seems to have an effect on metabolic, hormonal and inflammatory aspect of PCOS.

**DESIGN:** Systematic review and meta-analysis of randomized controlled trials (RCTs). Electronic databases (MEDLINE, Scopus, EMBASE, ScienceDirect, The Cochrane Database of Systematic Reviews and [ClinicalTrials.gov](http://ClinicalTrials.gov)) were searched from their inception until May 2018. The review protocol was registered in PROSPERO before starting the data extraction (CRD42018111534).

**MATERIALS AND METHODS:** Randomized controlled trials (RCTs) of PCOS's women undergoing a therapy at least of eight weeks with probiotics or synbiotics or without therapy. Primary outcomes were changes in anthropometric parameters, glucose/insulin metabolism, lipid profile, sex hormones profile, inflammation markers. Studies were assessed using the Cochrane Risk of Bias tool.

**RESULTS:** Nine RCTs were included; 294 women were assigned to the intervention group and 293 to the control group. The intervention was associated with a significant improvement in FPG, FBI, HOMA I-R, BMI and modified Ferriman-Gallway, serum triglycerides, serum testosterone, hs-CRP, NO, TAC, GSH and MDA. Subgroup analysis on the type of intervention showed that probiotics were associated with greater testosterone and FPG reduction, synbiotics administration resulted in a more pronounced decrease of FBI. Subgroup analyses on the duration of therapy showed that in the women with 12-weeks of therapy had a significantly greater effect on QUICK-I than the 8-weeks therapy, whilst no significant difference was observed in terms of FBI, HOMA-IR and FPG.

**CONCLUSIONS:** There is a clear need to structure a robust and well driven RCT that analyses pregnancy-related outcomes in PCOS women being treated with these substances to check their fertility-related effects, since previously available evidences point to recommend use of probiotic/synbiotic in the clinical practice.

**SUPPORT:** no financial support.

**P-722** Wednesday, October 16, 2019 6:30 AM

#### **CAN ANTI-MULLERIAN HORMONE (AMH) LEVELS PREDICT RESPONSE TO OVULATION INDUCTION TREATMENTS IN WOMEN WITH POLYCYSTIC OVARIAN SYNDROME (PCOS)?**

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**OBJECTIVE:** To assess whether AMH levels can predict response to ovulation induction (OI) regimens [clomiphene citrate (CC), letrozole (LTZ) or follicle-stimulating hormone (FSH)] among women with PCOS.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** 517 OI/intrauterine insemination (IUI) cycles from 172 women with PCOS, that took place at a single academic center between 8/2013 and 12/2018, were analyzed. Ovarian response to OI regimen was the primary outcome.

**Statistical analysis:** Parametric and non-parametric tests were used as appropriate to compare AMH levels between regimens. Predicted probabilities of response to treatment [adjusted for age and body mass index (BMI)] were determined using mixed effects linear regression modeling, controlling for the potential of multiple cycles per woman. The level of statistical significance was set at 0.05.

**RESULTS:** CC, LTZ, and FSH were administered in 204, 108, and 205 cycles, respectively. Overall, cycles in which response was noted had significantly lower AMH levels (ng/ml) when compared to cycles with no response [mean (SD): 10.5 (6.8) vs 14.4 (7.7), respectively,  $p < 0.05$ ]. When taking into consideration the type of OI treatment, cycles characterized by response to either CC or LTZ, when compared to those with no response, had significantly lower AMH levels [mean (SD): 9.3 (7.4) vs. 13.9 (7.9) ng/mL,  $p < 0.05$ ; 11.4 (5.5) vs. 14.5 (6.3) ng/mL,  $p < 0.05$ ; for CC and LTZ respectively, for response vs. non-response]. On the contrary, no such trend was noted in FSH cycles.

Overall, after adjusting for age and BMI, the probability of response to treatment decreased as the AMH levels increased, dropping from 0.99 (95%CI: 0.90-1.08) among women with AMH  $\leq 10^{\text{th}}$  percentile (3.1 ng/ml) to 0.86 (0.78-0.95) among those with AMH  $\geq 90^{\text{th}}$  percentile (20.0 ng/ml). A similar trend was noted in both CC and LTZ cycles, with the probability of response being: 0.86 (95%CI: 0.69-1.00) and 0.57 (95%CI: 0.33-0.81), for CC and LTZ respectively, for patients with AMH values  $\geq 90^{\text{th}}$  percentile (20ng/ml).

On the contrary, in FSH induced cycles the probability of response to treatment did not follow a similar trend; even at the highest AMH values ( $\geq 90^{\text{th}}$



percentile, 20ng/ml), the predicted response to treatment was 0.96 (95%CI: 0.90-1.00) in models adjusting for age and BMI.

**CONCLUSIONS:** Among PCOS patients, higher serum AMH levels are associated with significantly lower probability of response to either CC or LTZ but not to FSH, even after adjusting for age and BMI.

Our findings suggest that such select groups of women might benefit from stimulation with gonadotropins.

**SUPPORT:** None.

**P-723** Wednesday, October 16, 2019 6:30 AM

**ELEVATED ANTIMULLERIAN HORMONE IS DUE TO INCREASED FOLLICLE NUMBER IN POLYCYSTIC OVARY SYNDROME COMPARED TO CONTROLS.**



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**OBJECTIVE:** Although it is known that antimullerian hormone (AMH) levels are higher in polycystic ovary syndrome (PCOS), it is unclear if the elevated AMH is related to increased follicle number, over-production of AMH per follicle, or both. Thus, we sought to compare the AMH to AFC ratio in a population of PCOS and community-based controls.

**DESIGN:** Cross-sectional cohort study

**MATERIALS AND METHODS:** Study participants were recruited at the multidisciplinary PCOS clinic following a diagnosis of PCOS by Rotterdam criteria between July 2010 and September 2015. Controls included healthy, normo-ovulatory women from a community-based cohort (Ovarian Aging Study) between November 2006 to November 2010. Clinical and laboratory data were collected for all patients. Serum AMH was assayed for both cohorts at a central laboratory. T-tests were used to assess for significance between demographics variables. AMH, AFC, and AMH/AFC were compared between the PCOS and control cohorts using analysis of covariance (ANCOVA), while controlling for age, body mass index (BMI), smoking status, and race. Pairwise comparisons were adjusted using the Bonferroni method when necessary.

**RESULTS:** 160 patients with a diagnosis of PCOS and 310 community-based controls were identified for inclusion. The PCOS patients were younger on average by 7 years ( $p < 0.001$ ), had a higher BMI ( $p = 0.038$ ), as well as significantly higher total cholesterol, fasting insulin, AMH, total AFC, and AMH/AFC compared to controls. In ANCOVAs controlling for age, BMI, smoking, and race, a diagnosis of PCOS was an independent predictor of AMH and AFC but not of AMH/AFC. Age and BMI have a negative effect on AMH and AMH/AFC. African-Americans and Asians have a significantly lower AFC while Asians have a significant increase in their AMH/AFC compared to Caucasians.

**CONCLUSIONS:** PCOS is an independent predictor of AMH and AFC but not of the AMH/AFC ratio when compared with community-based controls. Age and BMI are significant independent negative predictors of the AMH/AFC ratio. Interesting racial comparisons can be made while holding other factors stable: AFC is lower in African-Americans and Asians and AMH/AFC is higher in Asians compared to Caucasians.

TABLE 2. Predictors of Primary Outcome in Analysis of Covariance Multivariate Models

Factor	Coefficient	95% Confidence Interval	p-value
<b>AMH/AFC</b>			
Have PCOS Diagnosis	0.04	-0.01, 0.08	0.162
Age (years)	-0.005	-0.008, -0.002	<b>0.002</b>
BMI (kg/m <sup>2</sup> )	-0.004	-0.007, -0.001	<b>0.005</b>
Ever Smoked	-0.03	-0.08, 0.02	0.254
Race			<b>&lt; 0.001</b>
Caucasian	-	-	-
African-American	-0.03	-0.10, 0.04	1.000
Asian	0.14	0.07, 0.21	<b>&lt; 0.001</b>
Hispanic	-0.005	-0.08, 0.07	1.000

**P-724** Wednesday, October 16, 2019 6:30 AM

**POLYCYSTIC OVARY SYNDROME IS ASSOCIATED WITH A SLOWER DECLINE IN ANTIMULLERIAN HORMONE PER ANTRAL FOLLICLE WITH INCREASING AGE.**



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**OBJECTIVE:** It is well-known that antimullerian hormone (AMH) and antral follicle count (AFC) are higher in women with polycystic ovary syndrome (PCOS) and that age generally has an inverse relationship to AMH and AFC, however it is not understood if a relationship exists in the AMH produced per follicle between aging patients with PCOS and controls. Hence, we sought to compare the AMH to AFC ratio in a population of PCOS and community-based controls with respect to increasing age.

**DESIGN:** Cross-sectional cohort study.

**MATERIALS AND METHODS:** Study participants were recruited at the multidisciplinary PCOS clinic following a diagnosis of PCOS by Rotterdam criteria between July 2010 and September 2015. Controls included healthy, normo-ovulatory women from a community-based cohort (Ovarian Aging Study) between November 2006 to November 2010. Clinical and laboratory data were collected for all patients. Serum AMH was assayed for both cohorts at a central laboratory. T-tests were used to assess for significance between demographics variables. AMH, AFC, and AMH/AFC were compared between the PCOS and control cohorts using analysis of covariance (ANCOVA), while controlling for age, body mass index (BMI), smoking status, and race.

**RESULTS:** 160 patients with a diagnosis of PCOS and 310 community-based controls were identified for inclusion. The PCOS patients were younger on average by 7 years ( $p < 0.001$ ), had a higher BMI ( $p = 0.038$ ), as well as significantly higher total cholesterol, fasting insulin, AMH, total AFC, and AMH/AFC compared to controls. In ANCOVAs controlling for BMI, smoking, and race, increasing age in the control cohort shows an appropriately significant decline in AMH, AFC, and AMH:AFC ratio. However in the PCOS cohort, only AFC shows an apparent decline with increasing age while changes in AMH and AMH:AFC are not significantly altered.

**CONCLUSIONS:** Increasing age is associated with a significantly lower AMH:AFC ratio in controls, which is not seen in patients with PCOS. Aging controls have a larger drop in AMH per antral follicle, while those with PCOS are able to maintain a lower decline in AMH per antral follicle despite the decline in AFC with ovarian aging. PCOS may serve as a model for delayed aging with respect to ovarian markers as evidenced by the absence of decline in AMH and AMH:AFC ratio with age.

Predictors of Primary Outcomes in Analysis of Covariance Multivariate Models with Respect to Increasing Age

Factor	PCOS		Controls	
	Coefficient	p-value	Coefficient	p-value
AMH	-0.038	0.794	-0.328	<b>&lt; 0.001</b>
AFC	-0.806	<b>0.034</b>	-0.916	<b>&lt; 0.001</b>
AMH:AFC	+0.00013	0.972	-0.007	<b>&lt; 0.001</b>

**P-725** Wednesday, October 16, 2019 6:30 AM

**DIVERGENT INFLAMMATORY PATHWAYS MODULATE KEY ANDROGENIC GENE EXPRESSION IN OVARIAN THECA-INTERSTITIAL CELLS.**



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**OBJECTIVE:** Polycystic ovary syndrome is characterized by low-grade systemic inflammation and excessive androgen production by ovarian theca cells. This study evaluated the molecular mechanism through which inflammatory stimuli increase androgenic gene expression in theca-interstitial cells (TIC).

DESIGN: In vitro study exploring the mechanism of action of pro-inflammatory lipopolysaccharide (LPS) on androgenic gene expression in TIC.

MATERIALS AND METHODS: Isolated rat TICs were cultured in chemically defined media for 48 hours with or without LPS (100ng/mL) and/or TAK-242 (1uM; an inhibitor of TLR4), MCC950 (1uM; an inhibitor of the NLRP3 inflammasome) or ibuprofen (10<sup>-4</sup>M), a non-selective inhibitor of cyclooxygenase (COX) enzymes. RNA was isolated and qPCR was performed to evaluate mRNA expression of *Cyp17a1*, *Cyp11a1*, *Hsd3b*, *Ptgs2*, *Cebpd* and *Hprt* (reference gene).

RESULTS: Compared to control cultures LPS increased *Cyp17a1*, *Cyp11a1*, *Hsd3b*, *Ptgs2*, and *Cebpd* by 4.7 fold (p<0.001), 7.1 fold (p<0.0001), 2.7 fold (p<0.0001), 5.6 fold (p<0.0001) and 3.2 fold (p<0.0001) respectively. These effects on androgenic gene expression were abrogated by ibuprofen (p<0.001) and TAK-242 (p<0.0001) treatment. The effect of LPS on *Cyp17a1* expression was also abrogated by MCC-950 (p<0.0005); in contrast, effects of LPS on *Cyp11a1*, *Hsd3b*, *ptgs2*, and *cebpd* were not significantly altered by MCC-950.

CONCLUSIONS: Collectively, our data demonstrate inflammatory stimuli affect androgen-synthesis and that the upregulation of key enzymes involved in androgen synthesis is mediated via activation of TLR4, and downstream effects mediated in part by NLRP3 inflammasome (MCC-950-sensitive pathway) and in part by other, NLRP3 independent pathway(s) including up-regulation of *Cebpd* (transcription factor involved in regulation of *Ptgs2*) and *Ptgs2*(COX-2). This data provides a mechanism through which inflammatory stimuli modulates androgen production in theca-interstitial cells.

SUPPORT: T32 HD007203 Training in Reproductive Sciences Grant.

P-726 Wednesday, October 16, 2019 6:30 AM

### SERUM OF POLYCYSTIC OVARY SYNDROME PATIENTS FROM THE PPCOSII TRIAL HAS HIGHER GONADOTROPIN RELEASING HORMONE RECEPTOR AUTOANTIBODY ACTIVITY THAN UNEXPLAINED INFERTILE CONTROLS FROM THE AMIGOS TRIAL.

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OBJECTIVE: Polycystic Ovary Syndrome (PCOS) is a complex disease of unknown etiology. We previously identified activating autoantibodies (AABs) to the second extracellular loop of the gonadotropin-releasing hormone receptor (GnRHR) in the serum of infertile PCOS patients. This AAB may provide a screening/diagnostic test for PCOS. We aimed to (1) confirm the increased GnRHR AAB activity in PCOS patients from a large, well-defined cohort, and (2) demonstrate the effectiveness of GnRH antagonist in suppressing GnRHR AAB activity.

DESIGN: Cross-sectional, matched case-control study.

MATERIALS AND METHODS: Sera from 200 PCOS patients from the Pregnancy in Polycystic Ovary Syndrome II (PPCOS II) trial and from 200 race, parity, age, and body mass index (BMI) matched ovulatory, unexplained infertile control patients from the Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation (AMIGOS) trial were obtained. All serum samples were tested with and without cetrorelix, a GnRH antagonist, for GnRHR AAB activity using the GeneBLAzer cell-based fluorescence resonance energy transfer (FRET) assay. AAB activity values are expressed as fold increase over buffer baseline to normalize the individual values. Statistical analyses in R included paired T-tests and linear regression.

RESULTS: There were no statistically significant differences between groups for race (91% white) or parity (65% nulliparous), however, significant differences within pairs remained, including Anti-Mullerian hormone (AMH), despite matching for age and BMI, Table 1. GnRHR AAB activity levels in the PCOS group were significantly higher than in the control group, p=0.0017, Table 1. With cetrorelix, GnRHR AAB activity was largely suppressed in the PCOS group (p<0.0001) but not in controls (p=0.93). These differences remained significant after adjusting for within pair differences in age, BMI, and AMH.

CONCLUSIONS: We have confirmed higher GnRHR AAB activity levels in the serum of an independent cohort of PCOS patients compared to un-

TABLE 1

Measure	PCOS	Control	PCOS vs Control, p-value
Age [years]	29 ± 4	31 ± 4	<0.0001
BMI [kg/m <sup>2</sup> ]	31.5 ± 8.0	28.9 ± 7.4	<0.0001
AMH [ng/ml]	9.3 ± 8.8	2.9 ± 2.1	<0.0001
GnRHR AAB level			
Baseline	3.66 ± 0.84	3.45 ± 0.99	0.0017
With cetrorelix	3.17 ± 0.82	3.46 ± 0.78	*0.0029
			<0.0001
			*<0.0001
p-value	<0.0001	0.93	

\*adjusted for pair differences in age, BMI, and AMH

plained infertile controls. Addition of cetrorelix resulted in significant suppression of AAB activity levels in PCOS patients but controls were unaffected. The GnRHR AABs we have identified may provide a future screening/diagnostic test for PCOS or a target for treatment.

SUPPORT: College of Medicine Alumni Association (COMAA) Grant, University of Oklahoma Health College Medicine.

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### PREGNANCY OUTCOMES WITHIN A PROSPECTIVE COHORT OF WOMEN WITH POLYCYSTIC OVARY SYNDROME (PCOS). Avanthi S. Ajjarapu, BA,<sup>a</sup> Karen M. Summers, MPH CHES,<sup>a</sup> Bradley J. Van Voorhis, MD,<sup>b</sup> Rachel Mejia, D.O.<sup>a</sup> <sup>a</sup>University of Iowa, Iowa City, IA; <sup>b</sup>University of Iowa, Iowa City, OR.



OBJECTIVE: To assess treatment course, treatment outcomes and time to pregnancy following participation in the “Combined Letrozole and Clomid in Women with infertility and PCOS Randomized Control Trial” (NCT02802865) among women who were not pregnant following the study treatment cycle.

DESIGN: Prospective Cohort Study.

MATERIALS AND METHODS: 63 participants in the NCT02802865 who did not conceive, had a biochemical pregnancy, or miscarriage at the end of the active study period were followed for 9 months after first menses following the treatment cycle. Chart abstraction was completed to follow fertility treatment, type and number of treatment cycles, ongoing clinical pregnancy, as well as time to pregnancy for those participants that had positive results. SPSS was used for statistical analysis.

RESULTS: The cohort consisted of women with a mean age of 30±4.1 with a mean BMI of 34 ±7.3. Within the cohort, the treatments received and the per cycle clinical pregnancy rate of those treatments are presented in Table 1. For oral ovulation induction, many of the participants used letrozole monotherapy 44/63 (70%). During the follow up window 37/63 (59%) of the participants conceived one or more pregnancies. Of these 6 (16%) resulted in miscarriage, 2 (6%) had biochemical pregnancies, 1 (3%) ectopic pregnancy. The clinical pregnancy rate per participant was 31/63 (49%).

TABLE 1

Treatments Received	Frequency (%)	Clinical Pregnancy Rate/Per cycle
Oral Ovulation Induction medication (w/ or w/o IUI)	53/63 (84%)	13/128 (10%)
Gonadotropins (w/ or w/o IUI)	15/63 (24%)	7/18 (39%)
IVF	7/63 (11%)	4/10 (40%)
Stopped seeking treatment after study cycle	3/63 (5%)	-

Six participants conceived spontaneously with no treatment. The mean time to clinical pregnancy was 3 ± 2.5 months.

CONCLUSIONS: This prospective cohort provides valuable information for patient counseling for women with PCOS. About half of the women

conceived within a 9-month period with mean time to clinical pregnancy of 3 months. This demonstrates an overall good prognosis for patients with PCOS and continued efforts with low intervention treatments such as oral ovulation induction can still be effective and worth pursuing.

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### INFLUENCE OF OBESITY ON CLINICAL OUTCOME IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME.

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**OBJECTIVE:** To determine the effect of obesity in patients with Polycystic Ovary Syndrome (PCOS).

**DESIGN:** Analysis of clinical outcome, immune status of serum and endometrium between obesity and normal weight patients with PCOS.

**MATERIALS AND METHODS:** A total of 1738 normal weight patients with PCOS and obesity patients with PCOS who received routine IVF or intracytoplasmic sperm injection (ICSI) in the first cycle from August 2015 to March 2017 at Reproductive and Genetic Hospital of CITIC-XIANGYA were included in the study. The clinical outcome was analyzed between obesity and normal weight patients with PCOS. Meantime, There were 67 patients to examine C-reactive protein, Interleukin-18, and white blood cell index from the blood samples. Interleukin-18 and percentage of endometrial Natural Killer cells were also examined from the endometrium sample. Two independent samples were compared using the *t* test. If the normal distribution is not met, the median (interquartile range) is used, and the two independent samples were compared using the Wilcoxon rank-sum test. Count data were used to describe the rate, comparative by chi-square test. A correlation between the indicators was drawn using linear correlation analysis.

**RESULTS:** There were no differences in first-trimester rate (6.91% vs. 5.63%,  $p = 0.443$ ), ectopic rate (1.1% vs. 0.47%,  $p = 0.304$ ) and live birth rate (65.4% vs. 65.3%,  $p = 0.954$ ) between normal body weight group and obesity group. However, The clinical pregnancy rate in the PCOS normal body weight group was higher (78.2% vs. 72.2%,  $p = 0.017$ ) in the PCOS normal body weight group. Serum levels of C-reactive protein and White blood cell in the early follicular and secretory phases were significantly higher in the PCOS obesity group ( $n = 24$ ) compared with the PCOS normal body weight group ( $n = 43$ ). However, there was no significant difference in serum and endometrial Interleukin-18 between the two groups in the early follicular phase and the luteal phase. uNK cells in the PCOS obesity group were significantly lower than those observed in the PCOS normal body weight group ( $P < 0.05$ ). No correlation was found between serum and endometrium of inflammatory status.

**CONCLUSIONS:** Clinical pregnancy rate decreased in obese patients with PCOS, whose serum inflammatory response and endometrial immune status may be disrupted by obesity.

Reference: N/A.

**SUPPORT:** This study was funded by the National Science Foundation of China (81501328).

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### ASSESSMENT OF PSYCHOLOGICAL DISTRESS IN POLYCYSTIC OVARIAN SYNDROME INFERTILE PATIENTS AT A TERTIARY LEVEL INFERTILITY CARE CENTRE IN INDIA.

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**OBJECTIVE:** Polycystic ovarian syndrome (PCOS) is the commonest endocrine disease affecting young women. Thirty percent of infertile women are diagnosed with PCOS as a cause of infertility. Apart from infertility, PCOS has huge long term metabolic consequences that affects patient's quality of life (QOL). Co-existing psychological distress have also been shown to impair patient's QOL. However it is difficult to say if they are particularly attributable to some clinic-biochemical features of PCOS per se. The present study was undertaken to assess the prevalence of psychological distress among PCOS infertile patients and its association with clinical-biochemical features of the syndrome

**DESIGN:** A single centre cross sectional study was carried out at a tertiary care infertility centre in India from 1<sup>st</sup> January 2018 through 31<sup>st</sup> March 2018. Three hundred infertile patients consented to participate in the study.

One hundred and fifty PCOS infertile patients were matched to one hundred and fifty infertile controls.

**MATERIALS AND METHODS:** Hamilton's Rating Scales (HAM-A and HAM-D) were used for assessing levels of anxiety and depression. Fertility and Quality of Life Questionnaire (Ferti QoL) was used to index the quality of life. Body Image distress was measured by Feel Ideal Discrepancy (FID) Score using Stunkard Figure rating Scale. Hirsutism score (calculated using Modified Ferriman Gallwey score) and body mass index (BMI) were determined. Primary outcome measured was the prevalence of psychological disorders in PCOS infertile patients and their comparison with non PCOS infertile controls. Secondary outcome was association between psychological distress with BMI and hyperandrogenism.

**RESULTS:** The baseline prevalence of anxiety in PCOS infertile patients was 40.32% and in non PCOS infertile controls was 28.86% ( $p = 0.039$ ); baseline prevalence of depression in PCOS patients was 38.2% and in controls was 24.82% ( $p = 0.018$ ), both were statistically significant. The HAM-A scores in PCOS and non-PCOS infertile controls (14.58 $\pm$ 7.46 vs. 11.95 $\pm$ 7.45;  $p = 0.002$ ) and HAM-D scores (14.18 $\pm$ 7.16 vs. 11.39 $\pm$ 6.95;  $p < 0.001$ ) in PCOS and non-PCOS infertile controls; the difference was clinically significant. There was no difference in FertiQoL scores for both the groups. Both groups showed comparable reduced quality of life and increased overall life stress. FID scores were higher in PCOS patients (1.2  $\pm$  1.4) compared to non PCOS infertile controls (0.5  $\pm$  1.4,  $p < 0.001$ ). BMI and Hirsutism score were associated with depression in these patients ( $p < 0.001$ ).

**CONCLUSIONS:** PCOS is a complex disorder associated with alarming levels of psychological distress which is much greater when compared to infertile controls. Clinicians should routinely evaluate all infertile patients, especially PCOS from a mental health perspective otherwise their hidden psychological stress would remain undiagnosed. Psychotherapy in addition to pharmacotherapy would help improve quality of life thus helping patients cope up with financial and emotional burden of their treatment.

References: 1. Berni et al. Polycystic ovary syndrome is associated with adverse mental health and neurodevelopmental outcomes. The Journal of Clinical Endocrinology & Metabolism Dec 2017.

2. Enjezab et al. Association between severity of depression and clinico-biochemical markers of polycystic ovary syndrome. Electronic Physician Nov 2017.

SUPPORT: NIL.

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### HOMOZYGOUS ANTIMULLERIAN HORMONE (AMH) GENE MUTATION rs10417628 IN A POLYCYSTIC OVARY SYNDROME (PCOS) WOMAN WITH EXAGGERATED HYPERANDROGENISM.

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**OBJECTIVE:** Gene mutations of anti-Müllerian hormone (AMH) have been reported in approximately 3% of women with polycystic ovary syndrome (PCOS), in whom impaired AMH inhibition of CYP17 transcription could occur, leading to enhanced androgen production (1, 2). We report an AMH gene mutation in a normal-weight PCOS woman with undetectable serum AMH levels. The purpose of this study was to determine whether the AMH mutation in this PCOS woman was associated with hyperandrogenemia in excess of that from a cohort of normal-weight PCOS women and, if so, whether it was accompanied by exaggerated LH hypersecretion.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Twelve normal-weight PCOS women (by 1990 NIH criteria), ages 18-35 years, and 19 age- and body mass index (18.5-25 kg/m<sup>2</sup>)-matched controls underwent serum hormone and metabolic measures as part of a NIH-funded study. Serum AMH levels were measured by ELISA (Ansh Labs, Webster, TX) in all subjects and were undetectable in the PCOS woman with a homozygous missense gene variant in exon 5 (T/C [Ala515Val]; rs10417628). Serum androgen and LH levels were measured by LC-MS/MS (Quest Diagnostics, San Juan Capistrano, CA) and chemiluminescence, respectively. Outcome variables between the cohorts of PCOS and control women were compared by the Wilcoxon rank-sum test. The same outcome variables of the PCOS woman with the AMH gene mutation were ranked in order of magnitude relative to those of the cohort of PCOS women.

**RESULTS:**

**CONCLUSIONS:** Undetectable serum levels of AMH immunoreactivity occurred in this PCOS woman with homozygous AMH gene mutation

	Controls (Median IQR)	PCOS (Median IQR)	P-value PCOS cohort vs. controls	AMH mutation (% rank of PCOS cohort)
	N=19	N=11		N=1
AMH (ng/ml)	3.9 (2.0-6.1)	10.5 (6.5-13.4)	<0.001	0.1 (<10th%)
Antral follicle count	16 (14-21)	44 (21-50)	0.003	43 (50th%)
LH (mIU/ml)	7.8 (5.5-12.9)	11.7 (6.5-18.0)	0.094	23.9 (>90th%)
Total Testosterone (ng/dl)	29.5 (24.5-34.5)	55.5 (49.5-62.5)	<0.001	89 (>90th%)
Free Testosterone (pg/ml)	2.2 (1.6-2.5)	6.2 (3.9-7.3)	<0.001	7 (75th%)
Androstenedione (ng/dl)	110.0 (89.0-137.0)	191.0 (112.0-230.0)	0.009	380 (>90th%)
DHEAS ( $\mu$ g/dl)	168.0 (101.0-213.0)	217.0 (187.0-272.0)	0.07	239 (67th%)

rs10417628 and could reduce its bioactivity to exaggerate the PCOS phenotype through impaired AMH inhibition of CYP17 transcription, promoting androgen induced loss of steroid negative feedback on LH.

References: A

1. Gorsic LK, Kosova G, Werstein B, Sisk R, Legro RS, Hayes MG, et al. Pathogenic Anti-Mullerian Hormone Variants in Polycystic Ovary Syndrome. *J Clin Endocrinol Metab.* 2017;102(8):2862-72.

2. Atlas THP. AMHR2 2018 [Version 18.1:[Data available from v18.1.proteinatlas.org]. Available from: <https://www.proteinatlas.org/ENSG00000135409-AMHR2/tissue>.

SUPPORT: NIH P50 HD071836; NIH P51 OD011092; UL1TR001881; Santa Monica Bay Woman's Club.

P-731 Wednesday, October 16, 2019 6:30 AM

#### EXPRESSION PROFILES OF miRNA -369-5P AND miRNA-671-3P IN THE PLASMA OF PREGNANT WOMEN WITH POLYCYSTIC OVARY SYNDROME VERSUS NORMAL PREGNANCIES.



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**OBJECTIVE:** Women with polycystic ovary syndrome (PCOS) exhibit increased risk of pregnancy complications (1). MicroRNA-369-5p (miRNA) and miR-671-3p were associated with adipogenic differentiation of mesenchymal stromal cells (2), diabetes (3) and insulin secretion (4, 5). The aim was to determine if there are differences in expression levels of miR-369-5p and miR-671-3p in the cell free plasma samples between pregnant women with PCOS and healthy controls.

**DESIGN:** A pilot prospective cohort study.

**MATERIALS AND METHODS:** Singleton and spontaneous pregnancies were enrolled this study. Using real-time quantitative PCR, the expression level of miR-369-5p and miR-671-3p in the plasma were analyzed in pregnant women with PCOS were diagnosed before pregnancy according to the Rotterdam criteria, and had no obstetrical or medical complications (n=14) compared to healthy pregnant women (n=12). The relative expression of the target miRNAs in samples was compared to the calibrator and the results were expressed as relative quantification (RQ) values.

**RESULTS:** The characteristics of patients are listed in (Table 1). The expression levels of miR-671-3p were significantly increased in the pregnant patients with PCOS (RQ value= 5.53±2.98) versus healthy controls (RQ value= 1.20±0.64) (p=0.0001). Onreceiving operator characteristic analysis, areas under the curve of the expression ratio of miR-671-3p in PCOS was 0.96 (95%CI: 0.88-1.00). At a cut-off value of 1.89 for this miRNA, sensitivity and specificity values were 92% and 93%, respectively. There was no difference in the expression levels of miR-369-5p between PCOS and controls.

**CONCLUSIONS:** Our results indicated miR-671-3p has a candidate for diagnosis for PCOS and could be a potential biomarker during pregnancy.

References: 1. Palomba, S., De Wilde, M. A., Falbo, A et al. Pregnancy complications in women with polycystic ovary syndrome. *Human reproduction update*, 2015;21:575-592.

2. Bork, Simone, et al. Adipogenic differentiation of human mesenchymal stromal cells is down-regulated by microRNA-369-5p and up-regulated by microRNA-371. *Journal of cellular physiology*, 2011;226.9:2226-2234.

3. Guay, Claudiane; Regazzi, Romano. Role of islet microRNAs in diabetes: which model for which question? *Diabetologia*, 2015;58.3:456-463.

4. Ntoumou, Eleni, et al. Serum microRNA array analysis identifies miR-140-3p, miR-33b-3p and miR-671-3p as potential osteoarthritis biomarkers involved in metabolic processes. *Clinical epigenetics*, 2017;9.1:127.

5. Murri, Mora, et al. Non-targeted profiling of circulating microRNAs in women with polycystic ovary syndrome (PCOS): effects of obesity and sex hormones. *Metabolism*, 2018, 86: 49-60.

SUPPORT: None.

TABLE 1. Patient demographics, clinical and biochemical characteristics

Index Characteristics	PCOS (n=14)	Control Group (n=12)	P value
	Mean ± SD	Mean ± SD	
Age (years)	29.6 ± 4.34	26.6 ± 4.9	0.561
Gestational age at sampling (weeks)	29.1 ± 5.1	28.9 ± 0.2	< 0.0001
Pre-Pregnancy BMI, kg/m <sup>2</sup>	28.3 ± 6.1	23.6 ± 2.9	< 0.0001
BMI (kg/m <sup>2</sup> )	31.2 ± 5.7	26.4 ± 2.9	0.004
Systolic blood pressure at sampling (mmHg)	106.4 ± 10.1	101.7 ± 5.8	0.028
Diastolic blood pressure at sampling (mmHg)	62.8 ± 7.3	60.0 ± 6.0	0.106
HbA1c (%)	5.3 ± 0.2	5.1 ± 0.3	0.844
Fasting glucose (mg/dl) at sampling	76.0 ± 9.1	75.9 ± 8.7	0.670
1-h glucose (mg/dl) at sampling following a 75-g OGTT	139.7 ± 27.5	122.7 ± 28.9	0.952
2-h glucose (mg/dl) at sampling following a 75-g OGTT	108.0 ± 24.4	97.5 ± 19.0	0.386

PCOS, polycystic ovary syndrome; SD, standard deviation; BMI, body mass index; GA, gestational age; OGTT, oral glucose tolerance test Data presented as mean ± SD and compared using unpaired t-test \*p < 0.05.

PRACTICE MANAGEMENT

P-732 Wednesday, October 16, 2019 6:30 AM



**THE IMPACT OF A BRIEF INTERVENTION ON RETENTION RATES WITH PATIENTS WHO DID NOT RETURN TO CARE AFTER AN INITIAL PHYSICIAN VISIT.** Alice D. Domar, Ph.D., Kristin L. Rooney, BA, Dan W. Duvall, Jr., BA, Denny Sakkas, PhD. Boston IVF, Waltham, MA.

**OBJECTIVE:** The goal of this study was to determine a) if a follow-up email to selected patients who had an initial consult with an infertility specialist, but did not return for a second visit, would change return to care behavior and b) why patients had not returned.

**DESIGN:** Controlled prospective trial.

**MATERIALS AND METHODS:** From July 2017 to March 2018 all patients who had attended an initial visit with an infertility specialist at the clinic, but had not returned for at least three months were selected to receive a follow up email. Those selected for an email excluded patients who we knew had achieved a pregnancy, already had a plan for treatment, had visited for an egg freeze and all LGTPQ patients. The email asked if the patient had any questions about that visit, offered support to the patient and included contact information for the patient liaison sending the email. The email also asked each participant to indicate why they had not returned and were provided 4 options and an opportunity to write in a response. From April 2018 to December 2018 no emails were sent to patients. No other change of patient contact practice was initiated during the trial period. All patients were then followed for 11 months after their initial visit to observe return to care behavior.

**RESULTS:** A total of 647 patients were selected to be sent 301 emails (Group 1) and 657 did not receive an email (Group 2). Forty-one percent of the patients in Group 1 returned to care, compared to 32% who did not (Group 2) (P<.0014). Of the Group 1 patients 116 replied (38.5%). For those who gave a reason why they hadn't returned, 32% of the respondents conceived on their own, 3% transferred care to another infertility center, 31% were taking a break/holding off from treatment, and 3% were unhappy with their care at the first visit. A total of 31% made a follow-up appointment.

**CONCLUSIONS:** A simple follow-up email sent to patients who had an initial visit with an infertility specialist but did not return to the clinic within three months was associated with a significant increase in return to care when compared to patients who did not receive an email.

P-733 Wednesday, October 16, 2019 6:30 AM



**EFFECT OF MANDATED COVERAGE FOR FERTILITY PRESERVATION ON CONVERSION RATE TO TREATMENT.** Eric Han, MD,<sup>a</sup> Amanda Nicole Kallen, MD,<sup>b</sup> Pasquale Patrizio, M.D.<sup>c</sup> <sup>a</sup>Yale School of Medicine, New Haven, CT; <sup>b</sup>Yale University, New Haven, CT; <sup>c</sup>Yale Fertility Center, New Haven, CT.

**OBJECTIVE:** In January 2018, Connecticut became the first state to mandate coverage for fertility preservation in patients diagnosed with cancer and facing medically necessary but potentially gonadotoxic therapies. We assessed the impact of this legislation on conversion rate to fertility preservation (FP) treatment for patients seen at our clinic.

**DESIGN:** A retrospective chart review on patients seen for FP consultation at the Smilow Cancer Center.

**MATERIALS AND METHODS:** A chart review was conducted on all patients seen in the oncofertility clinic in two-time periods: from April 2016 to December 2018 (pre-legislation) and from January 2018 to January 2019 (post-legislation). Patients currently receiving, or planning to initiate, potentially gonadotoxic therapies were included in the study. Data was analyzed using the unpaired t-test and chi square test. A p-value of less than 0.05 was considered to be statistically significant.

**RESULTS:** A total of 98 male and female patients were included in the study (59 seen pre-legislation and 39 post-legislation). The two groups did not differ in mean age or gender composition (mean age 30.2 years). The overall pre-legislation conversion rate from initial consultation to initiation of FP was 28.8%; post-legislation conversion rate was 46.2%, a change which approached but did not achieve statistical significance (p = 0.079). The rate of sperm cryopreservation significantly increased, from 14.3% to 71.4% (p = 0.031). The rates of oocyte and/or embryo cryopreservation were not statistically different, 26.9% vs 28.1% (p = 0.905). There were no differences in the number of patients who chose to self-pay for FP (23.5% vs 22.2%, p = 0.927) or who indicated that they did not pursue treatment specifically due to financial constraints (11.9% vs 14.3%, p = 0.789).

**CONCLUSIONS:** Mandated insurance coverage for FP was associated with an increase in sperm cryopreservation rates. However, this may be more reflective of differences in cancer types between the groups as there were more aggressive malignancies in the pre-legislation male patients. Overall, there was a nonsignificant but clinically relevant increase in patients pursuing FP after initial consultation, suggesting increased access and utilization as a result of this legislation in Connecticut. Further efforts are needed to reduce the time to get approval from insurances and to expand coverage to include patients with self-funded health plans or state insurance.

P-734 Wednesday, October 16, 2019 6:30 AM



**INFERTILITY PATIENT CLINICAL JOURNEY OUTCOME DEPENDS ON INITIAL TREATMENT, STARTING WITH OVULATION INDUCTION (OI) VS IN VITRO FERTILIZATION (IVF): RESULTS FROM A LARGE REAL-WORLD DATABASE.** Mary Mahony, PhD,<sup>a</sup> Gilbert L. Mottla, MD,<sup>b</sup> Kevin S. Richter, PhD,<sup>c</sup> G. David Ball, PhD,<sup>d</sup> Soudeh Ansari, PhD,<sup>e</sup> Brooke Hayward, SM, MBA.<sup>f</sup> <sup>a</sup>US Medical Affairs, EMD Serono, Inc., Rockland, MA; <sup>b</sup>Shady Grove Fertility Center, Annapolis, MD; <sup>c</sup>Fertility Science Consulting, Silver Spring, MD; <sup>d</sup>Seattle Reproductive Medicine Center, Seattle, WA; <sup>e</sup>Prometrika LLC, Cambridge, MA; <sup>f</sup>EMD Serono, Inc., Rockland, MA.

**OBJECTIVE:** In January 2018, Connecticut became the first state to mandate coverage for fertility preservation in patients diagnosed with cancer and facing medically necessary but potentially gonadotoxic therapies. We assessed the impact of this legislation on conversion rate to fertility preservation (FP) treatment for patients seen at our clinic.

Patient group	Initial treatment	Patients, n	Pregnancy		Cycles per pregnancy		Discontinued treatment			
			Total, %	From IVF, %	Mean	Standard deviation	Total without pregnancy, %	≥ 12 months, %	<12 months, %	
All treatment-naïve patients (n=78958)	OI oral	29450	49.2	36.4	3.5	2.30	50.8	33.6	17.2	
	OI Gn	18015	52.7	42.7	3.1	2.03	47.3	33.9	13.4	
	IVF	31493	64.3	100.0	1.8	1.23	35.7	25.1	10.6	
Prognosis examples										
	Poor; diminished ovarian reserve ≥35 years (n=8532)	OI oral	1179	31.5	41.8	3.7	2.47	68.5	47.1	21.5
		OI Gn	2286	34.4	47.6	3.3	2.34	65.6	46.1	19.5
IVF		5067	54.4	100	2.0	1.48	45.6	31.6	14.0	
Intermediate; unexplained infertility, all ages (n=13787)	OI oral	5085	55.1	46.2	3.4	2.05	44.9	28.4	16.5	
	OI Gn	5068	59.6	46.6	3.2	1.95	40.4	28.0	12.4	
	IVF	3634	69.4	100	1.8	1.21	30.6	22.9	7.7	
Best; ovulatory disorder/polycystic ovarian syndrome <35 years (n=9819)	OI oral	6013	56.6	26.1	3.4	2.35	43.4	29.0	14.4	
	OI Gn	2340	67.4	34.5	2.9	2.00	32.6	23.6	8.9	
	IVF	1466	78.1	100.0	1.6	0.93	21.9	14.7	7.2	

**OBJECTIVE:** To provide cumulative clinical pregnancy rates, time to pregnancy, and treatment discontinuation rates depending on initial fertility treatment and prognosis.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** Electronic medical records data from 78958 treatment-naïve infertile patients whose initial treatments were OI (with or without intrauterine insemination) with oral medication (clomiphene or letrozole), OI with gonadotropins (Gn), or IVF (fresh or cryopreserved embryo transfer) between Jul 2009–;Sep 2015 were analyzed. The overall population and prognosis subgroups were studied and stratified by initial treatment type.

**RESULTS:** Patients with a good prognosis were more likely to begin treatment with OI orals, while patients with a poor prognosis were more likely to start with IVF. Regardless of prognosis, the proportion of patients who achieved a pregnancy was highest among those who initiated treatment with IVF rather than OI. Patients who started treatment with OI required more cycles to achieve pregnancy than those who started with IVF (mean 3.5 vs 1.8 cycles). A large percentage (26–48%) of pregnancies among patients who started with OI resulted from IVF after OI failed. Treatment discontinuation without a pregnancy was most common among patients who started with OI oral.

**CONCLUSIONS:** Beginning clinical treatment with IVF rather than OI resulted in higher cumulative pregnancy rates, fewer total treatment cycles, and lower rates of treatment discontinuation without a pregnancy. The advantage of IVF was greater among patients with a poorer prognosis (eg, diminished ovarian reserve  $\geq 35$  years). These results favor initiating treatment with IVF unless a patient has a strong personal preference for less invasive approaches.

**SUPPORT:** Study sponsored by EMD Serono, Inc. (a business of Merck KGaA, Darmstadt, Germany), Rockland, MA, USA.

**P-735** Wednesday, October 16, 2019 6:30 AM

**THE FREQUENCY AND IMPLICATIONS OF "CLINIC SWITCHING" AMONGST US IVF PATIENTS.** Deborah Anderson, JD FertilityIQ, San Francisco, CA.



**OBJECTIVE:** To ascertain how often US IVF patients change clinics, what their underlying motivations are and how they perceive how much time and expense their initial choice of clinic may have cost.

**DESIGN:** 28,000 US IVF patients were surveyed on the number of clinics they received IVF treatment from. A sub-segment (1,000) of those who were treated at multiple clinics were re-surveyed on their reasons for switching and their perception of how being treated at multiple clinics impacted their financial and emotional well-being.

**MATERIALS AND METHODS:** Patients were surveyed at [www.FertilityIQ.com](http://www.FertilityIQ.com) and thereafter followed-up by email, whereby additional questions were posed using the Qualtrics survey tool.

**RESULTS:** 48% percent of surveyed fertility patients are treated at one clinic, 27% at two clinics and 25% at three-or-more clinics. Of those who were treated at more than one clinic, 32% left their first clinic due to inadequate attention or service, 30% due to inadequate clinical results, 18% due to personality conflicts with the doctor or staff, 6% due to cost considerations ("Other" comprised the remaining responses). Patients who were treated at multiple clinics believe they needlessly suffered a 6.3 month in delay in treatment and needlessly spent over \$12,725 in care. 84% of patients who went to multiple clinics believe having been dissatisfied with their primary choice of clinic negatively impacted their emotional well-being and 58% believe it impacted their ability to work.

**CONCLUSIONS:** "Clinic switching" is a meaningful phenomenon amongst US fertility patients, non-medical and non-cost factors may drive the preponderance of such choices and patients perceive the medical, financial and emotional consequences associated with such moves to be meaningful.

**P-736** Wednesday, October 16, 2019 6:30 AM

**RECONSIDERING THE WEEKEND FREE IN-VITRO FERTILIZATION TREATMENT. ARE RESULTS COMPROMISED BY A FIVE-DAY WORKWEEK?** Amir Weiss, MD,<sup>a</sup> Shira Baram, MD,<sup>b</sup> Simon Nothman, MD,<sup>c</sup> Yoel Geslevich, MD.<sup>c</sup> <sup>a</sup>Rappaport School of Medi-



cine, Technion Israel, Haifa, Israel; <sup>b</sup>CREATe Fertility Centre, Toronto, ON, Canada; <sup>c</sup>Emek Medical Center, Afula, Israel.

**OBJECTIVE:** To determine if treatment results differ as a function of the weekday of the first day of menstruation when implementing a five-day workweek.

**DESIGN:** A retrospective study covering 676 antagonist cycles from November 2010 to April 2017 at a public in-vitro fertilization unit serving the general public and operating five days a week.

**MATERIALS AND METHODS:** Included were women and couples with infertility requiring in-vitro fertilization at a public unit serving the general local population. Cycle data was recorded on an excel spread sheet prospectively and analyzed retrospectively following local ethics committee approval. Patient, treatment and outcome parameters were compared as a function of the day of the week menstruation began. Only antagonist cycles were included, each patient was included only once (the first treatment) and freeze all cycles were excluded.

SAS9.4 software was used for statistical analysis. Categorical data was analyzed with the chi-squared test while continuous data were compared between groups with the Kruskal-Wallis test.  $P < 0.05$  was considered significant.

**RESULTS:** Included were 676 cycles. The live birth rate for each weekday menstruation began is as follows: weekday1 – 14.56%, weekday2 – 14.56%, weekday3 – 22.02%, weekday4 – 26.09%, weekday5 – 27.78%, weekday6 – 15.38%, weekday7 – 12.90% ( $P=0.0407$ ). The treatment groups did not differ for age, infertility duration, BMI, FSH, parity, cause of infertility. The groups differed significantly for number of days of gonadotropin stimulation ( $P=0.0066$ ), though they did not differ for the amount of gonadotropins administered, the numbers of oocytes aspirated, or the fertilization rate ( $P=0.0742$ ). They differed significantly for pregnancy rate ( $P=0.0143$ ), and clinical pregnancy rate (0.0292) as well.

In a subgroup of 363 ICSI cycles, the percent of M2 oocytes differed significantly among treatment groups ( $P=0.0383$ ) but the live birth rate did not.

**CONCLUSIONS:** The study is limited by its retrospective nature. We are unaware of prospective randomized trials which compare a five-day work week to a six- or seven-day work week. Since the day menstruation begins is a rather random occurrence, the finding that there are differential results suggests that the lack of flexibility in scheduling the OPU after an ideal stimulation duration, may be compromising results.

Adding weekend OPU to the workschedule may improve outcomes but at considerable cost to the public sector. Alternatively, cycle scheduling using hormonal therapy may be considered. More research should be invested in exploring how work schedules may impact results.

**P-737** Wednesday, October 16, 2019 6:30 AM

**INSIGHTS INTO INFERTILITY PATIENT DISCONTINUATION OF CARE: RESULTS OF A NATIONWIDE SURVEY.** Barbara Collura, MA,<sup>a</sup> Brooke Hayward, SM, MBA,<sup>b</sup> Krysten Modrzejewski, PharmD,<sup>b</sup> Gilbert L. Mottla, MD,<sup>c</sup> Kevin S. Richter, PhD,<sup>d</sup> Allison B. Catherino, PhD.<sup>b</sup> <sup>a</sup>RESOLVE: The National Infertility Association, McLean, VA; <sup>b</sup>EMD Serono, Inc., Rockland, MA; <sup>c</sup>Shady Grove Fertility Center, Annapolis, MD; <sup>d</sup>Fertility Science Consulting, Silver Spring, MD.



**OBJECTIVE:** To illustrate perspectives of patients on the infertility treatment journey, and their motivations for treatment discontinuation and return to care.

**DESIGN:** Online, cross-sectional, quantitative–qualitative patient survey.

**MATERIALS AND METHODS:** Participants were recruited from the infertility patient community and invited to complete the survey, administered in March–April 2019. Descriptive statistics were calculated for all survey items.

**RESULTS:** Among 359 respondents from 40 US states, 99% were female. Forty-one percent had earned a graduate degree (master's or doctoral), and an additional 41% had a bachelor's degree. Most (69%) were 31–40 years of age, with 18% being 30 years or younger, and 13% being older than 40 years. The majority (51%) reported an annual household income of \$100k or greater, while 7% reported an income below \$50k. Of 180 patients who reported that they were done with treatment, 62% ( $n=111$ ) completed treatment with a live birth and 38% ( $n=69$ ) ended treatment without a live

Patient expectation	Actual time to pregnancy					Still on journey	Completed family without live birth	Total
	0.5 years	1 year	2 years	>2 years				
I did not have an expectation	<i>1</i>	<i>5</i>	<i>5</i>	<b>21</b>		<b>11</b>	0	43
I would be pregnant in <1 year	<i>2</i>	<i>3</i>	<b>14</b>	<b>78</b>		<b>83</b>	5	185
I would be pregnant in 1–2 years	<i>0</i>	<i>2</i>	<i>2</i>	<i>8</i>		<b>40</b>	4	56
I would never be pregnant	<i>0</i>	<i>1</i>	<i>1</i>	<i>3</i>		<i>2</i>	0	7
Total	3	11	22	110		136	9	291

Italic = patient expectation aligned with actual time to pregnancy; bold = expectations and actual time to pregnancy not aligned.

birth. Of the 200 respondents who considered discontinuation of care, 30% (n=60) continued without ever stopping, 36% (n=71) stopped for a period of time and then restarted, and 35% (n=69) stopped with no plan to restart. Commonly cited reasons (patients could choose multiple reasons) for treatment discontinuation were financial (62%), psychological burden/treatment fatigue (58%), poor prognosis (26%), and natural conception (6%); the reasons most often cited for staying in treatment were patient's desire for a family (47%), hope (21%), and partner's desire for a family (13%). The expected vs actual time to pregnancy was vastly different. Of patients who thought it would take <1 year to become pregnant, 42% (78/185) reported it took >2 years before pregnancy while 45% (83/185) reported still being on their treatment journey.

**CONCLUSIONS:** Fertility patients predominantly cite psychological burden/treatment fatigue and cost as reasons for discontinuation, and hope and desire for family as reasons for staying in treatment. Fostering more realistic patient expectations by fertility providers regarding the time it often takes to achieve pregnancy may play a role in reducing treatment discontinuation and dropout.

**SUPPORT:** Study sponsored by EMD Serono, Inc. (a business of Merck KGaA, Darmstadt, Germany), Rockland, MA, USA.

**P-738** Wednesday, October 16, 2019 6:30 AM

#### DETERMINING THE REASONS WHY INSURED WOMEN DROP OUT OF IVF TREATMENT AFTER ONE UNSUCCESSFUL CYCLE.

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**OBJECTIVE:** To determine reasons why insured patients discontinue in vitro fertilization (IVF) treatment after a single unsuccessful cycle.

**DESIGN:** Cross-sectional study.

**MATERIALS AND METHODS:** Women whose first autologous IVF cycle began June 2014–October 2018, who did not have a live birth, and who did not return for treatment for at least four months were eligible. Women completed a survey regarding treatment termination either online or via phone. Results were compared to those from a prior study of 237 insured patients who discontinued treatment after more than one unsuccessful cycle, the last of which began January 2010–May 2014, and who did not return to care for a one-year period.

**RESULTS:** Of 262 eligible women, 93 (36%) completed surveys. Of these, 25 (27%) did not have insurance coverage for IVF treatment and were excluded. Of the remaining 68 participants, 14 (21%) sought care elsewhere after their single unsuccessful cycle, which was significantly fewer than participants who completed more than one unsuccessful cycle (37%; P=0.02). Those who sought care elsewhere after a single unsuccessful cycle reported doing so because they were unhappy with their care (50%), they had moved away (29%), they wanted a second opinion (21%), or they had heard good things about another center (21%); these reasons were similar to those who did multiple unsuccessful cycles, with the exception that those doing multiple cycles were more likely to want a second opinion (60%; P=0.01). Of the 54 participants who had not sought additional care after one unsuccessful cycle, over half (52%) reported that they were taking a break from treatment, and nearly one-quarter reported that they could not afford the out-of-pocket costs (24%). Other reasons included losing insurance coverage (22%) and conceiving spontaneously (22%). Participants not seeking care after multiple unsuccessful cycles were more likely to be pursuing or have adopted a child (23% vs. 4%; P=0.002), to report that further treatment was too stressful (45% vs. 20%; P=0.001), and to report that they had been advised to stop treatment (15% vs. 4%; P=0.03).

**CONCLUSIONS:** Half of participants who did not return to care within four months of a single unsuccessful IVF cycle reported that they were taking a break from treatment, and despite having partial or full insurance coverage, nearly one-quarter reported not returning due to financial difficulties. Treatment stress was less of an issue for participants who had undergone a single unsuccessful cycle compared to those who had undergone multiple unsuccessful cycles.

**P-739** Wednesday, October 16, 2019 6:30 AM

#### DOES IN VITRO FERTILIZATION (IVF) INSURANCE COVERAGE CHANGE PRACTICE PATTERNS?

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**OBJECTIVE:** On January 1, 2015, a single, large academic institution implemented self-directed IVF insurance coverage for employees and students with an infertility diagnosis; however intrauterine insemination (IUI) remained an uncovered benefit. The insurance benefit mandated single embryo transfer ≤35 years, single or double embryo transfer >35 years, and imposed a lifetime limit on gonadotropin prescription coverage, regardless of whether the gonadotropins were used for IVF. The objective of this study was to examine practice patterns within the context of this insurance change. We hypothesized that patients with IVF coverage would undergo fewer injectable (gonadotropin or hybrid) cycles prior to IVF, fewer IUI cycles prior to IVF, and have less embryos transferred overall, compared to patients without IVF coverage.

**DESIGN:** Retrospective chart review.

**MATERIALS AND METHODS:** We used ICD-9/10 and CPT codes to identify patients who underwent IVF from 1/1/15 through 4/28/18 (n=568). Exclusion criteria included initial evaluation prior to 1/1/15, history of IVF treatment at an outside facility, IVF for fertility preservation, severe male factor or tubal factor infertility necessitating IVF treatment, and use of preimplantation genetic testing for aneuploidy (PGT-A). Primary outcome was number of embryos transferred. Secondary outcomes included number of injectable cycles prior to IVF and number of IUI cycles prior to IVF. Descriptive statistics and Student's t-test were used to characterize these distributions.

**RESULTS:** 321 patients met inclusion criteria (142 without insurance coverage and 179 with insurance coverage). Mean age in both the uncovered and covered groups was 33.3 years (SD = 4.26, NS). Mean number of embryos transferred was similar between uncovered and covered patients (1.42 vs 1.47, NS). Number of injectable cycles prior to IVF was similar between groups (3.82 vs 2.33, NS), as was the number IUIs prior to IVF (2.68 vs 2.64, NS). In patients with unexplained infertility, number of IUI cycles prior to IVF was similar between groups (3.31 vs 3.13, NS). In patients with a diagnosis of unexplained infertility, diminished ovarian reserve (DOR), or endometriosis, there were no significant differences in number of IUI cycles prior to IVF between groups (2.83 vs 2.6, NS).

**CONCLUSIONS:** Number of embryos transferred, number of IUI cycles prior to IVF, and number of injectable cycles prior to IVF was similar between patients with and without insurance coverage for IVF. These data provide reassurance that coverage status is unlikely to alter infertility provider practice and treatment strategy. Despite IUI being an uncovered benefit, providers still followed standards of care for treatment of conditions such as unexplained infertility and endometriosis.

**DECREASING THE BURDEN OF PROGRAMMED FET CYCLES.** Kathleen M. Doody, MD,<sup>a</sup> Martin Langley, MS,<sup>b</sup> Kevin J. Doody, M.D.<sup>a</sup> <sup>a</sup>CARE Fertility, Bedford, TX; <sup>b</sup>Center for Assisted Reproduction, Bedford, TX.



**OBJECTIVE:** Current management of FET cycles in programmed cycles generally entails monitoring with sonography and / or hormonal measurements. Endometrial thickness has shown correlation with implantation rate, prompting many clinicians to attempt to detect and correct poor endometrial response. To date, however, no studies have demonstrated that monitoring and active management of endometrial response is beneficial. This is important because the monitoring requirements associated with FET cycles add to the complexity and decrease the predictability of the day of embryo transfer. In an effort to increase access to care, our clinic has recently implemented a programmed cycle protocol performed without monitoring for patients undergoing FET following intravaginal culture (IVC). We examined the pregnancy outcomes of these cycles compared to contemporaneous programmed cycles with conventional monitoring.

**DESIGN:** FET cycles between April 2017 and March 2019 were retrospectively analyzed. 74 patients underwent a FET cycle with no monitoring and 143 underwent an FET cycle with monitoring. Non-monitored cycles: Patients began 6 mg of oral estradiol on cycle day 1 and were instructed to call the IVF coordinator to schedule their FET. IM progesterone (PIO) was begun after 12 or more days of estradiol. No ultrasounds or hormonal testing was performed during the cycle. Monitored cycles: The cycle was timed with oral contraceptives. A sonogram was performed prior to starting leuprolide SQ daily. OCPs were discontinued after 5 days of leuprolide overlap. A second sonogram was performed after 7 to days of leuprolide to assess endometrial thickness and ovarian suppression. Additionally, serum was obtained for an estradiol level. Oral estradiol 6 mg/d was begun daily if the serum E2 < 85 pg/ml and endometrial thickness < 5 mm. A third sonogram was performed after 12 to 14 days of estradiol supplementation. If the endometrium was 8 mm in thickness and trilaminar in appearance, PIO was begun. If not, a fourth sonogram was performed 7 days later. All FETs were performed on the 6<sup>th</sup> day of PIO.

**MATERIALS AND METHODS:** Fisher Exact Test.

**RESULTS:**

	Non-monitored				Monitored			
	<35	35-37	>37	All Ages	<35	35-37	>37	All Ages
Age	<35	35-37	>37	All Ages	<35	35-37	>37	All Ages
Average Age	30.9	36.0	39.7	32.4	30.8	36.6	39.8	34.6
# FET	56	12	6	74	73	25	44	142
Positive bHCG	37 (66.1%)	8 (66.7%)	5 (83.3%)	50 (67.6%)	45 (61.6%)	10 (40.0%)	19 (43.2%)	74 (51.1%)
BC	3	0	0	3	5	1	2	8
SAB	3	0	0	3	5	0	2	7
Ectopic	0	0	0	0	0	0	1	1
Ongoing/Delivery	31 (55.4%)	8 (66.7%)	5 (83.3%)	44 (59.5%)	35 (48.0%)	9 (36.0%)	14 (31.8%)	*58(40.9%)

\* P < 0.01 for all ages.

**CONCLUSIONS:** Streamlined IVF processes offer the possibility to increase patient access to care through fewer visits and lower patient cost. FET cycles with no monitoring have comparable outcomes to FET cycles with conventional monitoring.

**IMPACT OF INSURANCE COVERAGE FOR FERTILITY TREATMENT ON PATIENT PREFERENCE FOR SINGLETON GESTATION.** Seth J. Barishansky, MS,<sup>a</sup> Anne Hutchinson, M.D.,<sup>a</sup> Dana B. McQueen, MD, MAS,<sup>b</sup> Rafael Confino, BS,<sup>a</sup> Angela K. Lawson, Ph.D.,<sup>a</sup> Mary Ellen Pavone, MD, MSCI.<sup>a</sup> <sup>a</sup>Northwestern University, Chicago, IL; <sup>b</sup>Northwestern University Feinberg School of Medicine.



**OBJECTIVE:** To evaluate predictors for patient preference regarding multifetal or singleton gestation among women presenting for infertility care.

**DESIGN:** Cross-Sectional Study.

**MATERIALS AND METHODS:** IRB approval was obtained. Couples undergoing treatment at a university-based infertility clinic between February

	Desire Multifetal Gestation (n=24)	Desires Singleton Gestation (n=48)	P value
Mean Age (years)	34.5 (SD 4.6)	35.0 (2.9)	0.59
Mean Partner Age (years)	36.8 (5.6)	35.9 (4.7)	0.50
Married	75% (18/24)	83% (40/48)	0.53
Religious	67% (16/24)	75% (36/48)	0.58
Annual household income >100,000	63% (15/24)	85% (41/48)	0.04
Reported close friend/relative with twins	63% (15/24)	54% (26/48)	0.50
Ideal family size	2.54 (SD 0.7)	2.2 (0.6)	0.03
History of prior pregnancy	38% (9/24)	29% (14/48)	0.48
Mean number of children	0.2 (SD 0.4)	0.2 (SD 0.5)	0.60
History of miscarriage	33% (8/24)	19% (9/48)	0.17
Previously seen by a Reproductive Endocrinologist	33% (8/24)	25% (12/48)	0.46
Would not consider multifetal reduction	42% (10/24)	38% (18/48)	0.80

2019 and April 2019 participated in a 40-question previously validated digital survey (Ryan et al, 2004). All patients received treatment in a location with state mandated infertility insurance coverage. The desire for singleton versus multifetal gestation was recorded. Baseline characteristics and demographic data compared between groups.

**RESULTS:** 72 women completed the survey, with 33% reporting a multifetal gestation of twins or greater as the ideal treatment outcome and 67% preferring a singleton gestation. There were no significant differences in mean age, partner age, marital status, education or religious affiliation between groups (Table). The ideal family size was significantly higher in women desiring a multifetal gestation, 2.5 children vs 2.2, p=0.03. Women who preferred multifetal gestation were less likely to have an income >

\$100,000 per year, 63% vs 85%, p=0.04. Women with insurance coverage for infertility who were aware of their benefits (n=32) were more likely to prefer a singleton gestation, with 22% desiring a multifetal gestation and 78% desiring a singleton.

**CONCLUSIONS:** Multifetal gestations have a significantly increased risk of maternal and neonatal morbidity and mortality. Despite this, one third of women presenting for infertility care reported multifetal gestation as the ideal treatment outcome. Interestingly, patients with a higher income and insurance coverage for fertility care are more likely to desire singleton gestation. This data suggests that patients perceive multifetal gestation as a cost-effective treatment strategy. Future research should evaluate if improved access to insurance coverage decreases multiple pregnancy rates.

**CUTTING THE COST OF PARENTHOOD: THE EFFICACY AND COST SAVINGS OF COMPOUNDED FOLLICLE STIMULATING HORMONE.** Alexander J. Tatem, MD,<sup>a</sup> J. Abram McBride, MD,<sup>b</sup> Joie Guner, MD, MSc,<sup>c</sup> Jonathan A. Beilan,



Clinical Analysis of Ovarian Stimulation	Patient Groups	Total (N)	Cycles Cancelled		Mature Eggs	
			Due to Poor Response (total ((%))		(mean (range))	
	Women Pursuing OOC	9	1 (11.1%)		16.8 (5-39)	
	Women Pursuing IVF	25	4 (16%)		12.7 (4-36)	
	Women Pursuing OOC and IVF Combined	34	5 (14.7%)		13.9 (4-39))	
Clinical Analysis of IVF Outcomes	Women with IVF and Pregnancy Outcomes	Fertilized Eggs Per Cycle (%)	Blasts/Embryos (mean (range))	Embryo Transfers	Pregnancy	Live Birth
	21	74.1%	4.3 (0-13)	8	5 (3 ongoing)	1
Cost Analysis		B-FSH	C-FSH			
	Price per unit	\$2.2	\$0.32			
	Units per vial	450	1500			
	Price per vial	\$988.38	\$480			
	Price per average IVF cycle (2800 IU)	\$6,160	\$896.00			

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**OBJECTIVE:** Injectable follicle stimulating hormone (FSH) provides ovarian stimulation (OS) in women undergoing both in vitro fertilization (IVF) and oocyte cryopreservation (OCP). Brand FSH (B-FSH) is prohibitively expensive and can be a major roadblock to couples pursuing fertility treatment. Introduced in late 2017, compounded FSH (C-FSH) offers a cost-effective alternative to B-FSH with promising early clinical results. Here we provide the first analysis of the clinical efficacy and cost-effectiveness of C-FSH in women pursuing OCP and IVF.

**DESIGN:** Retrospective chart review identified all females receiving C-FSH for IVF or OCP from late 2017 to present.

**MATERIALS AND METHODS:** Clinical outcomes including oocyte retrieval rates, fertilizations rates, blast/embryo yield, pregnancies and live births were evaluated. All C-FSH prescriptions were obtained through the same specialty compounding pharmacy in Houston, TX. The average cost of B-FSH was derived from the top 9 commercial pharmacies listed on [www.GoodRx.com](http://www.GoodRx.com) and compared to C-FSH to determine the estimated cost of a typical course of therapy.

**RESULTS:** 34 female patients (mean age 35.3) initiated IVF or OCP. 29 women showed good response resulting in a mean retrieval of 12.75 (4-36) mature oocytes. Of the 21 women pursuing IVF, we observed a 74% fertilization rate and a mean yield of 4.3 (0-13) mature blastocysts per cycle. 8 total embryo transfers were performed. 6 of these were frozen transfers with single cryopreserved day 6 blastocysts. 1 transfer consisted of 3 fresh day 3 embryos and another consisted of 2 fresh day 5 embryos. These have resulted in 5 singleton pregnancies with 3 ongoing and 1 live birth. Cost analysis demonstrated significantly lower cost for C-FSH (\$0.32) compared to B-FSH (\$2.20).

**CONCLUSIONS:** In this novel analysis, C-FSH therapy showed excellent OS of women undergoing IVF and yielded several pregnancies, validating the clinical effectiveness of C-FSH. Compared to B-FSH, C-FSH provides unprecedented cost savings to patients undergoing FSH therapy and may allow some couples to achieve parenthood who otherwise would be prohibited by cost.

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**OBJECTIVE:** To identify factors correlated with fertility patients' likelihood of stopping, or considering stopping, treatment before achieving pregnancy.

**DESIGN:** Online, quantitative-qualitative survey of patients' experience with infertility treatment.

**MATERIALS AND METHODS:** Patients with a history of infertility treatment (ovulation induction with or without intrauterine insemination [OI/IUI], or *in vitro* fertilization [IVF]) were invited to take the survey. In addition to questions about considering or discontinuing treatment before achieving pregnancy, the survey addressed demographic factors suspected of affecting this decision. Associations between demographic factors and treatment discontinuation (or consideration) were evaluated by chi-square or logistic regression with independent variable ordinally coded, as appropriate.

**RESULTS:** Among 291 completed surveys, 91 (31%) patients never considered discontinuing treatment, 69 (24%) patients discontinued fertility treatment without pregnancy, and 131 (45%) considered quitting but did not or resumed treatment after a break of <1 year. Compared with patients treated with OI/IUI only, patients who underwent ≥1 IVF cycle were less likely to consider quitting (64% vs 77%; p=0.014) or to quit treatment unsuccessfully (40% vs 58%; p=0.004). Higher education level was associated with a decline in the probability of considering treatment discontinuation (91%, high school only vs 58%, doctoral degree; p=0.014) but was unrelated to actual discontinuation (p=0.97). Patients with annual household income ≤\$50,000 were somewhat more likely to consider discontinuing treatment (80% vs 68%; p=0.25) or to actually do so (54% vs 39%; p=0.32). A diagnosis of diminished ovarian reserve was not associated with considering quitting or doing so, despite a poorer prognosis than other patients of comparable age. There were also no trends associated with age, extent of insurance coverage (for IVF, for OI/IUI only, or for none), starting treatment with Ob/Gyn or at IVF center, or number of OI/IUI or IVF cycles completed. Most patients discontinuing treatment (76%) did so for financial reasons (58%), psychological reasons, including treatment fatigue, (64%), and 46% indicated both psychological and financial reasons.

**CONCLUSIONS:** IVF patients are less likely to discontinue treatment before achieving pregnancy than patients who undergo OI/IUI only, with most discontinuations due to financial and/or psychological reasons.

**SUPPORT:** Study sponsored by EMD Serono, Inc. (a business of Merck KGaA, Darmstadt, Germany), Rockland, MA, USA.

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#### FACTORS ASSOCIATED WITH A PATIENT'S DECISION TO DISCONTINUE FERTILITY TREATMENT BEFORE ACHIEVING PREGNANCY.

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## PREGNANCY LOSS

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### PROTEOMIC SIGNATURES OF EPIGENETIC AND TRANSCRIPTION REGULATORS ARE PIVOTAL IN CONTROLLING PATERNAL FACTORS IN RECURRENT PREGNANCY LOSS.

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**OBJECTIVE:** Recurrent pregnancy loss (RPL) is a problem often experienced with embryo loss within first trimester of gestation while in fifty percent of cases the cause remains unknown [1]. The epigenetic machinery of the spermatozoa is tailored in a way to meet the demands of the highly specialised sperm cell while the integrity of the sperm epigenome is essential for initiation and maintenance of a successful pregnancy. Increased oxidative stress has been a cause for damaged chromatin, proteins and lipids [2]. The objective is to investigate and identify altered proteomic signatures that control paternal factors post-fertilisation in RPL patients.

**DESIGN:** Comparative proteomic analysis to identify epigenetic and transcriptional proteins in RPL patients.

**MATERIALS AND METHODS:** After excluding subjects with any kind of known abnormalities as well karyotype abnormalities, a total of 20 well phenotyped male partners of women with RPL less than 10 weeks of gestational age were included in this study. 16 known fertile donors were included as controls. The samples were homogenised and subjected to high-throughput proteomic analysis using in-gel digestion through 2D-DIGE MALDI-TOF as well as in-solution Q-TOF analysis. A p value < 0.05 was considered statistically significant. Key proteins after pathway analysis were validated by western blotting.

**RESULTS:** The findings of the study indicate six proteins to be differentially expressed based on 2D-DIGE analysis that includes (HSPA2, GPX4, GSTM5, CC7A, TF3C1, ZN248 and JIP4) while based on Q-TOF proteomic analysis a total of 23 proteins were differentially expressed in RPL patients mainly includes (CLUS, CATSPER1, DAZ1, IGF2 mRNA binding protein 1,2 and 3).

**CONCLUSIONS:** Although low sample size may be considered as a limitation of study, our data suggests that altered proteins identified play a pivotal role in epigenetic programming and transcriptional regulation of paternal factors both during spermatogenesis and early development. In addition, although we cannot confirm these signature differences at proteomic level as an independent cause for unexplained RPL, the findings of the study are interesting and impose a better understanding of their biological implications.

Department of Science and Technology, Govt. of India.

Council for Scientific and Industrial Research.

**References:** 1. Practice Committee of the American Society for Reproductive Medicine., Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril, 2012;98:1103-11.

2. Mohanty G, Swain N, Goswami C, Kar S, Samanta L., Histone retention, protein carbonylation, and lipid peroxidation in spermatozoa: Possible role in recurrent pregnancy loss. Syst Biol Reprod Med, 2016;62:201-12.

**SUPPORT:** University Grants Commission, Govt. of India.

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### PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) REDUCES MISCARRIAGE AND IMPROVES LIVE BIRTH RATES IN RECURRENT PREGNANCY LOSS PATIENTS.

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**OBJECTIVE:** Recurrent pregnancy loss (RPL) is a diverse syndrome with many causes, the most common being embryonic aneuploidy. PGT-A should thus improve outcomes in RPL patients, but studies conflict as to whether

	Infertile w/ PGT-A N=3975	RPL w/ PGT-A N=660	RPL w/o PGT-A N=101	P-value
Mean oocyte age in years (SD)*	36.2 (3.4)	36.7 (3.4)	34.9 (4.8)	< .01
<b>Outcome †</b>				
Clinical Pregnancy per ET	2859 (72%)	480 (73%)	62 (61%)	0.01
Ongoing Pregnancy per ET	2670 (67%)	431 (65%)	56 (55%)	0.03
Live birth per ET	2524 (63%)	408 (62%)	42 (41%)	< .01
Clinical loss per pregnancy	335 (12%)	72 (15%)	20 (32%)	< .01
Total loss per pregnancy	641 (20%)	124 (23%)	34 (44%)	< .01

\*ANOVA analysis.

†Chi-squared analysis.

PGT-A reduces clinical losses or improves live birth. This study seeks to determine if PGT-A improves outcomes in RPL patients.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Patients undergoing their first IVF cryo-all cycle followed by single embryo transfer (SET) from 2012 to 2018 were reviewed. Patients with  $\geq 2$  losses were considered to have RPL independent of other diagnoses. Outcomes from first cryo-SETs were recorded. Clinical pregnancy was defined as having a gestational sac. Ongoing pregnancy was defined as delivery or ongoing gestation beyond 8 weeks. There were 3 patient groups: Infertile using PGT-A, RPL using PGT-A, and RPL not using PGT-A. These divisions empower fundamental questions: 1. Does PGT-A use in RPL reduce clinical loss rates and/or raise delivery rates; 2. Does it normalize outcomes to the same level as infertile controls?

**RESULTS:** 3975 infertile and 660 RPL patients had euploid SETs. An additional 101 RPL patients underwent frozen SET without PGT-A. Clinical pregnancy rates were higher in RPL patients using PGT-A (73%) than those not (61%) demonstrating a positive impact of selection. In fact, they normalized to that of infertile controls (72%). Most importantly, PGT-A reduced clinical loss risk amongst RPL patients. (32% vs 15%). The risk remained higher than infertile controls (15% vs 12%) indicating that aneuploidy is not the lone source of RPL. Ultimately the higher initial pregnancy rate and lower loss risk increased live birth rates in the RPL PGT-A group to 62% compared to those not using PGT-A (41%).

**CONCLUSIONS:** PGT-A use in RPL patients significantly raises clinical pregnancy rates while reducing loss rates and provides a 50% relative increase in delivery rates (62% vs 41%). While clinical loss rates are reduced, they remain 3% higher than in infertile controls. These findings reflect that much of RPL may be attributed to aneuploidy, but that other factors also lead to RPL. PGT-A is a useful adjunct in the care of patients with RPL. While our large sample size provides powerful insight into this question, definitive resolution awaits class I data.

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### HIGH ODDS OF ANEUPLOIDY IN RECURRENT PREGNANCY LOSS POPULATION IRRESPECTIVE OF FINDINGS ON TRADITIONAL EVALUATIONS.

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**OBJECTIVE:** To predict the probability of aneuploidy in the products of conception (POC) in the recurrent pregnancy loss (RPL) population based on the gestational age (GA) of pregnancy loss, maternal age, and positive findings on traditional RPL investigations.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Women with 2 or more pregnancy losses that underwent cytogenetic testing on their POC were eligible for analysis. Exclusion criteria included induced abortions and pregnancies missing data on GA at time of loss. Cytogenetics on the POC was performed using 24-chromosome microarray analysis. Abnormal results on traditional RPL investigations included TSH >4.0µIU/mL, hemoglobin A1c >6.4%, prolactin >23.3ng/mL, positive anti-phospholipid antibodies (lupus anticoagulant, anticardiolipin, and anti-β2 glycoproteins antibodies), abnormal parental karyotypes, or uterine anatomical defects on hysterosalpingogram (HSG) or hysteroscopy (congenital uterine anomalies or intrauterine lesions). Mixed-effects logistic regression was used to compare the probability of

aneuploidy by gestational age, maternal age, and positive findings on RPL investigations. A polynomial regression model was constructed based on the relationships of these variables.

**RESULTS:** A total of 604 miscarriages were included in the study. There was a significant relationship between the odds of aneuploidy and both maternal age and gestational age. There was a linear relationship between aneuploidy and maternal age, with a nearly 2-fold increase in the odds of aneuploidy with every 5-year increase in maternal age (OR 1.83, 95% CI 1.40-1.83). In contrast, the association between aneuploidy and gestational age was curvilinear, with a peak probability of aneuploidy with pregnancy losses at approximately 8 weeks gestation ( $p=0.02$ ). While women with positive findings on RPL investigations had a slightly lower odds of aneuploidy as compared to those with a normal work-up, this difference was minimal and did not reach statistical significance ( $p=0.18$ ).

**CONCLUSIONS:** There is an overall high rate of aneuploidy among the RPL population, even in women with positive findings on traditional RPL investigations. Across all maternal ages, the odds of aneuploidy significantly drop in pregnancy losses over 12 weeks gestation. These findings suggest that genetic testing on POC should be offered at the time of second and subsequent pregnancy losses <12 weeks gestation to all RPL patients.

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**PREGNANCY LOSS AFTER FROZEN EMBRYO TRANSFER OF BLASTOCYSTS, EXPLOID BY NEXT GENERATION SEQUENCING (NGS): IS IT THE STIMULATION FOR RETRIEVAL, THE UTERINE PREPARATION FOR FET, THE EMBRYO TRANSFER OR THE EMBRYO?** David H. McCulloh, Ph.D.,<sup>a</sup> Caroline McCaffrey, Ph.D.,<sup>b</sup> James A. Grifo, MD, Ph.D.,<sup>a</sup> NYU Langone Health, New York, NY; <sup>b</sup>New York Langone Health, NYU Fertility Center, New York, NY; <sup>c</sup>NYU Langone Prelude Fertility Center, New York, NY.



**OBJECTIVE:** The use of PGT-A and vitrification to select euploid embryos for transfer has led to improved live birth success in IVF; however, some euploid embryos fail to progress following implantation. Our objective was to compare parameters from 1) the retrieval cycle (IVF) in which blastocysts were biopsied and vitrified, 2) the frozen embryo cycle (FET<sub>u</sub>) during which the uterus is prepared for transfer, 3) the embryo transfer (FET<sub>t</sub>), and 4) the embryology (Lab) records all consolidated to determine what best predicts pregnancy loss following establishment of pregnancy by euploid embryos.

**DESIGN:** Multivariate analysis of 45 parameters from IVF, FET<sub>u</sub>, FET<sub>t</sub>, and Lab and their association with loss of pregnancies after a positive pregnancy test (+hCG).

**MATERIALS AND METHODS:** Data were collected from our electronic records for patients with transfers of thawed single euploid embryos diagnosed as euploid by NGS during the IVF cycle. Parameters from IVF (17), FET<sub>u</sub> (5), FET<sub>t</sub> (4), and Lab (19) were considered. All cases of STEET using euploid embryos tested with Next Generation Sequencing (908) were considered for analysis. Transfers without +hCG (204) and clinical pregnancies without final outcomes (205) at the time of analysis were excluded. Those 499 remaining cases with a positive pregnancy test (hCG > 5 mIU/mL) and all the required fields. 144 cases failed to progress (75 biochemical pregnancies and 69 SAb). Stepwise multiple logistic regression (152 combinations of parameters) was performed using the Akaike Information Criterion (AIC) to select parameters associated with loss of pregnancy precluding live birth following +hCG. +hCGs were considered implantations since 1) patients believe they are pregnant when they have a +hCG result and 2) no interfering hCG was administered to these patients.

**RESULTS:** Parameters associated with increased pregnancy loss after positive hCG (in descending magnitude of standard partial regression coefficient) were: more expansion of the trophoderm prior to biopsy (IVF); more serum estrogen on the day prior to progesterone administration (FET<sub>u</sub>) and more difficulty of the embryo transfer procedure (FET<sub>t</sub>). Age at retrieval, embryo grades, as well as many other parameters were not associated with pregnancy loss.

**CONCLUSIONS:** Parameters from 3 categories were associated with loss of pregnancy as biochemical pregnancies or spontaneous abortions. Of these, some are under our control: serum estradiol levels on the day prior to progesterone administration and possibly the difficulty of the transfer and the expansion of the blastocyst prior to biopsy. However, it is possible that these parameters may be aliases for other features such as rate of blastocyst development, patient weight, and/or uterine contractions or presence of blood in the cervix or uterus. No Lab parameters were associated with pregnancy loss. Also notable was the lack of association between embryo grades and pregnancy loss.

**SUPPORT:** None.

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**CHRONIC INTERVILLOSITIS OF UNKNOWN ETIOLOGY (CIUE): A CAUSE FOR REPRODUCTIVE FAILURE.**

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**OBJECTIVE:** Chronic Intervillositis of Unknown Etiology (CIUE) is a poorly understood idiopathic process that leads to massive infiltration of monocytic cells into the intervillous space, leading to recurrent pregnancy loss (RPL) and adverse pregnancy outcomes, such as intrauterine growth restriction (IUGR) and intrauterine fetal demise (IUFD). One study suggests that a higher degree of CIUE leads to worse outcomes than a lower degree of infiltration<sup>1</sup>, and studies are conflicting on whether empiric treatment improves pregnancy outcomes or not<sup>2,3</sup>. The aim of this study was to calculate the incidence of CIUE at our institution, the one and only referral center for RPL in British Columbia, Canada, and to evaluate the pregnancy outcomes based on severity of lesions as well as different empiric treatments.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** The pathology database was queried for the keywords "intervillositis" and "CIUE" between February 2006 and December 2018. Cases with a diagnosis of acute intervillositis were excluded. The histology and medical records for the cases were reviewed and pathology was re-examined using the diagnostic criteria set forth by Bos et al<sup>4</sup> to confirm diagnosis. Cases that met the Bos et al<sup>4</sup> criteria were categorized as low grade (<50% of intervillous space involved) or high grade (>50% involvement) using a modified grading scheme based on grading proposed by Rota et al<sup>5</sup>. The study was approved by the Children's & Women's Health Centre of British Columbia Research Ethic Board (H18-03623).

**RESULTS:** A total of 84 patients were diagnosed with intervillositis in the 12 year study period. Of these, CIUE was confirmed in 46 patients (54.8%) using the Bos et al<sup>4</sup> diagnostic criteria. A total of 95 specimens had previously been diagnosed with CIUE, of which 51 (53.7%) met diagnostic criteria on review. Total incidence was 0.17% (51 cases out of 29592 specimens), with a significantly higher incidence seen in 1<sup>st</sup> trimester products of conception compared with 2<sup>nd</sup> and 3<sup>rd</sup> trimester specimens (0.34% vs 0.09%;  $p < 0.0001$ ). 8 specimens had an abnormal karyotype (15.7%). 20 specimens were low grade, 11 of which were in the context of a first trimester loss, and 11 were losses after 20 weeks gestation. 27 cases were high grade, of which 21 were in the context of a first trimester loss, and 6 were 2<sup>nd</sup> and 3<sup>rd</sup> trimester losses. 29 (56.9%) CIUE diagnoses were made in the context of RPL, of which 4 had abnormal karyotype and one case was associated with multiple fetal anomalies. Empiric treatment was administered in 10 patients, including acetylsalicylic acid (ASA), low molecular weight heparin (LMWH), prednisone, and/or hydroxychloroquine.

**CONCLUSIONS:** This is the largest original case series on CIUE reported to date. Incidence rate was lower than quoted in other studies, which may be due to our rigorous diagnostic criteria. This remains an important diagnosis to make especially in the early pregnancy loss population where the incidence is highest. The number of patients receiving empiric treatment was too low to make any generalized conclusions regarding treatment.

**References:** 1. Parant O, Capdet J, Kessler S, Aziza J, Berrebi A. Chronic intervillositis of unknown etiology (CIUE): Relation between placental lesions and perinatal outcome. *Eur J Obstet Gynecol Reprod Biol.* 2009;143(1):9-13. <https://doi.org/10.1016/j.ejogrb.2008.06.012>.

2. Contro E, DeSouza R, Bhide A. Chronic intervillositis of the placenta: A systematic review. *Placenta.* 2010;31(12):1106-1110. <https://doi.org/10.1016/j.placenta.2010.10.005>.

3. Mekinian A, Costedoat-Chalumeau N, Masseau A, et al. Chronic histiocytic intervillositis: Outcome, associated diseases and treatment in a multi-center prospective study. *Autoimmunity.* 2015;48(1):40-45. <https://doi.org/10.3109/08916934.2014.939267>.

4. Bos M, Nickels PGJ, Cohen D, et al. Towards standardized criteria for diagnosing chronic intervillositis of unknown etiology: A systematic review. *Placenta.* 2018;61:80-88. <https://doi.org/10.1016/j.placenta.2017.11.012>.

5. Rota C, Carles D, Schaeffer V, Guyon F, Saura R, Horovitz J. Perinatal prognosis of pregnancies complicated by placental chronic intervillitis. *J Gynecol Obstet Biol Reprod (Paris).* 2006;35(7):711-719. [https://doi.org/10.1016/S0368-2315\(06\)76468-7](https://doi.org/10.1016/S0368-2315(06)76468-7).

**SUPPORT:** N/A.

**IS ANTIMULLERIAN HORMONE PREDICTIVE OF OUTCOMES AFTER PGT-A IN PATIENTS WITH RECURRENT PREGNANCY LOSS?**



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**OBJECTIVE:** Serum biomarkers of ovarian reserve have been utilized in non-RPL cohorts to stratify patients who may benefit from PGT-A. The goal of this study was to determine if AMH levels are predictive of outcomes in RPL patients pursuing PGT-A.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Unexplained RPL patients undergoing PGT-A at two fertility centers from 2009-2018 were included. All patients with the intent to perform PGT-A (trophectoderm biopsy and 24 chromosome screening) were included regardless of final cycle outcome. Pregnancy loss was defined as loss of pregnancy from conception (bHCG level >5mIU/mL) through twenty weeks gestation.

**RESULTS:** 157 patients underwent 191 retrievals (RET), 146 of which completed PGT-A. Patient demographics and outcomes stratified by AMH <1 ng/mL and AMH ≥ 1 ng/mL are shown in **Table 1**. Patients with AMH < 1 ng/mL were significantly older with similar BMI and number of prior losses compared to patients with AMH ≥ 1 ng/mL. Patients with AMH <1 ng/mL had fewer oocytes (p<0.01) and a higher average aneuploidy rate (p=0.02) compared to patients with AMH ≥ 1 ng/mL. In a regression model adjusting for age, AMH is not a significant predictor of having at least one euploid blastocyst (p=0.10, CI 0.97-1.43), reaching ET (p=0.97, CI 0.84-1.18), achieving pregnancy (p=0.42, CI 0.82-1.09), achieving live birth (p=0.12, CI 0.86-1.02) or undergoing pregnancy loss (p=0.42, CI 0.90-.28).

**CONCLUSIONS:** Although ovarian reserve is associated with IVF success rates, we report that RPL patients with diminished ovarian reserve (DOR) have similar likelihood of achieving pregnancy and live birth with PGT-A compared to RPL patients with AMH > 1 ng/mL. Future studies should incorporate total cycle potential in evaluation of clinical outcomes and consider a lower AMH cutoff for evaluating DOR.

Reference: None.

SUPPORT: None.

**DESIGN:** We performed a secondary analysis of a randomized trial of 300 participants<sup>1</sup> comparing mifepristone-misoprostol to misoprostol alone for EPL treatment.

**MATERIALS AND METHODS:** We tested the ability of characteristics associated with misoprostol success in a previous study<sup>2</sup>, vaginal bleeding and parity of 0 or 1, to discriminate successful from failed treatment in each arm of our study population and in the combined cohort using receiver-operating characteristic curves. We calculated the area under the curve (AUC) to quantify the ability of the score to discriminate between treatment success or failure in each arm as well as in the entire cohort. Using multivariable logistic regression, we then assessed our study population for other predictors of treatment success in both treatment groups, with and without mifepristone.

**RESULTS:** The clinical characteristics of vaginal bleeding and parity of 0 or 1 did not predict success above chance alone in the misoprostol-alone arm (AUC=0.55, 95% CI 0.44-0.65), the mifepristone pretreatment arm (AUC=0.59, 95% CI 0.45-0.72) or the combined cohort (AUC=0.56, 95% CI 0.48-0.64). No other baseline clinical factors predicted treatment success in the misoprostol-alone or mifepristone pretreatment arms individually. In the full cohort, randomization to pretreatment with mifepristone was a positive predictor of treatment success (aOR 2.51, 95% CI 1.43-4.43), while smoking was a negative predictor (aOR 0.47, 95% CI 0.23-0.97).

**CONCLUSIONS:** Pretreatment with mifepristone is a more useful intervention than applying baseline clinical factors to maximize treatment success in women undergoing medical management of EPL with misoprostol.

References: 1. Schreiber, CA, Creinin, MD, Atrio, J, Sonalkar, S, Ratcliffe, SJ, Barnhart, KT. Mifepristone pretreatment for the medical management of early pregnancy loss. *N Engl J Med.* 2018;378(23):2161-70.

2. A Creinin, MD, Huang, X, Westhoff, C, Barnhart, K, Gilles, JM, Zhang, J, et al. Factors related to successful misoprostol treatment for early pregnancy failure. *Obstet Gynecol.* 2006;107(4):901-7.

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	AMH <1 ng/mL n=42 RET	AMH ≥ 1 ng/mL n=149 RET	P-value
Age, yrs (mean ± SD, range)	37.6±4.2 (28-44)	36.2±3.6 (29-43)	0.03 <sup>1</sup>
No. of prior losses (mean ± SD, range)	3.1±1.2 (2-6)	3.1±1.0 (2-7)	0.86 <sup>1</sup>
BMI, kg/m <sup>2</sup> (mean ± SD, range)	24.1±3.6 (18-31)	23.2±3.5 (17-39)	0.15 <sup>1</sup>
No. of oocytes (mean ± SD, range)	11.1±9.2 (1-41)	18.8±8.5 (4-43)	<0.01 <sup>1</sup>
% of cycles reaching euploid ET (% , n)	48% (n=20/42)	59% (n=88/149)	0.18 <sup>2</sup>
% of cycles transferring untested embryos (% , n)	21% (n=9/42)	13% (n=20/149)	0.20 <sup>2</sup>
% of cycles not reaching ET (% , n)	31% (n=13/42)	28% (n=41/149)	0.66 <sup>2</sup>
PR per RET (% , n)	40% (n=17/42)	49% (n=73/149)	0.95 <sup>2</sup>
Avg. aneuploidy rate (mean ± SD)	69% ± 84%	53% ± 28%	0.02 <sup>1</sup>
PR per PGT-A cycle (% , n)	52% (n=14/27)	50% (n=60/119)	0.89 <sup>2</sup>
PR per euploid ET (% , n)	70% (n=14/20)	68% (n=60/88)	0.82 <sup>2</sup>
Pregnancy loss rate per pregnancy (% , n)	35% (n=6/17)	30% (n=22/73)	0.46 <sup>2</sup>
LBR per RET (% , n)	26% (n=11/42)	34% (n=51/149)	0.33 <sup>2</sup>

<sup>1</sup>Student's T Test, 2-tailed, unpaired.

<sup>2</sup>Chi-squared analysis.

**MANAGEMENT OF EARLY PREGNANCY LOSS WITH MIFEPRISTONE AND MISOPROSTOL: CLINICAL PREDICTORS OF SUCCESS FROM A RANDOMIZED TRIAL.**



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**OBJECTIVE:** To evaluate characteristics associated with treatment success in women receiving medical management for early pregnancy loss (EPL).

**THE CELLULAR ROLES OF RPL-PROTEASE A IN THE RECURRENT PREGNANCY LOSS.**



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**OBJECTIVE:** To investigate cellular functions of RPL-serine protease A on cell apoptosis, invasion, and proliferation that lead to recurrent pregnancy loss (RPL).

DESIGN: RPL-serine protease A was investigated to identify its putative substrates using proteomics and bioinformatics tools. XIAP was identified to interact with the RPL-serine protease A. XIAP was differentially expressed in a dose-dependent manner of RPL-serine protease A. To check the effect of RPL-serine protease A on cell proliferation and cell invasion, RPL-serine protease A and its mutant form were transfected into BeWo cells, and knock-out BeWo cell line was established.

MATERIALS AND METHODS: Immunoprecipitation: Flag-RPL-serine protease A and myc-XIAP were transfected into 293T cells for performing immunoprecipitation. GST pull-down assay: Recombinant GST and GST-RPL-serine protease A proteins were incubated in cell lysates overexpressed with Myc-XIAP. The bound proteins were analyzed with an anti-Myc antibody. Cell Counting Kit (CCK-8): CCK-8 assay was performed to investigate effect of RPL-serine protease A on cell proliferation. Invasion assay: Overexpressed RPL-serine protease A and its mutant form, and knock-out of RPL-serine protease A BeWo cells were divided into trans-well with the same numbers of cells to check the effect of RPL-serine protease A on cell invasion. To study the functions of RPL-serine protease A, CRISP-Cas9 system was applied for making the knock-out of RPL-serine protease A in reproductive cell lines.

RESULTS: In a previous study, we identified that an RPL-serine protease A gene is more expressed in chorionic villi from normal controls than in those from RPL patients. In this study, XIAP selected from candidate proteins identified from proteomics and bioinformatics tools, interacted with the RPL-serine protease A. Immunoprecipitation assay revealed that putative substrates such as XIAP and CPBP interacted with the RPL-serine protease A. Further investigation with GST pull-down assay indicates that RPL-serine protease A directly binds to XIAP. Exogenous and endogenous expression levels of XIAP were decreased by the RPL-serine protease A in a dose-dependent manner. It is of interest that RPL-serine protease A suppresses cell proliferation in vitro, and the proliferation rate of RPL-serine protease A knock-out cells was significantly higher than that of wild type cells. Overexpressed RPL-serine protease A stimulates BeWo cell invasion.

CONCLUSIONS: RPL-serine protease A interacts with XIAP, and expression of XIAP was decreased by RPL-serine protease A. Through these mechanisms, trophoblast apoptosis and proliferation may be regulated in placenta. The molecular functions of RPL-serine protease A in promoting cell proliferation needs to be investigated.

SUPPORT: This study was supported by the Ministry of Health & Welfare of the Republic of Korea (grant numbers, HI18C0378) through the Korea Health Industry Development Institute.

TABLE 1. RISK OF SAB STRATIFIED BY AMH LEVEL

AMH GROUP	UNADJUSTED IRR (95%CI)	ADJUSTED IRR (95%CI)
Low	1.9 (1.0, 3.6)*	1.6 (0.8, 3.1)
Normal (reference)	-	-
High	1.0 (0.5, 2.2)	1.3 (0.6, 2.8)
<b>AMH PERCENTILE</b>		
≤10 <sup>th</sup> (AMH≤0.4)	2.1 (1.1, 3.9)*	1.7 (0.9, 3.4)
≤25 <sup>th</sup> (AMH≤1.1)	1.5 (0.9, 2.7)	1.2 (0.7, 2.2)
≥75 <sup>th</sup> (AMH≥6.0)	0.7 (0.4, 1.4)	0.9 (0.4, 1.9)
≥90 <sup>th</sup> (AMH≥13.0)	0.9 (0.4, 2.2)	1.2 (0.5, 2.8)

\*P<0.05.

had lower incidence of SAB (15.6% and 16.3%, respectively) compared to those in the LOW AMH group (29.7%). However, after adjusting for age, the risk difference was no longer statistically significant.

Table 1 summarizes the adjusted and unadjusted IRR for SAB utilizing the NORMAL group as a reference. After adjusting for age, AMH was not associated with risk of SAB. There was also a trend toward higher SAB risk in women with AMH below the 10<sup>th</sup> percentile (AMH≤0.4), a finding that lost its significance in the adjusted models.

CONCLUSIONS: In women pursuing Gn-IUI treatment, lower AMH does not appear to be an independent risk factor for SAB. Therefore, younger women with lower ovarian reserve should not be counseled that they are at risk of worse early pregnancy outcomes compared to their age-matched counterparts with normal or high ovarian reserve.

References: 1. Gleicher, N., et al., *Definition by FSH, AMH and embryo numbers of good-, intermediate- and poor-prognosis patients suggests previously unknown IVF outcome-determining factor associated with AMH.* J Transl Med, 2016. 14(1): p. 172.

2. Hsu, J.Y., et al., *Müllerian-Inhibiting Substance/Anti-Müllerian Hormone as a Predictor of Preterm Birth in Polycystic Ovary Syndrome.* J Clin Endocrinol Metab, 2018. 103(11): p. 4187-4196.

3. Steiner, A.Z., et al., *Association Between Biomarkers of Ovarian Reserve and Infertility Among Older Women of Reproductive Age.* JAMA, 2017. 318(14): p. 1367-1376.

SUPPORT: None.

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### ANTI-MULLERIAN HORMONE (AMH) AND SPONTANEOUS ABORTION (SAB): IS AMH AN INDEPENDENT RISK FACTOR FOR SAB IN GONADOTROPIN-IUI CYCLES?

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OBJECTIVE: Emerging studies suggest that in infertile women undergoing IVF, AMH levels are associated with adverse obstetric outcomes, though the data remain inconclusive [1, 2]. Our objective was to evaluate if AMH is independently associated with risk of SAB among women undergoing gonadotropin-intrauterine insemination (Gn-IUI) cycles.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: *Intervention:* 1861 Gn-IUI cycles from 821 women were analyzed (11/2007 to 3/2019). Cycles were stratified by the following AMH (ng/ml) serum concentration cutoffs, based on previously published literature [3]: *LOW* (<0.7), *NORMAL* (0.7-8.4), and *HIGH* (≥8.5).

*Outcome measures:* Rate of SAB, defined as pregnancy loss following sonographic confirmation of clinical pregnancy, within each AMH group.

*Statistics:* Fisher's exact or  $\chi^2$  tests were used as appropriate. Multilevel mixed-effects Poisson regression models, adjusted for age, were used to determine the incidence risk ratios (IRR) for SAB within each AMH group. P-value <0.05 was considered significant.

RESULTS: The mean (SD) age of the study population was 35.4 (4.0) years with mean body mass index (BMI): 25.1 (5.2) kg/m<sup>2</sup>. The median (IQR) AMH value was 1.9 ng/mL (0.7,4.5) with 24%, 64%, and 12% of the women categorized into the *LOW*, *NORMAL*, and *HIGH* AMH groups, respectively.

Clinical pregnancy rates/cycle were: 8.2% 12.4%, and 19.0% for *LOW*, *NORMAL*, and *HIGH* AMH groups, respectively (p<0.001). The overall SAB rate was 18.1%. Women in the *NORMAL* and *HIGH* AMH groups



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### CHARACTERISTICS OF FIRST TRIMESTER MISCARRIAGES ASSESSED BY CHROMOSOMAL ANALYSIS OF PRODUCTS OF CONCEPTION WITH NEXT GENERATION SEQUENCING.

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OBJECTIVE: Chromosomal abnormalities are the major cause of early pregnancy loss. Chromosome testing of products of conception (POC) provides valuable information for counseling and clinical managing of patients. We previously showed that next generation sequencing (NGS) can be utilized as a technique demanding lesser specimen with a lower failure rate, higher resolution, and shorter turnaround time than conventional karyotyping which is requiring labor-intensive and time-consuming cell culture with possible maternal cell contamination. We aimed to assess the efficacy of NGS method for chromosomal analysis of POC. In addition, we attempted to identify any associations between the incidence of chromosomal abnormalities and the profile of patients as well as fetal development in an assisted reproductive technology (ART) program.

DESIGN: Retrospective study with a single reference genetic laboratory.

MATERIALS AND METHODS: Total of 131 consenting patients with first trimester miscarriages after vitrified-warmed embryo transfer were involved. POC samples were obtained by dilation and curettage between 7 to 10 gestational weeks. Chorionic villi were isolated under a dissecting microscope, subsequently processed for NGS chromosomal analysis. Incidence of each chromosomal abnormality was reported and evaluated according to the patient profile, such as maternal age, previous history of miscarriage and fetal development. Finally, frequency of mosaics was also assessed.



**RESULTS:** After NGS analysis, 28 cases (21.4%) were found to be normal, and the remaining 103 (78.6%) were abnormal, including 10 (7.6%) mosaics. Among normal karyotypes, ratio of female to male was 1.15 (15/13). Trisomies were the most common abnormalities except for the chromosome X monosomy (10.7%). Aneuploidy of chromosome 22 (20/113, 17.7%), 15 (16/113, 14.2%), 16 (16/113, 14.2%), X (13/113, 11.5%) and 21 (11/113, 9.7%) including overlaps, were most frequently involved. Mean maternal age of chromosomally abnormal cases was significantly higher than that of normal karyotypes ( $39.0 \pm 18.5$  vs  $36.9 \pm 16.5$  years,  $P < 0.05$ ). Patients with more than equal 3 previous miscarriages showed a significantly lower rate of abnormalities than those with  $< 3$  miscarriages (28.6% vs 81.5%,  $P < 0.01$ ). Rate of abnormalities with positive fetal cardiac activity was not different from that of anembryonic pregnancies (80.0% vs 76.1%), although fetal cardiac activity was detected in all the 45 XO cases. Interestingly, however, mosaic abnormalities were significantly more often detected in anembryonic pregnancies than the other (15.2% vs 3.5%,  $P < 0.05$ ).

**CONCLUSIONS:** With more conclusive and accurate results and higher resolution by NGS, we were able to characterize early pregnancy loss after ART, demonstrating relatively high rate of abnormalities with gender ratio being close to 1. Patients with repeated pregnancy loss showed lower chromosomal abnormalities indicating other causes for miscarriages in this group of patients. A higher incidence of mosaics detected in anembryonic pregnancies warrants further investigation.

**SUPPORT:** None.

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#### **VITAMIN D INSUFFICIENCY IS THE RISK FACTOR FOR HYPERHOMOCYSTEINEMIA DERIVED FROM MTHFR C677T GENE POLYMORPHISM IN WOMEN WITH RECURRENT PREGNANCY LOSSES.**



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**OBJECTIVE:** Vitamin D insufficiency, methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism, and hyperhomocysteinemia have been reported as risk factors for recurrent pregnancy losses (RPL). However, the relationship among vitamin D, homocysteine, and MTHFR C677T gene polymorphism in women with RPL remain unknown. In the current study, we aim to investigate whether the MTHFR gene polymorphism affects the levels of homocysteine and 25 (OH) vitamin D as well as immune-parameters in women with RPL.

**DESIGN:** This study was a cross-sectional study of 837 women with RPL at a university hospital.

**MATERIALS AND METHODS:** Total 837 women with unexplained RPL were registered, and MTHFR C677T genotypes (homozygous (TT), heterozygous (CT) and wild (CC)) were investigated by PCR. Biochemical tests were used to determine plasma homocysteine and serum 25 (OH) vitamin D levels, and natural killer (NK) cell cytotoxicities were analyzed by the flow cytometry. Data were analyzed by MTHFR C677T genotypes.

**RESULTS:** The level of 25 (OH) vitamin D in the TT group was significantly lower compared to CT and CC groups ( $p < 0.05$ ), while the level of homocysteine in the TT group was significantly higher than the CT and CC groups ( $p < 0.01$ ). NK cytotoxicities of TT group was significantly higher than those of CC but not CT group ( $p < 0.01$ ). There was a significant negative correlation between the levels of 25 (OH) vitamin D and homocysteine in the TT group ( $r = -0.357$ ). In multivariate analysis, 25 (OH) vitamin D insufficiency ( $< 30$  ng/ml) was an independent risk factor for hyperhomocysteinemia (adjusted odds ratio 1.89, 95% CI 1.41–2.52).

**CONCLUSIONS:** Both MTHFR C677T gene polymorphism and vitamin D insufficiency may involve in the pathogenesis of unexplained RPL via hyperhomocysteinemia. It is speculated that lowering the homocysteine level may improve the reproductive outcome in women with RPL.

#### **PROFESSIONAL DEVELOPMENT**

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#### **SURGICAL SIMULATION SUPPLEMENTS REI FELLOWSHIP TRAINING.**



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**OBJECTIVE:** To characterize interest and skill in minimally invasive reproductive surgery among Reproductive Endocrinology and Infertility (REI) fellows and the utility of an intensive “boot camp” in improving performance of select surgical tasks.

**DESIGN:** Prospective evaluation of 40 REI fellows during the 2-day 2019 SRS-SREI boot camp.

**MATERIALS AND METHODS:** Surveys collected data on fellow demographics, prior surgical and IVF experience, and perceived competency with reproductive surgery. Surveys were administered before, immediately after, and 1 month after the boot camp. Simulations focused on laparoscopic suturing/knot tying using both box trainers and cadavers, robotic suturing, and operative hysteroscopy. Wilcoxon signed-rank tests and rank-sum tests were used to compare suturing times for a given fellow over time and changes in suturing time across fellows by year of training, respectively. Spearman correlation coefficients assessed associations between prior clinical experience and surgical skill.

**RESULTS:** Forty fellows (25 first, 11 second, and 4 third year) provided data, representing 72% of REI fellowship programs in the USA. Fellows reported an average of 15 hours of prior simulation experience for conventional laparoscopy, 8 hours for robotics and 5 hours for hysteroscopy. Prior to the boot camp, most fellows felt prepared to perform hysteroscopy (100%) and conventional laparoscopy (82%), but only a minority felt prepared to perform robotic surgery (46%) or tubal anastomosis (15%). Significant improvement was seen across all levels of training in laparoscopic suturing tasks (box trainers): by 44 seconds (sec) for running suture, 82 sec for intracorporeal knots, and 71 sec for extracorporeal knots ( $p < .0001$  for all comparisons). The magnitude of improvement was significantly higher for first year fellows as compared to their second and third year peers (60 sec vs 28 sec running suture improvement,  $p = .04$ ). There were no strong associations observed between fellowship IVF case volume and the surgical skill of the fellow (all Spearman correlation coefficients  $< 0.34$ ). Interest in incorporating reproductive surgery into subsequent clinical practice was high when assessed immediately after the boot camp using a 5-point Likert scale and did not change when reassessed one month later (all  $p > 0.36$ ).

**CONCLUSIONS:** Given the heterogeneous training in reproductive surgery among REI fellowship programs, a surgical boot camp may be useful in enhancing surgical skill among REI fellows. Improvements in laparoscopic suturing were most significant for first year fellows. Increasing IVF volume was not associated with less surgical skill.

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#### **CLINICAL EXPOSURE IN OB/GYN RESIDENT TRAINING PROGRAMS IN THE UNITED STATES TO INFERTILITY CARE FOR LOW RESOURCE AND UNDERSERVED COMMUNITIES.**



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**OBJECTIVE:** Assess exposure of US OB/GYN residents to the provision of clinical infertility care for low resource and underserved communities.

**DESIGN:** Cross-sectional survey.

**MATERIALS AND METHODS:** An anonymous, self-administered 28 question survey was emailed to REI division directors or REI residency rotation directors affiliated with ACGME accredited OB/GYN residency programs. Respondents answered questions regarding REI practice and residency demographics, the presence of clinical programs designed to improve access to care, resident involvement in such programs, and perceived barriers to expanding access to care. Responses were analyzed descriptively and through logistic regression analysis using STATA software, with significance defined as  $p < 0.05$ .

**RESULTS:** The response rate for the survey was 30% (80/270). Of respondents, average OB/GYN residency size was 6.1 graduating residents per year.

Residents spent an average of 7.2 weeks rotating through REI during a 4-year residency. 38% (n=30) of practices had an affiliated REI fellowship. Less than half of OB/GYN residency programs (39%, n=31) responded have an associated REI clinic in which OB/GYN residents provide direct infertility care to populations who are medically underserved or unable to afford infertility services. The majority of clinics were held either at a resident GYN clinic (52%, n=16) at their primary institution or at a county / public medical center (39%, n=12). Frequency of clinic and services offered in trainee clinics varied; 100% (n=31) offered some form of diagnostic evaluation, 84% (n=26) provided treatment with clomiphene / letrozole, 16% (n=5) with gonadotropins, 19% (n=6) IUI, and 10% (n=3) IVF. 74% (n=23) performed laparoscopy for tubal disease and 19% (n=6) offered tubal reversal surgery. Size of residency program, REI practice setting (academic / hybrid / private), size of practice, geographic region, location in an IVF insurance mandated state, or presence of a REI fellowship was not significantly predictive of the presence of a trainee clinic. Regarding barriers encountered in the provision of fertility services to patients who are medically underserved or unable to afford infertility care, the majority of respondents cite prohibitive cost of treatment (97%), lack of insurance or public health coverage (97%), difficulty for patients with low income to qualify for loans or other financing plans (61%), patient language (61%) and health literacy (58%) as barriers to expanding access to care. An additional percentage cite low level of interest or support from hospital administration (29%), clinicians in practice (6.5%) and limited availability of trainees (6.5%).

**CONCLUSIONS:** In addition to underscoring the limited infertility care available to low resource populations in the United States, the findings indicate significant educational gaps among OB/GYN residency programs in exposure to infertility and its clinical management in low resource and underserved communities.

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#### **CURRENT ONCOLOGY TRAINING PROGRAMS LACK ADEQUATE EDUCATION IN FERTILITY PRESERVATION COUNSELING.**

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**OBJECTIVE:** Given that oncologic treatments can cause damage to reproductive organs leading to infertility, multiple national organizations recommend that all health care providers counsel patients on risk of infertility and fertility preservation options. Studies demonstrate a lack of counseling and referral to reproductive services in this population. Our aim was to develop an original survey for resident and fellow physicians in oncology subspecialties to assess their attitudes, awareness and knowledge on fertility preservation and identify barriers in training.

**DESIGN:** Questionnaire-based observational study.

**MATERIALS AND METHODS:** An IRB-approved survey study was electronically distributed to all ACGME-accredited programs in gynecologic oncology (GO), radiation oncology (RO), surgical oncology (SO), hematology oncology (HO) and pediatric oncology (PO) in the United States. The survey was distributed between January and April 2019. The questionnaire assessed attitudes and knowledge about fertility counseling and preservation for the newly diagnosed oncology patient. Comparisons between groups were evaluated with chi-squared tests and pairwise comparisons with significance defined as  $p < 0.05$ .

**RESULTS:** Two hundred and sixty-eight surveys were completed (GO: n=25; HO: n=93; PO: n=66, RO: n=60; SO: n=6). All respondents agreed that oncologists should be responsible for disclosing treatment effects on fertility; however, only 51% (n=119) responded that they often or always personally counsel patients on the impact of treatment on fertility. GO was more likely to refer patients >50% of the time to fertility preservation counseling compared to HO ( $p = 0.017$ ) or RO ( $p = 0.022$ ). RO was also significantly more likely than HO ( $p = 0.009$ ) or PO ( $p = 0.007$ ) to consider a patient's future fertility when planning treatment. Among all respondents, the most common reason infertility risk was not discussed was poor prognosis (41.6%). Furthermore, 61% of all respondents reported that there is no specific person in their office setting responsible for these discussions. Most trainees did not feel their program prepared them for counseling on fertility preservation (55.5%, n=122). When asked what materials would

be helpful to increase learning, lectures (48%, n=105) and practice bulletins (43%, n=95) were the most common answers.

**CONCLUSIONS:** Our study demonstrates that while all residents and fellows in oncology training programs believe that oncofertility counseling is important, they lack the adequate education and resources to do so. There is also a significant difference between subspecialties in the level of comfort in completing these discussions. Curriculum for residents and fellows should address these disparities and focus on improving patient counseling.

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#### **FERTILITY AWARENESS AND ATTITUDES AMONG RESIDENT PHYSICIANS ACROSS DIFFERENT SPECIALTIES.**

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**OBJECTIVE:** Among the general population, knowledge about natural fertility decline and the opportunity to cryopreserve oocytes is limited<sup>1-4</sup>. This limitation contributes to age-related infertility, particularly among women who choose to postpone childbearing. Although resident physicians often delay family building, little is known about their fertility knowledge or attitudes. The primary objective of this study was to compare knowledge of age-related fertility decline and oocyte cryopreservation among resident physicians in different specialties. Our secondary objective was to examine their attitudes toward family building.

**DESIGN:** National online survey.

**MATERIALS AND METHODS:** A hyperlink to an online survey was sent to program directors of ACGME accredited residency programs in the United States for Obstetrics and Gynecology (Ob/Gyn), Internal Medicine, Emergency Medicine, Family Medicine, General Surgery, Pediatrics, and Psychiatry. They were asked to forward the survey link to their respective residents. The survey consisted of three sections: 1) fertility knowledge, 2) oocyte cryopreservation knowledge, and 3) attitudes toward family building and fertility preservation<sup>5</sup>. Outcomes were compared between Ob/Gyn residents and all other specialties, both combined and separately. Wilcoxon rank sum test or Chi-square test was used to compare variables, as appropriate. Multivariable logistic regression models were used to investigate the association between the number of correct answers and specialties with and without adjustment for age, gender, race/ethnicity, PGY year, marital status, preexisting children, and history of infertility.

**RESULTS:** Of the 2,828 completed surveys, 450 (15.9%) were by Ob/Gyn residents and 2,378 (84.1%) were by residents of other specialties. The median number of correct answers was 2 out of 5 on the fertility knowledge section and 1 out of 3 on the oocyte cryopreservation knowledge section among all survey participants. The adjusted and unadjusted models showed that specialties were not significantly associated with answering these questions correctly in either section. The proportion of residents who had a child during residency or planned to have a child during residency was similar between Ob/Gyn and all other specialties, 33.9% vs. 35.5%, respectively,  $P = .50$ . Ob/Gyn residents were significantly more likely than residents of other specialties to feel "somewhat supported" or "very supported" by their program to pursue family building goals (83.5% vs. 75.8%,  $P = .0005$ ).

**CONCLUSIONS:** Resident physicians have limited knowledge of natural fertility decline and the opportunity to cryopreserve oocytes. Knowledge of these topics is similar between Ob/Gyn residents and residents of other specialties. These data suggest a need for improved fertility education, particularly within Ob/Gyn residency programs. The majority of residents do feel supported to build their families during training, particularly Ob/Gyn residents.

References: 1. Daniluk JC, Koert E. Childless women's beliefs and knowledge about oocyte freezing for social and medical reasons. *Hum Reprod.* 2016;31(10):2313-2320.

2. Hodes-Wertz B, Druckenmiller S, Smith M, et al. What do reproductive-age women who undergo oocyte cryopreservation think about the process as a means to preserve fertility? *Fertil Steril.* 2013;100(5):1343-1349.

3. Milman LW, Senapati S, Sammel MD, et al. Assessing reproductive choices of women and the likelihood of oocyte cryopreservation in the era of elective oocyte freezing. *Fertil Steril.* 2017;107(5):1214-1222.

4. Stoop D, Cobo A, Silber S. Fertility preservation for age-related fertility decline. *Lancet.* 2014;384(9950):1311-1319.

5. Lampic C, Svanberg AS, Karlström P, et al. Fertility awareness, intentions concerning childbearing, and attitudes towards parenthood among female and male academics. *Hum Reprod.* 2006;21(2):558-564.

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P-759 Wednesday, October 16, 2019 6:30 AM

### THE EFFECT OF RESIDENT PHYSICIAN INVOLVEMENT ON SURGICAL OUTCOMES AND COMPLICATIONS OF FERTILITY SURGICAL.

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**OBJECTIVE:** We sought to determine the effect of resident physician involvement in fertility surgical procedures on patient surgical outcomes and complications.

**DESIGN:** A review of fertility-specific surgical procedures in the American College of Surgeons National Surgical Quality Improvement Program (NSQIP) database was performed, followed by statistical analyses.

**MATERIALS AND METHODS:** The NSQIP database was reviewed for fertility surgical procedures from 2006 to 2012. The procedures included were: epididymectomy, spermatocelectomy, varicocelectomy +/- hernia repair, ejaculatory duct resection, vasovasostomy, vasoepididymostomy, and unlisted procedure male genital system (to capture sperm retrieval procedures).

Patient factors analyzed were: patient age, race, body mass index (BMI), morbidity probability, mortality probability, American Society of Anesthesiologists physical status classification (ASA), smoker status, alcohol usage status, history of diabetes, chronic obstructive pulmonary disease, congestive heart failure, peripheral vascular disease, cerebrovascular accident, and/or steroid usage. Outcomes examined included operative time, length of hospital stay, superficial infection, deep wound infection, wound dehiscence, urinary tract infection (UTI), and reoperation rate. Resident and non-resident groups were compared by Wilcoxon rank sum tests, followed by logistic regression, univariate, and multivariate analyses.

**RESULTS:** 924 cases were included: 309 with residents, and 615 without residents. The median post-graduate resident year was 3 (range: 0-10). There was no difference in baseline demographics between groups. On univariate analysis, mean operative time was longer with resident involvement, even after controlling for other covariates (76.2 vs 49.5 minutes,  $p=0.00$ ). Length of hospital stay was also longer in cases with resident involvement (0.41 vs 0.35 days,  $p=0.02$ ). There was no difference in superficial infections ( $p=0.57$ ) or UTIs ( $p=1.00$ ) with or without resident involvement.

**CONCLUSIONS:** While resident physician involvement in fertility surgical procedures may lengthen operative time, there were no significant differences in length of hospital stay, superficial infections, deep wound infections, wound dehiscence, UTIs, and reoperation rates. This data is reassuring for attending physicians operating with residents.

P-760 Wednesday, October 16, 2019 6:30 AM

### INFERTILITY, FERTILITY PRESERVATION, AND ACCESS TO CARE DURING TRAINING: A NATION-WIDE MULTI-SPECIALTY SURVEY OF UNITED STATES RESIDENTS AND FELLOWS.

Ange Wang, MD,<sup>a</sup> Christopher N. Herndon, MD,<sup>b</sup> Evelyn Mok-Lin, MD,<sup>c</sup> Lusine Aghajanova, MD PhD.<sup>a</sup> <sup>a</sup>Stanford University School of Medicine, Stanford, CA; <sup>b</sup>University of Washington, Seattle, WA; <sup>c</sup>REI UCSF, Center for Reproductive Health, San Francisco, CA.



**OBJECTIVE:** To investigate the prevalence of and experience related to infertility and utilization of fertility preservation during training for United States (US) medical residents and fellows.

**DESIGN:** Cross-sectional survey study.

**MATERIALS AND METHODS:** An online-based survey distributed to US postgraduate residents and fellows across medical specialties, via program directors and graduate medical offices of residency/fellowship programs.

**RESULTS:** Respondents included 732 residents and fellows, with the highest percentage in Obstetrics & Gynecology (26.0%), Pediatrics (14.1%), and Internal Medicine (13.9%). 75.5% of respondents were residents and 73.2% were PGY1-4. Respondents were 75.4% female and 18.4% male, with the most common ethnicities Caucasian (61.2%) and Asian/Pacific Islander (10.4%). 75.8% of respondents reported being married or partnered. In total, over half of respondents (56.6%) reported delaying

childbearing plans due to medical training. 51 respondents (7.0%) reported infertility, while 11 (1.5%) reported recurrent pregnancy loss (RPL). For the infertility/RPL group, 19 respondents reported undergoing IVF, 11 reported undergoing IUI, and 14 reported using oral medications for fertility purposes. For the fertility preservation group, 18 respondents reported undergoing IVF for embryo or oocyte cryopreservation. Additionally, 208 respondents (28.4%) reported that they had considered oocyte or embryo cryopreservation, though only 46 respondents underwent a fertility consultation for this purpose. Of those seeking treatment, respondents most commonly reported their own insurance or partner's insurance as the source of financial support, in addition to salary and parents/friends. Only 13.1% reported living in a state where fertility coverage is mandated by insurance. Respondents reported lack of time/flexibility (35.4%) and financial concerns (29.4%) as the top reasons for being unable to pursue either fertility consultation or treatment. The majority of respondents (65.5%) experiencing infertility/RPL or desire for fertility preservation reported that colleagues and program administration were unaware of treatments or struggles. However, of those whose challenges were known, the majority felt some degree of support by their program administrators (80.8%) and colleagues (84.4%).

**CONCLUSIONS:** The majority of residents and fellows delay childbearing due to medical training. The reported infertility rate in postgraduate medical trainees is comparable to general population, though may be underestimated as individuals may further delay childbearing until established in practice. Time/flexibility and financial concerns were identified by residents and fellows as the greatest barriers to seeking and pursuing medical assistance while in training. Most trainees facing fertility-related challenges do not share their concerns with program administrators or colleagues, but most who did so felt supported. Specific measures and awareness are needed in order to increase access to fertility services for US medical trainees.

P-761 Wednesday, October 16, 2019 6:30 AM

### UTILIZING A NOVEL RESIDENT EDUCATION INITIATIVE TO PROVIDE REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY CARE FOR UNDERINSURED WOMEN.

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**OBJECTIVE:** Few reports exist in the literature describing infertility evaluations or care in patients covered by Medicaid, but disparities in access are well documented. The objective of this study was to describe the health care received by a cohort of underinsured women with infertility after implementation of a novel quality improvement project designed to increase access to reproductive endocrinology and infertility (REI) specialists.

**DESIGN:** Retrospective observational study.

**MATERIALS AND METHODS:** We created a system for provision of infertility consultations from the REI division at an academic tertiary care institution. The Obstetrics and Gynecology (OB/Gyn) resident clinic at our institution provides care for primarily African American underinsured women. Patients are screened in by Ob/Gyn residents staffing routine gyn clinic when a patient presents with infertility. As part of their REI curriculum, Ob/Gyn residents reviewed patient charts in virtual visits under supervision of REI faculty. Patients were then provided diagnostic guidance, management recommendations and access to cost-saving research programs. Patients receive their individualized recommendations and associated costs to make informed healthcare decisions. Patient charts are maintained on a shared electronic medical record list for ongoing management. Charts from the first year of the service were reviewed for demographic and clinical information. This project was reviewed by the University of Pennsylvania Institutional Review Board and determined to be exempt as a Quality Improvement project.

**RESULTS:** Twenty-eight consultations were performed for underinsured women in the first year of service. Of these patients, 22 patients (78%) had Medicaid insurance. Two patients (7%) were seen in the REI office after initial consultation. Nine (22%) completed bloodwork and 10 patients (35%) underwent pelvic ultrasounds. Six evaluations of fallopian tube patency were completed via either imaging (hysterosalpingogram) or surgery (chromopertubation). Eight patients (28%) initiated ovulation induction. Five patients (17%) achieved pregnancy. Pelvic ultrasounds and blood work were fully covered by all insurance.

**CONCLUSIONS:** Though our resident clinic remains unable to provide standard infertility treatments like IUI and IVF to our Medicaid patients, patients were still take advantage of the robust REI division at our institution. Academic institutions may be able to connect uninsured and underinsured

patients with advice and diagnostics by utilizing resident educational opportunities to offset a portion of financial barriers.

**P-762** Wednesday, October 16, 2019 6:30 AM

**DOES LONGER EDUCATION MEAN POSTPONED PREGNANCY?** Ecem Esencan, M.D.,<sup>a</sup> Burcin Simsek, Ph.D.,<sup>b</sup> Emre Seli, M.D.<sup>a</sup> <sup>a</sup>Yale School of Medicine, New Haven, CT; <sup>b</sup>University of Pittsburgh, Pittsburgh, PA.



**OBJECTIVE:** To delineate the continually increasing participation of women in education at bachelor's, master's, and doctoral degree levels and how it correlates with changes in age of marriage, pregnancy rate after age 35, and rates of diminished ovarian reserve (DOR) diagnosis and use of donor eggs, over time in the United States (US).

**DESIGN:** Population-based epidemiologic study.

**MATERIALS AND METHODS:** Education data (between 1970-2018) were collected from records and projections reported by National Center for Education Statistics, Institute of Education Sciences, and US Department of Education. Results on percent married and age at first marriage were gathered from Current Population Survey of U.S. Census Bureau. Data on mean age of mother, mean age of mother at first birth, pregnancy and birth rates were gathered from annual National Vital Statistics Reports of Center for Disease Control and Prevention (CDC). Information on rates of DOR diagnosis and donor oocyte use among assisted reproduction technology (ART) cycles were collected from annual National Summary Reports on ART of CDC.

**RESULTS:** In 2018, prospected proportion women earning bachelor's degrees (per 10,000 female citizens) almost doubled compared to 1970 (64.8 vs 32.6;  $p < .001$ ). In the same time period, percentage of bachelor's degrees awarded to females in a given year, increased significantly (57.5 vs 43.1;  $p < .001$ ), surpassing males. Moreover, percentage of total US female population who completed four years of college raised significantly between 1970 to 2018 (8.2 to 35). This trend was followed in postgraduate education, with significant increase in the proportion of women earning master's (27.3/10,000 vs 7.9/10,000;  $p < .001$ ) and doctoral degrees (5.7/10,000 vs 0.54/10,000;  $p < .001$ ) in 2018 compared to 1970. The percentages of master's (from 38.8 to 61.0;  $p < .001$ ) and doctoral degrees (from 9.6 to 52.7;  $p < .001$ ) awarded to females also increased in the study period, both surpassing males. In the same time period the percentage of married women and median age at first marriage demonstrated an opposite trend. Less women were married (50.8% vs 61.9%) and marriages occurred at a more advanced age (27.8 vs 20.8). In parallel with this finding, an increase in mean age at first birth from 21.4 to 26.8 was observed between 1970 and 2017. Similarly, pregnancy rates of women in ages 35-39 and 40-44 more than doubled between 1980 and 2010 (0.036 to 0.077 and 0.009 to 0.019/1,000 respectively). The rise in birth rates of 1<sup>st</sup> child in those age brackets was even more dramatic (0.002 to 0.01 and 0.0003 to 0.002/1,000, respectively). In parallel, rate of DOR diagnosis in women undergoing ART raised significantly from 12% to 31% in 2005 to 2016, and number of ART cycles using donor eggs increased from 16,161 to 24,300 in the same time period.

**CONCLUSIONS:** Since 1970, participation of women in education in the US has risen significantly. This trend is paralleled by decreased rates and later occurrence of marriage as well as increasing age for childbearing, which in turn are reflected in the dramatic increase in DOR diagnosis and utilization of donor eggs in ART.

**P-763** Wednesday, October 16, 2019 6:30 AM

**MEDICAL STUDENTS' KNOWLEDGE ABOUT THIRD-PARTY REPRODUCTION.** Kajal Khodamoradi, phd student,<sup>a</sup> Fardin Amidi, phd,<sup>b</sup> Zahra Khosravizadeh, phd student,<sup>c</sup> Ali Talebi, phd,<sup>d</sup> Zahra Rashidi, phd student,<sup>c</sup>



Mohammad Hossein Ayati, MD, PhD,<sup>e</sup> Parva Namiranian, MD.<sup>f</sup> <sup>a</sup>School of Medicine, Tehran University of Medical Sciences, Tehran, Iran, miami, FL; <sup>b</sup>School of Medicine, Tehran University of Medical Sciences, Tehran, Iran, Tehran, FL, Iran (Islamic Republic of); <sup>c</sup>School of Medicine, Tehran University of Medical Sciences, Tehran, Iran, tehran, FL, Iran (Islamic Republic of); <sup>d</sup>School of Medicine, Shahrood University of Medical Sciences, Shahrood, Iran, Shahrood, FL, Iran (Islamic Republic of); <sup>e</sup>School of Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran, Tehran, Iran (Islamic Republic of); <sup>f</sup>Department of Persian Medicine, School

of Persian Medicine, Tehran University of Medical Sciences, Tehran, Iran., tehran, Iran (Islamic Republic of).

**OBJECTIVE:** infertility affects 13-15 percent of couples worldwide during their reproductive lives and its prevalence among Iranian couples is about 8%. The several techniques usually used in ART however some couples cannot have a child who is genetically connected to both of them due to medical problems, congenital conditions and reasons other than subfertility. Therefore, third-party reproduction is a medical alternative choice that has provided new hopes for infertility treatment which refers to the use of third persons' gametes, embryo or uterus. The management of infertility treatment needs to good knowledge of healthcare professionals about relevant issues of third-party reproduction. In this study, we evaluated the knowledge of medical students about third-party reproduction in Tehran University of medical sciences.

**DESIGN:** This descriptive cross-sectional study was carried in Medical university of Tehran in 2018.

**MATERIALS AND METHODS:** The questionnaire consisted of 2 parts: demographic characteristics of research units and a 26 researcher-made questionnaire about medical students' Knowledge about third-party reproduction. The validity of the questionnaire was determined by the content and face validity and the test-retest reliability of the questionnaire was established using Cronbach's alpha coefficient (0.82).

**RESULTS:** Total study participants were 329. Most of the participants were female ( $n = 178$ , 54.10%). Preponderance of the age group  $< 20$  (34.04%) was observed and approximately one fourth of the respondents were 23-26 years old. Slightly less than one fourth of the respondents had started university in 2011-2013 (24.01%) and those who became university students  $< 2011$  counted for 22.19% of study participants. Kruskal-Wallis non-parametric test showed that the difference of knowledge among different years of entering university is significant ( $p = 0.004$ ). Knowledge was significantly higher in  $< 2011$  university entrants and with the increase in the entrance year, knowledge has decreased. Mann-Whitney U test on gender and knowledge has been done showing that knowledge is not significantly different between men and women ( $p = 0.246$ ). Kruskal-wallis test in different age groups with  $p = 0.006$  shows that knowledge level is significantly higher in older participants. Among the whole respondents levels of knowledge were divided to poor, moderate and good and was investigated according to all variables.

**CONCLUSIONS:** Concerning knowledge of third-party reproduction, the age and year of entering university in students of Medical universities of Tehran were significantly related to this knowledge level. The elder the respondents were, the higher the level of knowledge was. Also, the participants who had entered university earlier had better knowledge. But there was not a significant difference between knowledge level of male and female participants. A repeat of this study is needed to confirm the observed results.

## REPRODUCTIVE IMMUNOLOGY

**P-764** Wednesday, October 16, 2019 6:30 AM

**IL-1 $\beta$  IS ASSOCIATED WITH DECREASED EXPRESSION OF GREM1 IN OBESE WOMEN UNDERGOING IN VITRO FERTILIZATION WITH INTRACYTOPLASMIC SPERM INJECTION (IVF-ICSI).** Tana Kim, MD,<sup>a</sup>



Yulian Zhao, PhD,<sup>b</sup> Elizabeth Ann Enninga, PhD<sup>b</sup> <sup>a</sup>Division of Reproductive Endocrinology and Infertility, Rochester, MN; <sup>b</sup>Department of Obstetrics and Gynecology, Mayo Clinic, Rochester, MN.

**OBJECTIVE:** To investigate obesity associated inflammatory factors in follicular fluid that may affect gene expression in cumulus cells of women undergoing IVF-ICSI.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Follicular fluid and cumulus cells were collected during oocyte retrieval in women undergoing IVF-ICSI, and grouped based on body mass index (BMI). Cytokine levels were measured from the follicular fluid using enzyme-linked immunosorbent assay (ELISA). Expression of 4 genes (GREM1, HAS2, PTGS2, VCAN), which have been positively correlated with oocyte maturity and/or pregnancy outcome in cumulus cells, was determined by quantitative reverse transcription polymerase chain reaction (RT-qPCR). Mann Whitney tests were utilized to compare cohorts by BMI and reported as medians with interquartile ranges. Cumulus cells of normal BMI (21.1 kg/m<sup>2</sup> to 23.6 kg/m<sup>2</sup>) women were cultured with IL-1 $\beta$  to investigate its impact on gene expression. Change in gene

expression following IL-1 $\beta$  was analyzed by paired t test. P values <0.05 were considered significant.

**RESULTS:** A total of 68 women were included in the ELISA analysis. Women were grouped based on BMI with 57 women having BMI <35 kg/m<sup>2</sup> and 11 women having BMI  $\geq$  35 kg/m<sup>2</sup>. Women with BMI  $\geq$  35 kg/m<sup>2</sup> had increased levels of IL-1 $\beta$  in the follicular fluid as compared to women with lower BMI (5.18 pg/mL vs 1.92 pg/mL, p=0.02).

Gene expression from cumulus cells was measured from a representative cohort based on BMI, with 6 in the normal group (BMI 21.1 kg/m<sup>2</sup> to 23.6 kg/m<sup>2</sup>) and 6 in the obese group (35.6 kg/m<sup>2</sup> to 42.0 kg/m<sup>2</sup>). The obese group had a significantly lower relative expression of GREM1 compared to the normal group (0.51 [0.38, 0.74] vs 1.01 [0.66, 1.40], p=0.03). No differences were seen with HAS2 (0.73 [0.49, 1.17] vs 1.06 [0.65, 1.78], p=0.39), PTGS2 (1.54 [1.09, 3.11] vs 0.58 [0.47, 4.19], p=0.22), or VCAN (0.88 [0.61, 1.56] vs 0.93 [0.63, 1.56], p=0.82).

Given increased levels of IL-1 $\beta$  in follicular fluid of obese women, cumulus cells from women with normal BMI were cultured with IL-1 $\beta$  to investigate the impact on GREM1 expression. Following IL-1 $\beta$  incubation, GREM1 levels significantly decreased in cumulus cells of normal BMI women (p=0.02) similar to the obese cohort.

**CONCLUSIONS:** Compared to women with normal BMI, obese women had higher levels of pro-inflammatory IL-1 $\beta$  in the follicular fluid and had lower cumulus cell expression of GREM1. Decreased expression of GREM1 in cumulus cells of normal BMI women following culture with IL-1 $\beta$  suggests that this pro-inflammatory cytokine may play a role in suppressing GREM1 levels in obese women. These molecular discrepancies may give insight into physiologic differences in oocyte development and cycle outcomes in obese women undergoing IVF-ICSI. Further studies are required to correlate these molecular findings to clinical outcomes.

**P-765** Wednesday, October 16, 2019 6:30 AM

#### **IMPACT OF YOGA BASED LIFESTYLE INTERVENTION ON QUALITY OF LIFE, DEPRESSION AND SPERM OXIDATIVE DNA DAMAGE: A RANDOMIZED CONTROLLED TRIAL ON INFERTILE MEN WITH RHEUMATOID ARTHRITIS.**

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**OBJECTIVE:** Rheumatoid arthritis (RA), an auto-immune disease, shows a consecutive stimulus of proinflammatory cytokines, following a wide range of pathophysiological reactions, leading to increased synthesis of acute phase proteins like C - reactive protein (CRP) and dysregulation in levels of immunomodulatory soluble Human Leukocyte Antigen-G (sHLA-G) molecule. Toxic effects of Methotrexate (MTX), a major component of disease-modifying antirheumatic drugs (DMARDs), can cross blood-testis-barrier and can induce changes in sperm for men of reproductive age group like germ cell apoptosis, mutagenic changes in germline cells, permanent gonadal failure and impaired spermatogenesis. Hence, it's cytotoxic, mutagenic and teratogenic activities may have side-effects in various ways to reproductive health.

**DESIGN:** A randomized controlled trial to assess the impact of 8 weeks Yoga-based lifestyle intervention (YBLI) on quality of life (QoL), stress markers, immune and oxidative stress parameters of active RA infertile men group compared with usual-care control group.

**MATERIALS AND METHODS:** Forty six infertile males with RA were randomized into two groups: yoga (23): practicing Yoga based lifestyle intervention (YBLI) in addition to disease-modifying anti-rheumatic drugs (DMARDs) for 8 weeks; non-yoga (23): DMARDs only. All subjects were assessed pre and post intervention for erythrocyte sedimentation rate (ESR), C reactive protein (CRP), IL-6, IL-17A and soluble HLA-G levels for systemic inflammation as well as seminal reactive oxygen species (ROS), DNA fragmentation index (DFI) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels. QoL by WHO-QOL scale, depression severity by Beck Depression Inventory II scale (BDI-II), disease activity by disease activity scores (DAS28-ESR) and pain acuity i.e. visual analogue scale (VAS) were assessed.

**RESULTS:** YBLI participants showed significant improvements in disease activity, pain acuity, disability index, QoL over the control group. Mean levels of pro and anti-inflammatory cytokines showed significant reversal after YBLI. Reduction in seminal ROS levels even after 10 days of practice & these cases were followed up to 6 months which resulted in further decline in ROS, 8-OHdG and DFI as well.

**CONCLUSIONS:** Post yoga reduction in inflammatory markers results in fewer requirements of anti-inflammatory drugs. Yoga not only reduces dis-

ease severity, optimize oxidative stress levels, increases periods of remission, but also minimize usage of drugs with minimum side effects especially on sperm.

**SUPPORT:** Science & Technology for Yoga & Meditation, Department of Science & Technology, India.

**P-766** Wednesday, October 16, 2019 6:30 AM

#### **DECREASED EXPRESSION OF PD1 IN PERIPHERAL BLOOD Th17 CELLS IN WOMEN WITH UNEXPLAINED RECURRENT PREGNANCY LOSS.**

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**OBJECTIVE:** Programmed Death-1 (PD1) and PD-ligand (PDL)-1 have been reported to participate in the regulation of T cells homeostasis and peripheral tolerance and have an important role in fetomaternal tolerance during pregnancy. PD1 blockade leads to CD4+ T (especially Th1, Th17 cells) activation and proliferation, which in turn increases embryo resorption and reduces litter size in a mouse model. The goal of this study was to investigate the expression of PD1/PDL1 on CD4+ T cells of peripheral blood in women with recurrent pregnancy loss (RPL).

**DESIGN:** A prospective cohort study.

**MATERIALS AND METHODS:** Forty-five women with RPL and 12 fertile women who had at least one or more live-born infants were enrolled in this pilot study. The expression of PD1/PDL1 on CD4+ T cells in the peripheral blood, including Th1, Th17, and Treg cells were analyzed by flow cytometry. The expression of PD1/PDL1 was tested using monoclonal antibodies (mAb) to CD279 (PD1) and CD274 (PDL1). The expression of Tregs was tested using mAbs to CD45, CD3, CD4, CD25, and CD127. The expression of Th1 cells was tested using mAb to CD45, CD3, CD8, IFN-g or TNF- $\alpha$ . The expression of Th17 cells was tested using mAb to CD45, CD3, CD8, and IL-17.

**RESULTS:** The proportions of PD1+ Th17 cells (CD4+IL17+CD279+ cells out of total CD4+T cells) were significantly lower in the RPL group than controls (P<0.05). However, there are no differences in PD1+ Th1 (CD4+TNF- $\alpha$  CD279+ and CD4+IFN-g+CD279+) and Treg (CD4+CD25+CD127+CD279+) cells between the RPL group and controls.

The proportions of PDL1+ Th1 (CD4+IFN-g+CD274+ and CD4+TNF- $\alpha$ +CD274+), Th17 (CD4+IL-17+CD274+), and Treg (CD4+CD25+CD127+CD274+) cells are not different between the RPL group and controls (P>0.05, respectively).

In Th1 and Th17 cells, the proportions of PDL1+ (CD274+) cells were significantly higher than those of PD1+ (CD279+) cells in both the RPL group and controls (P<0.01 respectively). However, there were no differences in PDL1+ and PD1+ Treg cells in both groups.

**CONCLUSIONS:** Decreased expression of PD1 on Th17 cells may lead to enhanced Th17 immunity and result in the imbalance between Treg and Th17 cells in women with RPL.

**SUPPORT:** This work was partially supported by grants from the National Natural Science Foundation of China (grant numbers 81741027, 81300533, 81601276), Chinese Medical Association Clinical Medicine Research Special Fund-2017, Reproductive Medicine Young Physicians Research and Development project (17020160685, 16020220638), and Yantai Key research and development program (2017YT06000491).

**P-767** Wednesday, October 16, 2019 6:30 AM

#### **DOES SERUM ANTI-NUCLEAR ANTIBODY PREDICT OUTCOMES OF PREGNANCY AMONG WOMEN WITH A HISTORY OF RECURRENT PREGNANCY LOSS, WITHOUT COEXISTING ANTI-PHOSPHOLIPID ANTIBODY SYNDROME ? A SYSTEMATIC REVIEW AND META-ANALYSIS.**

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**OBJECTIVE:** The concept of immune-mediated Recurrent Pregnancy Loss (im-RPL) is gradually being accepted as a true clinical entity. However, there is no universally accepted screening test or a diagnostic test for this condition. Anti-nuclear antibody (ANA) screening is widely used for screening auto-immune diseases in clinical medicine. The objective of this study was to determine whether serum ANA would be a reliable marker to predict outcomes in women diagnosed with Recurrent Pregnancy Loss (RPL) without co-existing anti-phospholipid antibody syndrome (APS).

**DESIGN:** Systematic review and meta-analysis.

**MATERIALS AND METHODS:** This systematic review and meta-analysis was registered with PROSPERO. The search strategy was applied to Medline, EMBASE and Cochrane Central Register of Controlled Trials (from database inception to Oct 2018). Studies retrieved by the search and the reference lists of relevant studies were included in the review if; the study population was described as having a diagnosis of RPL; had serum ANA testing done; if there was a control group and if the outcomes of miscarriage (or live birth) were reported. Studies in women who had co-existing APS were excluded. There were no other restrictions, including ANA titer or co-interventions. The primary outcome was the miscarriage rate. Heterogeneity between studies was measured using  $I^2$  statistics. Subgroup analyses included ANA titer levels, women who had two or more previous miscarriages and those who had three or more previous miscarriages. Data were extracted using a piloted data extraction proforma and meta-analysis was performed based on a random-effects model using R Version 3.5.2 (R Core Team, 2018) and the meta (Version. 4.9-5; Schwarzer, 2019) package. We also conducted a quality assessment of all included studies.

**RESULTS:** Thirty-two studies involving 4,375 women fulfilled the inclusion criteria and were subjected to quantitative and qualitative analysis. All studies included in the analysis were case-control studies. There was a statistically significant increase in risk of miscarriages in women with positive ANA (Odds Ratio [OR] 2.99; 95% CI [2.22 – 4.04];  $I^2 = 67%$ ;  $P < 0.01$ ). Subgroup analysis also confirmed a statistically significant association of an increase in the risk of miscarriage. In women with three or more previous miscarriages, analysis confirmed OR 2.47; 95% CI [1.66 – 3.65];  $I^2 = 41%$ ;  $P < 0.01$ . In women who had two or more previous miscarriages, analysis confirmed OR 3.47; 95% CI [2.24 – 5.39];  $I^2 = 79%$ ;  $P < 0.01$ . The total heterogeneity was high with  $I^2 = 67%$ ,  $I^2 = 0.4$ ,  $p < 0.01$ .

**CONCLUSIONS:** This systematic review postulates that positive ANA in women with RPL increases the risk of further miscarriage by three-fold. This finding underscores the importance of the immune system in RPL and suggests that ANA could be useful in outcome prediction for APS negative women with RPL. This study also opens a new direction for future research into disease mechanisms, and potential 'personalized treatment option' for women for this otherwise, difficult to treat clinical condition.

**SUPPORT:** Funding support: A Statistical analysis for this project was supported by the Clinical and Translational Science Award (CTSA) from the National Center for Advancing Translational Sciences at the National Institutes of Health (NIH).

**P-768** Wednesday, October 16, 2019 6:30 AM

#### **NATURAL KILLER CELL-BASED PREDICTIVE ASSAY FOR PREGNANCY OUTCOME IN FROZEN EMBRYO TRANSFER CYCLES.**

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**OBJECTIVE:** To characterize the pregnancy-compatible phenotypic and functional changes in peripheral blood natural killer (NK) cells during frozen embryo transfer (FET) cycles.

**DESIGN:** Prospective nested cohort study.

**MATERIALS AND METHODS:** Peripheral blood was collected from patients undergoing FET cycles at three time points: 1) follicular phase, 2) day of the embryo transfer, and 3) day of quantitative serum  $\beta$ -hCG analysis. Serum progesterone, estradiol levels, and hCG were quantified. Peripheral blood NK cell phenotype and cytotoxicity were compared based on timing of the blood draw and then stratified by presence/absence of a clinical pregnancy as defined by fetal heartbeat at the time of ultrasound. For phenotypic

analysis, frozen whole blood was stained for CD45, CD3, NKp46, CD56, and CD16 and quantified by flow cytometry. Three-dimensional endovascular tube formation involving endothelial HUVECs and first trimester extravillous HTR8 cells and NK cell-specific K562 cell kill assay were used to compare NK cell cytotoxicity. ELISA assays were used to quantify VEGF-A and VEGF-C in sera from pregnant vs non-pregnant women. Continuous variables were compared by ttest or ANOVA if normally distributed and Mann-Whitney U or Kruskal-Wallis test if not normally distributed. Categorical variables were compared with Fischer's exact or Chi-square test.

**RESULTS:** 35 patients were enrolled, 15 with clinical pregnancies and 20 with negative serum  $\beta$ -hCG levels. There were no differences in age, gravidity/parity, BMI, infertility diagnosis, endometrial preparation, mode of progesterone supplementation, embryo age, number of embryos transferred, serum progesterone and estradiol, or number of PGT-A cycles in the pregnant vs. non-pregnant patient groups. When all samples were analyzed together, CD45<sup>+</sup>CD3<sup>-</sup>CD56<sup>+</sup> NK cell numbers did not change based on the timing of the FET cycle. When subjects were stratified by pregnancy status, there was an increase in CD45<sup>+</sup>CD3<sup>-</sup>CD56<sup>+</sup> NK cell population in the pregnant group on the day of serum  $\beta$ -hCG. In the tube formation assay, NK cells from non-pregnant patients caused significant tube disruption when compared to NK cells from pregnant patients. When serum from pregnant women was added to the assay, tube disruption by NK cells from non-pregnant patients was significantly reduced; whereas serum from non-pregnant women failed to protect tube formation. In the serum free K562 cell kill assay, NK cells from pregnant patients had significantly lower cytotoxicity potential compared to NK cells from non-pregnant patients. The addition of pregnancy serum decreased the cytotoxicity of non-pregnant NK cells. VEGF-A and VEGF-C levels were similar in pregnant vs non-pregnant serum; hCG was only found in serum from women who experienced clinical pregnancy.

**CONCLUSIONS:** Increase in CD45<sup>+</sup>CD3<sup>-</sup>CD56<sup>+</sup> NK cells was observed in women with detected hCG. Pregnancy status and pregnancy serum have a significant impact on the cytotoxic potential of peripheral blood NK cells. In this regard, hCG, not VEGF-A or VEGF-C, may impart a non-cytotoxic phenotype on peripheral blood NK cells.

**SUPPORT:** Supported by NIH P20 GM121298.

## **REPRODUCTIVE SURGERY**

**P-769** Wednesday, October 16, 2019 6:30 AM

#### **WEEKEND PRESENTATION IMPACTS TIMING OF ECTOPIC PREGNANCY SURGICAL MANAGEMENT AND OUTCOMES.**

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**OBJECTIVE:** To evaluate whether weekend presentation of ectopic pregnancy impacts admission from the emergency department (ED), surgical timing, and morbidity in a national sample.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** This study utilized the Nationwide Emergency Database Sample (NEDS, 2006-2011) and Nationwide Inpatient Sample (NIS, 2002-2011). Ectopic pregnancies and subsequent surgeries were identified utilizing ICD-9-CM codes stratified by day of admission (weekend vs. weekday). Time to surgery was calculated from time of admission, using variables provided by the NIS. Multivariable log-linear analyses adjusting for patient (age, race, payer status, comorbidities, income) and hospital (region, bed-size, teaching status, location) factors were utilized to assess the relationship between day of admission and our outcomes of interest. Outcomes included admission from the emergency department, same-day surgery, surgery within one day, and the need for transfusion (adjusting additionally for time to surgery). Measures of association were reported as adjusted risk ratios (RR) with 95% confidence intervals (CI).

**RESULTS:** We analyzed 296,071 ED evaluations and 376,092 inpatient surgical admissions for ectopic pregnancy. Of ED evaluations, 25.4% were seen on the weekend, with an admission rate of 43.8% compared to 42.5% on a weekday. Once admitted, weekend admissions had lower same day surgery rates compared to weekday admits (78.4% vs. 82.4%,  $p < 0.05$ ). Weekend admissions also had increased blood transfusion rates (15.1% vs. 9.2%,  $p < 0.05$ ). In multivariable analysis, patients seen on the weekend (RR 1.04, 95% CI: 1.03, 1.05  $p < 0.01$ ), older patients (RR 1.07, 95% CI: 1.04, 1.10  $p < 0.01$ ), and those with co-morbidities (RR 2.06, 95% CI: 1.98, 2.15

p<0.01) were more likely to be admitted to the hospital. Of patients admitted, those on the weekend (RR .96 95% CI: .95, .97 p<.01), black patients (RR .97 95% CI: .95, .98 p<.01), younger patients (RR .92 95% CI: .89, .94 p<.01), and those with co-morbidities (RR .86 95% CI: .84, .88 p<.01) were less likely to undergo same-day surgery for ectopic pregnancy. Similarly, patients admitted on the weekend (RR .97 95% CI: .95, .97 p<.01) and those with co-morbidities (RR .88 95% CI: .86, .89 p<.01) were also less likely to receive surgery within one day. Furthermore, patients on the weekend (RR 1.49, 95% CI: 1.46, 1.53 p<0.01) and those with co-morbidities (RR 3.90, 95% CI: 3.73, 4.09, p<.01) were more likely to have a blood transfusion during admission.

**CONCLUSIONS:** Ectopic pregnancies evaluated in the ED during the weekend are more likely to be admitted to the hospital, but less likely to undergo same day or surgery within one day of admission. Weekend admissions were independently at significantly higher risk for blood transfusions even after adjustment for timing of surgical management. Further studies are needed to understand factors such as provider staffing which may contribute to this weekend effect, and to work to mitigate this impact.

**P-770** Wednesday, October 16, 2019 6:30 AM

**NORMAL SALINE SOLUTION IS AS EFFECTIVE AS POVIDONE IODINE IN PREOPERATIVE VAGINAL CLEANSING BEFORE SHORT DURATION GYNECOLOGICAL LAPAROSCOPY.**



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**OBJECTIVE:** Laparoscopy is a minimally invasive method used for diagnostic and therapeutic purposes. Our objective was to compare the postoperative vaginal irritation symptoms and infection rates after using povidone-iodine (PI) and normal saline (NS) solution in vaginal cleansing before short duration gynecological laparoscopy.

**DESIGN:** Randomized, single-blind clinical trial.

**MATERIALS AND METHODS:** All eligible participants who scheduled for short duration gynecologic laparoscopic procedures included diagnostic laparoscopy, bilateral ovarian drilling, and tubal sterilization were invited to participate in the study. Eligible participants were randomly allocated in a 1:1 ratio to two groups. Group I "PI group" where they subjected to PI for vaginal cleansing before laparoscopy and group II "NS group" where they subjected to the standard saline solution for vaginal cleansing. Two sponges of the same size and type were used for cleansing by both preparations. The primary outcome of the study was the difference in the rate of self-reported postoperative vaginal irritation symptoms after using PI and NS for vaginal cleansing. The secondary outcomes included the rate of postoperative fever  $\geq 38^\circ\text{C}$  during the first 24 hours, persistent vaginal irritation symptoms, urinary tract infection, candidal vaginitis and bacterial vaginosis and endometritis at one-week post-procedure. The outcome variables were calculated using an unpaired t-test and chi-square test.

**RESULTS:** Two-hundred forty-four women were analyzed in both groups (121 women in the arm). Both groups were similar regarding the mean age, residency, woman's education, parity, BMI and operative time. Diagnostic laparoscopy was the most common laparoscopic procedure performed during

Variables	HSC + LSC	HSC + LSC	HSC only	HSC only
	Tubal patency (N=69)	Tubal occlusion (N=12)	Tubal patency (N=83)	Tubal occlusion (N=4)
Fluid deficit (FD) ml	438.96	141	307.48	375.75
Peritoneal fluid ml	175.61	0	N/A	N/A
Proposed FD ml	281.09	90.18	N/A	N/A

the study period (84.29%), tubal sterilization (7.85%) then bilateral ovarian drilling (7.43%). The mean overall vaginal irritation symptoms in PI group were significantly more than that observed in the NS group (p=0.0001). The overall infection rates in the PI group were 15.9%, while in the NS group was 10.16 % without a statistically significant difference in both groups (p=0.567). Both groups were quite similar in the rate of postoperative fever (p=0.505), urinary tract infection (p=0.654), vaginal candidiasis (p=0.254), bacterial vaginosis (p=0.366) and postoperative endometritis (p=0.749).

**CONCLUSIONS:** Being less irritant, normal saline can substitute iodide solution as a vaginal cleansing tool before short duration gynecologic laparoscopy without increasing the risk of postoperative infection.

**SUPPORT:** None.

**P-771** Wednesday, October 16, 2019 6:30 AM

**FLUID DEFICIT CALCULATION AT HYSTEROSCOPY IN PATIENTS WITH AND WITHOUT TUBAL OCCLUSION: COULD CONSIDERATION OF TUBAL PASSAGE CHANGE SAFETY LIMITS?**



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**OBJECTIVE:** Hysteroscopy (HSC) fluid management guidelines (1) are not well-defined regarding the contribution on the fallopian tube patency to the fluid deficit (FD) during HSC and most surgeons attribute the entire FD to intravasation (2). Women with patent tubes undergoing HSC have accumulation of distension media in the pelvis which can be seen during laparoscopy (LSC) and could be in part due to transtubal passage (3). We explored whether FD could be in part due to transtubal passage.

**DESIGN:** Prospective observational study.

**MATERIALS AND METHODS:** We studied 164 patients aged 20-45 years, who underwent HSC using normal saline as distension media between January 2014 and August 2017. Tubal patency was previously assessed at sonohysterogram. FD and, in LSC cases, the amount of fluid found in the pelvis, were prospectively recorded. Whitney U test was used to compare distributions with a p value <0.05 defining statistical significance (SPSS v25 for Windows; Chicago, Illinois).

**RESULTS:** 164 patients were included in the study. 77 underwent HSC prior to LSC and 87 patients underwent HSC only. In the LSC group, 69 had at least one patent tube with an average FD of 438.96 ml and a calculated FD due to extravasation of 175.61 ml; 8 patients had bilateral tubal occlusion and all were found to have 0 ml of peritoneal fluid with an average FD of 141. In the HSC only group, 83 had at least one patent tube with an average FD of 307.48 ml; 4 patients had bilateral tubal occlusion with an average FD of 375.75 ml. There was no correlation between intrauterine fluid pressure and the amount of FD, or the presence of peritoneal fluid.

**CONCLUSIONS:** Most women with patent tubes undergoing HSC have accumulation of distension media in the pelvis and transtubal passage was not correlated with the intrauterine fluid pressure. FD in patients with tubal occlusion appears to be entirely attributed to intravasation. These findings add new insight to our understanding of fluid dynamics during operative hysteroscopy that can help develop more accurate and patient-centered safety protocols.

**References:** (1) Munro MG et al. AAGL Practice Report: Practice Guidelines for the Management of Hysteroscopic Distending Media. J Minim Invasive Gynecol, 2013 Mar-Apr;20(2):137-48. <https://doi.org/10.1016/j.jmig.2012.12.002>.

(2) Kumar A et al. New hysteroscopy pump monitor real-time rate of fluid intravasation. J Minim Invasive Gynecol, 2012 May-Jun;19(3):369-75. <https://doi.org/10.1016/j.jmig.2012.01.018>. Epub 2012 Mar 16.

(3) Solima E et al. Hysteroscopy in endometrial cancer: new methods to evaluate transtubal leakage of saline distension medium. Am J Obstet Gynecol, 2008 Feb;198(2): 214.e1-4. <https://doi.org/10.1016/j.ajog.2007.07.035>.

**P-772** Wednesday, October 16, 2019 6:30 AM

**SEVERE HAEMATOPERITONEUM AFTER TRANSVAGINAL OOCYTE RETRIEVAL RELATED OVARIAN BLEEDING COULD BE MOSTLY MANAGED BY CONSERVATIVE TREATMENT: 8332 CASES OF ONE CLINICIAN'S EXPERIENCE IN 5 YEARS.**



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**OBJECTIVE:** TVOR is the most common surgical procedure during in vitro fertilization (IVF) cycles. One of the most serious complications of the

procedure is intra-abdominal bleeding. Hospitalization is reported to be needed with an incidence between 0.06% and 0.35%. According to the literature, the rate of cases requiring abdominal surgery with severe haematoperitoneum (SHP) is very different, between 40% to 70% in large series. The aims of the study were to compare single clinician's complication rate for SHP caused by ovarian bleeding after TVOR with literature and to compare the outcome of treatment strategies.

**DESIGN:** This retrospective cohort study includes a total of 8332 consecutive TVOR procedures performed by a single clinician (65.2%) among a total of 12776 TVORs, between June 2014 and March 2019 and in one IVF center. All the suspected SHP cases who were hospitalized were enrolled in the study group. This "complication" group was categorized according to the need for a conservative or surgical treatment. General SHP rates and the treatment approaches were compared with the literature.

**MATERIALS AND METHODS:** The complications of SHP included in the study were grouped into two: Group I included patients in whom conservative treatment with or without red blood cell (RBC) transfusion was performed; Group II consisted of patients who were indicated for surgical treatment. Patients with non-ovarian bleedings were excluded. Number of RBC units for transfusion, duration of hospitalization of SHP patients, general body mass index (BMI) and women ages in TVOR were considered.

**RESULTS:** A total number of 79097 oocytes (8832 TVOR) were retrieved by the same clinician between June 2014 and March 2019. The mean female age was 35.04±5.67, the mean body mass index was 24.92±4.49, the mean number of retrieved oocytes and metaphase II oocytes was 9.50±8.35 and 7.92±6.97 respectively. The number of SHP related ovarian bleeding complications during TVOR was 17 out of 8332 (0.2%). The mean duration of hospitalization was 1.76 days/patient. The mean RBC units administered was 1.65 U/patient. Whereas 15 patients (88.23%) needed only conservative treatment, only two (11.77%) needed a laparoscopic intervention. None of the patients (17) had severe infections such as pelvic abscess or sepsis after the treatment.

**CONCLUSIONS:** The real complication rates of SHP after TVOR and especially their treatment methods are variable in the literature. Difficulties of origin of intra-abdominal bleeding after TVOR and diagnosis of severity of haematoperitonium also make the therapeutic approaches more complicated in IVF patients. We report here a very low complication rate (0.2%) of SHP in a large series performed by a single clinician in nearly five years. Our data showed that most of the ovarian bleeding related SHP after TVOR could be managed without adverse outcome by conservative treatment (88.23%) not by surgery in contrast to the published data.

Reference: No.  
SUPPORT: No.

P-773 Wednesday, October 16, 2019 6:30 AM

### ANALYSIS OF THE PREGNANCY OUTCOMES OF HETEROTOPIC FALLOPIAN TUBAL PREGNANCY AND HETEROTOPIC INTERSTITIAL PREGNANCY AFTER IN VITRO FERTILIZATION - EMBRYO TRANSFER.

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**OBJECTIVE:** To investigate the intrauterine pregnancy (IUP) outcomes of heterotopic interstitial pregnancy and heterotopic fallopian tubal pregnancy after in vitro fertilization - embryo transfer (IVF-ET).



**DESIGN:** A retrospective study.

**MATERIALS AND METHODS:** Women who underwent IVF-ET and transvaginal sonography (TVS) in our reproductive center between January 2005 and December 2017 were included. All pregnancies were diagnosed by TVS and were confirmed by surgery and pathological analysis. The outcomes of IUP after surgical treatment of the ectopic pregnancies were compared between the heterotopic fallopian tubal pregnancy group (n=347) and the heterotopic interstitial pregnancy group (n=160).

**RESULTS:** The two groups were statistically similar with respect to maternal age, body mass index, cause of infertility, insemination methods, transfer cycle and endometrial thickness on transfer day (p > 0.05). The early pregnancy loss rate (28.5% vs. 26.9%, p=0.700), the late miscarriage rate (0.6% vs. 0%, p = 0.613), preterm delivery rate (7.5% vs. 6.3%, p=0.613), perinatal mortality (1.6% vs. 0.8%, p=1.000), live birth rate (69.2% vs. 72.5%, p = 0.445), live birth weight (3.2 ± 0.6 vs. 3.2 ± 0.5 kg, p=0.747) and the gestational age at delivery (38.3 ± 2.3 vs. 38.4 ± 2.3 weeks, p=0.818) were statistically similar. However, the cesarean section rate (77.1% vs. 86.3%, p < 0.001) in the heterotopic interstitial pregnancy group was significantly higher than that in the heterotopic fallopian tubal pregnancy group.

**CONCLUSIONS:** After surgical treatment of ectopic pregnancies, the IUP of both heterotopic fallopian tubal pregnancy and heterotopic interstitial pregnancy can achieve a good IUP outcome.

P-774 Wednesday, October 16, 2019 6:30 AM

### THE IMPACT OF IPSILATERAL TESTICULAR ATROPHY ON SEMEN ANALYSIS AND DNA FRAGMENTATION RESPONSE TO VARICOCELE REPAIR.

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**OBJECTIVE:** The purpose of this study is to assess the response in semen parameters and sperm DNA fragmentation index (DFI) in men with ipsilateral testicular atrophy secondary to a varicocele in comparison to men without testicular atrophy.

**DESIGN:** A retrospective chart review was performed.

**MATERIALS AND METHODS:** Men who underwent varicocele repair for subfertility were categorized into 2 groups, those with testicular atrophy (TA) in the ipsilateral testicle and those with no testicular atrophy (NTA). Semen parameters and DFI in both groups were compared preoperatively and 3 months postoperatively. Morphology was not included due to lack of standardization of criteria in different labs with some using WHO 4th edition criteria and others using strict Kruger morphology criteria. From 10/2010 and 1/2019, 359 varicocele repairs were performed by a single microsurgeon and 141 varicocele repairs met inclusion criteria. Exclusion criteria included men who underwent bilateral varicocele repairs, men with bilateral testicular atrophy, men with a history of cryptorchidism or testicular torsion, men who underwent varicocele repair for hypogonadism or orchialgia and not for fertility, men who were azoospermic preoperatively, and men who did not obtain a 3-month postoperative semen analysis because they achieved a pregnancy prior to then or who did not follow up. Student's test was used with a p value of < 0.05 considered statistically significant. Results were expressed as means ± standard deviations.

TABLE A. comparison of the IUP between the heterotopic fallopian tubal pregnancy and heterotopic interstitial pregnancy

Pregnancy outcomes	Heterotopic fallopian tubal pregnancy (n=347)	Heterotopic interstitial pregnancy (n=160)	P-value	OR (95%-CI)
Early pregnancy loss rate, % ( n )	28.5% ( 99/347 )	26.9% (43/160 )	0.7	1.086 (0.714-1.653)
Late miscarriage rate, % ( n )	0.6% (2/347)	0	1	1.464 (1.379-1.553)
Preterm delivery rate, % ( n )	7.5% (26/347)	6.3% (10/160)	0.613	1.215 (0.571-2.584)
Term delivery rate, % ( n )	62.8% (218/347)	66.9% (107/160)	0.377	0.837 (0.564-1.242)
Labor induction rate, % ( n )	0.6% (2/347)	0	1	1.464 (1.379-1.553)
Babies born	255	119		
Perinatal mortality rate, % ( n )	1.6% (4/255 )	0.8% (1/119)	1	1.880 (0.208-17.009)
Live birth rate, % ( n )	69.2% (240/347)	72.5% (116/160 )	0.445	0.851 (0.562-1.289 )
Caesarean section rate, % ( n )	77.1% (188/244 )	86.3% (101/117 )	0	0.334 (0.180-0.618 )
Gestational age at delivery	38.345 ± 2.322	38.406 ± 2.271	0.818	
Live birth weight ( kg )	3.2 ± 0.6	3.2 ± 0.5	0.747	

**RESULTS:** Of the 141 men who were included, 20 were in the TA group and 121 were in the NTA group. There was no statistically significant difference in age between the 2 groups, 34.3 (6.5) in TA group and 34.1 (5.8) in the NTA group. The grades of varicoceles were similar in both groups: TA group had 10% grade 1, 55% grade 2, 35% grade 3; while the NTA group had 6.7% grade 1, 58.7% grade 2, and 34.7% grade 3. There was no statistically significant difference in preoperative semen parameters between the two groups including semen volume, sperm concentration, motility, forward progressive motility (FP), and total motile count (TMC). The NTA group had a higher preoperative DFI than the TA group: 35.3% vs 29.7% respectively. Although both groups revealed an improvement in semen parameters postoperatively, the TA group only showed a statistically significant improvement in DFI from 29.7% (5) to 22% (0), whereas the NTA group showed statistically significant improvements in concentration, motility, FP, TMC, and DFI. The mean change in preoperative to postoperative parameters when comparing groups only revealed a significant difference in TMC and DFI, with a larger mean improvement in the NTA group than the TA group.

**CONCLUSIONS:** Men with ipsilateral testicular atrophy secondary to varicoceles have improved overall semen parameters and DFI after varicocele repair, but do not get as significant of an improvement as men without testicular atrophy. However, only TMC and DFI have a significantly greater mean change in preoperative to postoperative response in the NTA group compared to the TA group.

**SUPPORT:** None.

**P-775** Wednesday, October 16, 2019 6:30 AM

#### **EFFICACY AND SAFETY OF ORAL VERSUS VAGINAL MISOPROSTOL IN CERVICAL PRIMING BEFORE HYSTEROSCOPY: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS.**

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**OBJECTIVE:** Hysteroscopy is common to be used in the diagnosis and management of many problems related to gynecology. Most of the complications of hysteroscopy occur throughout the cervical entry including cervical lacerations, false tract and uterine rupture. Cervical priming can be used to decrease the incidence of these problems and hazards before performing hysteroscopy. Our objective is to evaluate the evidence from published randomized clinical trials (RCTs) about the efficacy and safety of oral versus vaginal misoprostol in cervical priming before hysteroscopy.

**DESIGN:** Systematic review and meta-analysis.

**MATERIALS AND METHODS:** We searched in different electronic databases including PubMed, Cochrane Library, Scopus, [ClinicalTrials.gov](http://ClinicalTrials.gov) and Web of Science using the relevant keywords ((misoprostol OR Cytotec) AND (hysteroscopy OR uteroscope OR metroscope)). All RCTs are assessing the effect of oral versus vaginal misoprostol before hysteroscopy were considered. The extracted outcomes were; width of the cervical canal, ease of dilatation, the time needed for cervical dilatation; adverse effects and any complications during the procedure. For continuous data, efficacy outcomes were pooled as weighted mean difference (MD) or Standardized mean difference (SMD). For dichotomous data of safety outcomes, we used pooled risks ratios (RR) using the Mantel-Hansel method with 95% confidence intervals (CI). All statistical analyses in this study were completed by the RevMan software package

**RESULTS:** Our search found 110 studies from the electronic databases out of which, 35 were duplicates. Out of the remaining 75 studies, 65 studies were excluded based on the title, and abstract screening and ten studies were excluded during the full-text screening. About eight studies met our inclusion criteria. The quality of the included RCTs was from moderate to high quality according to the Cochrane risk of bias assessment tool. Both groups did not differ significantly in terms of cervical width diameter (MD= -0.25 mm, 95% CI [-0.92, 0.42], p=0.47). However, the vaginal route significantly superior to the oral route of misoprostol in reducing the time needed for cervical dilatation (SMD=0.17, 95% CI [0.02, 0.32], P=.03). We found no significant difference in any of the two routes regarding ease of dilatation (MD= 0.00, 95% CI [-0.15, 0.15], P= 0.96). Regarding safety profile, no significant differences between oral and vaginal misoprostol groups except for diarrhea which favored vaginal more than oral misoprostol (RR= 2.48, 95% CI [1.17, 5.26], p= 0.02). No significant difference was found in both oral and vaginal route of administration for increasing the risk of any other complications (RR=1.7, 95% CI [0.74, 3.92], P= 0.21).

**CONCLUSIONS:** Oral and vaginal misoprostol administration are similar regarding efficacy and safety in cervical priming before hysteroscopy, except that the vaginal route is associated with a lower incidence of diarrhea.

**SUPPORT:** None.

**P-776** Wednesday, October 16, 2019 6:30 AM

#### **OUTCOME OF LAPAROSCOPIC REPAIR OF CESAREAN SCAR DEFECT.**

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**OBJECTIVE:** To evaluate the gynecologic and obstetric outcomes of laparoscopic repair of symptomatic cesarean scar defect.

**DESIGN:** Prospective clinical study.

**SETTING:** University hospital and private gynecologic endoscopy center.

**MATERIALS AND METHODS: Patients:** A total of 52 women (age between 25 – 35 years) with symptomatic cesarean scar defect, who wish to conceive, and the remaining myometrial thickness at the site of defect is less than 3 mm. according to vaginal US examination and/or MRI.

**Intervention:** Laparoscopic excision and repair of the defective cesarean scar.

**Main Outcome measures:** Relief of relevant symptoms, restored myometrial thickness at the site of repair, achievement of pregnancy in infertile patients, obstetric outcome in those who become pregnant, and incidence of operative complications.

**RESULTS:** The mean thickness of the myometrium increased significantly from 1.62 ± 0.8 before surgery to 9.0 ± 2.1 mm after surgery.

Among the 47 patients presented with menstrual abnormalities and/or pelvic pains, 34 patients (72.34%) demonstrated complete relief of symptoms, 8 patients (17.02%) demonstrated partial improvement, and 5 patients (10.64%) stated no improvement.

Among the 25 patients who tried pregnancy 17 patients (68%) became pregnant, 12 patients demonstrated healthy pregnancy courses and delivered healthy babies by cesarean section at term (48%). There were no relevant major obstetric complications like scar dehiscence, placenta accreta, or cesarean scar ectopic pregnancy. There were no operative complications.

**CONCLUSIONS:** In women with symptomatic cesarean scar defect who wish to conceive, the laparoscopic approach for excision and repair of the defective scar is safe and efficient technique, ensures satisfactory symptoms relief, adequate restoration of sufficient myometrial thickness and strength, and results in good reproductive outcome.

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#### **PREDICTIVE VALUE OF HORMONES IN SPERM RETRIEVAL SURGERY.**

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**OBJECTIVE:** Non-obstructive azoospermia (NOA) causes male factor infertility in about 10% of cases. Multiple techniques have been described to obtain sperm from the testicle for use with assisted reproductive technologies. Conventional testicular sperm extraction (cTESE) is the most common but some argue that microdissection testicular sperm extraction (mTESE) is preferred for its superior sperm retrieval rates (SRR) and decreased microvascular damage to the testicle. However, mTESE is generally more expensive, time consuming, and requires more equipment. Previous work has attempted to identify variables that predict positive SRR with mTESE versus cTESE. The objective of this review was to create a model comparing the commonly evaluated variables; follicle stimulation hormone (FSH), testicular volume (TV), and testosterone (T), to better predict SRR.

**DESIGN:** The authors included 29 studies in the data analysis, with 9 studies including data on cTESE for a total of 1227 patients and 20 studies including data on mTESE for a total of 4760 patients. Not all studies included data for each variable.



**MATERIALS AND METHODS:** While not all studies included data for each variable, the authors were however able to create a weighted-means values of SRR, FSH, testosterone, and volume for the 29 studies. The authors then used weighted linear regression to describe associations between SRR, type of procedure, FSH, T, and volume.

**RESULTS:** Weighted-means values of SRR, FSH, testosterone, and volume were calculated and demonstrated mTESE to be superior to cTESE with a SRR of 51.9% versus 40.1% when there were no significant differences in FSH, T, or TV. Multiple weighted linear regressions were created to describe associations between SRR, procedure type, FSH, T, and TV. Model A demonstrated that one may expect an 11.8% increase in SRR when utilizing mTESE compared to cTESE. Model B showed that for every 1.19 IU/mL increase in FSH there will be a significant decrease in SRR by 1%. FSH values were then divided into low, medium, and high categories (FSH <10, 10-19, and > 20 IU/mL respectively). The model demonstrated that for an index patient undergoing cTESE retrieval rates would be 57%, 44%, and 31% for values low, medium, and high respectively.

**CONCLUSIONS:** Based upon pooled available data, mTESE is more successful than cTESE for sperm retrievals in NOA patients. The models generated in this study demonstrated an ability for FSH to predict SRR using mTESE and cTESE however the models were not suggestive for a correlation regarding SRR and T and TV. FSH alone can be predictive of retrieval success and used to counsel patients. More standardized data collection and publication will be useful for future modeling to allow improved outcomes and counseling for patients.

**SUPPORT:** None.

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**PREGNANCY RATES AFTER LAPAROSCOPIC OVARIAN DRILLING IN POLYCYSTIC OVARY SYNDROME PATIENTS FOLLOWING UNSUCCESSFUL OVULATION INDUCTION.** Shrutti Agarwal, DO,<sup>a</sup>

Mark P. Trolice, MD,<sup>b</sup> <sup>a</sup>UCF College of Medicine/HCA Consortium of Greater Orlando, Kissimmee, FL; <sup>b</sup>Fertility CARE: The IVF Center; University of Central Florida College of Medicine – Associate Professor, Winter Park, FL.



**OBJECTIVE:** Polycystic ovary syndrome (PCOS) is one of the most common endocrine pathologies and is a frequent cause of anovulatory infertility affecting 5-20% of reproductive age women<sup>1</sup>. Medical induction of ovulation is considered the first-line treatment option for infertile PCOS women. Laparoscopic ovarian drilling (LOD) is currently accepted as a successful second-line treatment in drug-resistant PCOS<sup>2</sup>. Many authors have reported high ovulation (~80%) and pregnancy (~60%) rates following LOD<sup>3</sup>. The aim of this study was to evaluate the efficacy of laparoscopic ovarian drilling (LOD) on the reproductive outcome of anovulatory PCOS women. The results of our study were compared to historical controls from literature.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Women unable to achieve pregnancy with ovulation drugs who underwent LOD from 2013-2018 were included. One physician followed a standard technique for LOD and performed all surgeries. Age, BMI, years of infertility, tobacco or alcohol use, pre and post surgery ovulation rates, pre and post surgery pregnancy rate, time until ovulation and pregnancy after surgery were documented.

**RESULTS:** 136 patients who underwent LOD were included in this study. Demographics were divided as follows: mean age 28.9; mean BMI 22.2 kg/m<sup>2</sup>, and mean duration of infertility 2.9 years. Race was divided as follows: 58.8% Caucasian, 22.8% Hispanic, 14% African American, 3.7% Asian, 0.7% other. Tobacco use was reported by 4.4% and social alcohol use was reported by 37.5%. 39.0% reported successful ovulation with drugs prior to surgery but were unable to achieve a pregnancy. Ovulation after LOD was reported by 77.2% with a live birth rate of 47.1% and mean time until ovulation of 79.0 days. Statistical analysis showed a woman was 2.27% (95% CI 1.83 - 2.82) more likely to ovulate following LOD. This was considered significant on a Chi Square test ( $X^2 = 69.8, P < 0.05$ ). Furthermore, a patient was 1.95% (95% CI 1.48 - 2.58) more likely to achieve a live birth following LOD with a mean time of 241.1 days until pregnancy. This was also considered significant on a Chi Square test ( $X^2 = 22.9, P < 0.05$ ). (Table 1).

**CONCLUSIONS:** Our study demonstrates significantly improved ovulation and pregnancy rates in drug resistant PCOS women after LOD. Although our rates are lower than those reported in previous published studies, this may be attributed to 37 out of 136 patients (27.2%) being lost to follow up. Some patients also did not achieve desirable results after the surgery due to other factors like male infertility, marital issues and diagnosis of cancer.

TABLE 1

Results	Yes	No	Unknown
Ovulation prior to LOD	39.0%	58.1%	2.9%
Ovulation after LOD	77.2%	7.4%	15.4%
Pregnancy rate prior to LOD	33.1%	66.9%	n/a
Pregnancy rate after LOD	47.1%	25.7%	27.2%

References: 1. Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol.* 2011;7:219–231.

2. Farquhar C, Lilford RJ, Marjoribanks J, Vandekerckhove P. Laparoscopic ‘drilling’ by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. *Cochrane Database Syst Rev.* 2007;3:CD001122.

3. S.A.K. Amer, T.C. Li, W.L. Ledger, Ovulation induction using laparoscopic ovarian drilling in women with polycystic ovarian syndrome: predictors of success, *Human Reproduction*, Volume 19, Issue 8, August 2004, Pages 1719–1724.

**SUPPORT:** NONE.

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**IMPACT OF SAFETY PROTOCOL IN AN AMBULATORY SURGICAL SETTING VS A HOSPITAL SETTING FOR LAPAROSCOPIC-ASSISTED MYOMECTOMY (LAM).**

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**OBJECTIVE:** Ambulatory surgery center (ASC) for major gynecological surgery improves efficiency and decreases cost compared to a hospital setting. Protocols to ensure safety when performing major gynecologic surgeries are critical in the ASC setting. The objective of this study is to assess whether protocols do ensure safety when performing major gynecological surgeries such as laparoscopic-assisted myomectomy (LAM) in a high-volume ASC and compare it with protocols and outcomes in a hospital setting.

**DESIGN:** This is a descriptive / retrospective study.

**MATERIALS AND METHODS:** This paper descriptively outlines the similarities and differences of a surgical safety protocol in an ambulatory surgical center compared to a hospital setting. Furthermore there is retrospective analysis of LAM outcomes that are commonly considered as safety standards in both settings including intraoperative and postoperative complications.

**RESULTS:** The protocols were similar with regards to preoperative patient selection and checklist, surgical precautions including prevention of retained surgical items, DVT prophylaxis, infection control, surgical wound classification, vaginal and genital antisepsis for the surgical patient, postoperative care in PACU and discharge criteria for surgical management.

The major preoperative differences from hospital protocol were transfusion criteria preoperatively. In the ASC, a cut-off of 9.0 g/dl was used, and a cut-off of < 7.5 g/dl was used in the hospital setting. LAM cases are only scheduled as morning cases. 23 hour observation is available at ASC. Additionally myomectomy patients at the ASC have ISTAT (blood analysis system to check Hemoglobin/Hematocrit) prior to procedure and at 1 and 2 hours after the procedure in the PACU to detect any signs of bleeding. Any patients that did require blood transfusion postoperatively were transferred to the local hospital from the ASC.

There were 588 patients that underwent LAM at the ASC compared to 228 patients at the hospital. There was no significant difference in case complexity factors between settings including BMI, number of previous abdominal and pelvic surgeries or other comorbidities. Intraoperative complication rate was 3.4% (95% CI 1.8-5.0) at the ASC compared to 4.9% (95% CI 1.7-8.1),  $p = 0.4430$ . There were no significant differences in postoperative complications between the ASC and the hospital setting including infections and thromboembolic events. Blood transfusion was required in 1.7% of the cases at ASC compared to 8.8% at the hospital setting. The estimated blood loss and average fibroid weight were not statistically different between the two groups.

**CONCLUSIONS:** The LAM safety protocol at a free-standing ASC allows for patient complication outcomes that are comparable to an in-hospital setting without apparent limitations in patient complexity.

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#### **OVARIAN CYSTS REQUIRING SURGERY AND INFERTILITY.**

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**OBJECTIVE:** Benign ovarian cysts are a common condition in reproductive-aged women. The long-term effect of surgery for ovarian cysts on fertility remains unknown.

**DESIGN:** Women aged 22-45 years were interviewed about their reproductive histories (n=2,219), including whether they ever had infertility (defined as 12 months of unprotected sex without getting pregnant) or surgery for ovarian cysts. Women who reported a hysterectomy prior to ovarian cyst surgery were excluded. A subset of women (n=717) was invited to attend a clinic visit where markers of ovarian reserve (anti-Müllerian hormone [AMH], antral follicle count [AFC]) were measured. Women who reported surgery for benign ovarian cysts were compared with those who did not report ovarian cyst surgery.

**MATERIALS AND METHODS:** To account for age at surgery, each woman with a history of ovarian cyst surgery was randomly matched to a woman without surgery, who was then assigned an artificial age at surgery equal to that of her match. This matching was repeated 1000 times. For each matching iteration, adjusted Cox models were fit examining time to infertility after surgery; the median hazard ratio (HR) and 95% simulation intervals (SI) are reported. Log-transformed and negative log binomial models were fit for AMH and AFC, respectively, to examine the relationship between ovarian reserve and history of ovarian cyst surgery; AMH and AFC were predicted for a woman at the median age at clinic visit.

**RESULTS:** Approximately 6.6% of women reported ovarian cyst surgery. The median age at surgery was 26 years. Women with and without ovarian cysts requiring surgery were similar with regards to race, level of education, relationship status at the interview, income, health insurance status, and body mass index. Infertility after age at surgery was more common for women reporting ovarian cyst surgery than those without surgery after adjusting for age, history of cancer, race, body mass index, parity before surgery age, and history of infertility before surgery age (median HR 1.74, 95% SI 1.06-2.94). This difference remained after also adjusting for history of endometriosis (median HR 1.79, 95% SI 1.02-3.23). The difference was more marked amongst those who reported attempting pregnancy (median HR 2.49, 95% SI 1.16-6.40). The model-based predicted mean level of AMH for a 39 year old woman was lower among those with a history of ovarian cyst surgery (AMH 0.65 ng/mL, 95% CI 0.34-1.24) versus those without (AMH 0.90 ng/mL, 95% CI 0.69-1.17), but AFC levels were similar (AFC 13.7, 95% CI 10.5-17.8 vs. AFC 12.1, 95% CI 10.8-13.5).

**CONCLUSIONS:** Women with a history of ovarian cysts requiring surgery were more likely to experience infertility after surgery compared to women with no history of ovarian cyst surgery. Women having surgery for ovarian cysts may be less fertile after surgery than women who have not had surgery for ovarian cysts. This association was more marked, but less precise, among those actually attempting pregnancy.

**SUPPORT:** Funding provided by NICHD 1R01HD066059.

**P-781** Wednesday, October 16, 2019 6:30 AM

#### **COMPLETE ROBOTIC LIVING DONOR HYSTERECTOMY FOR UTERUS TRANSPLANTATION.**

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**OBJECTIVE:** Uterus Transplantation is proven to be a successful treatment for women with uterine factor infertility (UFI). Most cases of living donor hysterectomies have so far been performed with an open technique with only a few attempts of robotic-assisted donor hysterectomies. In this report, we describe the technique of the first successful complete robotic living donor hysterectomy with vaginal extraction of the uterine graft.

**DESIGN:** Clinical prospective study.

**MATERIALS AND METHODS:** Both the donor and the recipient were part of an institutional review board-approved study (No. NCT02656550). Prior to the donation/transplantation, the donor and the recipient underwent thorough medical and psychological evaluation. The donor had no desire for further childbearing and approached our center to donate her uterus for uterus transplantation. She has no relation to the recipient and presented as a non-directed donor. She gave full informed consent after being thoroughly counseled regarding the facts that: our team had never previously performed a robotic donor hysterectomy and that there was a strong possibility that we may need to convert to an open laparotomy.

The living donor hysterectomy was performed using a Intuitive system, Da Vinci Xi. The subsequent recipient transplantation was done with an open technique.

**RESULTS:** Preoperative MR angio, CT angio and ultrasound showed a normal appearing uterus with patent uterine arteries (1 mm in diameter). The right utero-ovarian vein was dominant (7 mm in diameter) whereas the left utero-ovarian vein and bilateral uterine veins were harder to identify on imaging.

The donor surgery time was 9 hours and 25 min. The uterine graft was procured with bilateral uterine arteries, bilateral utero-ovarian veins and unilateral uterine artery. The specimen was extracted transvaginal. Estimated blood loss was 100 mL. The duration of warm ischemia was 1 h and cold ischemia 2 hours and 31 min. The recipient surgery time was 4 hours and 30 min. Estimated blood loss was 600 mL. Blood transfusions were not required during any surgery. The postoperative recovery of the donor and the recipient was uncomplicated with no adverse events, and they were discharged from the hospital 3 and 6 days postoperatively, respectively.

**CONCLUSIONS:** This is the report of the first of a complete robotic hysterectomy for living donor uterus transplantation. The major difference with the previously reported cases are significant in the vaginal extraction of the uterus graft. Although this report demonstrates the technical feasibility and the safety of the robotic approach for living donor hysterectomies, only the birth of a healthy child from the recipient should be considered as the proof of complete success of this surgical approach.

**P-782** Wednesday, October 16, 2019 6:30 AM

#### **VISUALIZATION AND INJECTION OF RETE TESTIS FOR GERM CELL TRANSPLANTATION AND SPERM RETRIEVALS IN RHESUS MONKEYS AND HUMANS.**

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**OBJECTIVE:** To test the feasibility of rete testis ultrasound guided puncture in humans for either sperm retrieval or spermatogenic stem cell injection and colonization.

**DESIGN:** Testis ultrasound exploration first in rhesus monkeys, and later in humans undergoing vasectomy reversal or micro-surgical testis sperm retrieval (TESE).

**MATERIALS AND METHODS:** 7 Rhesus monkeys were anesthetized and subjected to ultrasound guided exploration of each testis. In one animal with large testis, it was difficult to visualize the rete. But in all animals, a 25 gauge needle was used to puncture the rete under ultrasound guidance. Then the same technique was applied to humans undergoing conventional micro-TESE or microsurgical vasectomy reversal.

**RESULTS:** Germ cells were successfully retrieved from all 6 Rhesus monkeys in which the rete could be visualized. Visualization depended on the proper settings (Musculo-Skeletal) not the factory settings. In smaller testes, the rete was easier to see. The rete was easiest to visualize in the smaller testes. The anatomy of the human and Rhesus rete was different from what is depicted in most textbooks, which base their description and drawings on rodent testes. Actually the rete of the human and Rhesus is a linear collecting system from top to bottom right in the center of the testis, similar to an apple core.

**CONCLUSIONS:** The rete testis is the perfect collecting point for TESE in non-obstructive azoospermia, because it will contain sperm from every single seminiferous tubule, and it is easy to visualize with the proper ultrasound settings, which makes it accessible to simple needle puncture.

**P-783** Wednesday, October 16, 2019 6:30 AM

#### **OUTCOME OF HYSTEROSCOPIC REPAIR OF SYMP-TOMATIC CESAREAN SCAR DEFECT.**

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**BACKGROUND:** Cesarean section scar defect is currently a more frequently detected problem due to increased rate of cesarean section deliveries worldwide. Cesarean scar defect may be presented by: Postmenstrual uterine bleeding, chronic pelvic pain, dysmenorrhea and or dyspareunia.

**OBJECTIVE:** Evaluation of efficacy and safety of hysteroscopic treatment of symptomatic cesarean scar defect.

**DESIGN:** Prospective clinical observational study.

**MATERIALS AND METHODS:** • **Setting:** University hospital and private gynecologic endoscopy center.

• **Patients:** 40 patients with symptomatic cesarean scar defect who do not desire future pregnancy with myometrial thickness  $\geq 3$  mm at site of cesarean scar by transvaginal ultrasonography.

• **Intervention:** Hysteroscopic repair of cesarean scar defect.

• **Main Outcome Measure:** Relief of symptoms, occurrence of operative related complications and adequacy of repair of the defect evaluated by transvaginal ultrasonography.

**RESULTS:** Among 40 patients postmenstrual bleeding was completely resolved in 29 patients (72.5%) of patients and partially improved in 5 patients (12.5%). On the other hand, complete relief of chronic pelvic pain was reported in 23 patients (69.7%) out of 33 patients, while partial relief was recorded in 5 patients (15.2%). As regard dysmenorrhea, complete improvement was recognized in 15 patients (60.9%) out of 23 patients, and partial improvement in 4 patients (17.4%).

**CONCLUSIONS:** Hysteroscopic repair of symptomatic cesarean scar defect is an efficient minimally invasive safe procedure suggested for management of this lesion.

**P-784** Wednesday, October 16, 2019 6:30 AM

#### POST-CESAREAN SECTION VENTRAL UTERINE ADHESIONS. CLINICAL AND LAPAROSCOPIC CHARACTERISTICS OF 167 CASES. A PRELIMINARY REPORT OF UTEROLYSIS.

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**OBJECTIVE:** Rising cesarean section (CS) rate increase the possibility of pelvic adhesions. A recognized type is ventral adhesions between the anterior wall of the uterus and anterior abdominal wall. The current study aims to estimate the link between post CS ventral uterine adhesions and female fertility.

**DESIGN:** A case control study included patients undergoing laparoscopy for secondary infertility after previous CS.

**MATERIALS AND METHODS:** Patients were described as "cases" if there were abnormal adhesions between the uterus and anterior abdominal wall, while "control" patients had no such adhesions. Lysis of pelvic adhesions was done up to the maximum restoration of anatomical relationship between different pelvic organs. Patients were followed for 6 months after the procedure waiting for pregnancy to occur. Quantitative variables were presented in terms of mean and standard deviation. They were compared using a Student's t test. Qualitative variables were presented as frequency and percentage. Chi-square test was used for comparison between groups. For analysis,  $p < 0.05$  was considered to be significant.

**RESULTS:** The study included 167 cases (study group) and 40 patients in the control group. Adhesion between the uterus and anterior abdominal wall were mainly grade 2. Satisfactory uterolysis was achieved in 56% of cases. Pregnancy occurred in 71% of cases. Among a total of 134 patients who got pregnant over the 6 months follow up period, 88.1% were cases and only 12 % were control ( $P=0.000$ ). The extent of uterine adhesions had a definite effect on the occurrence of pregnancy; most cases had either grade 1 or 2. Associated severe adnexal adhesions were commoner in patients who didn't get pregnant than pregnant ones (19 % vs. 0.2%).

**CONCLUSIONS:** Ventral adhesions between the uterus and anterior abdominal wall secondary to CS seem to have a significant impact on fertility and can be successfully treated by laparoscopic uterolysis.

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#### DETERMINING THE IMPACT OF SURGICALLY RETRIEVED SPERMATOZOA FROM AZOOSPERMIC MEN ON EMBRYONIC DEVELOPMENT BY GENOMIC PROFILING.

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**OBJECTIVE:** To assess the ability of surgically retrieved spermatozoa to support a viable pregnancy by whole-exome sequencing.

**DESIGN:** To determine the impact of surgically retrieved spermatozoa on embryonic development, we assessed gene mutations of epididymal and testicular spermatozoa from men with acquired azoospermia (OA) and non-obstructive azoospermia (NOA), respectively, as compared to ejaculated sperm from donors.

**MATERIALS AND METHODS:** DNA was extracted and amplified from at least 500 spermatozoa (DNA concentration,  $741 \pm 519$  ng/ul; quality,  $1.7 \pm 0.1$  nm) obtained through surgical retrieval. Following NGS, gene mutations, duplications, and deletions were detected using the CLC Genomic Server 9.0. Genes were considered duplicated or deleted when the read depth was  $>1.5$  or  $<0.5$  times the median read depth for at least 70% of the exons in the control. Gene mutation profiles of the OA and NOA men were then assessed in relation to their ability to generate a pregnancy.

**RESULTS:** Of the 23 couples (paternal age,  $41.3 \pm 9$  yrs) included in this study, 14 OA men underwent surgical sperm retrieval with a concentration of  $1.3 \pm 3 \times 10^6$ /ml and  $7 \pm 14\%$  motility. Nine NOA men yielded spermatozoa with a concentration of  $0.02 \pm 0.1 \times 10^6$ /ml and  $0.5 \pm 0.5\%$  motility. NGS assessment did not show a significant difference in overall sperm aneuploidy between the two groups (OA, 1.6%; NOA, 1.8%).

In the OA group overall, 3 genes were mutated (ATP4A, SLC17A7, and OR1D4), which were classified as housekeeping genes unrelated to embryonic development. In the NOA patients, 5 genes were found to be mutated. These were involved in RNA transcription (POLR2L), apoptosis (AP5M1), and protein sorting (APIS2, APIG2, and APOE).

The OA patients were treated in 14 ICSI cycles (maternal age,  $38.7 \pm 5$  yrs), resulting in a pregnancy and delivery rate of 50% (7/14). While no relevant mutations were identified in the male partners, the couples that failed to achieve a pregnancy were older ( $P < 0.05$ ).

When NOA men were treated in 9 ICSI cycles (maternal age,  $32.9 \pm 3$  yrs), the pregnancy rate was 66.7% (6/9). While the fertile cohort displayed 1 mutated gene (MPIG6B), related to stem cell lineage differentiation, the infertile NOA cohort had 5 mutated genes involved in apoptosis and early embryonic development.

**CONCLUSIONS:** Compared to fertile donors, OA men did not have any meaningful gene mutations, as expected by their post-vasectomy status. The NOA men who were able to generate a pregnancy had only 1 affected gene, apparently responsible for the reduced number but albeit competent spermatozoa. Those men unable to procreate carried gene mutations responsible for apoptosis and impaired embryonic development. Although this study still contains a limited number of observations, screening men for gene mutations can help characterize the spermatogenic function and competence of their germinal epithelium.

#### STEM CELLS

**P-786** Wednesday, October 16, 2019 6:30 AM

#### INDUCTION OF SPERMATOGENESIS UNDER 3-DIMENSIONAL TISSUE CULTURE CONDITIONS BY IN VITRO TRANSPLANTATION OF SPERMATOGENIAL STEM CELLS ISOLATED FROM HUMAN FROZEN-

THAWED TESTIS TISSUE. Mahdi Mohaqiq, Ph.D,<sup>a</sup> Mansoureh Movahedin, Ph.D,<sup>a</sup> Zohreh Mazaheri, Ph.D,<sup>b</sup> Naser AmirJannati, MD<sup>a</sup> <sup>a</sup>Medicine Faculty, Kateb University, Kabul, Afghanistan; <sup>b</sup>Histogenotech company, Tehran, Iran, Tehran, Iran (Islamic Republic of).

**OBJECTIVE:** Sperm production is one of the most complex biological processes in the body. In vitro production of sperm is one of the most important goals of researches in the field of male infertility treatment, which is very important in male cancer patients treated with gonadotoxic methods and drugs. In this study, we examine the progression of spermatogenesis after

transplantation of spermatogonial stem cells under conditions of testicular tissue culture.

**DESIGN:** Testicular tissue samples from azoospermic patients were obtained and then these were freeze- thawed. Spermatogonial stem cells were isolated by two enzymatic digestion steps and the identification of these cells was confirmed by detecting the *PLZF* protein. These cells, after being labeled with DiI, were transplanted in azoospermia adult mice model. The host testes were placed on agarose gel as tissue culture system. After 8 weeks, histomorphometric, immunohistochemical and molecular studies were performed.

**MATERIALS AND METHODS:** For each experimental group, 3 to 5 NMRI mouse were used at the age of 4 weeks. These mice are treated with Busulfan 40 mg/kg and after 4 weeks, the Azoospermia model is developed. This study is based on 5 biopsy samples taken from different obstructive azoospermic patients. SSCs were isolated by Mirzapour *et al* (2012) protocol under two steps of enzymatic digestion. SSCs were transplanted into the host testes below the stereo microscope then they were cut into small pieces and placed under 3-D tissue culture conditions on the agarose support layer.

**RESULTS:** The results of histomorphometric studies showed that the mean number of spermatogonial cells, spermatocytes and spermatids in the experimental group was significantly more than the control group (without transplantation) ( $P < 0.05$ ) and most of the cells responded positively to the detection of DiI. Immunohistochemical studies in host testes fragments in the experimental group express the *PLZF*, *SCP3* and *ACRBP* proteins in spermatogonial cells, spermatocyte and spermatozoa, respectively, which confirmed the human nature of these cells. Also, in molecular studies of *PLZF*, *Tekt1* and *TP1*, the results indicated that the genes were positive in the test group, while not in the control group.

**CONCLUSIONS:** These results suggest that the slow freezing of SSCs can support the induction of spermatogenesis to produce haploid cells under the 3-Dimensional testicular tissue culture.

**References:** Geens M, De Block G, Goossens E, Frederickx V, Van Steirteghem A, Tournaye H. Spermatogonial survival after grafting human testicular tissue to immunodeficient mice. *Hum. Reprod* 2006; 21: 390– 396.

Keros V, Rosenlund B, Hulthenby K, Aghajanova L, Levkov L, Hovatta O. Optimizing cryopreservation of human testicular tissue: comparison of protocols with glycerol, propanediol and dimethylsulphoxide as cryoprotectants. *Hum. Reprod* 2005; 20: 1676–1687.

Hovatta O. Cryobiology of ovarian and testicular tissue. *Best Pract. Res. Clin. Obstet. Gynaecol* 2003; 17:331–342.

Yokonishi T, Ogawa T. Cryopreservation of testis tissues and in vitro spermatogenesis. *Reprod Med Biol.* 2016; 15: 21- 28.

Tuuri T, Moilanen J, Kaukoranta S, Mäkinen S, Kotola S, Hovatta O. Testicular biopsy gun needle biopsy in collecting spermatozoa for intracytoplasmic injection, cryopreservation and histology. *Hum. Reprod* 1999; 14: 1274–1278.

Hovatta O. Cryopreservation of testicular tissue. *Mol. Cell. Endocrinol* 2000; 169: 113–115.

Keros V, Hulthenby K, Borgström B, Fridström M, Jahnukainen K, Hovatta O. Methods of cryopreservation of testicular tissue with viable spermatogonia in pre-pubertal boys undergoing gonadotoxic cancer treatment. *Hum Reprod.* 2007; 22:1384–95.

Song H, Wilkinson M. In vitro spermatogenesis a long journey to get tails. *Spermatogenesis.* 2012; 2(4): 1-7.

Gohbara A, Katagiri K, Sato T, Kobuta Y, Kagechika H, Araki Y, Araki Y, Oqawa T. In vitro murine spermatogenesis in an organ culture system. *Biol Reprod.* 2010; 83: 261-267.

Uchida A, Dobrinski I. Germ cell transplantation and neospermatogenesis. Springer international publishing AG. 2018: 361- 375.

Yokonishi T, Sato T, Katagiri, K, Ogawa T. In vitro spermatogenesis using an organ culture technique. *Methods Mol Biol.* 2013: 927, 479–488.

Sato, T, Katagiri K, Kubota Y, Ogawa T. In vitro sperm production from mouse spermatogonial stem cell lines using an organ culture method. *Nat prot.* 2013; 8 (11), 2098-2104.

Sato T, Katagiri K, Gohbara A, Inoue K, Ogonuki N, Ogura A, Kubota Y, Ogawa T. In vitro production of functional sperm in cultured neonatal mouse testes. *Nature.* 2011; 471: 504–507.

Goodyear S, Brinster R. Spermatogonial stem cell transplantation to the testis. *Cold Spring Harb Protoc.* 2017; 10: 299- 305.

Zeng W, Snedaker A, Megee S, Rathi R, Chen F, Honaramooz A, Dobrinski I. Preservation and transplantation of porcine testis tissue. *Reprod Fertil Dev.* 2009; 21(3): 489- 497.

Mirzapour T, Movahedin M, Tengku Ibrahim TA, Koruji M, Haron AW, Nowroozi MR, Rafeian SH. Effects of basic fibroblast growth factor and leukaemia inhibitory factor on proliferation and short-term culture of human spermatogonial stem cells. *Andrologia.* 2012; 44(1); 41-55.

Ibtisham F, Wu J, Xiao M, An L, Banker Z, Nawab A, Zhao Y, Li G. Progress and future prospect of in vitro spermatogenesis. *Oncotarget.* 2017;A 8(39); 66709- 66727.

Chalkley HW. Methods for quantitative morphologic analysis of tissues. *J National Can Inst.* 1943; 4(1): 47-53.

Anjamrooz SH, Movahedin M, Mowla SJ, Beiranvand SP. Assessment of Morphological and Functional Changes in the Mouse Testis and Epididymal Sperms Following Busulfan Treatment. *Iran Biomed Jour.*2007; 11(1): 15-22.

Lee Y W, Lee R, Park J H, Tae J, Park C, Jhun H, Lee J, Hur T, Song H. Characterization of male germ cell markers in canine testis. *Anim Reprod Sci.* 2017: 182; 1-8.

Tardif S, Guyonnet B, Cormier N, Cornwall G. Alteration in the processing of the ACRBP/sp32 protein and sperm head/ acrosome malformations in pro-protein convertase 4 (PCSK4) null mice. *Mol Hum Reprod.* 2012; 18(6); 298-307.

Mohaqiq M, Movahedin M, Mokhatri Dizaji M, Mazaheri Z. Upregulation of  $\alpha 6$  and  $\beta 1$  integrins genes in mouse spermatogonial stem cells after continuous and Pulsed low intensity ultrasound stimulation. *Yakhteh.* 2018: 19 (4); 634- 639.

Jahnukainen K, Hou M, Petersen C, Setchell B, Soder O. Intratesticular transplantation of testicular cells from leukemic rats causes transmission of leukemia. *Cancer Res.* 2001; 61: 706–10.

Fujita K, Tsujimura A, Miyagawa Y, Kiuchi H, Matsuoka Y, Takao T. Isolation of germ cells from leukemia and lymphoma cells in a human in vitro model: potential clinical application for restoring human fertility after anticancer therapy. *Cancer Res.* 2006; 66: 11166–71.

Patience C, Takeuchi Y, Weiss RA. Zoonosis in xenotransplantation. *Curr Opin Immunol.* 1998; 10: 539–42.

Staub Ch, Hue D, Nicolle J, Saporì MH, Segretain D, Durand Ph. The whole meiotic process can occur in vitro in untransformed rat spermatogenic cells. *Exp Cell Res.* 2000; 260: 85- 95.

Shinohara M, Takehashi M, Takashima S, Lee J, Morimoto H, Chuma S, Raducanu A. Homing of mouse spermatogonial stem cells to germline niche depends in B1-integrin. *Cell Stem Cell.* 2008; 3: 533- 542.

Mohaqiq M, Movahedin M, Mazaheri Z, Amir Janati N. Following in vitro spermatogenesis with long-term preserved spermatogonial stem cells. *Pathobiology Research.* 2016;A 19(3); 1- 15.

Mohaqiq M, Movahedin M, Mazaheri Z, Amirjannati N. Successful human spermatogonial stem cells homing in recipient mouse testis after in vitro transplantation and organ culture. *Cell J.* 2019 Jan;20(4):513-520.

Humter D, Anand-Ivell R, Danner S, Ivell R. Model of in vitro spermatogenesis. *Spermatogenesis.* 2012; 2(1); 32- 43.

**P-787** Wednesday, October 16, 2019 6:30 AM

**AUTOLOGOUS STEM CELL OVARIAN TRANSPLANTATION (ASCOT) REVITALIZED THE AGED BLOOD-BORNE SECRETOME IN POOR RESPONDER (PR) WOMEN.** Nuria Pellicer, MD,<sup>a</sup> Anna Buigues, B.Sc.,<sup>b</sup> Francisco Dominguez, Ph.D.,<sup>c</sup> Susana Martinez-Cuenca, M.D.,<sup>a</sup> Antonio Pellicer, MD,<sup>d</sup> Sonia Herraiz, Ph.D.<sup>e</sup> <sup>a</sup>Hospital Universitario y Politécnico La Fe, Valencia, Spain; <sup>b</sup>IVI Foundation, Innovation, Valencia, Spain; <sup>c</sup>IVI Foundation - ISSLaFe Biomedical Research Institute, Valencia, Spain; <sup>d</sup>IVI-RMA, IVI Rome, Rome, Italy; <sup>e</sup>IVI Foundation Innovation - Reproductive Medicine IIS La Fe, Valencia, Spain.



**OBJECTIVE:** Do the non-cellular components of ASCOT optimize impaired ovarian reserve and allowed spontaneous pregnancies in PR, by reverting the aged-associated plasma secretome profile?

**DESIGN:** Plasma samples were obtained from 17 PR women (35-40yr) recruited in the ASCOT prospective pilot study developed at La Fe University Hospital (NCT02240342). Three samples of peripheral blood were collected per patient, at recruitment (PRE), during aphaeresis for stem cell collection (APHAERESIS) and 3 months after ASCOT (POST). Plasma was obtained by centrifugation following standard procedures.

**MATERIALS AND METHODS:** PRE-, APHAERESIS and POST paired plasma samples from same patient underwent protein relative quantitation by SWATH LCMS/MS analysis. The protein areas were calculated and normalized by the total sum of the areas of all quantified proteins. Then, statistical tests of reduction of the dimensionality, Principal component Analysis (PCA) and discriminant analysis (DA) (with Pareto scaling) were performed. Linear regression analysis was then applied to identify relevant proteins involved in differential proteomic profile between samples.

**RESULTS:** The dimensionality reduction tests PCA and DA showed a clear separation of PRE, ASCOT and POST samples (PC1:38.9%, PC2 16.8% and D1 50%, D2 50%).

Proteomic analysis identified a total of 296 proteins in our plasma samples. Eleven proteins (3.7%, PPI enrichment  $p=1.56e^{-06}$ ) were found differentially expressed in aphaeresis when compared to previous samples, while increased to 70 (23.6%, PPI enrichment  $p < 1.0e^{-16}$ ) in samples collected 3 months after ASCOT. The differentially regulated proteins were common in the two comparisons highlighting the reliability of the stem cell effect and were mainly involved in vascularization, stem cell regenerative effects, anti-apoptosis, anti-inflammation and niche protection. We found that Endothelial protein C receptor (EPCR), Thrombospondin 1 (TSP-1), Vascular Cell Adhesion Molecule (VCAM1) and Serpin-7 (THBG;  $p=0.04$ ) were upregulated after ASCOT, being VCAM1 and THBG previously described as decreased with aging. Of the downregulated proteins, we identified the Apolipoprotein C3 and Vitamin D binding protein, which were previously described as increased with aging.

Presence of higher TSP-1 aphaeresis levels were found in patients whom AMH increased ( $p=0.04$ ).

**CONCLUSIONS:** Non-cellular components of aphaeresis could be crucial on the ovarian reserve optimization observed after ASCOT in PR. These results allowed us the identification of specific proteins related to tissue regeneration and raised the possibility of long-term systemic effects induced by stem cell, according to several spontaneous pregnancies reported up to 6 months after ASCOT treatment. Nevertheless, this is a descriptive analysis of the proteomic profile modifications induced by stem cell ovarian transplant that should be confirmed in a larger population of patients with advanced maternal age or diminished ovarian reserve.

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**MAINTAINING ENDOMETRIAL STROMAL STEM CELLS IN A NON-SCAFFOLD BASED 3D CULTURE SYSTEM.** Sule Yildiz, MD, Bahar D. Yilmaz, MD, Stacy A. Kujawa, BS, Serdar Bulun, MD. Northwestern University, Chicago, IL.



**OBJECTIVE:** Endometrial stem cells are crucial for the cyclical regeneration of endometrium under the orchestration of steroid hormones. Currently, there are no standardized in-vitro systems to maintain endometrial mesenchymal stem cells (eMSC) in culture. We hypothesize that culturing endometrial stromal cells in a 3-dimensional (3D) system would mimic psychobiological environment more closely and overcome problems arising from contact inhibition in monolayer culture. The objective of this study is to identify a novel culturing method for endometrial stromal cells to maintain the percentage and gene expression profile of eMSCs in vitro.

**DESIGN:** Prospective experimental design.

**MATERIALS AND METHODS:** Endometrial tissues obtained from hysterectomies were enzymatically digested and cell suspension was filtered through 70 and 20 micron-sieves consecutively for isolating the stroma-enriched portion. Cells were cultured in conventional monolayer culture method till confluency and then were seeded to 96 well round-bottom ultra-low attachment plates for 3D culture and 6-well monolayer plates for 2-dimensional (2D) culture. Cell aggregates were grown in either regular culture media (DMEMF12 with 10% FBS) or a mesenchymal stem cell media (MSC media). Spheroid formation was monitored every 24 hours for 3D plates via live-cell imaging. Samples were harvested at day 6 for RNA extraction, RNA expression profile was investigated by RT-qPCR.

**RESULTS:** Stromal cells in the 3D system formed spheroids consistently within the first 24 hours. While we did not observe significant differences in spheroid size between regular vs. MSC media, spheroids cultured in regular media had a darker center which suggests higher viability with MSC media. mRNA expression of steroid hormone receptors ESR1, ESR2, PGR, previously proposed endometrial stem cell markers ABCG2, SUSD2, and endometrial differentiation marker HOXA10 were analyzed. When compared to 2D, PGR expression was decreased significantly in 3D culture with both media ( $n=3$ ,  $p<0.0001$ ). SUSD2 expression was also significantly decreased in 3D cultures ( $n=3$ ,  $p<0.0006$ ). Besides, we saw a trend for HOXA10 showing decreased expression in 3D culture methods ( $n=3$ ,  $p=0.06$ ). Our results demonstrated that both the type of culturing plate (2D vs 3D) and the type of supplementing media are crucially important to provide optimal conditions and maintaining gene signature of endometrial stromal cell culture.

**CONCLUSIONS:** We suggest that a non-scaffold based 3D culture method with ultra-low attachment plates can be a promising method to maintain endometrial stem cells in culture. Decreased expression of mature endo-

metrial stromal markers PGR and HOXA10 suggest a less differentiated stage. We, however, have not observed an increase in the expression of known stem cell markers in eMSCs cultured 3D. Our findings are the first steps in advancing consistent and accessible 3D culture system for altering the phenotype of eMSCs in-vitro and exploring their stemness potential.

**SUPPORT:** National Institutes of Health Grant R37-HD36891, USA.

**P-789** Wednesday, October 16, 2019 6:30 AM

**AUTOLOGOUS PRP FOR THE MANAGEMENT OF THIN ENDOMETRIUM IN FROZEN EMBRYO TRANSFER CYCLES: WOULD IT IMPROVE THE OUTCOME?** Siddhartha Nagireddy, MCh(Reproductive medicine and Surgery),<sup>a</sup> N. Sanjeeva Reddy, MD (Obstetrics and Gynaecology), DGO,<sup>b</sup> Monna Pandurangi, MD (Ob & Gyn),<sup>c</sup> Radha Vembu, DGO, DNB (Obstetrics and Gynaecology), MNAMS, FICS, FIGOG, PhD,<sup>c</sup> Manjula Daniel G, PhD,<sup>d</sup> Sindhuja Namboori Srinivasan, MBBS, M.Sc Clinical Embryology, PhD Research Scholar,<sup>e</sup> Lahari Katneni, MS (Ob & Gyn),<sup>f</sup> Assistant Professor, Sri Ramachandra Institute of Higher Education and Research, Chennai, India; <sup>b</sup>Professor and Head, Department of Reproductive Medicine and Surgery, Sri Ramachandra Institute of Higher Education and Research, Chennai, India; <sup>c</sup>Associate Professor, Department of Reproductive Medicine and Surgery, Sri Ramachandra Institute of Higher Education and Research, Chennai, India; <sup>d</sup>Embryologist, Chennai, India; <sup>e</sup>Bachelor of medicine, bachelor of surgery(MBBS), Msc Clinical Embryology, Chennai, India; <sup>f</sup>Postgraduate in MCh Reproductive Medicine and Surgery, Sri Ramachandra Institute of Higher Education and Research, Chennai, India.



**OBJECTIVE:** Autologous platelet rich plasma (PRP) has emerged as a newer modality of treatment to improve endometrial thickness (ET) in cases of thin endometrium. Platelet activation would release growth factors from the alpha granules such as VEGF, EGF, PDGF, TGF and other cytokines, which may facilitate endometrial development. The present study was aimed to study the effect of autologous PRP on endometrial development in cases of thin endometrium in frozen embryo transfer cycles.

**DESIGN:** Non-randomized single arm trial.

**MATERIALS AND METHODS:** All women aged 20 - 40 years, presenting with thin endometrium (<7mm) on day 11 of HRT (hormone replacement therapy) for FET (frozen embryo transfer) were included in the study. Patients with previous endometrial disease such as asherman syndrome, tubercular endometritis, mullerian anomalies, and premature ovarian failure were excluded. Endometrial preparation was performed by GnRHa down regulation and HRT by estradiol valerate at 6mg/day. PRP was prepared by two step centrifugation method, and administered intrauterine by IUI catheter. Repeat USG evaluation of endometrium was performed on Day 15 ( 4 days after PRP instillation). Statistical analysis was performed by Paired sample T test and Chi square test through SPSS version 17 software.  $P<0.05$  was considered statistically significant.

**DESIGN:** Non-randomized single arm trial.

**MATERIALS AND METHODS:** All women aged 20 - 40 years, presenting with thin endometrium (<7mm) on day 11 of HRT (hormone replacement therapy) for FET (frozen embryo transfer) were included in the study. Patients with previous endometrial disease such as asherman syndrome, tubercular endometritis, mullerian anomalies, and premature ovarian failure were excluded. Endometrial preparation was performed by GnRHa down regulation and HRT by estradiol valerate at 6mg/day. PRP was prepared by two step centrifugation method, and administered intrauterine by IUI catheter. Repeat USG evaluation of endometrium was performed on Day 15 ( 4 days after PRP instillation). Statistical analysis was performed by Paired sample T test and Chi square test through SPSS version 17 software.  $P<0.05$  was considered statistically significant.

Parameter	Result
Age	32 ± 3.79
Male factor	13 (46.4%)
PCOS	03 (10.7%)
Tubal factor	05 (17.9%)
Fibroid uterus	01 (3.6%)
Endometriosis	01 (3.6%)
Decreased ovarian reserve	03 (10.7%)
Unexplained	02 (7.1%)
Endometrial thickness (ET) before PRP	6.3 ± 1.0
Endometrial thickness (ET) after PRP	7.0 ± 1.1
No. of patients with good endometrial vascularity (Grade II & III) before PRP	11 (39.3%)
No. of patients with good endometrial vascularity after PRP	12 (42.8%)
No. of patients with improved ET (≥ 7 mm)	20 (71.4%)
No. of patients with cycle cancellation	08 (28.6%)
Pregnancy rate in transferred patients	35% (7/20)
Implantation rate	14.2%
Miscarriage rate	14.2% (1/7)
Ongoing pregnancy	02 (10.0%)
Live birth rate	04 (20.0%)

**RESULTS:** Of the 28 women who presented with thin endometrium, 20 patients (71.4%) had increased ET to  $\geq 7$  mm, and underwent frozen embryo transfer. Eight patients (28.6%) had cycle cancellation due to persistent thin ET. There was a significant increase in the ET after PRP instillation: from  $6.3 \pm 1.0$  to  $7.0 \pm 1.1$  mm;  $P=0.003$ . In transferred cycles, the pregnancy rate was 35% and implantation rate was 14.2%. The ongoing pregnancy and live birth rates were 14.2% and 20% respectively.

**CONCLUSIONS:** 1. Autologous PRP significantly improves endometrial thickness in cases of thin endometrium in FET cycles.

2. Intrauterine instillation of autologous PRP considerably reduces cycle cancellation in FET cycles.

**References:** 1. Chang Y, Li J, Wei LN, Pang J, Chen J, Liang X. Autologous platelet-rich plasma infusion improves clinical pregnancy rate in frozen embryo transfer cycles for women with thin endometrium. *Medicine (Baltimore)*. 2019 Jan;98(3):e14062.

2. Eftekhari M, Neghab N, Naghshineh E, Khani P. Can autologous platelet rich plasma expand endometrial thickness and improve pregnancy rate during frozen-thawed embryo transfer cycle? A randomized clinical trial. *Taiwan J Obstet Gynecol*. 2018 Dec;57(6):810-813.

3. Bos-Mikich A, de Oliveira R, Frantz N. Platelet-rich plasma therapy and reproductive medicine. *J Assist Reprod Genet*. 2018 May;35(5):753-756.

4. Chang Y, Li J, Li X, Yang X, Liang X. Platelet-rich plasma administration has benefit for infertile women with thin endometrium in frozen blastocyst-stage embryos transfer program. *Fertil Steril*. 2017 Sep;108(3):e77.

5. Sunita R, Tandulwadkar, Manasi V, Naralkar, Akash D, Surana, M. Selvakarthick, Avinash H. Kharat. Autologous Intrauterine Platelet-Rich Plasma Instillation for Suboptimal Endometrium in Frozen Embryo Transfer Cycles: A Pilot Study. *J Hum Reprod Sci*. 2017 Jul-Sep; 10(3): 208–212.

6. Leila Nazari, Saghar Salehpour, Sedighe Hoseini, Shahrzad Zadehmo-darres, Ladan Ajori, M.D. Effects of autologous platelet-rich plasma on implantation and pregnancy in repeated implantation failure: A pilot study. *Int J Reprod Biomed (Yazd)*. 2016 Oct; 14(10): 625–628.

**SUPPORT:** Self funded.

**P-790** Wednesday, October 16, 2019 6:30 AM

#### **DEVELOPING METHODS FOR CO-CULTURE OF HUMAN TESTICULAR CELLS AND PLURIPOTENT STEM CELLS.**

Marina V. Pryzhkova, PhD, Philip W. Jordan, PhD. Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.



**OBJECTIVE:** Human Sertoli and Leydig cells are of great value for research community as tools for studying testicular physiology and effects of environmental pollutants. However, traditional cell culture conditions containing serum pose limitations for evaluation of the role of active molecules on cell functions. Therefore, our first objective was to establish serum-free culture conditions to maintain somatic testicular cell lines. The ability to perform live cell microscopy to model testicular physiology is of great importance. Thus, our second objective was to develop methods for genetic manipulation of human somatic testicular cells and establish cell lines that express fluorescent markers. Our long term goal is to use human pluripotent stem cells (hPSCs) to generate testicular somatic and germ cells. Our final objective was to co-culture our genetically modified testicular cell lines with hPSCs in 3D microenvironment.

**DESIGN:** Using de-identified donor testes we established cell lines, which were used for testing serum-free culture conditions and genetic modification. Characterized human embryonic stem cell (hESC) line was used for comparison in genetic manipulations and in co-culture with testicular cells.

**MATERIALS AND METHODS:** De-identified adult donor testes were mechanically dissociated and plated into serum-containing medium. Suspension cells were removed, and adherent cells were further propagated to establish cell lines, which were further characterized for testicular somatic cell marker expression by immunocytochemistry and RT-PCR. For comparative studies, cells were grown in serum-containing medium or under serum-free conditions for consecutive passages and evaluated for increase in cell number. Fluorescent markers were introduced into testicular cells and hESCs using lipofection. Transfected cells were further selected for antibiotic resistance and subcloned. Genetic modification was confirmed by genotyping, and transgene expression was assessed using live cell imaging. In co-culture studies, fluorescent hESCs were first induced to differentiate in embryoid bodies. Formed embryoid bodies and fluorescent testicular cells were embedded into extracellular matrix for stationary culture and trans-

ferred for culture in spin bioreactors. Cell co-cultures were assessed by live imaging.

**RESULTS:** Our study has shown, that established cell lines are represented by cells expressing Sertoli cell markers with a minor contribution of other testicular cell types. We have shown that cells can be maintained under serum-free conditions without the loss of proliferative activity. Established cell lines can be genetically modified to express a marker of interest. Importantly, we have demonstrated that testicular cells and hESCs can be cocultured for prolonged time in a 3D microenvironment.

**CONCLUSIONS:** Our research extends possible applications of human testis-derived somatic cells for further studies of human male reproductive biology and shows that these cells can be successfully adapted for PSC research.

**SUPPORT:** KY Cha Award in Stem Cell Technology.

**P-791** Wednesday, October 16, 2019 6:30 AM

#### **EXOSOMAL MIR-664-5p DERIVED FROM HUMAN BONE MARROW MESENCHYMAL STEM CELLS IMPROVE OVARY FUNCTION OF PREMATURE OVARIAN FAILURE BY TARGETING p53 SIGNALING PATHWAY.**

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**OBJECTIVE:** Although many reports show that various kinds of stem cells have the ability to recover the function of premature ovarian failure (POF), few studies are associated with the mechanism of stem cell treatment of POF. We designed this experimental study to investigate whether human bone marrow stem cell-derived exosomes (hMSC-Exos) retain the ability to restore ovarian function and how hMSC-Exos work in this process.

**DESIGN:** A POF mouse model was established and Cisplatin-damaged granulosa cells (GCs) were prepared to illuminate the mechanism of hMSCs in curing POF.

**MATERIALS AND METHODS:** The hematoxylin and eosin assay method was employed to assess the number of follicles. Enzyme-linked immunosorbent assay (ELISA) was used to detect the serum levels of sex hormones. Cellular activity and apoptosis were measured by flow cytometry and CCK-8. Real-time PCR were used to determine and western blot assays were used to determine protein expression levels of p53 and exosomal miRNA secreted by hMSCs. Real-time PCR was used to detect the expression of p53 mRNA and the expression of in ovarian and granulosa cells. Site-directed mutagenesis was used to establish p53 3'UTR mutant granulosa cells; miRNA mimics and miRNA inhibitor were used to target regulating the expression of hMSCs exosome-derived miR-664-5p. Western blot assays were used to test the protein expression levels of apoptosis genes (p53, Fas, FasL, caspase-3, and caspase-9).

**RESULTS:** After the hMSCs-Exos were transplanted into the POF mice model, they exerted better therapeutic activity on mouse ovarian function, improving follicle numbers during four stages. ELISA results showed that hMSCs-Exos elevated the hormone levels to the normal levels. In addition, after hMSCs-Exos were cocultured with POFGCs, our results showed that hMSCs-Exos significantly promoted the proliferation rate and inhibited the apoptosis rate. Besides, mRNA and protein assays demonstrated that hMSCs-Exos downregulated the expression of p53 in vivo and in vitro. A series of microRNAs targeting p53 were screened by bioinformatics, and the expression of miR-664-5p was significantly increased in MSC exosomes. Western blot assay demonstrated that hMSCs-Exos inhibited expression of the apoptosis genes in POFGCs.

**CONCLUSIONS:** These findings demonstrate for the first time the molecular cascade and related cell biology events involved in the mechanism by which exosomal miR-664-5p derived from hMSCs improved ovarian function of POF disease via regulation of the p53 signaling pathway.

**References:** • Joop S. E. Laven. Premature ovarian insufficiency. *Semin Reprod Med* 2016; 34(04): 230-234

• Jagarlamudi K, Reddy P, Adhikari D, Liu K. Genetically modified mouse models for premature ovarian failure (POF). *Mol Cell Endocrinol*. 2010; 315:1-10.

• Torrealday S, Kodaman P, Pal L. Premature Ovarian Insufficiency- an update on recent advances in understanding and management. *F1000Res*, 2017, 6:2069-2083.

• Kozub MM, Prokopiuk VY, Skibina KP. Comparison of various tissue and cell therapy approaches when restoring ovarian, hepatic and kidney's function after chemotherapy-induced ovarian failure. *Exp Oncol*, 2017, 39(3): 181-185.

**IN VITRO STUDY FOR STIMULATION EFFECT OF HUMAN BONE MARROW MESENCHYMAL STEM CELL SECRETOME ON HUMEN GRANULOSA CELL.** Hang-Soo Park, PhD,<sup>a</sup> Abdeljabar El Andaloussi, PhD,<sup>b</sup> Rishi Man Chugh, PhD,<sup>a</sup> Amro Elsharoud, MD,<sup>a</sup> Mara Ulin, MD,<sup>a</sup> Hajra Takala, MD., MPH.,<sup>a</sup> Ayman Al-Hendy, MD PhD.<sup>a</sup> <sup>a</sup>The University of Illinois College of Medicine, Chicago, IL; <sup>b</sup>The University of Illinois College of Medicine Department of Pathology, Chicago, IL.



**OBJECTIVE:** Primary ovarian insufficiency (POI) refers to ovarian loss of function under the age of 40 years and lead those patients to usually present by amenorrhea and infertility. One of the reasons of POI is chemotherapy for cancer patients. It is broadly believed that chemotherapy drugs may vastly eliminate granulosa cells which are essential for oocyte survival and follicular development. Our previous study shows that transplantation of human bone marrow derived mesenchymal stem cell (hBM-MSC) in chemotherapy induced POI mouse ovary can reverse POI through correction of serum hormonal level, promote follicular generation in ovary, increase in granulosa cell population, and achieving pregnancy. According to this research, BM-MSC is a promising cell source to treat POI patients, however, it is still not clear how hBM-MSC reverse POI. BM-MSC is already known that secreting various type of cytokines including growth factors and anti-inflammatory factors and some of those factors could be contribute on granulosa cell function or population in ovary. Understanding the mechanism of hBM-MSC secretome on granulosa cells will explain, how BM-MSC work to treat on chemotherapy induced POI ovary.

**DESIGN:** Secretome from hBM-MSC can increase the proliferation and function of human granulosa cells.

**MATERIALS AND METHODS:** In this study, we used hBM-MSC conditioned media (hBM-MSC CM) for secretome treatment. HGrC1 human non-luteinized granulosa cell line (RRID:CVCL\_KB28) were plated in culture flask and cultured 24 hours and treated with hBM-MSC CM. The hBM-MSC CM treated HGrC1 cells were compared with control CM treated HGrC1 cells. We examined proliferation of HGrC1 cells by cell doubling time and Ki67 positive population. We also analyzed the expression of granulosa cell markers such Cyp19, and StAR at mRNA and protein level by real time RT-PCR, FACS analysis and western blot.

**RESULTS:** We found that hBM-MSC CM treated HGrC1 cells shows higher cell number in cell counting and more Ki67 positive cells in FACS analysis. We also found that hBM-MSC CM treated HGrC1 cells shows higher expression of Cyp19, StAR, and FOXL2 gene quantified by real-time RT-PCR. The higher expression of Cyp19 and StAR also confirmed in protein level by FACS and Western blot.

**CONCLUSIONS:** Our data reveal that hMSC CM treated human granulosa cells shows higher proliferation and marker gene expression. It suggests that some factors in hBM-MSC secretome can stimulate granulosa cell proliferation and function which can explain the therapeutic effect on chemotherapy induced POI animal model. Our study suggests that using BM-MSC secretome may present a novel treatment modality for POI patients.

**SUPPORT:** Startup fund of University of Illinois at Chicago.

P-793 Wednesday, October 16, 2019 6:30 AM

**EMBRYONIC DEVELOPMENT KINETICS AFTER AUTOLOGOUS BONE MARROW MESENCHYMAL STEM CELL- DERIVED MITOCHONDRIA TRANSFER INTO COMPROMISED OOCYTES : A PROSPECTIVE SELF-CONTROLLED STUDY.** Xiaolan Li, MD, Lei Jia, MD, Zhiqiang Zhang, master's degree, Shihui Zhang, bachelor's degree, Xiao-Yan Liang, MD, PhD The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.



**OBJECTIVE:** We aimed to explore whether autologous bone marrow mesenchymal stem cell (BMSC)-derived mitochondria transfer into compromised oocytes change their embryonic development kinetics and improve outcomes in women with multiple in vitro fertilization (IVF)/ Intracytoplasmic sperm injection (ICSI) failures due to low oocyte quality

**DESIGN:** A prospective self-controlled study

**MATERIALS AND METHODS:** This prospective self-controlled study was conducted at the Department of Assisted Reproduction of the sixth affiliated hospital of Sun-Yet san university from January 2018 to June 2018. Patients were voluntarily enrolled meeting the following criteria: [1] undergoing ICSI program, [2]<42 years of age, [3] body mass index <30 kg/m2, [4]at least one previous failed IVF with Low embryo quality. Low

• Li J, Yu Q, Huang H, Deng W, Cao X, Adu-Frimpong M, Yu J, Xu X. Human chorionic plate-derived mesenchymal stem cells transplantation restores ovarian function in a chemotherapy-induced mouse model of premature ovarian failure. *Stem Cell Res Ther.* 2018 Apr 3;9(1):81.

• He Y, Chen D, Yang L, Hou Q, Ma H, Xu X. The therapeutic potential of bone marrow mesenchymal stem cells in premature ovarian failure. *Stem Cell Res Ther.* 2018 Oct 4;9(1):263.

• Badawy A, Sobh MA, Ahdy M, Abdelhafez MS. Bone marrow mesenchymal stem cell repair of cyclophosphamide-induced ovarian insufficiency in a mouse model. *Int J Womens Health.* 2017 Jun 15; 9:441-447.

• Khanmohammadi N, Sameni HR, Mohammadi M, Pakdel A, Mirmohammadkhani M, Parsaie H, Zarbakhsh S. Effect of Transplantation of Bone Marrow Stromal Cell- Conditioned Medium on Ovarian Function, Morphology and Cell Death in Cyclophosphamide-Treated Rats. *Cell J.* 2018 Apr;20(1):10-18.

• Mohamed SA, Shalaby SM, Abdelaziz M, Brakta S, Hill WD, Ismail N, Al-Hendy A. Human Mesenchymal Stem Cells Partially Reverse Infertility in Chemotherapy-Induced Ovarian Failure. *Reprod Sci.* 2018 Jan;25(1):51-63.

• Nelson LR, Bulun SE. Estrogen production and action. *J Am Acad Dermatol.* 2001; 45(3 Suppl):S116-S124.

• Massin N, Méduri G, Bachelot A, Misrahi M, Kuttann F, Touraine P. Evaluation of different markers of the ovarian reserve in patients presenting with premature ovarian failure. *Mol Cell Endocrinol.* 2008 Jan 30;282(1-2):95-100.

• Fu X, He Y, Wang X. Overexpression of miR-21 in stem cells improves ovarian structure and function in rats with chemotherapy-induced ovarian damage by targeting PDCD4 and PTEN to inhibit granulosa cell apoptosis. *Stem Cell Res Ther.* 2017; 8(1): 187-199.

• Ding L, Yan G, Wang B, Xu L, Gu Y, Ru T, Cui X, Lei L, Liu J, Sheng X, Wang B, Zhang C, Yang Y, Jiang R, Zhou J, Kong N, Lu F, Zhou H, Zhao Y, Chen B, Hu Y, Dai J, Sun H. Transplantation of UC-MSCs on collagen scaffold activates follicles in dormant ovaries of POF patients with long history of infertility. *Sci China Life Sci.* 2018 Dec;61(12):1554-1565.

• Su J, Ding L, Cheng J, Yang J, Li X, Yan G, Sun H, Dai J, Hu Y. Transplantation of adipose-derived stem cells combined with collagen scaffolds restores ovarian function in a rat model of premature ovarian insufficiency. *Hum Reprod.* 2016 May;31(5):1075-86.

• Cordonnier M, Chanteloup G, Isambert N, Seigneuric R, Fumoleau P, Garrido C, Gobbo J. Exosomes in cancer theranostic: Diamonds in the rough. *Cell Adh Migr.* 2017 Mar 4;11(2):151-163.

• Huang B, Lu J, Ding C, Zou Q, Wang W, Li H. Exosomes derived from human adipose mesenchymal stem cells improve ovary function of premature ovarian insufficiency by targeting SMAD. *Stem Cell Res Ther.* 2018 Aug 9;9(1):216.

• Sun L, Li D, Song K, Wei J, Yao S, Li Z, Su X, Ju X, Chao L, Deng X, Kong B, Li L. Exosomes derived from human umbilical cord mesenchymal stem cells protect against cisplatin-induced ovarian granulosa cell stress and apoptosis in vitro. *Sci Rep.* 2017 May 31;7(1):2552.

• Xiao GY, Liu IH, Cheng CC, Chang CC, Lee YH, Cheng WT, Wu SC. Amniotic fluid stem cells prevent follicle atresia and rescue fertility of mice with premature ovarian failure induced by chemotherapy. *PLoS One.* 2014 Sep 8;9(9): e106538.

• Xiao GY, Cheng CC, Chiang YS, Cheng WT, Liu IH, Wu SC. Exosomal miR-10a derived from amniotic fluid stem cells preserves ovarian follicles after chemotherapy. *Sci Rep.* 2016 Mar 16; 6:23120.

• Wang X, Simpson ER, Brown KA. p53: Protection against Tumor Growth beyond Effects on Cell Cycle and Apoptosis. *Cancer Res.* 2015 Dec 1;75(23):5001-7. Jagarlamudi K, Reddy P, Adhikari D, Liu K. Genetically modified mouse models for premature ovarian failure (POF). *Mol Cell Endocrinol.* 2010; 315:1-10.

• Wu YY, Liang CY, Liu TT, Liang YM, Li SJ, Lu YY, Liang J, Yuan X, Li CJ, Hou SZ, Lai XP. Protective roles and mechanisms of polysaccharides from *Dendrobium officinale* on natural aging-induced premature ovarian failure. *Biomed Pharmacother.* 2018 May;101:953-960.

• Xiong Y, Liu T, Wang S, Chi H, Chen C, Zheng J. Cyclophosphamide promotes proliferation and inhibition of mouse ovarian granulosa cells and premature ovarian failure by activating the lncRNA-Meg3-p53-p66Shc pathway. *Gene.* 2017 Jan 5; 596:1-8.

• Zhang T, He WH, Feng LL, Huang HG. Effect of doxorubicin-induced ovarian toxicity on mouse ovarian granulosa cells. *Regul Toxicol Pharmacol.* 2017 Jun;86:1-10.

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embryo quality was understood as 1) no fertilized MIIs; 2) deficient-quality embryos according to morphologic criteria; 3) arrested embryos. Patients with abnormal chromosome were excluded. BMSCs were isolated from 20ml bone marrow and cultured. Three days before oocyte retrieval, mitochondria were isolated by differential centrifugation from BMSCs. The retrieved oocytes were randomly and averagely divided into two groups i.e. Mitochondria transfer (MT) groups and control group. In MT group, 4000-5000 copies mitochondria DNA were injected into each oocyte during intracytoplasmic sperm injection. By means of a time-lapse system (EmbryoScope; Unisense FertiliTech, Aarhus, Denmark), this study determined the timing of a number of developmental parameters including cleavage timing from a zygote to a 8-cell embryo (t2, t3, t4, t5, t6, t7, t8) and assessed fragmentation at each stage. The intervals between two consecutive cleavages were also analyzed. Duration of the second cell cycle ( $cc2 = t3 - t2$ ) is the time from the division into a two-blastomere embryo until the time to the division into a three-blastomere embryo, and second synchrony ( $s2 = t4 - t3$ ) is the time from this division into a four-blastomere embryo.

**RESULTS:** A total of 25 patients were included and we got 231 oocytes in total. Their average age was 33.00 years old. The average antimullerian hormone level and antral follicles was 3.86 ng/ml and 14.68 respectively. Most of patients was primary infertility (72.22%) and the major cause of infertility was tubar factor (64.00%). We observed that the timings of all embryo cleavage stages (from t2 to t8) together with fragmentation values showed no significant differences between embryos deriving from oocytes with MT or without MT. It was noteworthy that  $s2$  was shorter in MT group, although difference didn't reach statistical significance (4.20 vs. 5.90). In addition, Oocytes of MT groups had little higher fertilization rate (89.06% vs. 88.35%,  $P = 0.865$ ).

**CONCLUSIONS:** This study demonstrated that BMSC-derived autologous mitochondria transfer didn't alter embryonic development kinetics. It might help to improve embryo development synchrony and fertilization.

## THE WEB

**P-794** Wednesday, October 16, 2019 6:30 AM

**HASHTAGS AND HATCHING: AN ANALYSIS OF INFORMATION AND INFLUENCE IN FERTILITY-RELATED SOCIAL MEDIA.** Arielle H. Bayer, MD,<sup>a</sup> Jennifer K. Blakemore, MD,<sup>a</sup> Meghan B. Smith, MD,<sup>b</sup> James A. Grifo, MD, PhD,<sup>c</sup> <sup>a</sup>NYU Langone School of Medicine, New York, NY; <sup>b</sup>University of Southern California, Los Angeles, CA; <sup>c</sup>NYU Langone Prelude Fertility Center, New York, NY.



**OBJECTIVE:** 79.9% of patients surveyed in a fertility clinic felt social media (SM), the use of electronic communication to share information, benefited the patient experience.<sup>1</sup> Up to 40% of Americans doubt professional opinion when it conflicts with web-based findings.<sup>2</sup> We examined fertility-related SM accounts and factors that contribute to influencer status (credibility to a large SM audience).

**DESIGN:** Cross-sectional analysis.

**MATERIALS AND METHODS:** The search function of Twitter (TW) and Instagram (IG) was used on 3/26/19 to generate a list of accounts with the terms: fertility, infertility, etc, egg freezing, ivf, endometriosis and reproductive. Accounts not in English, private, no posts in > 1 year, or content unrelated to search terms were excluded. Between 3/31/19 - 4/7/19, accounts were assessed for: author type; REI board certification (REI-BC); influencer (INF) status (>10K followers on IG; verified check mark on TW); age of account (mo); number (n) of followers; n of posts; hashtags and content in last 5 posts. Statistical analysis included unpaired t-tests and a classification and regression tree (CART) using n of posts per month (ppm) and most frequent content to determine factors associated with INF status.

**RESULTS:** 710 accounts (347 TW, 363 IG) were identified, of which 537 (278 TW, 259 IG) were included. There were 4 academic/professional societies (4 TW/1 INF, 0 IG/0 INF), 90 REI clinics (42 TW/1 INF, 48 IG/0 INF, 24 REI physicians (15 TW/1 INF, 9 IG/0 INF), 28 allied health professionals (18 TW/1 INF, 10 IG/2 INF), 8 organizations with an MD advisor (5 TW/1 INF, 3 IG/1 INF), 162 patients (67 TW/4 INF, 95 IG/7 INF), 123 support groups (75 TW/23 INF, 48 IG/7 INF), 23 wellness accounts (9 TW/0 INF, 14 IG/4 INF), and 75 classified as others (43 TW/7 INF, 32 IG/6 INF). Mean n of TW posts was 9,329 (10 - 251,000) with mean 16,947 for INFs. Mean n of IG posts was 284 (1 - 2,784) with mean 915 INF. Mean n of TW followers was 9,728 (77 - 561,000) with mean 15,915 INF. Mean n of IG followers was 3,706 (6 - 55,900) with mean 20,514 INF. INFs were more likely to be awareness and support ac-

counts (59.8% TW, 25.0% IG), patients (12.8% TW, 25% IG), or other (17.9% TW, 21.0%IG). Only 7.7% TW and 7.1% IG INFs were REI-BC. Mean age of INFs was older than non-INFs (TW 102.3 ± 26.5 vs 84.2 ± 34.5,  $p < 0.0017$ ; IG 39.1 ± vs 21.0 ± 17.2,  $p < 0.0001$ ). IG content (1290 posts reviewed) was primarily personal stories (31.7%) or inspiration/support (23.7%). TW content (1390 posts reviewed) was mostly promotion (28.2%) and research/education (20.2%). Top hashtags included #infertility (128) and #ttc (54), with #infertile (2) and #tryingtoconceive (5) less common. CART analysis showed that the best predictor for classification as an INF was high activity (>50ppm TW, >10ppm IG). Inclusion of the most frequent content by platform did not accurately classify INFs.

**CONCLUSIONS:** As patients increasingly utilize SM to obtain and engage with health information, it is critical for REI physicians and clinics to understand the fertility-related SM landscape in order to successfully enhance relationships with patients and ensure dissemination of accurate and evidence-based information.

**References:** 1. Broughton D, Schelble A, Cipolla K, Cho M, Franasiek J, Omurtag KR. Social media in the REI clinic: what do patients want? *J Assit Reprod Genet.* 2018 Jul;35(7): 1259-1263.

2. Kane G et al. *Community Relations 2.0.* Harv Bus Rev. 2009 Nov;87(11): 45-50, 132.

**SUPPORT:** None.

**P-795** Wednesday, October 16, 2019 6:30 AM

**UNDERSTANDING THE ROLE OF SOCIAL MEDIA FOR PHYSICIANS.** Natalie M. Crawford, MD, MSCR,<sup>a</sup> Emily Evans-Hoeker, MD,<sup>b</sup> <sup>a</sup>Aspire Fertility Austin, Austin, TX; <sup>b</sup>Virginia Tech Carilion School of Medicine, Roanoke, VA.



**OBJECTIVE:** To evaluate current use and perceptions of physician usage of social media among users.

**DESIGN:** Survey study.

**MATERIALS AND METHODS:** Social media users, recruited via multiple social media platforms (blogs, Facebook, Instagram, Twitter), were asked to complete an electronic survey evaluating the role of social media in medicine.

**RESULTS:** A total of 3,080 people participated in the survey. A majority of respondents were Caucasian (81%), highly educated (87.5% completion of 4 year degree or more), women (98.9%) of reproductive age (18-44 years, 92.8%).

Platforms utilized included Facebook (91.4%), Instagram (85.2%), Snapchat (54.6%) and Twitter (41.9%). Instagram and Facebook were noted to have the highest daily engagement (46.4% and 43.6% respectively, vs 3.2% with Twitter). Instagram was cited as the most enjoyable platform for obtaining medical information (44% vs 18.1% for Facebook and 3.1% for Twitter). Most participants enjoyed learning about medical information on social media (84.8%), reported following at least one physician (75.3%), and indicated they would schedule an appointment with a physician they follow (74.6%), even if it required travel (54.4%). Of those who do not enjoy medical information on social media, 74.4% don't trust the accuracy of the information, and 41.6% only want to use social media for fun.

Most consumers (59.8%) enjoy seeing their doctor on social media due to finding medical information interesting (66.1%), understanding what their

TABLE 1. Interest in Topics Posted by Physicians

Medical facts	91.2%
Behind the scenes as a physician	88.1%
News-worthy research	87.6%
Work-life balance	86.6%
Clinical cases	84.6%
Behind the scenes personal	78.3%
Motivational posts	77.4%
Medical pictures	73.8%
Educational videos	73.7%
Local activities	68.7%
Live Q&A	62.7%
Path to becoming a physician	62.6%
Giveaways	50.4%

doctor does (55.6%), getting to know their doctor better (48.6%), understanding their health better (41.1%), and feeling like their doctor is up to date with technology (33.1%). Topics of highest interest included medical facts, behind the scenes, and news-worthy research (Table 1). Of the 24.7% of respondents who do not follow physicians, 44.2% reported they did not know physicians to follow and 23.3% felt like physicians were advertising.

**CONCLUSIONS:** As consumers have a high interest in utilizing social platforms for access to medical information, physicians have an opportunity to reach potential patients through utilization of popular platforms for education and patient recruitment. Given that Instagram is currently the platform with the highest usage and interest for medical information, physicians and medical practices should consider initiating or expanding use of this platform.

**P-796** Wednesday, October 16, 2019 6:30 AM

**NATIONAL INFERTILITY AWARENESS WEEK AND INTERNET SEARCH VOLUME: A GOOGLE TRENDS ANALYSIS.**

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**OBJECTIVE:** National Infertility Awareness Week (NIAW) aims to raise awareness among the general public regarding infertility and “remove the stigmas and barriers that stand in the way of building families.” While the success of other health awareness campaigns, most notably breast cancer, have been well documented, the efficacy of infertility awareness campaigns is less well characterized. Using internet search volume as a surrogate for public interest, we sought to assess the efficacy of NIAW.

**DESIGN:** Retrospective, cross-sectional study examining internet search trends.

**MATERIALS AND METHODS:** Using Google Trends, the relative search volumes (RSV) were determined for each year from 2010 – 2018 for “infertility” and “breast cancer,” the latter serving as a comparison campaign with well-documented success. Baseline annual RSV was calculated by determining the median weekly RSV for each year. The RSV was then determined for NIAW and Breast Cancer Awareness Month (BCAM). Awareness campaign RSV was then compared with the yearly baseline RSV. Significant increase was defined as a two-fold rise from baseline.

**RESULTS:** Search volumes for “infertility” increased from a mean RSV of 77.5 at baseline to 97.98 during NIAW with a mean yearly search volume increase of 27.1% during the study period, not meeting the definition of significance. In contrast, BCAM led to a significant increase in mean RSV for “breast cancer” from 28.1 at baseline to 100 during the awareness month with a mean increase of 263.1%.

**CONCLUSIONS:** NIAW is associated with an increase in internet search volume for the term “infertility,” but this was substantially less than the increase for “breast cancer” seen during BCAM. Many parameters might influence this disparity, including duration of the campaign and resources expended for campaign promotion. While additional metrics are needed to evaluate the efficacy of public health campaigns, the current data suggest there is opportunity to further increase public awareness of infertility through the NIAW campaign.

**SUPPORT:** None.

TABLE 1. Percent rise in relative search volume (RSV) for the terms “breast cancer” and “infertility” during Breast Cancer Awareness Month and National Infertility Awareness Week from 2010 – 2018. \*Denotes significant rise in RSV from baseline.

	National Infertility Awareness Week (%)	Breast Cancer Awareness Month (%)
2010	23.5	284.6*
2011	1.8	257.1*
2012	37.0	334.8*
2013	12.8	308.2*
2014	19.0	316.7*
2015	37.9	257.1*
2016	34.2	194.1*
2017	44.9	203.0*
2018	32.5	212.5*

**P-797** Wednesday, October 16, 2019 6:30 AM

**CONTENT ANALYSIS OF AN ONLINE MALE INFERTILITY COMMUNITY ON THE SOCIAL MEDIA WEBSITE REDDIT.**

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**OBJECTIVE:** As social media plays an increasingly influential role in healthcare, we aimed to understand the concerns and experiences of discussants in an online male infertility community and to provide insight into their perceptions of interactions with healthcare professionals.

**DESIGN:** We performed a qualitative and quantitative content analysis of posts in an online male infertility community on the anonymous social media website Reddit, which represents the 3<sup>rd</sup> most visited website in the United States and 6<sup>th</sup> in the world.

**MATERIALS AND METHODS:** We extracted all posts from the “MaleInfertility” Reddit community from 11/2017 to 10/2018 and used an inductive approach to perform content analysis to identify major themes and subthemes. For semantic analysis, the Language Inquiry and Word Count 2015 program was used; Mann-Whitney U tests were employed to identify differences in the linguistic attributes of text authored by men versus their partners, when it was possible to identify author gender.

**RESULTS:** We analyzed 97 posts. Notable themes and subthemes emerged: 72% shared personal experiences/emotions, including feeling emasculated or alone, or describing a negative (29%), positive (13%), or neutral (58%) experience with a healthcare professional; 35% indicated searching for shared experiences, such as with microdissection testicular sperm extraction or use of donor sperm; 19% posed questions about personal semen analysis (SA) results; and 14% shared resources or information. Men authored 53 posts (55%), women 21 (22%), and gender was not identifiable among 23 posts (24%). Based on semantic analysis, posts by men had higher authenticity scores (Z=3.44, p<0.001), suggesting more honest or personal text, but lower clout scores (Z=-4.57, p<0.001), suggesting a more tentative or anxious style of writing, compared to posts by women.

**CONCLUSIONS:** To our knowledge, this study represents the first evaluation of a social media community focused on male infertility. Despite the prevalence of male factor infertility in the general population, many patients anonymously express feeling alone in their struggles with infertility, are searching for others who have gone through similar experiences, and may tie their self-worth to their ability to conceive a child. Perceived poor physician communication may compound these feelings, as nearly 20% of posts involve a question related to interpretation of SA results, even after a recent visit with a fertility specialist. These results suggest a potential role for physicians on social media to engage with patients and connect them to accurate resources. Moreover, the data also indicate opportunities to improve in-office patient education.

**P-798** Wednesday, October 16, 2019 6:30 AM

**MALE INFERTILITY WEBSITES: WHAT ARE OUR PATIENTS READING?**

English Margaret, BS,<sup>a</sup> Wesley Yip, MD,<sup>b</sup> Manan Darshan Mehta, BS,<sup>c</sup> Mary Katherine Samplaski, MD.<sup>d</sup> <sup>a</sup>Keck School of Medicine, University of Southern California, Los Angeles, CA; <sup>b</sup>University of Southern California, Los Angeles, CA; <sup>c</sup>Keck USC School of Medicine, Los Angeles, CA; <sup>d</sup>Keck School of Medicine, University of Southern California, Los Angeles, CA.



**OBJECTIVE:** People ages 20-45 years (reproductive age) have been shown to use the Internet as a source of health information more frequently than their older counterparts. We sought to evaluate the quality and readability of highly visible websites on male infertility.

**DESIGN:** Structured website review.

**MATERIALS AND METHODS:** Using Google, the first 60 relevant websites from the search “male infertility” were classified by source and analyzed. Content was evaluated by 4 blinded reviewers. We chose Google because it is the most widely used search engine, comprising 74.5% of Internet searches. Website quality of information was evaluated using the DISCERN score (assesses the quality of written information on treatment choices for a health problem), JAMA benchmark criteria (uses four core standards to evaluate web sites: authorship, attribution, disclosure, currency), and Health on the Net code (HONcode) accreditation status (assesses the reliability and credibility of online information). Readability was assessed using the Dale-Chall and Flesch Reading Ease indexes.

**RESULTS:** Websites were classified as: 43% hospital based, 12% fertility clinic based, 5% other clinic based, 33% society/association based, and 7% government based. The mean total DISCERN score was  $44 \pm 12$  (maximum score 80). 75% (45/60) of websites had clear aims or achieved their aims (scores of 4 or 5 [good]), but only 15% (9/60) described areas of clinical uncertainty. 25% (15/60) described the benefits of treatments, but only 5% (3/60) described the risks of treatments. 72% (43/60) provided unbiased information. 68% (41/60) websites made it clear that there was more than one possible treatment choice. 27% (16/60) of websites encouraged shared decision making. Overall, 60% (36/60) of websites were "poor quality" (score 1-2-3) on the final question of the DISCERN instrument. Only 4/60 (6.7%) websites met all four JAMA benchmark criteria. The mean Dale-Chall score was  $9.53 \pm 1.30$ , indicating a college or graduate degree level of readability. The mean Flesch Reading Ease index was  $34.01 \pm 16.26$ , indicating a graduate degree level of readability. 20% (12/60) of websites were HONcode certified.

**CONCLUSIONS:** Websites on "male infertility" are of low quality, and only 6.7% met JAMA benchmark criteria. Minimal information on treatments was present, with only 25% of websites describing treatment benefits, but only 5% describing treatment risks. Only 15% of websites described areas of clinical uncertainty. Despite that these websites were written at a college to graduate degree level of reading, only 27% encouraged shared decision making. Reassuringly, most of these websites were hospital based, and 72% provided unbiased information. Patients should be cautioned that incomplete and potentially biased information on male infertility is prevalent online.

**P-799** Wednesday, October 16, 2019 6:30 AM

**ONLINE PATIENT EDUCATION INCREASES USE OF SINGLE EMBRYO TRANSFER.** Deborah Anderson, JD  
FertilityIQ, San Francisco, CA.



**OBJECTIVE:** To ascertain to how online patient education impacts a US patient's decision of whether to transfer one embryo per transfer (eSET).

**DESIGN:** 62 US patients were surveyed who met two strict criteria: #1 Would soon undergo a transfer whereby multiple embryos were available for transfer and B. Had completed a 10-minute online video course on the trade-offs of "Single or Multiple Embryo Transfer."

**MATERIALS AND METHODS:** Surveys were sent to patients electronically following their date of expected embryo transfer. Results were compiled using Qualtrics Surveys & regression was run to account for patient age, embryo stage, PGT-A results and insurance coverage.

**RESULTS:** Of the 62 surveyed patients, 33 (79%) elected for a single-embryo transfer, of whom 72% believed the online course was "critically influential" in their decision of how many embryos to transfer. By contrast, less than 42% of surveyed patients believe their doctor provided an "in-depth discussion" on the subject. Results persisted after accounting for potential confounders such as patient age, embryo stage, PGT-A results or insurance coverage.

**CONCLUSIONS:** Online patient education may compliment societal and clinical efforts to encourage patients to consider the benefits of elective single embryo transfer.

**P-800** Wednesday, October 16, 2019 6:30 AM

**UTILIZATION OF SOCIAL MEDIA FOR PERSONAL BRANDING BY PHYSICIANS.** Natalie M. Crawford, MD, MSCR,<sup>a</sup> Emily Evans-Hoeker, MD,<sup>b</sup> <sup>a</sup>Aspire Fertility Austin, Austin, TX; <sup>b</sup>Virginia Tech Carilion School of Medicine, Roanoke, VA.



**OBJECTIVE:** To evaluate the current usage of social media by physicians in medical practice.

**DESIGN:** Survey study.

**MATERIALS AND METHODS:** Physician users of social media, recruited via private physician Facebook groups and Instagram, completed an electronic survey evaluating the use of social media in medical practice and marketing.

**RESULTS:** 200 physicians participated in the survey. Most were Caucasian (81.9%) and of reproductive age (18-44 years, 80.4%). A majority of physicians (58.8%) were in private practice and had been out of training for 5 years or more (68%). Social media platforms most commonly utilized included Facebook (100%), Instagram (51.2%),

Twitter (38.4%), and Snapchat (20%). Few physicians had a personal blog or website (8.8%).

Most physicians believed that consumers would enjoy posts about medical information (83%) and would like to see their doctor on social media (66.4%). In addition, most physicians believed that patients would schedule appointments with physicians they follow on social media (92%) and would even travel to see a doctor they follow on social media (79.5%). However, only 49.2% of physicians had a professional social media account, and many indicated they would not post medically related information (50.8%). The majority of those who did not have a professional account felt that professional and personal lives should remain separate (83.5%). Of the 45% of physicians who would post information about being a physician on social media, most would do so because they "like educating the public about medical facts" (48%) and they "want others to understand what life as a physician is like" (27%).

Of physicians who do currently post professionally on social media, most post on Facebook (88.4% versus 6.7% on Twitter and 2.1% for Instagram cite medical facts and news worthy research as the information they share most commonly (76.6% and 71.2%, respectively). Most physicians take their own pictures (70.4%) and attempt to grow their following by commenting on other physicians' posts (56.6%), following other physicians (41.7%), posting on other media sources (guest blogs/interviews, 37.7%), and posting with specific hashtags (17%), though social media accounted for only 27.4% of marketing efforts, while practice websites (54.8%) and print ads (32.3%) were the most common.

**CONCLUSIONS:** Opportunities for personal branding and educating target populations via social media are likely underutilized by current physicians. Although the majority of physicians believe consumers enjoy medically related content on social media, there is a reluctance to posting medical content on a social platform. Efforts for personal branding and marketing could be improved by targeting popular platforms and topics preferred by the ideal audience.

## IVF OUTCOME PREDICTORS

**P-813** Wednesday, October 16, 2019 6:30 AM

**PATERNAL FACTORS AND EMBRYO ANEUPLOIDY: IS SOMETHING RELATED?.** Thiago F. Nunes, MD,<sup>a</sup>

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**OBJECTIVE:** The high incidence of aneuploidy observed in preimplantation embryos is one of the most significant factors affecting the clinical outcomes in assisted reproduction treatments. We investigate the correlation between paternal factors that could have impact on embryo aneuploidy in an oocyte donation program, including male age, sperm concentration, morphology and DNA sperm fragmentation.

**DESIGN:** Retrospective analysis of Preimplantation Genetic Testing for Aneuploidy (PGT-A) data of biopsied embryos from an oocyte donation program in a private clinic in Sao Paulo, Brazil.

**MATERIALS AND METHODS:** The present study analyzed cycles from an oocyte donation program, which minimized the impact of aneuploidies arising from the female gamete. Between January 2017 and March 2019, a total of 229 biopsied embryos from 75 cycles have been analyzed by NGS (next generation sequencing) for numerical and structural abnormalities in chromosomes. Embryo biopsies were performed at blastocyst stage (day 5 or 6), and were allocated according to paternal age in two groups:  $\leq 41$  years ( $n = 26$ ) and  $\geq 42$  years ( $n = 49$ ); sperm concentration in normozoospermic ( $n = 67$ ) and oligospermic ( $n = 8$ ); morphology according to Kruger's strict criteria  $\geq 4$  ( $n = 17$ ) and  $< 4$  ( $n = 58$ ). DNA sperm fragmentation has been assessed in 29 cases.

**RESULTS:** The results show a median paternal age of 44.7 years, with an average number of fertilized embryos of 6.2 and 1.9 of blastocysts at day 5 and 6. Of the 229 biopsied embryos, 143 were normal and 86 altered embryos

(37.55%), including 79 numerical and 7 structural abnormalities. Comparing the variables, the advanced paternal age was not related to an increase in the absolute number of embryo aneuploidy ( $p=0.15$ ). The sperm concentration showed no statistical difference between normo and oligospermic males ( $p=0.70$ ). According to strict morphology, Kruger  $< 4\%$  had 36% of aneuploidy comparing with 42.5% in Kruger  $\geq 4$  ( $p=0.38$ ). Comparing sperm DNA fragmentation and aneuploidy, we did not observe difference between the groups using a cut-off of 15% in the fragmentation rate ( $p=0.08$ ).

**CONCLUSIONS:** Therefore, these results suggested that the paternal factors, including age, sperm count, strict morphology and DNA sperm fragmentation were not related to the aneuploidy rate in preimplantation embryos in an oocyte donation program.

**P-814** Wednesday, October 16, 2019 6:30 AM

#### **THE NUCLEAR AND CYTOPLASMIC MATURITY OF RETRIEVED OOCYTES CONTRIBUTE TO ICSI OUTCOME.**

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**OBJECTIVE:** To evaluate whether the proportion of mature oocytes retrieved affects clinical intracytoplasmic sperm injection (ICSI) outcome.

**DESIGN:** Consenting couples with a female partner  $\leq 35$  years of age treated by ICSI at our center between 1993 and 2017 were included in this study. Cycles were allocated to four groups based on the proportion of metaphase-II (MII) oocytes at the time of injection: optimal (100-76%), adequate (75-51%), partial (50-26%), and minimal (25-1%). Clinical outcome was compared among the four groups.

**MATERIALS AND METHODS:** Couple age, body mass index, smoking and drinking habits, and demographics were controlled for in our study. Oocyte retrieval and the ICSI procedure were performed in the standard fashion. Cycles without oocytes injected on the day of retrieval were excluded. Embryology and clinical outcome were recorded.

**RESULTS:** In total, there were 7,672 ICSI cycles included; 4,838 in the optimal group, 2,252 in the adequate group, 518 in the partial group, and 64 in the minimal group. There was no difference in the average number of oocytes retrieved per cycle.

Among the four groups, a decreasing proportion of MII oocytes lowered the fertilization rate from 78% to 71% ( $P < 0.0001$ ) while raising the rate of 3PN embryos from 2% to 4% ( $P < 0.01$ ). There was a concurrent reduced number of good-quality embryos ( $P < 0.0001$ ) that resulted in a decreasing number of blastocysts cryopreserved ( $P < 0.0001$ ).

The implantation rate fell from 33% in the optimal group to as low as 17% in the minimal group ( $P < 0.0001$ ); thus, the clinical pregnancy rate dropped from 63.6% in the optimal group to 60.9% in the adequate, 52.1% in the partial, and 37.5% in the minimal groups ( $P < 0.0001$ ). Consequently, the live birth rate decreased from 49.2% in the optimal group to 26.6% in the minimal group ( $P < 0.0001$ ), whereas pregnancy loss rose inversely with oocyte maturity, from 22.6% in the optimal group to 29.1% in the minimal group ( $P = 0.001$ ).

**CONCLUSIONS:** The different ICSI outcomes seen with the use of MII oocytes can only be explained by differences in ooplasmic maturity. Achieving an optimal proportion of mature oocytes may enhance fertilization and consequent embryo development and implantation.

**P-815** Wednesday, October 16, 2019 6:30 AM

#### **LONG-ANTAGONIST PROTOCOL; A NEW PROTOCOL WHERE A BOLUS LUTEAL DOSE OF LONG-ACTING GNRH-ANTAGONIST DEGARELIX CAN EFFICIENTLY DOWNREGULATE LH DURING OVARIAN STIMULATION FOR IVF ADDRESSING FLEXIBILITY IN AN ANTAGONIST PROTOCOL.**

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erans Hospital of Athens, ATHENS, Greece; <sup>e</sup>3rd Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Thessaloniki, Greece.

**OBJECTIVE:** The purpose of that study was to examine whether a bolus luteal dose of a new long-acting GnRH-antagonist can be compared with the classical short with follicular multiple doses antagonist protocol.

**DESIGN:** In this randomized control trial, did participate 129 infertile women  $\leq 39$  years of age prepared to undergo IVF treatment in Assisting Nature Centre. Trial registration number was NCT03684421 and performed between January 2017-January 2019. Two groups of patients were compared: Control-Group (Short Antagonist group) consisted of 69 women, who followed a classic fixed day-6 GnRH-antagonist protocol whereas, Study-Group (new Long Antagonist Group) involved 60 women undergoing the new long-antagonist protocol.

**MATERIALS AND METHODS:** The new protocol was as follows: in late luteal phase (day-24) a bolus injection of 0.5 ml Degarelix was administered subcutaneously. After menses, initiation of ovarian stimulation was flexible, with gonadotropins (200-300IU) could be initiated from cycle-day-2 to cycle-day-10 and no other dose of antagonists was allowed. In the classical short antagonist-group gonadotropins 200-300IU started on day-2 or 3 of the cycle and 0.25 mg of antagonist (ganirelix) was administered daily from stimulation day-6 in a fixed way. Ovulation triggering was administered when 3 follicles of 18mm were present and rechCG was used (unless more than 14 follicles were present then agonist triggering was proffered). Oocyte pick-up performed 36h later. Only blastocyst transfer was allowed and fresh/frozen embryotransfer was decided upon response and progesterone rise.

**RESULTS:** No LH rise was noticed first of all in any patient. The mean age (33,3 vs. 33,0) and AMH (2,4 vs. 2,1) were not different among groups. Nevertheless, duration of stimulation ranged from 9-10 days in control group, whether in study-group ranged from 10-11 days. Similar number of oocytes retrieved (10.8 vs. 11.8) and similar mean number of blastocysts produced in both groups (5.0 vs. 5.5). No OHSS case was reported. Fresh embryotransfer was performed in 30/69 patients in control-group and the rest 39 patients underwent frozen embryotransfer in a Freeze-All strategy. Similarly, fresh embryotransfer was performed in 20/60 patients in study-group and the rest 40 patients underwent frozen embryotransfer in a Freeze-All strategy. Cumulative ongoing/delivery rate was 44.9% ( $n=31/69$ ) in classic antagonist (Control-group) and 50.0% (30/60) in the new Long Antagonist (Study-group),  $p < 0.05$ .

**CONCLUSIONS:** This pure novel concept combines the flexibility of the long agonist protocol, the security of the antagonist protocol, and eventually similar pregnancy efficacy as both of them used to. This new Long-Antagonist protocol addresses cycle programming that was missing with antagonist protocols and at the same time minimizes the risk for OHSS. It is for first time that a single dose of long-acting antagonist Degarelix, during luteal phase is described to efficiently down-regulate LH, produce mature eggs and implantable embryos. However, larger studies are required to confirm the success of this protocol.

**P-816** Wednesday, October 16, 2019 6:30 AM

#### **EUPLOID EMBRYOS WHERE ONLY 1PN OR NO PRONUCLEI (PN) WERE SEEN HAVE DELIVERY RATES COMPARABLE TO EUPLOID 2PN EMBRYOS.**

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**OBJECTIVE:** To determine the incidence of euploidy and implantation and delivery of Blastocysts derived from 0PN and 1PN compared with 2PN embryos.

**DESIGN:** Single center retrospective review of PGT-A cases over a 4 year period (2015-2018) where a biopsy and ploidy determination was performed on blastocysts (blasts) derived from zygotes where pronuclei (PNs) were either not evident (0 PN) or only 1 pronucleus (1 PN) was evident at the time of fertilization check.

**MATERIALS AND METHODS:** At our center fertilization checks are routinely conducted  $\sim 18$  hours post insemination or ICSI. The number of PN in each egg is recorded and zygotes cultured individually. Cases where  $\leq 50\%$  of the mature eggs exhibit 2PN are routinely rechecked later on Day 1 and omitted from this study if additional PNs seen. In cases for PGT-A, all viable inseminated eggs excluding those with  $\geq 3$  PN remain in culture to Day 6/7. Good quality blastocysts with a distinct Inner cell mass and cohesive trophectoderm are considered for PGT-A regardless of

TABLE 1.

	All Patient Ages	2PN	1PN	0PN	Sig
<b>Conventional insemin</b>	Rate of Good Quality Blast form rate	11287/21819(51.7%)	428 / 1538 (27.8%)	147 / 7657 (1.9%)	<0.001
	Number Blasts Bx'd	11287	428	147	<0.001
	Number Euploid Blastocysts (% Bx'd)	3864 (34%)	114 (27%)	35 (24%)	<0.001
	Ratio XX:XY	1820:2044	69:45	19:16	<0.005
<b>ICSI</b>	Rate of Good Quality Blast form rate	6553/12533 (52.2%)	76/490 (16%)	31/ 2219 (1.4%)	<0.001
	Number Blasts Bx'd	6553	76	31	<0.001
	Number Euploid (% Bx'd)	2188 (33%)	29 (38%)	11 (35%)	NSD
	Ratio XX:XY	1092: 1096	25:4	7:4	<0.001
<b>Insemin+ICSI</b>	IR (sac/ ET) (%)	1235/1809 (68%)	24/40 (60%)	8/10 (80%)	NSD
	LB / ET with known outcome (%)	756/1452 (52%)	16 /36 (44%)	6/9 (67%)	NSD
	LB Ratio XX:XY	362:394	11:5	4:2	NSD

whether they were 0PN, 1PN or 2PN at fertilization check. PGT-A results are shown in Table 1 along with PGT-A sex of blasts derived from each group.

#### RESULTS:

**CONCLUSIONS:** Prior to utilization of PGT-A and/or timelapse zygotes not exhibiting 2PN at fertilization check were routinely discarded. However, it is now obvious that a percentage of these, albeit small, are fertilized normally and are euploid. Though they account for only a small percentage these may be the only euploid blasts available. Implantation rates and LB rates following transfer of these blasts are similar to those for 2PN blastocysts. Of interest, ratios of XX:XY blasts derived from 1PN and 0PN zygotes were skewed towards female while those from 2PN zygotes were ~1:1. It should be noted that NGS cannot detect pure haploidy (23, X) or triploidy (69, XXX) thereby possibly misdiagnosing these as euploid although our IR and LB results indicate otherwise.

SUPPORT: None

References: None

**P-817** Wednesday, October 16, 2019 6:30 AM

#### SPERM MOTILITY IS ASSOCIATED WITH THE NUMBER OF GOOD QUALITY EMBRYOS PRODUCED IN WOMEN WITH DIMINISHED OVARIAN RESERVE.

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**BACKGROUND:** Previous data suggests that semen parameters do not predict pregnancy rates after in vitro fertilization (IVF) in egg donation (OD) cycles. Less is known about the impact of semen parameters on IVF laboratory outcomes with good quality eggs and among women with diminished ovarian reserve (DOR) for who may be more sensitive to subtle differences in semen parameters.

**OBJECTIVE:** To determine if semen parameters are associated with (1) IVF laboratory outcomes among egg donors with multiple cycles (2) IVF outcomes among women with DOR.

**DESIGN:** Retrospective cohort study

**MATERIALS AND METHODS:** We performed a chart review of all repeated egg donation cycles with different sperm sources and for women with DOR (<9 eggs collected) between January 1, 2010 and March 31<sup>st</sup>, 2019. The impact of semen parameters (volume, motility, and concentration) on the IVF laboratory outcomes including the number of normally fertilized eggs, good quality embryos (embryos(s) transferred + cryopreserved) and euploid rate were assessed in donor cycles and DOR with a mixed effects Poisson regression adjusting for age, eggs collected and repeated cycles.

**RESULTS:** 465 egg donation cycles and 2,456 DOR cycles were reviewed. The number of normally fertilized eggs differed in egg donation cycles in a univariate and multivariate model but these were not explained by semen parameters or male or female age (p<0.01 for both) There were no difference in the number of good quality embryos produced (p= 0.700). Among women with DOR, in a bivariate model adjusting for the number of eggs inseminated, sperm concentration was predictive of the number of normally fertilized embryos (p=0.037). After adjusting for male and female age and the method of sperm production (ejaculation vs surgical), concentration was not predictive of the number of normally fertilized eggs (p=0.082).

In a bivariate model, adjusting for the number of normally fertilized eggs, motility was predictive of the number of good quality embryos (p=0.042). Adjusting for male and female age and the method of sperm production, motility continued to be marginally predictive of the number of good quality embryos produced (p=0.046). A 10% increase in motility was associated with a predicted 0.012 increase in the number of good quality embryos.

**CONCLUSIONS:** When adjusting for within patient and between patient differences, semen parameters do not impact donor egg cycles but other male related predictors yet to be elucidated that impact fertilization. Among women with DOR, increased sperm motility is marginally associated with an increasing number of good quality embryos. Eggs from women with DOR may be less able to compensate for abnormal sperm function.

SUPPORT: None

**P-818** Wednesday, October 16, 2019 6:30 AM

#### EVIDENCE-BASED EVALUATION OF REPEATED CYCLES OF OOCYTE DONATION BY THE SAME WOMAN: TREATMENT OUTCOMES ARE NOT ADVERSELY AFFECTED BY MULTIPLE PRIOR DONATIONS.

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**OBJECTIVE:** To evaluate the quality of oocyte cohorts retrieved from healthy young women undergoing repeated cycles of oocyte donation.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Records of oocyte donation cycles performed at a single ART center from November 2011 through April 2019 were reviewed. The associations between oocyte donation cycle number and pre-treatment antral follicle count (AFC), total dosage of follicle-stimulating hormone (FSH) administered during ovarian stimulation, days of ovarian stimulation, follicles > 14 mm at trigger, retrieved oocyte number, mature (MII) oocyte number, the number of good quality blastocysts, and the percentage of euploid blastocysts among cycles undergoing PGT-A analysis by NGS were evaluated by linear regression analysis.

**RESULTS:** A total of 91 oocyte donation cycles among 18 oocyte donors (3 to 6 cycles each) were available for analysis. The total dosage of FSH administered per cycle remained constant across treatment cycle numbers. However, a higher number of prior oocyte donation cycles was associated with significantly increasing numbers of antral follicles, mature follicles (>14mm) at trigger, and mature (MII) oocytes retrieved.

**CONCLUSIONS:** Our investigative efforts focus on providing more couples the opportunity to have healthy children. One such group is egg donor recipients. There are limited available data on repeated ovulation stimulation cycles. It has been suggested that FSH stimulation may hasten ovarian aging by increasing recruitment of small growing follicles, thereby accelerating the depletion of follicle reserve. It has not been reported if repetitive oocyte donation affects average follicles recruited, oocyte maturity, blastocyst rate or ploidy status.

Increasing numbers of prior oocyte donation cycles are associated with a better response to ovarian stimulation, rather than adversely affecting treatment outcomes. Egg donation cycles 3-6 are associated with higher AFC,

Donation number	1	2	3	4	5	6	P-value for linear trend (regression)
Number of cycles	18	18	18	14	12	11	
AFC	27.9	29.3	31.4	35.3	38.2	39.3	$p=0.0005$
Total FSH dosage	2983.3	2950.0	2616.7	2580.4	2718.8	2718.2	$p=0.52$
Days of stimulation	14.2	12.7	16.6	12.6	12.6	13.0	$p=0.43$
Follicles > 14mm	24.4	25.8	26.3	31.6	30.5	29.3	$p=0.033$
Retrieved oocytes	25.8	28.3	29.9	30.9	33.7	30.8	$p=0.061$
MII oocytes	18.9	23.3	26.1	25.2	26.4	26.0	$p=0.0096$
Good quality blastocysts	8.9	11.9	13.4	13.1	12.3	11.6	$p=0.18$
Percent euploid	54%	73%	64%	61%	75%	74%	$p=0.30$

more follicles > 14 mm at trigger, and retrieval of approximately one third more mature (MII) oocytes, relative to cycle 1. A reevaluation of the ASRM guidelines regarding numbers of oocyte donation cycles per woman may be warranted.

References: Repetitive oocyte donation does not decrease serum anti-Müllerian hormone levels. Bukulmez, Orhan et al. *Fertility and Sterility*, Volume 94, Issue 3, 905 - 912

American Society for Reproductive Medicine. Repetitive oocyte donation. *Fertil Steril*. 2008; 90: S194-S195

Richardson, S.J. and Nelson, J.F. Follicular depletion during the menopausal transition. *Ann N Y Acad Sci*. 1990; 592: 13-20 (discussion 44-51)

P-819 Wednesday, October 16, 2019 6:30 AM

#### DOES LH SUPPLEMENTATION IN POOR RESPONDERS AFFECT GRANULOSA CELL APOPTOSIS RATE IN ART?

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**OBJECTIVE:** To compare the granulosa cells apoptosis rate with or without luteinizing hormone (LH) supplementation in poor ovarian responders during controlled ovarian stimulation (COS) for assisted reproductive technologies (ART).

**DESIGN:** Prospective randomized controlled clinical trial comparing LH supplementation versus FSH only in poor ovarian response patients.

**MATERIALS AND METHODS:** A total of 40 women with poor ovarian response according to Bologna criteria enrolled. Patients were randomly separated into two clinical trial groups: 20 patients were in group A, in which ovarian stimulation included rFSH and LH, and group B, in which 20 patients received rFSH without further LH addition. After oocyte retrieval, the oocytes were extracted with hyaluronidase treatment for ART procedure by the embryologist. The rest of follicular fluid was transferred to the HLA Typing Laboratory within the same day. To eliminate the effect of hyaluronidase treatment on granulosa cell viability and apoptosis, the validation of the cytometry protocol has been performed initially.

The verified flow cytometry protocol analyzing with Annexin V-FITC/Propidium Iodide has been applied to all 40 women to determine the apoptosis rate of granulosa cells. A sufficient number of cells required for evaluation could not be obtained from 5 samples of study group, 4 samples of control group and were excluded from the study.

Primary outcome measure was granulosa cells apoptosis rate in terms of viability, early apoptosis, late apoptosis and necrosis. Secondary outcomes were total r-FSH dose, metaphase II oocytes retrieved, clinical pregnancy rate.

**RESULTS:** No statistically significant differences were determined in mean age, BMI, duration of infertility, FSH level, AMH level and AFC between the groups. Mean values of viability were 93.30 and 74.74 for groups A and B respectively ( $p<0.001$ ). The granulosa cells apoptosis rates were compared as early apoptosis, late apoptosis and necrosis. Late apoptosis rates

were significantly lower in group A (mean value= 4.2975) than group B (mean value=17.3473)( $p<0.001$ ). Interestingly, although early apoptosis rates were 3.0656 and 6.8267 for group A and B respectively, these differences did not reach statistical significance ( $p=0.04$ ). Similarly, when clinical pregnancy rates were analyzed, no significant difference was observed; the rate of clinical pregnancy was %25 for group A whereas %20 for group B.

**CONCLUSIONS:** The results of this prospective and randomized trial show that the supplementation of LH in COS for ART decreases the late granulosa cell apoptosis rate in poor ovarian responder patients. Although LH supplementation seems necessary in poor responders to decrease the late granulosa apoptosis rates, this does not improve clinical pregnancy rates.

**SUPPORT:** This study was supported by grants from Giresun University Scientific Research Projects Department (SAG-BAP-A-2302)

P-820 Wednesday, October 16, 2019 6:30 AM

#### EFFICACY OF A MODIFIED MICROSECURE VITRIFICATION (MS-VTF) PROCEDURE WITH DMSO-FREE SOLUTIONS APPLIED TO A DEDICATED BLASTOCYST BIOPSY/VITRIFICATION-ALL/PGT-A PROGRAM: OPTIMIZING SINGLE HEALTHY TERM LIVE BIRTHS.

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**OBJECTIVE:** MicroSecure vitrification ( $\mu$ S-VTF) evolved as a non-commercial, FDA compliant device integrating sterile flexipettes (300 $\mu$ mID; Cook Medical) into CBS™ embryo straws. The mean cooling rate (1391°C/min) and warming rate (6233°C/min) of this closed, aseptic VTF system were verified by Dr. Greg Fahy (Schiewe et al., 2015). In 2014, CBS removed traditional hydrophobic plugged embryo straws from the worldwide marketplace, being replaced by 0.3ml semen straws. In turn, a modification of our  $\mu$ S-VTF procedure (Schiewe et al., 2017) was necessary to prevent wicking of flexipette contents. The goal of this study was to clinically validate the overall performance of our  $\mu$ S-VTF technique for human blastocysts over a 4-year duration.

**DESIGN:** Retrospective analysis of  $\mu$ S-VTF application with an apriori arrangement of FET cycle year (2015-2018) and number of euploid embryos transferred (1 or 2) were statistically analyzed by Chi-square analysis ( $p<0.05$ ).

**MATERIALS AND METHODS:** The  $\mu$ S technique modification of semen straws involved creating a mid-straw weld seal in front of the inner PVA plug, thus eliminating any contact with the fluid-embryo filled flexipette tip once loaded prior to sealing the ends closed and LN<sub>2</sub> immersion. Human blastocysts (>2CC grade) subject to trophectoderm biopsy on Day 5 to 7, were vitrified by  $\mu$ S-VTF using a Glycerol/EG-based solution ( $\geq 7.9$ M; Innovative CryoEnterprises, NJ). Straw enclosed, individual embryos contained in an open flexipette (3 $\mu$ l volume) were warmed in a 37°C 0.5M Sucrose media bath (60 $\mu$ m dish, >15ml). Following a 4-step dilution and 1-4h of tri-gas in vitro culture (37°C), ET was performed by transvaginal ultrasound guidance.

**RESULTS:** Between 2015-2018, 12,583 blastocysts were vitrified in 2,139 of 2,289 IVF patient cycles (94.2%; mean=5.7 blastocysts/cycle; mean patient age=37 years old). Of 2660 blastocysts warmed, 2640 survived completely (99%), as revealed by their cellular responsiveness to osmotic changes and intact membrane/cellular integrity. When single (n=2130) or multiple (n=237; mean=2.1 embryos/ET) vitrified-warmed ET was performed, 1500 (70.4%) and 260 (52.3%) blastocysts implanted, respectively. These implantation rates were different ( $p<0.05$ ), even though ongoing/live birth rates were similar (65.5% versus 67.1%, respectively). Overall, vitrified embryo efficiency was significantly improved transferring a single euploid blastocyst, independent of patient age, having a monozygotic twinning rate of 0.61% in contrast to a potentially unhealthy 38.8% multiple birth rate transplanted  $\geq 2$  euploid embryos.

**CONCLUSIONS:** Since 2014, our clinical practice has predominantly (>85%) applied blastocyst biopsying/preimplantation genetic testing for aneuploidy (PGT-A) and >99% VTF-all cycles. Vitrified embryo transfer cycles have become our clinical norm. In turn,  $\mu$ S-VTF using non-DMSO, glycerol based solutions has been associated with some of the highest % healthy single term live birth rate as reported by the annual CDC/ART Surveillance survey (2014-2017).  $\mu$ S-VTF has continued to be safe, secure, simple and inexpensive technique.

**SUPPORT:** None

References: Schiewe, MC, S Zozula, RE Anderson, GM Fahy. Validation of microSecure vitrification ( $\mu$ S-VTF) for the effective cryopreservation of human embryos and oocytes. *Cryobiology* 2015; 71:264-272. [A org/10.1016/j.cryobiol.2015.07.009](http://dx.doi.org/10.1016/j.cryobiol.2015.07.009)

Schiewe, MC, S Zozula, N Nugent, K Waggoner, J Borba, L Gamboa and JB Whitney. Modified microSecure vitrification: A safe, simple and highly effective cryopreservation procedure for human blastocysts. *J Vis Exp* 2017; 121: e54871. [doi:10.3791/54871](https://doi.org/10.3791/54871).

**P-821** Wednesday, October 16, 2019 6:30 AM

**CAN THE MOST FREQUENT IMBALANCE SEGREGATION MODE FOR RECIPROCAL TRANSLOCATION CARRIERS BE PREDICTED BY THE STENDEL-RUTKOWSKI METHOD OR HC-FORUM WEB SITE IN PRE-IMPLANTATION GENETIC TESTING FOR STRUCTURAL REARRANGEMENT?.** Toshiaki Endo, M.D.,<sup>a</sup> Tsuyoshi Baba, M.D.,<sup>b</sup> Takema Kato, Ph.D.,<sup>c</sup> Hiroki Kurahashi, M.D.<sup>d</sup> <sup>a</sup>Assistant Professor, Sapporo, Japan; <sup>b</sup>Sapporo Medical University, Sapporo, Japan; <sup>c</sup>Research assistant, Nagoya, Japan; <sup>d</sup>Div. Molecular Genetics, ICMS, Fujita Health University, Nagoya, Japan.



**OBJECTIVE:** Thus far, predicting segregant outcomes has been done using a diagram of the presumed pachytene configuration of the quadrivalent to deduce which modes of segregation are likely to lead the formation of embryos in reciprocal translocation carriers. However, it is very difficult to predict segregation outcomes precisely. As the Stengel-Rutkowski method (S-R method) (1998) and HC-Forum web site (HC-F site) (Cohen et al. 2001) are well known to predict risks of having “a liveborn aneuploid child” due to imbalance from 3 modes (adjacent-1 segregation (ADJ-1), adjacent-2 segregation (ADJ-2) and 3:1 segregation (3:1)), these methods have not been utilized for predicting segregation outcomes of embryos of IVF by preimplantation genetic testing for structural rearrangement (PGT-SR). It is important to know if the most frequent imbalance segregation mode for reciprocal translocation carriers can be predicted by the S-R method or HC-F site in PGT-SR.

**DESIGN:** Chromosome segregations in embryos of 33 female and 20 male reciprocal translocation heterozygotes were studied by PGT-SR as reported in Table 5-3 in the 5th edition of Gardner and Southerland’s “Chromosome abnormalities and genetic counseling.” Although Table 5-3 indicates that, in 53 cases, alternate segregation, ADJ-1, ADJ-2, and 3:1, each carrier had different mode patterns of segregation due to their different breakpoints. We tried to predict most frequent imbalance mode in embryos.

**MATERIALS AND METHODS:** We predicted “the most frequent imbalance mode” instead of risks of having “a liveborn aneuploid child” in embryos of 53 reciprocal translocation carriers using the S-R method and HC-F site. Then, we compared the most frequent modes predicted by these 2 method with the actual most frequent modes detected by PGT-SR. We also compared the results of the S-R method with the results from the HC-F site.

**RESULTS:** There were multiple modes of segregation in embryos in 61% female, and in 55% male carriers. The S-R method predicted that the risk of having a liveborn aneuploid child was  $1.81 \pm 0.60\%$  (mean  $\pm$  SE) and the HC-F site predicted  $18.8 \pm 1.82\%$  in female carriers. In male carriers the risks were  $3.35 \pm 1.70$ , and  $17.60 \pm 3.82$  by 2 methods. Thus, these risk figures were quite different. However, the most frequent segregation mode determined by the 2 methods was the same in 82% of the subjects in female carriers and 90% in male carriers. The most frequent segregation modes predicted by the S-R method and by HC-F site were the same in 85% and 86%, respectively, as those of the actual most frequent mode determined by PGT-SR. On the contrary, those modes by S-R method and by HC-F site were the same as 55% and 60%, respectively, as that by PGT-SR. Unfortunately, the HC-F site has been closed since December 31, 20018.

**CONCLUSIONS:** In PGT-SR for reciprocal translocation carriers, prediction of the most frequent imbalance segregation mode is quite important for genetic counseling. In this study, it is proposed that the S-R method and HC-F site are both good tools to predict the most frequent segregation mode of embryos for reciprocal translocation female carriers, not for male carriers.

**SUPPORT:** no support

**References:** no references

Groups	Group 1	Group 2	p-value
Number of MII	202	202	N/A
tPB2	3.6 $\pm$ 0.9	3.9 $\pm$ 1.1	0.15
tPNa	7.2 $\pm$ 1.2	7.8 $\pm$ 2.3	0.2
tPNf	24.6 $\pm$ 3.3	25.3 $\pm$ 2.6	0.12
t2	26.9 $\pm$ 3.7	29.0 $\pm$ 4.1	0.01*
t3	37.0 $\pm$ 4.4	38.7 $\pm$ 4.3	0.02*
t4	39.7 $\pm$ 4.6	40.9 $\pm$ 5.5	0.07
t5	50.0 $\pm$ 5.4	51.1 $\pm$ 6.2	0.31
cc2 (t3-t2)	9.7 $\pm$ 3.4	9.9 $\pm$ 2.3	0.68
s2 (t4-t3)	2.3 $\pm$ 2.5	1.8 $\pm$ 3.1	0.44
tM	95.0 $\pm$ 8.0	94.6 $\pm$ 9.0	0.84
tSB	105.0 $\pm$ 8.3	102.2 $\pm$ 8.3	0.03*
tB	111.4 $\pm$ 9.7	109.9 $\pm$ 8.3	0.38
tEB	117.9 $\pm$ 8.4	119.2 $\pm$ 10.6	0.31
Fertilization (%)	81.80%	80%	0.68
Euploidy (%)	36%	42%	0.09
Aneuploidy (%)	45%	31%	0.11
Mosaicism (%)	17%	22%	0.11
Irregular Division (%)	17%	21%	0.27
Day 5 Blastulation (%)	58%	52%	0.38

**P-822** Wednesday, October 16, 2019 6:30 AM

**THE MORPHOKINETIC EFFECTS OF CULTURE MEDIA WITH LOW LACTATE DURING EARLY EMBRYONIC DEVELOPMENT.** Sule Dogan, PhD,<sup>a</sup> Mike Urich, BSc,<sup>a</sup> Fang Li, MD/ PhD,<sup>a</sup> Ahmad Hammoud, MD,<sup>a</sup> Hanh N. Cottrell, MD,<sup>a</sup> Iqbal Khan, PhD,<sup>a</sup> Nicholas Shamma, MD<sup>b</sup> <sup>a</sup>IVF Michigan Fertility Clinics, Bloomfield Hills, MI; <sup>b</sup>IVF Michigan Fertility Centers, Bloomfield Hills, MI.



**OBJECTIVE:** To investigate the morphokinetic effects of culture media in early embryonic development.

**DESIGN:** Retrospective randomized study.

**MATERIALS AND METHODS:** Data used in this study were collected from our routine IVF-PGT patients who used either autologous and/or donor oocytes between February and December 2018. All cases whose embryos were incubated in conventional incubators were excluded. Embryoscope slides were prepared using two different media where well 1-6 contained one media vs. well 7-12 had the second media and equilibrated overnight. On the day of retrieval, patients with at least 10 MII oocytes were randomly selected for this study. After ICSI, MII oocytes (n=35 patients, n=404 MII oocytes) were divided into two groups, and cultured in the same embryoscope (Vitrolife) slides including two different media; Group #1 (Global total by Life global) vs Group #2 (Low lactate: 1mM, CSCM-NX by Irvine). The embryos were hatched on Day 3 and trophoctoderm biopsies (n=212) were performed accordingly. The biopsied materials were sent to CooperGenomics for Next Generation Sequencing [NGS] testing. There was no bias between two groups in this study because, the sibling oocytes were incubated under the same condition. Morphokinetic parameters were analysed using t-test. Fertilization, Irregular division, Blastulation and Euploidy rates were analysed using  $\chi^2$ -test among the groups.

**RESULTS:** According to our findings, all results were shown in Table 1. The differences were considered statistically significant once p-values are  $< 0.05$ .

**CONCLUSIONS:** In this study, we only demonstrated that the t2 and t3 divisions were earlier, and blastulation was later in Group #1 than Group #2. Although the euploidy rate was higher in media with low lactate (Group #2), this difference was not statistically significant. In conclusion, a larger sample size is needed to conclude the positive effects of culture media with low lactate.

**SUPPORT:** None

**References:** Cross PC, Brinster RL. The sensitivity of one-cell mouse embryos to pyruvate and lactate. *Exp Cell Res*. 1973;77(1):57-62.

Summers MC, Bird S, Mirzai FM, Thornhill A, Biggers JD. Human pre-implantation embryo development in vitro: a morphological assessment of sibling zygotes cultured in a single medium or in sequential media. *Hum Fertil (Camb)*. 2013;16(4):278-285.

Embryo Results Stratified by Age				
Embryo Classification	Age (yrs)	PGT w/SC (N=522)	PGT w/AI (N=568)	P value
Euploid	≤ 35	42.7% (94)	56.3% (130)	< 0.01
	> 35	26.2% (79)	38.6% (130)	< 0.01
Low Level Mosaic	≤ 35	18.2% (40)	6.5% (15)	< 0.01
	> 35	8.6% (26)	4.2% (14)	0.02
High Level Mosaic	≤ 35	8.2% (18)	8.2% (19)	0.99
	> 35	7.9% (24)	5.6% (19)	0.24
Abnormal/ Complex Abnormal	≤ 35	24.5% (54)	23.4% (54)	0.86
	> 35	55.0% (166)	46.6% (157)	0.03
Other	≤ 35	6.4% (14)	5.6% (13)	0.74
	> 35	2.3% (7)	5.0% (17)	0.07

Lane M, Gardner DK. Lactate regulates pyruvate uptake and metabolism in the preimplantation mouse embryo. *Biol Reprod.* 2000;62(1):16-22.

P-823 Wednesday, October 16, 2019 6:30 AM

**IMPACT OF CONCOMITANT INFERTILE FACTORS ON IVF OUTCOMES OF YOUNG WOMEN WITH DIMINISHED OVARIAN RESERVE.** Yujie Li, Doctor Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.



**OBJECTIVE:** Whether concomitant infertile factors impact the IVF outcomes of young infertile women with diminished ovarian reserve (DOR)?

**DESIGN:** Retrospective cohort study of cycles from the Reproductive Center Of Sixth Hospital of Sun Yat-sen University

**MATERIALS AND METHODS:** 1952 young patients (<37 years old) with DOR undergoing their first IVF or ICSI cycles from 2010.6-2018.12 were recruited. These cycles were categorized as those having an isolated diagnosis of DOR ("DOR Only"), DOR plus at least one other concomitant diagnosis ("DOR Plus Tubal factor", "DOR Plus Endometriosis" and "DOR Plus Male factor"). The outcomes of interest included oocyte yield, fertilization rate, cleavage rate, high quality embryo rate, HCG positive rate, implantation rate, clinical pregnancy rate, live birth rate.

**RESULTS:** In cycles of patients with DOR, 31.1% reported an isolated diagnosis and a concomitant diagnosis with tubal factor, endometriosis, male factor accounted for 35.9%, 9.2%, 8.4%, respectively. Women with "DOR Plus Endometriosis" had higher fertilization rate (69.6% vs 62.5%,  $P=0.001$ ), higher cleavage rate (68.8% vs 61.0%,  $P<0.001$ ) and higher cumulative live birth rate of one oocyte retrieval cycle (31.1% vs 27.3%,  $P<0.001$ ) compared to those with "DOR Only". However, women with "DOR Plus Tubal factors" or "DOR Plus Male factor" had similar outcomes compared to those with "DOR Only".

**CONCLUSIONS:** DOR is associated with lower oocyte yield, lower implantation rates, and lower pregnancy rates after IVF. However the association of DOR and IVF outcomes is confounded by other infertility diagnoses. For women with DOR plus endometriosis, better IVF outcomes are demonstrated.

## REPRODUCTIVE GENETICS

P-824 Wednesday, October 16, 2019 6:30 AM

**REDUCING THE FREQUENCY OF EMBRYO MOSAICISM THROUGH ARTIFICIAL INTELLIGENCE.** Annemieke Wilcox, MD,<sup>a</sup>

Jeffrey Thorne, MD,<sup>b</sup> John Nulsen, MD,<sup>c</sup> Claudio Benadiva, MD,<sup>c</sup> Daniel R. Grow, MD<sup>d</sup> <sup>a</sup>Center for Advanced Reproductive Services, University of Connecticut School of Medicine, Farmington, CT; <sup>b</sup>Center



for Advanced Reproductive Services, Farmington, CT; <sup>c</sup>Center for Advanced Reproductive Services, University of Connecticut, Farmington, CT; <sup>d</sup>University of Connecticut Health Center, Center for Assisted Reproductive Services, Farmington, CT.

**OBJECTIVE:** With the advent of Next Generation Sequencing (NGS) used in Preimplantation genetic testing (PGT), identifying mosaicism within a sample biopsy of trophectoderm has become increasingly common. While reports show that transfer of these embryos can result in live births, the implantation of mosaic embryos remains controversial. With advancement in artificial intelligence (AI) algorithms, it may be possible to detect embryo mosaicism with higher efficiency and accuracy. This study seeks to determine the difference in identification of embryo mosaicism between subjective calling (SC) by trained technicians and the use of AI algorithms at a single academic center.

**DESIGN:** Retrospective cohort study

**MATERIALS AND METHODS:** PGT data of 1,090 blastocysts from 351 patients at our center was obtained through CooperGenomics. 522 embryos evaluated using PGT with SC (4/2018 to 11/2018) were compared to 568 embryos evaluated using PGT with AI (11/2018 to 4/2019). PGT results were reported as euploid, low level mosaic (20-40% mosaicism), high level mosaic (40-80% mosaicism) and abnormal or complex abnormal (containing 3 or more aneuploid or mosaic chromosomes). Embryos listed as other contained polyploid embryos and those with insufficient data. Embryo data was further stratified by age. Chi-square test was used to assess categorical variables. A p-value <0.05 was considered statistically significant.

**RESULTS:** Overall, PGT using AI technology identified a significantly higher percentage of euploid embryos compared to SC (45.8% vs. 33.1%,  $p < 0.05$ ) as well as a lower percentage of low level mosaic embryos (5.1% vs. 12.6%,  $p < 0.05$ ). The overall percentage of high level mosaics and abnormal/ complex abnormal embryos identified were similar (8% vs 6.7% and 42.2% vs 37.1%, respectively). These differences persisted after stratifying by age, except in the >35yo group in which AI identified fewer abnormal/ complex abnormal embryos than SC (46.6% vs. 50.0%).

**CONCLUSIONS:** AI technology identified a higher number of euploid embryos and fewer low level mosaic embryos, leading to the identification of more embryos suitable for transfer. Further analysis of pregnancy outcomes is needed to determine if this translates into a clinically significant increase in livebirths.

**References:** Munné S, Blazek J, Large M, Martinez-Ortiz PA, Nisson H, Liu E, et al. Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected with the use of high-resolution next-generation sequencing. *Fertil Steril* 2017;108(1):62-71.e8.

Greco E, Minasi MG, Fiorentino F. Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts. *N Engl J Med* 2015;373(21):2089-2090.

## VIDEO PROGRAM PRIZE SESSION

V-1 Monday, October 14, 2019 4:30 PM

**TECHNIQUES FOR SUCCESSFUL VAGINAL ANASTOMOSIS IN THE UTERINE TRANSPLANT PATIENT.** Jenna M. Rehmer, MD, Elliott G. Richards, MD, Cecile A. Ferrando, MD, MPH, Rebecca Flyckt, MD. Cleveland Clinic Foundation, Cleveland, OH.



**OBJECTIVE:** To demonstrate our techniques for successful vaginal anastomosis in the uterine transplant patient.

**METHODOLOGY:** We report the case of a recent uterine transplant from a deceased multi-organ donor and highlight the vaginal anastomosis portion of this multi-step surgery. This video uses live action footage from surgery and detailed descriptions review our techniques for successful vaginal anastomosis in the uterine transplant patient.

**CONCLUSIONS:** Following uterine transplantation, access to the donor allograft cervix is important for many reasons. Vaginal strictures pose a unique problem in this surgical population. We believe strictures result from difficulty in approximating donor vaginal mucosa to recipient vaginal mucosa, and that this is paramount in reducing this untoward postop complication. Given the difficulty of surgery and tendency for the recipient vaginal mucosa to retract, our teams has employed techniques from vaginal reconstructive surgery to reduce the occurrence of postoperative vaginal strictures.

**Reference:** None.

**SUPPORT:** No financial support to disclose.

V-2 Monday, October 14, 2019 4:42 PM

**SURGICAL MANAGEMENT OF DEEP INFILTRATING ENDOMETRIOSIS INVOLVING THE RECTOSIGMOID COLON.**

Natalia C. Llarena, MD,<sup>a</sup> Anup B. Shah, MD, MS,<sup>a</sup> Hermann Kessler, MD, PhD,<sup>b</sup> Tommaso Falcone, M.D.,<sup>c</sup> Rebecca Flyckt, MD.<sup>a</sup> <sup>a</sup>Cleveland Clinic Foundation, Cleveland, OH; <sup>b</sup>Cleveland Clinic, Cleveland, OH; <sup>c</sup>Cleveland Clinic, Cleveland, OH.



**OBJECTIVE:** To discuss the surgical management of deep infiltrating endometriosis involving the rectosigmoid colon.

**METHODOLOGY:** Here we demonstrate a case of a 34-year-old female with chronic pelvic pain, infertility, and a 1-cm rectosigmoid endometriotic implant noted on operative MRI. She underwent segmental bowel resection of the involved rectosigmoid colon with colorectal reanastomosis.

**CONCLUSIONS:** There are several surgical approaches to managing endometriosis involving the rectosigmoid colon, including rectal shaving, disc resection, and segmental resection. Segmental resection allows for complete resection of endometriotic lesions and histologic analysis of the specimen.

**Reference:** None.

**SUPPORT:** None.

V-3 Monday, October 14, 2019 4:53 PM

**NO-SCALPEL VASECTOMY: PUNCTURE-FIRST WITH MULTI-OCCLUSION TECHNIQUE.**

Khushabu Kasabwala, MD,<sup>a</sup> Helen Levey Bernie, DO MPH,<sup>b</sup> Soo Jeong Kim, MD,<sup>a</sup> Vanessa L. Dudley, MSHS,<sup>c</sup> Marc Goldstein, MD.<sup>d</sup> <sup>a</sup>New York Presbyterian - Weill Cornell Medical Center, New York, NY; <sup>b</sup>Memorial Sloan Kettering Cancer Center, New York, NY; <sup>c</sup>Weill Cornell Medicine, New York, NY; <sup>d</sup>Weill Cornell Medicine, New York Presbyterian Hospital, New York, NY.



**OBJECTIVE:** Vasectomy is a widely utilized, permanent contraceptive method. Vasectomy failure may occur secondary to recanalization (1-10%) and is a major cause of malpractice suits and pregnancy. The no-scalpel vasectomy (NSV) is a minimally invasive technique where failure rates depend on occlusion techniques. We describe a puncture-first NSV with four occlusion techniques to optimize outcomes and minimize technical difficulty.

**METHODOLOGY:** Men who had a puncture-first NSV by a single surgeon (MG) over 25 years (1993 - 2018) were included in this study. The procedure begins administering local anesthesia to the skin overlying the vas. The vas, excluding vasal vessels and nerves, is then delivered through a single midline puncture hole. After securing two ends of the vas and hemi-transecting them, the first occlusive step, intraluminal cautery, is performed on the

testicular end of the vas by rotating the cautery for 10 seconds to ensure a 360-degree burn. A hemoclip is lightly placed on the testicular end to prevent sperm leakage until cautery causes a permanent seal. The abdominal end of the vas is cauterized intraluminally, completed transected, and allowed to retract into the vasal sheath. The sheath is grasped and sealed over the abdominal end with a hemoclip, accomplishing fascial interposition. A 5mm vas segment is excised. The ends are dabbed with betadine before retraction into the scrotum. The contralateral vas is accessed through the same puncture hole and occluded identically. No antibiotics are administered before or after the procedure. Post-vasectomy semen analysis (PVSA) is performed 6-8 weeks or 15 ejaculations after the procedure. Complications were graded using the Clavien-Dindo classification scale.

Over 25 years, 819 vasectomies were performed. The mean age of the patient and partner was 41.6 years (+/- 5.8 years) and 38.6 years (+/- 3.8 years), respectively. At least one PVSA was performed in 484 (59%) of men, at a median of 53 days post-procedure. Nearly half of those, 222 men (45%) required a second PVSA to confirm vasal occlusion. No pregnancies were reported after vasectomy. Three complications occurred including one abscess requiring incision and drainage (Grade IIIa) and two hematomas managed conservatively (Grade I). No chronic pain or orchitis was reported.

**CONCLUSIONS:** Vasectomy failure and complications can be minimized by utilizing a combination of four occlusion techniques: 1.) intraluminal cautery for 10 seconds; 2.) testicular end occluding clip; 3.) fascial interposition and 4.) removal of 1/2 to 1 cm segment of vas. Additionally, the puncture-first technique eliminates the need to grasp the scrotal skin with the vas in the ring clamp, decreasing technical difficulty. This technique has minimal complications and a 100% success rate in our patient cohort.

V-4 Monday, October 14, 2019 5:06 PM

**DEVELOPMENT OF AN AUTOMATIC PRONUCLEAR DETECTION SYSTEM FOR HUMAN EMBRYOS USING DEEP LEARNING TECHNOLOGY.**

Hiroyuki Watanabe, M.S.,<sup>a</sup> Noritaka Fukunaga, Ph.D.,<sup>a</sup> Sho Sanami, Ph.D.,<sup>b</sup> Hiroya Kitasaka, Ph.D.,<sup>a</sup> Yuji Tsuzuki, M.S.,<sup>b</sup> Yuta Kida, M.S.,<sup>a</sup> Seiji Takeda, M.S.,<sup>b</sup> Yoshimasa Asada, M.D., Ph.D.,<sup>a</sup> Asada Ladies Clinic, Nagoya, Aichi, Japan; <sup>b</sup>Research & Development Center, Dai Nippon Printing Co., Ltd., Kita-ku, Tokyo, Japan.



**OBJECTIVE:** Fertilization is generally evaluated by pronuclear number. Correct judgment may sometimes be difficult due to morphology and number of pronuclei of pronuclear embryos.

Correct evaluation of the pronuclei number is important in order to reduce the possibility of transferring abnormal embryo.

Therefore, in this study, we aimed to develop an automatic pronuclear detection system by deep learning technology using time lapse embryo images.

Deep Learning technology is an information processing system using Deep Learning Neural Networks (DLNN). DLNN is a multi-layered combination of neural networks that mimics a cranial nerve network. This technology has several important features such as a) high-precision learning is possible, b) currently it is the highest performance image recognition method", and c) it makes effective use of all time-lapse embryo images.

**METHODOLOGY:** 70-80 images before and after pronuclear formation of one embryo were extracted from the time-lapse incubator.

Using these 70-80 images as one set, each 400 sets of 2PN, 1PN and 0PN images that were evaluated by an embryologist were prepared in order to construct the automatic pronuclei detection system.

The automatic pronuclear detection system used DLNN which outputs the number of pronuclei to the inputted embryo image.

Of the 400 sets of images, 300 sets of each were input to the DLNN with labels of 2 PN, 1 PN and 0PN, and the DLNN learned from these images by Deep Learning.

The remaining 100 sets of images that were not used for learning, were entered without labels to the DLNN which completed the learning, and the DLNN detected the number of pronuclei from these images.

**CONCLUSIONS:** In 2PN embryos, the rate of correctly detected 2PN was 97% and the rate of incorrectly detected 1PN, 0PN was 3%, 0% respectively, with respect to the input of 100 unlabeled time-lapse images.

In 1PN embryos, the rate of correctly detected 1PN was 68%, and the rate of incorrectly detected 2PN, 0PN was 20%, 12% respectively, with respect to the input of 100 unlabeled time-lapse images.

In 0PN embryo, the rate of correctly detected 0PN was 78%, and the rate of incorrectly detected 2PN, 1PN was 4%, 18% respectively, with respect to the input of 100 unlabeled time-lapse images.

As a result of this study, we succeeded in constructing a system for automatic detection of pronuclear number from embryo images using Deep Learning technology.

The correct answer rate was 97% in 2PN embryos, but the rate was lower in 1PN and 0PN embryos compared to 2PN embryos.

These results are very promising, but it is necessary to improve the detection system further and apply this technology to embryos with 3 or more PN number.

Reference: None.

SUPPORT: None.

V-5 Monday, October 14, 2019 5:18 PM

**TRANSGENER YOUTH: EXPLAINING HORMONAL AFFIRMATION TREATMENT VIA POWTOON FORMAT-(MTF).** Gloria Bachmann, md, mms, Ian Marshall, MD. Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ.



**OBJECTIVE:** To explain to transgender youth in an animated format containing auditory, visual and written explanations, the pros and cons of hormone use that may be prescribed to them in order to achieve their desired gender changes.

**METHODOLOGY:** Written materials on hormonal therapy used for affirmation of gender may not be comprehensively read by young individuals seeking this intervention. To address this issue, an animated presentation was developed that addresses the pros and cons of hormonal use for gender affirmation. The script was developed by clinicians who care for transgender individuals with the input of learner and community groups. The Powtoon format was utilized for creating the animated presentations.

**CONCLUSIONS:** The appropriate Powtoon is being shown to transgender individuals being cared for by a pediatric endocrinologist, who has over 200 transgender youth in his practice. Parents and guardians are also encouraged to watch them. Comments have been overall positive both from the individuals being managed with hormonal therapy and the parents/guardians.

Reference: None.

SUPPORT: None.

## VIDEO SESSION 2

V-6 Tuesday, October 15, 2019 4:15 PM

**CONTINUOUS MONITORING OF THE EMBRYO DEVELOPMENT: A LEAP TOWARDS AUTOMATED SYSTEMS.** Lorena Bori, PhD,<sup>a</sup> Raquel Del Gallego, PhD,<sup>a</sup> Lucia Alegre, PhD,<sup>a</sup> Antonio Pellicer, MD,<sup>b</sup> Marcos Meseguer, PhD<sup>c</sup> <sup>a</sup>IVIRMA Global, Valencia, Spain; <sup>b</sup>IVI Foundation Innovation - Reproductive Medicine IIS La Fe, Valencia, Spain; <sup>c</sup>IVIRMA Global, Valencia, Spain, Tel Aviv, Israel.



**OBJECTIVE:** To illustrate the introduction of automated systems to assess the embryos in daily clinical practice in IVF laboratories.

**METHODOLOGY:** More than 80,000 embryos have been cultured in time-lapse incubators since 2009 at IVIRMA Valencia. Abnormal embryo development has been observed thanks to the high number of videos available. The best examples of zygotes with one or three pronuclei and embryos with irregular divisions that achieved good quality blastocysts were gathered. Blastocysts that changed their quality few hours before embryo selection were also selected for the project. Automated systems based on image analysis technology have been introduced in our laboratory to assess embryo development. A step by step video demonstration of the automatic annotations performed by EmbryoScope® and Geri Connect and Assess 2.0® was conducted. Additionally, a table of the comparison between the manual annotations performed by an embryologist team and the automated annotations performed by the software Geri Assess 2.0 in 1360 embryos (10,880 development events) at IVIRMA Valencia is shown. The parameters included were: pronuclear fading (tPNf), division time to 2 cells (t2), division time to 3 cells (t3), division time to four cells (t4), division time to five cells (t5), division time to six cells (t6), appearance of morula (tM) and expanded blastocyst (tEB). High accordance was found between both, showing a struggle in the detection of late parameters by the embryologist team. Finally, a demonstration of a method to measure novel embryo parameters, impossible to assess with automated systems, were performed by embryologists through

the drawing tools provided by the EmbryoViewer®. The parameters included were: blastocyst expanded diameter, inner cell mass area and trophectoderm cell cycle length. Additionally, the impact of these parameters over the implantation rate was analyzed and illustrated through graphs.

**CONCLUSIONS:** Time-lapse technology allows monitoring of unusual patterns of embryo development and makes it possible to progress towards automated and objective systems to assess the embryos. The use of big data technology as a tool to detect embryo development events combined with embryologist skills are a promising approach towards the improvement of IVF treatments.

V-7 Tuesday, October 15, 2019 4:23 PM

**COMPARISON OF SPERM RETRIEVAL TECHNIQUES FOR MEN WITH OBSTRUCTIVE AZOOSPERMIA.** Joshua N. Bitran, BS,<sup>a</sup> Premal Patel, MD,<sup>b</sup> Ranjith Ramasamy, M.D.<sup>c</sup> <sup>a</sup>University of Miami, Miami, FL; <sup>b</sup>University of Miami Miller School of Medicine, Miami, FL; <sup>c</sup>University of Miami.



**OBJECTIVE:** To compare the Percutaneous Epididymal Sperm Aspiration (PESA) and Microsurgical Epididymal Sperm Aspiration (MESA) techniques, requirements, and outcomes for men with obstructive azoospermia.

**METHODOLOGY:** Intra-operative video highlights the main steps for performing PESA and MESA, along with their complications and sperm retrieval outcomes. Intra-operative table microscope was used to visualize sperm from PESA and MESA.

**CONCLUSIONS:** PESA and MESA are both effective means for obtaining sperm for in-vitro fertilization with differences in technique, equipment required, complications, and sperm quality outcomes.

V-8 Tuesday, October 15, 2019 4:28 PM

**POST-ABLATION RESIDUAL DISEASE: HISTOLOGICAL ASSESSMENT OF EXCISED PERITONEAL ENDOMETRIOSIS.** Christine Hur, MD,<sup>a</sup> Tommaso Falcone, M.D.,<sup>b</sup> Rebecca Flyckt, MD.<sup>c</sup> <sup>a</sup>Cleveland Clinic, Cleveland, OH; <sup>b</sup>Cleveland Clinic, Cleveland, OH; <sup>c</sup>Cleveland Clinic Foundation, Cleveland, OH.



**OBJECTIVE:** The objective of this video is to present a case of post ablation residual peritoneal endometriosis while also highlighting surgical techniques of excision of endometriosis.

**METHODOLOGY:** This video presents a case of a 25 year old para 2 who had a history of a prior diagnostic laparoscopy with ablation of peritoneal endometriosis two months prior. The surgical case presented is her second laparoscopy, which was performed with the intention of excision of endometriosis per patient preference. Surgical findings were significant for remaining endometriosis in areas of previous ablation. The histological findings included residual endometriosis deep to prior superficial ablation.

**CONCLUSIONS:** Superficial ablation may not treat deeper forms of endometriosis. Ablation without appropriate dissection and mobilization risks injury to the bowel, ureters and bladder. For these reasons, excision of endometriosis may be a superior form of treatment for deep peritoneal endometriosis.

References: • Falcone, T. and Wilson, J. Surgical Treatment of Endometriosis: Excision Versus Ablation of Peritoneal Disease. *JMIG*. 2019; 26(1): pp 1-2.

• Duffy J.M., Arambage K., Correa F.J., et al: Laparoscopic surgery for endometriosis. *Cochrane Database Syst Rev* 2014.

• Healey M., Ang W.C., and Cheng C.: Surgical treatment of endometriosis: a prospective randomized double-blinded trial comparing excision and ablation. *Fertil Steril* 2010; 94: pp. 2536-2540.

V-9 Tuesday, October 15, 2019 4:34 PM

**TEACHING SURGERY FOR MASSIVE ADENOMYOSIS WHICH PRESERVES THE UTERUS.** Sherman Silber, MD,<sup>a</sup> Yuting Fan, M.D.,<sup>b</sup> Sierra Goldsmith, B.S.<sup>a</sup> <sup>a</sup>Infertility Center of St. Louis, Chesterfield, MO; <sup>b</sup>University of Michigan, Ann Arbor, MI.



**OBJECTIVE:** To determine the feasibility of teaching massive adenomyectomy surgery with favorable results.

**METHODOLOGY:** 4 patients with massive adenomyosis in China who wished to get pregnant and have a baby were enlisted for the teaching of the Chinese surgical team. Subsequently 20 more such patients were operated on in China by the surgical team we taught. For the first two teaching cases, we did the surgery, and the Chinese team assisted. For the next two cases, the Chinese team did the surgery, and we assisted. We described the quintuple flap reconstruction with no overlapping suture lines to prevent uterine rupture. Video documentation was performed.

**CONCLUSIONS:** Following this intense two day training in China, the Chinese team did 20 more cases on their own over the next six months, with a live baby rate of 55%, and uneventful pregnancies with no uterine rupture.

V-10 Tuesday, October 15, 2019 4:53 PM

**LAPAROSCOPIC RELOCATION OF THE OVARIES AFTER PRIOR TRANSPOSITION.** Jessica Traylor, M.D., Jaclyn Friedman, M.D., Magdy P. Milad, M.D., MS. Northwestern University Feinberg School of Medicine, Chicago, IL.



**OBJECTIVE:** To describe the indications, surgical approaches and expected outcomes for ovarian transposition and highlight a case of ovarian relocation to the pelvis in a patient who underwent prior transposition.

**METHODOLOGY:** A 34 year old patient with a history of metastatic spinal ependymoma underwent laparoscopic ovarian transposition prior to craniospinal radiation. Eleven years after her transposition, she was seen by reproductive endocrinology and infertility for preconception counseling and evaluation. Her follicle stimulating hormone levels were within normal limits, but her hysterosalpingogram demonstrated bilateral tubal isthmic occlusion. She was referred to minimally invasive gynecologic surgery for surgical consultation.

The patient was taken to the operating room for operative laparoscopy, ovarian relocation to the pelvis and evaluation of her fallopian tubes. Intra-operative findings were notable for transposition of the bilateral ovaries and fallopian tubes to the lateral abdominal peritoneum. Adhesiolysis was performed to mobilize each ovary on its vascular pedicle. Without compromise to the ovarian blood supply, and in a tension-free manner, each ovary was sutured to the ipsilateral round ligament.

**CONCLUSIONS:** Laparoscopic ovarian transposition is an important surgical technique to aid preservation of ovarian function in reproductive aged women undergoing pelvic radiation. Gynecologic surgeons should be aware of the techniques to perform ovarian transposition, as well as relocation of the ovaries to the pelvis for future spontaneous or assisted reproduction. Knowledge of abdominal and pelvic anatomy, as well as proficiency in laparoscopic suturing are essential to perform ovarian transposition and relocation in a minimally invasive fashion.

**References:** Arian SE, Goodman L, Flyckt RL, Falcone T. Ovarian transposition: a surgical option for fertility preservation. *Fertil Steril*. 2017;107(4):e15.A

Barahmeh S, Al Masri M, Badran O, et al. Ovarian transposition before pelvic irradiation: indications and functional outcome. *J Obstet Gynaecol Res*. 2013;39(11):1533-1537.

Bisharah M, Tulandi T. Laparoscopic preservation of ovarian function: an underused procedure. *Am J Obstet Gynecol*. 2003;188(2):367-370.

Friedman J, Butler S, Milad M. Laparoscopic ovarian transposition – a review of indications, techniques and expected outcomes, highlighted by a successful case report. ASRM, Denver, Colorado, October 2018.

Hoekman EJ, Knoester D, Peters AAW, Jansen FW, de Kroon CD, Hilders C. Ovarian survival after pelvic radiation: transposition until the age of 35 years. *Arch Gynecol Obstet*. 2018;298(5):1001-1007.

Hoekman EJ, Broeders E, Louwe LA, Nout RA, Jansen FW, de Kroon CD. Ovarian function after ovarian transposition and additional pelvic radiotherapy: A systematic review. *Eur J Surg Oncol*. 2019.

Husseinzadeh N, Nahhas WA, Velkley DE, Whitney CW, Mortel R. The preservation of ovarian function in young women undergoing pelvic radiation therapy. *Gynecol Oncol*. 1984;18(3):373-379.

Moawad N, Santamaria E. Ovarian transposition. YouTube: Green Journal, 2016. Available from: [https://youtu.be/q-DAYY\\_gsBq](https://youtu.be/q-DAYY_gsBq). Accessed April 7, 2019.

**SUPPORT:** None.

V-11 Tuesday, October 15, 2019 5:01 PM

**OPTIMIZING FERTILITY PRESERVATION USING MULTIPLE MODALITIES IN A YOUNG PATIENT WITH CERVICAL CANCER.** Natalia C. Llarena, MD, Bouran Kilany, MD, Mariam Alhilli, MD, Rebecca Flyckt, MD. Cleveland Clinic, Cleveland, OH.



**OBJECTIVE:** Oocyte cryopreservation is the mainstay of fertility preservation in patients with malignancy; however, live birth rates after oocyte cryopreservation are lower in cancer patients than in healthy patients. Multiple modalities of fertility preservation can be combined to optimize success rates.

**METHODOLOGY:** We demonstrate a case of a 24-year-old female who was diagnosed with cervical clear cell adenocarcinoma and was advised to undergo treatment with cisplatin and radiation therapy. She strongly desired fertility preservation and opted to proceed with oocyte cryopreservation, ovarian tissue cryopreservation, and ovarian transposition.

**CONCLUSIONS:** Ovarian transposition is a surgical approach to fertility preservation that preserves both fertility and gonadal function. Different modes of fertility preservation may be combined to optimize live birth rates in patients with malignancy.

**SUPPORT:** None.

V-12 Tuesday, October 15, 2019 5:09 PM

**LEFT OVARIAN TRANSPOSITION OF UNDESCENDED OVARY WITH UNICORNATE UTERUS.** Kirsten Sasaki, M.D.,<sup>a</sup> Charles E. Miller, M.D.<sup>b</sup>



<sup>a</sup>Advocate Lutheran General Hospital, Naperville, IL; <sup>b</sup>The Advanced Gynecologic Surgery Institute/The Advanced IVF Institute, Charles E. Miller, MD & Associates, Naperville, IL.

**OBJECTIVE:** To review the presentation, symptoms, diagnosis and treatment of an undescended ovary, and to demonstrate a laparoscopic technique for ovarian transposition to facilitate trans-vaginal oocyte monitoring and retrieval.

**METHODOLOGY:** Left ovarian transposition and ovarian drilling.

**CONCLUSIONS:** Laparoscopic ovarian transposition is a feasible, safe option to facilitate oocyte retrieval in cases of undescended ovaries.

**References:** • Barton S, Politch J, Benson C et al. Transabdominal follicular aspiration for oocyte retrieval in patients with ovaries inaccessible by transvaginal ultrasound. *Fert Steril* 2011;95:1773-6.

• Dabirashrafi H, Mohammad K, Moghadami-Tabrizi N. Ovarian malposition in women with uterine anomalies. *Obstet Gynecol* 1994;83:293-4.

• Dietrich J, Hertweck S, Bond S. Undescended ovaries: A clinical review. *J Pediatr Adolesc Gynecol* 2007;20:57-60.

• Gorgen H, Api M, Delikara N. Undescended fallopian tubes and ovaries: a rare incidental finding during an infertility investigation work up. *Acta Obstet Gynecol Scand* 2002;81:371-4.

• Ireo E, Haruna M, Gandhi P. Laparoscopic management of mal-descended ovary presenting with recurring acute abdomen. *Gynecol Minim Invasive Thera* 2018;7:74-7.

• Ombelet W, Grieten M, DeNeubourg P et al. Undescended ovary and unicornuate uterus: simplified diagnosis by the use of clomiphene citrate ovarian stimulation and magnetic resonance imaging (MRI). *Hum Reprod* 2003;18:858-62.

• Ombelet W, Verswijvel G, Vanholsbeke C et al. Unicornuate uterus and ectopic (undescended) ovary. *F, V, & V in ObGyn* 2011;3(2):131-4.

• Van Voorhis B, Dokras A, Syrop C. Bilateral undescended ovaries: association with infertility and treatment with IVF. *Fertil Steril* 2000;74:1041-3.

**SUPPORT:** None.

V-13 Tuesday, October 15, 2019 5:15 PM

**EXCISION OF A PELVIC SIDE-WALL FIBROID.** Alexander Kotlyar, MD,<sup>a</sup> Pinar Kodaman, MD/PhD.<sup>b</sup> <sup>a</sup>Yale University, New Haven, CT; <sup>b</sup>Yale School of Medicine, New Haven, CT.



**OBJECTIVE:** To share our experience in excising a retroperitoneal pelvic fibroid.

**METHODOLOGY:** This video describes the essential steps for resecting a retroperitoneal pelvic fibroid. These fibroids are rare and do not typically arise from the uterus or cervix and carry a higher risk of sarcoma compared to typical uterine fibroids. In this video, we review a case presentation including pre-operative MRI imaging, and outline the key steps in retroperitoneal fibroid excision. These steps include proceeding with lateral to medial excision, systematic identification of the retroperitoneal structures and use of bipolar electrosurgery to transect key vascular connections to the fibroid. Once this has been completed, the fibroid is then removed and linearized within a surgical containment system.

**CONCLUSIONS:** Retroperitoneal fibroids are a rare type of pelvic tumor that carry an increased risk of sarcoma. The resection of these tumors requires pre-operative imaging to assess their relationship to adjacent pelvic structures and judicious dissection of the retroperitoneum.

**References:** 1. Jeong G. Retroperitoneal Leiomyoma of the Uterus Mimicking Sarcoma in Perimenopausal Woman: A Case Report. *J Menopausal Med.* 2014; 20(3): 133-137.

2. Tantitami T, Hamontri S, Randiratanakul L, Suksamarnwong m. Pelvic Retroperitoneal Cellular Leiomyoma: A Case Report. *J Med Assoc Thai.* 2015; 98 Suppl 9: S160-4.

3. Sewell CA, Russo ML. Retroperitoneal leiomyoma: a case report. *J Reprod Med.* 2011; 56(11-12): 515-7.

4. Poliquin V, Victory R, Vilos GA. Epidemiology, presentation, and management of retroperitoneal leiomyomata: systematic literature review and case report. *J Minim Invasive Gynecol.* 2008; 15(2): 152-60.

5. Sayer RA, Amundsen CL. Giant pelvic retroperitoneal leiomyoma arising from the rectal wall. *Obstet Gynecol.* 2003; 101(5 Pt 2): 1132-4.

**SUPPORT:** None.

### VIDEO SESSION 3

V-14 Wednesday, October 16, 2019 3:45 PM

#### 2- PORT MYOMECTOMY TECHNIQUE FOR UTERUS ADHERENT TO THE ANTERIOR ABDOMINAL WALL.

Hadi Ramadan, M.D., Jerri A. Waller, M.D., Traci E. Ito, M.D., Joseph L. Hudgens, M.D., Eastern Virginia Medical School, Norfolk, VA.



**OBJECTIVE:** To describe a 2 port myomectomy technique in the setting of extensive adhesions aiming for fertility preservation.

**METHODOLOGY:** Our patient is a 40 year old female G3P2012 presenting with symptomatic fibroids with a history of prior C-section. Her MRI showed extensive adhesions requiring specific surgical techniques. Using a 2 port method and gel point mini, lysis of adhesions was performed. Enucleation of fibroids followed. This multistep process ensures hemostatic and efficient myomectomies without jeopardizing the integrity of the endometrium.

**CONCLUSIONS:** This case is an example of how reduced port technique can be utilized in complex fertility preserving techniques.

**SUPPORT:** None.

V-15 Wednesday, October 16, 2019 3:53 PM

#### PROOF OF CONCEPT FOR AN AUTOMATED TANK STORING FROZEN EMBRYOS AND GAMETES IN AN ART LABORATORY.

Timothy Allen Sharp, B.S.,<sup>a</sup> William N. Garbarini Jr., MBA,<sup>b</sup> Chad A. Johnson, PhD,<sup>a</sup> Ann Watson, B.A.,<sup>a</sup> Kathryn J. Go, PhD.<sup>a</sup> <sup>a</sup>TMRW Life Sciences, New York, NY; <sup>b</sup>TMRW Life Sciences, Inc., New York, NY.



**OBJECTIVE:** We explored proof of concept for an automated, cryogenic robot for the storage of human embryos and gametes.

**METHODOLOGY:** Adapt an automated, electronically-monitored storage system to ART.

**CONCLUSIONS:** Proof of concept was obtained to apply a robotic, electronically-monitored automated tank for ART.

**SUPPORT:** Financial support was provided by TMRW Life Sciences, Inc.

V-16 Wednesday, October 16, 2019 3:56 PM

#### LAPAROSCOPIC RESECTION OF FUNCTIONAL, NON-COMMUNICATING UTERINE HORN.

Rachel M. Whynott, M.D.,<sup>a</sup> Rachel Mejia, D.O.<sup>b</sup> <sup>a</sup>University of Iowa Hospitals and Clinics, Iowa City, IA; <sup>b</sup>University of Iowa, Iowa City, IA.



**OBJECTIVE:** To review a common presentation of unicornuate uterus with a functional, non-communicating rudimentary uterine horn and a laparoscopic method of management, highlighting laparoscopic surgical techniques.

**METHODOLOGY:** A 13-year-old G0 was referred to the clinic for severe, cyclic right lower quadrant pain during menses. A transvaginal ultrasound revealed a left unicornuate uterus with a right-sided, non-communicating rudimentary horn measuring 4.8 x 4.7 x 4.6 cm, containing blood consistent with hematometra. Kidneys were bilaterally present and normal by ultrasound. Due to the patient's worsening pain and presence of hematometra, decision was made to proceed with diagnostic laparoscopy and removal of the rudimentary uterine horn. The entire procedure was performed laparoscopically, with an estimated total blood loss of 20 cc. She had no complications or readmissions. Her severe menstrual pain was resolved at her follow up appointments.

**CONCLUSIONS:** In patients with severe menstrual pain from outflow obstruction from a non-communicating rudimentary uterine horn with functional endometrium, laparoscopic resection can be a safe and effective method of treatment.

**References:** 1. Wozniakowska E, Stepniak A, Czuczwar P, Milart P, Paszkowski T. Secondary dysmenorrhea due to a rudimentary, non-communicating functional uterine horn. *Ginekologia polska* 2017;88:404-5.

2. Pados G, Tsolakidis D, Athanatos D, Almaloglou K, Nikolaidis N, Tarlatzis B. Reproductive and obstetric outcome after laparoscopic excision of functional, non-communicating broadly attached rudimentary horn: a case series. *European journal of obstetrics, gynecology, and reproductive biology* 2014;182:33-7.

3. Chan YY, Jayaprakasan K, Tan A, Thornton JG, Coomarasamy A, Raine-Fenning NJ. Reproductive outcomes in women with congenital uterine anomalies: a systematic review. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology* 2011;38:371-82.

**SUPPORT:** None.

V-17 Wednesday, October 16, 2019 4:03 PM

#### MEIOTIC SPINDLE AVOIDANCE USING A POLARIZING FILTER AT THE TIME OF ICSI, A STEP CLOSER TOWARDS INDIVIDUALIZED ICSI.

Alejandro Chavez-Badiola, MD,<sup>a</sup> Rodolfo Garcia-Sánchez, MSc,<sup>b</sup> Erika L. Iniguez-Arteaga, Biol,<sup>b</sup> Dante Josué Sánchez González, Biol,<sup>a</sup> Carmen Ortega Madera, Biol,<sup>a</sup> Sarahi Vazquez-Pacheco, Biol.<sup>a</sup> <sup>a</sup>New Hope Fertility Center Mexico, Mexico City, EM, Mexico; <sup>b</sup>New Hope Fertility Center Mexico, Guadalajara, JA, Mexico.



**OBJECTIVE:** To assess the impact of routine meiotic spindle identification during intra-cytoplasmic sperm injection (SI-ICSI), on fertilization and blastocyst formation rates when compared against conventional intra-cytoplasmic sperm injection (ICSI).

**METHODOLOGY:** All ICSI cycles undertaken between February 2015 and December 2016 in two similarly run IVF centers were included. At February 2016, spindle identification was introduced into routine practice. ICSI was performed following standard protocols: polar body was positioned either at 6 or 12 o'clock and used as reference for sperm injection at 3 o'clock. SI-ICSI oocyte identification was performed just before ICSI under 40 times magnification and then rotated until polar body was positioned at 12 o'clock. At this point a polarizer filter (Olympus IX2), was inserted while light turned to maximum intensity. Oocytes were rotated until a birefringent spindle was identified. Spindle position and intensity was recorded by embryologist as 0/+/++/+++, with 0 being assigned to spindle absence and +++ to maximum spindle intensity when compared against the most birefringent area in zona pellucida. Sperm injection was performed at 3 o'clock. Fertilization and blastocyst formation rates were recorded blind to spindle characteristics. Comparison was performed using Fisher's Exact test.

**CONCLUSIONS:** With an increased 5% normal fertilization rate and 7% higher blastocyst rate, this study suggests that visualizing the oocyte meiotic spindle using an inexpensive polarizing filter at the time of ICSI can avoid inadvertent damage and leads to improved normal fertilization and blastocyst formation. Other potential benefits could include better timing for injection in accordance with cytoplasmic maturation. Prospective studies would be needed to validate this later concept.

V-18 Wednesday, October 16, 2019 4:21 PM

### LAPAROSCOPIC MANAGEMENT OF TUBAL DISEASE TO IMPROVE FERTILITY OUTCOMES.

Aarathi Cholkeri-Singh, M.D.,<sup>a</sup> Charles E. Miller, M.D.,<sup>b</sup> <sup>a</sup>The Advanced Gynecologic Surgery Institute, Naperville, IL; <sup>b</sup>The Advanced Gynecologic Surgery Institute/The Advanced IVF Institute, Charles E. Miller, MD & Associates, Naperville, IL.



**OBJECTIVE:** This video demonstrates decision making and surgical techniques for tubal disease to improve fertility outcomes. Three scenarios are shown demonstrating surgical techniques of fallopian tubes deemed to have good, intermediate and poor prognosis.

**METHODOLOGY:** Private infertility practice in the suburbs of Chicago, Illinois performing laparoscopy to manage tubal disease.

**CONCLUSIONS:** Tubal disease is responsible for 25-30% of all female infertility. Several risk factors exist. The utilization of preoperative assessment tools as well as intraoperative findings, can allow a surgeon to make an informed decision of whether to repair and restore versus remove the fallopian tube to improve fertility outcome.

**References:** Ajonuma L, Ng E, Chan H. New insights into the mechanisms underlying hydrosalpinx fluid formation and its adverse effect on IVF outcome. *Hum Reprod Update* 2002;8:255-64.

Camus E, Poncelet C, Goffinet F, et al. Pregnancy rates after in-vitro fertilization in cases of tubal infertility with and without hydrosalpinx: a meta-analysis of published comparative studies. *Hum Reprod* 1999;14:1243-9.

Nackley A, Muasher S. The significance of hydrosalpinx in in vitro fertilization. *Fertil Steril* 1998;69:373-84.

Boer-Meisel M, te Velde E, Habbema J, et al. Predicting the pregnancy outcome in patients treated for hydrosalpinx: A prospective study. *Fertil Steril* 1986;45:23-9.

Chanelles O, Ducarme G, Sifer C, et al. Hydrosalpinx and infertility: what about conservative surgical management? *Eur J Obstet Gynecol Reprod Bio* 2011;159:122-6.

**SUPPORT:** None.

V-19 Wednesday, October 16, 2019 4:27 PM

### ROBOTIC ASSISTED LAPAROSCOPIC RESECTION OF ANTERIOR ADENOMYOMA.

Papri Sarkar, MD,<sup>a</sup> Anthony N. Imudia, MD,<sup>b</sup> <sup>a</sup>OBGYN Resident, Tampa, FL; <sup>b</sup>University of South Florida, Tampa, FL.



**OBJECTIVE:** This video demonstrates a unique technique of anterior adenomyoma resection and repair of the defect laparoscopically.

**METHODOLOGY:** We performed a robotic assisted laparoscopic resection of anterior adenomyoma where we excised the adenomyoma leaving approximately 1 cm of myometrium to the endometrium and serosa and then closed the defect by the overlap flap technique in a 40 yo G 3P0020 with secondary infertility who had multiple failed euploid frozen embryo transfers. She had an anterior adenomyoma and decision was taken to excise the adenomyoma to optimize the fertility outcome.

**CONCLUSIONS:** Due to lack of tactile sensation performing adenomyoma resection laparoscopically is more challenging than by laparotomy. This video demonstrates how we have delineated the endometrium by injecting methylene blue prior to surgery and then excised the adenomyoma maintaining the endomyometrial and myoserosal plane. We used the overlap technique to close the uterine defect and reinforced it with a third layer to prevent any hematoma formation. The advantages of this technique of closure includes: 1. good myometrial thickness, hence less chance of uterine rupture during pregnancy, 2. Prevention of deadspace to decrease the chance of hematoma formation, 3. Resection of adenomyotic is optimal while maintaining good myometrial thickness.

**SUPPORT:** None.

V-20 Wednesday, October 16, 2019 4:35 PM

### SPECIALIZED PIEZO-ICSI FOR LOW QUALITY OOCYTES.

Atsushi Tanaka, M.D., Ph.D., Motoi Nagayoshi, M.D., Izumi Tanaka, Ph.D., Takashi Yamaguchi, M.D., Ph.D., Motoharu Ohno, M.D., Saint Mother Hospital, Kitakyushu, Japan.



**OBJECTIVE:** In 1995, Kimura and Yanagimachi reported the usefulness of ICSI using Piezo-micro manipulator by applying a Piezo pulse which produced ultra-fast sub-micron forward momentum using uniquely shaped flat-tipped micropipettes with no bevel or spike (Piezo-ICSI).

Hiraoka et al reported that Piezo-ICSI has advantage of high fertilization rate, low damage rate of oocyte at ICSI and high clinical outcome in 2015. However, we have worried about one problem in Piezo-ICSI, that is the volume of injected medium at ICSI. So, we developed a newly specialized Piezo-ICSI with sperm with a shortened sperm tail after cutting the tails to lessen the damage to the cytoplasm of these low-quality oocytes. We then investigated the effect of the specialized Piezo-ICSI.

**METHODOLOGY:** Prospective study to improve clinical outcome of Specialized Piezo-ICSI for Low quality oocytes.

The sperm tail was cut with injection pipette a little below the mid piece then aspirate it injection pipette head first. The zona pellucida was penetrated using a weak piezo pulse (speed 1.5, intensity 1) and the tip of injection pipette was introduced forward to stretch the cytoplasmic membrane. A weaker pulse (speed 1.0, intensity 1) was added to break it and the sperm injected simultaneously. Pushing the sperm forward and aspiration of the medium injected at ICSI were unnecessary.

**CONCLUSIONS:** Oocytes that received ICSI. Oocytes that survived after ICSI (%), Oocytes fertilized (%), ood quality day-3 embryos (%), Blastocysts (%), Clinical pregnancies (%) between conventional Piezo-ICSI and Specialized Piezo-ICSI were [512, 124] [435 (85), 112 (90)] [409 (80), 103 (83)] [266 (52), 69 (56)] [230 (45), 60 (48)] [27, 31] respectively.

This newly developed Piezo-ICSI, using tail-cut shortened sperm through tail first was successful in making the injection easier. The reduction in injected volume of medium resulted in production of high-quality embryos.

V-21 Wednesday, October 16, 2019 4:42 PM

### NOVEL UTERINE CLOSURE TECHNIQUE TO PREVENT INTRAUTERINE ADHESIONS.

Clarissa J. Lam, MD, Anthony N. Imudia, MD. University of South Florida, Tampa, FL.



**OBJECTIVE:** The purpose of this video is to describe a novel uterine closure technique to prevent intrauterine adhesions after myomectomy. This is an important topic in the field as intrauterine adhesions can result in infertility, recurrent pregnancy loss, and future pregnancy complications, such as morbidly adherent placenta.

**METHODOLOGY:** In this video, we first discussed the epidemiology and the existing techniques that have been studied regarding the prevention of intrauterine adhesions. We then used an illustration to describe the suturing technique. We concluded with video clips demonstrating the technique from three of our myomectomy cases.

**CONCLUSIONS:** This novel intraoperative uterine closure technique is one method that can potentially reduce the risk of intrauterine adhesion formation.

**References:** 1. Capmas P, Pourcelot AG, Fernandez H. Are synechiae a complication of laparotomic myomectomy?. *Reprod Biomed Online*. 2018;36(4):450-454.

2. Conforti A, Krishnamurthy GB, Dragamestianos C, et al. Intrauterine adhesions after open myomectomy: an audit. *Eur J Obstet Gynecol Reprod Biol*. 2014;179:42-5.

3. Bhandari S, Ganguly I, Agarwal P, Singh A, Gupta N. Effect of myomectomy on endometrial cavity: A prospective study of 51 cases. *J Hum Reprod Sci*. 2016;9(2):107-11.

4. Asgari Z, Hafizi L, Hosseini R, Javaheri A, Rastad H. Intrauterine synechiae after myomectomy; laparotomy versus laparoscopy: Non-randomized interventional trial. *Iran J Reprod Med*. 2015;13(3):161-8.

5. Gambadauro P, Gudmundsson J, Torrejón R. Intrauterine Adhesions following Conservative Treatment of Uterine Fibroids. *Obstet Gynecol Int*. 2012;2012:853269.

6. Yang JH, Chen MJ, Wu MY, Chao KH, Ho HN, Yang YS. Office hysteroscopic early lysis of intrauterine adhesion after transcervical resection of multiple apposing submucous myomas. *Fertil Steril*. 2008;89(5):1254-9.

7. Acunzo, M. Guida, M. Pellicano et al., "Effectiveness of auto-cross-linked hyaluronic acid gel in the prevention of intrauterine adhesions after hysteroscopic adhesiolysis: a prospective, randomized, controlled study," *Human Reproduction*, vol. 18, no. 9, pp. 1918–1921, 2003.

8. Guida, G. Acunzo, A. Di Spiezio Sardo et al., "Effectiveness of auto-crosslinked hyaluronic acid gel in the prevention of intrauterine adhesions after hysteroscopic surgery: a prospective, randomized, controlled study," *Human Reproduction*, vol. 19, no. 6, pp. 1461–1464, 2004.

9. K. Roy, S. Singla, J. Baruah, J. B. Sharma, S. Kumar, and N. Singh, "Reproductive outcome following hysteroscopic myomectomy in patients with infertility and recurrent abortions," *Archives of Gynecology and Obstetrics*, vol. 282, no. 5, pp. 553–560, 2010.

10. Guida M, Acunzo G, Di Spiezio sardo A, et al. Effectiveness of auto-crosslinked hyaluronic acid gel in the prevention of intrauterine adhesions after hysteroscopic surgery: a prospective, randomized, controlled study. *Hum Reprod*. 2004;19(6):1461-4.

11. Yang JH, Chen MJ, Wu MY, Chao KH, Ho HN, Yang YS. Office hysteroscopic early lysis of intrauterine adhesion after transcervical resection of multiple apposing submucous myomas. *Fertil Steril*. 2008;89(5):1254-9.

12. Giatras K, Berkeley AS, Noyes N, Licciardi F, Lolis D, Grifo JA. Fertility after hysteroscopic resection of submucous myomas. *J Am Assoc Gynecol Laparosc*. 1999;6(2):155-8.

13. Taskin O, Sadik S, Onoglu A, et al. Role of endometrial suppression on the frequency of intrauterine adhesions after resectoscopic surgery. *J Am Assoc Gynecol Laparosc*. 2000;7(3):351-4.

14. De milliano I, Twisk M, Ket JC, Huirne JA, Hehenkamp WJ. Pre-treatment with GnRHa or ulipristal acetate prior to laparoscopic and laparotomic myomectomy: A systematic review and meta-analysis. *PLoS ONE*. 2017;12(10):e0186158.

15. Evans-hoeker EA, Young SL. Endometrial receptivity and intrauterine adhesive disease. *Semin Reprod Med*. 2014;32(5):392-401.

16. Conforti A, Krishnamurthy GB, Dragamestianos C, et al. Intrauterine adhesions after open myomectomy: an audit. *Eur J Obstet Gynecol Reprod Biol*. 2014;179:42-5.

17. Gupta S, Talaulikar VS, Onwude J, Manyonda I. A pilot study of Foley's catheter balloon for prevention of intrauterine adhesions following breach of uterine cavity in complex myoma surgery. *Arch Gynecol Obstet*. 2013;288(4):829-32.

18. Xiao S, Wan Y, Xue M, et al. Etiology, treatment, and reproductive prognosis of women with moderate-to-severe intrauterine adhesions. *Int J Gynaecol Obstet*. 2014;125(2):121-4.

19. Tonguc EA, Var T, Yilmaz N, Batioglu S. Intrauterine device or estrogen treatment after hysteroscopic uterine septum resection. *Int J Gynaecol Obstet*. 2010;109(3):226-9.

20. Liu L, Huang X, Xia E, Zhang X, Li TC, Liu Y. A cohort study comparing 4â€‰mg and 10â€‰mg daily doses of postoperative oestradiol therapy to prevent adhesion reformation after hysteroscopic adhesiolysis. *Hum Fertil (Camb)*. 2018;:1-7.

cebo in women with uterine fibroid (UF)-associated heavy menstrual bleeding (HMB).

DESIGN: Multinational phase 3 randomized, double-blind, placebo-controlled trial

MATERIALS AND METHODS: Premenopausal women (18-50 years) with menstrual blood loss (MBL) volume  $\geq$  80 mL/cycle assessed by the alkaline hematin method and ultrasound-confirmed UF, were eligible to participate in the study. Women were randomized 1:1:1 to one of 3 arms: once daily treatment with relugolix 40 mg + E2 1 mg/NETA 0.5 mg for 24 weeks (Group A), relugolix 40 mg alone for 12 weeks followed by relugolix 40 mg + E2 1 mg/NETA 0.5 mg for 12 weeks (Group B), or placebo for 24 weeks (Group C). The primary efficacy endpoint was the proportion of women in Group A vs Group C who achieved an MBL of  $<$  80 mL and  $\geq$  50% reduction from baseline MBL over the last 35 days of treatment. Secondary endpoints included mean % reduction in MBL, amenorrhea rate, improved anemia, and reduced UF-associated pain. Group B was included to explore the impact of E2/NETA on the anticipated hypoestrogenic effects of relugolix. Adverse events (AEs) and bone mineral density (BMD) changes by dual-energy X-ray absorptiometry were assessed.

RESULTS: In LIBERTY 1, 388 women were randomized and 308 (79%) completed the study. In Group A 73.4% met the primary endpoint vs 18.9% in Group C ( $p < 0.0001$ ). Mean % reduction in MBL from baseline at Week 24 was 84.3% for the Group A and 23.2% for Group C ( $p < 0.0001$ ). The proportion of women who achieved amenorrhea was 52.3% vs 5.5% in Groups A vs C, respectively ( $p < 0.0001$ ). In women with anemia (hemoglobin  $\leq$  10.5 g/dL) at baseline who completed 24 weeks treatment, 50.0% experienced a 2 g/dL increase in Group A vs 21.7% in Group C ( $p < 0.05$ ). Among the 50% of women reporting moderate/severe UF-associated pain (based on a maximum daily diary pain score of  $\geq$  4 at baseline where 0=no pain, 10=worst pain ever) 43.1% in Group A reported minimal/no pain (maximum pain score  $\leq$  1) in the last month of treatment vs. 10.1% in Group C ( $p < 0.0001$ ). Efficacy results in Group B were similar to those of Group A. Incidence of AEs was comparable between Groups A and C (62% vs 66%, respectively) and higher in Group B (73%), including the most common AE, hot flushes (11% and 8% in Groups A and C, respectively, vs 36% in B). The mean % change from baseline to Week 24 in lumbar spine BMD was -0.36%, -1.82%, and 0.05% in Groups A, B, and C, respectively. The distribution of the % change in BMD was similar between Groups A and C, including outliers.

CONCLUSIONS: In this Phase 3 pivotal study, relugolix combination significantly reduced MBL in women with UF-associated HMB and was generally well tolerated. Additional benefits were observed including a clinically meaningful reduction of UF-related pain, a high rate of amenorrhea, and improved anemia. Coadministration of E2/NETA maintained BMD and mitigated vasomotor symptoms. Relugolix combination with E2/NETA represents a potential long-term treatment option for women with UF.

SUPPORT: The Phase 3 LIBERTY clinical trial was funded by Myovant Sciences, Inc.

## O-266

### OPEN-LABEL PHASE IV CLINICAL TRIAL TO EVALUATE THE EFFECT OF NASAL TESTOSTERONE GEL ON REPRODUCTIVE HORMONES AND SEMEN PARAMETERS IN HYPOGONADAL

MEN. Thomas A. Masterson, III, MD,<sup>a</sup> Joshua N. Bitran, BS,<sup>b</sup> Manuel Molina, MD,<sup>b</sup> Emad Ibrahim, MD, HCLD(ABB),<sup>a</sup> Ursula Kaiser, MD,<sup>c</sup> Ranjith Ramasamy, M.D.<sup>a</sup> University of Miami Miller School of Medicine, Miami, FL; <sup>b</sup>University of Miami, Miami, FL; <sup>c</sup>Brigham and Women's Hospital, Division of Endocrinology, Boston, MA.

OBJECTIVE: Low testosterone (low T) affects around 12% of men under age of 40 and prescriptions for testosterone replacement therapy (TRT) to men of reproductive age are increasing. Unfortunately, most commonly prescribed TRT as gels, patches, injections and pellets are long-acting and can cause azoospermia in up to 65% of men. Therefore, it is imperative to identify an option for increasing T while minimizing effects on semen parameters. Natesto® (125 uL/nostril, 11.0 mg testosterone/dose, three times a day (TID)) is a short-acting, FDA approved nasal TRT available since 2015. We hypothesized that Natesto can preserve spermatogenesis by maintaining release of gonadotropins. We evaluated the effect of Natesto on reproductive hormones (Testosterone (T), Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH)), hypogonadal symptoms, and semen parameters in hypogonadal men.

DESIGN: Open-label single center phase IV clinical trial

MATERIALS AND METHODS: We prospectively enrolled men aged 18-55 years with two T levels  $<$  300 ng/dL (drawn before 10 AM) with

## LATE-BREAKING ABSTRACTS

### O-265

#### TREATMENT OF SYMPTOMS OF UTERINE FIBROIDS WITH RELUGOLIX COMBINATION THERAPY: EFFICACY AND SAFETY RESULTS FROM THE PHASE 3 LIBERTY 1 CLINICAL TRIAL.

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OBJECTIVE: To evaluate the efficacy and safety of 24 weeks of treatment with the oral GnRH receptor antagonist, relugolix, in combination with estradiol (E2) and norethindrone acetate (NETA) compared with pla-



hypogonadal symptoms and 2 semen analyses (SA) with total motile sperm counts (TMSC) > 5 million. Eligible men began Natesto TID for 3 months. T, LH, FSH, and 2 semen analyses were collected at baseline and after 3 months of therapy. Symptoms were evaluated using the international index of erectile function 6 (IIEF-6) and the short form 36 (SF-36) questionnaires. The primary endpoints were change in T, LH, FSH, sperm concentration, sperm motility and TMSC. Secondary end points were change of symptoms and adverse events (AEs). Data are presented as means (SD), students t-test was used to compare changes after 3 months, p<0.05 was considered significant. The study was adequately powered to detect a decline in 30% of gonadotropin levels at 80% with alpha set at 0.05.

**RESULTS:** In total, 55 men (age 19-55 years) were eligible and enrolled into the trial. Of the 55 who enrolled, 38 completed the trial and 17 dropped out (nasal irritation was a common cause of dropout). Among the men that completed the trial, mean T increased from 230(62) to 605(278) ng/dL (p=0.005), LH and FSH decreased but remained within the normal range (2-5 IU/mL). Most importantly, semen parameters remained unchanged; sperm concentration 26.6(15.2) vs 26.0(21.2) million/cc p=0.6, sperm motility 49.6(12.4) vs 48.9(22.5)% p=0.8, TMSC 40.8(36.7) vs 41.9(65.4) million p=0.9. There was improvement across all domains of the IIEF scores in erectile function, libido, intercourse satisfaction, orgasm, and overall sexual satisfaction as well as improvement in questions related to energy in the SF-36. Only 3 (7.9%) men developed severe oligospermia and one (2.6%) became azoospermic. All of these 4 men recovered spermatogenesis after discontinuation. The only adverse events were nasal irritation in 10 men.

**CONCLUSIONS:** This single center phase IV clinical trial demonstrated that Natesto increases serum T and improves hypogonadal symptoms while simultaneously maintaining gonadotropins and semen parameters. Natesto® appears to be a safe and effective treatment for men with hypogonadism who wish to preserve fertility.

**SUPPORT:** Aytu Biosciences provided drug free to patients.

## O-267

### DECREASED LIVE BIRTH RATE WITH LONGER OVARIAN STIMULATION IN FRESH BUT NOT FREEZE-ALL IN VITRO FERTILIZATION (IVF) CYCLES: ANALYSIS OF 17,830 CYCLES FROM THE SART REGISTRY.

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**OBJECTIVE:** To evaluate the effect of controlled ovarian stimulation length on first transfer live birth rates in fresh and freeze-all antagonist IVF cycles.

**DESIGN:** Historical cohort study of the SART CORS database

**MATERIALS AND METHODS:** Patient data was obtained for all gonadotropin antagonist IVF cycles (n=17,830) from 2014 to 2015 in which a single embryo transfer was completed as part of a fresh embryo transfer (n=14,866) or the first frozen embryo transfer in freeze-all non-preimplantation genetic testing-aneuploidy cycles (n=2964). Days of ovarian stimulation, patient and cycle characteristics, and pregnancy outcomes were extracted in both fresh and freeze-all cycles. Binomial regression models estimated the relative risk of live birth with respect to days of stimulation singularly, and after adjustment for *a priori* confounders.

**RESULTS:** In fresh cycles, days of ovarian stimulation ranged from 4 to 40 days, with 24% of patients having 4-8 days, 62% 9-11 days, 8% 12 days, and 6% with 13 or more days. In fresh transfer cycles, live birth rates decreased significantly with each additional day of stimulation from ≤ 8 days to > 12 days by univariate analysis ranging from 47.36% to 41.49%, p-value (trend) = <0.0001. These findings were validated in a multivariable model controlling for age, gravidity, BMI, Max FSH, and etiology of infertility with a p-value (trend) = 0.005. In freeze-all cycles, a decline in the live birth rate with increasing days of stimulation was observed with p(trend)=0.01, however this trend was not statistically significant in the adjusted model p(trend) = 0.46. (Table 1)

**CONCLUSIONS:** Increasing length of ovarian stimulation negatively affects live birth rates in fresh but not freeze-all antagonist IVF cycles. In fresh transfer cycles, live birth rate is highest with stimulation of ≤ 8 days (47.4%) and was observed to decline with increasing days of stimulation (41.5%) with 13 or more stimulation days. This points to an endometrial cause for the adverse impact on live birth rates with longer stimulation in fresh transfer cycles that may not be relevant in freeze-all cycles.

TABLE 1. Association between Days of Stimulation and Live Birth Rate Stratified by Type of Transfer

Days of Stimulation	Fresh (n=14,866)	Frozen (n=2,964)
4-8	1716 47.36%	308 50.24%
9	1756 45.97%	349 48.61%
10	1413 44.17%	304 46.34%
11	931 43.79%	192 40.85%
12	489 42.67%	112 44.27%
>12	395 41.49%	115 45.28%
p-value (trend) <sup>1</sup>	<0.0001	0.01
adjusted (trend) <sup>2</sup>	0.005	0.46

<sup>1</sup> Corresponding to a non-zero estimate for linear trend across days of stimulation categories in a univariate binomial regression model

<sup>2</sup> Corresponding to a non-zero estimate for linear trend across days of stimulation categories in a multivariable binomial regression model

## O-268

### IN VITRO MATURATION (IVM) VERSUS IN VITRO FERTILIZATION (IVF) IN WOMEN WITH HIGH ANTRAL FOLLICLE COUNT (AFC): A RANDOMIZED CONTROLLED TRIAL (NCT03405701).

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**OBJECTIVE:** IVM has been proposed as an alternative to IVF for women at increased risk of ovarian hyperstimulation syndrome (OHSS) due to a high antral follicle count (AFC) and/or polycystic ovary syndrome (PCOS). Here, we compare the effectiveness and safety of one IVM and one IVF cycle in women with infertility and high AFC.

**DESIGN:** A single-center noninferiority randomized controlled trial (NCT03405701) in Vietnam.

**MATERIALS AND METHODS:** Women scheduled for assisted reproductive technology (ART) with an AFC ≥ 24 were randomized (1:1 ratio) to IVM or IVF. In the IVM group, oocyte pick-up was performed 42 hours after the last injection of highly purified human menopausal gonadotropin (hp-hMG) 150 IU/day; all oocytes were cultured in capacitation pre-maturation medium for 24 h and then transferred to maturation culture for 30 h. Women allocated to IVF underwent ovarian stimulation using a hp-hMG/gonadotropin

	IVM (n=273)	IVF (n=273)	Rate difference (95% CI)
Embryos transferred, n	1.9±0.3	2.0±0.2	
Clinical pregnancy, n (%)	138 (51)	154 (56)	-5.9% (-14.6%, 2.9%)
Ongoing pregnancy, n (%)	104 (38)	126 (46)	-8.1% (-16.7%, 0.6%)
Singleton	71/104 (68)	79/126 (63)	
Twins	33/104 (32)	47/126 (37)	
OHSS, n (%)	0	2 (0.7)	

releasing hormone (GnRH) antagonist protocol and oocytes were retrieved 36 h after GnRH agonist trigger.

In both groups, mature oocytes were fertilized using intracytoplasmic sperm injection, and all embryos were frozen on day 3;  $\leq 2$  embryos were transferred in a subsequent frozen cycle. The primary outcome was live birth after first embryo transfer of the started treatment cycle. The planned sample size was 546, assuming an expected live birth rate of 45% in the IVF group, a noninferiority margin of  $-10\%$ , 90% power and 15% loss to follow-up. While follow-up for live birth is ongoing, we report ongoing pregnancy in this abstract.

**RESULTS:** Between January 2018 and December 2018, we randomized 546 women (273 in each group). Baseline characteristics were comparable (mean age 30 years, BMI 22 kg/m<sup>2</sup>). The ongoing pregnancy rates after the first embryo transfer were 38% and 46%, respectively (difference  $-8.1\%$  [ $-16.7\%$ ,  $0.6\%$ ]). Other fertility outcomes after first embryo transfer were also not statistically significant between the groups (Table). All laboratory outcomes favoured IVF over IVM: oocytes retrieved (19.8 vs 14.1), MII oocytes (15.7 vs 8.9), maturation rate (79% vs 64%), fertilized oocytes (13.7 vs 7.3), top-quality embryos (7.9 vs 3.2), and freezable embryos (7.6 vs 4.0) were significantly higher in the IVF vs IVM group (all  $p < 0.001$ ).

**CONCLUSIONS:** Among women undergoing ART with an AFC  $\geq 24$ , IVM did not result in significantly lower ongoing pregnancy rates than IVF. Live birth data will be available by October 2019.

## O-269

### ANTI-MULLERIAN HORMONE (AMH) IN THE CALIPER COHORT OF HEALTHY COMMUNITY CHILDREN AND ADOLESCENTS: IMPROVING CARE IN ONCOFERTILITY.

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**OBJECTIVE:** Serum AMH is an excellent biomarker of ovarian reserve (1,2). The assessment of AMH pre- and post gonadotoxic treatment helps define reproductive potential in young adults facing cancer treatment. Normative childhood and adolescent AMH levels are not well defined despite the potential for high clinical utility (3). Most studies have been limited by sample size, varying and less sensitive manual assays, and with some conflicting trends (4-12). Our objective is to establish accurate reference intervals (RIs) for AMH in the pediatric population that can be used to assess AMH in pediatric/adolescent survivors.

**DESIGN:** This cross-sectional study examined AMH in serum samples from healthy pediatric subjects in the Greater Toronto Area.

**MATERIALS AND METHODS:** 300 samples were collected from healthy females age 6 to  $< 19$  years, which were previously drawn and stored as part of a collaboration with the Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER), an internationally recognized initiative developing normative reference values for clinically important biomarkers of paediatric health and disease (13). Samples were analyzed using the automated Beckman Dxl AMH assay in one batch. Basic demographics and menstrual data on sample subjects was also noted.

Results were divided into four predetermined age groups (6 to  $< 9$  years, 9 to  $< 12$ , 12 to  $< 15$ , 15 to  $< 19$ ). Statistical significance between each age group was determined by the Harris & Boyd method. Outliers were removed from each age partition separately using the Tukey method and the adjusted Tukey method. Mean  $\pm$  SD, associated quartiles and reference intervals were calculated in alignment with CLSI guidelines. The effect of menstrual status and ethnicity on study cohort was assessed using a one-way analysis of variance.

**RESULTS:** 300 patient samples were included with a range of 7.0 to 56 pmol/L. Forty-one percent of the study cohort was postmenarcheal. Serum AMH concentrations varied across age ranges with females 6 to  $< 9$  years demonstrating significantly lower AMH concentrations compared to females aged 9 to  $< 12$  years (Mean  $\pm$  SD of  $22.7 \pm 11.6$  and  $29.7 \pm 18.2$  pmol/L respectively). Additionally, females aged 9 to  $< 12$  years demonstrated significantly higher AMH concentrations compared to those aged 12 to  $< 15$  years (Mean  $\pm$  SD of  $22.7 \pm 12.0$  pmol/L).

Menstrual status did not significantly impact AMH serum concentrations ( $p=0.787$ ). Similarly, ethnicity did not significantly impact AMH concentrations ( $p=0.0965$ ).

**CONCLUSIONS:** Our results demonstrate comparable trends to other studies with respect to rising AMH in childhood. This contributes to the limited literature on normative AMH values throughout childhood and adolescence. This study presents reliable reference ranges, from a single batched assay on healthy children. This is also the largest series of its kind using an automated AMH assay. These normative values for AMH will be a major adjunct to counseling pediatric cancer patients and their families who require or have completed fertility damaging therapies.

**SUPPORT:** AMH assay kits were provided by Beckman Coulter (Lot 971017)

**References:** 1. Lindhardt Johansen M, Hagen CP, Johannsen TH, Main KM, Picard J-Y, Jørgensen A, et al. Anti-müllerian hormone and its clinical use in pediatrics with special emphasis on disorders of sex development. *Int J Endocrinol* 2013;2013:198698.

2. Broer SL, Broekmans FJM, Laven JSE, Fauser BCJM. Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. *Human Reproduction Update* 2014 Sep;20(5):688-701.

3. Lie Fong S, Visser JA, Welt CK, de Rijke YB, Eijkemans MJC, Broekmans FJ, et al. Serum anti-müllerian hormone levels in healthy females: a nomogram ranging from infancy to adulthood. *J Clin Endocrinol Metab* 2012 Dec;97(12):4650-5.

4. Lie Fong S, Visser JA, Welt CK, de Rijke YB, Eijkemans MJC, Broekmans FJ, et al. Serum anti-müllerian hormone levels in healthy females: a nomogram ranging from infancy to adulthood. *J Clin Endocrinol Metab* 2012 Dec;97(12):4650-5.

5. Lee MM, Donahoe PK, Hasegawa T, Silverman B, Crist GB, Best S, et al. Müllerian inhibiting substance in humans: normal levels from infancy to adulthood. *J Clin Endocrinol Metab* 1996 Feb;81(2):571-6.

6. Guibourdenche J, Lucidarme N, Chevenne D, Rigal O, Nicolas M, Luton D, et al. Anti-Müllerian hormone levels in serum from human foetuses and children: pattern and clinical interest. *Mol Cell Endocrinol* 2003 Dec 15;211(1-2):55-63.

7. Hagen CP, Aksglaede L, Sørensen K, Main KM, Boas M, Cleemann L, et al. Serum levels of anti-Müllerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. *J Clin Endocrinol Metab* 2010 Nov;95(11):5003-10.

8. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WHB. A validated model of serum anti-müllerian hormone from conception to menopause. *PLoS ONE* 2011;6(7):e22024.

9. Ahmed SF, Keir L, McNeilly J, Galloway P, O'Toole S, Wallace AM. The concordance between serum anti-Müllerian hormone and testosterone concentrations depends on duration of hCG stimulation in boys undergoing investigation of gonadal function. *Clin Endocrinol (Oxf)* 2010 Jun;72(6):814-9.

10. Sanders RD, Spencer JB, Epstein MP, Pollak SV, Vardhana PA, Lustbader JW, et al. Biomarkers of ovarian function in girls and women with classic galactosemia. *Fertility and Sterility* 2009 Jul;92(1):344-51.

11. Hagen CP, Aksglaede L, Sørensen K, Mouritsen A, Andersson A-M, Petersen JH, et al. Individual serum levels of anti-Müllerian hormone in healthy girls persist through childhood and adolescence: a longitudinal cohort study. *Human Reproduction* 2012 Mar;27(3):861-6.

12. Lashen H, Dunger DB, Ness A, Ong KK. Peripubertal changes in circulating antimüllerian hormone levels in girls. *Fertility and Sterility* 2013 Jun;99(7):2071-5.

13. Adeli K, Higgins V, Trajcevski K, White-Al Habeeb N. The Canadian laboratory initiative on pediatric reference intervals: ACALIPER-white paper. *Critical Reviews in Clinical Laboratory Sciences* 2017;54(6):358-413.

14. Á Cui L, Qin Y, Gao X, Lu J, Geng L, Ding L, et al. Antimüllerian hormone: correlation with age and androgenic and metabolic factors in women from birth to postmenopause. *Fertility and Sterility* 2016 Feb;105(2):481-485.e1.

15. Jopling H, Yates A, Burgoyne N, Hayden K, Chaloner C, Tetlow L. Paediatric Anti-Müllerian Hormone measurement: Male and female reference intervals established using the automated Beckman Coulter Access AMH assay. *Endocrinology, Diabetes & Metabolism* 2018;1(4):e00021.

### DAMAGED SPERM PARAMETERS AND SPERMIA-TION FAILURE IN VENLAFAXINE-TREATED RATS: A CORRELATION WITH HIGH TESTICULAR AROMATASE IMMUNOEXPRESSION AND REDUCED EPIDIDYMAL V-ATPASE.



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**OBJECTIVE:** The antidepressant venlafaxine (Serotonin Norepinephrine Reuptake Inhibitor-SNRI) has impaired sexual function in male patients. We investigated the impact of this SNRI on sperm parameters, relating them to testicular and epididymal histophysiological markers. The recovery of sperm and testicular changes was also evaluated following the interruption of treatment.

**DESIGN:** Adult male rats were grouped: Venlafaxine-35 days (VFG-35; n=6) and Venlafaxine-65 days (VFG-65; n=6) received venlafaxine (30mg/kg BW) by gavage for 35 days; Control-35 days (CG-35; n=6) and Control-65 days (CG-65; n=6) received saline. After treatment, the animals from CG-35 and VFG-35 were killed while the animals from CG-65 and VFG-65 were maintained without treatment for 30 days to evaluate reversibility of changes. In these groups, sperm parameters were evaluated in association to seminiferous epithelium integrity, steroidogenesis, testicular aromatase and epididymal V-ATPase immunoeexpression.

**MATERIALS AND METHODS:** Rats received 30mg/kg (therapeutic dosage) of venlafaxine for 35 days (minimal period for the antidepressant effect). The concentration, morphology and mitochondrial cytochemical activity (MCA) of sperm from cauda epididymis were analyzed. In epididymal and testicular sections, the following parameters were evaluated: epididymal duct diameter, frequency of tubules with spermiation failure, number of Sertoli cells (NSC), viability of germ cells by TUNEL, Leydig cells nuclear diameter (LCn), StAR immunoeexpression (steroidogenesis),

TABLE 1. demonstrates significant increase in relative costs per MII oocyte and potential LB with age. In our model, cost per potential LB at 30-32 is 2.18x less the cost at 37-39 and 1.28x less the cost at 34-36 (p<0.001). The difference equals 6-12 years of storage.

	≤34 N=415	35-37 N=524	38-40 N=234	41-42 N=51	>42 N=17	p
# Cycles	1.28±0.60	1.46±0.77	1.60±0.93	1.76±0.97	2.29±1.49	<0.001
MIIs	18.16±9.52	16.18±9.06	13.88±8.43	12.35±10.7	11.23±9.99	<0.001
GND (IUs)	4254.22 ±3302.98	5473.56 ±3830.88	6239.31 ±4238.65	7703.92 ±5061.78	9798.53 ±6590.56	<0.001
Relative increase: Cost per MII	Ref	1.34 (0.80-2.23)	1.78 (1.11-2.78)	2.71 (1.33-5.46)	3.70 (1.83-6.13)	<0.001
Relative increase: Cost per potential LB	Ref	1.51 (0.90-2.50)	3.25 (2.01-5.07)	8.89 (4.36-17.91)	12.13 (6.01-20.12)	<0.001

testicular aromatase (Cyp19) and epididymal V-ATPase immunofluorescent intensity. Serum and testicular testosterone levels were also measured. Data were submitted to two-way ANOVA with Tukey *post-hoc* test.

**RESULTS:** In VFG-35, the epididymal duct diameter, sperm concentration and MCA decreased, and a high frequency of sperm tail abnormalities was found. Changes in seminiferous epithelium and high frequency of post-spermiation tubules with retained spermatids were found. The NSC decreased whereas the number of TUNEL-positive germ cells and Cyp19 immunoeexpression increased in this group. In VFG-35, LCn was larger than CG; a high immunoeexpression of StAR and elevated serum and testicular testosterone levels were observed. Venlafaxine also impaired the epididymal V-ATPase immunoeexpression. Except for the tail changes and MCA, sperm concentration and testicular parameters were improved following the interruption of treatment.

**CONCLUSIONS:** Venlafaxine stimulates LC steroidogenesis and increases aromatase levels, impairing spermiation and sperm concentration and quality. Therefore, the evaluation of fertility together with a careful analysis of spermatogenesis and hormonal status of patients treated with SNRI is useful. The changes in sperm parameters may also be associated with disturbs in the acid/basic milieu of epididymal lumen due to reduction in V-ATPase. The improvement of sperm parameters following the interruption of treatment is, at least in part, due to recovery of aromatase/estrogen levels and the restoration of spermiation process.

**SUPPORT:** FAPESP (2017/19829-6; 2018/13590-4; 2018/25353-7)

### THE AGE TAX: OOCYTE CRYOPRESERVATION (OC) AGE-BASED COST ANALYSIS.



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**OBJECTIVE:** The degree to which OC costs increase with age is not known. Data regarding OC outcomes and associated costs are limited. With increasing demand for OC, there is a need for evidence-based counseling tools for age-related costs. The primary aim of this study is to quantify relative age-based increase in OC costs with an evidence-based cost evaluation model of OC cost, incorporating age, medication, oocyte yield, and potential for LB.

**DESIGN:** Cost analysis. Nested retrospective cohort study.

**MATERIALS AND METHODS:** All women undergoing OC at Extend Fertility Medical Practice from 4/2016-12/2018 were included in the cohort. Demographic and cycle data were abstracted from the electronic medical record. Cycle and storage fees were calculated for 135 U.S. practices from Freeze.Health's public dataset. Medication pricing was calculated using first 15 listings in a national online database ([fertilitydrugcalculator.com](http://fertilitydrugcalculator.com)).

Mathematical model for cost per cryopreserve MII oocyte and cost per potential LB were developed with age, MII oocyte per cycle, total cycles, total medication utilization, mean national cycle and medication fees, and per oocyte potential LB rates from Doyle et al. Using the <34 group as a reference, relative increase in median cost per MII and cost per potential LB were calculated as multiples of the median (MoM) and 25-75%tiles. Associations were analyzed using ANOVA and Kruskal-Wallis, where appropriate.

**RESULTS:** 1241 subjects with a total of 1791 cycles were included. Mean age =35.6±3.3, mean # cycles 1.45±0.79, mean MII cryopreserved oocytes = 16.19±9.34, median cost per MII oocyte cryopreserved = \$1170.99 (\$708.19-2051.69) and median cost per potential LB = \$17,041.34 (\$9589.29-33253.79).

**CONCLUSIONS:** The relative cost of OC significantly increases with age.

Women considering OC should be counseled about the drastic increase in cost with age. This study represents the most robust analysis to date of OC costs with data collected from OC cycles.

**References:** Successful elective and medically indicated oocyte vitrification and warming for autologous in vitro fertilization, with predicted birth probabilities for fertility preservation according to number of cryopreserved oocytes and age at retrieval. Doyle, Joseph O. et al. *Fertility and Sterility*, Volume 105, Issue 2, 459 - 466.e2

### MENSTRUAL CYCLE REGULARITY AND LENGTH AND RISK OF MORTALITY: A PROSPECTIVE COHORT STUDY.



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**OBJECTIVE:** To prospectively assess the associations of menstrual cycle characteristics in adolescence and adulthood with risk of all-cause and cause-specific mortality.

DESIGN: Prospective cohort study.

**MATERIALS AND METHODS:** We followed 93,775 women participating in the Nurses' Health Study II between 1991 and 2013 who had no prior history of cardiovascular disease, cancer, or diabetes and who reported the usual length and regularity of their menstrual cycles at ages of 14-17, 18-22, and 28-48 years. We obtained hazard ratios (HR) and 95% confidence intervals (CI) for the relationships between menstrual cycle characteristics and mortality from Cox proportional hazards models adjusted for relevant confounders including body mass index, race/ethnicity, and physical activity, and lifestyle factors.

**RESULTS:** We documented 1679 deaths, including 828 from cancer, and 166 from cardiovascular disease, during 1,729,410 person-years of follow-up. After adjustment for various covariates, women reported that their menstrual cycles were always irregular between the ages of 14-17 and 18-22 were 21% [HR=1.21 (95% CI: 1.04, 1.40)] and 34% [HR=1.34 (95% CI: 1.08, 1.66)], respectively, more likely to die from any causes during follow-up than women reporting very regular menstrual cycles in the same age range. A similar relation was observed with irregular menstrual cycles between the ages of 28-48 years. Likewise, women reporting a current usual cycle length of 32-39 days or of  $\geq 40$  days were more likely to die from any causes during follow-up than women whose current usual cycle length was 26-31 days [HRs=1.23 (95% CI: 1.04, 1.45), and 1.28 (95% CI: 1.05, 1.55), respectively]. Elevated HR for cardiovascular and cancer mortality was also associated with longer menstrual cycle lengths ( $>32$  days) between the ages of 28-48 years.

**CONCLUSIONS:** Irregular and long menstrual cycles are associated with an increased risk of mortality.

### O-273

#### THE EFFECT OF EXTENDED BLASTOCYST EXPOSURE OF HYALURONAN ENRICHED TRANSFER MEDIA ON IMPLANTATION RATE IN FROZEN EMBRYO TRANSFERS.

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**OBJECTIVE:** Hyaluronan enriched transfer media (EmbryoGlue®, Vitro-life, Inc) published reports demonstrating conflicting results on improving clinical pregnancies and implantation rate. This study was designed to determine if extended embryo exposure of hyaluronan enriched transfer media improved clinical pregnancy and implantation rate outcomes in frozen embryo transfers (FET).

**DESIGN:** Prospective randomized study

**MATERIALS AND METHODS:** A total of one hundred nineteen FET patients were included in this study. Frozen blastocysts were thawed following the Dallas Fertility Center laboratory thaw protocol and randomly divided into four treatment groups. Blastocyst transfer dishes were prepared using 2 ml (1 ml in inner well and 1 ml in outer well) of EmbryoGlue® in organ well dishes for all experimental groups. These transfer dishes were equilibrated overnight. Embryos in the control group were transferred in embryo glue following the manufacture's guidelines. Treatment groups consisted of one-hour, two-hour and three-hour post thaw exposure to EmbryoGlue® before blastocyst transfer. Clinical pregnancy, ongoing pregnancy, and implantation rate after embryo transfer were compared among the groups. Clinical pregnancies were confirmed by the presence of an intrauterine gestational sac. Chi square analysis was used to analyze data.

**RESULTS:** No statistical difference was seen between the control group and one-hour treatment group in terms of clinical pregnancy, ongoing preg-

nancy and implantation rate. In contrast, statistical difference was presented after exposure increment of blastocysts to EmbryoGlue®.

**CONCLUSIONS:** Blastocysts with extended exposure of hyaluronan enriched medium before embryo transfer showed an improved implantation rate in frozen embryo transfers. This procedure before embryo transfer provides better clinical outcomes when thawed blastocysts were exposure to embryo glue for more than 2 hours.

### O-274

#### THE RATE OF TRUE RECURRENT IMPLANTATION FAILURE (RIF) IS LOW: RESULTS OF THREE SUCCESSIVE FROZEN EUPLOID SINGLE EMBRYO TRANSFERS (SET).

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**OBJECTIVE:** RIF is one of the more challenging areas of reproductive medicine. Despite a significant interest in RIF, its definition has not yet been agreed upon, and etiologic factors responsible for RIF have not been fully characterized. One of the most common causes of pregnancy failure is chromosomal abnormality of the embryos. Therefore, it is likely that a number of RIF cases are due to aneuploidy. Other potential causes of RIF include immune and endometrial factors; however, the relative contribution of these factors to RIF have not yet been established. In this study, we aimed to determine the true prevalence of RIF in women undergoing successive SET.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** To answer this question we analysed all patients (n=4,515) with up to three consecutive euploid frozen SETs taking place from January 2012 to July 2018, excluding cycles with donor eggs or gestational carriers. We analysed the cumulative outcomes from these cycles in order to determine what percentage of them had causes of RIF that were not related to the ability to achieve a euploid blastocyst. All embryos underwent PGT-A at the blastocyst stage using qPCR or NGS-based platforms. All embryos were vitrified at the blastocyst stage after a trophoctoderm (TE) biopsy was performed. Endometrial preparation was achieved with oral E2 and intramuscular progesterone supplementation with transfer of a single euploid blastocyst performed on the 6<sup>th</sup> day of progesterone exposure. The primary endpoint was implantation as determined by the presence of a gestational sac with fetal cardiac activity. A logistic regression model was employed to assess the differences of outcomes between first, second, and third euploid SET and a Kaplan-Meier curve as utilized to analyze cumulative implantation rate.

**RESULTS:** The mean age of the patients included in the study was of 35.4±4.2. The implantation rates of the first, second and third frozen euploid SET were 69.4%, 59.3%, and 59.2% per transfer, respectively. Of those who failed to achieve implantation after the first euploid SET (n=1381), 799 (57.9%) underwent a second euploid SET and of those who failed to achieve implantation after the second euploid SET (n=325), 142 (43.7%) patients underwent a 3<sup>rd</sup> euploid SET. The second (OR=0.638, 95% CI 0.547-0.746) and third (OR=0.627, 95% CI 0.446-0.886) frozen euploid SET provided a slightly decreased implantation when compared to the first frozen euploid SET. The cumulative implantation rates after up to three consecutive frozen euploid SET was 94.9% (95% CI: 93.7%-95.9%).

**CONCLUSIONS:** Our findings suggest that true RIF is rare. For those patients with the ability to make euploid blastocysts, 94.9% would achieve clinical pregnancy with 3 embryos transferred. The implantation rates decline minimally with increasing transfers, but the fact that they remain high



Treatment	n	Age (Average)	Exposure Time to Embryo Glue Hours (Average)	Embryos Transferred (Average)	Clinical Pregnancy Rate	Implantation Rate	Ongoing Pregnancy Rate
Control	26	33	0	1.2	46% <sup>a</sup>	47% <sup>a</sup>	45% <sup>a</sup>
One-hour	26	33	1.3	1.3	54% <sup>a</sup>	48% <sup>a</sup>	46% <sup>a</sup>
Two-hour	31	33	2.2	1.3	68% <sup>b</sup>	60% <sup>b</sup>	59% <sup>b</sup>
Three-hour	36	34	3.3	1.2	81% <sup>b</sup>	73% <sup>c</sup>	75% <sup>c</sup>

<sup>a,b,c</sup> Different superscripts within columns indicate significant differences (P<0.05)

suggests that 5% who fail to implant after three attempts may largely be victims of simple probabilities/statistics and that additional transfers offer hope of a good outcome.

**O-275**

**A SINGLE INJECTION OF LONG ACTING GnRH-ANTAGONIST -DEGARELIX- DOWNREGULATES HYPOPHYSIS DURING OVARIAN STIMULATION. A RANDOMIZED CONTROLLED TRIAL.**



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**OBJECTIVE:** Study's objective was to examine if the use of a novel long acting, single dose GnRH antagonist, Degarelix, can cause efficient pituitary downregulation during ovarian stimulation in oocyte donors.

**DESIGN:** This RCT (Trial Registration Number: NCT03861715) recruited healthy young oocyte donors (<35yrs) between January 2017-January 2019 in Assisting Nature, Centre of Assisted Reproduction and Genetics, Thessaloniki, Greece. Two groups of patients were examined; the first group (study group) the received a single Day-6 follicular dose of degarelix (Firmagon, Ferring Pharmaceuticals); the second group (control group) received daily 0.25mg of ganirelix as is the standard antagonist protocol. Study Group (Degarelix group) consisted of 80 women, who followed the new protocol, whereas, 93 donors followed the classical fixed Day6 GnRH-antagonist protocol.

**MATERIALS AND METHODS:** Ovarian stimulation was initiated on cycle Day2 or 3 with gonadotropins 225 IU (200-300), daily, in both groups. In Control group 0.25 mg of antagonist ganirelix was administered daily from stimulation Day6 in a fixed manner. In the new study group, on the same day, day-6, a single bolus injection of 0.1 ml Degarelix was administered subcutaneously. Agonist triggering (Triptorelin 0.3ml) was employed for all and OPU performed at 36h. Fresh or frozen blastocyst-only transfer was performed following recipient endometrial estrogen and progesterone priming.

**RESULTS:** No LH rise or any OHSS was noticed in any groups. Mean age (27.1 vs 27.9 years), mean AMH (4.1 vs. 3.6ng/ml) and total gonadotropin dose (2400 vs 2508 IU) of participants were not different among Control-group- and Study-group respectively. Similar number of oocytes retrieved (18.1 vs.17.1, p>0.05) with degarelix short antagonist group, and similar number of blastocysts produced in both groups (6.6 in Control-group-A vs. 6.9 in Study-group). All recipients underwent 2 blastocysts transfer. Pregnancy is expressed per donor. Initial positive HCG per donor was significantly higher (p<0.05) in the Degarelix Short Antagonist (Study-Group) 78.7% (63/80) as compared with 65.5% (n=61/93) in classic short antagonist (Control-Group). Cumulative delivery rate was higher 60.0% (48/80) in the new single shot Degarelix short antagonist group as compared to 50.5% (n=47/93) in classic antagonist group, however not significant (p>0.05).

**CONCLUSIONS:** The new long-acting GnRH antagonist in a single bolus dose of 0,1 mg carries no risk for LH, produce mature oocytes and achieve comparable pregnancy outcome to the classical short multiple dose antagonist protocol. This new protocol is first described by us, it is more patient friendly decreasing the number of injections that a patient receives. This is an ongoing study, dose of degarelix was arbitrarily chosen by our team, and more degarelix doses can be tested in future studies.

**O-276**

**EFFICACY OF LF111 IN OBESE WOMEN, AN INVESTIGATIONAL PROGESTIN-ONLY ORAL CONTRACEPTIVE.**



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**OBJECTIVE:** Obesity is common in North American women and may be associated with lower contraceptive efficacy. As a sub-analysis of a phase 3 safety and efficacy trial, we evaluated the efficacy, stratified by age and obesity status, of LF111, a progestin-only oral contraceptive regimen with drospirenone (dosage: 4.0 mg x 24 days and 4 placebo days).

**DESIGN:** An open-label trial in women ≥ 15 years desiring contraception to evaluate efficacy and safety during 13 consecutive 28-day cycles.

**MATERIALS AND METHODS:** We calculated the Pearl Index (PI) in non-breastfeeding women who received at least one dose of LF111 (modified Full Analysis Set [mFAS]). We defined obesity as body mass index (BMI) ≥ 30 kg/m2.

**RESULTS:** Among 1006 subjects, 354 (35.2%) were obese, a proportion similar to the overall US population (36.5%). This analysis included 352 obese subjects of all ages. Four confirmed pregnancies occurred among 352 obese subjects versus 8 among 641 non-obese subjects. The PI in 2283 exposure cycles in obese subjects was 2.3 (95% CI 0.6 - 5.8) versus 2.4 (95% CI 1.0 - 4.8) in 4283 exposure cycles in non-obese subjects. No women >35 years had a confirmed pregnancy; thus, the PI in women ≤35 age was 2.9 (95% CI 0.8 - 7.3) in 1817 evaluable cycles in obese participants versus 3.0 (95% CI 1.3 - 5.8) in 3520 evaluable cycles in 590 non-obese participants. No cases of venous or arterial thromboembolism, myocardial infarction, stroke, or pulmonary embolism were reported in the clinical trial.

**CONCLUSIONS:** Drospirenone 4.0 mg 24/4 provides effective contraceptive protection in obese women.

Pearl Index; LF111		
BMI	< 30 kg/m2	≥ 30 kg/m2
Confirmed pregnancies n (%)	8 (1.2%)	4 (1.1%)
Exposure cycles	4283	2283
Pearl Index	2.4	2.3
95% CI Lower Limit		
95% CI Upper Limit		
Pearl Index women ≤35 years		
BMI	< 30 kg/m2	≥ 30 kg/m2
N	590	325
Confirmed pregnancies n (%)	8 (1.4%)	4 (1.2%)
Exposure cycles	3520	1817
Pearl Index	3.0	2.9
95% CI Lower Limit	1.3	0.8
95% CI Upper Limit	5.8	7.3

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Chen, S.	Create Fertility Centre <sup>3</sup>	Dominguez, J.	Ferring <sup>7</sup> ; Gedeon-Richter <sup>7</sup> ; Merck <sup>6,7</sup> ; MSD <sup>6</sup>
Chen, S. H.	Cooper Genomics <sup>6</sup> ; Hologic <sup>6,7</sup> ; MedAnswers <sup>2</sup> ; Ohana <sup>6</sup> ; Phosphorus <sup>6</sup>	Doody, K. J.	Ferring Pharmaceuticals <sup>6,7</sup> ; INVO Bioscience <sup>1,2</sup>
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Choudhary, K.	Previvo Genetics inc. <sup>3</sup>	Dukhovny, D.	Clearview Consulting <sup>6</sup> ; Vermont Oxford Network <sup>5,6,7</sup>
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Clemente-Ciscar, M.	Igenomix <sup>3</sup>	D'Hooghe, T.	Merck Healthcare KGaA <sup>3</sup>
Clementi, C.	Celmatix <sup>3</sup>	Edelman, A.	Merck <sup>4,8</sup> ( <i>Trainer</i> )
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Copel, J. A.	Jubel LLC <sup>6</sup>	EL Kasmi, I.	Clinique Ovo <sup>3</sup>
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	Estetra SPRL <sup>8</sup> ( <i>research support to</i>	Lanes, A.	BORN Ontario <sup>6</sup>
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	Belgium <sup>8b</sup> ( <i>This company</i>		Merck Serono <sup>4</sup>
	<i>provided the assay kit</i> )	Lazorwitz, A.	Merck & Co <sup>4</sup>
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Jukic, A.	Theralogix <sup>8</sup> ( <i>Vitamin D supplements</i>		Guerbet <sup>4</sup>
	<i>from this company were donated</i>	Leppert, P.	Duke Univesrity <sup>8</sup> ( <i>I am listed on</i>
	<i>for a clinical trial of which I am</i>		<i>a patent for CCH and the</i>
	<i>PI.</i> )		<i>treatment of fibroids. It is signed</i>
Kadoch, I.	Yadtech <sup>2</sup>	Lesaint, C.	<i>over to Duke Unversity</i> )
Kalaghan, L.	CCRM Boston <sup>3</sup>	Letterie, G. S.	Clinique ovo <sup>3</sup>
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Kim, J. J.	Abbvie <sup>6</sup> ; Intuitive <sup>5</sup>	Maalouf, W.	Parallabs <sup>6</sup>
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	<i>royalties from the Company, co-</i>	Maisenbacher, M. K.	EMD Serono <sup>3</sup>
	<i>founded the Company.</i> )	Malik, M.	Natera <sup>2,3</sup>
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Labarta, E.	Ferring <sup>6,7</sup> ; FINOX <sup>4b</sup> ; IBSA/	Mateu-Brull, E.	Salveo Health <sup>6</sup>
	Angelini <sup>7</sup> ; MSD <sup>7</sup> ; OvaScience <sup>5</sup>		IGENOMIX S. L. <sup>3</sup>

Mathur, R.	Bausch <sup>5a,6a,7a</sup> ; Bausch Health <sup>8a</sup> ( <i>Cedars sinai has a licensing agreement with Bausch</i> ); Gemelli Biotech <sup>2b</sup> ; Naia Pharmaceuticals <sup>2a,6a,8a</sup> ( <i>Cedars sinai has a licensing agreement with Naia</i> ); Shire <sup>6a</sup> ; Synthetic Biologics <sup>2a,6a,8a</sup> ( <i>Cedars Sinai has a licensing agreement with Synthetic Biologics</i> )	Modest, A. M. Modrzejewski, K. Mohebbi, L. Mol, B. Montag, M. H.	<i>the use of aromatase inhibitors for infertility treatment and other reproductive disorders</i> Renovia Inc. <sup>6</sup> ; Tissue Regenix <sup>6</sup> EMD Serono <sup>3</sup> Celmatix <sup>3</sup> Guerbet <sup>4,6</sup> ; Merck <sup>6</sup> ; ObsEva <sup>6</sup> Ferring AG <sup>5</sup> ; Merck GmbH <sup>5</sup> ; Vitrolife AB <sup>6</sup> Androvia LifeSciences <sup>3</sup> Yale University <sup>3,8</sup> ( <i>Patent rights owner</i> ) Arthrex <sup>6a</sup> ; Probility Physical Therapy <sup>6a</sup> ; Team Rehab Physical Therapy <sup>6a</sup> Igenomix <sup>8</sup> ( <i>Part-time employee</i> ) Animated Dynamics, Inc. <sup>1,2,3</sup> Assist university <sup>8</sup> ( <i>Coauthor</i> ) EMD Serono <sup>5</sup> AbbVie <sup>4</sup> CooperGenomics <sup>6</sup> Life Whisperer <sup>2,3</sup> PerkinElmer <sup>3</sup> Previvo Genetics <sup>3,4</sup> Myriad Genetics <sup>3</sup> Varinos Inc <sup>1</sup> Cooper-Surgical / Origio <sup>6</sup> ; Prelude <sup>2</sup> Elsevier <sup>8</sup> ( <i>Paid Web Content Editor</i> ) Punta Mita Hospital <sup>2,8</sup> ( <i>Principle Investigator</i> ) Previvo Genetics, Inc. <sup>6</sup> National Institute of Health <sup>4</sup> Future Fertility <sup>6</sup> Novo Nordisk <sup>4</sup> Invitae <sup>2,3</sup> Agile Therapeutics <sup>4</sup> ; AMGA Pharma <sup>6</sup> ; Avion <sup>5,6,7</sup> ; Bayer <sup>5,6,7</sup> ; ContraMed/Sebela <sup>4,6</sup> ; CooperSurgical <sup>5,6,7</sup> ; Evofem <sup>4</sup> ; FHI MonaLisa <sup>4</sup> ; Merck <sup>4,5,6,7</sup> ; Pharmanest <sup>6</sup> Ferring <sup>4,6,7</sup> ; Merck <sup>7</sup> ; Roche <sup>4,7</sup> Ferring Pharmaceuticals <sup>3</sup> Invo Bioscience <sup>2,6</sup> Medicines360 <sup>3</sup> Medical Electronic Systems <sup>6</sup> ; Nestle <sup>6</sup> New Hope Fertility Center <sup>3</sup> Androvia LifeSciences <sup>2,3</sup> AbbVie Inc. <sup>3</sup> IVI MADRID <sup>3a</sup> BioIncept, LLC <sup>8</sup> ( <i>research funding, scientific advisory board member, stock options</i> ); CSL Behring <sup>4</sup> ; GestVision <sup>4</sup> ; NovoNordisk <sup>8</sup> ( <i>consultant, webinar</i> ); Progenity <sup>4</sup> ; rEVO Biologics <sup>4</sup> Abbott <sup>8</sup> ( <i>Advisory Board Member</i> ); AMAG <sup>7</sup> ; FLOHEALTH <sup>6</sup> ; GLG <sup>6</sup> ; Natera <sup>8</sup> ( <i>Advisory Board Member</i> ) NOVAGEN <sup>8</sup> ( <i>Medical Director</i> ) Merck Serono and Ferring <sup>4</sup> Celmatix <sup>3</sup> Cooper srugical, Irvine Fujifilm <sup>4</sup> ; NTERILIZER <sup>2</sup> Parryscope Fertility <sup>8b</sup> ( <i>Intellectual property rights relating to a surgical technique and device</i> ) Bayer AG <sup>7</sup> ; Endo Pharmaceuticals <sup>4,6,7</sup> ; Woven Health <sup>1,2</sup>
Matorras, R.	Angelini <sup>6</sup> ; Ferring <sup>6</sup> ; Merck Serono <sup>6</sup>	Moody, M. A. Mor, A.	
Matsuo, K.	Chugai <sup>5</sup> ; Springer <sup>8</sup> ( <i>text book contribution compensation</i> ); VBL therapeutics <sup>8</sup> ( <i>Expense for meeting attendance</i> )	Moravek, M. B.	
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McDaniel, L. D.	Progenity, Inc. <sup>3</sup>	Nakajima, S. T.	
McKenna, G. J.	Novartis <sup>6</sup>	Natarajan, L.	
McQueen, D. B.	Innovative Drive Corporation <sup>6a</sup>	Nayot, D.	
Mellinger, U.	Bayer AG <sup>3</sup>	Neff, L. M.	
Merhi, Z.	HOCATT LLC <sup>6</sup>	Neitzel, D.	
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Merrion, K.	Natera, Inc. <sup>2,3</sup>		
Meseguer, M.	Ferring <sup>8</sup> ( <i>Speaker fee</i> ); MERCK <sup>8</sup> ( <i>Speaker fee</i> ); MSD <sup>8</sup> ( <i>speaker fee</i> ) clinique ovo <sup>3</sup>		
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Mills, S. A.	WiserCare, Inc. <sup>8</sup> ( <i>Freelance illustration for online patient decision aid modules</i> )	Parente Barbosa, C.	
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Mitwally, M. F.	Mohamed F. Mitwally <sup>8</sup> ( <i>Own patent on the use of aromatase inhibitors for the treatment of ectopic pregnancy, and other patents for</i>	Parry, P.	
		Pastuszak, A. W.	

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Prusinski Fernung, L.	DePuy Synthes <sup>8a</sup> ( <i>Contractor</i> )	Sharara, F. I.	Promescent <sup>2</sup>
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Richter, E.	EMDSerono <sup>6</sup>		
Richter, K. S.	Abbvie <sup>7</sup>		
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	Bayer <sup>5</sup> ; Cooper Surgical <sup>5</sup> ; Merck <sup>5</sup>		
	Tempus Labs <sup>6</sup>		
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Rubio, C.	UCSF <sup>3</sup>		
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	Igenomix <sup>8</sup> ( <i>Par-time Company Employee</i> )		

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