

**Proposed authors:**

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**Abstract title (25 words)**

Low serum Progesterone the day of embryo transfer is associated with diminished ongoing pregnancy rate in artificial endometrial preparation cycles. A prospective study.

**Study question (25 words)**

Is there a relationship between serum Progesterone (P) and endometrial volume the day of transfer with the ongoing pregnancy rate in artificial endometrium preparation cycles?

**Summary answer (25 words)**

Patients with serum P <9.2 ng/ml the day of embryo transfer had significantly lower ongoing pregnancy rate than the rest of patients.

**What is known already (100 words)**

A window of optimal serum P levels during the embryo implantation period (e.g. after 7 days of P administration) has been described in artificial endometrium preparation cycles. This interval of P concentration has been defined to be between 70 and 99 nmol/L (22.0-31.1 ng/mL), while serum P levels < 50 nmol/L (15.7 ng/mL) and > 100 nmol/L (31.4ng/mL) are related to significantly lower pregnancy rates. Also, it has been shown that an endometrial volume <2.5 ml is related to a poorer outcome, and that a volume < 1 ml is associated with a null pregnancy rate.

**Study design, size, duration (75 words)**

Prospective cohort study including 244 patients undergoing embryo transfer after an artificial endometrial preparation cycle with estradiol valerianate and vaginal micronized progesterone (400 mg/12 hours). The study was performed between February 22<sup>nd</sup>, 2016 and October 25<sup>th</sup>, 2016 (8 months). Sample size was calculated to detect a 20% difference (35-55%) between 2 groups according to serum P levels, in a two sided test with a statistical power of 80% and a confidence level of 95%.

**Participants/materials, setting, methods (75 words)**

Patients undergoing their 1<sup>st</sup>/2<sup>nd</sup> oocyte donation cycle, aged <50, BMI <30 Kg/m<sup>2</sup>, with a normal uterine cavity in 3D ultrasound, a triple layer endometrium >6.5 mm, and being transferred 1-2 good quality blastocysts; in a private infertility centre. Serum P determination and 3D ultrasound of the uterine cavity were performed the day of embryo transfer. Endometrial volume measurements were done using VOCAL system. Primary endpoint was ongoing pregnancy rate beyond the 12<sup>th</sup> week of pregnancy.

**Main results and the role of chance (200 words)**

Of 244 patients recruited, 211 fulfilled all the inclusion/exclusion criteria. In 27 patients a Müllerian abnormality was diagnosed with the 3D ultrasound (19 T-shaped, 6 septate partial/complete, 1 hemiuterus and 1 bicorporeal uterus). In 6 cases there was a protocol violation. The mean age of the included women was 41.3±4.4; BMI: 22.3±2.6; Endometrial thickness: 8.9±1.7 mm. Serum P the day of embryo transfer was 12.7±5.4 ng/mL. (p25: 9.2; p50:11.8; p75:15.8). The ongoing pregnancy rates according to serum P levels were: <p25: 32.7%; p25-p50: 49.1%; p50-p75: 58.5%; >p75: 50.9%. Women with serum P <p25

(<9.2 ng/mL) had a significantly lower ongoing pregnancy rate compared to the rest of patients: 32.7% vs. 52.8%;  $p=0.016$ ; RR (95% CI): 0.62 (0.41-0.94). Endometrial volume was  $3.2\pm 1.3$  ml. Serum P the day of embryo transfer did not correlate with the endometrial volume. Only women with a very low endometrial volume ( $n=10$ ), ( $p=1.4$  ml) were associated with a poor ongoing pregnancy rate (22.2% vs. 48.3%).

A logistic regression analysis adjusting for all potential confounders showed a statistically significant relationship between serum P the day of embryo transfer and the likelihood of ongoing pregnancy (OR: 1.10; 95%CI: 1.02-1.19),  $p=0.01$ .

**Limitations, reasons for caution (50 words)**

Only women with a normal uterine cavity, an appropriate endometrial thickness and good quality blastocysts transfer were included. Extrapolation to an unselected population needs to be validated. The role of endometrial volume could not be fully defined, as only a few patients presented a very low volume.

**Wider implications of the findings (50 words)**

The present study suggests that there is a minimal threshold of serum P values the day of embryo transfer that needs to be reached in artificial endometrial preparation cycles to optimize the outcome. An upper threshold could not be defined.

**Study funding/competing interest(s):**

None

**Trial registration number**

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