

O-116 Tuesday, October 9, 2018 11:00 AM

ULTRA-LOW OXYGEN (O₂) TENSION AFTER DAY 3 OF IN VITRO DEVELOPMENT DOES NOT ALTER BLASTOCYST TRANSCRIPTOME : A COMPARISON OF 2% VERSUS 5% O₂ TENSION IN EXTENDED CULTURE.

S. Morin,^{a,b} T. Wang,^{c,d} X. Tao,^d M. Earnhardt,^e E. Seli,^{c,a} R. Scott.^{a,b} aIVI RMANewJersey, Basking Ridge,NJ; bThomas JeffersonUniversity, BaskingRidge,NJ; cYale School of Medicine,NewHaven, CT; dFoundation for Embryonic Competence, Basking Ridge, NJ; eRutgers RobertWood Johnson Medical School, Morristown, NJ.

OBJECTIVE: Preliminary data suggest that reducing oxygen (O₂) tension in embryo culture from 5% to 2% after day (d) 3 produces a greater number of blastocysts (blasts) compared to culture at 5% throughout. These findings fit with observations that 1) the embryo crosses the uterotubal junction on d3 in vivo, 2) the O₂ tension in the uterus is lower than the oviduct, and 3) the embryo's metabolic strategy shifts from oxidative phosphorylation to aerobic glycolysis around d3. However, modifications in the culture environment may induce untoward changes in the transcriptome that alter reproductive competence. This study sought to compare gene expression between human embryos cultured continuously at 5% versus embryos cultured at 5% from d1 - 3, then 2% through d6.

DESIGN: Experimental study.

MATERIALS AND METHODS: Patients were recruited as part of a randomized controlled trial comparing live birth rates between a sequential O₂ tension culture system versus 5% O₂ throughout. Aneuploidy screening (PGT-A) was performed on all blasts in the study. All embryos were vitrified while awaiting PGT-A results. Patients with aneuploid blasts were recruited for this subanalysis. After obtaining consent, blasts were warmed and additional biopsies were taken from the trophectoderm (TE) and inner cell mass (ICM). cDNA was amplified using the Smart-seq v4 Ultra Low Input RNA Kit (Clontech). RNA sequencing libraries were constructed using Nextera XT library preparation kit (Illumina) and were sequenced by Yale Center for Genome Analysis on Illumina's HiSeq 2500 with paired-end 75 bp reads. Gene expression values were calculated as FPKM using Cufflinks 2.1.1. Genes were deemed differentially expressed between different conditions if they showed a FDR (adjusted p-value) of <0.05.

RESULTS: TE and ICM biopsies were obtained from 6 blasts cultured in 2% O₂ and 5 blasts cultured at 5% O₂. RNAseq analysis revealed significant differences in gene expression between the TE and ICM, with a total of 480 genes differentially expressed, including epigenetic regulatory histone modification genes (HIST1H1T, HIST1H4B, HIST3H3, HIST4H4). However, RNAseq analysis revealed no difference in gene expression between embryos cultured at 2% versus 5% (p>0.05) in either the TE or ICM.

CONCLUSIONS: Reducing O₂ tension to 2% after d3 does not significantly alter the transcriptome in the ICM or TE. The increased efficiency in blast conversion previously

observed appears related to different mechanisms mediating the biosynthetic activity and metabolic health of preimplantation embryos, potentially through translational and post-translational regulatory pathways. These findings also suggest that a culture system that decreases O₂ levels after d3 does not result in significant perturbations in the expression of genes involved in mitochondrial function over standard 5% O₂ systems. References: 1. Kaser et al. Randomized controlled trial of low (5%) vs. ultralow (2%) oxygen tension for in vitro development of human embryos. *Fertil Steril* 2016;106(3):Suppl e4. Supported by: Foundation for Embryonic Competence.