Epigenetic and Physiological Outcomes in a Mouse Model of Assisted Reproductive Technologies

Alexandra J. Savage[1], Cassidy N. Hemphill[2], Eric A. Rhon-Calderon[2], Marisa S. Bartolomei[2]

[1]School of Arts and Sciences, University of Pennsylvania (2026), Philadelphia, PA; [2]Epigenetics Institute, Department of Cell and Developmental Biology, Perelman School of Medicine, Philadelphia, PA

Email: savageal@sas.upenn.edu

Introduction

Assisted reproductive technologies (ART) are noncoital methods of conception involving donor or non-donor eggs and sperm, usually used to treat infertility. ART, including in vitro fertilization (IVF), have resulted in over 9 million births worldwide since 1978 and it is expected that the use of ART will continue to increase. However, ART are associated with a higher risk for adverse outcomes, including low fetal weight, hypertensive disorders such as preeclampsia, preterm birth, and imprinting disorders. Genomic imprinting is an epigenetic modification in which one of the parental alleles is controlled by methylation, which results in disorders that impact growth, development, and metabolism. This project consists of two aims: to examine the differences between Natural, IVF, and TEBx placentas and to determine the effect of 2% versus 5% oxygen environments on in vitro generated blastocysts, both using mouse models.

Methods

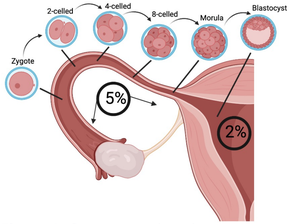

Figure 1. Oxygen tensions in the uterus.

It is suggested that oxygen tension in the uterus is lower, approximately 2%, than in the oviduct which is around 5% (Figure 1) and hypoxia might be more effective for blast development. Additionally, it is already known that IVF shows differences from natural placentas and embryos, so the effect of TEBx will be examined, as it is an invasive procedure.

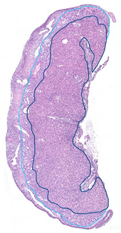

Figure 2. The **labyrinth zone** contains the maternal/fetal vasculature needed for nutrient and gas exchange. The **junctional zone** is responsible for endocrine function.

Hematoxylin and eosin (H&E) staining: Shown in Figure 2. This stain allows for visualization of placental layers and varying tissue types. Placentas were cut, stained, and imaged, using Fiji analysis to measure the areas of the junctional and labyrinth zones.

CD34 staining is an immunohistochemical stain that serves as a marker for fetal blood vessel endothelial cells. Placentas were cut, stained, and sent to the CHOP pathology core for analysis.

DNA methylation is an epigenetic process that controls gene expression. It occurs at critical points in embryonic development. Two methylation assays were used to measure DNA methylation.

LUMA (Luminometric Methylation Assay): Used to measure genome-wide DNA methylation. The assay involves restriction cut sites on a small amount of DNA isolated from placental or fetal tissue and polymerase extension of the overhangs by pyrosequencing.

Bisulfite pyrosequencing: Used to measure DNA methylation of specific sequences of genes such as imprinting control regions (ICRs), relying on the fact that DNA methylation commonly occurs at CpG sites. DNA was isolated from placental or fetal tissue, bisulfite converted, and PCR were performed using primer sequences for the selected imprinted genes.

Results

Natural vs. IVF vs. TEBx Placentas

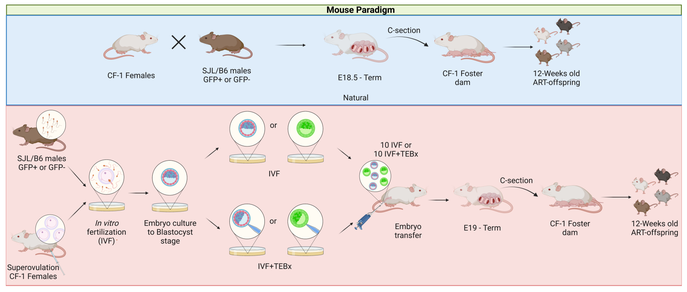

Figure 3. Mouse model for Natural, IVF, and IVF + TEBx. **TEBx**: Trophectoderm (TE) biopsy. Several TE cells are removed from a blastocyst for genetic testing of the E3.5 pre-implantation embryo.

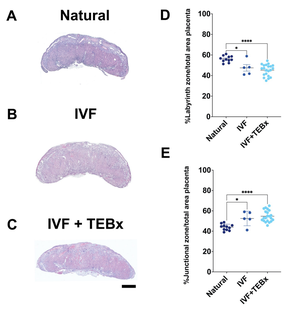

Figure 4. IVF + TEBx placentas from the 12 weeks cohort show no difference compared to IVF with respect to junctional and labyrinth zone areas.

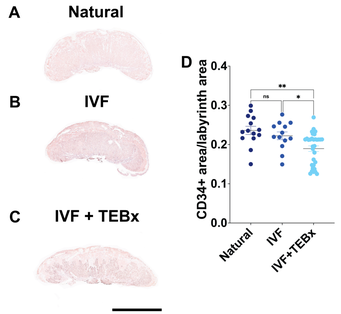

Figure 5. IVF + TEBx placentas from the 12 weeks cohort show a difference compared to IVF with respect to labyrinth area.

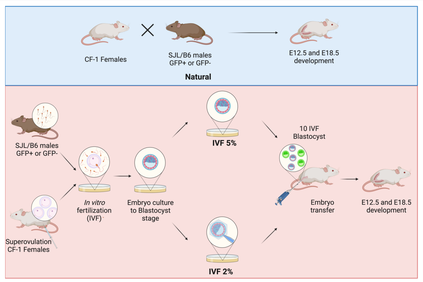

Figure 6. Mouse model for culturing blastocysts in 5% oxygen or 2% oxygen.

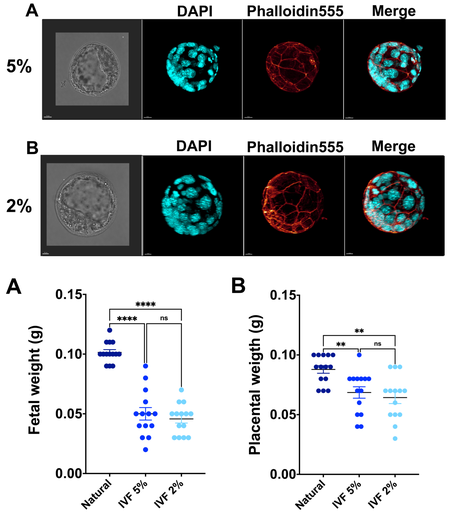

Figure 7. (A) Imaged blastocysts from 5% oxygen culture. (B) Imaged blastocysts from 2% oxygen culture. (C) Blastocysts cultured in 2% oxygen have increased cell numbers compared to 5%.

Figure 8. At E12.5, IVF 2% did not show differences with IVF 5% in terms of fetal weight, placental weight, and the fetal to placental weight ratio.

5% vs. 2% O_2 Environment

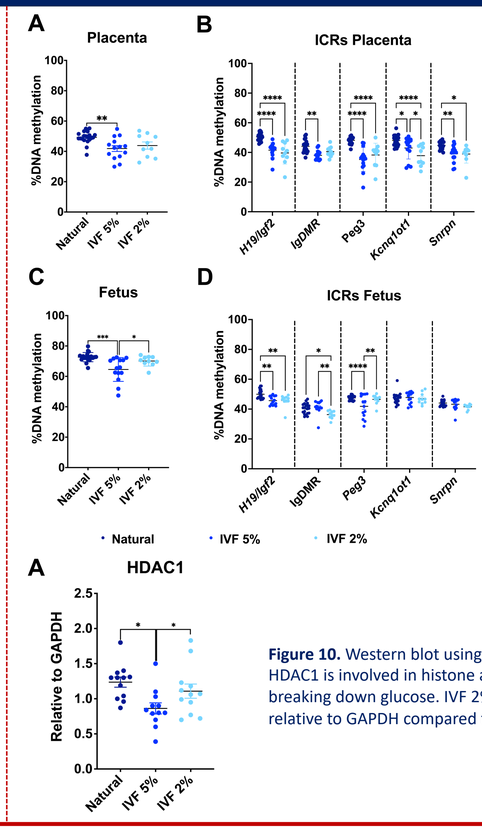

Figure 9. Global DNA methylation compared for natural, IVF 5%, and IVF 2% in both the placenta (A) and fetus (C). Imprinting control region methylation was compared in five known imprinted genes: H19, IgDMR, Peg3, Lit1, and Snrpn in both the placenta (B) and fetus (D). At E12.5, IVF 2% did not prevent the loss of DNA methylation globally or at ICRs in the placenta. IVF 2% partially prevented the loss of DNA methylation at ICRs in fetal tissue.

A, C. Global DNAm by LUMA
B, D. ICR DNAm by pyrosequencing

Figure 10. Western blot using HDAC1 and GAPDH as control proteins. HDAC1 is involved in histone acetylation and GAPDH is involved in breaking down glucose. IVF 2% showed an increased level of HDAC1 relative to GAPDH compared to IVF 5%.

Conclusions

IVF + TEBx showed difference from IVF in terms of placental histology from the analysis from CD34 staining, but no difference by analysis from H&E staining. There is some evidence that embryo culture in 2% oxygen is more advantageous than 5% oxygen, including increased blastocyst cell count.

Future Directions

To further this research, we aim to determine the effect of reducing the oxygen tension during IVF embryo culture from 5% to 2% to align with the stages of development. We will also perform histological and molecular analyses on the junctional and labyrinth zones of the placenta separately.

Acknowledgments

I would like to thank the **Bartolomei Lab** for their exceptional support, the CDB Microscopy Core and CHOP Pathology Core for their imaging and analysis resources, **CURF,** and the **Penn Undergraduate Research Mentoring Program (PURM)** for funding my research.