School of Medicine Greenville

Comparison of USP34 expression in preimplantation blastocoel fluid from IVF-generated embryos

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Introduction

According to the CDC, 12.7% women have received infertility services, including in vitro fertilization (IVF). Beyond the psychological and emotional cost of IVF, the median out-of-pocket expenses for one cycle are $16,069 with many patients needing additional cycles. Currently, preimplantation genetic testing (PGT) is used to identify euploid embryos based on sequencing of genomic DNA obtained from 5-7 trophectoderm cells from the day 5 IVF-embryo. Additionally, embryo morphology is graded looking at expansion, the quality of the inner cell mass, and the quality of the trophectoderm. PGT and embryo grading allow REI physicians to help patients select the IVF embryo for uterine transfer, but this does not yield 100% success rates. Other embryo quality metrics are needed for improvement.

Aim

Our project seeks to identify molecular markers associated with implantation outcomes found in blastocoel fluid obtained from IVF-embryos.

Methods

Blastocoel fluid-conditioned media was collected from day 5-6 human embryos undergoing PGT-A. cDNA synthesis was performed on individual fluid samples. Real-Time qPCR was performed to obtain mRNA levels for genes of interest, relative to GAPDH, the housekeeping gene. The genes of interest (USP28, USP22, KAT2A, UBR5, USP34, and MYSM1) were identified prior using RNA-Seq analysis of blastocoel-fluid conditioned media from embryos with known implantation status. Preliminary results then led to analysis of USP34 in 15 media samples from euploid embryos from mothers under age 35. The gene expression of USP-34 was analyzed regarding implantation outcomes of the embryo via Welch's t-test.

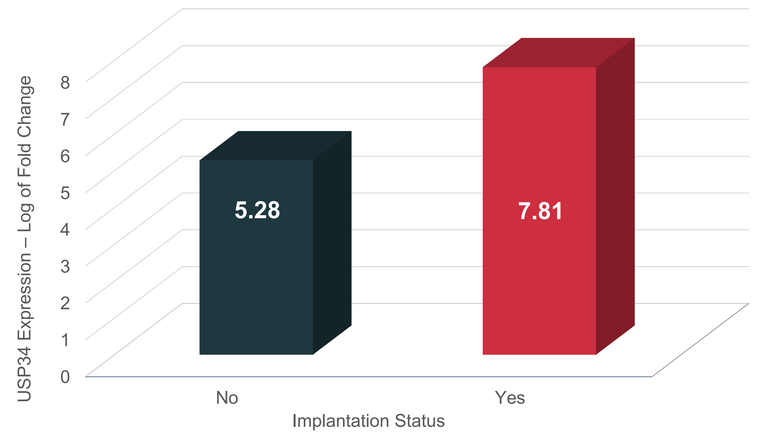
Results

Table 1: Embryo Grade, Implantation, and USP34 Expression

Sample ID	Pt ID Number	Age	Embryo Grade	Implant (Yes/No)	USP34 Expression
1	1	30	6AA	NO	4.91265489
2	1	30	5BC	NO	6.72862861
3	1	30	4BB	NO	4.20767325
4	2	28	3BC	YES	5.99645209
5	2	28	4AB	YES	14.2209757
6	2	28	4BB	YES	10.3287553
7	3	32	4AA	YES	3.4657359
8	4	34	3BB	YES	3.52636052
9	5	31	4AA	YES	3.4339872
10	6	32	5BA	YES	4.53152365
11	6	32	4AA	YES	26.494707
12	7	31	4AB	YES	5.65248918
13	8	34	3BC	YES	5.38449506
14	1	30	5AA	YES	3.34990409
15	9	32	4AB	YES	7.38398946

Expression of the USP34 gene was assessed in 15 blastocoel fluid-conditioned media samples from euploid embryos. Table 1 details the patient age at time of egg retrieval, embryo grade, implantation status, and USP34 expression level for these media samples from women under age 35. Red represents embryos with negative implantation status. Green represents embryos with positive implantation status. The average USP34 expression was higher in implantation positive samples compared to implantation negative samples (Figure 1). However, statistical analysis comparing USP34 expression in embryos associated with negative outcomes versus those with positive implantation outcomes revealed no statistically significant difference between the two groups ($p=0.123$).

Figure 1: Average USP34 Expression



Discussion

USP34, or ubiquitin specific peptidase 34, is located on chromosome 2p15 and encodes a deubiquitinating enzyme. This group of enzymes removes the ubiquitin moiety from ubiquitin-modified proteins resulting in functional changes. USP34 positively regulates the Wnt signaling pathway, which plays roles in gender differentiation and folliculogenesis, as well as other processes involved in reproduction. USP34 has known somatic variations in ovarian tumors and polycystic ovary syndrome.

We assessed USP34 prevalence in 15 samples from mothers under age 35. Although we found no statistically significant difference between the implantation outcome groups, UPS34 or other DUBs may play role in early embryo development that is related to overall embryo viability.

Future Directions

Since we utilized a limited sample size, we plan to continue to analyze USP34 expression in more fluid samples. Our initial findings also suggest a need to assess the expression of more genes in blastocoel fluid when looking for indicators of potential implantation success.

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