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SEPT12 EXPRESSION IN HUMAN TROPHECTODERM CELLS: INSIGHT INTO EMBRYONIC ARREST

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OBJECTIVE:

SEPT12, a member of the Septin gene family, has expression restricted to the testis and is critical for normal spermatogenesis. Septin genes code for polymerizing guanosine triphosphate (GTP) binding proteins whose homologs have been conserved throughout evolution from yeast to humans (1). Roles assigned to Septin genes include cell division, cytoskeletal organization, and membrane-remodeling events including the epithelial-mesenchymal transition in metastatic cancers (2). A study that investigated azoospermia and teratozoospermia in a mouse knockout model demonstrated oocytes fertilized via ICSI with sperm targeted *SEPT12* antisense alleles resulted in embryo arrest by the morula stage (3). The application of RNA sequencing to elucidate the expression profile of *SEPT12* in human preimplantation embryos may unlock insights into the transcriptional events of early embryogenesis.

DESIGN:

Prospective cohort study on human, donated embryos

MATERIALS AND METHODS:

The study included patients who donated fresh embryos at the blastocyst stage during an IVF cycle between January, 2016 and June, 2016. Embryos were biopsied, and approximately 2-4 cells were removed for preimplantation genetic testing for aneuploidy (PGT-A) by next generation sequencing (NGS) using the ReproSeq assay to assess copy number variants (CNVs). The remaining cells of the embryo were designated for RNA Sequencing. Read counts per gene

were summed across embryo cohorts and normalized using the median of ratios. Differential gene expression between embryo cohorts was calculated using DESeq2, in order to estimate variance-mean dependence and evaluate differential gene expression using a negative binomial distribution. A likelihood ratio test was used to account for heterogeneity due to patient, batch, and ploidy and growth status (arrested/ongoing). The adjusted threshold for significance was $p<0.05$.

RESULTS:

43 blastocysts underwent PGT-A assessment and RNA sequencing. 36 showed expression of *SEPT12*, 6 of the 7 blastocysts that failed to show *SEPT12* expression had poor trophectoderm morphology grade. The expression of *SEPT12* was further examined in 15 embryos, 9 were enriched (>90%) for trophectoderm cells (TE) and 6 enriched (>80%) for inner cell mass cells (ICM). *SEPT12* expression was significantly higher in TE cells than ICM cells, where $P<0.0001$.

CONCLUSIONS:

Septins were first discovered nearly fifty years ago however their function remains poorly understood (4). The importance of this gene family has been indicated by the conservation of the functional domains throughout evolution. *SEPT12* has been shown to be critically important in spermatogenesis. This study is the first to characterize *SEPT12* expression in the human embryo. Our data supports the findings that wild type *SEPT12* expression was preferentially associated with blastocyst formation compared to arrest at the morula stage for embryos that contained the antisense *SEPT12* allele (3). Our current studies are focused on characterizing *SEPT12* expression in cleavage and morula stage human embryos to further elucidate this gene's role in early embryogenesis.

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