American Society for Reproductive Medicine 2020 Virtual Congress

October 17-21, 2020

X INACTIVE SPECIFIC TRANSCRIPT (XIST) AND ANTISENSE TSIX EXPRESSION IN SIBLING EUPLOID HUMAN BLASTOCYSTS: INSIGHTS INTO HUMAN X CHROMOSOME INACTIVATION

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OBJECTIVE:

Within a mouse model, X chromosome inactivation has been shown to be tightly regulated in early embryo development (4 cell stage), and remains so throughout adulthood (1). The RNA of two non-coding genes (*Xist* and *Tsix*) coats the X chromosomes within the cell nucleus (2). Mouse *Xist* is expressed by the inactive paternal X, yet, future activity of the X chromosome is protected from *Xist* inactivation by antisense RNA *Tsix* (2). While the human homologs of *Xist* and *Tsix* have been described, their roles in human X chromosome inactivation remains misunderstood. Only studies utilizing human embryonic stem cells and / or placental tissue have attempted to elucidate the roles of *XIST* and *TSIX* in the human embryo. The objective of this study was to compare *XIST* and *TSIX* gene expression in sibling euploid male and female human blastocysts.

DESIGN:

Prospective cohort study on human embryos for research

MATERIALS AND METHODS:

After receiving IRB consent, eleven sibling embryos were donated for research by an infertile couple. These embryos had previously undergone embryo biopsy, with approximately 2-4 cells removed for preimplantation genetic testing for aneuploidy (PGT-A) by next generation sequencing (NGS) prior to vitrification. The embryos were derived from an anonymous oocyte

donor and the sperm from the couple's male partner. After standard warming procedures, the blastocysts underwent 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 batch. The adjusted threshold for significance was $p<0.05$.

RESULTS:

11 euploid blastocysts, 5 male and 6 female were analyzed. The two embryo cohorts compared were male versus female. Both *XIST* and *TSIX* were expressed in all blastocysts. Male blastocysts showed significantly lower expression of *XIST* than females, -3.36 fold change where $p=0.0003$. *TSIX* was expressed at significantly lower levels in males compared to female blastocysts, -3.30 fold change where $p=0.0005$.

CONCLUSIONS:

The inherent variability in human embryos makes studying the molecular processes of X inactivation difficult. The strongest feature of this study is the known ploidy status of the sibling embryos derived from a young, healthy oocyte donor. Unlike studies using a mouse model, ours demonstrated expression of both the *XIST* and *TSIX* genes within the human male blastocysts. Our finding suggests the *XIST* and *TSIX* genes are co-expressed allowing expression of both in male blastocysts. Again, we diverge from studies utilizing a mouse model, wherein the *Xist* gene is expressed only from the inactive X and the *Tsix* gene from the active X chromosome only seen in mouse female embryos. Our study shows that *XIST* and *TSIX* are active in both male and female human blastocysts. The expression of both genes in male embryos indicates the roles that these genes play may be different in the human than the mouse.

REFERENCES:

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