O-187 Wednesday, October 10, 2018 10:45 AM

DNA METHYLATION IS ASSOCIATED WITH PLOIDY STATUS AND PATIENT AGE IN HUMAN EMBRYOS.

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OBJECTIVE: During pre-implantation embryo development, DNA methylation (DNAme) is a fundamental epigenetic regulatory mechanism that guides differentiation of cells into their future lineages. As its accuracy and efficiency is essential for embryo viability, DNAme has the potential to be a maker for embryonic reproductive competence. This study aims to establish whole genome-wide DNA methylome analysis for embryo trophoectoderm (TE) biopsies.

DESIGN: Experimental study.

MATERIALS AND METHODS: Two TE biopsies from each of the previously diagnosed euploid (n¼10) and aneuploid (n¼20) embryos were analyzed using Whole Genome Bisulfite Sequencing (WGBS). Bisulfite conversion was performed using EZ DNA Methylation-Direct Kit (Zymo). Methylome sequencing libraries were constructed using TruSeq DNA Methylation Library Prep (Illumina) with 18 cycles of amplification. Sequencing was performed on Illumina HiSeq 2500 with paired-end 150 bp reads, and sequencing reads were aligned to human genome reference using Bismark software. Duplicates were removed, unconverted reads were filtered, and genome-wide cytosine methylation at single base resolution was determined. Statistical analysis was carried out using a linear model to assess the relationship of DNAme levels with ploidy status, maternal age (range 29.5 to 41.1), and time of blastulation (day 5 [n¼16] vs. day 6 [n¼14]).

RESULTS: The average CpG coverage achieved byWGBS in TE samples was 30% of the sites predicted in the genome. Analysis revealed that 20%- 30% CpG sites in TE samples were methylated, consistent with levels reported in studies using animal models. The two TE samples from the same embryo showed significantly higher similarity of overall methylation rate compared to the unrelated embryos ($p<0.0001$), which demonstrated the reproducibility and feasibility of assessing DNAme from blastocyst TE biopsies. Aneuploid embryos showed significantly higher DNAme levels compared to euploid embryos ($p<0.0001$), and increased patient age was correlated with elevated DNAme levels in blastocysts ($p<0.0001$). Whole chromosomal aneuploidy was predicted by calculating the fraction of read count from each chromosome, and all the karyotypes showed 100% consistent with previous aneuploidy screening. The chromosomes involved in monosomy embryos (-4,-13,-16, -17, -21, and -22) showed reduced methylation rates compared to the other chromosomes.

CONCLUSIONS: DNA methylation levels detected in trophoectoderm biopsies from human blastocysts correlate with ploidy status and maternal age, and may provide a foundation for the development of epigenetic biomarkers of reproductive competence.