

F-214 - DNA Methylation is Associated with Ploidy Status, Delayed Blastulation, and Patient Age in Human Embryos

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Abstract

Introduction: DNA methylation (DNAm) is a fundamental epigenetic control mechanism that occurs by the addition of methyl (CH₃) groups to cytosine residues and guides differentiation during embryonic development. We hypothesized that DNAm may be associated with viability of human embryos and compared genome-wide DNAm profile in human blastocysts with different ploidy status, developmental stage, and maternal age.

Methods: Two trophoectoderm (TE) biopsies from each of the previously diagnosed euploid (n=10) and aneuploid (n=10) embryos were analyzed using Whole Genome Bisulfite Sequencing (WGBS). Bisulfite conversion was performed using EZ DNA Methylation-Direct Kit (Zymo). Unmethylated *E. coli* and methylated pUC19 DNA were added as negative and positive controls, respectively, to monitor bisulfite conversion efficiency. 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 DNAm levels with ploidy status, maternal age (range 29.5 to 41.1), and time of blastulation (day 5 [n=8] vs. day 6 [n=12]).

Results: The average CpG coverage achieved by WGBS 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 unmethylated *E. coli* and methylated pUC19 DNA controls showed 1% and >98% methylation rate, respectively. 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 DNAm from blastocyst TE biopsies. Aneuploid embryos showed significantly higher DNAm levels compared to euploid embryos (p<0.005), and increased patient age was correlated with elevated DNAm levels in blastocysts (p<0.0001). In addition, blastocysts cryopreserved on day 6 had significantly higher DNAm compared to those that were cryopreserved on day 5 (p<0.0001).

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