77th ASRM Scientific Congress & Expo

October 16-20, 2021 // Baltimore, MD, USA

SINGLE CELL DNA SEQUENCING OF WHOLE HUMAN BLASTOCYSTS: NOVEL INSIGHTS INTO THE PHENOMENON OF EMBRYONIC MOSAICISM

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OBJECTIVE: Conventional wisdom suggests that viable human embryos are the result of faithful cloning of euploid zygotes. However, study of blastocysts grown in vitro often identifies mosaicism that is likely the result of post-fertilization mitotic errors[1]. Yet, the true incidence of embryonic mosaicism remains poorly understood. Although prior work has studied cleavage stage embryos or blastocyst biopsies, to our knowledge, whole embryonic analysis via single cell sequencing of every cell from a human blastocyst has yet to be performed. Our objective was to isolate and sequence every cell from a dissociated human blastocyst in order to define the precise genetic composition of human embryos.

MATERIALS AND METHODS: We analyzed 9 vitrified human blastocysts donated for research. All blastocysts previously underwent trophectoderm biopsy and preimplantation genetic testing for aneuploidy (PGT-A). Blastocysts were thawed and dissociated into single cells using Accutase® solution and individually placed in 96 well plates. DNA amplification and library preparation was performed using the Ion SingleSeq™ 96 Kit from the Ion ReproSeq™ PGS Kit with Ion 530™ Chips. Libraries were sequenced via next generation sequencing (NGS) using an Ion S5 XL sequencer, and aneuploidy analysis of each cell was performed using the Reproseq PGS w1.1 workflow on Ion Reporter using standard parameters.

RESULTS: Nine blastocysts were dissociated and sequenced: 5 PGT-deemed euploid, 4 PGT-deemed aneuploid. Sequencing was attempted on 433 cells, of which 320 cells were successfully sequenced (216 from euploid embryos, 104 from aneuploid embryos). Among euploid embryos, 73.2% of cells were successfully sequenced (26.8% dropout) on average; among aneuploid embryos, 79.2% of cells were successfully sequenced (20.8% dropout). The majority (4/5) of PGT-deemed euploid embryos were predominantly euploid, consisting of an average of 73.4% euploid cells. One euploid embryo was composed of 49.1% euploid cells. In addition, the majority (3/4) of aneuploid embryos were found to be 100% aneuploid on single cell analysis. One PGT-deemed aneuploid embryo consisted of a majority (75.9%) of euploid cells. In euploid embryos, an average of 31.4% of embryonic chromosomal mosaicism was detected.

CONCLUSIONS: This is the first study in which single cell DNA sequencing was performed of the whole human blastocyst. Our results showed striking differences between euploid and aneuploid embryos. Although the majority of cells in euploid embryos were identified as euploid, an average of 31.4% of embryonic chromosomal mosaicism was identified in all euploid embryos. In contrast, in the majority of aneuploid embryos, aneuploidy appears to be present from the moment of the fertilization and the union of genetic material from sperm and egg, likely representing meiotic rather than post-fertilization mitotic errors.

IMPACT STATEMENT: Single cell sequencing of the whole human blastocyst expands our current understanding of the genetic composition of human embryos. Some degree of embryonic mosaicism may be a part of normal human embryonic blastocyst development.

REFERENCES:

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