



**AMERICAN SOCIETY FOR
REPRODUCTIVE MEDICINE**



American Society for Reproductive Medicine 2018 Scientific Congress & Expo
October 6 to 10, 2018 • Denver, Colorado, USA

Title:

SINGLE-CELL MITOCHONDRIAL STAINING AND RIBONUCLEIC ACID (RNA) SEQUENCING OF HUMAN BLASTOCYSTS

Authors:

T.G. Nazem^{1,2}; K.G. Beaumont³; C. Briton-Jones²; Joseph A. Lee²; B. Sebra^{3,4}; A.B. Copperman^{1,2,4}

Affiliations:

1. Department of OBGYN and Reproductive Science, Mount Sinai School of Medicine, Klingenstein Pavilion, 9th Floor 1176 Fifth Ave. New York, NY, United States, 10029
2. Reproductive Medicine Associates of New York, 635 Madison Ave, (10th Floor), New York, NY, United States, 10022
3. Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1498, New York, NY, US 10029
4. Sema4, 333 Ludlow Street, South Tower, 3rd Floor, Stamford, CT, United States, 06902

Objective:

Physician scientists are looking beyond chromosomal copy number to understand why some euploid embryos fail to implant. Studies on embryo gene expression and transcriptional profiles are growing within the field of reproductive medicine, however, current findings are limited by sequencing platforms that rely on large populations of cells (>1000). Given that a blastocyst consists of approximately 100-200 cells, drawing conclusions on the cellular or even embryo level is challenging with current sequencing methodologies. Additionally, there is an abundance of studies investigating the role of mitochondria in embryonic competence, particularly focusing on differences in mitochondrial (mt) DNA and implantation potential. However, mtDNA research is an indirect method of understanding the role of mt in blastocyst viability, and the majority of the work on mtDNA has been restricted to analysis of a trophectoderm (TE) biopsy (5-8 cells). The aim of the study is to characterize mitochondrial activity at the single cell level in human blastocysts as well as to determine whether single-cell RNA sequencing (scRNA-seq) is feasible on cells with different levels of mitochondrial activity.

Design:



Prospective cohort study



AMERICAN SOCIETY FOR
REPRODUCTIVE MEDICINE



Materials and Methods:

The study included previously vitrified human blastocysts that had undergone TE biopsy and were subsequently found to be aneuploid based on next-generation sequencing between 2017-2018. Embryos were re-warmed following routine clinical protocol and immersed in trypsin at 37 degrees Celsius for 15 minutes to dissociate cells. These cells were then washed with blastocyst culture media and suspended in fully-supplemented RPMI (with 10% FBS). Cells from individual embryos were stained at room temperature for 30 minutes with either Cytopainter MitoRed or JC-1. Cells were then loaded onto the Beacon single cell handling platform and positioned individually or in groups of up to 5 cells into numbered, tracked pens. They were imaged using exposure times of 1500 ms and 25 ms for FITC and TRed channels respectively. Analysis was performed on images in ImageJ. ScRNA-seq was initiated using barcoded, mRNA capture beads on the Beacon platform. Single beads were panned with cells of interest. Cells were lysed and reverse transcription was performed on the Beacon chip. Barcoded beads were exported off of the Beacon chip for further processing. Complementary DNA (cDNA) was cleaved off of the bead, amplified by PCR and characterized on a 2100 BioAnalyzer using the High Sensitivity DNA assay.

Results:

Two aneuploid embryos biopsied for PGT-A on day 5 of development were included in the study. Embryo A had a morphologic grade of 4BC and embryo B had a morphologic grade of 4AB. A total of 120 cells from embryo A were stained with Cytopainter, of which 32 cells (26.7%) retained staining. Of the successfully stained cells, 13 (40.6%) had high intensity, 11 (34.4%) had moderate intensity, and 8 (25.0%) had low intensity. A total of 259 cells from embryo B were also panned for comparison by scRNASeq. A selection of 32 cells (stained and unstained) from embryo A and 22 cells from embryo B were utilized for scRNASeq. From embryo A, 10 ng of cDNA was obtained, and 286 pg of cDNA was obtained from embryo B, with fragments in the 200-600 base pair size range.

Conclusion:

This study is one of the first to show mitochondrial activity on a single cell level as well as confirm the ability to obtain cDNA from a single human blastocyst cell. Microfluidic-based single cell isolation followed by bead-based scRNAseq is a unique approach that enables the correlation of cellular phenotype to sequencing data on a heterogeneous population of cells. Future work will focus on comparing transcriptome profiles among cells with varying mt content, differences in ploidy, and clinical dissimilarities. Perhaps further research evaluating mt



**AMERICAN SOCIETY FOR
REPRODUCTIVE MEDICINE**



**Icahn
School of
Medicine at
Mount
Sinai**

activity and gene expression on the single cell level will offer insight into the functional elements of the genome that alter embryonic development and competence.

Support:

None