American Society for Reproductive Medicine 2019 Scientific Congress & Expo
October 12 to 16, 2019 ● Philadelphia, PA, USA

Title:

SINGLE-CELL MITOCHONDRIAL STAINING OF HUMAN BLASTOCYSTS

Authors:

Taraneh Gharib Nazem, MD[1,2], Kristin Beaumont, PhD[1], Christine Briton-Jones, PhD, HCLD[1,2], Joseph A. Lee, BA[2], Robert P. Sebra, PhD[3] and Alan B Copperman, MD[1,2,3]

Affiliations:

1. Icahn School of Medicine at Mount Sinai, Klingenstein Pavilion 1176 Fifth Avenue 9th Floor New York, New York, United States, 10029

2. Reproductive Medicine Associates of New York, 635 Madison Ave 10th Floor New York, New York, United States, 10022

3. Sema4, a Mount Sinai Venture, Stamford, CT

Objective:

Physician are looking beyond chromosomal copy number to understand why some euploid embryos fail to implant. Studies investigating the contribution of mitochondrial (mt) DNA levels on implantation have shown some association between mt and embryonic competence, but, these studies are limited, as DNA count has been estimated based on limited sampling of trophectoderm (TE). As mt are the primary energy source of embryonic cells, characterization of mt activity rather than DNA copy number might offer insight into embryonic competence. This study aimed to characterize mt activity on the single cell level in human blastocysts as a marker of embryonic quality.

Design:

Prospective cohort

Materials and Methods:

Previously vitrified human blastocysts that had undergone TE biopsy and next-generation sequencing from 2017-2018 were included. Embryos were thawed and immersed in trypsin to dissociate cells. Cells were washed with blastocyst culture and suspended in RPMI (with 10% FBS). Cells were incubated at room temperature with Cytopainter mitochondrial stain and loaded

onto the Beacon single cell-handling platform into tracked pens. Cells were imaged in the Texas Red channel. Analysis was performed using Fiji and Prism 8.

Results:

A total of 515 cells were analyzed from seven embryos in four patients. Two embryos biopsied for PGT-A on day 6 of development from a 35 yo woman were analyzed: embryo A (grade 4AB) was euploid and embryo B (grade 4BA) was aneuploid. Three euploid embryos from a 33 yo patient, each biopsied on a different day of development, were also included: embryo C (Grade 4BB) was biopsied on day 5, embryo D (grade 6BB) was biopsied on day 6, and Embryo E (grade 6BB) was biopsied on day 7. Two euploid embryos biopsied on day 5 with grades of 4BB were included: embryo F from a 33 year old (yo) patient and embryo G from a 40 yo patient.

For embryo A, 72 cells penned and 66 retained staining (91%). Of the 91 cells that penned for embryo B, 78 (86%) stained. Comparing embryo A to B, a higher level of staining was observed with embryo A ($P<0.0001$). Of the 68 cells that penned for embryo C, 49 showed staining (72%). Embryo D had 58 cells penned, of which 52 cells stained with Cytopainter (90%). Embryo E had 18 cells penned with 10 cells retaining staining (56%). When comparing embryos C, D, and E, there was a significantly higher level of mitochondrial staining in the earlier stage embryos ($p=0.0013$). A total of 77 cells from embryo F penned and 36 showed staining (47%). Embryo G had 131 cells penned and 68 (52%) stained. Of the stained cells, embryo F was observed to have higher intensity staining than embryo G ($p=0.6$).

Conclusion:

This study is one of the first to demonstrate single cell mt activity from a human embryo. We found that cells from euploid, faster growing embryos derived from younger patients possess higher mt content, perhaps representing an increase in embryonic competence. Mt activity might therefore be a useful clinical marker of embryonic quality. Future study will focus on correlating mt activity and gene expression profiles on the single cell level to gain insight into genomic markers and drivers of embryonic development and competence.