P-547 Single-cell RNA sequencing identifies molecular regulations associated with poor maturation performance on rescue in vitro matured oocytes

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Study question: What is the transcriptome signature associated with rescue*in vitro*matured (rIVM) oocytes?
Summary answer: GATA-1/CREB1/WNT signaling axis was repressed in rIVM oocytes of poor quality.
What is known already: rIVM aims to produce mature oocytes (MII) for *in vitro* fertilization (IVF) through IVM of immature oocytes collected from stimulated ovaries. It is less popular due to limited success rate in infertility treatment. Genetic aberrations, cellular stress, and the absence of cumulus cell support in oocytes could account for the failure of rIVM.
Study design, size, duration: We applied single-cell RNA sequencing (scRNA-seq) to capture the transcriptomes of human *in vivo* (IVO) oocytes (n = 10) from 7 donors and rIVM oocytes (n = 10) from 10 donors, followed by

studying the maternal age effect and ovarian responses on rIVM oocyte transcriptomes.

Participants/materials, setting, methods: Human oocytes were collected from donors aged 28-41 years with a body mass index of <30. RNA extraction, cDNA generation, library construction and sequencing were performed in one preparation. scRNA-seq data were then processed and analyzed. Selected genes in therIVM vs. IVO comparison were validated by quantitative real-time PCR.

Main results and the role of chance: The transcriptome profiles of rIVM/IVO showed distinctive differences. A total of 1559 differentially expressed genes (DEGs, genes with at least two-fold change and adjusted $p<0.05$) were found to be enriched in metabolic processes, biosynthesis, and oxidative phosphorylation. Among these DEGs, we identified a repression of WNT/αcatenin signaling in rIVM when compared with IVO oocytes. We found that estradiol level exhibited a significant age-independent correlation with the IVO mature oocyte ratio (MII ratio). rIVM oocytes with higher MII ratio showed over-represented cellular processes such as anti-apoptosis. To further identify targets that contribute to the poor outcomes of rIVM, we compared oocytes collected from young donors with high MII ratio versus donors of advanced maternal age and revealed *CREB1* was an important regulator in rIVM. Our study identified GATA-1/CREB1/WNT signaling was repressed in both rIVM condition and rIVM oocytes of low-quality.

Limitations, reasons for caution: In the rIVM oocytes of high- and low-quality comparison, the number of samples was limited after data filtering with stringent selection criteria. For the oocyte stage identification, we were unable to predict the presence of oocyte spindle so polar body extrusion was the only indicator.

Wider implications of the findings: This study showed that GATA-1/CREB1/WNT signaling and antioxidant actions were repressed in rIVM condition and was further downregulated in rIVM oocytes of low-quality, providing us the foundation of subsequent follow-up research on human subjects.

Trial registration number: not applicable