How the oocyte detaches from the zona pellucida at ovulation

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Introduction:

Fully-grown oocytes are tightly attached to the zona pellucida (ZP). Within the first four hours after ovulation is triggered or upon oocyte isolation from the follicle, the oocyte progressively detaches from the ZP. Detachment during meiotic maturation is part of the process by which oocytes first become capable of independently regulating their cell volume, yet how this occurs remains unknown. We hypothesized that oolemma-zona release is mediated by a peptidase cleaving the ZP proteins that remain in their transmembrane form, attaching the oocyte to the ZP. We therefore sought to determine whether proteinase activity was required for oocyte-ZP detachment and to identify the proteinase.

Methodology and Results:

We performed an initial bioinformatics screen using published RNAseq datasets for mouse oocytes (GSE70116) to reveal oocyte transcripts encoding peptidases in the MEROPS peptidase database. These were further refined using Gene Ontology terms indicating localization in the plasma membrane or extracellular space. This led to the identification of thirty-nine candidate extracellular peptidases in oocytes. Inhibitors matched to each class of candidates were screened for their ability to prevent oocyte detachment from the ZP using an osmotic shock assay. We found that only inhibition of the matrix metalloproteinases class of peptidases significantly inhibited oocyte-ZP detachment. Oocytes remained strongly attached to the ZP in the presence of the metalloproteinase inhibitors batimistat and marimistat for at least 24 hours, persisting even through first polar body formation. Immunofluorescence imaging using a monoclonal antibody that recognizes an epitope just distal to the putative cleavage site of the ZP3 protein showed an initial increase in uncleaved ZP3 protein at the oolemma of oocytes whose ZPs had been removed with acid Tyrode solution, followed by progressive loss of transmembrane ZP3 within the first four hours of maturation. Further study of the kinetics of the putative cleavage site and the role of metalloproteinases is ongoing and includes using a fluorescent ZP3 construct (ZP3::EGFP) expressed in oocytes (a gift from Jurrien Dean, NIH).

Conclusion:

Here we have identified a family of peptidases responsible for the detachment of the oolemma and ZP following isolation from the follicle. We hope that further study will elucidate connections to other key oocyte processes contributing to oocyte quality.

Genomic Insights Into IVF Failure; Dysregulated Inflammation In Stimulated Follicles.

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This study aims to understand IVF failure and see if markers could be used to characterize the patient response to the treatment and identify the failure cause. This could provide clues on how the stimulation protocol of subsequent cycles could be modified and personalized to turn unproductive cycles into successful ones. We first performed a microarray analysis to identify the granulosa gene expression profile of women (n=32) that didn't get pregnant after an IVF cycle and identify potential failure causes. 165 differentially expressed genes (DEGs) were found between cells from follicles associated with failed and successful IVF cycles. The biological functions significantly affected in the negative patients were mainly related to immune and inflammatory responses. Indeed, many of the DEGs encode pro-inflammatory cytokines (e.g. IL1B and EGR1) or other inflammation-related factors that have been seen transcriptionally active in granulosa cells. Overexpression of several factors, including some acting upstream from VEGF, also indicates increased permeability and vasodilation. Another mechanism that appears more pronounced in the negative group is the recruitment of immune cells to ovarian tissues. Additionally to this sustained pro-inflammatory response, the anti-inflammatory mechanisms normally helping restoration of homeostasis seem to be impaired in the negative group supporting the hypothesis that the ovarian environment created by hormonal stimulation is prone to dysregulation. Using these inflammation markers and genes indicative of follicular status that could also be indicative of failure (e.g. growth phase or over-differentiated follicles) we analyzed an enlarged cohort of negative patients (n=63) by qRT-PCR. A hierarchical cluster analysis showed that the negative patients could cluster into 3 groups. Moreover, these groups mainly differ on the expression of genes indicative of different failure causes. These results highlight the possibility of creating a simple diagnostic tool to identify the probable cause of failure and means to improve the next cycle.

Immunomodulatory cell therapeutic approaches for psychiatric disorders: Dampening peripheral innate immune activation to protect the brain.

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Introduction: Prolonged or severe psychosocial stress is a major risk factor linked to the development of mood disorders, including major depressive disorder (MDD) and anxiety. Recently, clinical and animal studies have demonstrated a pronounced, stress-induced, innate immune response detectable in circulation via quantification of pro-inflammatory cytokines and monocytes. Novel approaches targeting inflammatory processes may provide improved or adjunctive therapeutic opportunities in the treatment of MDD. In that regard, mesenchymal stromal cells (MSC), isolated from diverse sources, have demonstrated immunomodulatory capabilities in the context of infection and injury but have yet to be tested in stress-based preclinical models of psychiatric disorders. We sought to test the ability of first trimester human umbilical cord perivascular cells (FTM-HUCPVCs), a readily available source of young MSC, to modulate stress-induced inflammation and depressive behaviors in an established stress-based murine model of MDD / anxiety known as repeated social defeat (RSD).

Materials and methods: Peripheral and central RSD-induced innate immune activation as well as depressive and anxiety-like behaviors were assessed in unstressed control, RSD and RSD + MSC groups using ELISA, flow cytometry, immunohistochemistry and sociability / anxiety behavioral assays. Detailed, unbiased, whole-body biodistribution and fate studies were performed using pre-labelled FTM-HUCPVCs to investigate potential mechanisms of action.

Results: MSCs decreased stress-induced circulating pro-inflammatory cytokines and monocytes. MSCs also reduced neuroinflammation and reduced social avoidance and anxiety-like behaviours. However, biodistribution and IHC analyses revealed that tail vein infused MSCs distributed entirely within peripheral organs without homing to the brain. Fate studies indicated that infused MSCs provoked transient recruitment of recipient neutrophils and monocytes to the lungs. Infused MSCs and recruited neutrophils were subsequently cleared by macrophages which accumulated in the lungs and spleen throughout RSD. Clearance of both MSCs and recruited neutrophils, promotes a phenotypic switch towards anti-inflammatory macrophages and ultimately, resolution of systemic inflammation, associated with detectable increases in circulating anti-inflammatory mediators.

Conclusion: Recruitment of host innate immune cells towards infused MSCs led to resolution of inflammation. This effect may provide downstream protection to distal organs in preclinical disease models in which peripherally generated innate immune cells contribute to pathogenesis, including RSD. These data represent a novel avenue for translational MSC research and potentially identify unexpected targets in the periphery towards improved treatment of psychiatric disorders with an inflammatory component.

Application of a novel telomere-specific assay for human spermatozoa offers new insight into the dynamics of male factor infertility

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Introduction

Telomeres in spermatozoa and their role in male fertility has been a topic of interest in recent years. Our recent publication (Kurjanowicz et al, 2018) showed the presence of two distinct telomere populations in human sperm: chromosomal (chr) telomeres that flank intact chromosomes, and extra-chromosomal, free-floating fragments of telomere repeat-containing DNA (EC). We have applied a novel single-cell assay, HaloFISH, to simultaneously detect the length and number of both types of telomeres, analyzing the relationship between sperm telomeres and current identifiers of male factor fertility.

Materials & Methods

Male patients of the CReATe Fertility Center were recruited to the study following informed consent (n=17). Motile sperm were separated via two-layer density gradient centrifugation, each fraction was cryopreserved according to standard methods. Sperm were subjected to HaloFISH according to Komosa et al. (2015), telomeric DNA was hybridized with telc-CY3 probes (PNA Bio), nuclei were stained with DAPI, slides were imaged on an epifluorescence microscope (Zeiss), single cell quantification was performed on 20-30 cells per patient using Imaris software (Bitplane), and data was analyzed via Graphpad Prism.

Results

Extrachromosomal (EC) telomeric-repeat containing DNA was present in all spermatozoa. Generally, Chr telomeric DNA was significantly longer than EC (mdn=6.037, 4.955-7.088 vs mdn=1.645, 1.457-1.928; $U=1608$, $p<0.0001$). Sperm from normal men had significantly longer Chr telomeres than men with oligozoospermia (mdn=6.298, 5.366-7.244 vs mdn=3.988, 2.859-5.474; $U=2643$, $p<0.0001$, n=13 vs 3 patients). Patients with high DFI had significantly shorter Chr telomeres than patients with low DFI (mdn=6.757, 4.358-6.757 vs mdn=6.617, 5.645-7.273; $U=7232$, $p<0.0001$), and significantly more EC telomeric DNA fragments (mdn=27, 9.25-62 vs mdn=16, 8-27; $U=8027$, $p=0.0008$, n=7 vs 8 patients). The additional fragments were significantly shorter than those present in low DFI patients (mdn=1.556, 1.390-1.776, mdn=1.705, 1.518-2.129; $U=7278$, $p<0.0001$), indicating telomere-specific damage in patients with high DFI.

Conclusions

Spermatozoa of oligozoospermic patients have chromosomes with short telomeres. Simultaneous detection of Chr and EC telomeric DNA revealed telomere-specific DNA damage in spermatozoa.

Minimally-invasive Transcriptomic Profiling of the Endometrium to Identify Markers of Endometrial Receptivity

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Introduction:
To omit the need for an invasive biopsy for transcriptomic analysis of the endometrium, we have developed a minimally-invasive technique of uterine fluid aspiration (UFA) that allows for molecular testing of the endometrium during an active conception cycle. This approach was applied to identify genes differentially-expressed during the window of implantation in both natural cycle and stimulated IVF cycles, which are putative biomarkers of endometrial receptivity.

Methods:
In this prospective translational cohort study, fertile women (Group 1) with regular menstrual cycles, and women (Group 2) undergoing their first or second IVF cycle were recruited. In Group 1, UFA sampling was performed on LH+2 (pre-receptive, N=17) and LH+7 (receptive, N=17) in natural cycles. In Group 2, UFA sampling was performed on hCG+2 (pre-receptive, N=40) and hCG+7 (receptive, N=26) in IVF cycles. Total RNA was extracted from the endometrial pellet of UFA samples and transcriptomic profiling was carried out by microarray or RNA-Seq for bioinformatic analysis.

Results:
For both cycles, unsupervised hierarchical clustering revealed a clear segregation of samples into two major branches: the first included all “pre-receptive” samples and the second consisted exclusively of “receptive” samples. A predictor cassette containing 53 genes that can separate “pre-receptive” and “receptive” samples was generated and validated in both cycles. A large number of transcripts (6983 in natural cycle, and 11421 in stimulated cycle) were differentially expressed between receptive phase and pre-receptive phase (adjusted P <0.05). Cross-referencing them with genes in the panel of ERA (IGENOMIX) revealed 146 overlapping genes, among which, expression of genes encoding secreted extracellular matrix (ECM) proteins were validated by ELISA and immunohistochemistry (IHC).

Conclusions:
We have identified a distinct gene expression signature that can distinguish the receptive endometrium by a non-invasive sampling approach. This approach may supplant previously established invasive testing.

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The First Live Birth Following Aneuploidy Testing using Spent Embryo Medium combined with Blastocoel Fluid in Parallel with Trophectoderm Biopsy

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Introduction: Non-invasive preimplantation genetic testing for aneuploidy (NIPGT-A) using cell-free embryonic DNA (cfeDNA) of spent embryo culture medium (SEM) combined with blastocoel fluid (BF) could eliminate the need for invasive embryo biopsy in the future. In addition, cfeDNA may be more representative the entire embryo compared to trophectoderm (TE) biopsy. Several important issues still need to be addressed before clinical application of NIPGT: minimization of maternal DNA contamination risk, test accuracy, optimization of the whole genome amplification (WGA) protocol.

Materials and Methods: 23 patients (89 blastocysts) were included in the study. On Day 4, each laser zona-opened embryo was transferred to a fresh 15µl droplets of Global HP medium with HSA. On Day 5/6 the blastocysts were collapsed by a laser pulse, followed by collection of a mixture of leaked BF together with SEM as one D4-D5/6 NIPGT sample. Corresponding TE samples were used as controls. WGA products were assessed with the Qubit 3.0 Fluorometer and VeriSeq™ PGS kit on the MiSeq system (Illumina). Results were statistically evaluated using Chi Square/Fisher exact testing.

Results: Informative NIPGT-A results were obtained from 97.8% of TE biopsies (87/89) and 86.5% of SEM+BF samples (77/89). Concordance rate per sample for whole chromosome copy number between SEM+BF and corresponding TE biopsies was: 1) Ploidy status (euploid/aneuploid) - 98.7% (77/78); 2) Gender – 100% (78/78); 3) Euploid samples - 98.0% (48/49); 4) Whole/segmental chromosome aneuploidy – 96.7% (29/30) with one false positive result for segmental aneuploidy. Concordance rate for mosaicism between NIPGT (n=11) and TE biopsy (n=15) samples was 52.9% (9/17, euploid-mosaic samples). Transfer of euploid blastocyst in November 2017 tested by both TE biopsy and cfeDNA (SEM+BF) at the CReATe Fertility Centre, Toronto, resulted in a healthy boy born at full term. To our knowledge, this is the first report where two sources of DNA (SEM+BF and corresponding TE biopsy) were analysed in parallel for clinical PGT-A. In this case, results of cfeDNA analysis from SEM+BF were concordant with TE biopsy.

Conclusions: Spent embryo culture medium combined with blastocoel fluid has great potential as a source for NIPGT-A. If used in parallel with trophectoderm biopsy it may improve testing efficacy by acting as a backup source of embryonic DNA in cases of inconclusive TE biopsy results, potentially avoiding the need for re-biopsy.