Endometrial Microbiota of Women with Endometriosis is more Diverse than that of Symptomatic Controls

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Introduction: Endometriosis is a chronic, estrogen-dependent gynecological condition for which there is no cure. It is characterized by the growth of endometrial glands and stroma outside the uterus, and women with endometriosis often suffer debilitating pelvic pain and infertility. Endometriosis occurs in up to 10% of women world-wide (~176M) and 50% of women with infertility, yet the cause of endometriosis-associated infertility remains poorly understood. Recent reports profiling the reproductive microbiotas challenge the notion of a sterile uterine cavity, and the endometrial microbiota of women with endometriosis remains poorly defined.

Materials and Methods: Twenty-one women (N=21) attending McMaster University Medical Centre who were undergoing gynecological laparoscopy for pelvic pain were selected for the present study from a larger study on endometriosis. The study was approved by the Research Ethics Board, McMaster University, and all participants provided written informed consent prior to participation. In our prospective cohort study we collected endometrial biopsies and profiled the endometrial microbiota using 16S rRNA gene sequencing, in women with surgically confirmed endometriosis (N=12) and surgically confirmed controls (symptomatic controls, N=9). Alpha and beta diversity metrics were calculated using our in-house data pipeline (sl1p), and graphs were generated in R and GraphPad. Linear discriminant analysis (LDA) effect size (LEfSe) was used to determine if there were significant taxonomic differences between the endometrial microbiota of cases and controls.

Results: Women with endometriosis had an endometrial flora that verged on being significantly different from the endometrial microbiota of surgically confirmed, symptomatic controls (β-diversity, $P=0.09$, PERMANOVA). Furthermore, the endometrial microbiota of women with endometriosis had a significantly greater bacterial diversity (α-diversity, $P<0.05$, Shannon Diversity Index) at all levels of rarefaction as compared to controls. LEfSe analysis revealed greater enrichment of several taxa including bacteria belonging to the *Actinobacteria* phylum, *Oxalobacteraceae* and *Streptococcaceae* families, and *Tepidimonas* genus in women with endometriosis, while symptomatic controls were found to have greater enrichment of the *Burkholderiaceae* family, and *Ralstonia* genus. Finally, some clustering of endometrial microbiota by disease status was evident during Bray-Curtis principal coordinates analysis (PCoA), and three clusters were present in the data, as assessed by k-means clustering.

Conclusions: Taken together, our results suggest the endometrial microbiota is altered in women with endometriosis, as compared to symptomatic control women; who have pelvic pain but do not have endometriosis. Furthermore, an altered endometrial microbiota in women with endometriosis may be a factor contributing to endometriosis-associated infertility.

Validation of metabolomics technology to evaluate the presence of metabolites in embryo culture medium

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Introduction

Pregnancy rates with IVF remain low despite embryo selection with morphologic grading. Methods have not been perfected on how to increase live birth rates without increasing multiple births. With the standard of care being single embryo transfer (SET), additional methods are needed for embryo selection. Metabolomic profiling with proton nuclear magnetic resonance (^{1}H NMR) spectroscopy is a novel technique used to identify metabolites rapidly. Little is known about the detection of metabolites in embryo culture medium but studies suggest there are metabolic differences in embryos that result in a successful pregnancy and those that do not.

Materials and Methods

Our primary objective was to assess the feasibility of undertaking a metabolomics study among IVF patients in Halifax. Our secondary objective was to determine which metabolites could be identified in day-5 embryo culture medium by ^{1}HMR and the proportion of samples in which each metabolite is detectable.

This was a feasibility study designed to define the metabolomic profile of day-5 embryo culture medium prior to embryo transfer into the endometrium. Eligible patients included females undergoing first or second round IVF via SET at Atlantic Assisted Reproductive Therapies (AART). Twenty samples of used culture medium were analyzed with ^{1}H NMR initially. Untouched culture media was used as a negative control. Sample volumes were too low to detect concentrations with ^{1}H NMR. Therefore, mass spectrometry was then used to successfully yield results reported in concentrations. Patient charts were reviewed after 8-weeks post embryo transfer to determine if pregnancy occurred.

Results

Of the 20 samples analyzed, 8 were associated with a viable pregnancy on 8-week ultrasound, 6 were associated with a biochemical pregnancy and 6 were associated with no pregnancy. ^{1}H NMR was unable to detect metabolites due to the low sample volumes. Mass spectrometry identified over 110 metabolites, which varied in concentration between each sample.

Conclusions

^{1}H NMR spectroscopy was not feasible to analyze the metabolomic profile of day-5 culture medium at AART due to small sample volumes. However, mass spectrometry was able to detect metabolites and provide concentrations for each. This suggests that growing embryos exchange metabolites with the culture medium they grow in. Our

future goal is a to determine metabolomic profiles of embryo culture medium on a larger scale at AART.

References

Nadal-Desbarats, L., Veau, S., Blasco, H., Emond, P., Royere, D., Andres, C.R., Guerif, F. (2013). *Is NMR metabolomic profile of spent embryo culture media useful to assist in in vitro human embryo culture selection*. Magn Reson Mater Phys, 26, 193-202.

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Title

IVF patients and Peer Relationships: Applying the Disclosure Decision-Making Model to Their Communication and Understanding Their Social Support Outcomes.

Introduction

Assisted reproductive treatment (ART) has traditionally been approached through a biomedical lens (Cousineau et al. 2006). The psychosocial dimensions of infertility, such as the impact of social relationships are often overlooked by the medical community (Cousineau et al., 2006). It takes a village to raise a family, but in today's society it can be argued that it takes a village to start one.

Psychosocial support can be understood through the lens of House's (1981) categories of social support: informational, emotional, instrumental and appraisal. ART patients revealed that other women with shared experiences had ability to offer a high quality connection and respond to their social support needs (Bell, 2012). However how these relationships unfold and are approached from a self-disclosure perspective and how ART patients perceive the capacity of their peers to fulfill these support functions remain unknown.

The disclosure of a health condition is seen as a critical step that "facilitates access to social support" (Greene et al., 2012, p. 356). Greene (2009) developed the Disclosure-Decision Making Model (DD-MM) that breaks down the process according to three contemplation steps: information assessment, receiver assessment and disclosure efficacy. This model has never been applied to infertility context.

Method

The researcher has just completed her fieldwork and has had face to face interviews with 22 IVF patients. A combination of purposeful and snowball sampling with the support of two Toronto-based fertility clinics, TRIO and Create, has given the research a robust study population that includes a mix of both first-time and recurring IVF patients.

Results

The following thematic results will be described during the presentation, and synthesized by presenting a model that encapsulates the process (Creswell, 2013).

1. IVF Patient -Peer Self-Disclosure Process According to the DD-MM

   Sub-themes:
   - evaluating the information
     - specific information sharing patterns and topics of conversation
   - assessing the receiver and disclosure recipient profiles
   - disclosure efficacy including
     - conditions and influencers that lead to self-disclosure

- communication style and modalities

2. Perceived Social Support and Positive Relational Outcomes

   Sub-themes:
   - examples of social support (emotional, informational, instrumental and appraisal)
   - strategic actions
   - feelings of self-efficacy

3. Perceived Negative Relational Outcomes

   Sub-themes:
   - social comparison
   - one-sided success
   - advice overload
   - feelings of incompetence
   - risks of oversharing
   - the fertility-centric relationship
   - circumspection

References

Bell, K. (2012). An exploration of women's psychosocial support needs in the context of assisted reproduction. *Social Work in Health Care*, 51(8), 695-709.

Cousineau, T.M., & Domar, A.D. (2007). Psychological impact of infertility. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *21*(2), 293-308.

Cousineau, T.M., Green, T.C., Corsini, M.S., Barnard, T., Sebring, A.R., & Domar, A. (2006). Development and validation of the infertility self-efficacy scale. *Fertility and Sterility, 85*(6), 1684-96.

Creswell. J. (2013). *Qualitative inquiry & research design. Choosing among five approaches*. (3rd Ed.). Thousand Oaks: Sage.

Greene K. An integrated model of health disclosure decision-making. In: Afifi TD, Afifi WA, editors. Uncertainty and information regulation in interpersonal contexts: Theories and applications.New York, NY: Routledge; 2009. pp. 226–253

Greene K, Magsamen-Conrad K, Venetis MK, Checton MG, Bagdasarov Z, Banerjee SC. Assessing health diagnosis disclosure decisions in relationships: Testing the disclosure decision-making model. Health Communication. 2012;27:356–368.

House, J. S. (1981). Work stress and social support. Reading, MA: Addison-Wesley.

Detection of Follicular Fluid Phytocannabinoids Before and After Canadian Legalization of Cannabis, and Impact of Phytocannabinoids on Oocyte Quality and Embryo Development

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Introduction: Cannabis is the third most commonly consumed substance by women of childbearing age. Data regarding its effect on fertility treatment outcomes is sparse and is mostly based on self-reporting. The objectives of this study were: 1.measure Δ9-THC, the psychoactive component of cannabis, and its metabolites, in follicular fluid (FF), 2.assess the impact national legalization of Phytocannabinoids (PC) has had on consumption in the ART population attending a single clinic, situated in a downtown urban area, and 3.assess the impact of PC on oocyte quality.

Materials and Methods: We retrospectively performed liquid chromatography–mass spectrometry (LC-MS/MS) on 161 FF samples from dominant and subordinate follicles of consenting patients between Jan 2018-April 2019. All assays were conducted at the Hospital for Sick Children (Toronto, ON). Proteins were precipitated using a 1:1 methanol/water ratio and the protein-depleted supernatant was subjected to LC-MS/MS (QTRAP 5500 and Agilent 1290 HPLC) for the following PCs; Δ9-THC, 11-OH- and 11-COOH-Δ9-THC. Results were correlated with demographic features and self-reporting. Linear regression was applied to explore interactions between PC levels and oocyte quality, as well as embryo development.

Results: 80% of patients (13/161) tested positive for PCs; this increased from 6% to 14% after legalization of cannabis in Canada on October 2018 (chi^2; p=0.56). PC concentrations per FF testing positive, did not differ after legalization. The correlation between self-reporting and detection of PCs in FF increased after legalization. Of note, one patient reported once a month consumption, but no PCs were detected. There was no significant difference between the concentrations of PCs in dominant or subordinate follicles (paired t-test). Using linear regression,

none of the PCs were associated with oocyte quality (oocyte maturation and fertilization rate) or embryo development (cleavage and blastulation rates).

Conclusions: To our knowledge, this is the first study measuring PC levels in human FF. The proportion of patients consuming cannabis increased following legalization, but concentrations per follicle remained the same. Measuring PCs in FF was more reliable than self-reporting. Utilizing LC-MS/MS allowed detection down to 0.001ng. Study limitations include potential sampling bias and small sample size. Future studies will aim at determining the functional impact cannabis has on oocyte maturation, as well as assess a larger sample size to determine if there is an impact on pregnancy outcomes.

Androgen-induced granulosa cell-derived exosomal mir-379-5p regulates macrophage polarization

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Introduction: Polycystic ovarian syndrome (PCOS) is associated with hyperandrogenemia and ovarian follicle growth arrest. Studies with an androgenized (DHT) rat PCOS model demonstrate that androgen reduces the granulosa cell mir-379-5p content in pre-antral follicles through the induction of exosome release, leading to up-regulation of granulosa cell phosphoinositide-dependent kinase-1 (PDK1; as target gene), activation of the PI3K/AKT pathway and cell proliferation. Macrophages play a key role in inflammation and the balance between M1 (inflammatory) and M2 (anti-inflammatory) macrophages determines their physiological/pathological responses. DHT increases M1 macrophages abundance, but reduces M2 polarization in rat antral and pre-ovulatory follicles *in vivo*. We have also observed a significantly higher M1/M2 ratio in the ovary of PCOS subjects. Follicular fluid extracellular vesicles, including exosomes and microvesicles, facilitate cellular cross-talk in the follicular antrum by selective packaging and transfer of miRNAs. However, the role of exosome secretion in determining the cellular content and function of miRNAs in exosome-secreting and -receiving cells is largely unknown. Our objective was to determine the regulatory role of miRNAs (mir-379-5p targeting PDK1 in macrophages) derived from granulosa cells on macrophage polarization.

Methods: Rat bone marrow cells were differentiated into macrophages under M-CSF stimulation for 7 days *in vitro*. Pre-antral follicle granulosa cells were cultured on trans-well inserts and transfected with CD63-GFP tagged (to label exosomes released from granulosa cells) and alexa-647-labelled mir-379-5p mimic. To determine if granulosa cell-derived exosomes were taken up by macrophages, transfected granulosa cells were transferred and co-cultured with bone-marrow derived macrophages for 24 hours.

Results: The assessment of GFP (granulosa cell-derived exosome) and alexa-647 (granulosa cell-derived mir-379-5p) signals indicate that macrophages engulf granulosa cell derived exosomes (CD63-GFP tagged) containing mir-379-5p (Alexa-647). The transfection of macrophage with mir-379-5p mimic reduced the cellular content of PDK1

and IL-4-M2 induced PDK1 in macrophages, suggesting that PDK1 is potential target gene for mir-379-5p. PI3K/Akt signaling is involved in the control of M2 macrophage polarization and its inhibition increases the M1/M2 ratio (Vergadi E., 2017 J Immunol). However, the regulatory role of androgen-induced granulosa cell-derived exosomal miRNAs in macrophage polarization remains to be determined.

Conclusions: Our preliminary results suggest that androgen-induced granulosa cell-derived exosomal mir-379-5p regulates macrophages polarization and is involved in ovarian immune regulation (Supported by a grant from CIHR and the Lalor Foundation Postdoctoral Fellowship).

Development and validation of a new personalized molecular test based on endometrial receptivity and maternal-fetal dialogue

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Introduction:

Establishment and maintenance of pregnancy is a complex process which depends on several factors including successful implantation, a competent embryo, a receptive endometrium and a synchronized mother-embryo crosstalk. It has been suggested in a few studies that up to two-thirds of implantation failures are due to defects in ER. An endometrium is receptive to an embryo in a spatially and temporally restricted period called the window of implantation (WOI) which is usually more or less delayed in recurrent implantation failures. Therefore, it appears essential to identify inadequate endometrial receptivity to offer personalized care management. Genomic diagnostic tools for human endometrial receptivity based on the transcriptomic signature currently available to characterize this process are very limited. In this study, a new Molecular diagnostic tool for endometrial receptivity and embryo implantation is presented for the first time.

Methods:

The development and clinical validation of a new test, Adhesio RT, allowed us to analyze 215 biopsies of which 50 biopsies were collected in natural cycle during the optimal theoretical implantation window LH+7 to LH+11 (35 with successful clinical pregnancy 15 with implantation failure). Similarly, a total of 29 co-culture biopsies were performed on autologous- endometrial co-culture (14 endometrial cells cultured in absence of embryo, 5 in presence of good-quality embryo successfully transferred, 10 with good quality embryo but with implantation failures). Samples were analyzed using microarrays and selected biomarkers were assessed using RT-qPCR. Finally 130 biopsies from IVF-patients with a known pregnancy outcome were used for clinical validation. These

clinical results were compared with previously known outcome using ERA arrays and Win-test.

Results:

Adhesio RT included 10 new selected genes by using a new approach that incorporates two specific transcriptomic signatures obtained by different bioinformatics and statistical technologies applied to microarray analyses:

A first specific transcriptomic signature of 1717 genes specifically modulated associated to biopsies from patients with successful clinical pregnancy *versus* biopsies from patients with implantation failure. Gene ontology analyzes revealed that cell division, cellular proliferation, cell adhesion and mitotic cycle are the most over-represented biological terms in this group of genes.

A second specific transcriptomic signature of 60 genes associated to endometrial co-culture successfully transferred was obtained using class prediction approach. Gene expression was validated by RT–qPCR. Clinical validation was performed on 130 biopsies from IVF-patients with a known pregnancy outcome.

Conclusions:

This molecular diagnostic tool can be used clinically in reproductive medicine and gynecology. It can predict IVF success and may help in the management of endometrial preparation for embryo transfer and optimizes chances of successful pregnancy for many couples.