The Human Embryo RNA Secretome

Stewart J. Russell[1], Karen Menezes[1], Clifford L. Librach[1,2,3]

1. Create Fertility Centre, Toronto, Ontario, Canada. 2. Department of Obstetrics and Gynecology, University of Toronto, Toronto, Ontario, Canada 3. Department of Physiology, University of Toronto, Toronto, ON, Canada

Introduction

- The current standard of practice for most clinics is single frozen embryo transfer of a blastocyst (day 5-6)
- This provides a key opportunity for clinics and staff to prioritize embryos when multiple blastocysts are available
- However, current rates of implantation range from 40-60% even in cases where the embryo is genetically normal, and the window of implantation has been identified
- This leaves considerable room for improvement in identifying embryos with the greatest implantation potential
- During culture, embryos release RNA, DNA, proteins, and metabolites into the surrounding **embryo conditioned culture media (ECCM)**
- We have previously shown a rich small non-coding RNA complement is released into the ECCM

Primary Aim

To determine the feasibility and sensitivity of sequencing large RNA from ECCM to evaluate their potential use as biomarkers.

Secondary Aim

To characterize the diversity of large RNA sequences present in the ECCM which may illuminate RNA-mediated embryo-maternal communication

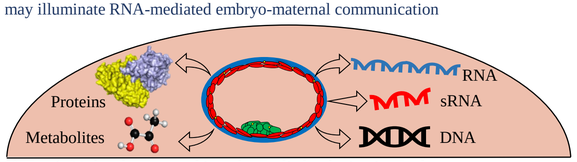


Figure 1. Molecules secreted into the media by the in vitro embryo

Hypothesis

RNA secreted by the embryo will be significantly different than those detected from control media, and will be detectable by qRT-PCRECCM samples

Materials and Methods

- Embryos cultured for 5-6 days in Sage 1-Step culture media
- RNA Extracted with Norgen Total RNA Micro kit, including ERCC Spike-in
- cDNA Libraries prepared with NEB Single Cell/Low Input RNA kit
- Libraries were sequenced on the NextSeq 550 platform at 2x150 bp
- Data was analyzed in-house using available software: Cutadapt, STAR aligner, TEtranscripts, DESeq2

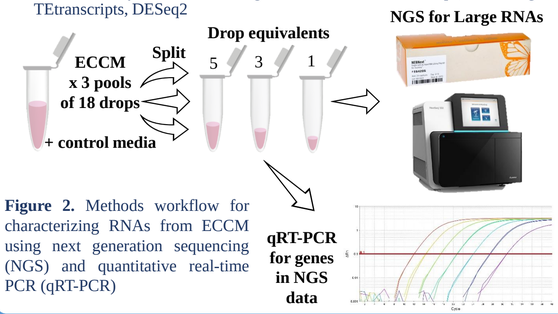


Figure 2. Methods workflow for characterizing RNAs from ECCM using next generation sequencing (NGS) and quantitative real-time PCR (qRT-PCR)

Results

Next Generation Sequencing Identifies Embryo-Secreted RNAs

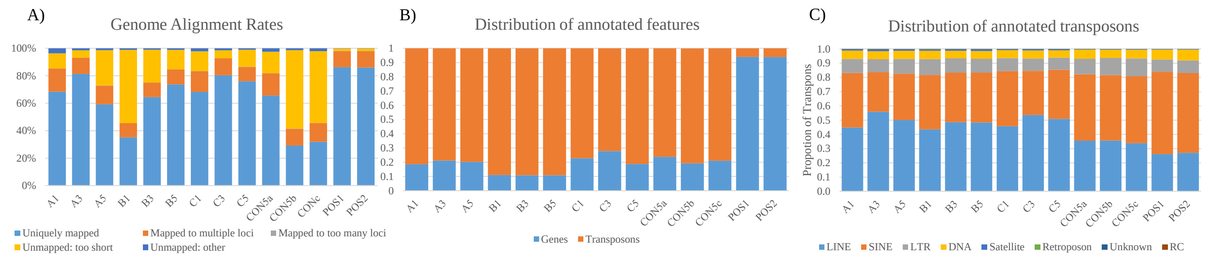


Figure 1. Next Generation Sequencing of ECCM for large RNA. Samples A1-C5 are ECCM bioreplicates at various inputs (1-5 droplets), CON are control drops (no embryo), and POS are cellular positive controls. A) Genome alignment rates for sequenced reads using STAR show a significant portion of uniquely mapped reads, as well as many reads that are too short which represent gene fragments and sequencing artifacts. These are discarded from further analysis. B) The software TEtranscripts is used to annotate genes and transposons (TEs). ECCM and control media contain mostly (80-90%) TE transcripts. C) The most prevalent TEs identified were LINEs, SINEs, and LTRs.

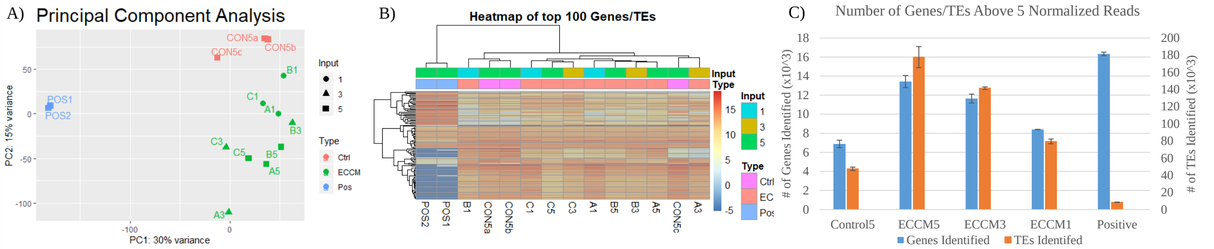


Figure 2. Exploratory Analysis of RNA Sequencing Data. A) PCA was performed to reduce the dimensionality of the data and identify sample clusters. Control media (CON), ECCM (A1-C5), and cellular RNA (POS) cluster separately. B) Unsupervised hierarchical clustering of the top 100 expressed genes/TEs show clustering by sample type. C) The number of genes (left y-axis) and TEs (right y-axis) above a threshold of 5 **normalized** reads in each input amount were compared, showing a clear decrease in detection with input amount in the ECCM samples.

Genes Secreted From Developing Embryos

Table 1. Differential Expression Summary

Top 20 Genes in ECCM Only		Differential Expression Summary
DPPA3	SDE2	DE Genes Total (ECCM vs CON) 632
CENPA	PARL	
CYSTM1	TUBB8P7	Upregulated 614
TTC9C	TOMM6	
OOEP	GABARAP	Downregulated 18
HSD3B2	BCAR4	
HIST1H1A	ACTL8	Present only in ECCM 123
CXCL2	GCA	
HMGN5	H1FOO	Present only in CON 3
TUBB7P	OOSP2	

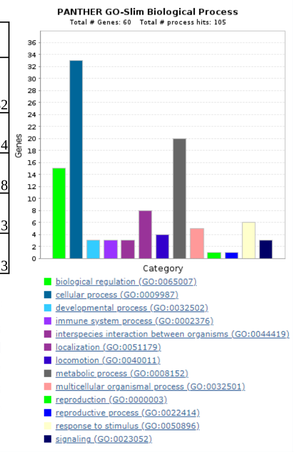


Figure 2. PANTHER classifications of ECCM-specific genes. Biological processes represented by genes specifically secreted from embryos into the media. These processes provide insight into their functions and potential for communication with the endometrium.

Gene Expression Validation

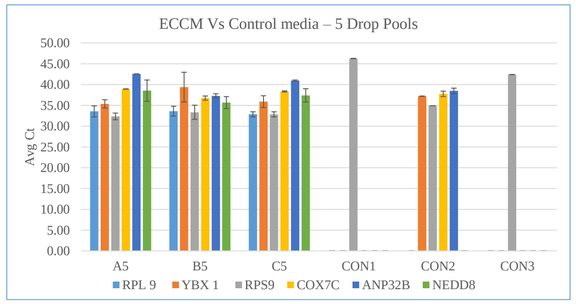


Figure 2. qRT-PCR analysis for RNA present in ECCM compared with control. 6 Genes were selected based on high levels of detection in NGS of ECCM samples and low or absent levels in controls. Due to lack of endogenous control genes, relative quantification to an internal reference gene was not possible. All assayed targets were present in ECCM and inconsistent or absent in control media droplets. Higher Ct represents lower transcript levels. Values presented are mean ± SD.

Discussion and Conclusions

Discussion

- Genome alignment rates are lower from media samples as compared to control samples which was expected.
- The large portion of reads mapping to Transposons in the ECCM was unexpected, and not previously reported. However, it is known that pre-implantation embryos express high levels of transposons (Ge 2017; Muñoz-Lopez et al.)
- LINEs are the most active transposons in the human genome, which may explain their abundance in the media
- PCA and clustering of the sequencing data show a clear delineation of the ECCM from control, confirming that we can effectively profile the media with this NGS method
- There is a direct relationship between the number of genes and transposons identified in the media and the input amount (5, 3, or single droplets), suggesting greatest sensitivity at higher inputs
- Several of the top genes present in ECCM but not detected in the control media have known roles in embryo development (Azizollah Bakhtari 2014; Zhao et al. 2013; Smoak et al. 2016.)
- Quantitative RT PCR validation is challenging due to variations in RNA fragment lengths and low input. However, we consistently detect gene targets in 5 ECCM drop pools which are low abundance or absent in control drop pools

Conclusions

- We can sequence and quantify RNA from single embryo culture media droplets, and the RNA signature is unique from control media
- There is a significant fraction of transposable elements present in the media
- Gene and transposons transcripts secreted by the embryo into the culture media may be assayed for biomarkers of implantation

Conclusions

- Develop assays for single ECCM droplet quantification of RNA biomarkers
- Probe transposable elements present in the media for potential functions in implantation and embryo-maternal signalling
- Compare gene transcript abundance between embryos which implant and fail to implant to develop a panel predictive of implantation potential

References

1. Abu-Halima M, Häusler S, Backes C, Fehlmann T, Staib C, Nestel S, Nazarenko I, Meese E, Keller A. Micro-ribonucleic acids and extracellular vesicles repertoire in the spent culture media is altered in women undergoing In Vitro Fertilization. Sci Rep 2017;7:13525. Nature Publishing Group.
2. Azizollah Bakhtari PJR. DPPA3 prevents cytosine hydroxymethylation of the maternal pronucleus and is required for normal development in bovine embryos. Epigenetics 2014;9:1271. Taylor & Francis.
3. Evan M Smoak, Paula Stein, Richard M Schultz, Michael A Lampson, Ben E Black. Long-Term Retention of CENP-A Nucleosomes in Mammalian Oocytes Underpins Transgenerational Inheritance of Centromere Identity. Curr Biol 2016;26:1110–1116. Cell Press.
4. Ge SX. Exploratory bioinformatics investigation reveals importance of ``junk" {DNA} in early embryo development. BMC Genomics 2017;18:200. BMC Genomics.
5. Muñoz-Lopez M, Vilar R, Philippe C, Rahbari R, Richardson SR, Andres-Anton M, Widmann T, Cano D, Cortes JL, Rubio-Roldan A, et al. LINE-1 retrotransposition impacts the genome of human pre-implantation embryos and extraembryonic tissues. bioRxiv [Internet] 2019; http://dx.doi.org/10.1101/522623.
6. Rosenbluth EM, Shelton DN, Wells LM, Sparks AET, Van Voorhis BJ. Human embryos secrete microRNAs into culture media--a potential biomarker for implantation. Fertil Steril 2014;101:1493–1500. Elsevier Inc.
7. Zhao B, Cun YX, He XC, Zheng P. Maternal-effect Floped gene is essential for the derivation of embryonic stem cells in mice. Dongwuxue Yanjiu [Internet] 2013;34.: Dongwuxue Yanjiu.

Acknowledgements

This project was funded by the CReATe Fertility Centre. The authors would like to thank the CReATe Biobank for their tremendous help obtaining samples and data for this project.

Bisphenols and Reactive Oxygen Species in Oocytes, Sperm and Granulosa Cells

Mimi Nguyen, Reem Sabry, Kirstin Surbek, Elizabeth J. St. John, and Laura A. Favetta
Reproductive Health and Biotechnology Lab, Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph; Guelph, ON
mnguye06@uoguelph.ca | lfavetta@uoguelph.ca

Introduction

- Bisphenol A (BPA) is an endocrine disrupting chemical with negative impact on fertility.
- Our lab has found poor oocyte and embryo quality (spindle abnormalities[1], increased DNA fragmentation[2], altered miRNAs[3]) after BPA exposure.
- BPS and BPF have been introduced as "safer" alternatives – but research is limited[4,5].
- Its weak affinity to estrogen receptors suggest possible alternative mechanisms at play, such as through **oxidative stress**[6].

Hypothesis:

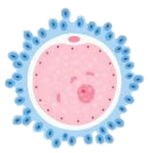
BPA, BPS and BPF affect reproductive potential in COCs, granulosa cells and sperm by altering oxidative stress levels.

Objective:

Quantify key reactive oxygen species (ROS) genes (SOD1, SOD2, CAT, GPX1, GPX4) to assess oxidative stress in 3 experimental models: COCs, granulosa cells, and sperm.

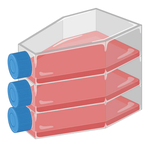
Methods

1



COCs
24hr matured

1. Control
2. Vehicle
3. BPA*
4. BPS*
5. BPF*



Granulosa Cells
12hr & 48hr culture

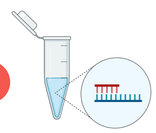
1. Control
2. Vehicle
3. Estradiol
4. BPA High*
5. BPS High*
6. BPF High*
7. BPA Low**
8. BPS Low**
9. BPF Low**

Frozen-thawed, Percoll washed

Sperm
4hr incubation

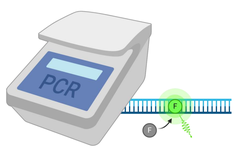
1. Control
2. Vehicle
3. BPA*
4. BPS*
5. BPF*

2



RNA Extraction & Reverse Transcription

3

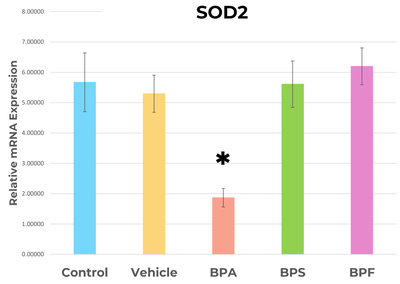


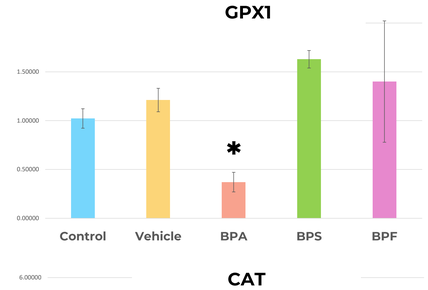
Quantitative PCR (qPCR)

*** LOAEL dose: 50 µg/mL**
**** 100X less than LOAEL: 0.5 µg/mL**

Results

COCs





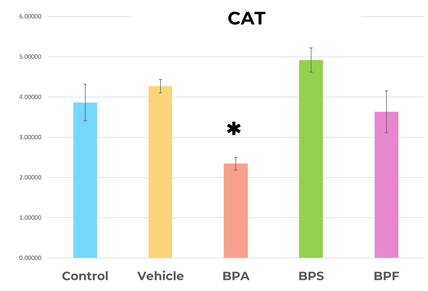
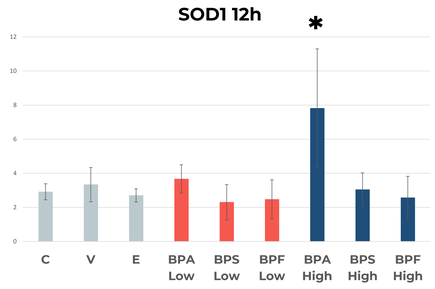
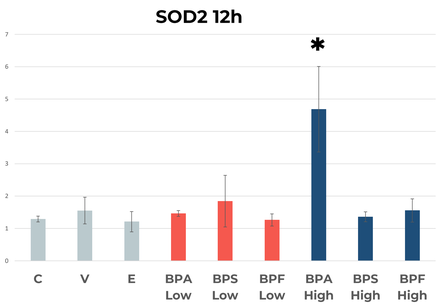
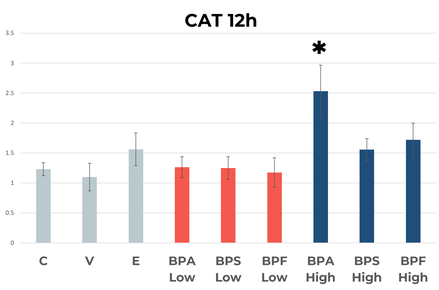


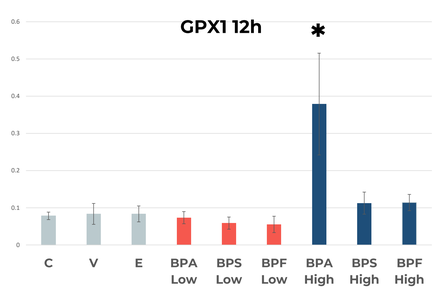
Figure 1: mRNA expression of SOD2, GPX1 and CAT in COCs after 24hrs incubation with BPA, BPS and BPF (0.05mg/ml). ***** $p < 0.05$.
No significant changes across groups for SOD1 and GPX4 in COCs.

Granulosa Cells









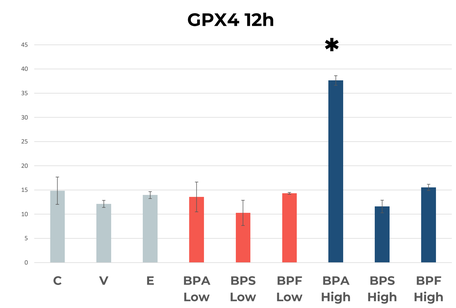
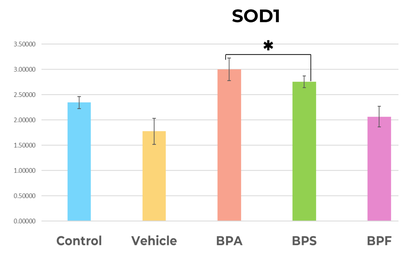
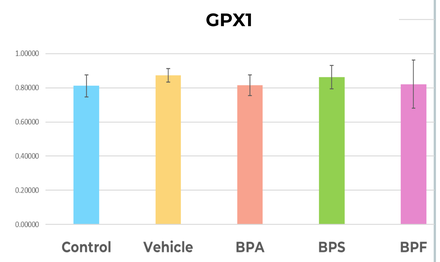


Figure 2: mRNA expression in bovine granulosa cells after *12hr* culture in bisphenols (0.05mg/ml). ***** $p < 0.05$.
No significant changes across groups for all antioxidants at 48hr in granulosa cells.

Results

Sperm





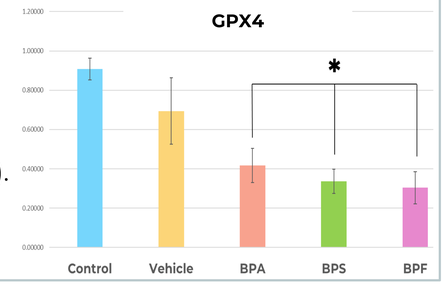


Figure 3: mRNA expression of SOD1, GPX1 and GPX4 in sperm after 4hrs incubation in BPA, BPS and BPF (0.05mg/ml). ***** $p < 0.05$.
SOD2 and CAT were not detected in sperm by qPCR.

Conclusion

Significant mRNA expression changes include:

- COCS: ↓ in CAT, GPX1 and SOD2 in BPA group only
- GC: ↑ in all 5 antioxidants in the BPA group only
- Sperm: ↓ GPX4 in all bisphenol groups; ↓ SOD1 in BPA & BPS groups.

These data suggests that BPF likely acts through a different mechanism, not involving oxidative stress.

Future Directions:

- Confirm mRNA data at the protein level via Western Blot
- Determine overall oxidative stress levels after treatment

Overall, this research aims to understand non-traditional mechanisms of bisphenols affecting both female and male fertility.

Acknowledgements

NSERC CRSNG
University of Guelph
Ontario Veterinary College
Department of Biomedical Sciences
Ontario Graduate Scholarship (OGS)

Special thanks to: Dr. Mastromonaco, Dr. Kalisch, Dr. Antenos, & Allison Mackay.

References

1. Ferris, J., Favetta, L., & King, W. (2015). Bisphenol A Exposure during Oocyte Maturation in vitro Results in Spindle Abnormalities and Chromosome Misalignment in Bos taurus. *Cytogenetic And Genome Research*, 145(1), 50-58. doi: 10.1159/000381321
2. Saleh, A., Sabry, R., Mastromonaco, G., & Favetta, L. (2021). BPA and BPS affect the expression of anti-Mullerian hormone (AMH) and its receptor during bovine oocyte maturation and early embryo development. *Reproductive Biology And Endocrinology*, 19(1). doi: 10.1186/s12958-021-00773-6
3. Sabry, R., Saleh, A., Stalker, L., LaMarre, J., & Favetta, L. (2021). Effects of bisphenol A and bisphenol S on microRNA expression during bovine (Bos taurus) oocyte maturation and early embryo development. *Reproductive Toxicology*, 99, 96-108. doi: 10.1016/j.reprotox.2020.12.001
4. Rochester, J. R., & Bolden, A. L. (2015). BPS and F: a systematic review and comparison of the hormonal activity of bisphenol A substitutes. *Env Health Per*, *123*(7), 643-650.
5. Žalmanová, T., Hošková, K., Nevoral, J., Adámková, K., Kott, T., Sulc, M., ... & Petr, J. (2017). Bisphenol S negatively affects the meiotic maturation of pig oocytes. *Sci Reports*, *7*(1), 1-11.
6. Gassman, N. R. (2017). Induction of oxidative stress by bisphenol A and its pleiotropic effects. *Environmental and molecular mutagenesis*, *58*(2), 60-71.
7. Guo, J., Zhao, M. H., Shin, K. T., Niu, Y. J., Ahn, Y. D., Kim, N. H., & Cui, X. S. (2017). The possible molecular mechanisms of bisphenol A action on porcine early embryonic development. *Sci Reports*, *7*(1), 1-9.

Human Platelet Lysate Increases Attachment of Trophoblast Spheroids to Primary Endometrial Epithelial Cells from Patients with Recurrent Implantation Failure

[1,3]Tina Tu-Thu Ngoc Nguyen, [1]Yat Sze Sheila Kwok, [1]Stewart J. Russell, and [1,2,3,4]Clifford Librach

[1]CReATe Fertility Centre, Toronto, Ontario, Canada; [2]Department of Obstetrics and Gynaecology, [3]Department of Physiology, and [4]Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada

INTRODUCTION

- Implantation failure is associated with ~75% of failed embryo transfers and remains one of the largest challenges for assisted reproductive technologies.
- Recurrent implantation failure (**RIF**) is roughly defined as the absence of implantation after ≥2 failed transfers of good quality embryos.
- Inadequate endometrial receptivity and thickness have been proposed as major causes of RIF, but current therapies remain relatively ineffective.
- Recent clinical studies suggest that intrauterine infusion with autologous platelet-rich plasma (**aPRP**) prior to transferring an embryo may improve pregnancy outcomes for unexplained RIF and/or thin endometrium (**TE**) patients by promoting endometrial growth and improving implantation rates.
- Our own *in vitro* studies also support a mitogenic effect of aPRP and the non-autologous PLUS™ human platelet lysate **(HPL)** product (Compass Biomedical) on endometrial cell proliferation.

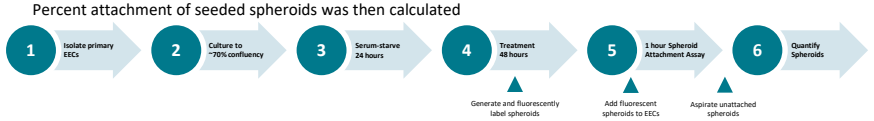
OBJECTIVE AND HYPOTHESIS

- **Objective:** Investigate the efficacy of PLUS™ HPL to increase endometrial receptivity and embryo implantation *in vitro.*
- **Hypothesis:** In addition to cell proliferation, *in vitro* treatment with PLUS™ HPL will improve embryo attachment to primary EECs isolated from patients with a history of RIF.

MATERIALS AND METHODS

1. Endometrial tissue was collected from nine RIF patients at the CReATe Fertility Centre, Toronto, Canada (**Veritas REB#16580**).
2. EECs were isolated and treated with serum-free culture media (**SFM**) or 1% HPL (PLUS™, Compass Biomedical) for 48 hours.
3. Trophoblast spheroids (**HTR-8/SVneo**) were generated, calcein labelled, and size-selected (70-100 µm) to be similar in size to a human blastocyst.
4. Spheroids were seeded on pre-treated EEC monolayers and calcein fluorescence was immediately measured by a spectrophotometer.
5. Following the 1-hour incubation, unattached spheroids were aspirated, and calcein fluorescence was measured again.
6. Attached spheroids were quantified by measuring calcein fluorescence and spheroid count by fluorescent microscopy and ImageJ™ software. Percent attachment of seeded spheroids was then calculated

Nine Consented Patients with a History of RIF	
≥2 failed transfers with euploid/good quality embryos	
RIF+TE (N=5)	**RIF only** (N=4)
Thin Endometrium (<7 mm)	Adequate Endometrial Thickness (≥7 mm)
Age: 31-45	Age: 32-42



ACKNOWLEDGEMENTS

This project was funded by the CReATe Fertility Centre. Special thanks to the clinical staff, especially Dr. Noga Weizman, Dr. Shira Bar-Am, Mai Sherif, the Biochemistry Department, and the CReATe Biobank. The authors confirm that there are no conflicts of interest.

RESULTS

HTR-8/SVneo Spheroids (70 – 100 µm)

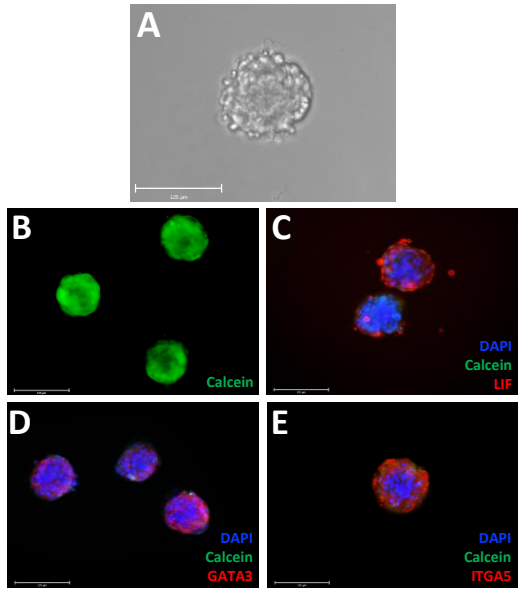


Figure 1 – HTR-8-Svneo trophoblast spheroids express EVT and implantation biomarkers.

Representative phase contrast **(A)** and immunocytochemistry images **(B-E)** for the expression (**red**) of the implantation biomarker LIF **(C)**, and trophoblast phenotypic markers transcription factor GATA3 **(D)** and surface adhesion protein ITGA5 **(E)** in HTR-8/SVneo spheroids labelled with green-fluorescent calcein **(B).** Nuclei were counter-stained with DAPI (blue) and imaged at 200x magnification.

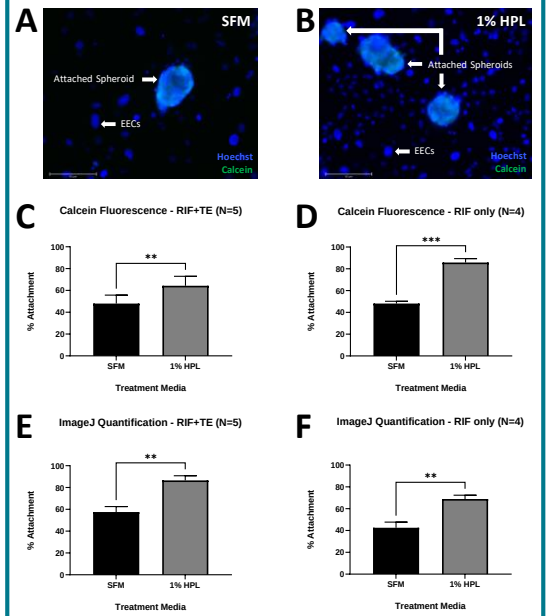


Figure 2 – Treatment of endometrial cells from patients with RIF+TE (N=5) or RIF only (N=4) with HPL improves trophoblast attachment.

Primary EECs treated with 1% HPL **(B)** showed a significant increase in spheroid attachment compared to SFM **(A)** in both RIF patient cohorts, regardless of endometrial thickness. Nuclei were Hoechst 33342 counter-stained (**blue**) and imaged at 100x magnification. Spheroid attachment was quantified by percent (%) attachment, as measured by calcein fluorescence **(C&D)** or by quantification of individual spheroids with ImageJ™ **(E&F)**. The data is presented as mean ± SEM. Statistical analysis was performed using the two-tailed paired t test (* = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$).

- The HTR-8/SVneo cell line, derived from human first-trimester extravillous trophoblast cells (**EVT**), has been shown to be a suitable model to assess adhesion and invasion *in vitro*.
- Trophoblast spheroids visually resembled a blastocyst and maintained expression of the EVT biomarkers GATA3 & ITGA5, and implantation biomarker LIF (**Figure 1**).
- $\% \, Attachment = \left(\frac{attached\ spheroids}{seeded\ spheroids}\right) \times 100$
- Primary EECs, treated for 48 hours with SFM supplemented with 1% commercially sourced and non-autologous HPL, overall exhibited increased attachment to HTR-8 spheroids (**Figure 2**).
- The percentage of spheroid attachment, as measured by calcein fluorescence alone, significantly increased by **16%** ($P<0.01$) of seeded spheroids in RIF+TE EEC cultures, and by **38%** ($P<0.001$) of seeded spheroids in RIF only EEC cultures.
- Quantification of spheroid count by ImageJ™ software revealed a significant increase in spheroid attachment, by **39%** ($P<0.01$) in RIF+TE EEC cultures, and by **26%** ($P<0.01$) in RIF only EEC cultures.

CONCLUSIONS

- **Summary of Results:** Increased quantity of HTR-8 spheroids attached to primary EECs, isolated from patients with RIF, suggests *in vitro* treatment with non-autologous PL could improve endometrial receptivity. There are two explanations for the observed increase in attachment: **1)** HPL stimulates cell proliferation, therefore, there is an increase in the surface area available for spheroid attachment; **2)** HPL stimulates increased expression of receptivity biomarkers that are important for embryo implantation in primary EECs.
- **Limitations:** Although there was a positive correlation between calcein fluorescence and spheroid quantity, quantification by fluorescence alone may be unreliable due to the variable numbers of cells in each spheroid. Our data suggest a more precise increase in attachment is detected when spheroid count was quantified by fluorescent microscopy and ImageJ™ software.
- **Significance:** We report a method to functionally assess endometrial receptivity *in vitro*. Commercial non-autologous HPL appears to promote implantation in RIF patients in a model of embryo attachment. We hypothesize that HPL can potentially be used as an alternative for aPRP and standardize future clinical treatments (intrauterine infusions). We also predict that the observed increase in attachment is due to increased endometrial receptivity gene expression, which will be our next investigative avenue.

Advancements in the CReATe Biobank; the certified Resource for Reproductive Biology Research

Sahar Jahangiri[1], Zeinab Sadrosadat[1], Sydney Wells[1], Malaika Gomes[1], Clifford Librach[1,2,3]

[1]CReATe Fertility Centre; [2]Department of Obstetrics and Gynecology, University of Toronto; [3]Departments of Physiology and the Institute of Medical Sciences, University of Toronto , Toronto, Ontario; Canada.

Objectives

The CReATe Biobank is Canada's first biobank to focus entirely on human reproductive biology-related samples. It has been certified as adhering to best practices by the Biobank Resource Centre. Currently, we bank a variety of donated samples, including; seminal fluid, spermatozoa, follicular fluid, granulosa/cumulus cells, arrested, aneuploidy, euploid and untested embryos, embryo culture conditioned media, unfertilized eggs, serum, plasma, buffy coat and urine samples, Figure 1.

The biobank lab receives, processes and stores a variety of biospecimens daily

- **Waste materials**
  - **IVF egg retrieval Waste Material**: follicular fluid, granulosa cells, cumulus cells, GV oocytes, oocytes that failed to fertilize
  - **Seminal fluid**
  - **Excess semen samples**
  - **Arrested embryos, aneuploid embryos, normal embryos**
  - **Embryo culture media**
  - **Endometrial cells and fluid**
- **Non-waste materials**
  - **Urine**
  - **Blood; serum, plasma, buffy coat**
  - **Buccal Swabs**

Figure 1- Samples collected by CReATe Biobank

Materials

Upon receiving informed consent from the patient, the biobank collects and stores materials. The collected samples can then be used by researchers to improve the diagnosis and treatment of infertility and increase scientific knowledge. Samples are collected from consented participants for institutional REB-approved studies, together with corresponding anonymized clinical data. Sample processing and storage are monitored for quality control and to maintain integrity and security. Concurrently, a database has been developed to organize sample storage and corresponding patients' clinical information.

Results

From April 2015 to present, we have collected more than 13000 follicular fluid and granulosa/cumulus cell samples, over 3000 seminal fluid and sperm samples, 333 embryos, 252 immature/unfertilized oocytes, and over 3000 embryo culture media droplets from consented patients. Recently, we started the collection of urine and blood samples and collected about 40 urine and 150 blood samples. Our in-house developed database currently manages more than 20,000 samples and contains information about biospecimens, patients, data and everything related to repository management (users, and access restrictions) in which all personal identifiers are coded. The web portal has two graphical interfaces for two groups of authorized users, biobank staff and researchers to manage and access data, respectively. The researcher portal is designed for the internal researchers only to view de-identified samples and data and make their request orders, Figure 2. By incorporating the latest technology in the biobanking industry, our program can provide multitude of services: 1. Patient recruitment and screening 2. Biospecimen collection and processing 3. Storage and shipping 4. Sample data management 4. Consulting services, Figure 3. Biobanking is now a more carefully controlled and "professionalized" scientific endeavor. Furthermore, more reviewers and editors are aware of details about specimen collection and processing and how they may affect the quality of the study and the resulting manuscript. To maintain the quality, we've established a quality management system that systematically monitors and evaluates all aspects of our biobanking processes. Our goal is to offer unbiased and high-quality samples and data using well-designed and documented procedures. We've had continuous improvements in the infrastructure, and the operational set-up. We also record and report various pre-analytical variables including, ischemia times, number of eggs in each follicle; 0-1 egg, annotating and labeling, bloody samples (FF vs Plasma), transportation condition (cold vs RT), processing variables (Type/Duration/Method), storage and retrieval condition (Temperature, Duration).

Security Measurements:

1. Data encryption strategies
2. Data back-up at geographically disparate locations
3. Complete audit trail, along with a date and time stamp
4. Necessary server and auditing guidelines to ensure complete data security and privacy

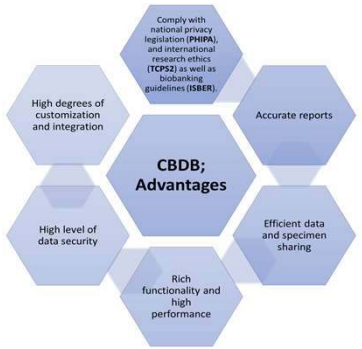


Figure 2. CReATe Biobank database

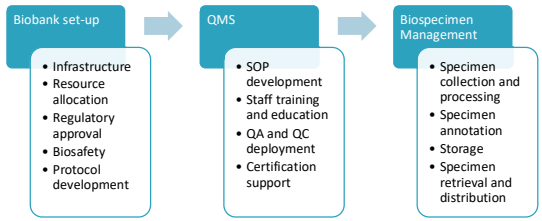


Figure 3. Consulting services

Mission statement

For Patients

To represent our patients by providing researchers access to biospecimen and data supporting the development of novel diagnostic tests and treatments

For Researchers

To empower research teams by ethically collecting, processing, storing, analyzing, and distributing high-quality biospecimens and data.

For Our Community

To be standard for the reproductive biology biobanking and through stakeholder relationships, we serve the scientific community to implement novel research and improve personalized medicine.

Conclusion

The CReATe Biobank has become a hybrid biobank and has developed a comprehensive library of annotated patient samples. It supports an increasing number of national and international collaborations. In order to access the samples for REB approved research projects, researchers can submit an application form to request samples, Figure 4. We are also able to prospectively collect samples tailored to specific research study requirements.

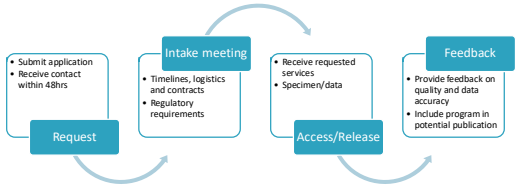


Figure 4. Requesting specimens/services

References

1. Canadian Tumour Repository Network (CTRNet). Available at https://www.ctrnet.ca. Accessed 8 April 2015.
2. ISBER (International Society for Biological and Environmental Repositories). 2012 Best Practices for repositories.

ACKNOWLEDGEMENTS

We thank the clinical and research personnel at CReATe Fertility Centre for their cooperation as well as our patients for their generous donations.

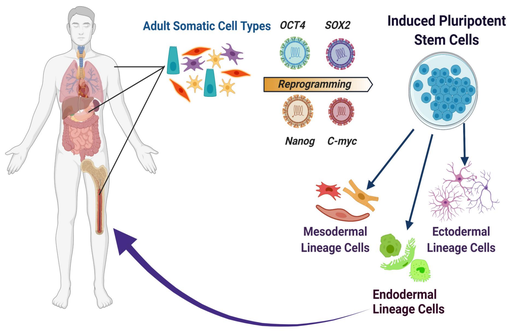
Human Induced Pluripotent Stem Cells Reprogrammed from First Trimester Human Umbilical Cord Perivascular Cells: A Novel Disease-Modelling Platform

Mirabelle Ho[1,*], Miriel Ho[1,*], Siwei Chen[1], Ran Antes[1], Svetlana Madjunkova[1], Clifford L. Librach [1,2,3,4]

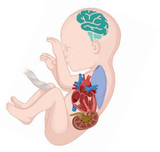
[1] CReATe Fertility Center, Toronto, Canada, [2] Department of Obstetrics and Gynecology, Faculty of Medicine, University of Toronto, Toronto, ON, Canada, [3] Department of Physiology, University of Toronto, 1 King's College Circle, Toronto, ON Canada, Canada, [4] Institute of Medical Sciences, University of Toronto, 1 King's College Circle, Toronto, ON Canada, Canada

* Authors contributed equally to the work

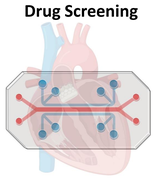
Induced Pluripotent Stem Cells



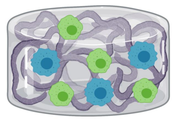
Developmental/Disease Modelling



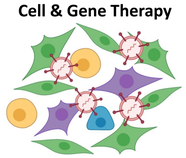
Drug Screening



Bioengineering



Cell & Gene Therapy

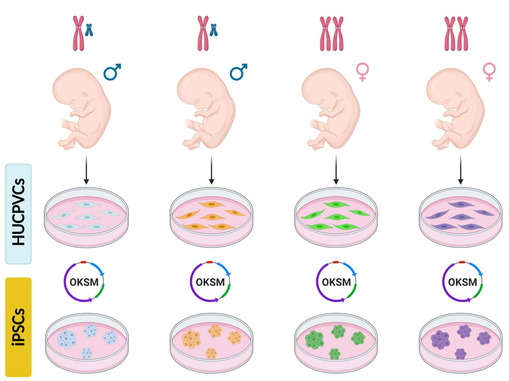


Hypothesis: FTM-HUCPVCs from male and female donors may be reprogrammed to generate FTM-iPSCs

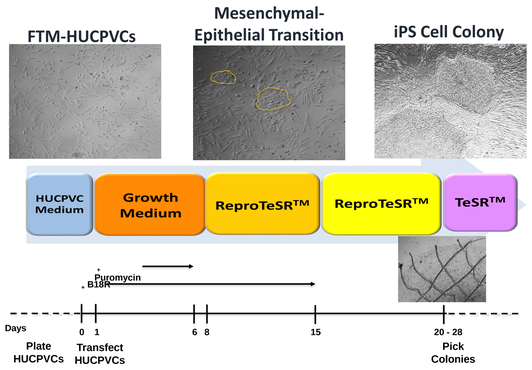
Objective 1: Reprogram FTM-HUCPVCs into FTM-iPSCs

Objective 2: Characterize FTM-iPSC lines

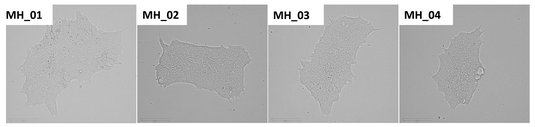
Objective 3: Utilize FTM-iPSC lines as a disease-modeling platform



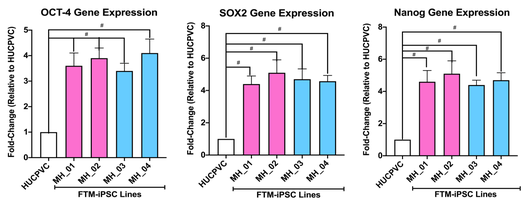
Reprogramming Schematic



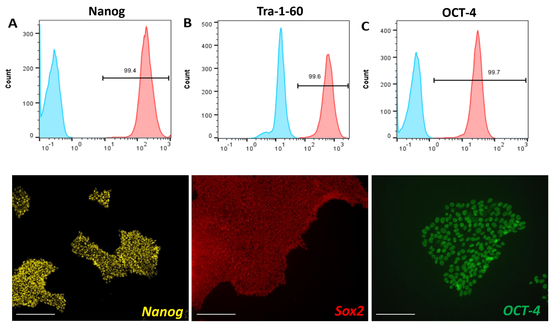
Reprogrammed FTM-iPSCs Characterization



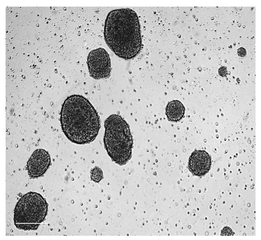
RESULTS			
Sample #	Sample ID	Results	Interpretation
MH-1	CELL-LINE-MH1-NextSeq	46, XX	Euploid, female
MH-2	CELL-LINE-MH2-NextSeq	46, XX	Euploid, female
MH-3	CELL-LINE-MH3-NextSeq	46, XX	Euploid, female
MH-4	CELL-LINE-MH4-NextSeq	46, XX	Euploid, female
MH-5	CELL-LINE-MH5-NextSeq	46, XX	Euploid, female
MH-6	CELL-LINE-MH6-NextSeq	46, XX	Euploid, female
MH-7	CELL-LINE-MH7-NextSeq	46, XX	Euploid, female
MH-8	CELL-LINE-MH8-NextSeq	46, XX	Euploid, female
MH-9	CELL-LINE-MH9-NextSeq	46, XX	Euploid, female
MH-10	CELL-LINE-MH10-NextSeq	46, XX	Euploid, female
MH-11	CELL-LINE-MH11-NextSeq	46, XX	Euploid, female
MH-12	CELL-LINE-MH12-NextSeq	46, XX	Euploid, female
MH-13	CELL-LINE-MH13-NextSeq	46, XY	Euploid, male
MH-14	CELL-LINE-MH14-NextSeq	46, XY	Euploid, male
MH-15	CELL-LINE-MH15-NextSeq	46, XY	Euploid, male

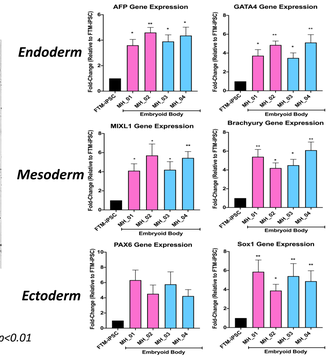


Graph represent mean ± SEM, N=3, # p<0.05

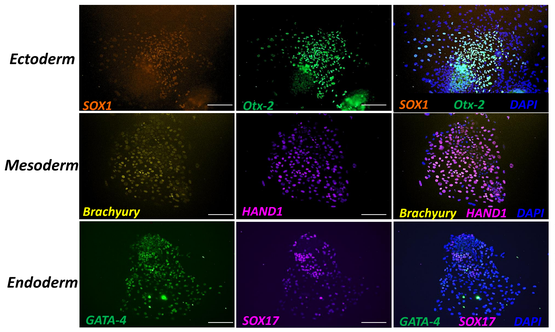


FTM-iPSCs Are Capable of Spontaneous Tri-lineage Differentiation

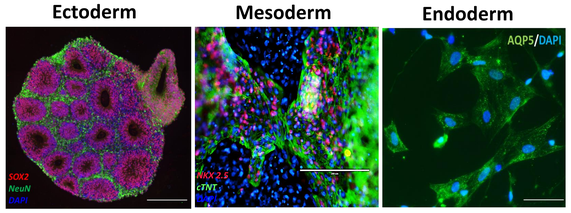




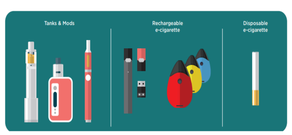
*Graph represent mean ± SEM, N=3, *p<0.05, ** p<0.01*

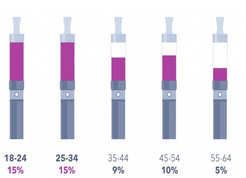


FTM-iPSCs Are Capable of Directed Tri-lineage Differentiation



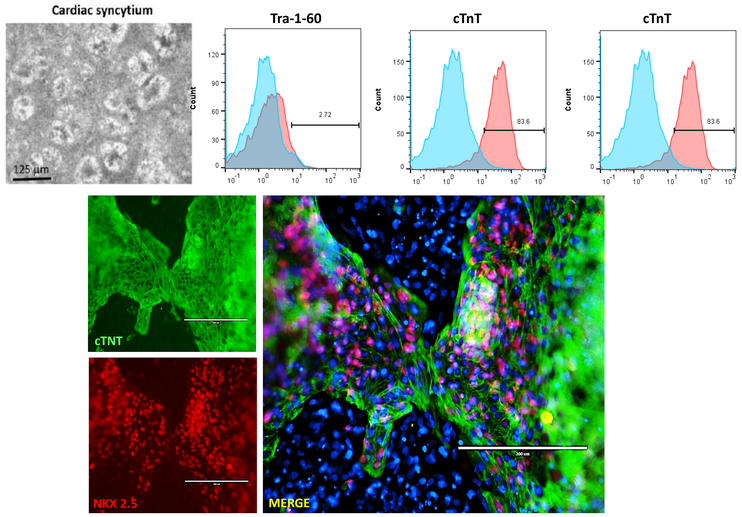
Effects of Vaping During Pregnancy on Early Fetal Organ Development



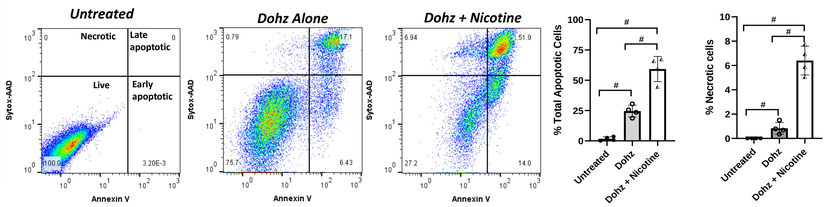


- Increased popularity of vaping (due to misconception)
- Flavorants in e-liquids (>7000) release toxic aldehydes
- Long term health effects are unknown
- Literature draws on studies of nicotine in traditional cigarettes to infer their effect on pregnancy complications and fetal development

Differentiation of FTM-iPSCs into Cardiomyocytes

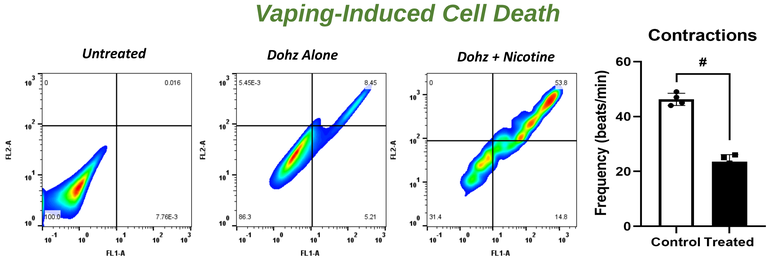


Acute Vaping Exposure Triggers Two Distinct Forms of Cell Death



Graph represent mean ± SEM, N=4, # p<0.05

Disruption in Mitochondria Membrane Potential is Implicated in Vaping-Induced Cell Death



Conclusion

Characterized to be :-

- ❖Karyotypically Normal
- ❖High Pluripotency Gene and Protein Expression
- ❖Capable of Spontaneous and Directed Tri-lineage Differentiation
- ❖ Novel Models of Disease and Development

Canadian Fertility Nurses' Learning Needs

Danielle Dubois[1], Anne-Laure Nivet[2], Alayna Lea[2]

[1]Ottawa Fertility Centre [2]EMD Inc., Mississauga, Canada; a business of Merck KGaA, Darmstadt, Germany.

INTRODUCTION: There are no standardized Fertility Nurse training programs or certification courses available in Canada. Fertility related skills and knowledge are learned through independent fertility centre onboarding, CFAS – ASRM resources and events, and learning programs offered by Industry.

An understanding of the effectiveness of the current learning systems is not known, and would be helpful for improvements in curriculum design and delivery. It would allow the CFAS NSIG and industry partners to address specific nursing needs in their preferred format, increasing nurses' comfort and competency which could ultimately lead to improved patient care.

PURPOSE: To establish fertility nurse topic knowledge, skills confidence and weaknesses, as well as to define levels of interest for further education/training on relevant topics in desired learning formats.

METHODS: A steering committee formed of medical experts (EMD Serono and fertility nurses from independent centres), developed a survey based from the CFAS-ASRM Nursing Competency Framework.

The survey was circulated to all fertility nurses across Canada through CFAS NSIG communications, and completed by nurses between February – May 2020.

A 4-point Likert scale was used for majority of questions, and descriptive statistical data analysis was used to interpret results.

Three Sections included: Demographics, Learning Topics and Tasks, and Preferred learning Formats.

DEMOGRAPHICS: Approximately 69.9% of the nurses who answered the question are practicing in Ontario, 10.7% in the West (British Columbia), 10.7% in the prairies (Alberta, Manitoba, Saskatchewan), 5.8% in Quebec, and 2.9% in the Atlantic (New Brunswick, Newfoundland, Nova Scotia, Prince Edward Island). The regional proportions are relatively representative of the approximate proportion of Canada fertility centre numbers per area.

TABLE 1: Demographics of Survey Respondents

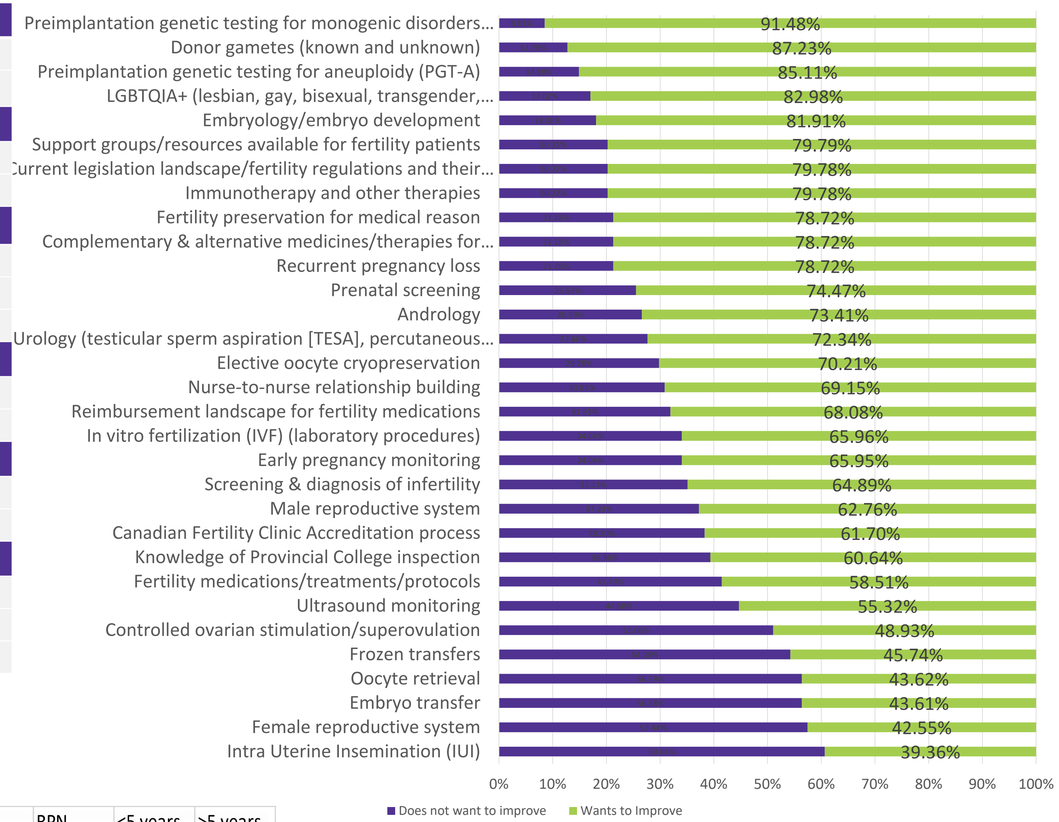
Designation	% (n=106*)
Registered Nurse	70 (n=74)
Registered Practical Nurse (RPN)/ Licensed practical nurse (LPN)	30 (n=32)
# Years of Practice	
≤5 years	57 (n=59)
>5 years	43 (n=44)
Clinic Setting	
Private	86 (n=87)
Hospital-based	14 (n=14)
Other (not specified)	n=2
Clinic Type	
Main Centre	89 (n=92)
Satellite Centre	11 (n=11)
Procedures Performed	
IUI	98 (n=101)
IVF	92 (n=95)
Membership	
CFAS	43 (n=44)
Other (ASRM)	9 (n=9)
Completed ASRM Fertility Nurse Certificate Course	26 (n=27)

*N =106 (n=3 did not provide years of experience, location, services, clinic setting, or affiliations)

TABLE 2: Learning Topics Desire to Improve

Moderate or high desire for training per subgroup and topics	RN	RPN	<5 years	>5 years
Explaining the diagnosis to patients	70.18%	81.48%	80.00%	64.70%
Explaining diagnostic testing procedures to patients	49.12%	77.78%	66.00%	47.06%
Counselling patients on optimizing fertility through lifestyle changes	73.68%	74.07%	78.00%	67.65%
Assessing patient/partner's readiness to learn	38.59%	51.85%	46.00%	38.24%
Educating patients on the female reproductive system	38.59%	62.97%	52.00%	38.23%
Educating patients on the male reproductive system	64.91%	74.07%	74.00%	58.82%
Educating patients on intrauterine insemination (IUI)	26.32%	59.25%	44.00%	26.47%
Educating patients on in vitro fertilization (IVF)	40.35%	81.48%	66.00%	35.29%
Counselling patients undergoing IUI	33.34%	66.66%	52.00%	32.35%
Counselling patients undergoing IVF	43.86%	81.48%	64.00%	44.11%
Educating patients on the various fertility medications and how to use/administer them	24.57%	59.26%	44.00%	23.53%
Assisting patients in navigating the drug reimbursement process	56.14%	62.96%	60.00%	55.89%
Advising patients on support groups/where to go for additional information	80.71%	77.78%	80.00%	79.42%
Counselling patients during early pregnancy	82.45%	81.48%	86.00%	76.47%
Counselling patients on elective oocyte cryopreservation	75.44%	85.18%	86.00%	67.65%
Counselling patients on fertility preservation for medical reason	80.71%	74.07%	84.00%	70.59%
Counselling patients following a miscarriage or termination	80.70%	92.59%	92.00%	73.53%

TABLE 3: Learning Tasks Desire for Further Training



RESULTS: Nurses with ≤ 5yrs experience and RPN/LPNs have an overall higher interest for learning within all topics surveyed. Nurses' motivation for learning is topic-based, staying up to date, and earning CMEs. Onsite lunch & learns, conferences and regional events are the preferred learning formats. Case-based workshops, expert panels and best practices sharing are the preferred in-person learning formats. Lunch time events on Wednesdays/Thursdays are the preferred date and time. Interprofessional collaboration with REIs was rated as very confident, but also ranked as very high desire for further training.

CONCLUSION: This was the first Canadian fertility nurse educational needs assessment. It determined that different nursing groups have different learning needs. It also determined that there should be more ongoing education provided on "soft topics" and skills.

Canadian Fertility and Andrology Society Annual Conference, Vancouver, BC. September 2021

Identifying Factors Influencing Embryo Donation for Reproductive Uses and Gaps in Current Literature

Anushka Kuwar[a,b], Samantha Yee[a], Noga Fuchs Weizman[a], Clifford L. Librach[a,c,d]

[a]CReATe Fertility Centre, Toronto, ON, Canada; [b]University of Waterloo, Waterloo, ON, Canada;
[c]Departments of Obstetrics and Gynecology; and [d] Physiology, University of Toronto, Toronto, ON, Canada.

INTRODUCTION

Embryo donation (ED) is a form of third-party reproduction in which fertility patients donate their surplus embryos to others for reproductive use. When compared with other embryo disposal options such as donating to research, thawing and discarding and indefinite storage, ED is commonly known to be the least preferred option among patients (Provoost et al, 2011; Alexander et al, 2020).

This literature review aims to identify factors that influence patients' decisions to donate their surplus embryos for reproductive use by conducting a literature search to identify research articles related to the decision-making aspects of ED. The key findings of each article were synthesized and compiled into a list of factors that are influential in ED decision-making. Lastly the gaps in the existing literature and research findings were identified and suggestions for future research made.

METHODS

The online databases Scopus, Web of Science and PubMed were used to conduct a literature search on the topic of embryo donation. The search terms used were "Embryo dispos*" OR "Embryo donation" OR "Embryo adoption" AND ["reproduction" OR "family-building" OR "third party reproduction"].

Inclusion criteria:

1. Psychosocial-based research studies,
2. Centred around the attitudes, preferences and decisions regarding ED
3. Published between January 2000 to March 2021; and
4. Published in the English language

Exclusion criteria:

1. Centred around disposition options other than embryo donation for reproductive use; and
2. Conference abstracts, commentaries, opinion pieces, articles, thesis/dissertations or book chapters

RESULTS

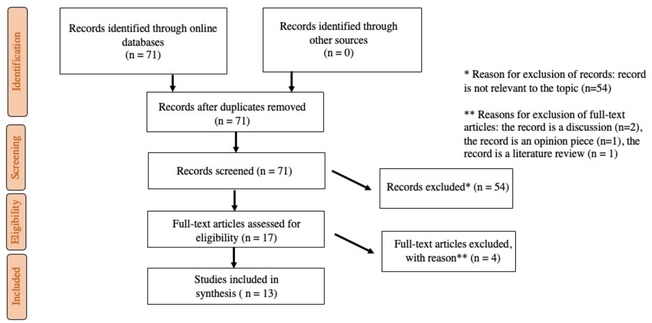


Figure 1: PRISMA Flow Diagram

Study Description:

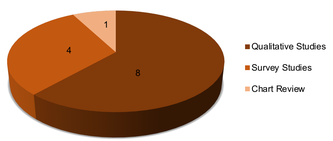


Figure 2: Methodology of studies included in literature review

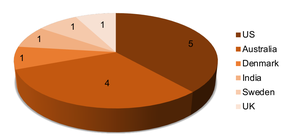


Figure 3: Location of studies included in literature review

- The samples comprised of of IVF patients who had stored embryos (n = 7), embryo donors and recipients (n = 2), infertile patient (n = 1) and patients who have already made a disposition decision (n = 3)
- 7 studies had a sample size of >100 individuals (from ~123 patients to 1020 patients), 6 studies had < 100 individuals

DISCUSSION

Key Findings:

Table 1: List of Motivating Factors Identified in the Literature Review

	Authors (Year, Location)	Altruistic desire to help other families	Embryos deserve a chance at life	Avoiding wasting a valuable resource	Emotional detachment with the embryos
1	de Lacey (2005), Australia	✓			
2	Hammarberg et al. (2005), Australia		✓		
3	Lyerly et al. (2006), USA		✓		
4	de Lacey (2007), Australia		✓	✓	✓
5	Nachtigall et al. (2009), USA	✓		✓	
6	Lyerly et al. (2010), USA	✓	✓		
7	Frith et al (2011), UK		✓		
8	Millbank et al. (2013), Australia	✓	✓	✓	✓
9	Wanggren et al (2013), Sweden	✓			
10	Bartholomaeus & Riggs (2018), Australia	✓	✓	✓	
11	Chandy et al. (2019), India/USA	✓			

I. Motivators

The following factors motivate people to choose ED for reproductive use over other disposal options (Table 1):

A. Altruistic desire to help
- Having experienced the fertility journey themselves, they know how stressful the process can be
- View embryos as a chance to help others start their families and give them a chance to experience parenthood
- Want to make the process easier for others with infertility struggles

B. Embryos deserve chance of life
- Having the potential to become full human beings, embryos are seen as precursors to life that deserves special consideration
- Donating to others would give the embryos a chance at life

C. Avoiding wastage of a valuable resource
- Embryos symbolize a substantial financial and emotional investment of a long fertility journey
- Donating to others would make good use of resource and help to preserve the original intention of creating embryos

D. Emotional detachment with the embryos
- Embryo donors view the offspring as legally and socially the recipients' child
- Trust that the recipients are good people with strong parenthood desire

Table 2: List of Inhibiting Factors Identified in the Literature Review

	Authors (Year, Location)	Concerns about offspring being raised by unknown parents	Genetic linkage of embryos to themselves	Genetic linkage of embryos to their children	Concern about being contacted by unknown offspring	Lack of understanding of the process
1	Bangsboll et al. (2004), Denmark		✓			
2	de Lacey (2005), Australia	✓	✓	✓	✓	
3	Hammarberg et al. (2005), Australia	✓			✓	
4	Lyerly et al. (2006), USA	✓				✓
5	de Lacey (2007), Australia		✓			
6	Nachtigall et al. (2009), USA	✓	✓	✓		✓
7	Lyerly et al. (2010), USA	✓	✓			
8	Lanzendorf et al. (2010), USA	✓	✓	✓		
9	Millbank et al. (2013), Australia	✓	✓	✓	✓	
10	Wanggren et al (2013), Sweden					✓
11	Bartholomaeus & Riggs (2018), Australia	✓				

II. Inhibitors

The following factors inhibit people from donating their embryos to others, preferring to choose other disposal options such as discarding or donating to research (Table 2):

A. Genetic linkage to themselves
- Due to genetic contribution of one parent or both parents to creating embryos, some patients view them as 'their children' and there is a deep emotional attachment
- The genetic linkage and associated attachment makes patients uncomfortable with the idea of unknown recipients raising the offspring born from their donated embryos

B. Genetic linkage to their children
- Surplus embryos are seen as genetic siblings to existing children born from embryos created in the same IVF cycle
- Patients dislike the idea of their children having unknown genetic siblings in the world due to concerns about them unknowingly forming romantic relationships with their genetic siblings

C. Concerns about offspring being raised by unknown parents
- The genetic linkage creates a sense of moral responsibility towards ensuring the continued safety and welfare of the embryo
- Unable to trust unknown recipients to raise the offspring born from their donated embryos
- Did not want the offspring to grow up in undesirable situations, feeling unloved or unwanted

D. Concern about being contacted by unknown offspring
- Anonymity cannot be guaranteed with the availability of home-based DNA testing kits that can track genetic linkage
- For some people there is a fear that the offspring resulting from the donation would track them down and ask why they were given away or ask to be a part of the donors' family

E. Lack of understanding of the process for informed decision
- Likelihood of donation is impacted by how much information and guidance the patients are given by the IVF clinics
- Giving too much information leaves patients overwhelmed and confused
- Giving too little information leaves them feeling lost and uncertain
- Both cases lead them to prefer the easiest, most convenient options such as discarding or storing indefinitely, without considering the benefits of ED

Avenues for future research

A thorough analysis has identified some gaps in the existing literature, highlighting areas that can benefit from future research

1. Dearth of research on motivating factors
- Research on motivating factors is considerably smaller than that on inhibiting factor
- Due to the fact that the number of patients opting for ED is consistently lower than those choosing not to donate
- More studies are needed to better understand the decision-making process of embryo donors

2. Lack of research on impact of missing genetic relatedness
- There are possible situations where the embryo is not genetically related to either parent
- Since the existence of a genetic linkage has an inhibiting effect on ED, it is possible that the opposite - a missing genetic link – is also influential to ED decision-making
- More studies are needed to understand the degree of genetic relatedness in influencing ED disposition decisions

3. Future longitudinal studies
- Most current research is cross-sectional, highlighting attitudes/preferences and the final decision at one point in the fertility journey
- Future studies should employ longitudinal design to investigate the changes of ED decisions and preferences throughout the fertility journey, and the factors these changes depend on and at what points in the journey these changes occur

CONCLUSION

This review has enumerated and discussed the factors that influence ED decision-making, providing a comprehensive list of motivators and inhibitors. Additionally, this review has also identified areas that have not been as thoroughly researched, providing avenues for future research to better understand the ED decision-making process.

What psychotherapy techniques do women prefer for the treatment of infertility-related distress?

Women's Mental Health RESEARCH UNIT

Jennifer L. Gordon, PhD,
Sabrina Lybeck, BSc, Loveness Dube, PhD
University of Regina, Regina, SK, Canada

University of Regina

Background

- 30-40% of infertile women experience clinically significant anxiety or depression.
- Currently available psychotherapeutic approaches have been of limited efficacy in treating infertility-related distress.
- It may be beneficial to consider what additional evidence-based therapy techniques may apply to infertility

Objectives

- **Identify psychotherapy techniques that could apply to infertility-related distress**
- **Assess women's impressions of their perceived usefulness**

Methods

- All psychotherapeutic approaches endorsed by the American Psychological Association as being evidence-based for the treatment of depression, anxiety, relationship problems, and chronic illness, were identified.
- Approaches were broken down into component techniques.
- Each technique was summarized for a lay audience in collaboration with patient advisors.
- Women with current or past infertility from online support groups were invited to rate each technique according to its perceived usefulness (/10) and to identify any 'most hated' techniques.
- Women also provided information pertaining to demographics and reproductive health history.
- Women currently struggling to conceive also completed questionnaires assessing depressive symptoms (PHQ-9), anxiety (GAD-7), fertility quality of life (FertiQoL), and relationship satisfaction (RDAS).
- Repeated measures ANOVA compared preference to the various techniques
- Repeated measures linear regression was used to examine whether participant characteristics predicted technique preference

Results

- Five psychotherapy approaches were identified: Cognitive Behavioural Therapy (CBT), Interpersonal Psychotherapy (IPT), Mindfulness-Based Cognitive Therapy (MBCT), Acceptance and Commitment Therapy (ACT), and Emotionally-focused therapy
- 644 women completed the survey: 449 currently struggling to conceive and 195 with a history of infertility
- Among those currently struggling, 76% scored in the clinical range for anxiety and 34% scored in the clinical range for depressive mood

Technique	Rating (/10) M (SE)
Addressing complicated grief (IPT)	6.4 (0.1)
Problem solving (CBT)	6.3 (0.1)
Increasing social support (IPT)	6.3 (0.1)
Mindfulness meditation (MBCT)	6.2 (0.1)
Values clarification & commitment (ACT)	6.2 (0.1)
Behavioural Activation (CBT)	6.1 (0.1)
Diaphragmatic breathing (CBT)	6.0 (0.1)
Cognitive restructuring (CBT)	6.0 (0.1)
Core beliefs (CBT)	5.9 (0.1)
Cognitive defusion (ACT)	5.8 (0.1)
Communication analysis (IPT)	5.6 (0.1)
Emotionally-Focused Therapy for couples	5.4 (0.1)
Scheduling worry Time (CBT)	4.7 (0.1)
Exposure to infertility reminders (CBT)	3.2 (0.1)

Table 1. Perceived usefulness of therapy techniques.
Note: Those in pink box are statistically equivalent, p>.05

- Neither depressive symptoms, anxiety, fertility quality of life, relationship satisfaction, nor time spent trying to conceive predicted technique preferences (ps >.05).
- The five most hated techniques were: exposure (20% identified it as a hated technique), scheduling worry time (15%), diaphragmatic breathing (8%), communication analysis (7%), and values clarification (6%).

Table 2. Brief description of top-rated psychotherapy techniques

Addressing Complicated Grief

- Address unresolved beliefs that prolong grief (e.g., blame of self or medical professional for loss/failure)
- Increasing communication between romantic partners about how partners can best support each other

Problem Solving

- Aimed at improving approach to solving infertility-related problems (e.g., financial strain, tx decision making)
- Involves psychoeducation about good problem solving practices and applying specific steps in one's approach

Increasing Social Support

- Increase the number of people one can approach for social support, either by forming new relationships or improving support from existing relationships.

Mindfulness Meditation

- Develop the practice of paying attention to one's thoughts and emotions without judgment
- Allows one to become aware of one's negative thoughts and feelings without becoming overwhelmed by them

Values Clarification

- Involves reflecting on one's overarching life values and how one could increase engagement in activities that are in service of those values (e.g., value of community or friendship), despite one's struggles with infertility

Behavioural Activation

- Increasing engagement in previously enjoyed activities into one's daily life (e.g., going out with friends) that may have been abandoned amidst infertility struggles

Conclusion

These findings can be used to develop a new tailored psychological intervention for infertility-related distress

Contact: Jennifer.Gordon@uregina.ca; **Funded by the Saskatchewan Health Research Foundation**

Fertilization and Blastocyst Rates in High DFI Patients and Normal DFI Patients Comparable with the use of Magnetic Sperm Enrichment Technology (MaSE).

[1] Abdul Munaf Sultan Ahamed, [1] R.M.Ruvana Fonseka, [1] Musarrath Begum, [1,2] Yaakov Bentov, [1,3] Marjorie Dixon

[1] Anova Fertility & Reproductive Health, Ontario Canada; [2] Juno Fertility, Ontario, Canada, IVF OBGYN Hadassah mount Scopus Jerusalem ; [3] University of Toronto, Ontario, Canada

Introduction

Natural conception is affected adversely by high DNA fragmentation in sperm. Reactive Oxygen Species (ROS) produced by sperm with high DNA fragmentation can unfavorably affect viable sperm, and in turn, affect fertilization and subsequent blastocyst formation.

•In semen samples with a high percentage of DNA fragmentation, it is beneficial to be able to separate viable sperm from the sperm with fragmented DNA, using nanotechnology. MaSE is a technology that utilizes nanoparticles to decrease DNA fragmentation.

•In our study, we attempted to look at fertilization and blastocyst rates when sperm with high DNA fragmentation was selected for Intracytoplasmic Sperm Injection (ICSI) using MaSE and compare with patients where MaSE was not used.

Methods

This study was done at Anova Fertility between Jan -Dec 2020 (n=307). Patients were split into two groups, based on DNA Fragmentation Index (DFI): normal DFI group (DFI<27) (n=284) and high DFI group (DFI>27) (n=23). In the high DFI group, inclusion criteria included a minimum concentration of 10M/ml and >50% total motility on the day of the Oocyte Pick Up (OPU). Fresh ejaculate was obtained in all cases on day of OPU, with all samples being prepared using density gradient for ICSI.

In the high DFI group, the sperm sample was subjected to MaSE post density gradient. Concentration was estimated using the microcell and for every 5 M/ml sperm present, 50ul of magnetic particles were added, as per manufacturer's instructions (5). Samples were maintained at room temperature and mixed by hand, gently, every 5 minutes for 20 minutes. The tube containing the sample was attached to the magnet, provided by manufacturer for 5 minutes. Supernatant, containing viable sperm, was aspirated and moved to clean tube while the magnet was still attached.

Samples were incubated for 2-3 hours, after preparation, in both groups prior to ICSI. Post ICSI, all eggs were placed in monophasic media (Vitrolife) and fertilized zygotes were group cultured in this medium till day 7.

Results

•In the high DFI group, total fertilization was 77%, normal fertilization (2PN) was 70% and total blastocyst rate was 49%. In the normal DFI group, total fertilization was 77%, normal fertilization was 72% and blastocyst rate was 48%.

Conclusion

Comparable fertilization and blastocyst rates were obtained in both high DFI and normal DFI groups. This may indicate that the use of MaSE was effective in decreasing DNA fragmentation to a level where equivalent fertilization and blastocyst development is obtained as in the normal DFI group.

Further studies using large and similar sample sizes may be beneficial in the future with a follow through up to live birth stage.

#121

Human Umbilical Cord perivascular cells (HUCPVC) for onco-fertility preservation: assessing effects in a tumor-bearing mouse model

Lianet Lopez[1], Hannah Shuster-Hyman[1], Eden Marco[1], Avishai Gasner[1,3], Hasna Khan[1], Samar Mouazz[1], Amanda Kauffman[1], Denis Gallagher[1], Andrée Gauthier-Fisher[1], Clifford Librach[1,2,3,4].

[1]Create Fertility Centre, Toronto, Ontario; [2]Department of Obstetrics and Gynecology, [3]Department of Physiology, [4]Institute of Medical Sciences, University of Toronto; Toronto, Ontario.

lianet@createivf.com

INTRODUCTION

- Mesenchymal stromal cells (MSC) have been studied as candidates for cell therapy in regenerative medicine, including for the treatment of anti-cancer therapeutic drug induced side-effects[1,2] .
- Human umbilical cord perivascular cells (HUCPVCs) derived from first trimester (FTM) and term (TERM) umbilical cords have been characterized as promising sources of MSCs[3].
- HUCPVC have the capacity to maintain their proliferative capacity and regenerative properties when exposed to cytotoxic chemotherapeutics *in vitro* and *in vivo*[4].

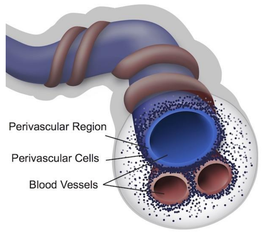


Figure 1. Human Umbilical cord-derived perivascular cells

Treatment with HUCPVC prior to administration of chemotherapy can prevent cyclophosphamide-induced ovarian damage[4] and busulfan-induced loss of male fertility *in rodent models*[5].

Given the discordant findings in the literature about the effects of MSCs on cancer[6], the effect of FTM and TERM HUCPVC on the properties of cancer cells must be studied to evaluate the safety of administering HUCPVC to cancer patients, before such a therapy can be clinically translated.

Our previous *in vitro* studies involving the co-culture of multiple breast cancer and melanoma cell lines with HUCPVC in a transwell co-culture system or with HUCPVC-derived conditioned media suggest that HUCPVC influence cancer cell survival and proliferation in a cell-dependent manner, where pro-, anti- and neutral cancer cell growth effects were observed (Table 1).

Cell line	FTM (TW)			TERM (TW)			FTM CM	TERM CM	UC-MSC Literature
	Growth	Viability	Invasion	Growth	Viability	Invasion	Growth	Growth	Concordant/discordant findings
Breast cancer cell lines									
SKBR3 (PR-, ER-, HER2+)		Not tested			Not tested				
MDA-MB-231 (PR-, ER-, HER2-)		Not tested			Not tested				Ayuzawa et al (Cancer Letters, 2010); Mandel et al (Stem Cells and Development 2013); Li et al (Oncology Reports, 2015); Li et al (Oncology Letters, 2017) Gunthaman et al (Journal of Cellular Biochemistry, 2012); Li et al (Oncology Letters, 2017); Ilie et al (Cell Death & Disease, 2018)
MCF-7 (PR+, ER+, HER2-)		Not tested			Not tested				Mirabdollahi et al (Cell and Tissue Banking, 2019); Mirabdollahi et al (Iranian Journal of Basic Medical Science. 2020) Li et al (Oncology Reports, 2015); Ma et al (Cell Transplantation, 2015)
Melanoma cell lines									
A375							Not tested	Not tested	Wang et al (Oncology Reports, 2018)
SK-MEL-28							Not tested	Not tested	

Pro-tumor effects
Anti-tumor effects
Neutral tumor effects

Table 1. Summary of findings from previous *in vitro* studies performed to determine the effects of FTM and term HUCPVC on 3 human breast cancer and 2 human melanoma cell lines. TW, transwell co-culture; CM, conditioned media culture; UC, umbilical cord)

HYPOTHESIS

FTM HUCPVC can be safely administered for cell therapy in cancer patients undergoing anti-cancer treatment

OBJECTIVE

To determine if HUCPVC modulate tumor growth when injected systemically in a tumor-bearing xenograft mouse model.

MATERIALS AND METHODS

1. ASSESSING EFFECTS OF FTM AND TERM HUCPVC ON MELANOMA TUMOR GROWTH USING A XENOGRAFT ASSAY

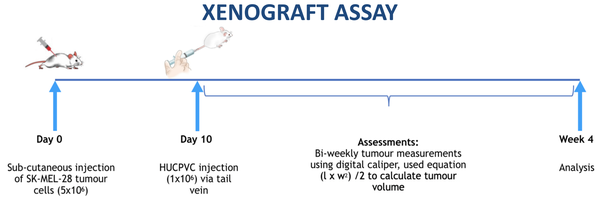


Figure 2. Time course and summary of experimental design to assess the effect of FTM and term HUCPVC in a human melanoma tumor-bearing mouse model.

Experimental details:

- An immunocompromised mouse model was used (NODSCID)
- SK-MEL-28 cells (ATCC, human melanoma tumor-derived) were expanded in culture and injected subcutaneously in Matrigel™ .
- 3 pathogen-free lines of 2 independent lines of FTM and 1 line of Term HUCPVC were expanded in αMEM supplemented with 2.5% HPL; Passage 6 cells were resuspended in Hank's buffered saline solution (HBSS) and administered systemically when tumors were palpable.
- Animals were randomized to each treatment group. HUCPVC injections and tumor measurements done using a caliper were performed by a technician blinded to treatment groups.
- **N=12 per group (from 3 independent experiments)**
- At endpoint, tumors were dissected for histological analysis

Treatment Groups:
FTM line 1
FTM line 2
Term line 1
HBSS (control)

2. ASSESSING THE LOCALIZATION AND FATE OF HUCPVC ADMINISTERED IN A MELANOMA XENOGRAFT MODEL

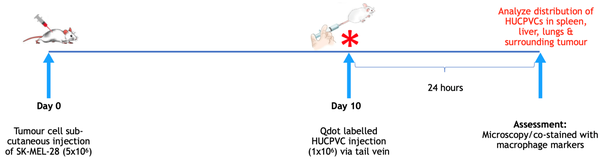


Figure 3. Time course and summary of experimental design to assess localization and fate of FTM and term HUCPVC in melanoma tumor-bearing mouse model.

Experimental details:

- Tumor xenograft assay and cell culture were performed as above
- HUCPVC were pre-labeled with fluorescent Qdot (Thermofisher) on day of injection
- **N=3 per group (24hrs)**
- At endpoint, animals were perfused with 4% PFA. Tumors, liver, lung and spleen were dissected for histological analysis using frozen tissue sections.
- Tissue sections were immunostained for CD68, a pan-macrophage marker
- Tissue sections (6 per tumor, 3 per lung and liver for each animal) were imaged using Evos Fluorescence Microscope.

Treatment Groups:
FTM line 1
FTM line 2
Term line 1
HBSS (control)

RESULTS

MELANOMA TUMOR GROWTH IS REDUCED IN FTM AND TERM HUCPVC-TREATED ANIMALS

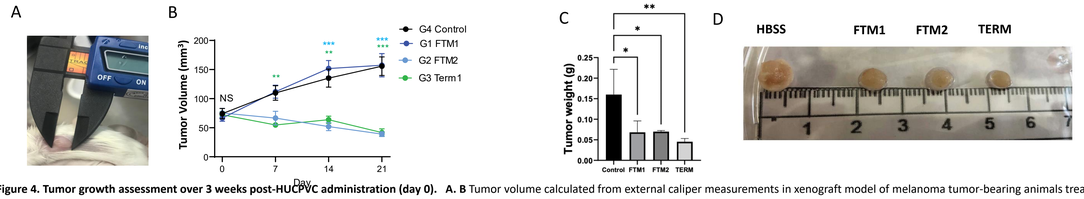


Figure 4. Tumor growth assessment over 3 weeks post-HUCPVC administration (day 0). **A. B** Tumor volume calculated from external caliper measurements in xenograft model of melanoma tumor-bearing animals treated with 2 FTM and 1 term HUCPVC lines or HBSS as a control. **, P<0.01; ***, P<0.001. **C, D** Weight and representative images of tumors after dissection (3 weeks).

THE PROPORTION OF PROLIFERATING CELLS IN TUMORS OF ANIMALS TREATED WITH HUCPVC IS DECREASED

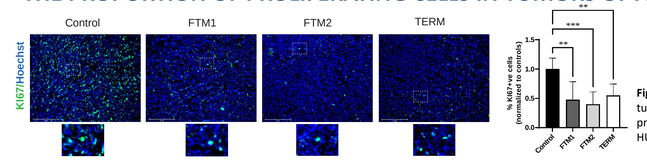
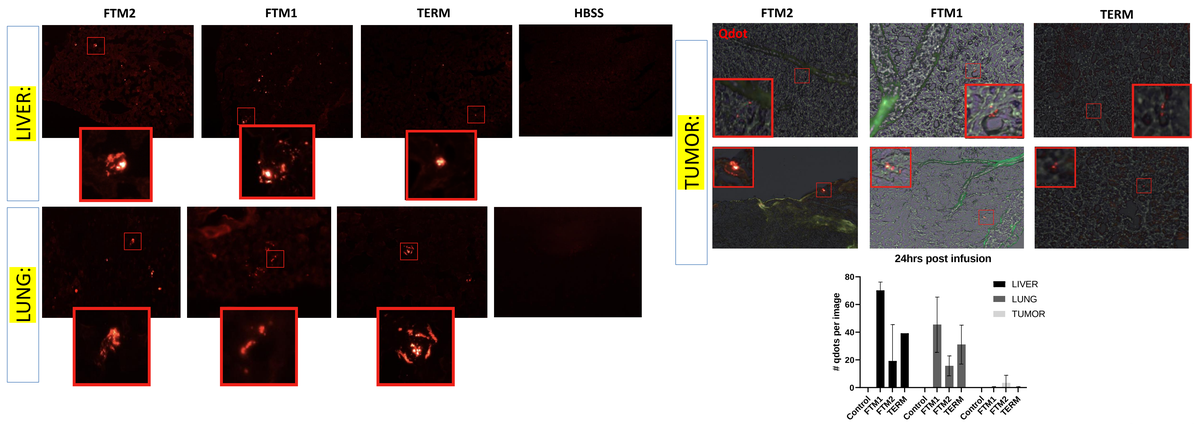


Figure 5. Assessment of Cell Proliferation. Representative images of KI67 immunostaining (green) in tumor tissue sections counterstained with Hoechst to visualize all nuclei (blue) (A) and quantification of proportion of Ki67 +ve cells (B), three weeks after animals were treated with FTM1, FTM 2, 1 term HUCPVC lines or HBSS as a control.

QDOT-LABELED HUCPVC LOCALIZE TO THE LIVER AND LUNGS AND ONLY RARE QDOT SIGNALS ARE DETECTED IN TUMORS 24HRS FOLLOWING INJECTION



~20-40% of QDOT SIGNALS IN THE LIVER AND 3-12% in LUNG CO-LOCALIZE WITH MACROPHAGE MARKER CD68 (24hrs)

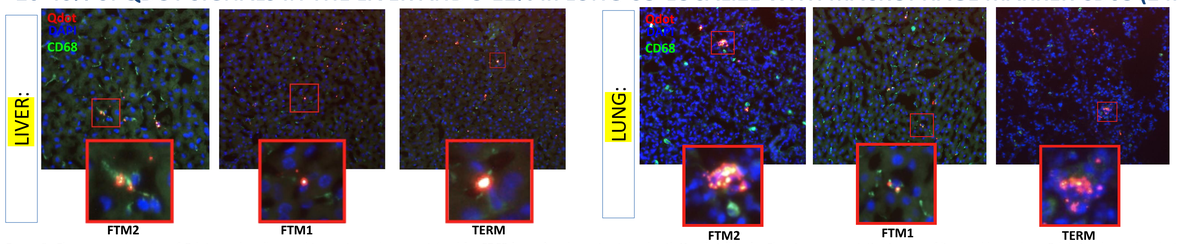


Figure 6. Fluorescence imaging of Qdot signals in liver and tumor tissue immunostained for CD68 (green) and counterstained with Hoechst (blue). Showing representative images of liver and lung where Qdot signal was abundant 24hrs after HUCPVC injection.

CONCLUSION AND DISCUSSION

Conclusions: Systemic administration of FTM and term HUCPVC can prevent melanoma tumor growth in a tumor bearing animal model. HUCPVC do not appear to home to tumors, and a large proportion are engulfed by macrophage in the liver and lungs at 24hrs. This suggests that HUCPVC may modulate tumor growth through paracrine and/or immunomodulatory effects.

This study suggests that FTM and term HUCPVC may represent a safe and effective cell-based therapy for fertility preservation in cancer patients receiving gonadotoxic therapies.

Future Directions: **1**. To assess the effects of HUCPVC in additional tumor models, including on chemotherapeutic drug-treated tumors; **2**. To assess the effect of multiple HUCPVC doses and timing of delivery on tumor growth; **3**. To determine the mechanism by which HUCPVC may modulate tumor growth.

REFERENCES

1. Samsonraj RM et al. Concise Review: Multifaceted characterization of human mesenchymal stem cells for use in regenerative medicine. *Stem Cells Translational Medicine*. 2017;6(12):2173-2185.
2. Zimmerlin L et al. Mesenchymal stem cell secretome and regenerative therapy after cancer. *Biochimie*. 2013; 95(12):2235-45.
3. Hong S.-H et al. Ontogeny of human umbilical cord perivascular cells: molecular and fate potential changes during gestation. *Stem Cells and Development*. 2013;22(17):2425–2439
4. Zohni *et al. Cancer Letters 2021*
5. Mashiach et al Fert Ster Sci 2021
6. Norozi F et al. Mesenchymal stem cells as a double-edged sword in suppression of progression of solid tumor cells. *Tumor Biol.* 2016; 37(9): 11679-11689.

ACKNOWLEDGEMENTS

This project was funded by the CReATe Fertility Centre. We thank Peter Szaraz, Alexander Johnston and Fyyaz Siddiqui for technical assistance, and Aleksandra Uzelac, Madhu Sangaralingam, Ariel Gorodonsky, and Joseph Fish for contributions to related *in vitro* work (data not shown).

Optimization of Microbial DNA Extraction from Follicular Fluid (FF) for Metagenomics Analysis

***Adi Kuperman*[1,2], *Svetlana Madjunkova*[3,4], *Ran Antes*[4] , *Clifford Librach*[1,2,5,6]**

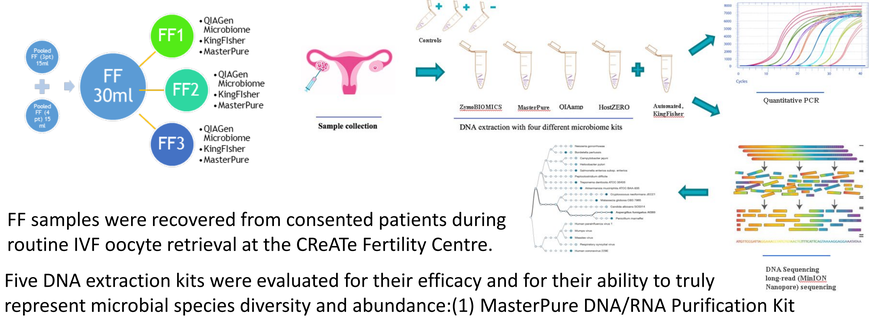
[1]CReATe Fertility Centre, Toronto, Canada; [2]Department of Obstetrics and Gynaecology,[3] CReATe Fertility Centre, Reproductive Genetics, [4]Department of Laboratory Medicine and Pathobiology, University of Toronto, [5]Institute of Medical Sciences and [6]Department of Physiology, University of Toronto, Toronto, ON, Canada;

INTRODUCTION

- Studies have suggested that the human microbiome plays a key role in regulating the pathophysiology of the reproductive tract(RT), and that dysbiosis can affect reproductive outcomes.
- A continuum of the microbiota along the female RT was demonstrated with declined abundance and increased diversity in the upper RT when compared to the lower RT.
- A major challenge of assessing the effects of the upper RT microbiome, is the low abundance of microbial DNA.
- High-throughput sequencing for characterizing the microbiota has led to a profounder understanding of the spectrum of these community structures and function.
- Method choice from sample collection to DNA extraction and sequencing, can greatly affect the microbial community classification, richness, diversity, and relative species abundance and therefore are essential for obtaining reliable data.
- Low biomass samples require modification and optimization of microbiome extraction protocols.

We aimed to identify the optimal DNA extraction kit that best represents the species abundance and diversity of the FF microbiome, with maximal extraction efficiency.

MATERIALS AND METHODS



- FF samples were recovered from consented patients during routine IVF oocyte retrieval at the CReATe Fertility Centre.
- Five DNA extraction kits were evaluated for their efficacy and for their ability to truly represent microbial species diversity and abundance:(1) MasterPure DNA/RNA Purification Kit , (2) ZymoBIOMICS DNA/RNA Mini Kit, (3) QIAamp DNA Microbiome Kit (Qiagen), (4) HostZERO Microbial DNA Isolation Kit(Zymo Research) and (5) KingFisher Flex Purification System (ThermoFisher Scientific).
- FF samples pool from 3 patients were extracted by each kit , together with a negative control (DNase/RNase free-water) and two positive controls - bacterial mock communities with defined species distribution and abundance, ZymoBIOMICS® Microbial Community: Standard-I with equal biomass of different species (- efficacy and selective efficacy), Standard II-Log distribution - species are in very low, medium and high concentration (-sensitivity).

MATERIALS AND METHODS

Extraction Kit	Sample volume needed	Host depletion step	Cell lysis method
MasterPure(MP) DNA/RNA Purification Kit	150µl		desalting process
ZymoBIOMICS DNA/RNA Mini Kit (ZDR)	250µl		combination of mechanical and chemical lysis
QIAamp DNA Microbiome Kit (Qiagen)	1000µl	✓	Bead beating system
HostZERO(HZ) Microbial DNA Isolation Kit(Zymo Research)	200µl	✓	Bead beating system
KingFisher(KF) Flex Purification System (ThermoFisher Scientific).	500µl	✓	Bead beating system

- Bacterial and human DNA in extracted samples were quantified in three replicates using the Femto™ Bacterial and Human DNA Quantification Kits (Zymo Research)
- Library preparation was conducted using the Oxford Nanopore Rapid-PCR Barcoding Kit(SQK-RPB004)
- **50-100fmol** of pooled libraries were then sequenced on a long-read nanonpore sequencing platform using the MinION™ handheld sequencer(Oxford-Nanopore Technology).
- Determine species and abundance of microbes present in samples using Oxford Nanopore's **What's In My Pot** and **EPI2ME** analysis pipeline.

CONCLUSION

- FF DNA amounts are ultra low, detected only with the most sensitive quantification measures.
- Different extraction method influences DNA and bacterial DNA yield.
- MasterPure was the most efficient kit, yielding the highest amount of DNA.
- Host depletion approaches during extraction are not suitable for FF samples, as they yield overall low genomic material available for downstream analysis.

RESULTS

EXTRACTION METHOD AFFECTS DNA YIELD

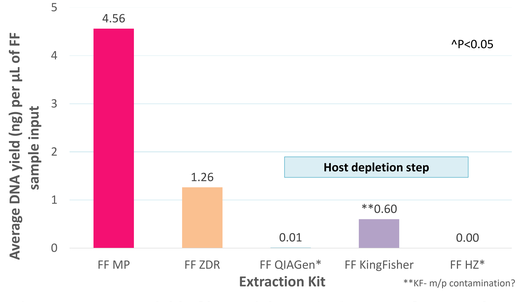


Figure 1: Average yield of bacterial DNA (ng) per µL of FF sample input. Showing overall low genomic material yield with host depletion extraction kits.

ADDITIONAL HOST DEPLETION STEP AFFECTS HUMAN AND BACTERIAL DNA CONCENTRATION

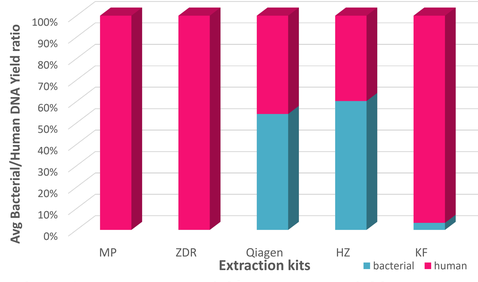


Figure 2: Average Bacterial/Human DNA Yield per µL of FF sample input. Kits with addition of host depletion step efficiently remove Human DNA though increase sensitivity for microbial detection.

LIBRARY PREPARATION FOR METAGENOMIC SEQUENCING

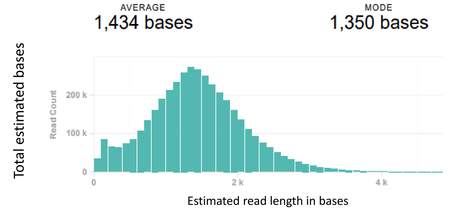


Figure 3: MinION sequencing: read length (bases) of samples prepared with Rapid PCR barcoding kit. Library preparation was successful for FF samples extracted using the Masterpure kits.

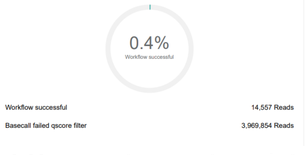


Figure 4: Metagenomic sequencing analysis using WIMP pipeline of the same FF sample extracted with Masterpure Microbiome. Low read with poor quality control, avert accurately detecting microbial genus and species.

Future Directions

- Optimize extraction method of FF DNA, to allow reliable sequencing approach while preserving the diversity and abundance of the FF microbiome.
- Approaches such as whole genome amplification of extracted material, targeted enrichment using 16S Sequencing and new 'Read Until sequencing' algorithms (eg "UNCALLED") may be better to enrich for bacterial genomes in FF.
- Using optimized methodological stream to characterize FF microbiota.

ACKNOWLEDGEMENTS

- This project was funded by the CReATe Fertility Centre
- The authors thank the staff at the CReATe Fertility Centre, especially Ran Antes, Siwei Chen and Rina Abramov for their assistance during this study.
- The authors confirm there are no conflicts of interest

BPA affects DNMT3A transcription, but not translation, in a miR-21-dependant manner in bovine granulosa cells

Reem Sabry, Nicholas Werry, Jonathan LaMarre, Laura A. Favetta
Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, ON Canada
Reem Sabry: rsabry@uoguelph.ca, Laura A. Favetta: lfavetta@uoguelph.ca

INTRODUCTION

Bisphenol A (BPA), one of the most widespread Endocrine Disrupting Chemicals, has been repeatedly linked to negative fertility outcomes (1).

Alternative mechanisms of action on epigenetic pathways have been documented, yet not fully characterized. MicroRNAs (miRNAs), are crucial epigenetic regulators of gene expression, vital for granulosa cell function, and ultimately, oocyte competence.

miR-21, a highly-conserved miRNA expressed in oocytes and granulosa cells, is consistently upregulated after BPA treatment with simultaneous repression of predicted target genes (2).

DNA methyltransferase 3A (DNMT3A) is a miR-21 predicted target gene responsible for regulating global gene expression during early development.

HYPOTHESIS and OBJECTIVES

We hypothesize that:

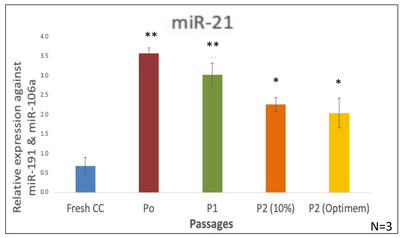
BPA - induced DNMT3A increase is miR-21 dependent in bovine granulosa cells

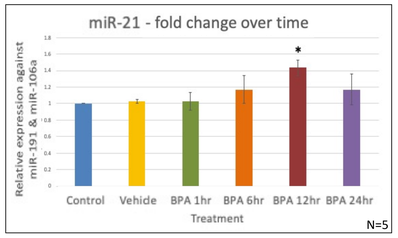
To test this hypothesis we aimed to:

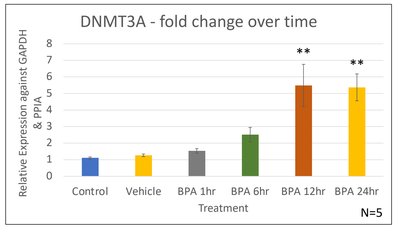
1. Quantify miR-21 levels in culture in the absence/presence of BPA at the LOAEL dose (0.05 mg/mL) at 1, 6, 12, and 24 hrs by qPCR.
2. Knockdown miR-21 using LNA inhibitors and confirm transfection/knockdown by confocal microscopy and qPCR.
3. Quantify DNMT3A mRNA and protein levels by qPCR and Western blotting.

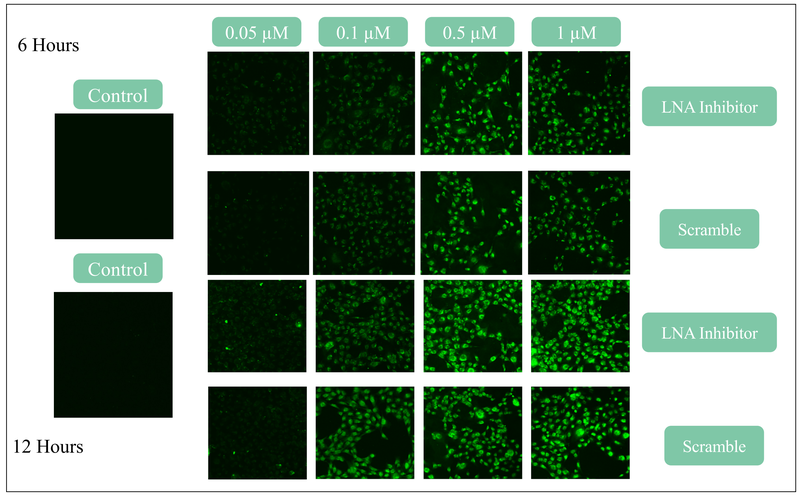
METHODS

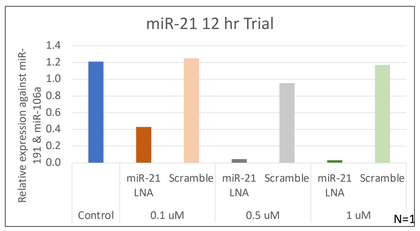
1. Granulosa cells were cultured *in vitro* at various passages, serum restricted, and treated with BPA at various time points.
2. Cells were transfected with either a miR-21 inhibitor or a negative scramble control
3. Knockdown was confirmed using confocal microscopy and qPCR
4. DNMT3A transcript and protein levels were quantified using qPCR and western blotting, respectively.

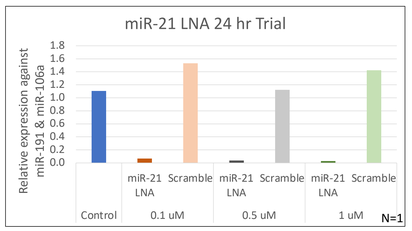


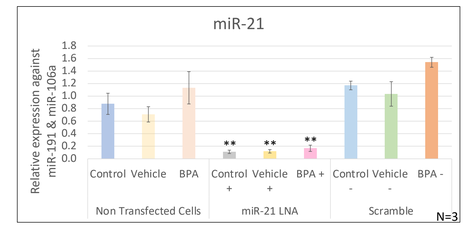


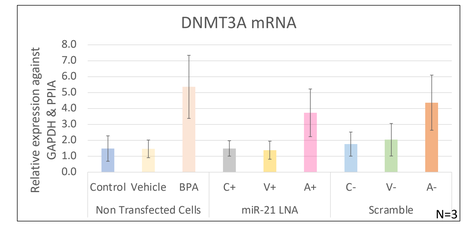


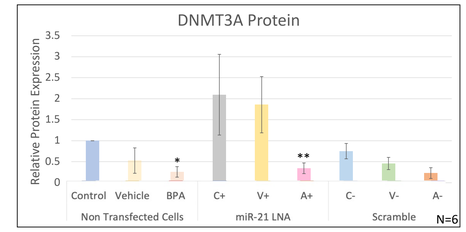


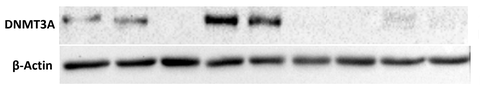












CONCLUSIONS

Our study shows an inverse correlation between two epigenetic regulators, miR-21 and DNMT3A, in bovine granulosa cells exposed *in vitro* to BPA.

These data hint at a possible novel mechanism of action of BPA in the oocyte surrounding environment (granulosa cells) that determines oocyte competence, ultimately affecting development and the achievement of a viable pregnancy.

This project is crucial for understanding the effects of bisphenol toxicity on fertility.

REFERENCES

1. Ferris, J., Mahboubi, K., MacLusky, N., King, W. A.,& Favetta, L. A. (2016). BPA exposure during *in vitro* oocyte maturation results in dose-dependent alterations to embryo development rates, apoptosis rate, sex ratio and gene expression. Reproductive Toxicology (Elmsford, N.Y.).
2. Sabry, R., Saleh, A. C., Stalker, L., LaMarre, J., & Favetta, L. A. (2021). Effects of bisphenol A and bisphenol S on microRNA expression during bovine (Bos taurus) oocyte maturation and early embryo development. *Reproductive toxicology (Elmsford, N.Y.)*, *99*, 96–108. https://doi.org/10.1016/j.reprotox.2020.12.001

ACKNOWLEDGMENTS

Liz St. John, Monica Antenos, Ed Reyes, Allison McKay, Makenna Williams, Ola Davis, Mimi Nguyen, Vivien Truong, Rushi Patel, Emilia Kourmaeva. Jaustin Dufour, Jyoti Sharma and the Department of Biomedical Sciences

Canadian Fertility & Andrology Society

NSERC CRSNG