Deep learning analysis of endometrial histology as a predictive tool for pregnancy outcome of frozen embryo transfer

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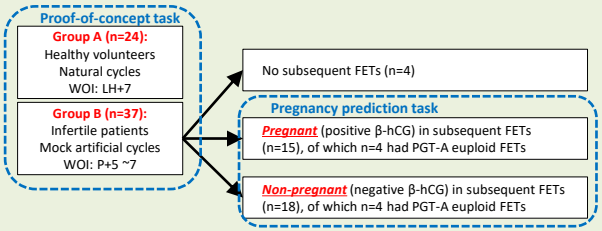
INTRODUCTION

Histology on hematoxylin and eosin (H&E) slide provides rich information associated with endometrial receptivity. However, traditional histological examination, especially Noyes' dating method, is of limited value as it is prone to high variability and is not correlated with pregnancy outcome. Analysis of endometrial histology by deep learning (DL) models such as convolutional neural networks (CNNs) may offer a promising approach to promote consistency of interpretation and maintain objectivity, while maximizing the extraction of morphological features associated with and predictive of for pregnancy.

Objective: To validate CNNs as a tool for endometrial histomorphological evaluation by correlating endometrial histology assessed by CNNs with pregnancy outcomes after frozen-thawed embryo transfers (FETs).

MATERIALS

1. Study population



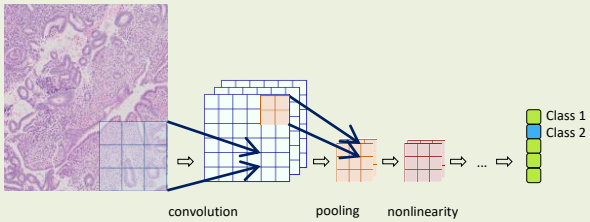
2. Characteristics of subjects with subsequent FETs

Characteristic	Pregnant (n=15)	Non-pregnant (n=18)	P value
Age (y), mean ± SD	34.3 ± 4.0	35.6 ± 3.0	0.27 [a]
BMI (kg/m^2), median (range)	23.6 (19.5 – 39.1)	24.35 (16.7 – 30.4)	0.72 [b]
AMH, mean ± SD	19.66 ± 10.80	26.43 ± 12.73	0.16 [a]
AFC, median (range)	13.5 (5 – 37)	21 (9 – 40)	0.10 [b]
Total failed fresh and frozen cycles	3 (1 – 8)	4 (2 – 9)	0.11 [b]
Total embryos transferred in failed cycles	4 (1 – 13)	5 (3 – 10)	0.19 [b]
Subsequent FET characteristic			
Number of subsequent FET cycles, median (range)	1 (1 – 2)	1 (1 – 3)	0.20 [b]
Number of embryos transferred in subsequent FET cycles, median (range)	1 (1 – 3)	1 (1 – 5)	0.64 [b]
Number of patients having PGT-A euploid embryo transfer, n (%)	4 (4/15=26.7%)	4 (4/18=22.2%)	> 0.99 [c]

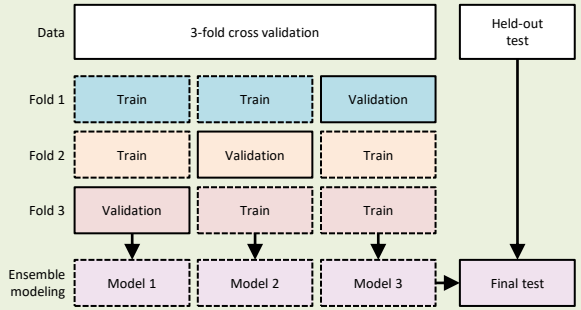
[a] Student t test; [b] Mann Whitney test; [c] Fisher's exact test

METHODS

1. CNNs for classification



2. Three-fold cross validation and held-out test



RESULTS - 1

1. Performance of the classifiers:
(1) Proof-of-concept task: classifier for Group A vs B
(2) Pregnancy prediction task: classifier for pregnant vs non-pregnant

Classifiers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Classifier: Group A vs. B 3-fold cross validation, mean ± SD	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Classifier: pregnant vs. non-pregnant 3-fold cross validation, mean ± SD	83.3 ± 28.9	73.3 ± 23.1	72.2 ± 25.5	86.7 ± 23.1	77.8 ± 22.2
Classifier: pregnant vs. non-pregnant held-out test	75.0	75.0	75.0	75.0	75.0

PPV: positive predictive value. NPV: negative predictive value. Accuracy = (true positive + true negative)/(true positive + false positive + true negative + false negative).

RESULTS - 2

2. Visualization of histopathological features related to pregnancy

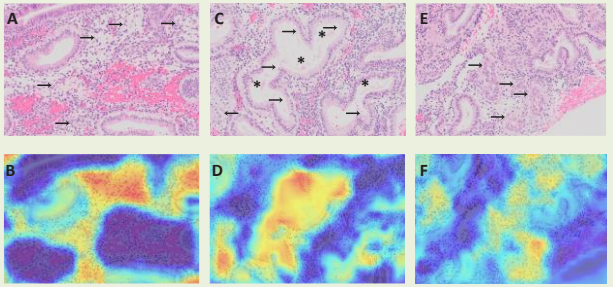


FIGURE 1: Representative pixel-level morphological characteristics related to positive pregnancy outcome visualized by the saliency map. Features that were highly related to positive pregnancy outcome were highlighted in color closer to red. Highlighted regions of interest in Figure 1B correlate "***edematous stoma*** (arrow)" in Figure 1A. Highlighted regions of interest in Figure 1D correlate "***secreting glands emptying the cytoplasmic vacuoles*** (arrow)" in Figure 1C. With secretions released into the lumen (asterisk), the glands become distorted with sawtooth appearance. Highlighted regions of interest in Figure 1F correlate "***enrichment of blood vessels*** (arrow)" in Figure 1E.

SUMMARY

- This study for the first time demonstrated the feasibility and excellence of DL model in predicting pregnancy outcome of FETs by analysis of endometrial histology.
- Our findings also suggest that important pregnancy-related DL features derived from CNNs could be interpreted as human recognizable histopathological characteristics.

ACKNOWLEDGEMENT

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Patient Perspectives in a Large Single Site Embryo Donation Program

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BACKGROUND

Embryo Donation (ED) is a form of third-part reproduction in which fertility patients donate their surplus embryos to others for reproductive use. In Canada, embryos can be cryopreserved indefinitely, and most IVF patient cryopreserve surplus embryos for future use; but a third never return to transfer the embryo. This study aims to explore the perspectives of known and anonymous donors and recipients following participation in a large embryo donation program. Our study has the largest cohort and is the only available Canadian study with a high response rate representative of the ED patient population.

METHODS

- Data were extracted from the counselling reports comprised of 69 patients participated in the CReATe Fertility Center ED program between 2016-2020.
- 67 online surveys were emailed, 45 surveys were collected (67% response rate).
- 43 fully completed surveys were used for analysis (Table 1).
- Quantitative data were analyzed by SPSS using cross-tabulation, Fisher's exact test and t-test. Qualitative free-text comments from the surveys were analyzed by thematic analysis.

RESULTS

Sample Characteristics

- Mean ages of female donors and recipients were 43.1 ± 5.2 (range: 34-54) and 41.2 ± 3.9 (range: 31-48), respectively.
- Mean of 5 embryos donated by each donor.

Table 1. Survey Population Breakdown

	Known Donors (KD)	Anonymous Donors (AD)	Known Recipients (KR)	Anonymous Recipients (AR)	Total
Chart Review of Counselling Reports	**24**	**11**	**24**	**10**	**69**
Surveys Sent	23	10	23	10	67
Surveys Received	15	7	14	9	45
Surveys Completed and Analyzed	**15**	**7**	**13**	**8**	**43**
Response Rate	65%	70%	58%	90%	67%

Source of Awareness of ED (Figure 1)

- The most common source recipients learned about ED was through a *medical professional* (60%) and for donors, an *online platform* (36%)

RESULTS (continued)

Factors Influencing ED Decisions (Figure 2)

- Giving others the *joy of parenthood*, giving *embryos a chance at life, family completion*, and their *own fertility journey* played a major role in the donation decision of KD and AD
- *Family completion* was significantly more important to KD than AD (p=.044)
- 70% of AD made their decision upon receiving the storage renewal form and 40% of KD made their decision during fertility treatment
- 43% of AD and 20% of KD had high feelings of attachment to the embryo

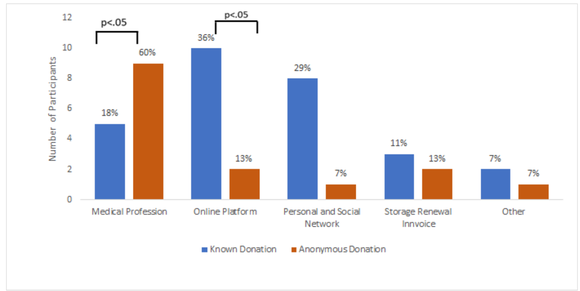

Figure 1. Factors Influencing Donors Decisions to Donate Embryos (15 Known Donors vs 7 Anonymous Donors)

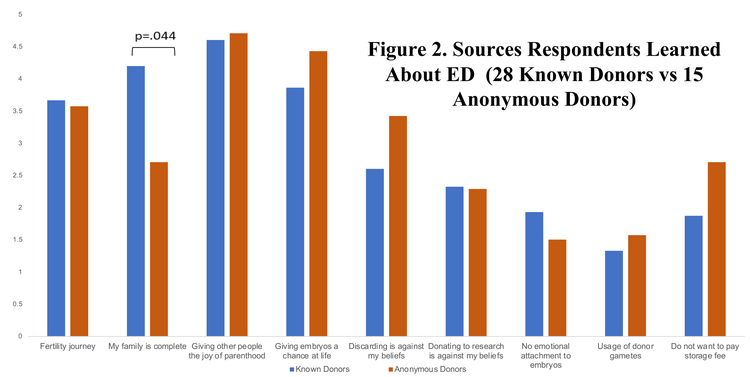

Figure 2. Sources Respondents Learned About ED (28 Known Donors vs 15 Anonymous Donors)

Known Donation Considerations

- Most known matches had no relation prior to ED and 54% of KD did not desire a future relationship
- *Shared values & capabilities* were the top matching criteria for KD and KR

Disclosure Preference

- 93% of known and 74% of anonymous participants were likely to disclose their ED participation with their child(ren) (p=.02)

Concerns Regarding Anonymity

- All AD had low concern and 38% AR were moderately or highly concerned of their child unknowingly having a romantic relationship with a biological sibling
- 86% of AD requested to be notified when a child is born from their donation

Table 2. Anonymous Participants Preference for Known Donation

Sub-themes	Illustrative quotes from free-text comments
a) Would have preferred known donation	"We would have felt more comfortable with a known/open donation."
b) Would have wanted to know the donor	"I would prefer known donation. And would love to be able to talk to our donors. So thankful for what they did."
c) Would have wanted to express gratitude to their donor	"Just would really like the donors to know how grateful we are."
d) Known donation is a better model	"As most research points to the importance of openness in embryo donation/adoption situations, we believe it's important that [clinic name] look at ways to offer more open donation. In this day and age it's difficult for anyone to remain anonymous and it would be great to have the support and resources of the clinic to support contact between children and donors."

DISCUSSION

- Anonymous participants learned about ED from medical professionals. On the other hand, most known participants discovered this option through the internet and only a few through medical professionals.
- 27% of respondents had used donor gametes; yet usage of donor gametes was the least important decision factor
- Storage fees and discarding embryos being against their beliefs played a stronger role in the donation decision of AD
- Most AD opted for identity release and wanted to be notified of a livebirth, despite identity disclosure not being mandated in Canada
- Known participants were significantly more satisfied with the ED process, and many anonymous participants indicated in free-text comments that they would have preferred known donation if given a choice (Table 2)

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 research, providing avenues for future research to better understand the ED decision-making.

BPA, but not BPS, affect AMH signaling pathway in bovine granulosa cells

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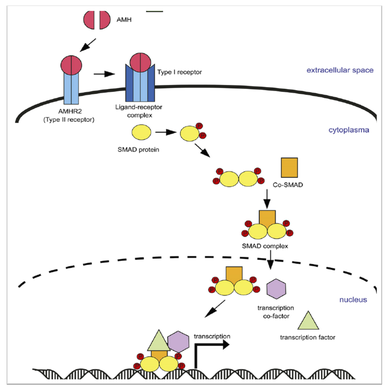
BACKGROUND

Bisphenol A (BPA) and Bisphenol S (BPS)

- Endocrine disrupting compounds (EDCs) capable of interfering with the endocrine system
- BPA activates the estrogen receptor and has been replaced by BPS in plastic production
- BPA exposure associated with adverse fertility outcomes:
  - Decreased implantation[1]
  - Decreased oocyte yield during IVF procedures[2]
  - Altered gene expression[3]

Anti-Mullerian Hormone (AMH) Receptor and SMAD Proteins

- AMH is produced by granulosa cells of pre-antral follicles[4]
- AMH is a marker of ovarian reserve
- AMH Receptor II stimulation activates SMAD proteins 1, 4, and 5
- Cascade will terminate in transcriptional alteration



HYPOTHESIS AND OBJECTIVES

Hypothesis

***In vitro* bisphenol exposure increases expression and activation of AMH signaling components (AMHRII and SMAD proteins) in bovine granulosa cells**

Objectives

1. Evaluate BPA and BPS effects on the transcript levels of AMH Receptor and SMADs in *in vitro* cultured granulosa cells

2. Determine optimal bisphenol treatment length to observe protein activation

3. Evaluate BPA and BPS effects on the protein levels of AMH receptor and SMADs in *in vitro* cultured granulosa cells

MATERIALS AND METHODS

Ovary Aspiration

Cumulus-Oocyte-Complexes (COCs) aspirated from bovine ovaries and granulosa cells are collected and cultured

MATERIALS AND METHODS

Granulosa Cell Culture

Objective 1

Cells are treated for 24 hours under four conditions;

- ***Control:*** *DMEM + 0.1% FBS*
- ***Vehicle:*** *0.01% ethanol*
- ***BPA:*** *0.05mg/mL in 0.01% ethanol*
- ***BPS:*** *0.05mg/mL in 0.01% ethanol*

→ ***Objective 1***

RNA extraction → Reverse transcription → RT-qPCR

- *Analysis of **AMHRII**, **SMAD1**, **SMAD4**, and **SMAD5** expression using GAPDH and PPIA as housekeeping genes*

Objective 2

Cells are treated for 0 (Control), 1, 6, 12, or 24 hours

- ***Control:*** *DMEM + 0.1% FBS*
- ***Vehicle:*** *0.01% ethanol*
- ***BPA:*** *0.05mg/mL in 0.01% ethanol*
- ***AMH:*** *25ng/mL*

→ ***Objective 2 & 3***

Protein extraction → Western Blotting

- *Analysis of **AMHRII**, **phosphorylated-SMAD1**, **total-SMAD1**, and **SMAD4** using Beta-Actin as loading control*

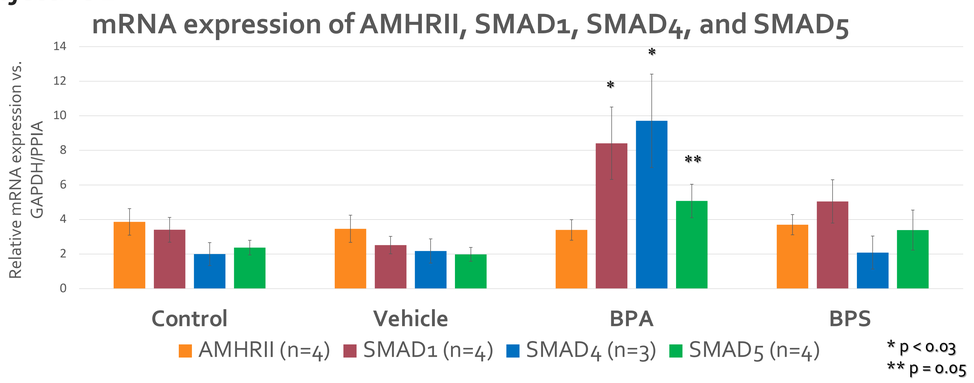
Objective 3

Cells are treated for 1 and 12 hours under 4 conditions from Objective 1

- ***Control, Vehicle, BPA, BPS***

RESULTS

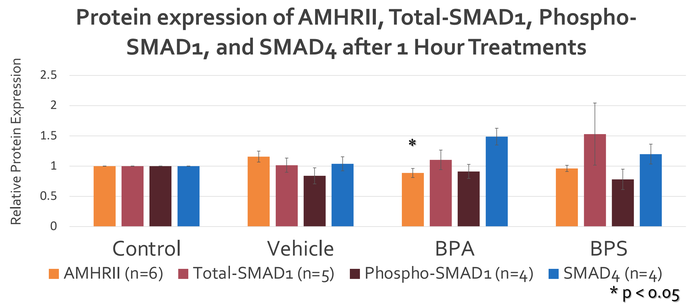
Objective 1

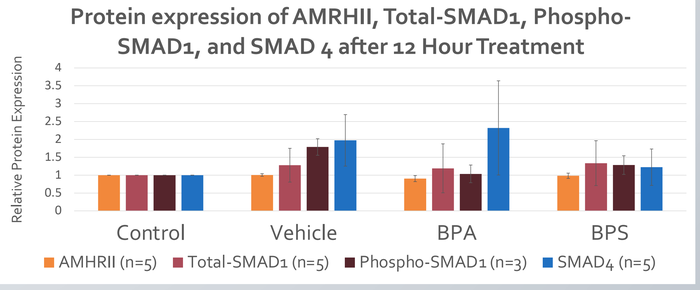


Objective 2:

RESULTS

Objective 3





CONCLUSIONS

BPA treatment of *in vitro* cultured bovine granulosa cells significantly decreased protein levels of AMHRII after 1hr exposure, while transcripts of SMAD1, SMAD4, and SMAD5 were significantly increased following 24hrs exposure.

No notable expression changes were seen following BPS exposure.

These results suggest that BPA affects AMH signaling pathway.

Future Directions

- Conduct functional experiments to evaluate AMH binding and receptor activation in the presence of BPA and BPS
- Assess effects of bisphenols on signaling components while AMHRII is knocked-down

ACKNOWLEDGEMENTS

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Mosaicism and aneuploidy concordance between cell-free embryonic DNA in the spent medium combined with blastocoel fluid and trophectoderm biopsy DNA

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BACKGROUND

Limitations of TE biopsy-based NGS PGT-A

- Blastocyst biopsy is labour intensive and require highly trained embryologists
- TE biopsy is invasive and may have a negative impact on implantation and clinical pregnancy
- TE biopsy may not accurately represent the inner cell mass (ICM) and the reminder of trophectoderm (TE)
- Long-term sequelae of TE biopsy are presently unknown (Zhang *et al., 2019;* Tocci *et al., 2020;* Makhijani *et al., 2021*)

NIPGT-A as an alternative approach to TE biopsy PGT-A

Challenges for NIPGT-A

- The quantity of embryonic DNA in BF and SEM samples is low, presenting challenges for genetic analysis
- Optimal methods for the collection of the samples, whole genome amplification (WGA), and analysis of cell-free embryonic DNA (cfeDNA) all remain to be defined
- It is not yet certain whether the cfeDNA in the SEM derived from inner cell mass, trophectoderm, or both?
- Risk of maternal DNA contamination from the residual corona radiata cells in the embryo culture medium
- Whether aneuploid cells in mosaic embryos undergo apoptosis more often than euploid cells

METHODS

Non-invasive and invasive PGT-A workflow

Day 0/1

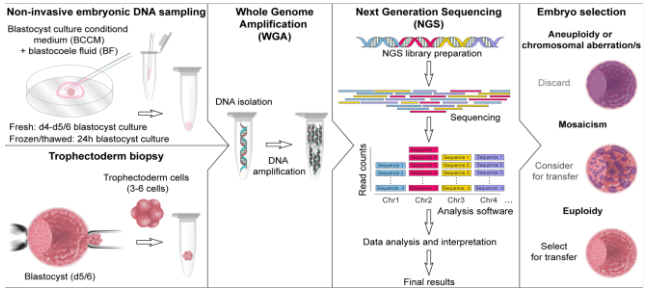
- Careful removal cumulus-corona cells and washing

Day 4

- Laser AH vs. no AH
- Washing and transfer embryo to a fresh medium droplet

Day 5/6

- Blastocyst collapse by single laser pulse at the junction of TE cells before TE biopsy
- Transfer blastocysts to a biopsy dish for TE biopsy
- Collection of all samples into PCR tubes
- Collection of the Blank medium as negative control



All WGA products were conducted PGT-A using NGS by Illumina NextSeq® 550 platform

RESULTS

We chose 50% as the mosaicism threshold for distinguishing aneuploid from euploid embryos for both SEM+BF and TE biopsy analysis

Type of samples	WGA rate (%)	WGA-DNA amount (ng/µL)	Informative NGS results (%)
NI, no AH	134/134 (100)	40.3 ± 17.76*	125/134*(93.3)
NI, AH on D4	65/65 (100)	36.8 ± 20.1*	56/65*(86.2)
NIPGT total	199/199 (100)	39.2 ± 18.61	181/199 (91.0)
TE biopsy	206/206 (100)	46.5 ± 7.67	204/206 (99.0)
NI euploid	100/100 (100)	40.8 ± 18.85**	
NI aneuploid	81/81 (100)	41.2 ± 17.11**	

*There is no significant difference

Concordance rate for euploidy (101/113, 89.4%) and aneuploidy (61/67, 91.0%) were not significantly different between SEM+BF and TE biopsy groups

CONCLUSIONS

NGS results showed high concordance rate for euploidy and whole/segmental chromosome aneuploidy between combined SEM+BF samples and corresponding TE biopsy samples, but not between mosaic samples. Combined SEM+BF has great potential to be used as a source of embryonic DNA for clinical NIPGT-A

#105

Human umbilical cord perivascular cells (HUCPVC) improve pregnancy rates in a mouse model of natural ovarian aging

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INTRODUCTION

- Many physiological and environmental factors contribute to ovarian aging, leading to ovarian dysfunction and/or diminished ovarian reserve and oocyte quality.

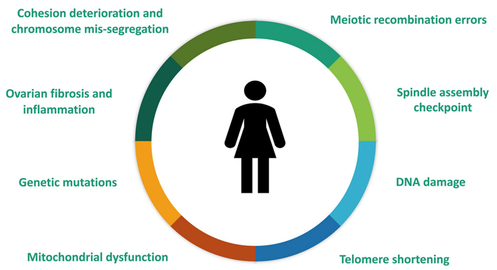


Figure 1. Mechanisms of ovarian aging *(From Park et al Reproduction 2021)*

- Mesenchymal stromal cells (MSC) have been studied as candidates for cell therapy in regenerative medicine, including for fertility preservation[1] and have recently started to be investigated for the prevention of ovarian aging[2,3].

- Human umbilical cord perivascular cells (HUCPVCs) derived from first trimester (FTM) and term (TERM) umbilical cords have been characterized as promising sources of MSCs[4].

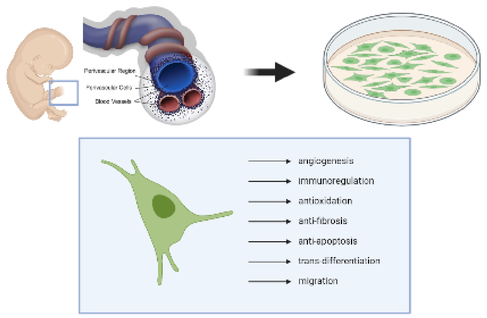


Figure 2. Human Umbilical cord-derived perivascular cells (HUCPVC) are a promising source of MSC, that are thought to act through multi-targeted mechanisms.

- Treatment with HUCPVC prior to administration of chemotherapy can prevent cyclophosphamide-induced ovarian damage[5] and busulfan-induced loss of male fertility in rodent models[6].

- When injected systemically in animal models, HUCPVC have been shown to have distal effects (for example on the brain) through immunomodulatory and anti-inflammatory mechanisms[7].

HYPOTHESIS

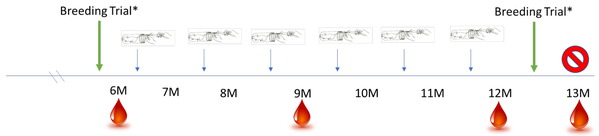
The systemic delivery of HUCPVC during the period of ovarian aging may delay the age related decline in ovarian reserve and oocyte quality via immunomodulatory mechanisms, also impacting overall aging

OBJECTIVE

To examine whether the repeated systemic administration of FTM and term HUCPVC has a protective effect against ovarian aging and may delay ovarian aging in a mouse model.

MATERIALS AND METHODS

STUDY DESIGN AND TIMELINE



6M ICR female mice were randomized in 5 groups (n=10 per group)
Control Group: Media injection (HBSS) *n=15
Group 1: FTM HUCPVC
Group 2: Term HUCPVC
Group 3: Fibroblast
*Group 4: FTM HUCPVC (single injection at 12M) *n=5*

Monthly Tail Vein Injections for 6 months – 1 million pathogen-tested cells per animal

Figure 3. 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:

- This study was approved by the animal care committee at the University Health Network.

- 5 month old female ICR mice were ordered from Charles River or aged from 6-8 week old females used in control mating experiments. Females were randomized to each group after baseline fertility assessments at 6M.

- Pathogen-free lines of previously characterized FTM and Term HUCPVC lines (REB #28889, UofT) were expanded in αMEM supplemented with 2.5% HPL; Passage 6 cells were resuspended in Hank's buffered saline solution (HBSS) and administered systemically at 1 month intervals. The last injection took place 2 weeks before the final mating trial.

- Serum was collected at 6, 9, 12 months and at endpoint.

ASSESSMENTS

		Study Timepoint (months)							
	Procedures Involved	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Treatment - cell/media administration	I.V. injection of 500,000 cells/animal	x	x	x	x	x	x		
Primary Endpoints Assessments									
AMH assessment	Blood Collection and ELISA	x			x			x	
Fertility Assessment	Mating Trials (Pregnancy rates, litter sizes)	x						x	
Histological Analysis of Ovaries	Endpoint dissection of ovaries & histology- follicle count & inflammatory markers (giant cells, fibrosis)								x
Secondary Endpoints Assessments									
Animal health	Body weight measurements, general observations	x	x	x	x	x	x	x	x
Inflammation - analysis of circulating pro-inflammatory monocytes and serum cytokines	Blood Collection AND Flow Cytometry/ ELISA	x			x			x	
Tumor formation	Full necropsy at time at endpoint								x
Analysis of aging markers in other tissues	Dissection of spleen, liver, heart, brain, uterus etc for RNA/protein analysis and								x

Table 1. Assessments and Timepoints.

Experimental details:

- All assays and data collection were performed by blinded observers

- **Fertility** was assessed by mating each female with 6-8 week old ICR males with proven fertility in triad breeding cages for 5 days. Pregnancies and litters were monitored daily.

- For **histological analysis**, ovaries were embedded in paraffin and serial 5µm sections spanning the entire tissue were collected on microscopy slides and stained with hematoxylin and eosin (H&E). Every 10th section was scanned using the Hamamatsu Whole Tissue Slide Scanner (Olympus) (The Toronto Centre for Modeling of Human Disease, Pathology Core, Mount Sinai Hospital) and analyzed by 2 independent blinded observers to quantify follicle stages and giant multinucleated cells.

- Anti-Mullerian hormone (AMH) (MyBiosource) and C-Reactive Protein (R&D Systems) **ELISA**s were assessed in serum following manufacturer instructions.

RESULTS

Repeated monthly injections of FTM and term HUCPVC in aging mice over 6 months improved age-related decline in pregnancy rates

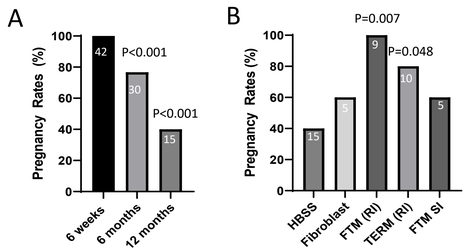


Figure 4. Pregnancy Rates in Young (6 weeks, 6months) and Aging (12 months) ICR females (**A**) and in Aging ICR females (12 months) treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC (RI) starting at 6 months, or a single injection of FTM at 12 months (**B**). Data was analyzed using a Chi-Square test and P values indicate significant differences with control group. RI = repeated injection; SI = Single injection. Animal # per group are indicated in each bar.

The age-associated decrease in litter size was not improved by HUCPVC treatment

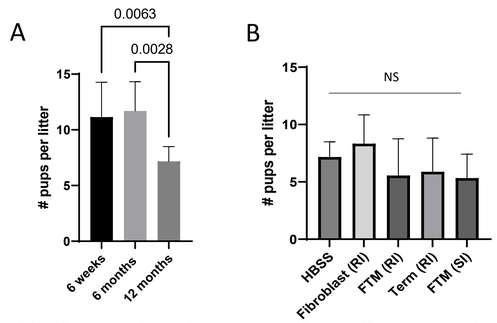


Figure 5. Litter Sizes in Young (6 weeks, 6months) and Aging (12 months) ICR females (**A**) and in Aging ICR females (12 months) treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC (RI) starting at 6 months, or a single injection of FTM at 12 months (**B**). Data was analyzed using One-Way Anova. RI = repeated injection; SI = Single injection. Animal # per group are indicated in each bar.

The age-associated loss of ovarian reserve was not significantly altered by repeated monthly HUCPVC treatment but AMH levels were restored by one dose of FTM HUCPVC at 12 months

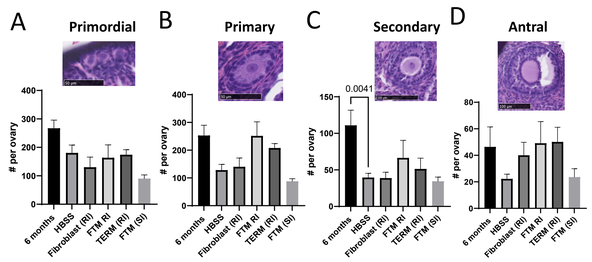


Figure 6. Preliminary analysis of follicles at primordial (A), primary (B), secondary (pre-antral) (C) and antral stages (D) in ovaries of 6months and aging (13 months) ICR females treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC (RI) starting at 6 months, or a single injection of FTM at 12 months. Data was analyzed using One-Way Anova.

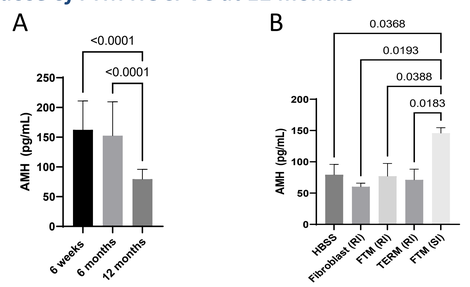


Figure 7. AMH ELISA in in Young (6 weeks, 6months) and Aging (12 months) ICR females (**A**) and in Aging ICR females (12 months) treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC (RI) starting at 6 months, or a single injection of FTM at 12 months (B). Data was analyzed using One-Way Anova.

An age-associated increase in giant multinucleated cells (GMNC) in ovarian tissue was not significantly altered by HUCPVC treatment

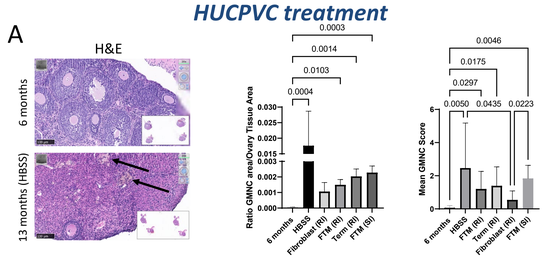


Figure 8. Preliminary analysis of giant multinucleated cells (GMNC) in ovaries of 6 months and aging (13 months) ICR females treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC (RI) starting at 6 months, or a single injection of FTM at 12 months. **A**) Representative images of 6 and 13 months ovarian tissue (arrow points to GMNC cluster). **B**) Quantification of GMNC to ovarian tissue surface ratios and **C**) GMNC score, reflecting # of islands per tissue section. Data was analyzed using One-Way Anova.

An age-associated increase in serum levels of C-Reactive Protein was significantly reduced in FTM HUCPVC-treated mice

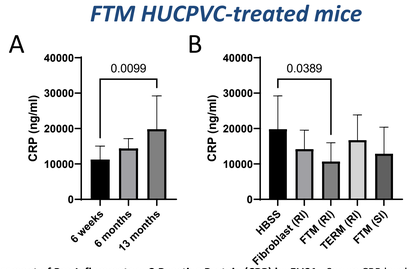


Figure 9. Assessment of Pro-Inflammatory C-Reactive Protein (CRP) by ELISA. Serum CRP levels in Young (6 weeks, 6months) and Aging (12 months) ICR females (**A**) and in Aging ICR females (12 months) treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC (RI) starting at 6 months, or a single injection of FTM at 12 months (**B**). Data was analyzed using One-Way Anova.

CONCLUSIONS

This is the first study to assess the safety of a repeated HUCPVC dose regimen and, to our knowledge, the first study to investigate MSC for the mitigation of inflammation in the context of aging.

Repeated doses of HUCPVC appear to be safe and well tolerated.

A repeated FTM and term HUCPVC dosing regimen improved pregnancy rates in a mouse model of age-related fertility decline but did not significantly alter ovarian reserve (primordial follicle count, AMH) or litter size.

A repeated dose regimen of FTM HUCPVC appears to reduce systemic inflammation in an aging mouse model.

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So is this going to hurt?

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The Montreal Fertility Centre

Objective:

- To **compare** how much pain patients **anticipated prior to a sonohysterogram** (SHG) with **actual pain experienced**

Materials & Methods:

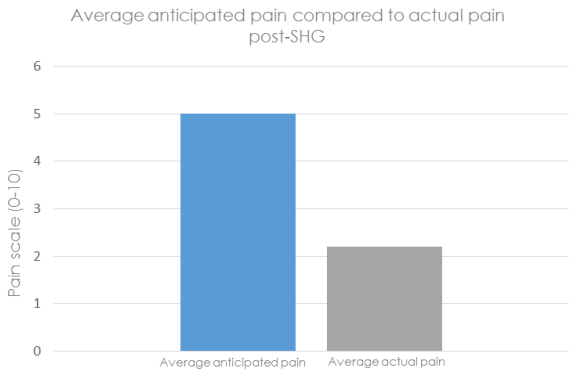
- All patients scheduled for an SHG at the Montreal Fertility Centre from November 2019-March 2020 were invited to participate in the survey;
- The Survey assessed expectations and actual experience of the SHG containing yes/no answers and pain scales from 0-10:
  - **1st section**: completed by patient before procedure;
    - Questions regarding medical history (e.g. pain level during speculum insertion, presence of dyspareunia, dysmenorrhea);
    - Questions regarding expectations for the SHG procedure.
  - **2nd section** : completed by patient after the procedure;
    - Questions regarding the experience of the SHG (i.e. pain level during SHG).
  - **3rd section** : completed by MD after procedure;
    - Assessment of procedure (e.g. duration, volume of balloon insufflation, quantity of saline liquid injected, etc.).

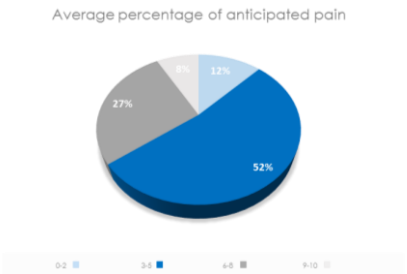
Results:

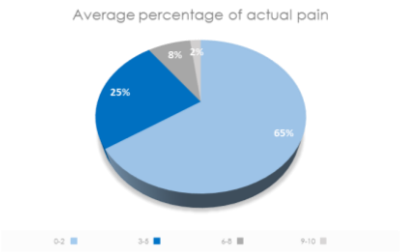
- Final sample: **163 patients** (22-48 years old) completed the survey;
- Average expected pain level = **5.0**; average actual pain level = **2.3**;
- Actual pain separated in two groups : Low (pain level 0-5) and High (pain level 6-10);
- **28%** patients **expected high pain level → 5% reported high pain level** after the SHG;
- Significant difference on anticipated pain between groups (↓ pain/ ↑ pain; $p < 0.01$);
- **Factors predicting actual pain level** : pain on speculum insertion ($p < 0.01$), dyspareunia ($p = 0.03$), and dysmenorrhea ($p < 0.01$);
- **Low positive correlations** between actual pain levels and **duration** ($r = 0.30$), **volume balloon** ($r = 0.24$) and **quantity of saline liquid injected** ($r = 0.24$).

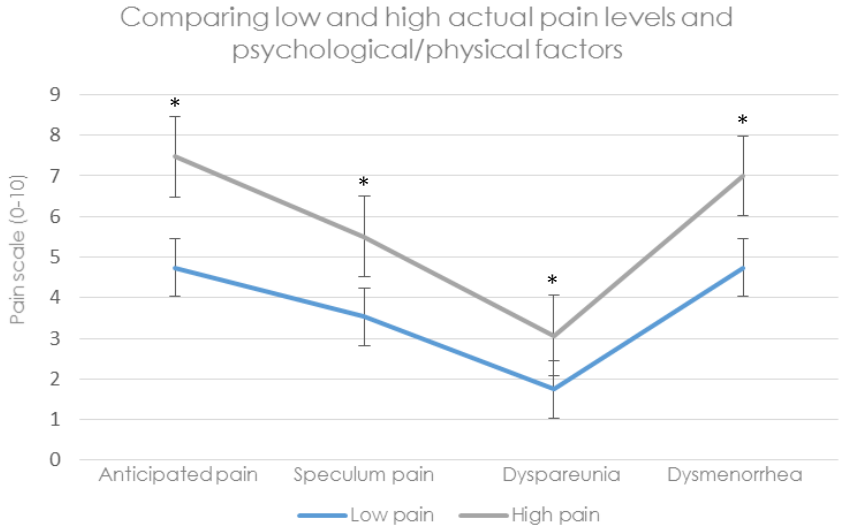
Groups	Age (mean)	BMI (mean)	Duration (minutes)	Volume of balloon (milliliters)	Saline injected (milliliters)
Low actual pain	22-48 (35.1)	16-38 (26.3)	4.7	1.4	6.5
High actual pain	25-45 (34.0)	19-36 (24.2)	5.3	1.5	7.0

Note. Averages of parameters measured during SHG









Conclusion :

- **Majority** of patients **anticipated moderate to severe pain** during SHG;
- However, the vast **majority** actually **experienced low pain levels** during the procedure;
- 90% with actual pain score <6;
- **Pain level** during the SHG are **influenced** by →

- Creating a patient **information pamphlet** conveying information relative to the actual experience of SHG could help ↓ pain anticipation.

#107

Intravenously infused first trimester human umbilical cord perivascular cells interact with innate immune cells and mediate a systemic reduction in inflammatory mediators in a murine model of systemic inflammation

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INTRODUCTION

- **Modulation of inflammation** is a major property underlying the therapeutic benefit of mesenchymal stromal cells (MSC)
  - Preclinical work has demonstrated that MSC can alleviate inflammation and reduce disease burden in a variety of contexts[1,2,3,4]
- **First trimester human umbilical cord perivascular cells (FTM HUCPVC):** highly proliferative MSC with superior functional properties[5]
- Hundreds of clinical trials using MSC therapy have been initiated in severe inflammatory conditions
  - Results of trials often **fail to reach efficacy** expected based on preclinical work[6,7]
- **Major barrier for clinical translation:** incomplete understanding of the mechanism by which MSC exert immunomodulatory effects
- **Predominant hypothesis:** active, paracrine-based[8]
  - Mediators, including PGE2[9], IDO[10], and TSG-6[11], have all been implicated
- **Alternative hypothesis:** passive process, whereby the death and clearance of MSC by immune cells is required to stimulate an anti-inflammatory effect[12,13,14]
- A lack of quantitative, multi-organ biodistribution and fate investigations is limiting our understanding of the relative roles of paracrine secretion and passive phagocytosis in MSC immunomodulatory action

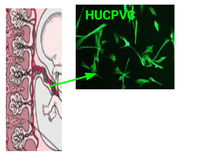

Figure retrieved from Kabat, M., Bobkov, I., Kumar, S., & Grumet, M. (2020). Trends in mesenchymal stem cell clinical trials 2004-2018: is efficacy optimal in a narrow dose range? *Stem Cells Translational Medicine, 9*(1), 17-27

OBJECTIVE

This study aims to investigate the biodistribution and fate of a young source of MSC, first trimester human umbilical cord perivascular cells (FTM HUCPVC), delivered intravenously in a murine model of systemic inflammation

MATERIALS AND METHODS

Male C57BL6 mice randomly allocated to treatment groups:

Treatment	
1	Vehicle control
2	LPS
3	FTM HUCPVC
4	LPS + FTM HUCPVC

LPS (lipopolysaccharide): *immune-stimulating bacterially-derived endotoxin*
Dosage: 0.83 mg/kg
Delivery route: intraperitoneal

FTM HUCPVC: *LL02 & LL05 cell lines previously established in Librach lab; used at P4-P6 for experiments*
Dosage: 1x10^6 cells/animal
Delivery route: intravenous via tail vein
Pre-label: qTracker-625

- **Endpoints:** 5 minutes, 5 hours, or 24 hours after FTM HUCPVC infusion
- **Collection & processing:** Lungs, liver, and spleen harvested and frozen in OCT (optimal cutting temperature compound) for serial sectioning; plasma collected for cytokine analysis

Immunohistochemistry

Target	Antibodies
Apoptotic FTM HUCPVC	Anti-cleaved caspase-3 (abcam)
Murine neutrophils	Anti-NIMP-R14 (abcam)
Murine alternatively polarized macrophages	Anti-CD206 (R&D systems)

Assessment of systemic inflammatory cytokine levels: Proteome Profiler™ Array Mouse Cytokine Array Kit Panel A (R&D Systems)

Data analysis: Results were analyzed in GraphPad Prism using one-way ANOVA with Tukey post-hoc tests, or t-test *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

RESULTS

FTM HUCPVC INCREASE NEUTROPHIL TRAFFICKING TO THE LUNGS IN LPS-TREATED MICE

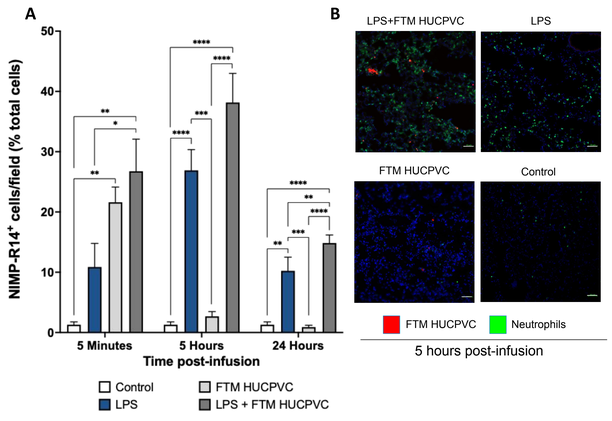

Figure 1. Neutrophil infiltration in the lungs in response to FTM HUCPVC infusion with and without inflammatory stimulus. (A) Quantification of neutrophils in the lungs at 5 minutes, 5 hours, and 24 hours after infusion. Neutrophils quantified as the percent of total cells per field positive for NIMP-R14. Mean and error bars representing SEM are shown, n=5 per group. Statistical significance indicated as **p<0.01, ***p<0.001, ****p<0.0001. **(B)** Representative images of lung cryosections showing FTM HUCPVC (red) and neutrophils (green) at 5 hours after infusion (scale bar = 50μm).

FTM HUCPVC INCREASE NEUTROPHIL TRAFFICKING TO THE LIVER IN LPS-TREATED MICE AT 5 HOURS AND 24 HOURS AFTER INFUSION

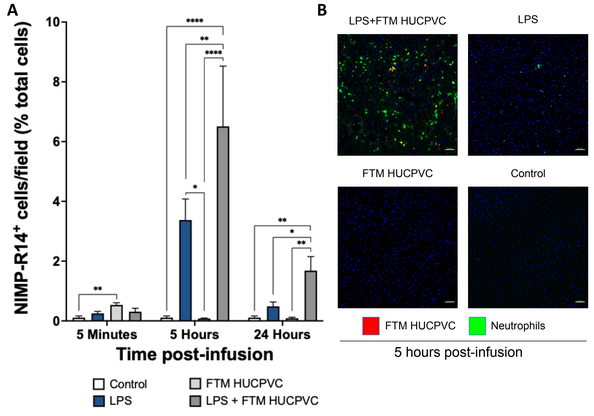

Figure 2. Neutrophil infiltration in the liver in response to FTM HUCPVC infusion with and without inflammatory stimulus. (A) Quantification of neutrophils in the liver at 5 minutes, 5 hours, and 24 hours after infusion. Neutrophils quantified as the percent of total cells per field positive for NIMP-R14. Mean and error bars representing SEM are shown, n=5 per group. Statistical significance indicated as *p<0.05, **p<0.01, ****p<0.0001. **(B)** Representative images of liver cryosections showing FTM HUCPVC (red) and neutrophils (green) at 5 hours after infusion (scale bar = 50μm).

NEUTROPHILS CO-LOCALIZE WITH FTM HUCPVC IN THE LUNGS AT 5 MINUTES AND 5 HOURS AFTER INFUSION

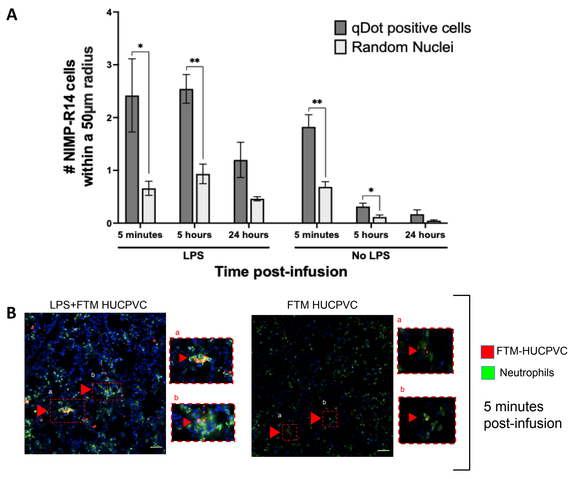

Figure 3. Co-localization of neutrophils and FTM HUCPVC in the lungs with and without inflammatory stimulus (A) Quantification of neutrophils within a 50 μm radius of either qTracker-625-labeled FTM HUCPVC or random nuclei within the same field. Mean and error bars representing SEM are shown, n=5 per group. Statistical significance indicated as *p<0.05, **p<0.01. **(B)** Representative images of lung cryosections showing FTM HUCPVC (red) and neutrophils (green) at 5 minutes after infusion (scale bar = 50μm). Inserts show neutrophils associated with FTM-HUCPVC.

FTM HUCPVC-ASSOCIATED APOPTOTIC PATHWAYS ARE ACTIVATED IN THE LUNGS EARLY AFTER INFUSION IN LPS-TREATED MICE

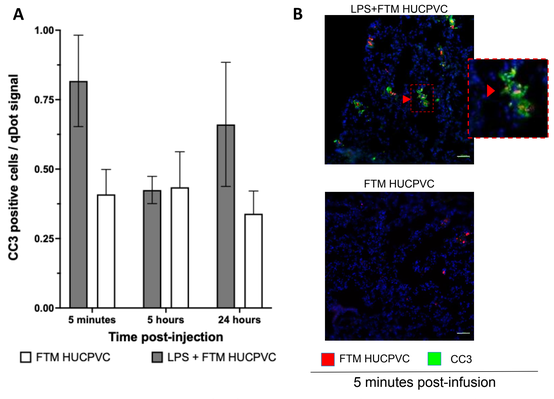

Figure 4. Human cleaved-caspase 3 (CC3) signal in the lungs after FTM HUCPVC infusion with and without inflammatory stimulus. (A) Quantification of activated human cell death pathways in the lungs quantified as the ratio of CC3-positive cells to qDot-positive cells. Mean and error bars representing SEM are shown, n=5 per group. **(B)** Representative images of lung cryosections showing FTM HUCPVC (red) and CC3 (green) at 5 hours after infusion (scale bar = 50μm). Insert shows FTM-HUCPVC surrounded by CC3 signal.

FTM HUCPVC INFUSION INCREASES LEVELS OF ANTI-INFLAMMATORY MACROPHAGES IN THE LUNGS

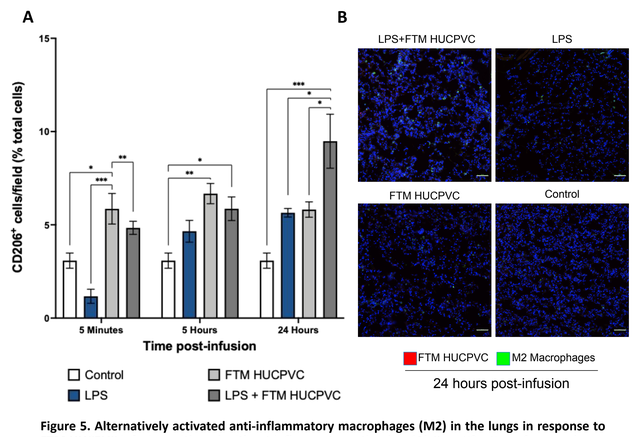

Figure 5. Alternatively activated anti-inflammatory macrophages (M2) in the lungs in response to FTM HUCPVC infusion with and without inflammatory stimulus. (A) Quantification of macrophages in the lungs at 5 minutes, 5 hours, and 24 hours after infusion. M2 quantified as the percent of total cells per field positive for CD206. Mean and error bars representing SEM are shown, n=5 per group. Statistical significance indicated as *p<0.05, **p<0.01, ***p<0.001. **(B)** Representative images of lung cryosections showing FTM HUCPVC (red) and M2 (green) at 24 hours after infusion (scale bar = 50μm).

FTM HUCPVC INFUSION ABROGATES LPS-INDUCED INCREASES IN SYSTEMIC LEVELS OF PRO-INFLAMMATORY CYTOKINES AT 24 HOURS AFTER INFUSION

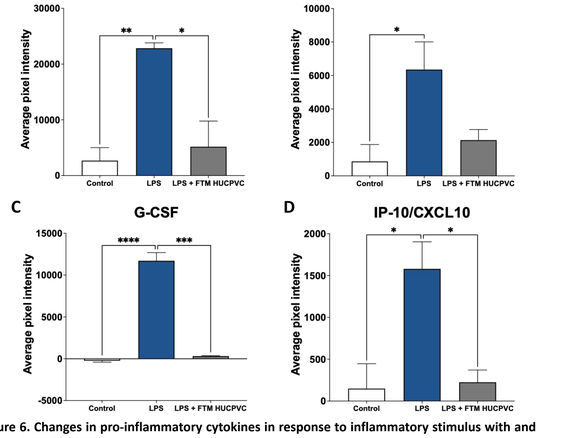

Figure 6. Changes in pro-inflammatory cytokines in response to inflammatory stimulus with and without FTM HUCPVC infusion. Inflammatory cytokine levels in serum assessed using a mouse cytokine panel array and quantified as average pixel intensity. **(A)** CXCL13/BLC; **(B)** TIMP-1; **(C)** G-CSF; **(D)** CXCL10/IP-10. Mean and error bars representing SEM are shown, n=2-3 per group. Statistical significance indicated as *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

CONCLUSION AND DISCUSSION

Our data: (1) indicate a close association between FTM HUCPVC and innate immune cells early after systemic infusion, specifically in the lungs; **(2)** suggest an impact of inflammatory priming on the biodistribution and fate of FTM HUCPVC *in vivo*; and **(3)** demonstrate the ability of FTM HUCPVC to increase levels of anti-inflammatory macrophages in the lungs, major players in inflammatory resolution, and mitigate LPS-induced increases in systemic pro-inflammatory cytokines.

Future Directions: further flow cytometry-based assessments are underway to investigate systemic and organ-specific changes in inflammatory cytokines in response to FTM HUCPVC infusion, with and without inflammatory stimuli. Additional areas of ongoing investigation include:

- Assessing the potential therapeutic effect of FTM HUCPVC-derived extracellular vesicles
- Assessing impacts of toll-like receptor activation on FTM HUCPVC interactions with immune cells
- Examining the mechanism by which peripheral infusion of FTM HUCPVC can mitigate neuroinflammation

ACKNOWLEDGMENTS

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Normalizing the Discontinuation of Fertility Treatment

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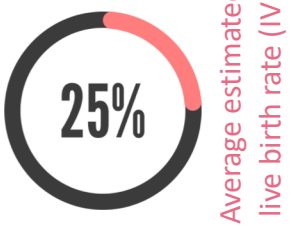
BACKGROUND

Many users will discontinue fertility treatment (ART) without a live birth - a difficult decision due to the high physical, emotional, and financial investment in the outcome of treatment.



RESEARCH QUESTION

How and why do women discontinue fertility treatment in Canada?

- What aspects made pausing/stopping easier or more difficult?
- What role do fertility clinics play in stories of discontinuation?

METHODS

36 qualitative, open-ended and follow-up interviews with women across Canada (n=22) who discontinued fertility treatment. Design and analysis were informed by feminist narrative theory and organized thematically.

PARTICIPANTS

22 women:

- Aged 25 to 45+
- Hetero (19), Queer (2), Bisexual (1)
- Majority Caucasian / white, Black, South East Asian, and Metis
- Household income CAD $100,000-225,000 annually
- Not all women were 'childless'

Discontinuation is the right choice for many. However, it is a marginalized experience in clinical environments.

FINDINGS

In their stories…

- Pausing, ending, or forgoing treatment was an ambiguous event.
- Participants lacked guidance and support from providers to discontinue fertility treatment.
- Participants navigated restrictive gendered & heteronormative language and felt pressured by the clinical imperative to facilitate pregnancy, which made it harder for them to discontinue.

RECOMMENDATIONS

- "Discontinuation discussions" become a regular part of treatment protocols
- Providers reflect on potentially restrictive language in order to support & normalize discontinuation

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Specific gene expression response in KGN human granulosa cells in response to different inflammatory stimuli

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Introduction

Ovulation is often compared to an inflammatory reaction. Previous studies performed in our lab showed that failure to conceive after in vitro fertilization could be associated with dysregulated or excessive inflammation. Understanding and controlling inflammation is primordial in the context of assisted reproductive technologies specially to target proper treatment or to be able to control inflammation in some patients. However, it is still unclear how the different cell types can respond to inflammatory stimulus or anti-inflammatory compounds. This study thus aimed to gain information on how pure granulosa cells respond to different inflammatory stimulus and if acetylsalicylic acid could act directly on these cells to regulate inflammation.

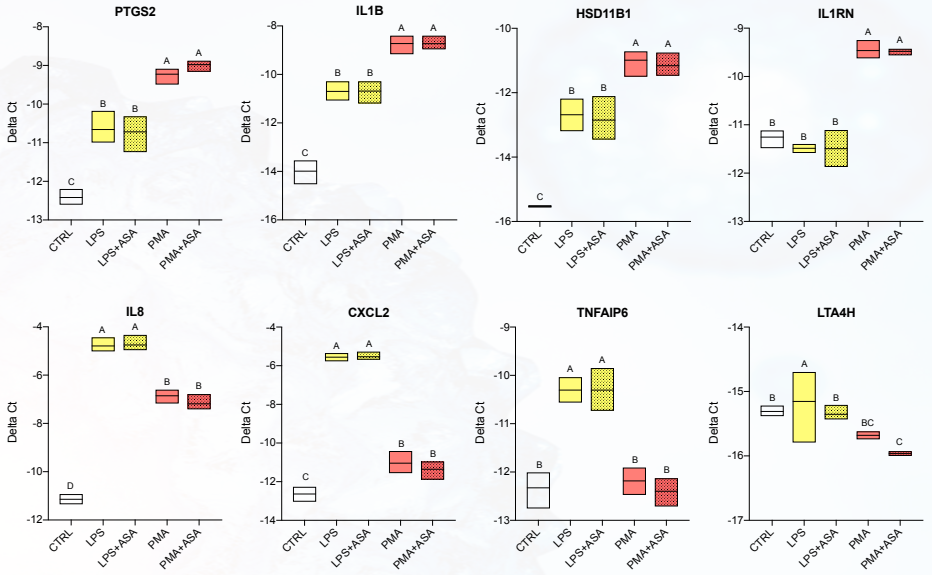
Material and methods

Chemicals and cell culture

The cell-stimulating compounds used in this study were 4-androstene-3,17-dione (androstenedione), phorbol 12-myristate 13-acetate (PMA), acetylsalicylic acid (ASA), and Escherichia coli lipopolysaccharide (LPS). The human ovarian granulosa cell line KGN was used for this study.

Treatments and analysis

- Cells were treated with PMA (20 nmol/L) or with LPS (2 µg/ml), alone or in combination with ASA (5 µmol/L) for 24 hours. The experiment was repeated three times (during different weeks).
- Total RNA was then isolated, purified and reverse transcribed.
- Eight inflammation-related genes were analyzed by quantitative RT-PCR (qRT-PCR), namely CXCL2, HSD11B1, IL1B, IL1RN, IL8, LTA4H, PTGS2 and TNFAIP6. These genes are known to be important inflammation mediators. They were selected based on results of our previous study and for their involvement in the ovulation inflammatory reaction. For normalization purpose, we also analyzed three reference genes: GAPDH, PPIA and YWHAZ. Relative expression levels for each gene were calculated using the delta Ct method.



Results and conclusion

Treatment with LPS increased the expression of all inflammatory genes (PTGS2; P=0.0008, IL1B, HSD11B1, IL1RN, IL8, CXCL2, TNFAIP6, LTA4H; P<0.0001) except IL1RN (P=0.6921). Adding ASA had no effect excepted for LTA4H for which the expression was increased in response to LPS alone while it remained similar to control when cells were treated with LPS and ASA (P=0.9837).

Treatment with PMA increased the expression of PTGS2, IL1B, HSD11B1, IL1RN, IL8 (P<0.0001) and CXCL2 (P=0.0023). PMA had no effect on TNFAIP6 (P=0.9786) and LTA4H (P=0.0720). Adding ASA had no effect excepted for LTA4H. The expression of LTA4H was lower than control when cells were treated with PMA and ASA (P=0.0070), but it was not different than treatment with PMA alone (P=0.2107).

In addition, the increase in the expression of PTGS2, IL1B, HSD11B1 and IL1RN was higher when cells were treated with PMA than with LPS. On the opposite, LPS induced a higher increase of expression than PMA for IL8, CXCL2, TNFAIP6 and LTA4H. Although both PMA and LPS treatments induced an inflammatory response, when looking at the two treatments, each stimulated genes related to a common function. When looking at the lower part of the figure, we can see that the genes that increased more in response to LPS (IL8, CXCL2, TNFAIP6 and LTA4H) are all genes related to immune cells.

On the other hand, when looking at the upper part of the figure, we can see that the genes that increased more in response to PMA (PTGS2, IL1B, HSD11B1 and IL1RN) were mainly related to prostaglandins production or inhibition and to IL1 signaling.

In this preliminary study, aspirin seems to generate a very limited effect on the inflammation induced in granulosa cells by PMA or LPS since the addition of aspirin negatively affected the expression of LTA4H but had no effect on the expression level of all the other inflammatory genes.

To conclude, this preliminary study showed that pure granulosa cells alone are able to respond to endogenous and exogenous inflammatory stimuli. However, the change in the expression of the inflammatory markers differs according to the type of stimulus with LPS response being more related to granulosa cells acting as immune cells while PMA being more related to a direct inflammatory response to LH. The results did not show that granulosa cell can directly respond to aspirin by reducing the expression of pro-inflammatory cytokines like IL1B or IL8 but suggest that aspirin could have anti-inflammatory effects through the regulation of leukocyte recruitment by granulosa cells. However, this hypothesis would need to be validated in vivo or in a context involving immune cells before recommendations can be made regarding the use of aspirin to control inflammation in some patients during ovarian stimulation.

In Vitro Transplantation: Novel Method in Infertility Treatment by Spermatogonial Stem Cell

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Background

Sperm production is one of the most complex biological processes in the body. In vitro production of sperm is one of the most important goals of researches in the field of male infertility treatment, which is very important in male cancer patients treated with gonadotoxic methods and drugs. In this study, we examine the progression of spermatogenesis after transplantation of spermatogonial stem cells under conditions of testicular tissue culture.

Results

Testicular tissue samples from azoospermic patients were obtained and then these were freeze- thawed. Spermatogonial stem cells were isolated by two enzymatic digestion steps and the identification of these cells was confirmed by detecting the *PLZF* protein in the colonies derived from them. These cells, after being labeled with DiI, were transplanted in azoospermia adult mice model. After 8 weeks of host testis culture, histomorphometric, immunohistochemical and molecular studies were performed. The results of histomorphometric studies showed that the mean number of spermatogonial cells, spermatocytes and spermatids in the experimental group was significantly more than the control group ($P<0.05$) and most of the cells responded positively to the detection of DiI. Immunohistochemical studies in host testes fragments in the experimental group express the PLZF, SCP3 and ACRBP proteins in spermatogonial cells, spermatocyte and spermatozoa , respectively, which confirmed the human nature of these cells. Also, in molecular studies of *PLZF*, *Tekt1* and *TP1*, the results indicated that the genes were positive in the test group, while not in the control group.

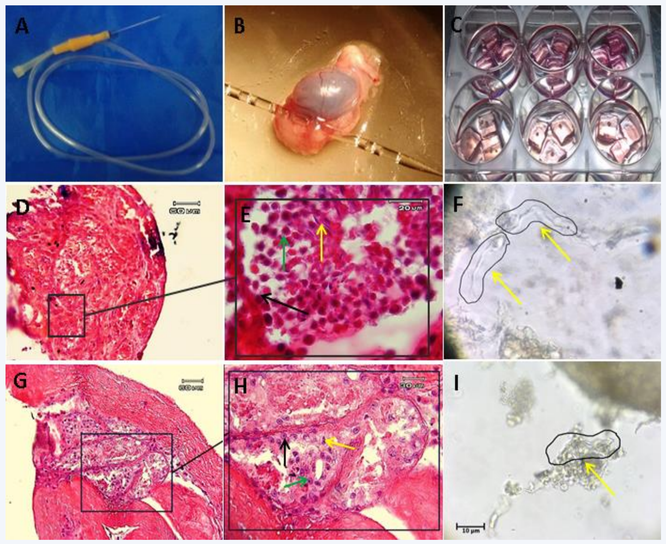

Figure 2. IVT of SSs to host testis and organ culture (A, B and C). H & E staining of tissue sections IVT group (D and E) and control group (G and H). Dynamic dissection of testis fragments after 8 weeks in IVT group (F) and control group (I). Black arrow: SCs, green arrow: spermatocyte and yellow arrow: Long spermatid or sperm like cells.

Conclusions

These results suggest that the conditions of the 3-Dimentional testicular tissue culture after long-term preserved spermatogonial stem cell transplantation can support the progress of spermatogenesis to produce haploid cells.

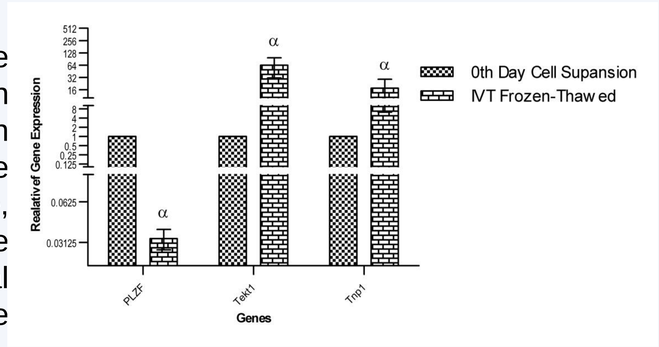

Chart2. The relative expression of human specific SSCs gene in the host testes after 8 weeks of tissue culture.
α: Significant different with other group in same gene (P<0.05).

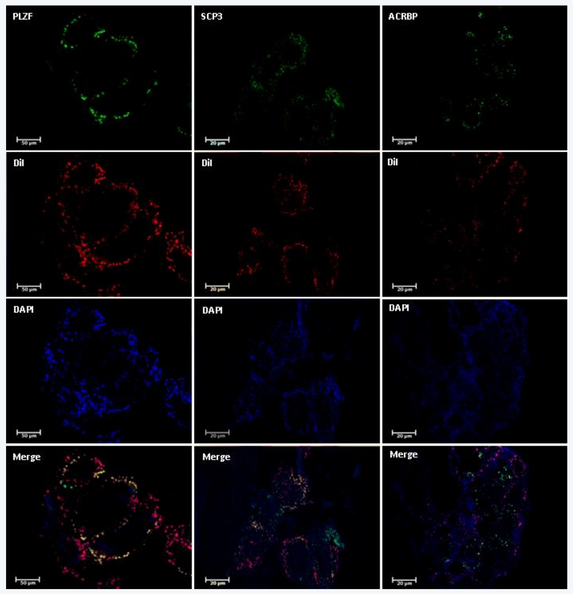

Figure 2. Expression of specific proteins of spermatogonial cells (PLZF), spermatocytes (SCP3) and spermatozoa (ACRBP) and detection of DiI in host testes after 8 weeks of tissue culture.

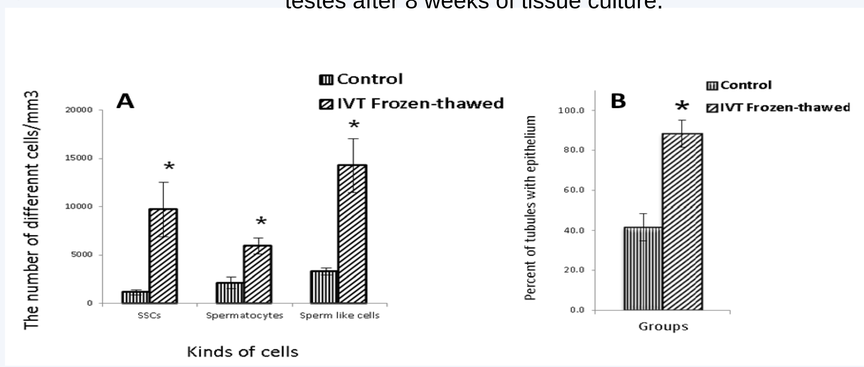

Chart. 1. Number of different types of cells of seminiferous tubule epithelium (A) and percentage of seminiferous tubules containing epithelium (B) after 8 weeks of tissue culture in different groups.
*: Significant different with control group (P<0.05)