CFAS 68TH
ANNUAL MEETING
Poster Presentation
TV3 & TV4

The Canadian Fertility and Andrology Society

Comparison of mtDNA & MAPD as determinants of sustained implantation of euploid blastocysts.

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OLIVE fertility centre

The Fertility Partners

SEQUENCE46 GENETIC TESTING LABORATORY

INTRODUCTION

Numerous factors are influential in contributing to sustained implantation rate (SIR) of euploid embryos, including those potentially indicated by technical signals that are unappreciated.

For example, intermediate CNV (aka mosaicism) has only relatively recently been appreciated as a common contribution to uncertain sustained implantation. Thus, it is plausible that other elements of NGS output could also be related to SIR.

Here, we review 2 other outputs of the sequencing workflow as possible determinants of SIR:

1) **Mitochondrial DNA (mtDNA) fraction,** which has been inversely correlated with implantation potential.
2) **Median absolute pairwise difference (MAPD**), a QC indicator of read coverage noise across amplicons, which is inversely correlated with reliability of CNV calls.

METHODS

- Retrospective review of 691 single euploid embryo transfer outcomes.
- Independent variables: Maternal age at freeze, blastocyst grade, day of freeze, MAPD, and mtDNA
- Outcomes: Viable (delivered, ongoing) vs Nonviable (failed implantation, biochemical or clinical miscarriage).
- Independent sample t-test, and chi-square for multiple comparisons

RESULTS

Table 1: Overall Results

Age at Freeze	36.6 +/-3.6 years
Implantation Rate	71%
Viability Rate	52.5%
mtDNA	0.00135 +/- 0.00086
MAPD	0.200 +/- 0.0199

Table 2: Outcome-Stratified Results

	Viable	Non-Viable	p-value
Age at Freeze	36.7 +/- 3.6	36.5 +/- 3.4	0.612
mtDNA	0.0013 +/- 0.0013	0.0013 +/- 0008	0.294
MAPD	0.2008 +/- 0.017	0.2003 +/- 0.022	0.917

Table 3: Embryo Characteristics Significantly Associated with Viability

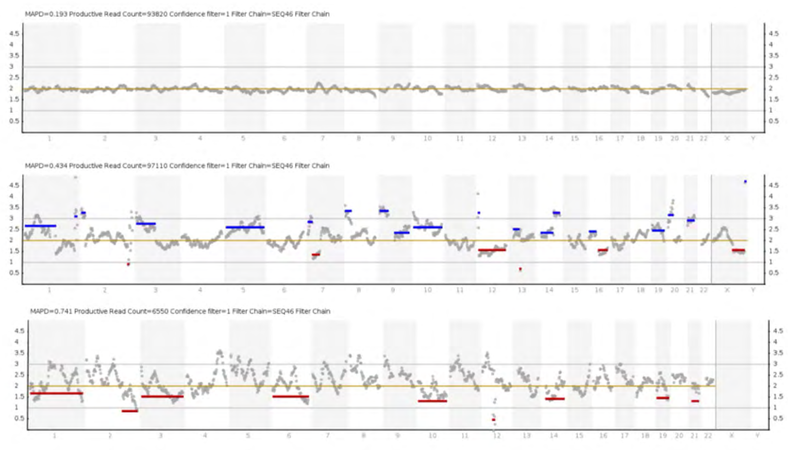
Day of Biopsy	Viable %
5	58.6
6	50.7
7	39
p-value	0.0086

ICM	Viable %
A	42.9
B	54
C	29
p-value	0.001

Figure 1: Example of NGS Workflow Output (TF Ion Reporter)

#	EID	MA	An(+)	An(-)	MAPD
1	1	0.0016			0.214
2	2	0.0019	22		0.213
3	3	0.0026			0.200
4	4	0.0006	21	13	0.229

Figure 2: Example of CNV Profiles & MAPD



CONCLUSIONS

According to our analysis, differences in 2 outputs of sequencing workflow, mtDNA fraction and MAPD, were not apparent when viable and nonviable embryos were compared. As has been previously demonstrated, features of embryo stage and grade are significantly associated with SIR.

Intravenous Human Umbilical Cord Perivascular Cells Treatment in a Mouse Model of Natural Aging Results in the Upregulation of Anti-aging Proteins in Lung Tissue.

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INTRODUCTION

- Aging, a process of the natural decline in physiological functioning that all living organisms endure, increases one's susceptibility to major pathologies and death (1).
- Aging is characterized by several biological hallmarks, including stem cell exhaustion (1).

Figure 1. *Biological hallmarks of aging.*

- Mesenchymal stem cells (MSC) have been studied as candidates for cell therapy in regenerative medicine, including for the treatment of different inflammatory and age-related diseases (2).
- Human umbilical cord perivascular cells (HUCPVC) are a rich and potent source of MSC which have been shown to have anti-inflammatory and anti-aging properties in vivo (3).

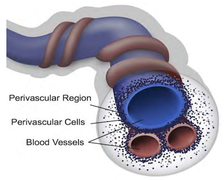

Figure 2. *Human umbilical cord-derived perivascular cells.*

- Preliminary results from an ongoing ovarian aging study being conducted in our lab have demonstrated the therapeutic potential of first trimester (FTM) HUCPVC on increasing pregnancy rates and decreasing ovarian fibrosis in aging animals.
- However, gaps remain in the current literature regarding
  - 1) The biological pathways targeted in the anti-aging action of HUCPVC
  - 2) The different effects produced by FTM versus term HUCPVC

HYPOTHESIS

- HUCPVC can prevent the aging process via upregulation of anti-aging and anti-inflammatory proteins.
- Due to their more naive state, FTM HUCPVC will have a more significant and stronger anti-aging effect compared to term HUCPVC.

OBJECTIVE

- The main objective of this study was to investigate the effects of term and FTM HUCPVC treatment on the presence and levels of aging markers (anti-aging-associated proteins) in lung tissue of young and aging mice.
- By examining specific proteins involved in the early stages of the cellular aging signaling pathway, we aim to elucidate how stem cell therapy could be useful in age prevention.

MATERIALS AND METHODS

An aging mouse model was used for this experiment. These mice were also used in the ongoing ovarian aging study.

ICR mice were randomly assigned to 5 groups

1. ***Young control group (6-month):*** Untreated group, sacrificed at 6 m.
2. ***Aged vehicle control group (12-month):*** Received an IV injection of vehicle (HBSS media) once a month starting at 6 m, and then were sacrificed after 12 m of age.
3. ***Cell therapy control (Fibroblast) group (12-month):*** Received IV injections of 1 million fibroblasts monthly starting at 6 m, sacrificed after 12 m of age.
4. ***FTM-HUCPVC group (12-month):*** Received IV injections of 1 million **FTM** HUCPVC monthly starting at 6 m, sacrificed after 12 m of age.
5. ***Term HUCPVC group (12-month):*** Received IV injections of 1 million **term** HUCPVC monthly starting at 6 m, sacrificed after 12 m of age.

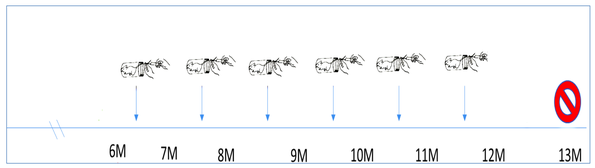

Figure 3. *Injection timeline for Groups 2-5 (Gauthier-Fisher, 2021).*

Thereafter multiple organs, including the lungs, were collected and flash-frozen for future protein analysis. Lung tissue was thawed on ice and homogenized using a bead homogenizer. Proteins were extracted using RIPA buffer. Proteins were measured using the BCA method. Levels of the anti-aging proteins FOXO, SIRT, PTEN, and inflammatory protein TNFα were assessed using Western Blotting. Signals were captured and quantified using the Odyssey Imaging System and Image J respectively (n = at least 3).

RESULTS

1. THE REPEATED AND MONTHLY INJECTION OF FTM AND TERM HUCPVC IN AGING MICE OVER 6 MONTHS HAS NO ADVERSE EFFECTS

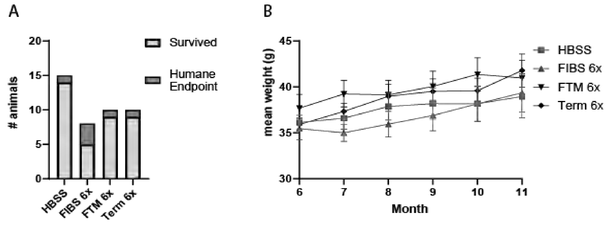

Figure 4. *A. Mortality rates in each group (P=0.2). B. Animal Weight (repeated injections only, excluding sick animals). While the weights of all groups showed a trend of increasing over 6 months, the increase in mean weight was only significant between the first time point (prior to the first injection) and each other timepoint in the 6x FTM -treated and 6x Term-treated groups (P<0.05 at all timepoints). There was no significant difference between the mean weight of any group at any of the timepoints (P>0.05), except for on the day of the 2nd injection, where the 6x FTM mean weight was significantly higher than the 6x FIBS group (P<0.05) (Dviri et al).*

RESULTS

2. SYSTEMIC ADMINISTRATION OF HUCPVC IN AN AGING MODEL PREVENTS THE REDUCTION OF FOXO3a PROTEIN IN LUNG TISSUE

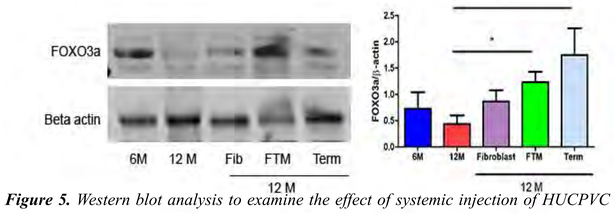

Figure 5. *Western blot analysis to examine the effect of systemic injection of HUCPVC cells on Forkhead box O3a (FOXO3a) protein level in lung tissue of aging mice. Bar diagrams represent the mean ± SEM level of FOXO3a protein. Beta-actin was used as a loading control. N=4 per group, *P < 0.05.*

3. HUCPVC TREATMENT PREVENTS THE REDUCTION OF SIRTUINE FAMILY PROTEINS IN AGING MICE

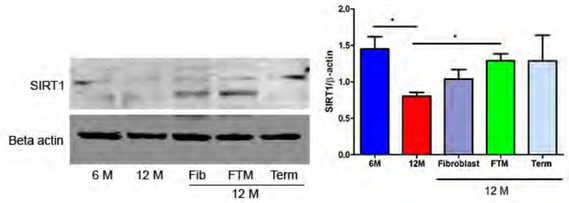

Figure 6. *Western blot analysis to examine the effect of systemic injection of HUCPVC cells on Sirtuin (Sirtuin 1 isoform) protein level in lung tissue of aging mice. Bar diagrams represent the mean ± SEM level of SIRT1 protein. Beta-actin was used as a loading control. N=3 per group, *P < 0.05.*

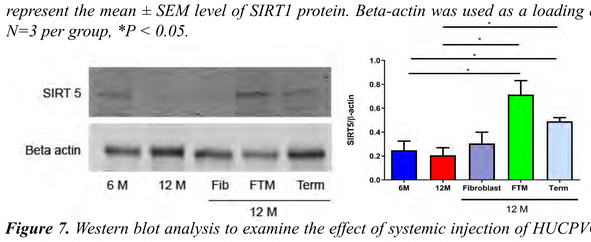

Figure 7. *Western blot analysis to examine the effect of systemic injection of HUCPVC cells on Sirtuin (Sirtuin 5 isoform) protein level in lung tissue of aging mice. Bar diagrams represent the mean ± SEM level of SIRT1 protein. Beta-actin was used as a loading control. N= 3 per group.*

4. HUCPVC TREATMENT PREVENTS THE REDUCTION OF PTEN PROTEIN IN AGING MICE

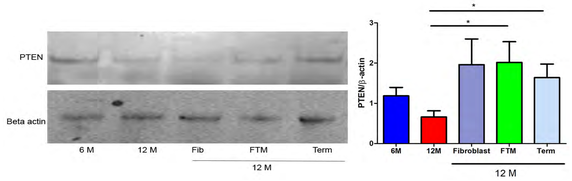

Figure 8. *Western blot analysis to examine the effect of systemic injection of HUCPVC cells on PTEN (anti-aging) protein level in lung tissue of aging mice. Bar diagrams represent the mean ± SEM level of the PTEN protein. Beta actin was used as a loading control. N=3 per group, *P < 0.05.*

5. AGING PROCESS REDUCES THE ACTIVATED (PHOSPHRYLATED) AKT PROTEIN HUCPVC SHOW A TREND OF PREVENTING THAT CHANGE OF AKT PHOSPHORYLATION

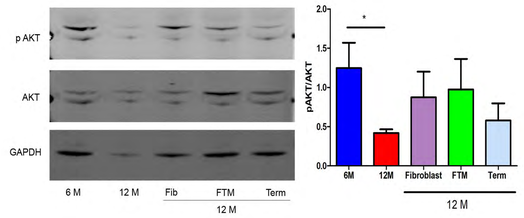

Figure 9. *Western blot analysis to examine the effect of systemic injection of HUCPVC cells on AKT phosphorylation level in lung tissue of aging mice. Bar diagrams represent the mean ± SEM level of the pAKT/AKT ratio GAPDH was used as a loading control. N=3 per group, *P < 0.05.*

6. HUCPVC TREATEMENT PREVENTS THE ACCUMULATION OF INFLAMMATORY PROTEIN TNF α IN AGING MICE

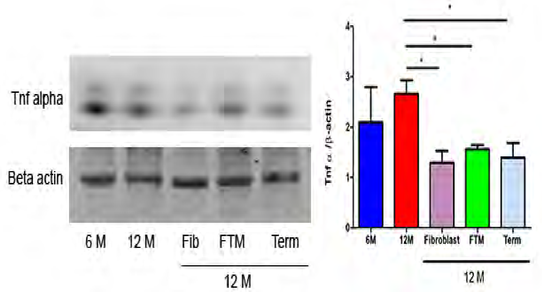

Figure 10. *Western blot analysis to examine the effect of systemic injection of HUCPVC cells on* TNFα *(anti-aging) protein level in lung tissue of aging mice. Bar diagrams represent the mean ± SEM level of the* TNFα *protein. Beta-actin was used as a loading control. N=3 per group, *P < 0.05.*

SUMMARY

- No adverse effects were observed on animal health after HUCPVC injection.
- HUCPVC injection can prevent the age-associated
  - reduction of anti-aging Sirtuin protein (mainly SIRT1).
  - reduction of anti-aging protein FOXO3a.
  - reduction of anti-aging protein PTEN
  - accumulation of inflammatory protein TNFα in aging mice.

CONCLUSIONS

The data generated so far suggest that HUCPVC can be safely administered in aging animals. As well, these cells may have anti-aging effects leading to upregulation of anti-aging proteins and downregulation of inflammatory proteins, specifically PTEN, FOXO3a, SIRT1 (anti-aging) and TNFα (inflammatory) in lung tissues.

SIGNIFICANCE AND FUTURE DIRECTIONS

This study has many potential therapeutic applications:

- If HUCPVC are found to have significant anti-aging effects in the lung ,liver, heart and brain, stem cell therapy could ultimately be used to prevent normal age-related health decline in these organs which have significant impacts on mortality and quality of life.
- Further, if continued investigation demonstrates the potential of this therapy to decelerate the overall aging process, it could potentially be explored for mitigation of aging disorders such as Hutchinson–Gilford Progeria Syndrome, a premature aging disease in which affected individuals have an average life expectancy of 14.6 years (4).

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An Increased Mitochondrial RNA/Nuclear RNA (mt/nRNA) Ratio is Predictive of Implantation Potential

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INTRODUCTION

- Mitochondria are maternally inherited and highly specialized organelles in eukaryotic cells.
- One of the key roles of mitochondria is the production of energy (ATP) through the process of oxidative phosphorylation.
- Mitochondria also have a crucial role in gamete formation, fertilization and embryo development.
- However, mutations that affect ATP production can cause bioenergetic defects and result in mitochondrial dysfunction.
- Mitochondrial dysfunction can lead to implantation failure of seemingly healthy, euploid embryos.
- Our group has previously shown that an increased mtDNA/nuclear DNA (mt/nDNA) ratio is associated with improved embryo implantation (Madjunkova S, 2017; Antes A, 2019).

OBJECTIVES

- The objectives of this study were to:
  - ➢ Further assess the association of mt/nDNA ratio with implantation outcome, using mtRNA expression.
  - ➢ Assess differences in mtRNA gene expression between implanted and non-implanted embryos.

MATERIALS AND METHODS

- This study received IRB approval.
- The mean age of patients included in the study was 35.6 ± 0.31 years
- There was a total of 150 embryos donated for research with known implantation outcome:
  - **a) Implanted (n=84)**
  - **b) Non-implanted (n=66)**
- The analysis was conducted on whole transcriptome sequencing from a trophectoderm (TE) biopsy (4-5 cells).

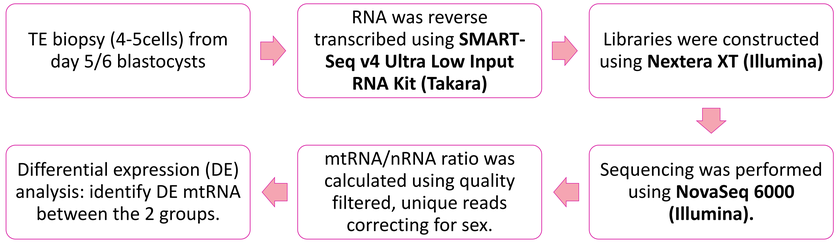

Figure 1: Flowchart outlining the workflow for assessing the association between mtRNA/nRNA ratio and implantation potential.

RESULTS

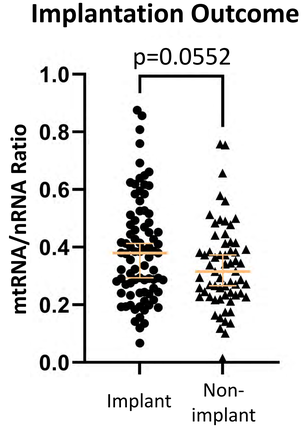

Figure 2: A trend towards increased mtRNA/nRNA ratio in implanted embryos (avg. 0.39±0.019) compared to the non-implanted group (avg. 0.33±0.018) was observed.

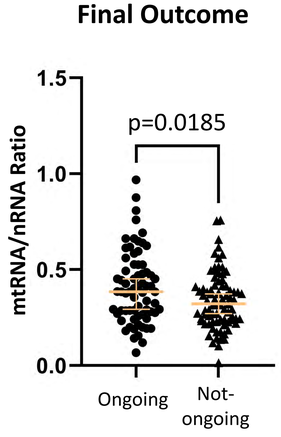

Figure 3: A significant increase in mtRNA/nRNA ratio in embryos that resulted in ongoing pregnancy (>12 GW) (avg. 0.41±0.022) compared to not-ongoing (avg. 0.34±0.016).

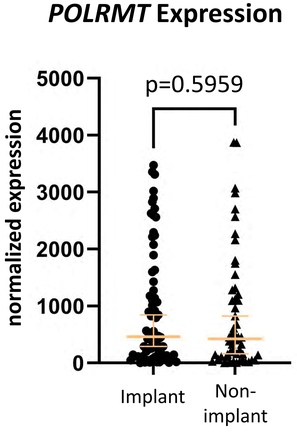

Figure 4: Expression of mtRNA specific RNA polymerase (*POLRMT*) was consistent between implanted and non-implanted groups (fold change = -0.18).

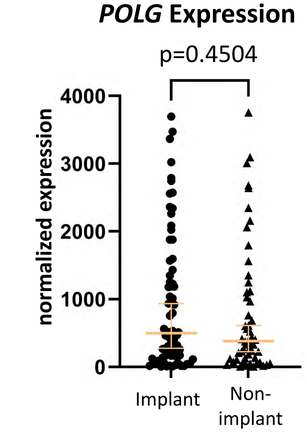

Figure 5: Expression of mtDNA specific DNA polymerase gamma (*POLG*) was consistent between implanted and non-implanted groups (fold change = -0.16).

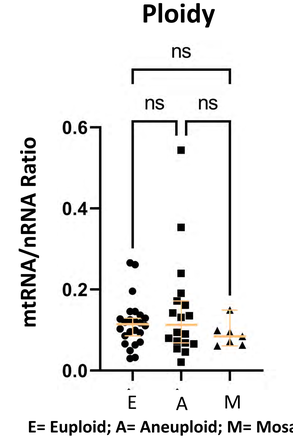

Figure 6: mtRNA/nRNA ratio was consistent between euploid, aneuploid and mosaic embryos. However, the small sample size limits the power for detection of difference..

CONCLUSION

- This study provides novel insight into the dynamics of mtRNA copy number and mtRNA expression in preimplantation embryos, as well as their association with implantation potential.
- Increased mtRNA/nRNA ratio may be a factor in predicting implantation and ongoing pregnancy.
- Increased mtRNA copies did not correspond to increased mtRNA transcription in implanted embryos, which highlights the importance of the mitochondrial content in the oocyte on early pregnancy outcomes.
- To determine the predictive value of the mtRNA/nRNA ratio larger studies are needed.

ACKNOWLEDGEMENTS

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- The authors have no competing interests to declare.

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Counseling oocyte cryopreservation patients: the impact of the time interval from counseling to retrieval on the concordance between pre-treatment predictions and post-retrieval parameters.

Lauren Whitehead, Gary Nakhuda

INTRODUCTION

Age and ovarian reserve are commonly used parameters to counsel patients on expectations of elective oocyte cryopreservation (EOC) outcomes. Intuitively, the delay in treatment would be expected to affect the accuracy of predictions given the temporal decay in ovarian reserve. We reviewed the outcomes for patients who presented for EOC consultation, then followed up for treatment to determine how the interval to retrieval affected the accuracy of pre-counseling predictions.

METHODS

Retrospective review of 283 EOC retrievals from Jan 1, 2021- May 9, 2022. Pre-treatment predictions were made with 2 step process:

A) A nomogram based on internal data, using AMH and age as the independent variables to predict oocytes retrieved.
B) A binomial regression model derived from published and in-house data to predict live birth.

Pre-treatment retrieval and LBR predictions were compared to number of M2 oocytes cryopreserved, and post-retrieval LBR estimates per the **Violet AI.**
The difference between pre- and post-treatment parameters were **analyzed as a function of the time interval from the initial AMH analysis to egg retrieval.**
Outcomes were stratified by age groups, and age as a continuous variable. Pearson's correlation coefficient, ANOVA, Chi-squared, independent sample and pairwise T-tests were calculated where appropriate.

Pre-Treatment Counseling:

A) AMH vs Age nomogram for oocyte retrieval prediction

	AMH stratified by percentiles									
ng/ml	0-0.57	0.57-0.88	0.88-1.19	1.19-1.50	1.50-1.87	1.87-2.37	2.37-2.97	2.97-3.75	3.75-5.1	>5.1
pmol/l	0-4.07	4.07-6.29	6.29-8.50	8.50-10.70	10.70-13.36	13.36-16.90	16.90-21.20	21.20-26.80	26.80-36.43	>36.43
<35	5	7	8	10	13	15	16	17	20	23
35-37	5	7	8	10	12	13	16	16	19	23
38-40	4	6	7	9	10	11	14	16	18	23
41-42	5	5	7	8	8	10	12	14	16	23
43+	2	4	6	8	8	9	10	11	15	19

B) LB Predictor

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Oocyte Age	35														
2	Live Birth Rate per Oocyte	0.0733														
3	Number of Oocyte Cryopreserved	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
4	Number of Oocyte Warmed&Survived	1	3	5	6	8	10	11	13	15	17	18	20	22	23	25
5	Probability of having 1+ live birth childr	7.33%	20.42%	31.66%	36.67%	45.61%	53.29%	56.72%	62.83%	68.08%	72.59%	74.60%	78.18%	81.26%	82.64%	85.09%
6	Probability of having 2+ live birth childr	N/A	1.53%	4.63%	6.61%	11.19%	16.35%	19.05%	24.61%	30.20%	35.72%	38.43%	43.67%	48.66%	51.05%	55.61%
7	Probability of having 3+ live birth childr	N/A	0.04%	0.35%	0.67%	1.67%	3.20%	4.16%	6.47%	9.23%	12.40%	14.11%	17.74%	21.59%	23.57%	27.62%

Post-Treatment Counseling:

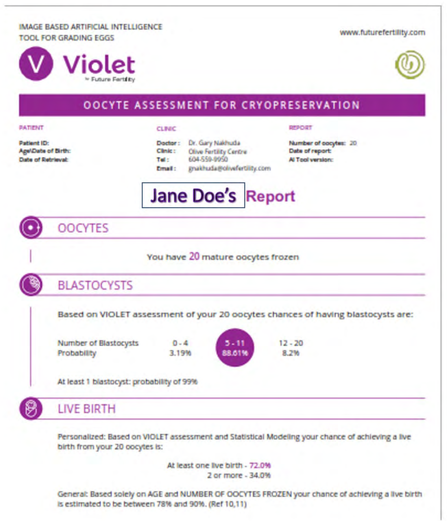
IMAGE BASED ARTIFICIAL INTELLIGENCE TOOL FOR GRADING EGGS

www.futurefertility.com

Violet

OOCYTE ASSESSMENT FOR CRYOPRESERVATION

PATIENT
Patient ID:
Age/Date of Birth:
Date of Retrieval:

CLINIC
Doctor: Dr. Gary Nakhuda
Clinic: Olive Fertility Centre
Tel: 604-559-9950
Email: gnakhuda@olivefertility.com

REPORT
Number of oocytes: 20
Date of report:
AI Tool version:

Jane Doe's Report

OOCYTES

You have 20 mature oocytes frozen

BLASTOCYSTS

Based on VIOLET assessment of your 20 oocytes chances of having blastocysts are:

Number of Blastocysts	0 - 4	5 - 11	12 - 20
Probability	3.19%	88.61%	8.2%

At least 1 blastocyst: probability of 99%

LIVE BIRTH

Personalized: Based on VIOLET assessment and Statistical Modeling your chance of achieving a live birth from your 20 oocytes is:

At least one live birth - 72.0%
2 or more - 34.0%

General: Based solely on AGE and NUMBER OF OOCYTES FROZEN your chance of achieving a live birth is estimated to be between 78% and 90%. (Ref 10,11)

RESULTS

Interval from Consultation to Retrieval	223.2 (+/- 192) Days
Age at Retrieval	35.6 (+/- 4.1) years
Predicted Oocytes vs. Actual Oocytes Vitrified	13.8 (+/- 5.2) vs. 10.4 (+/- 6.1) (p<0.001)
Predicted LBR Models: Pre- vs Post-treatment	52.1% vs. 43.6% (p<0.001)

- Overall, the interval to treatment was not correlated with a difference in pre- to post-treatment ratios.
- Variations in the pre- and post- treatment prediction models was not affected by time interval from initial consult to treatment.
- Stratified by age, the magnitude of differences between pre- and post-treatment increased with the largest differences in the 41-42, and 43+ age groups.

CONCLUSIONS

- Counseling patients about EOC is challenging due to the multiple variables that ultimately dictate the outcomes.
- Our internal modeling data estimated greater number of oocytes and LBR compared to actual M2's vitrified and LBR predicted by Violet.
- The differential in predictions could be due to errors and biases in the modeling, or to the time delay to treatment, which was on average 223 days.
- The impact of delay within the intervals of the study did not appear significant for patients under 40, but older patients had greater discrepancies between pre- and post- treatment parameters with increased durations of beyond 6 months.
- Consistent with other ART outcomes, the impact of advanced age impacts the expected outcomes of EOC, and delay to treatment accelerates the decline in treatment efficacy.

First Trimester Human Umbilical Cord Perivascular cells (HUCPVC) Modulate the Kynurenine Pathway and Glutamate Neurotransmission in an LPS-induced Mouse Model of Neuroinflammation & Depression

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1.CReATe Fertility Centre; 2. Department of Physiology, University of Toronto; 3. Institute of Medical Sciences; Canada; Toronto, 4. Department of Obstetrics and Gynecology, University of Toronto, Toronto, Canada;
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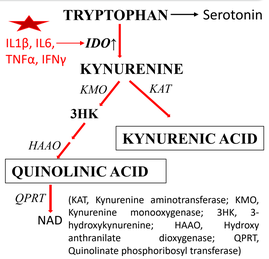
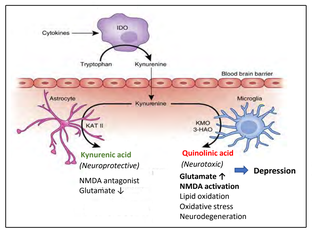
INTRODUCTION

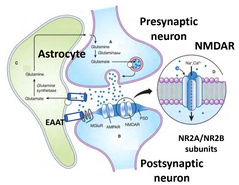
Synthesis of neuroactive metabolites by the activated kynurenine pathway (KP) is associated with a broad spectrum of neurological diseases and mental illnesses. Inflammation-induced activation of the enzyme IDO deviates tryptophan (TRY) metabolism away from serotonin synthesis to produce kynurenine (KYN) that crosses the blood-brain barrier. Astrocytes metabolize KYN to kynurenic acid (KYNA) whereas microglia forms many neurotoxic metabolites, notably, quinolinic acid (QUIN).

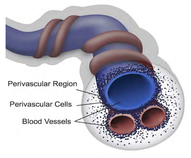




KYNA is an antagonist to the glutamate ionotropic receptor, NMDAR, while QUIN is an endogenous NMDAR agonist. Along with the synthesis of QUIN and other neurotoxic metabolites, microglial activation increases glutamate levels which further activates NMDAR. The deleterious effects of the resulting excitotoxicity are implicated in many brain pathologies, including depression.

NMDAR obligatory subunits, NR2A/NR2B, and astrocytic glutamate transporter, EAAT, are key players in NMDAR activation and glutamate toxicity respectively. Various KYNA analogs and NMDAR antagonists have been developed to target the aberrant kynurenine pathway metabolism and glutamate excitotoxicity. Some of these compounds, however, are known to have psychoactive side effects and cardiovascular toxicity.





The therapeutic potential of HUCPVC, a well-characterized and rich source of MSC, has been demonstrated in the context of various neuropathological conditions. Our lab previously reported modulation of depressive-like behavior by systemically administered HUCPVC in a stress-based *in-vivo* model of depression. Thus, in the current study, we seek to investigate if the modulation of depression by HUCPVC is the result of its immunomodulatory effect on the kynurenine metabolism.

HYPOTHESIS

Peripherally administered HUCPVC may modulate inflammation-associated depressive-like behavior by exerting influence on the kynurenine pathway and glutamatergic system in the brain.

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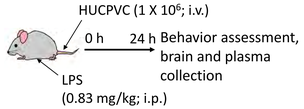
Human umbilical cord mesenchymal stem cells: a new era for stem cell therapy. Cell Transplant. 2015;24:339–347

Recent evidence for an expanded role of the kynurenine pathway of tryptophan metabolism in neurological diseases. Neuropharmacology; 2017; 112(Pt B):373-388.

Mesenchymal stromal cells modulate peripheral stress-induced innate immune activation indirectly limiting the emergence of neuroinflammation-driven depressive and anxiety-like behaviors. Biol Psychiatry, 86(9):712-724

MATERIALS AND METHODS

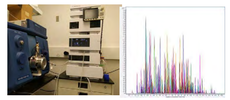
Animal treatment & groups



Experimental groups

LPS, LPS+HUCPVC and control

LC-MS/MS analysis

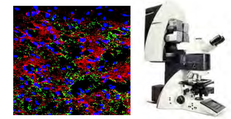


Sciex QTrap 5500 with SelexION + Agilent 1200 HPLC was employed at AFBM-SickKids, Toronto, to isolate and measure the plasma and brain levels of kynurenine metabolites

Forced Swim Test (Behavior assessment)

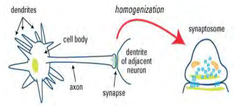
Each mouse was placed in a water-filled cylinder for 6 min and video recorded. Percent immobility Time was calculated and correlated to the depressive behavior

Immunohistochemistry & confocal microscopy



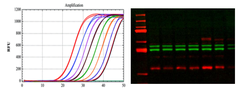
Immunohistochemistry was performed on the frozen cryosectioned whole brain sections. Imaging was done by Leica SP8 confocal microscopy.

Synaptosomal fractionation



An enriched fraction of synaptic proteins was obtained by gradient centrifugation of the whole brain using Syn-PER reagent (Thermo Fisher)

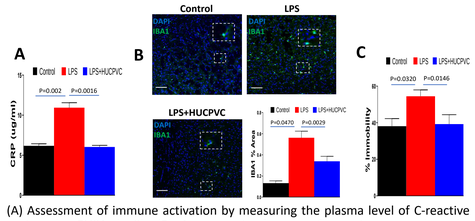
QPCR & Western blot



QPCR: performed using SYBR-Green reagent. Fold change analyzed by ΔΔCt method.
Western blot: Performed using Licor 2-antibodies and Odyssey dual Imaging system

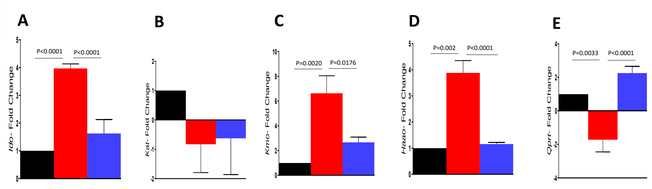
RESULTS

HUCPVC modulate LPS-induced immune activation, neuroinflammation & depressive-like behavior



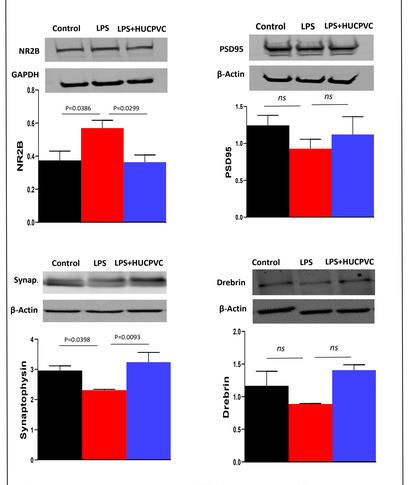
(A) Assessment of immune activation by measuring the plasma level of C-reactive protein by ELISA (n=3); (B) Assessment of neuroinflammation was done by immunostaining of microglial activation marker, IBA1, in the cerebral cortex (n=3). (C) Forced Swim Test was employed to assess depressive-like behavior (n=3).

HUCPVC modulates the LPS-induced dysregulation of brain kynurenine pathways enzymes



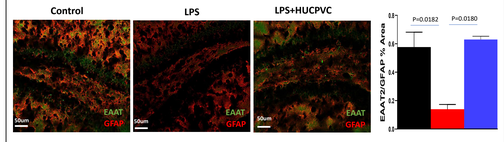
Real-time qPCR analysis for gene expression levels of (A) *Ido,* (B) *Kat*, (C) *Kmo,* (D) *Haao,* and (E) *Qprt* enzymes of the kynurenine pathway in the mRNA isolated from the brains of control, LPS, and LPS+HUCPVC groups (n=3).

Modulatory effect of HUCPVC on LPS-induced synaptic loss



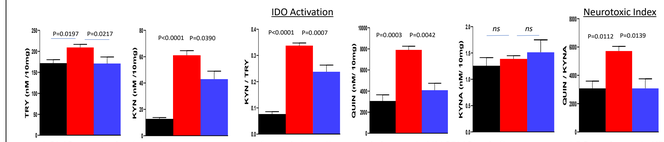
Western blot analysis to test HUCPVC's effect on LPS- induced loss of synaptic proteins in the isolated synaptosome fraction (n=3).

HUCPVC rescues the LPS-induced decline of the brain glutamate transporters



Confocal imaging and analysis of double staining of astrocyte marker, GFAP and glutamate transporter, EAAT2 in control, LPS, and LPS+HUCPVC groups (n=3)

Modulation of LPS-induced dysregulation of brain kynurenine pathways metabolites by HUCPVC



LCMS analysis for the assessment of peripherally administered HUCPVC's influence on LPS-activated KP metabolites in the brain homogenate obtained from control, LPS, and LPS+HUCPVC groups (n=5-8)

CONCLUSION

This is the first study that demonstrates the modulatory effect of systemically administered MSC on the inflammation-associated imbalance in the kynurenine pathway and glutamatergic system

ACKNOWLEDGEMENTS

The authors thank the contributions of Fatima Sultani, (AFBM, SickKids, Toronto), for her LCMS technical services and Subhendu Mukherjee. Research funded by the Create Fertility Centre.

Identifying Spermatogonial Stem Cells (SSCs) in Postnatal Development

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Department of Obstetrics and Gynecology, Research Institute of the McGill University Health Center (RI-MUHC)

Background

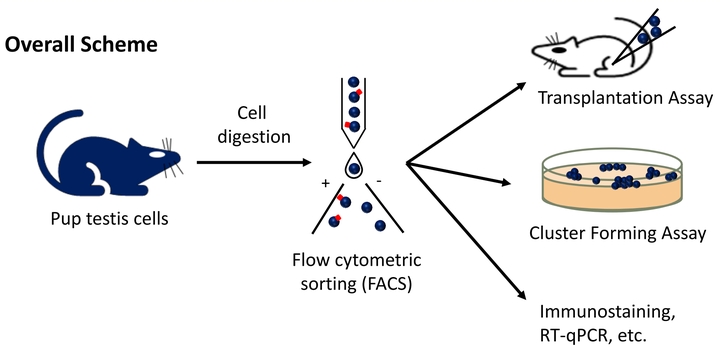
Stem cells can self-renew as well as generate other cell types, and therefore restore lost function in tissue. SSCs located in the seminiferous epithelium maintain spermatogenesis and are the only cell type capable of restoring fertility. They have many potential applications to medicine, such as treating infertility or cryopreserving SSCs in child cancer patients who need to undergo gonadotoxic treatments. While isolating and cryopreserving SSCs then transplanting them later is a reality in mice, it is not yet possible in humans. Our goal is to identify the surface phenotype of the SSC fraction, which will allow isolation and cryopreservation with minimal expansion. While many SSC surface markers have been identified, their exact surface phenotype is still unknown.

Objective

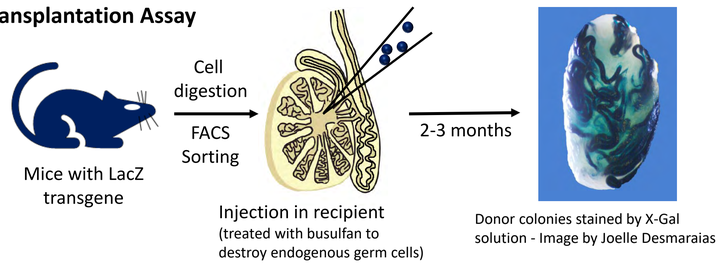
Identify the surface phenotype of SSCs in postnatal development, focusing on three selected stages: P0-1, P8-9, and P16-18 (P1 is Postnatal Day 1). Understand the fate map of SSC commitment in postnatal development using fate markers like GFRα1 and c-kit.

Methods

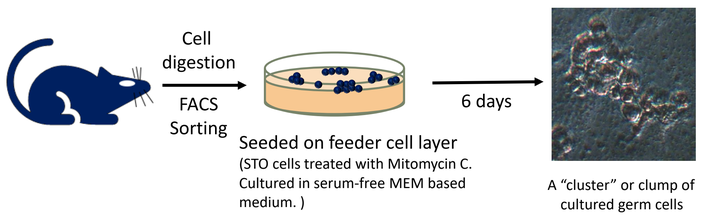
Overall Scheme



Transplantation Assay



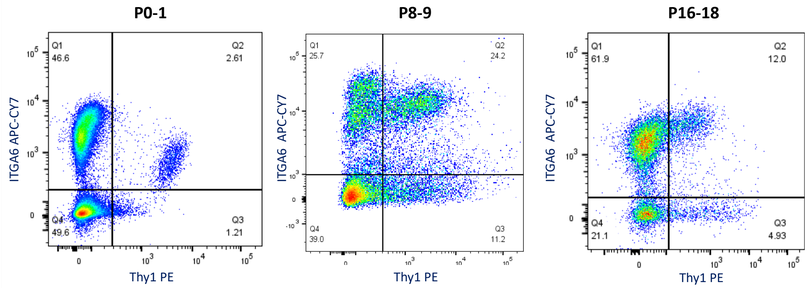
Cluster Forming Assay (CFA)



Results

Figure 1. Selected stages in postnatal development.

Data collected on BD LSRFortessa™ or BD FACSAria™ Fusion

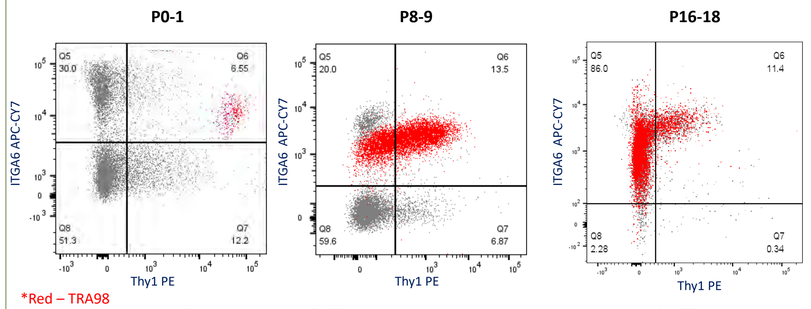


- Germ cells still in lumen
- Migration to basal membrane

- Immediately before meiosis onset

- After Blood-testis barrier formation

Figure 2. TRA98 (germ cell marker) expression measured by intracellular flow.

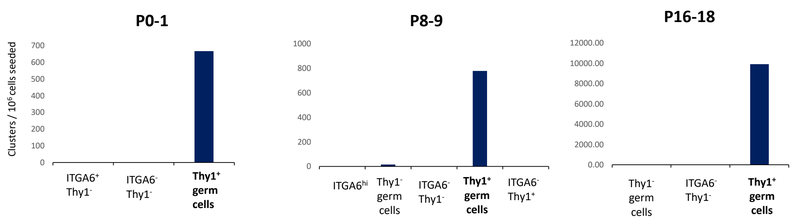


*Red – TRA98

Similar results in FACS followed by immunofluorescence staining for TRA98.
Shows that germ cells express Integrin-α6(ITGA6) throughout development.

Figure 3. Thy1+ germ cells have most self-renewal activity in vitro and in vivo.

(A) In vitro (Cluster forming assay)



(B) In vivo (Transplantation assay)

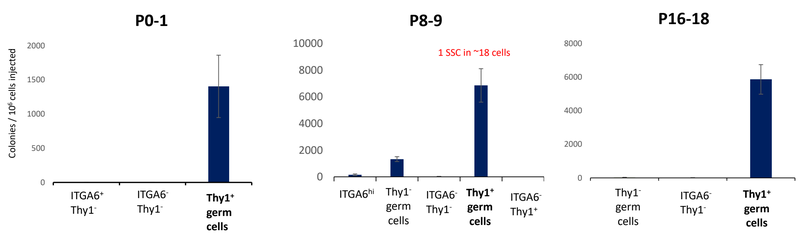


Figure 4. Fate marker (GFRα1, c-kit) expression in Thy1+ germ cells.

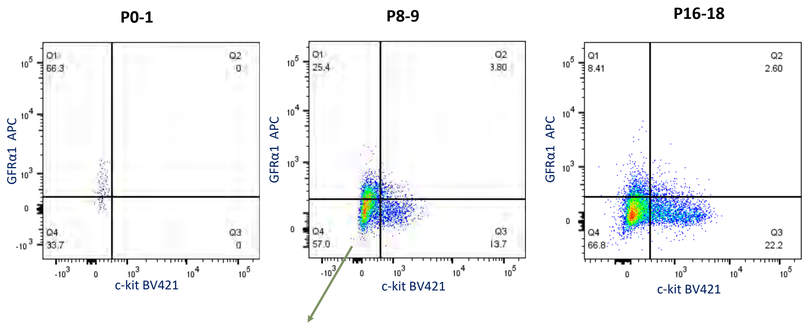
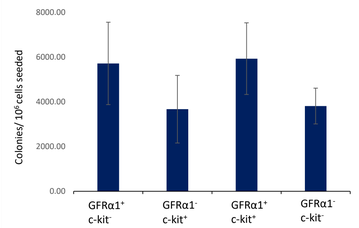
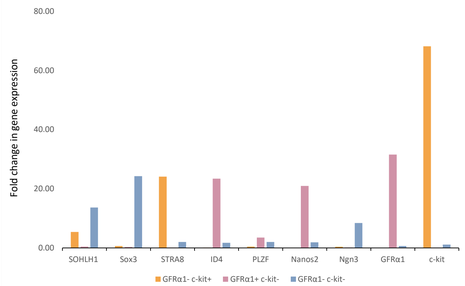


Figure 5a. P8-9 Thy1+ germ cells: GFRα1 and c-kit fractions (Transplantation Assay)



P8-9 Thy1+ ITGA6+ germ cells were further fractionated with fate markers GFRα1 and c-kit, then fractions were compared using the Transplantation Assay.
GFRα1⁺ fractions were similarly enriched in SSCs and showed a higher enrichment than c-kit⁺ fractions, which were also similarly enriched.
As germ cells progress in commitment and lose SSC potential, they likely transition from the GFRα1⁺ to c-kit⁺ state.

Figure 5b. P8-9 Thy1+ germ cells: GFRα1 and c-kit fractions (RT-qPCR)



Cell fractions were analyzed for marker expression by RNA extraction followed by cDNA synthesis and qPCR, performed with the Roche LightCycler® 96. Fold change in gene expression was quantified with the $2^{-\Delta\Delta CT}$ method and the amplification efficiency of each primer was verified to be between 90-110%. Reference gene was β-actin.

GFRα1⁺ c-kit⁻ fraction highly expressed SSC/self-renewal genes like ID4, Nanos2, and GFRα1. GFRα1⁻ c-kit⁺ fraction highly expressed commitment/differentiation genes like STRA8 and c-kit. GFRα1⁻ c-kit⁻ fraction highly expressed commitment genes like SOHLH1, Sox3, and Ngn3, but the identity of these genes suggest less commitment compared to the GFRα1⁻ c-kit⁺ fraction. GFRα1⁺ c-kit⁺ fraction was omitted due to insufficient RNA yield.

Conclusions

1. Germ cells express ITGA6 throughout postnatal development (Figure 2).
2. Thy1+ germ cells are the SSC-enriched fraction throughout development (Figure 3).

Hypothesis: Thy1+ germ cells transition to Thy1- state during fate commitment as they transition towards the point-of-no-return (loss of stem cell activity).

3. GFRα1 expressing cells tend to show higher regenerative activity and SSC marker (intracellular) expression compared to c-kit expressing cells (Figure 5a and 5b).

Furthermore, we suspect that fate commitment may progress from GFRα1⁺ c-kit⁻ → GFRα1⁻ c-kit⁻ → GFRα1⁻ c-kit⁺ based on qPCR data. In the future, we will further unravel how GFRα1 and c-kit expression of germ cells in postnatal development relates to progression in fate commitment by completing the analysis in other stages (P0-1 and P16-18).

Using just two markers (Thy1 and ITGA6) we were able to purify SSCs by a very high degree (6854 col./10^6 cells injected). Our ultimate goal is to isolate pure SSCs.

Acknowledgements: Research was supported by the RI-MUHC and NSERC. Presentation was supported by a RQR Travel Award.

Implication of Fragile-X Related Proteins and Neurotrophic Factors in Establishing Transzonal Projections

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[2]Centre de recherche en Reproduction, Développement et Santé Intergénérationnelle (CRDSI)
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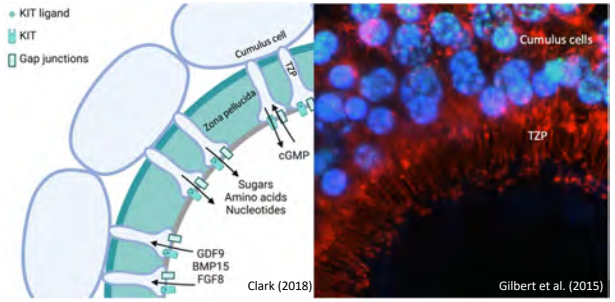
INTRODUCTION

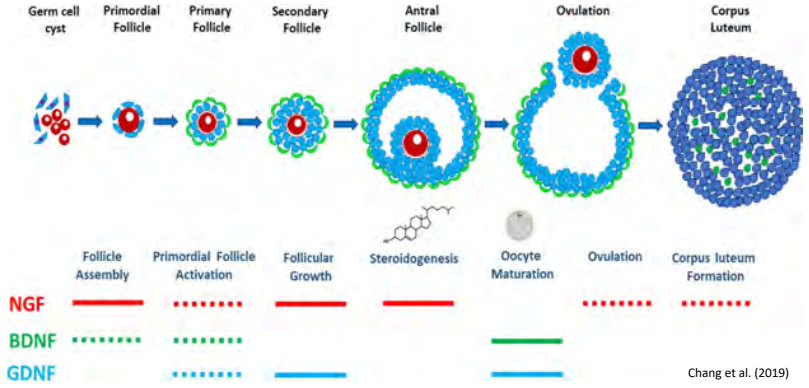
Transzonal projections (TZP) are essential in order to get a competent oocyte.



The TZP network has structural and functional similarities with neurons.

Fragile-X related proteins (FXR) contribute to the development of the neural network through their ability to bind mRNA and are involved in translational control.

About 20% of women who are fragile X premutation carriers have primary ovarian insufficiency (POI).



HYPOTHESIS

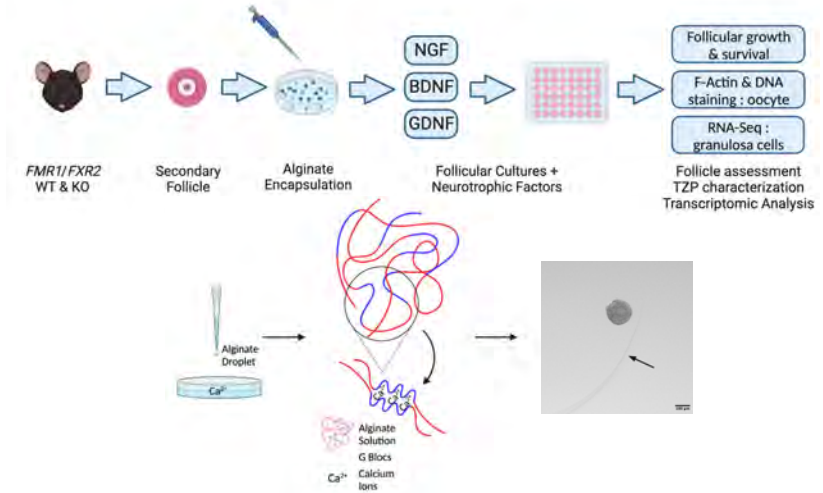
Neurotrophic factors support the development and function of TZP, and these effects are mediated by FXR proteins.

OBJECTIVES

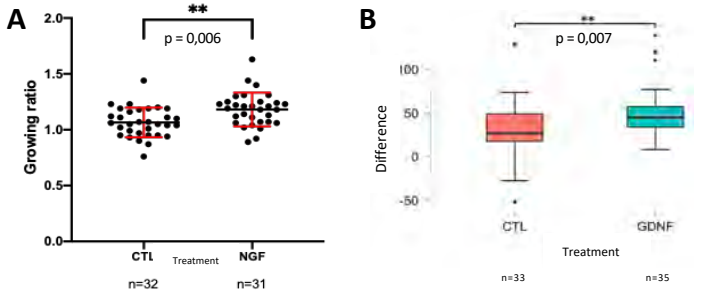
Evaluate the addition of neurotrophic factors (NGF, BDNF, GDNF) on follicular growth and the development of the TZP network.

Determine their impact on the expression of candidate genes, particularly FXRP.

METHODS



PRELIMINARY RESULTS



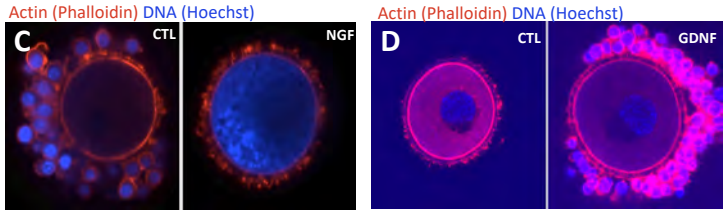


Fig. 1. Growth of bovine follicles with added treatment of (A,C) NGF (5 ng/ml) and (B,D) GDNF (50 ng/ml) over 8 (A,B,D) or 13 days (C). Dubuc, K. and Marchais, M.

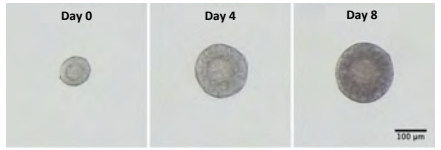


Fig. 2. Growth of a mouse ovarian follicle encapsulated in an 0,5% alginate hydrogel for 8 days.

CONCLUSION AND PERSPECTIVES

Bovine: NGF and GDNF had beneficial effects on follicular growth which resulted in a more developed TZP network.

Mice: Data on the addition of neurotrophic factors to follicular culture is being collected. Transcriptomic analysis will be carried out.

ACKNOWLEDGEMENTS

NSERC CRSNG
RQR Réseau Québécois en reproduction

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A Retrospective Study of the Feasibility, Safety, Pregnancy Rates and Accuracy of Hysterosalpingo-foam sonography (HyFoSy) in Tubal Patency Assessment.

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Background

- Assessment of tubal patency is a critical component of a fertility workup.
- The gold standard tubal patency assessment is diagnostic laparoscopy with chromotubation (LC). To avoid the risks of surgery, hysterosalpingography (HSG) has been established as a first line investigation. However, HSG requires utilization of hospital resources, patient exposure to radiation and can be uncomfortable to patients.
- HyFoSy is an office-based ultrasound procedure that allows for tubal patency assessment, as well as uterine cavity assessment

Objective

- To review the use of HyFoSy by examining its safety, completion rate, pregnancy rate following the procedure and when possible, its concordance with HSG or LC.

Materials and Methodology

- Retrospective chart review of 557 women between the ages of 18-50 years old who underwent an attempt at HyFoSy procedure for tubal patency assessment between January 1, 2019 – June 30, 2019 was conducted at a single academic fertility clinic in London, Ontario.
- Results of the HyFoSy were collected as well as results from secondary evaluations. Not all women underwent secondary evaluation
- HyFoSy was completed between menstrual cycle days 6-12 of a women's menstrual cycle either directly by the same four GREI physicians or by residents/fellows under their direct supervision.
- Patients were prescribed a two-day course of prophylactic antibiotics to be started the day before the ultrasound

Baseline Characteristics

Table 1. Baseline characteristics of the 557 women included in study

Demographic	
Average Age (IQR)	33 (29-36, range 19-49)
Mean BMI (SD) *	29.1 (SD ±7.5)
Median Parity **	0 (IQR = 0-2, range 0-9)
Diagnosis ***	(n=)
Primary infertility	287
Secondary Infertility	230
Other	37

* 44/557 did not have BMI recorded
** 5/557 did not have number of previous pregnancies recorded
*** 3/557 did not have diagnosis recorded

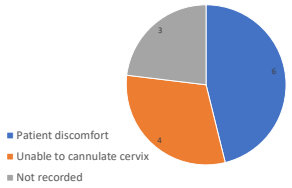
Results

Safety
- There were no documented infections, complications or allergic reactions within 1 month of completion of HyFoSy

Pregnancy Rate
- A pregnancy rate of 15% (81/544) was noted within 6 months of completion if HyFoSy

Completion Rate
- 97.7% (544/557) of women completed the HyFoSy
- 13 women did not complete HyFoSy

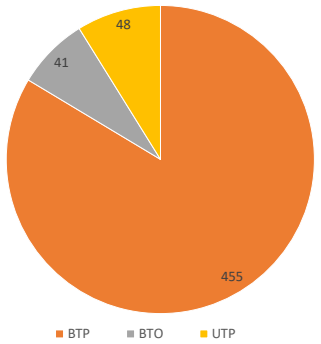
Fig 1. Reason for Inability to Complete HyFoSy



Results

Tubal patency assessment results of 544 women who completed HyFoSy
- Bilateral Tubal Patency (BTP) 83.6%
- Unilateral Tubal Patency (UTP) 8.8%
- Bilateral Tubal Occlusion (BTO) 7.5%

Fig 2. Tubal Patency Results of 544 who completed HyFoSy



HyFoSy concordance with secondary tests (HSG or LC).
- 8% (42/544) of women who completed HyFoSy had records of secondary evaluation.
- BTP – 10 women underwent secondary testing – 7 had LC, of which 1 had diagnosis changed to UTP. 3 underwent HSG and had BTP status confirmed.
- UTP – 10 women underwent secondary testing – 3 women had LC, of which 2 had diagnosis changed to BTP. 7 women had HSG of which 4 had diagnosis changed to BTP
- BTO – 22 women underwent secondary testing – 20 women had HSG of which 18 had diagnosis changed to BTP. 2 women had LC and diagnosis confirmed as BTO

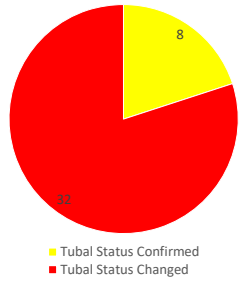
Table 2. Concordance of results between HyFoSy and secondary testing

Tubal Status per HyFosy	% diagnosis confirmed with secondary testing, (n)
BTP	90% (10)
UTP	40% (10)
BTO	18% (22)

Results

- Total: 32 women with non-BTP status based on HyFoSy underwent secondary evaluation, of which 25% (8/32) had diagnosis confirmed.

Fig 3. Tubal Status after Secondary Evaluation



- Based on the sample of 32 women with non-BTP status, PPV of HyFoSy is 20% (95% CI 19.6%-20.4%)
- Based on the sample of women with BTP status, the NPV of HyFoSy is 90% (95% CI 89.4% - 90.1%)

Limitations

- There were a number of charts where basic demographic information was unavailable
- Only 8% of women underwent secondary evaluation which limits ability to perform robust analysis of positive and negative predictive value, sensitivity and specificity.

Conclusions

- HyFoSy is a safe test
- HyFoSy has a high completion rate
- Pregnancy rate within 6 months of completing HyFoSy is 15%
- HyFoSy has a high NPV and a low PPV
- Patients diagnosed with non-BTP status should undergo secondary evaluation to confirm tubal status if it will change clinical decision making

Sperm DNA fragmentation is not correlated with the oxidation-reduction potential in men presenting for infertility evaluation.

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ABSTRACT

Traditional semen analysis remains the standard of clinical care to initially investigate and diagnose male infertility. However, the basic semen analysis does not provide information on the oxido-reductive potential (ORP) and nuclear integrity of semen and spermatozoa, respectively. Sperm DNA fragmentation (SDF) and oxidative stress are markers believed to be implicated in the pathogenesis of male infertility. Thus, here were prompted to evaluate the relationship between ORP and SDF in infertile men receiving antioxidants. The cohort study included 64 men undergoing infertility evaluation. Sperm DNA fragmentation index (DFI) was measured with Tunel assay and ORP in semen was assessed using the standardized MiOXSYS system. Patients were then divided into two study groups according to the use of antioxidant supplementation. There was no statistically significant difference in the mean abstinence period (3.0 ± 0.2 vs. 2.4 ± 0.1 days), sperm concentration (64.9 x 10^6/mL (8.1) vs. 55.4 x 10^6/mL (10.4)) and DFI (15.8% (1.8) vs. 19.0% (2.3)) between groups (p=> 0.05). However, mean ORP was significantly lower in the no antioxidant compared to the FertilPro antioxidant group (1.0mV/10^6sperm/mL ± 0.2 vs. 1.6mV/10^6sperm/mL ± 0.4). We observed no significant correlations between SDF and semen ORP in the no antioxidant group (r=-0.02; p=0.89) and the FertilPro groups (r=-0.11; p=0.58). When applying the established clinical cut-off (1.34 mV/10^6 sperm/ml), only 6% of patients had both an abnormal SDF and ORP in the no antioxidant group and 17% of patients in the FertilPro group. These findings suggest that monitoring these markers in men with infertility may provide us with a better understanding of the complex relationship between semen.

INTRODUCTION

Infertility is characterized by an inability to conceive, despite repeated attempts for a period of more than one year. It is a condition that affects approximately 15% of couples of childbearing age worldwide. Although it may be of female origin, there are also several male factors that may be involved. In general, it is possible to diagnose male infertility via a semen analysis. In fact, low sperm count, altered morphology, or a reduction in the amount of effective movement can harm a couple's chances of conception. Although sperm parameters remain important for the diagnosis of fertility in men, the methods used are highly criticized due to their high level of variability. Indeed, despite the attempt of the World Health Organization (WHO) to standardize the practices, the results remain subjective and vary greatly depending on the observers as well as the handling errors, precision and variability within the specimens themselves. Furthermore, in several cases (30 to 50%), sperm parameters are not sufficient to establish the infertility diagnosis.

STUDY QUESTION

Does an elevated ORP (≥1.34mV/10^6 sperm/mL) is associated with higher SDF amongst infertile patients and should it be measured routinely to assess the reproductive potential?

METHODS

Study design: The cohort study included 64 male patients undergoing infertility evaluation. Following the reception of written informed consent, the sperm DNA fragmentation index (DFI) was measured with terminal deoxynucleotidyl dUTP transferase nick-end labeling (TUNEL) assay (AccuriC6 Flow Cytometer) and ORP in semen was assessed using the standardized Male Infertility Oxidative System (MiOXSYS) system. Patients were then divided retrospectively into two study groups according to the use of antioxidant supplementation: no antioxidant or FertilPro antioxidant therapy group.

Participants: Out of the 64 males included in the study, 34 had no history of antioxidant therapy and 30 were taking FertilPro on a regular basis prior testing.

Settings: All sperm samples were analyzed according to the WHO criteria, and a cut-off value of 16.9% for DFI and 1.34mV/10^6sperm/mL for ORP were applied to classify normal vs abnormal samples.

Quality control: ZoBell's Solution, Oxidation-Reduction Potential Standard (988016, Ricca Chemical) was used as a positive control. A solution of ascorbic acid in SAGE 1-Step HSA medium (7701, Origio) served as a negative control. A calibration solution at pH 7 saturated with quinhydrone (ORPCALKIT, Myron L), was used as an extra control for the accuracy of the results.

Data analysis: Pearson's r was used for correlation analysis and continuous variables, or percentage results are expressed as mean ± (standard error).

RESULTS

Table 1. The variation coefficients of intra-assay and inter-assay reproducibility of ORP using MiOXSYS

	Mean ORP (mV)	SD	CV (%)
Intra-assay	226.1 (6)	1.2	0.52
Inter-assay lot 1	242.5 (20)	1.8	0.76
Inter-assay lot 2	222.7 (20)	1.5	0.66
Inter-assay lot 1 and 2	232.6 (40)	10.2	4.39

*Number of data in brackets

Figure 1. Correlation between seminal ORP and sperm DNA fragmentation in patients following up at a fertility clinic with no history of antioxidant therapy

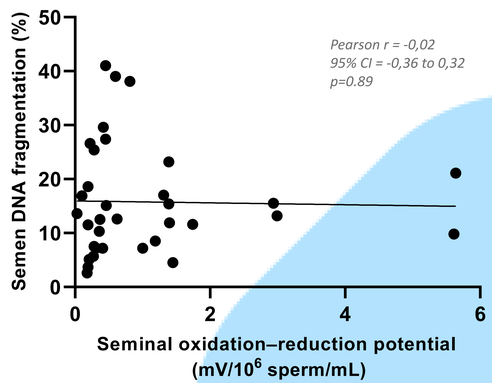
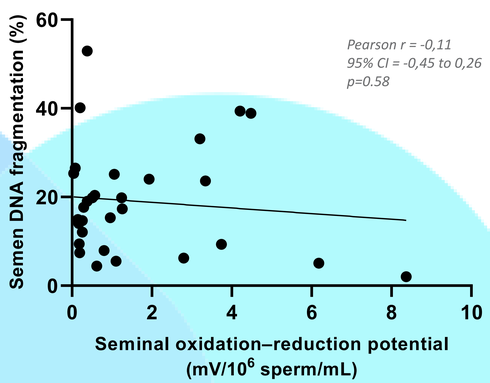


Table 2. Semen parameters in patients with no history of antioxidant therapy and in patients on FertilPro therapy

Patients	DFI (%)	ORP	Abstinence (days)	Concentration (mil/ml)
Control	15.8 (1.8)	1.04 (0.2)	3.0 (0.2)	64.9 (8.1)
FertilPro	19.0 (2.3)	1.63 (0.4)	2.4 (0.1)	55.4 (10.4)

*ORP is expressed in mV/10^6sperm/mL

Figure 2. Correlation between seminal ORP and sperm DNA fragmentation in patients following up at a fertility clinic and taking FertilPro supplement



CONCLUSIONS

Semen DFI doesn't correlate with ORP in patients undergoing routine screening for infertility or in patients on FertilPro antioxidant therapy. This finding highlights the importance of testing both semen ORP and DFI for screening, clinical diagnosis, and antioxidant therapy monitoring especially in patients with unexplained infertility.

Limitations: The sample size of the current study was moderate, despite similar observation between both study groups. The effect of antioxidants on both semen ORP and DFI should be confirmed in a prospective controlled trial.

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Obesity and PCOS radically alters the smallRNA composition of follicular fluid extracellular vesicles

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Introduction

Follicular Fluid and Extracellular Vesicles

- Follicular fluid (FF), derived from serum and somatic cell excretions, and somatic cells (granulosa and cumulus cells) provide the micro-environment for the developing and maturing oocyte[1]
- FF from equine[2], bovine[3], and humans[4] are rich in extracellular vesicles (FFEVs) which are thought to be reflective of the state of the granulosa cells (GC), cumulus cells (CC), and the oocyte (Oo)
- FFEVs have been shown to contain a myriad of proteins[5], metabolites[6], DNA and RNA[7], including miRNAs[4,8], a family of small-noncoding RNAs (snRNAs). Several miRNAs have been associated with various conditions including PCOS[9], endometrosis[10], and advanced reproductive age[11]
- However, assessing the whole snRNA cargo in FFEVs from a single follicle has not been explored, limiting the ability to use FFEV snRNAs as predictive biomarkers of in-vitro fertilization (IVF)

Obesity and PCOS

- Polycystic ovarian syndrome (PCOS) is a common infertility disorder and is estimated to affect 5-10% of women of reproductive age[12]. It is a complex and multifactorial disorder characterized by infertility, amenorrhea/oligomenorrhea, hirsutism (excessive hair growth), obesity, insulin resistance, hyperandrogenism, and polycystic ovaries by ultrasound[13,14].
- Obesity is a common comorbidity associated with PCOS[13,14] and affects all organ systems[15,16].
- There have been limited reports of the effect adiposity has on the snRNA profile of the follicle independent of PCOS[17].
- Adipose tissue is the largest source of circulating miRNAs, and is considered a new class of adipokine[18]

Objectives

1) Develop a novel sequencing method for the detection of snRNA in fractionated follicular fluid extracellular vesicles (FFEVs): microparticles (MP), exosomes (EX), depleted follicular fluid (FFD), and granulosa cells (GC) from a single follicle
2) Profile the snRNA profile of MP, EX, FFD, and GC from PCOS matched to non-PCOS patients
3) Elucidate the impact adiposity has on the snRNA profiles in both PCOS and matched non-PCOS patients
4) Identify specifically packaged snRNAs and the impact their secretion may have on folliculogenesis

Methods

FF Collection and FFEV Isolation

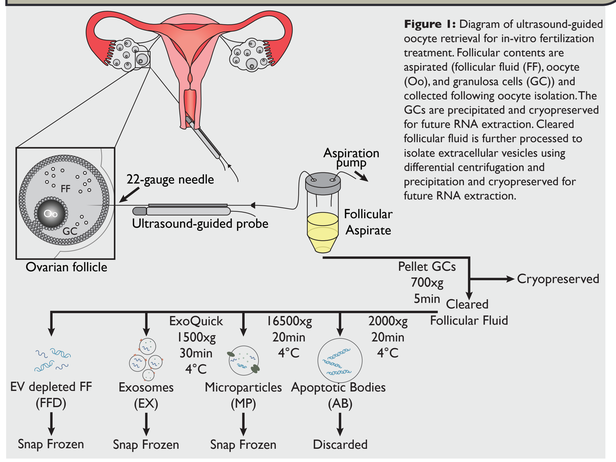

Figure 1: Diagram of ultrasound-guided oocyte retrieval for in-vitro fertilization treatment. Follicular contents are aspirated (follicular fluid (FF), oocyte (Oo), and granulosa cells (GC)) and collected following oocyte isolation. The GCs are precipitated and cryopreserved for future RNA extraction. Cleared follicular fluid is further processed to isolate extracellular vesicles using differential centrifugation and precipitation and cryopreserved for future RNA extraction.

RNA Isolation and smallRNA Sequencing

Participant Matching and Demographics

Table 1: Participant Demographics presented as mean±SEM (Range). Significant differences were determined using two-way ANOVA with multiple comparisons (Turkey) indicated by superscript letters.

	Obese PCOS (n=5)	Obese Non-PCOS (n=10)	Lean PCOS (n=10)	Lean Non-PCOS (n=10)
Age (years)	36.4±1.9 (31-42)	35.0±1.6 (27-45)	37.8±1.0 (30-41)	32.9±0.8 (31-40)
BMI (kg/m2)	32.5±1.2[a] (30.1-37.2)	33.8±1.1[b] (30.2-38.4)	21.5±0.5[a] (19.1-24.8)	21.7±0.4[b] (20.6-23.7)
AMH (pmol/L)	56.3±6.1[c] (43.3-81.9)	26.4±1.3[c] (19.6-32.2)	60.5±7.1[d] (30.2-101.0)	34.2±2.3[d] (26.4-46.2)
LH on Trigger (mIU/ml)	3.6±0.9 (1.3-7.0)	3.7±0.8 (0.5-8.0)	2.6±0.6 (0.1-5.5)	1.6±0.5 (0.2-4.0)
Estradiol on Trigger (pmol/L)	10702.2±2996.2 (1430-19875)	10792.0±1531.4 (6183-20688)	10218.8±1566.3 (4024-18857)	14154.2±1881.5 (3146-27064)

Results

Differences observed in FFEV smallRNA profiles are due to Adiposity

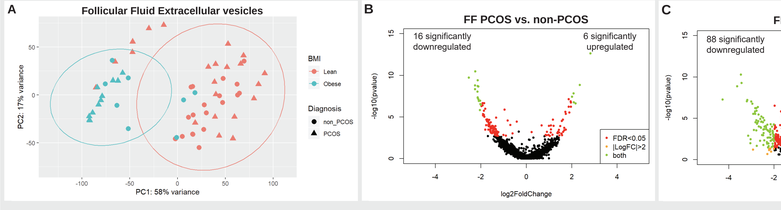

Figure 2: Principal component analysis (PCA) and differential expression. A) PCA of all follicular fluid libraries shows significant separation along PC1 by BMI, depicted by colour, and no apparent effect of PCOS diagnosis, depicted by the shape. B) DE analysis between PCOS and non-PCOS, regardless of particle type, using DESeq2; 22 snRNAs were differentially expressed (6 significantly upregulated (log2FC > 2 and FDR < 0.05), and 16 significantly downregulated (log2FC < -2 and FDR < 0.05)). C) DE analysis between Lean and Obese, regardless of particle type, using DESeq2; 2771 snRNAs were differentially expressed (2683 significantly upregulated (log2FC > 2 and FDR < 0.05), and 88 significantly downregulated (log2FC < -2 and FDR < 0.05)).

Results - Continued

miRNA is the most abundant biotype in extracellular vesicles

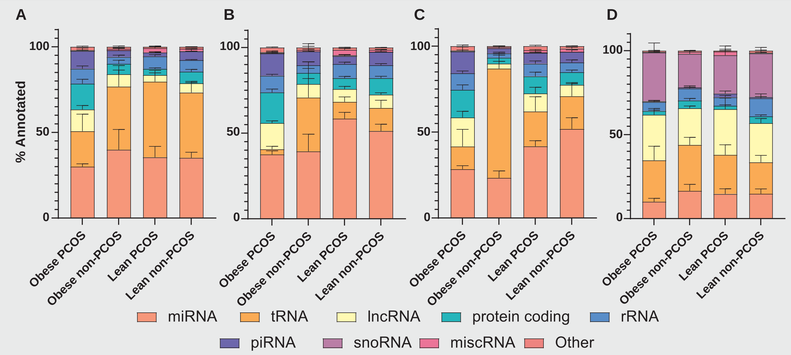

Figure 3: snRNA biotype distributions and differentially expressed snRNAs in subgroups. The proportion of annotated reads mapping to the indicated snRNA biotypes across the four subgroups A) Microparticles (MP), B) Exosomes (EX), C) Follicular fluid depleted (FFD), and D) Granulosa cells (GC).

Adiposity significantly alters the miRNA signature in all FFEVs but not in GCs

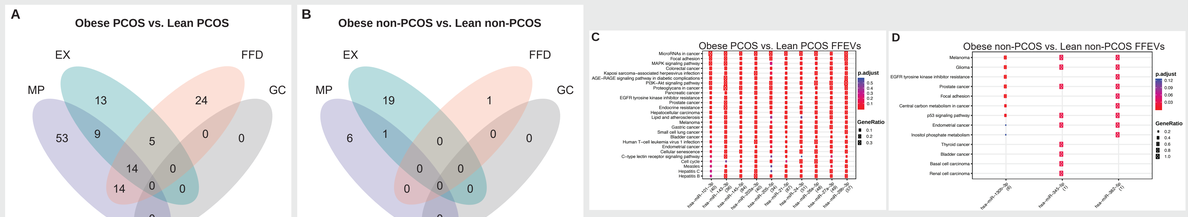

Figure 4: Venn diagram of differentially expressed miRNAs identified in each particle/cell type across two comparisons E) Obese PCOS vs Lean PCOS, F) Obese non-PCOS vs Lean non-PCOS. The colours correspond to the particle/cell type the miRNA was differentially expressed in. Overlapping regions indicate miRNAs that were differentially expressed in more than one particle/cell type, G) Functional analysis was conducted on the top 10 enriched targets and the associated pathways were determined using the KEGG database in Obese PCOS vs. Lean PCOS and H) Obese non-PCOS vs. Lean non-PCOS. The colour of the dot represents the adjusted p-value (FDR), whereas the size of the dots represents gene ratio (number of annotated targets in each KEGG annotation over the total number of recognized targets).

FFEVs are significantly enriched in miRNAs targeting genes involved in cell signalling and apoptosis

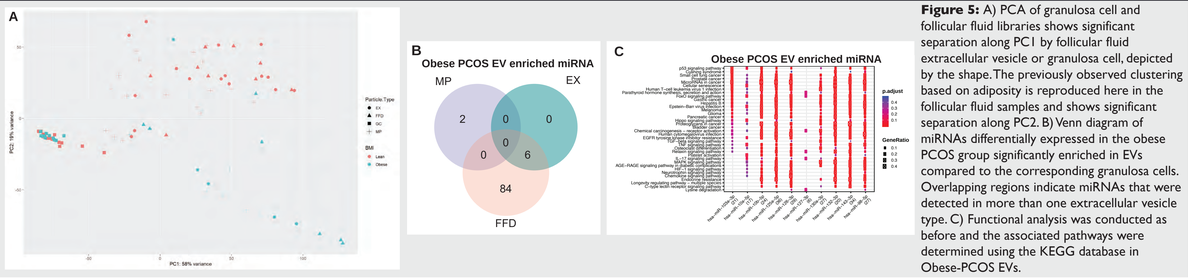

Figure 5: A) PCA of granulosa cell and follicular fluid libraries shows significant separation along PC1 by follicular fluid extracellular vesicle or granulosa cell, depicted by the shape. The previously observed clustering based on adiposity is reproduced here in the follicular fluid samples and shows significant separation along PC2. B) Venn diagram of miRNAs differentially expressed in the obese PCOS group significantly enriched in EVs compared to the corresponding granulosa cells. Overlapping regions indicate miRNAs that were detected in more than one extracellular vesicle type. C) Functional analysis was conducted as before and the associated pathways were determined using the KEGG database in Obese-PCOS EVs.

Conclusions

- Herein, using a novel sequencing method, this study is the first to profile the smallRNA profile of FFEVs from a single follicle. We successfully, and with high fidelity, profiled not only miRNAs, but all classes of snRNAs from MP, EV, FFD, and GCs from matched PCOS and non-PCOS patients.
- We also highlighted the significant impact adiposity has on snRNA profiles of PCOS and non-PCOS patients, with adiposity driving the observed snRNA differences.
- Finally, we identified miRNAs that are specifically packaged into FFEVs and secreted into the FF. We identified a potential mechanism by which PCOS GCs selectively package and release miRNAs targeting anti-apoptotic genes which may reduce the apoptotic pressure on the cell and delay early onset apoptosis observed in PCOS follicles.

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Sperm deoxyribonucleic acid integrity decreases with age and exhibits a rapid decline beyond the age of 35: a retrospective evaluation of 3446 semen samples

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INTRODUCTION

In recent decades, birth rates have substantially increased for men older than 30 years because of advanced age of marriage, rising life expectancy at birth, modern societal norms, and accessibility to assisted reproductive technology (ART). Advanced paternal age has been associated with a decline in conventional semen parameters (volume, concentration, motility, as well as, reduced fertility, increased risk of miscarriage, structural chromosomal aberrations and complex epigenetic disorders.

Multiple studies have underlined the significance of sperm DNA integrity test, as a part of routine semen analysis, a key component which affects functional competence of the sperm. Furthermore, several etiological factors such us obesity, smoking, genital tract infection, chemotherapy, varicocele, irradiation, leukocytospermia have been associated in the impairment of sperm DNA integrity.

Sperm with high DNA fragmentation may rise the possibility of transmitting the genetic aberrations to the conceptus and may affect the embryo and post natal development. Such transfer of aberrant sperm genome may result in increased rates of miscarriage or birth of offspring with major or minor congenital malformation.

The effect of paternal age on semen quality and integrity is controversial. Several studies with different measurement techniques (TUNEL, SCD, SCGE, SCSA) in the literature have recommended testing sperm DFI in infertile men with advanced age (≥40 years) as it may provide prognostic information for couple attempting natural and assisted reproduction.

STUDY QUESTION

Does advancing paternal age correlate with sperm DNA fragmentation index (DFI) and is there a cut-off age beyond which sperm DFI increases significantly?

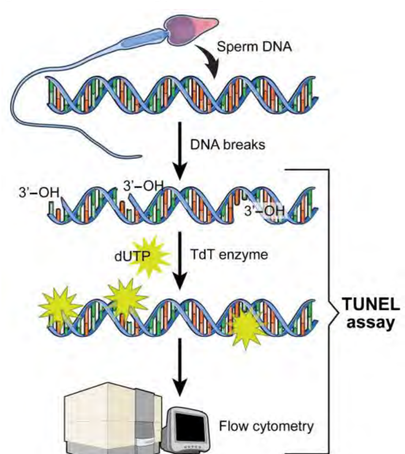
METHODS

Study design: This is a retrospective study of 3446 semen samples from patients under investigation for infertility between April 2016 and January 2022. Semen samples were obtained after 2-3 days of sexual abstinence. Patients were stratified into seven groups based on their age: patients ≤ 29 years (n=127; 3.7%), 30-35 years (n=868; 25.2%), 36-39 years (n=863; 25.0%), 40-45 years (n=1017, 29.5%), 46-49 years (n=321; 9.3%), 50-55 years (n=179, 5.2%) and ≥ 56 years (n=71, 2.1%).

Sperm DNA fragmentation analysis: Conventional semen parameters were assessed according to the WHO criteria and DFI was evaluated by TUNEL assay using the APODirect Kit run on BDAccuriC6 flow cytometer. A cut-off of 16.9% for DFI was applied to classify normal vs fragmented samples.

Statistics: Pearson's r was used for correlation analysis between sperm concentration, DFI and paternal age. DFI results for each stratified patient group were evaluated by one-way ANOVA, followed by Tukey pos-hoc multiple comparison test. Results are presented as the mean±standard error and a P-value of <0.05 was considered statistically significant.

Figure 1. Diagram of the DNA staining procedure using the TUNEL method

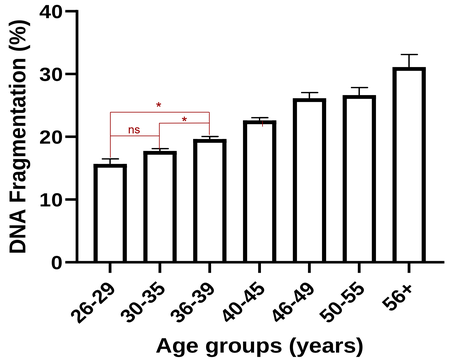


RESULTS

Table 1. Descriptive statistics of 3446 men attending OVO fertility clinic and sperm DNA integrity

Age group	26-29	30-35	36-39	40-45	46-49	50-55	56+
Number of values	127	868	863	1017	321	179	71
Minimum	1,9	0,4	0,8	1,0	3,0	2,9	0,9
Maximum	38,4	70,6	93,7	98,9	118,4	88,4	78,5
Range	36,5	70,2	92,9	97,9	115,4	85,5	77,6
Mean DFI	15,68	17,74	19,65	22,61	26,15	26,66	31,12
SD	9,07	11,55	12,05	13,7	16,21	15,77	16,94
SEM	0,81	0,39	0,41	0,43	0,91	1,18	2,01
Lower 95% CI of mean	14,09	16,97	18,84	21,76	24,37	24,34	27,11
Upper 95% CI of mean	17,28	18,51	20,45	23,45	27,93	28,99	35,13

Figure 2. Association between age and sperm DNA fragmentation in patients who follow up at the OVO fertility clinic



In this cohort of men with a mean age of 39.5 years ± 0.1 (range 23-76 years), sperm DNA fragmentation (21.1% ± 0.2) was positively correlated with age (r=0.23, p<0.001). In contrast, the correlation between sperm concentration and age was not significant (r=0.03, p=0.07). Mean %DFI level in the 26-29 and 30-35 age groups were not significantly different (p=0.65). However, mean %DFI level in the 36-39 age group was significantly higher than in the 26-29 and 30-35 age groups (p=0.02 and p=0.03, respectively). Mean %DFI level in the older age groups (40-45, 46-49, 50-55 and ≥ 56 years) were all significantly higher than in the 26-29 or 30-35 age groups (p<0.001). Using a %DFI threshold level of 16.9%, 46.0% of patients <36, 52.2% of men aged 36-39, 60.2% of men aged 40-45, 67.3% of men aged 46-49, 72.6% of men aged 50-55 and 74.7% of men aged ≥ 56 years had an elevated DFI.

CONCLUSIONS

Our results underline the relationship between paternal age and sperm DFI and demonstrate a significant increase in sperm DNA fragmentation in men over the age of 35 years. The data suggest that we may want to reconsider the age cut-off we traditionally use to define advanced paternal age.

Limitations: This is a retrospective analysis that did not account for confounding variables (e.g., clinical diagnosis, gonadotoxin exposure, febrile illness) that may affect conventional sperm parameters and DFI.

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