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TV1 & TV2

The Canadian Fertility and Andrology Society

Cost benefit analysis of PGT-A for embryo selection: a Canadian perspective

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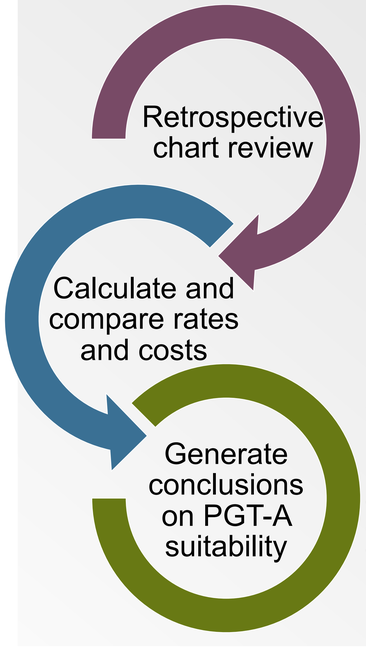
BACKGROUND

- Routine employment of preimplantation genetic testing for aneuploidy (PGT-A) has been a general topic of debate.
- Aneuploidy in embryos is a major cause of failed implantation and miscarriage cases (1).
- PGT-A has the potential to reduce the number of embryo transfer cycles and has been correlated with reduced miscarriage rates, however it can also be costly, increase time waiting, and may be associated with a risk to the embryo during the biopsy process.
- This along with the variable number of blastocysts achieved in an IVF cycle makes for a difficult decision whether to use PGT-A or not for the patient.

PURPOSE

Assess PGT-A benefits and costs in order to better advise patients on its use

MATERIALS AND METHODS



1. Perform a blinded retrospective chart review of 101 patients with autologous oocytes that underwent IVF with PGT-A
2. Identify number of blastocysts produced per patient, euploidy rates, pregnancy rates and PGT-A costs
3. Determine correlation between morphological assessment alone and assessment based on morphology and euploidy status
4. Evaluate rates, trends and data to develop an information guideline when counselling patients on PGT-A benefits

RESULTS

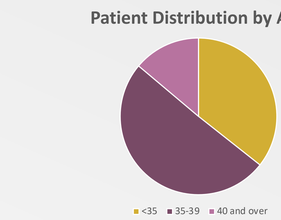


Figure 1. Patient distribution by age.101 patients were included in this study with data extrapolated from 415 embryos. Due to the small sample size in the 40 and over group, patients were ultimately categorized in two groups: <35 and 35 and over.

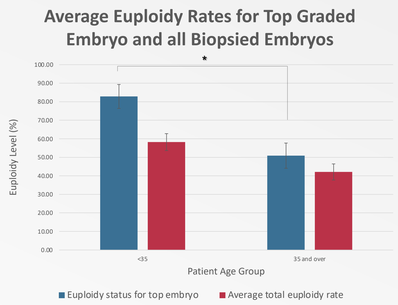


Figure 3. Average euploidy rates for top embryo and average euploidy for all embryos tested. Blue bars also represent the agreement between the embryologists' choice for the top embryo (based on Gardner's blastocyst grading system) and euploidy status. *p<0.05

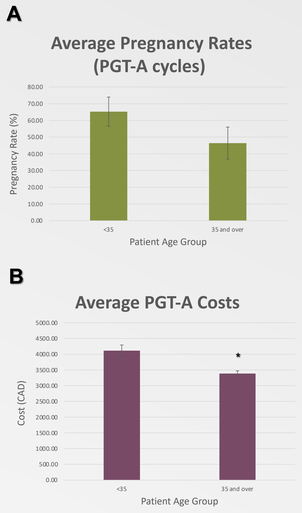


Figure 2. Average rates per age group. **A)** depicts average pregnancy rates and **B)** depicts average PGT-A costs. Both graphs indicate lower rates for > 35 age group. *p<0.05

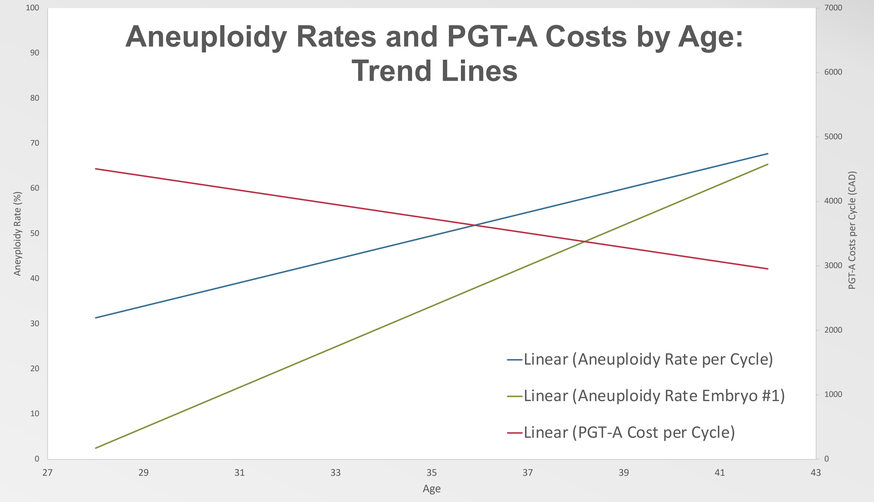


Figure 4. Trend lines, as patient age increases, for aneuploidy rates (both total per cycle and per top embryo only) and PGT-A costs per cycle. The blue and green trend lines represent the data set, by age, for the total aneuploidy rates per cycle and the aneuploidy rate for the top embryo (based on Gardner's grading system) respectively. Both show an upward trend for aneuploidy as patients age. On the other hand, PGT-A costs per cycle seem to decrease with age.

DISCUSSION AND CONCLUSIONS

- Our study saw a negative correlation between patient age and number of embryos produced, PGT-A costs, as well as euploidy and pregnancy rates per IVF cycle.
  - The association between age and lowered fertility and IVF success rates has been well-established in the literature (2,3,4).
  - PGT-A costs may be lower in the older age group per cycle; however, patients may have to perform multiple cycles.
- Morphology-alone ratings were consistent with ratings according to euploidy status 82% of the time, while there was 54% agreement for the older age group.
  - We speculate whether it's more beneficial to opt-in for PGT-A after a certain age.
- This topic remains largely debatable; however, current literature (including a CFAS commissioned study) do not recommend routine employment of PGT-A for every patient (5,6).
- Decision to have PGT-A or not remains complex and may be based on a multitude of factors.

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EXPLORING THE RELATIONSHIP BETWEEN TROPHECTODERM MITOCHONDRIAL DNA RATIO AND EMBRYO MORPHOLOGY

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INTRODUCTION

Approximately 16% of couples in Canada suffer from infertility. According to the society of assisted reproductive technologies (SART), the top fertility clinics have reported overall pregnancy rates of up to 50% in good prognosis patients [1]. Still in most clinics, at least 30% of euploid (chromosomally normal) embryos do not result in pregnancy resulting in stress and anxiety to patients, repeat cycles and increased cost of treatment[2]. Recently it was found that an increased mitochondrial DNA (mtDNA) ratio in human embryos correlated to lower pregnancy outcome[3].

OBJECTIVE

To explore the relationship between mtDNA ratio in human blastocysts and embryo morphology to determine whether mtDNA can be used as a biomarker for embryo development potential.

DESIGN

A single-center retrospective study of embryos created using standard IVF procedures.

METHODS

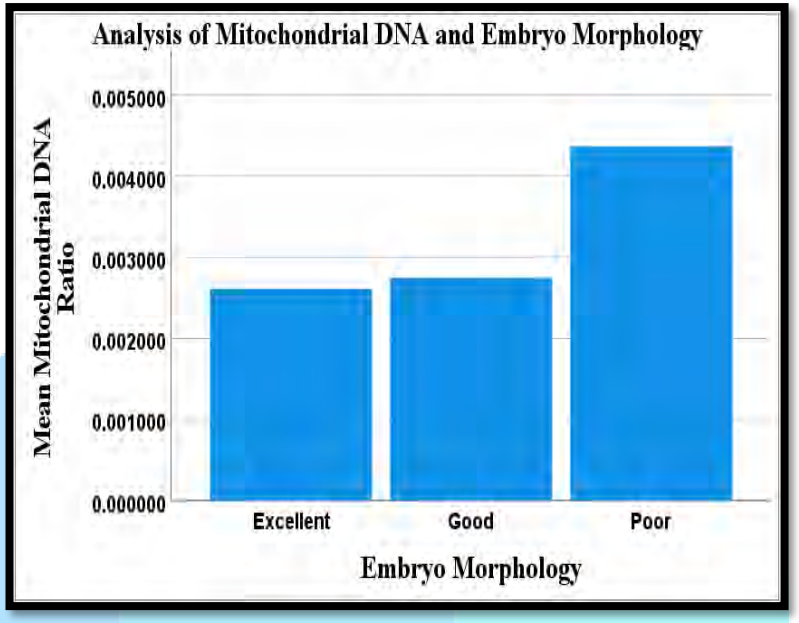
Trophectoderm specimens were obtained from 364 embryos obtained during routine clinical care of infertile couples at TIRM between March to December, 2021. mtDNA ratios were assessed with NGS-analysis conducted by Sequence 46 (California, USA). The mtDNA ratio is defined as the ratio of mtDNA to autosomal (chromosome 1-22) DNA. Embryos were graded based on the Gardner-Schoolcraft (1999) Blastocyst Grading System. Embryo morphologies were ranked into three categories: **Excellent** (3 to 6 AA and 4 to 6 AB); **Good** (any BB, 1 to 3 AB, and 1 to 2 AA); and **Poor** (any AC, CA, BC, CB, or CC). Statistical analysis was done using SPSS software, and ANOVA was used to determine the relationship between factors. $P<0.05$ was considered significant.

RESULTS

Most embryos consisted of good morphology (N=199, 55%), followed by excellent (N=114, 31%) and poor (N=51, 14%). Embryos with a higher mean mtDNA ratio were of poorer quality as seen in Figure 1.

However, there is no statistically significant differences between morphology groups (p=0.09).

Fig 1. Correlation of mtDNA ratio and embryo morphology



CONCLUSION

This study did not find a correlation between mtDNA ratio and embryo morphologic grade. Further study is needed to investigate a relationship between mtDNA ratio and embryo implantation rate.

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The Association Between Marijuana Use and Semen Quality: A Retrospective Study

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uOttawa

Marijuana & Male Fertility

- Reported marijuana use has been increasing among Canadians on a yearly basis since legalization in 2018
- Evidence on the impact of marijuana on male fertility potential is conflicting
  - Animal models have demonstrated a negative impact on spermatogenesis
  - A 2015 prospective study among healthy Danish men found significantly decreased sperm counts and concentration
  - Conflictingly, a 2019 longitudinal study in subfertile American men reported higher sperm counts and concentration
  - A 2021 prospective study among subfertile men suggested that marijuana may have a detrimental effect on morphology and volume, but a protective effect on sperm motility

Our goal was to evaluate the association between marijuana use and semen parameters among patients referred to an academic fertility clinic

Methods

Retrospective Chart Review (n = 2096)
- Men aged 18 to 50 y.o.
- Assessed at OFC in 2019

Excluded (n = 1231)
- Azoospermia
- Known cancer or history of gonadotoxic treatment
- Use of testosterone, anabolic steroids, androgen antagonist treatment within 1 year
- Known retrograde ejaculation
- Consultation > 3 months prior to semen analysis
- Prior treatment for infertility
- First semen analysis performed outside the OFC

Sample Size Calculations

Assuming 60% non-users will have a normal semen analysis, to detect a 15% difference in the primary outcome (alpha 0.05, power 80%)

n = 342 (171 per group)

Analyzed (n = 865)
No use (n = 636) All use (n = 229)

Primary Outcome

Normal semen analysis comparing non-users to all users as defined by concentration ≥ 16 M/mL and progressive motility ≥ 30% (WHO 6th Edition, 2021)

Secondary Outcomes
- Volume
- Total motile count
- Concentration
- Progressive motility

Marijuana use was not associated with a higher prevalence of abnormal semen parameters

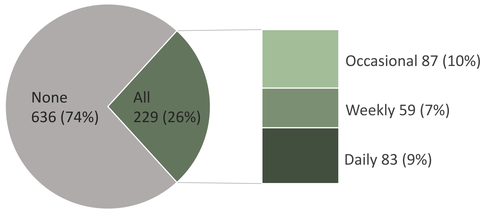

Figure 1. Self-reported marijuana use among men presenting to the Ottawa Fertility Centre for assessment in 2019.

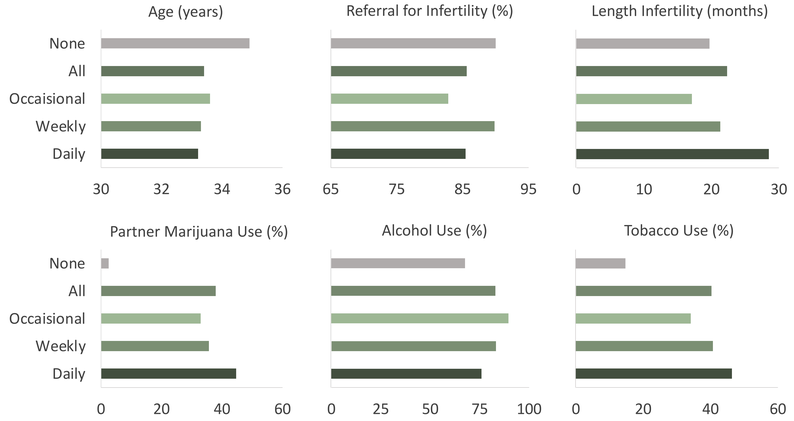

Figure 2. Descriptive characteristics of men presenting for assessment at the Ottawa Fertility Centre in 2019, stratified by self-reported marijuana use.

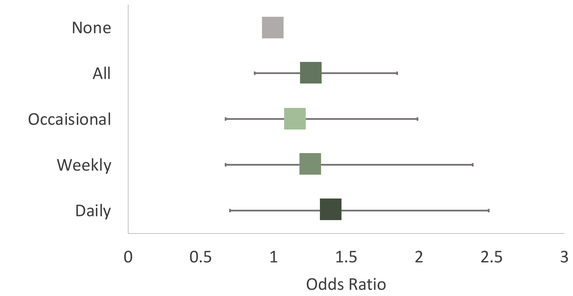

Figure 3. Logistic regression analysis for binary primary outcome of normal semen analysis, as defined by concentration ≥ 16 million/mL and progressive motility ≥ 30%. Error bars represent 95% confidence intervals. Models are adjusted for age, alcohol use, tobacco use, and length of abstinence.

Table 1. Regression analysis for continuous secondary outcomes compared to no marijuana use. Models are adjusted for age, alcohol use, tobacco use, and length of abstinence.

Outcome	Marijuana Use	Beta	Upper 95% CI	Lower 95% CI	p-value
Volume (ml)	All	-0.10	-0.35	0.15	0.43
	Occasional	0.06	-0.29	0.42	0.73
	Weekly	-0.39	-0.80	0.03	0.07
	Daily	-0.06	-0.42	0.30	0.74
Concentration (10^6/ml)	All	4.34	-2.12	10.80	0.19
	Occasional	2.30	-7.01	11.61	0.63
	Weekly	2.39	-8.47	13.26	0.67
	Daily	7.98	-1.58	17.54	0.10
Total Motile Count (10^6)	All	8.37	-5.68	22.42	0.24
	Occaisional	5.18	-15.02	25.38	0.61
	Weekly	-0.71	-24.25	22.83	0.95
	Daily	18.64	-2.18	39.47	0.08
Progressive Motility (%)	All	2.10	-1.26	5.45	0.22
	Occasional	-0.41	-5.23	4.41	0.87
	Weekly	0.38	-5.22	5.97	0.90
	Daily	6.13	1.16	11.11	0.02

Discussion

- Evidence on the impact of marijuana on semen parameters is conflicting
- Our findings that marijuana does not significantly impact semen quality in our patient population adds to the growing body of evidence
- Limitations of our study include that BMI is not routinely collected on male patients and could not be adjusted for in our findings, and that tobacco use is a large confounding factor difficult to control for
- When counselling patients it is important to consider the impact of marijuana on fertility and pregnancy as a whole, including early pregnancy loss and obstetrical complications

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- Dr. Julia Zhu-Pawlowski

Ottawa Fertility Centre / Centre de fertilité d'Ottawa
The Ottawa Hospital Research Institute | L'Hôpital d'Ottawa Institut de recherche

References:

The Impact of Magnetic Activated Sperm Sorting on Sperm DNA Fragmentation and HBA Binding

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ABSTRACT

Introduction: Separation of sperm from seminal plasma and debris is a pre-requisite for IVF/ICSI to ensure proper fertilization, embryo development and successful clinical outcomes. The isolated population of spermatozoa should have high quality motility and morphology, as well as decreased DNA Fragmentation Index (DFI) and improved hyaluronin binding compared to the raw specimen. A novel method to obtain this population is magnetic activated sperm sorting (MACS), in which apoptotic sperm are separated from nonapoptotic sperm using a microbead suspension and a magnetic separation column. This study compares sperm quality parameters following MACS to the widely used density gradient with swim-up procedure.
Design: Prospective trial

Materials and Methods: Semen samples from 20 men were obtained and samples were split and processed using both the magnetic activated sperm sorting device (CANSEP, Microptics, Spain) as well as density gradient followed by swim-up (DG+SU). The CANSEP separation device conjugate sperm to isolate non-apoptotic sperm. DG+SU entailed layering raw semen over the top of a 40%/80% gradient and spinning the sample at 300xg for 20 minutes followed by two 8 minute washes at 300xg. The pellet was then overlaid by GIVF medium and motile sperm isolated from the top fraction ~1hr later. Samples were assessed for HBA binding (HBA assay) and DNA fragmentation (DFI) using the TUNEL assay**.** Data were analyzed using ANOVA and Tukey analysis, p<0.05.

Results: The sperm separation techniques examined in this study both resulted in isolation of sperm with significantly improved sperm DFI compared to sperm from raw semen. There was no statistical difference in the resulting sperm DFI levels between CANSEP and DG+SU. HBA binding did not show improvement from the raw specimen with either of the test methods.

Conclusion: Magnetic activated sperm sorting (MACS) did not show improvement in DNA fragmentation (DFI) or HBA compared to sperm prepared by DG+SU. Future studies will examine impact of MACS on resulting fertilization, embryo development/aneuploidy and clinical outcomes.

BACKGROUND

- Separation of sperm from seminal plasma and debris is a prerequisite for IVF/ICSI proper fertilization
- Isolated sperm should have high quality, motility and morphology as well as decreased DNA Fragmentation (DFI) and improved hyaluronan binding compared to the raw sample
- Several sperm preparation protocols currently exist in mainstream clinical embryology: density gradient/swim up, microfluidics, magnetic activated sperm sorting (MACS)
- MACS protocol allows to separate apoptotic from non apoptotic sperm by using microbead suspension and magnetic separation

OBJECTIVE

Compare sperm quality parameters following MACS to the widely used density gradient with swim up procedure

MATERIALS & METHODS

20 ejaculate sperm samples split into two groups

MACS
CAN SEP(Microptics, Spain)

DG+SU
40/50% Density Gradient
20minute centrifugation
2x 8 minute wash
Swim up

Routine Semen Analysis
HBA
DFI (TUNEL)
ANOVA and Tukey analysis used

RESULTS

Sperm prep protocol	Concentration		Motility		DFI		HBA	
	Average	SEM	Average	SEM	Average	SEM	Average	SEM
Raw	93.0	9.4	44.0	2.4	12.8	0.9	82.9	4.3
DG+SU	39.4	3.8	91.1	5.0	6.8	0.8	84.9	4.5
CanSep	36.0	3.6	91.2	4.9	7.2	0.9	86.3	4.6
P value	0.177		0.300		0.530		0.143	

Figure 1: No statistical significance was found between any of the analysis groups in Raw vs DG+SU vs CAN SEP

RESULTS

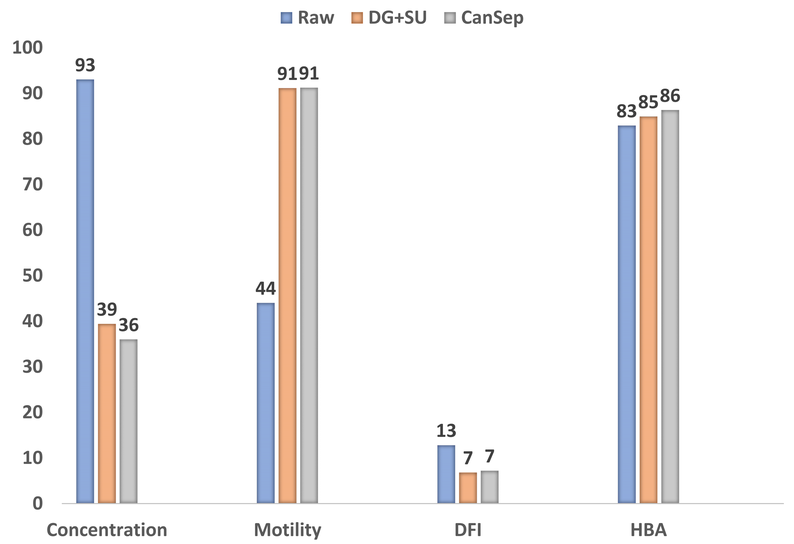

Figure 1. Graph representation of the data confirming no improvement in sperm quality post prep with CAN SEP device

CONCLUSIONS

- Magnetic activated sperm sorting (MACS) did not show improvement in DNA Fragmentation.
- MACS preparation also did not show improvement in HBA when compared to the well validated and clinically accepted DG+SU method.
- Future studies will examine impact of MACS preparation on embryo fertilization, embryo development, genetic stability as well as clinical pregnancy rates.

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Reproductive outcomes of ICSI in men with high sperm DNA fragmentation: Ejaculated sperm selected by MACS vs testicular sperm

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INTRODUCTION

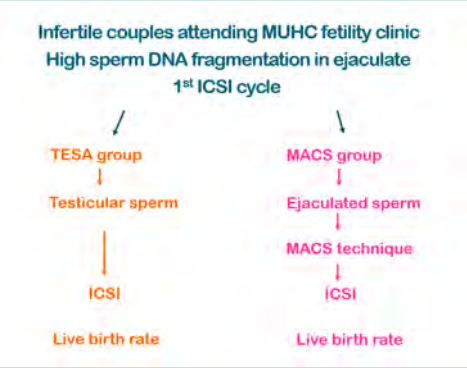
There has been a growing interest on the impact of sperm DNA fragmentation on reproductive outcomes, particularly in assisted reproduction. Even with advanced reproductive technologies such as ICSI, using sperm with high levels of DNA fragmentation may significantly impair the outcomes. Novel approaches such as the use of testicular sperm and various selection techniques beyond morphology and motility on ejaculated sperm have been proposed as adjunctive measures to improve reproductive outcomes with ICSI.

OBJECTIVE

The aim of this pilot study was to compare the outcomes of ICSI among infertile couples with elevated sperm DNA fragmentation rates using testicular sperm versus ejaculated sperm coupled with magnetic activated cell sorting (MACS).

METHODS & EXPERIMENTAL DESIGN

A cohort of infertile couples (n=56) who underwent ICSI at a university-based reproductive center from 2016-2020 were evaluated retrospectively. The male partners of all couples had elevated levels of DNA fragmentation (TUNEL ≥ 36, DFI by SCSA® ≥ 25) in ejaculated sperm. All couples underwent the first ICSI cycle using either testicular sperm (TESA) or ejaculated sperm processed with annexin V-MACS (MACS). The primary outcome measured was cumulative live birth rate per couple.



RESULTS: Demographic characteristics

Demographic characteristics	TESA	MACS	p value
Number of patients	42	14	
Male age (years) (mean ± SD)	38.0 ± 6.5	36.4 ± 3.5	NS
Female age (years) (mean ± SD)	36.5 ± 4.5	36.3 ± 3.7	NS
Infertility duration (years) (mean ± SD)	3.5 ± 2.6	3.3 ± 2.2	NS
Previous failed inseminations (IUI) (mean ± SD)	2.2± 1.8	4.4 ± 1.4	NS
Previous failed ejaculated ICSI cycles	0.3	0.4	NS

RESULTS: Embryological characteristics

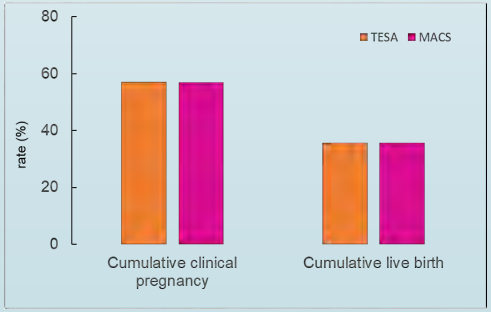
	TESA	MACS	p value
Number of matured oocytes (mean ± SD)	8.0 ± 4.7	7.5 ± 4.7	NS
Number of embryos transferred/fresh ET (mean ± SD)	1.3 ± 0.4	1.2 ± 0.4	NS
Number embryos transferred/frozen ET (mean ± SD)	1.1 ± 0.4	1.0 ± 0.2	NS
Number of embryos frozen (min-max #)	1.7 (0-6)	2.2 (0-8)	NS
Fertilization rate	63.4%	73.4%	NS
Miscarriage rate/couple (n)	42.8% (12/28)	37.5% (3/8)	NS

RESULTS: Sperm parameters

Sperm parameters	TESA	MACS	p value
Sperm conc. (min–max range)	50.6 M/ml (2.4-192.8)	75.2M/ml (21.7-192.8)	NS
Sperm motility	15.8%	27.6%	0.05
Sperm morphology	2%	2%	NS

Sperm DNA fragmentation	TESA	MACS	p value
TUNEL	51.6%	44.8%	NS
SCSA	33.5	26.5%	NS

RESULTS: Reproductive outcomes



Couples undergoing ICSI with TESA or MACS sperm had similar pregnancy and live birth rates.

CONCLUSIONS

Our preliminary data indicated that for infertile couples with high sperm DNA fragmentation in the male partners, using testicular sperm or ejaculated sperm processed with MACS offers comparable reproductive outcomes. Further investigations are required to confirm our findings and to assess characteristics of patients that can help clinicians and patients to choose a sperm selection strategy to minimize ICSI failure.

Permeable Cryoprotectant-free Vitrification of Human Testicular and Epididymal Spermatozoa: Fertilization Rate and Embryo Development

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INTRODUCTION

- Cryopreservation of testicular and epididymal spermatozoa is more complicated compared to ejaculated sperm due to much lower sperm count, lack of motility, and higher sperm sensitivity to cryoprotectants
- Sperm vitrification without using potentially toxic permeable cryoprotectants might be an attractive alternative to vapor freezing for testicular or epididymal spermatozoa, or severe oligozoospermic samples

STUDY OBJECTIVE

- To evaluate testicular and epididymal sperm motility and viability after using our novel technique of permeable cryoprotectant-free sperm vitrification and to analyze embryo development after ICSI with these vitrified spermatozoa

METHODS

- The testicular sperm extractions (TESE) and percutaneous epididymal sperm aspirations (PESA) were performed for obstructive azoospermia for in 2019-2022
- Vitrification of 20µL samples was performed in mHTF medium with protein and 0.25M sucrose
- The mixture was loaded into the Minitube OPS straw
- Minitube OPS straw inserted into a 0.5 ml high security CBS straw served as a closed vitrification device
- After sealing, CBS straws were plunged directly into the liquid nitrogen
- Vitrified sperm sample were warmed by direct placing of the OPS straws into the media at 37°C

OPS straw

Closed straw-in-straw vitrification device

CONCLUSIONS

- Our results demonstrate that human testicular and epididymal spermatozoa can be successfully vitrified in small volumes without any permeable cryoprotectants using a closed system, and this technique is efficient in clinical settings
- Moreover, without permeable cryoprotectants, a washing step is not required, and a warmed sperm sample can be placed directly into the ICSI dish

RESULTS

Total 91 samples have been vitrified, 49 samples have been used for ICSI

Sperm sample	Number of samples	Sperm count, x10⁶/mL	Motility, % Initial	Motility, % post-thaw	P
Testicular	21	0.6 ± 1.7	7.4 ± 9.2	6.7 ± 9.0	0.73
Epididymal	22	12.7 ± 21.8	21.7 ± 16.4	12.9 ± 13.8	0.001
Ejaculate	6	9.2 ± 17.1	9.0 ± 4.2	3.9 ± 5.7	0.15

ICSI outcomes using vitrified sperm samples, average oocyte age 37.1 ± 4.9

Sperm sample	Number of oocytes	Fertilization rate n (%)	≥6-cell embryo rate n (%)	Blastocyst ate n (%)	Euploidy rate in 29 PGT-A cycles n (%)
Testicular	172	121 (70.3)	97 (80.2)	60/118 (50.8)	6/28 (21.4)
Epididymal	163	118 (72.4)	93 (78.8)	71 (60.2)	23/54 (42.6)
Ejaculate	107	80 (74.8)	73 (91.3)	49 (61.3)	17/49 (34.7)

- Fresh TESE, PESA or ejaculated sperm samples were used in 12 ICSI cycles 100 mature oocytes. The following fertilization, ≥6-cell embryo development, blastocyst development, and euploidy rates have been observed: 78%, 74.4%, 48.7% and 22.9% (9 PGT-A cycles) respectively.
- **After performing of 44 FETs with the blastocysts created with the use of vitrified PESA, TESE or ejaculated sperm**, 34% resulted in clinical pregnancies: 6/18 (33.3%), 7/17 (41.2%), and 2/9 (22.2%) respectively

An Extended Culture System Mimicks Early Human Embryo Development *in vitro*

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INTRODUCTION

- Early **human embryogenesis** starts from the single-celled zygote to the lineage-specific architecture of the post-implantation embryo.
- Multiple sophisticated mechanisms and signaling pathways are involved in coordinating the molecular and cellular transformations.
- Modeling the developmental hallmarks, especially during the **post-implantation** stage, is both technically and ethically difficult.

OBJECTIVE AND HYPOTHESIS

- **Objective:** to build upon the technique of **extended culture culture** from pre- to post-implantation stages and investigate dynamics of the day 5-14 human **embryo transcriptome** and DNA **copy number variation** through single-cell sequencing.
- **Hypothesis:** Post-implantation chromosomally normal embryos grown in our extended culture system show consistent expression patterns of **lineage-specific** genetic markers as a result of self-organization and morphogenic differentiation.

MATERIALS AND METHODS

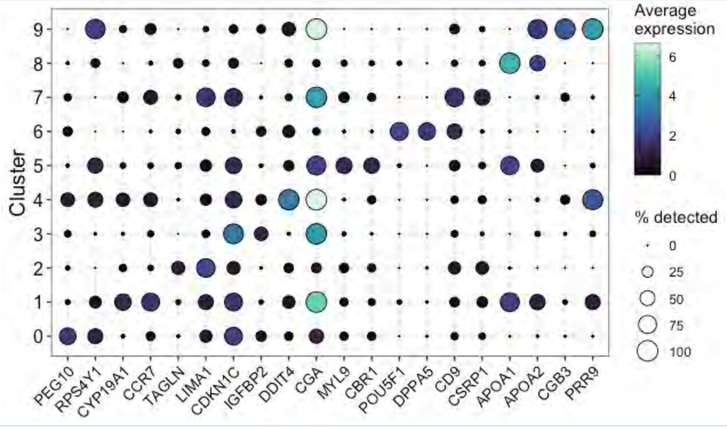
- Human blastocysts donated for research are obtained from the **CReATe Fertility Centre Biobank** (IRB protocol # 16580).
- Eight embryos were thawed and recovered in Global-HP medium. The zona pellucida was removed and the embyos were further cultured as described by **Shahbazi *et al*** (2016).
- On embryonic Day 10-12, the culture was terminated, and embryos were dissociated into single cells using TrypLE and mechanical disruption with a glass pipette.
- Single cells were barcoded using the **Mutli-Seq** method described by **McGinnis *et al*** (2019).
- Cells are pooled and loaded onto the **10x Chromium** to generate a cDNA library, followed by sequencing using the **Illumina NextSeq 550**.
- Sequencing results are processed and analyzed using **Cell Ranger** from **10x Genomics**.

RESULTS

Figure 1. Feature Dotplot of top-2 expressed genes for each cell type from the 8 extended cultured embryos.

10 unique clusters were detected from grouping **1737** single cells based on their gene expression profiles. Each cluster corresponds to a specific cell type in early human embryo. **Differential gene expression (DGE) analysis** was performed to identify enriched genes in each cluster based on the **$\log_2$ fold change** values of the genes.

Cell Ranger-processed sequencing results were further analyzed via **R Studio** (version 4.1.2) using **Seurat** (version 4.1.1). cells with > 200 features and < 20.0% mitochondrial reads were retained in the analysis



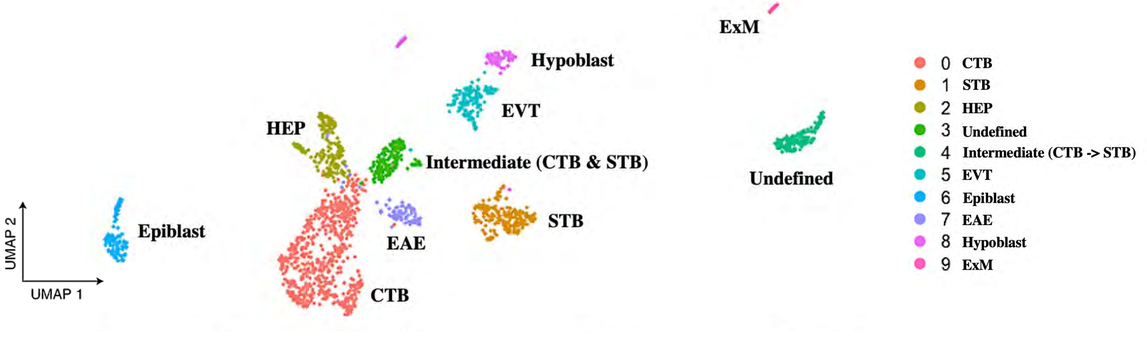


Figure 2. UMAP projection of single-cell RNA sequencing results from the 8 extended cultured embryos.

Cluster identities were concluded by referring the **differentially-expressed genes** in each cluster to the scRNA-seq data from **1)** Petropoulos *et al* (2016), **2)** Zhou *et al* (2019), **3)** Mole *et al* (2021) and **4)** Tyser *et al* (2021). Genetic markers corresponding to both **embryonic** and **extra-embryonic** lineages that arise from **pre-implantation**, **post-implantation**, or **peri-gastrulation** stage are all detected.

CTB, cytotrophoblast; **STB**, syncytiotrophoblast; **HEP**, haemato-endothelial progenitors; **EVT**, extravillous trophoblast; **EAE**, embryonic/extraembryonic ectoderm; **ExM**, extraembryonic mesoderm.

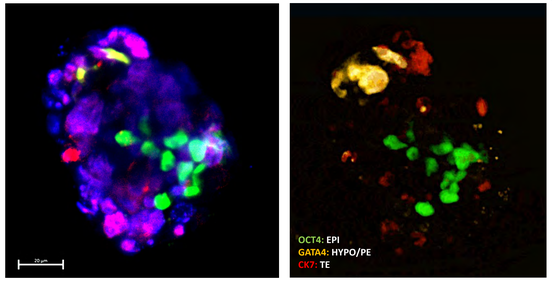


Figure 3. Immunofluorescence staining of a Day-9 euploid embryo after extended culture. The embryo was fixed and stained for markers specific to the three essential lineages: **OCT-4** (green) for **epiblast** (embryo proper); **GATA-4** (yellow) for **hypoblast** (yolk sac); **CK7** (red) for **trophectoderm** (placenta); **DAPI** (blue) for **nuclei**. The images were taken with Zeiss LSM710 Confocal/Two-Photon microscope (20x, NA=1.0).

CONCLUSIONS

- Our culture system can support human embryo development from the **pre- to post-implantation** stage.
- The cultured embryos underwent segregation of the **embryonic** and **extraembryonic lineages**, which recapitulates the timing and outcomes of **self-organization** to mimic human embryogenesis *in vivo*.
- The expression of specific genetic markers indicates the emergence of **peri-gastrulation** events within the embryos after extended culture.

ACKNOWLEDGEMENT

This project was funded by the **CReATe Fertility Centre**.
Special thanks to CReATe Biobank and the research team.
The authors confirm there are no conflicts of interest.

Permeable Cryoprotectant-free Vitrification of large Volume of Human Ejaculated Spermatozoa

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INTRODUCTION

- Despite the widespread utilization of sperm cryopreservation, little progress has been made to modify freezing protocols or to improve the rates of sperm survival and motility
- Vitrification of the large volumes of human sperm suspension without the use of potential toxic permeable cryoprotectants (CPAs) is an attractive alternative to the vapour freezing technique

STUDY OBJECTIVE

The purpose of this study was to develop a technique for vitrification of large volume (150 µl) of sperm suspension without using potentially toxic permeable cryoprotectants

METHODS

- Density gradient isolated semen samples were evaluated by a SQA Vision machine (Automated Sperm Quality Analyzer)
- Vitrification of 150 µL samples was performed in mHTF medium with protein and 0.25M sucrose
- The mixture was loaded into the 0.25ml CBS straws
- 0.25mL CBS straw inserted into the 0.5 mL high security CBS straw, which was heat-sealed at both ends, served as a closed sperm vitrification device
- The straws were plunged directly into the liquid nitrogen
- Vitrified sperm samples were warmed by direct placing of the 0.25ml straw into the media at 37ºC
- Viability was assessed with the hypo-osmotic swelling test (HOST)

RESULTS

10 donated ejaculates were obtained from 10 normozoospermic patients. Average neat sperm concentration ($\times 10^6$/mL) and motility (%) were 187.5 ± 15.9 and 49 ± 1.7, respectively

Parameters	Post wash/Pre-vitrification, %	Post thaw, %	P-values
Motility	84.3 ± 5.2	58.9 ± 5.7	p=0.006
Immotile	15.7 ± 5.2	41.1 ± 5.7	p=0.006
Progressive	70.9 ± 8.6	48.7 ± 5.4	p=0.02
Non-progressive	13.4 ± 5.9	10.2 ± 0.8	p=0.58
Velocity, mic/sec	62.6 ± 7.5	41.9 ± 2.2	p<0.01
Viability (HOS test)	n/a	68.7 ± 2.8	

CONCLUSIONS

- Our results demonstrate that 150 µL samples of human ejaculated spermatozoa can be successfully vitrified in a closed straw-in-straw system (aseptic vitrification) without using permeable cryoprotectants
- This technique can be potentially useful in the clinical setting for conventional *in vitro* fertilization (IVF) and even for intrauterine insemination (IUI)
- Moreover, with no involvement of permeable cryoprotectants, the wash/centrifugation step after the thaw is not required for IUI, and warmed sperm sample just need to be resuspended in the desired amount of medium

Evaluating the impact of total gonadotropin dose on live birth rate between fresh embryo transfer vs. frozen embryo transfer from the same IVF cycle

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INTRODUCTION

Munch et al. (1) found a 38% reduction in live birth rates amongst patients that received a high gonadotropin (Gn) dose (>2500 IU) during fresh embryo transfer cycles compared to frozen embryo transfer cycles from the same in vitro fertilization (IVF) cycle. However, once adjusted for important covariates like age and BMI, this difference was no longer significant. Compared to Europe, North American practice involves using significantly higher Gn doses. This is the first study to examine the impact of total Gn dose on live birth rate using North American Gn levels.

HYPOTHESIS

We hypothesized that a high Gn dose has a deleterious effect on embryo implantation during fresh embryo transfers, independent of age and ovarian reserve. An elevated Gn dose may be an indication to freeze all embryos during IVF.

OBJECTIVE

- The primary objective was to compare the live birth rate by Gn dose, among patients who underwent fresh and frozen embryo transfers from the same IVF cycle.
- The secondary objectives included comparing other pregnancy outcomes such as implantation rate and ongoing pregnancy rate.

METHODS

We established a retrospective cohort of patients who underwent IVF treatment with ovarian stimulation, followed by a fresh embryo transfer and then a subsequent frozen embryo transfer, using embryos from the same IVF cycle. The study period was January 2010 to December 2019.

Inclusion criteria: Female, aged 18-42 years; first IVF cycle; Autologous IVF stimulation; IVF with fresh embryo transfer, followed by at least one frozen embryo transfer; and single embryo transfers (both fresh and frozen).

For patients with multiple IVF cycles within the study period, only the first IVF cycle was included. For patients with multiple frozen embryo transfers, only the first frozen embryo transfer was included.

Patients were grouped based on the total Gn dose received during the IVF stimulation as follows: less than 1800 IU, 1800-3600 IU, 3601-5400 IU, more than 5400 IU.

STATISTICS

For comparisons between groups, ANOVA or Chi-square tests were used depending on the variable. To evaluate the association between high Gn doses and live birth rate, a binomial multivariate logistic regression model was constructed, adjusting for age, BMI, smoking status, antral follicular count (AFC), infertility diagnosis, length of ovarian stimulation, number of mature oocytes, and embryonic stage. Data were analyzed using R, version 4.0.2.

Table 1. Sociodemographic characteristics

Characteristic		Total Gonadotropin Dose (IU) < 1800 n = 200	1801-3600 n = 698	3601-5400 n = 430	> 5400 n = 179	P-value
Age (years)		30.3 ± 3.7	31.7 ± 3.7	33.9 ± 3.8	35.5 ± 3.3	<0.001
BMI (kg/m²)		24.5 ± 4.6	25.0 ± 4.8	25.8 ± 5.2	26.2 ± 5.5	0.002
Active smoking		13%	9%	10%	7%	0.23
Gravida	0	69.5%	66.6%	61.6%	58.1%	0.04
	1	20.0%	18.2%	18.8%	22.3%	0.63
	≥ 2	10.5%	15.0%	19.5%	19.6%	0.01
Para	0	89.1%	86.8%	79.5%	79.3%	<0.001
	1	8.6%	10.6%	14.9%	14.5%	0.04
	≥ 2	2.3%	2.4%	5.6%	6.1%	0.01
Spon. Abortion	0	87.7%	85.8%	82.6%	79.9%	0.08
	1	8.6%	9.2%	10.7%	13.4%	0.32
	≥ 2	3.6%	4.9%	6.7%	6.7%	0.28
D&C	0	96.8%	94.8%	91.6%	89.4%	0.003
	1	2.7%	3.2%	4.9%	5.6%	0.23
	≥ 2	0.5%	1.0%	2.8%	2.2%	<0.05
Ectopic preg.	0	97.3%	95.4%	95.3%	95.5%	0.66
	1	2.3%	3.2%	2.8%	3.9%	0.80
	≥ 2	0.0%	1.3%	1.9%	0.6%	0.17
Infertility diagnosis						
None		1.8%	0.7%	0.9%	0.6%	0.47
Tubular or endometriosis 3-4		6.4%	13.2%	13.3%	11.7%	0.04
Male factor		37.3%	41.4%	37.9%	26.3%	0.003
Ovulatory dys.		17.7%	9.9%	2.8%	2.8%	<0.001
Unexplained or diminished OR		30.0%	29.5%	38.8%	49.2%	<0.001
Multiple		6.8%	5.3%	6.3%	9.5%	0.22
Length of infertility (years)		2.6 ± 1.7	3.2 ± 2.5	3.1 ± 2.2	3.1 ± 2.4	0.02
AFC		29.0 ± 13.2	22.8 ±10.7	15.3 ± 8.0	11.6 ± 6.0	<0.001
AMH (ng/mL)		5.0 ± 4.1	3.7 ± 2.9	2.0 ± 2.0	1.3 ± 1.6	<0.001
FSH (IU/mL)		5.9 ± 1.6	6.0 ± 1.9	7.1 ± 4.2	7.5 ± 3.4	<0.001

Spon.: spontaneous; D&C: dilation and curettage; preg.: pregnancy.

Table 2. IVF Characteristics

	Total Gonadotropin Dose (IU) < 1800 n = 200	1801-3600 n = 698	3601-5400 n = 430	> 5400 n = 179	P-value
Protocol type					
Antagonist	89.1%	88.1%	83.3%	61.5%	<0.001
Short	1.4%	2.3%	11.4%	37.4%	<0.001
Long	9.5%	9.6%	5.3%	1.1%	<0.001
Stimulation time (days)	10.2 ± 1.7	10.8± 1.6	11.5± 1.5	13.5± 1.9	<0.001
Number of mature oocytes	12.1± 5.8	11.2± 4.7	8.5± 4.0	7.5± 3.8	<0.001
Embryonic stage					
Cleavage	49.1%	44.6%	50.0%	55.3%	<0.05
Blastocyte	50.9%	55.4%	50.0%	44.7%	<0.05
Number of frozen embryos	2.7±1.6	2.6±1.7	2.2±1.4	1.9±1.3	<0.001
Frozen embryo transfer protocol					
Substituted	93.6%	90.4%	88.1%	90.5%	0.17
Natural	6.4%	9.3%	11.9%	9.5%	0.15

Table 3. Pregnancy Outcomes - Adjusted

	Total Gonadotropin Dose (IU) < 1800 n = 200	1801-3600 n = 698	3601-5400 n = 430	> 5400 n = 179
Fresh Embryo Transfer				
Implantation rate	Reference	0.92 (0.67-1.27)	0.73 (0.49-1.08)	0.99 (0.58-1.69)
Pregnancy	Reference	0.91 (0.66-1.25)	0.72 (0.49-1.08)	0.99 (0.58-1.69)
Live birth	Reference	0.98 (0.70-1.37)	0.73 (0.48-1.11)	0.87 (0.49-1.57)
Frozen Embryo Transfer				
Implantation rate	Reference	1.52 (1.08-2.16)	1.45 (0.95-2.20)	1.21 (0.68-2.14)
Pregnancy	Reference	1.48 (1.04-2.11)	1.44 (0.94-2.21)	1.30 (0.72-2.33)
Live birth	Reference	1.52 (1.06-2.21)	1.38 (0.88-2.16)	1.39 (0.75-2.56)

Adjusted for: age, BMI, smoking status, antral follicular count (AFC), infertility diagnosis, length of ovarian stimulation, number of mature oocytes, and embryonic stage.

Odds ratio (95% confidence interval).

RESULTS

A total of 1527 patients were included in the cohort. As expected, there were significant differences in patient age, BMI, infertility diagnosis, length of infertility, ovarian reserve (AFC, AMH, FSH), IVF protocol type, duration of ovarian stimulation, number of mature oocytes, embryonic stage and number of frozen embryos between Gn dose groups (tables 1 & 2). Unadjusted regression analysis showed a significant negative association between total Gn dose and live birth rate in fresh embryo transfers, however once adjusted for relevant covariates, no significant association was detected (table 3).

DISCUSSION

Diminished ovarian reserve is associated with decreased IVF success, independently of age. This patient group typically needs higher Gn doses during ovarian stimulation, therefore, it is crucial to determine the impact of higher Gn doses on IVF outcomes, independently of age and ovarian reserve. To date, no RCTs have examined this relationships, however some retrospective studies have. Malizia et al. (2) found no association between the total Gn dose and live birth rate, consistent with our results.

Strengths: our study used live birth rate as the primary outcome, which is the ultimate goal of fertility treatments; we included many important covariates in our analysis, which is, to our knowledge, the most complete retrospective analysis to date; we have a large study sample (1527 patients); and fresh and frozen embryo transfers from the same IVF cycle were compared.

Limitations: our study is retrospective in nature and thus can only determine an association, high-quality RCTs are needed to fully understand this relationship.

CONCLUSION

Contrary to our starting hypothesis, a high total Gn dose in IVF is not associated with significant differences in live birth rate following fresh embryo transfer, independently of age and ovarian reserve. Therefore, a high total Gn dose in IVF is not an indication to freeze all embryos. Further prospective study is warranted in order to solidify this conclusion.

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#484

Human Umbilical Cord Perivascular Cells (HUCPVC) Reduce Ovarian Fibrosis and 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.

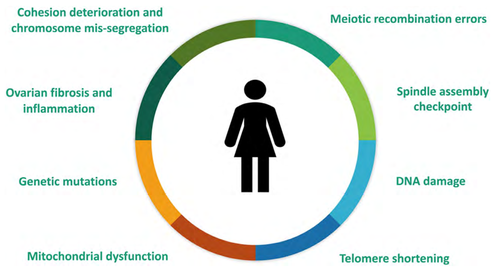

Figure 1. Mechanisms of ovarian aging *(From Park et al Reproduction 2021)*

- 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].

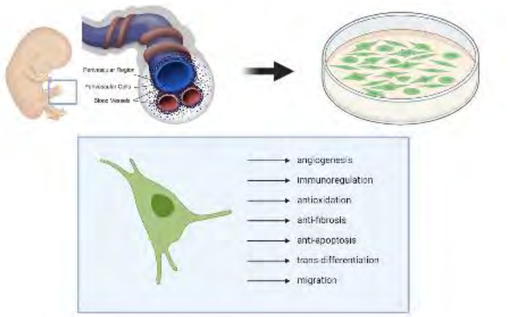

Figure 2. Human Umbilical cord-derived perivascular cells (HUCPVC) are a promising source of MSC, that are thought to act through multi-targeted mechanisms.

- 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 determine if the repeated administration of HUCPVC during the period of ovarian aging could prevent age-related fertility decline in a mouse model.

MATERIALS AND METHODS

STUDY DESIGN AND ASSESSMENTS

		Study Timepoint (months)							
	Procedures Involved	6	7	8	9	10	11	11 months + 2 weeks	12 months + 1 week
Treatment - cell/media administration	I.V. injection of 1,00,000 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

Table 1. Assessments and Timepoints.

Group 1: Control (vehicle, **HBSS**) n=15
Group 2: Human Fibroblast (cell control, **FIBS**) n=8.
Group 3: FTM HUCPCV, repeated injection (**6x FTM**) n=10
Group 4: Term HUCPVC, repeated injection (**6x Term**) n=10
Group 5: FTM HUCPVC, single injection at 11M (**1x FTM**) n=5

- 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 (3 cohorts) were randomized to each group after baseline fertility assessments.

- Pathogen-free lines of previously characterized male FTM and Term HUCPVC lines from single donors (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 endpoint.

- All procedures, 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. Picrosirius Red staining was performed to assess ovarian fibrosis and images were analyzed using ImageJ.

- Serum Anti-Mullerian hormone (AMH) (MyBiosource) and C-Reactive Protein (R&D Systems) were assessed by ELISA, following manufacturer instructions.

RESULTS

The repeated monthly injection of FTM and term HUCPVC in aging mice over 6 months has no adverse effects

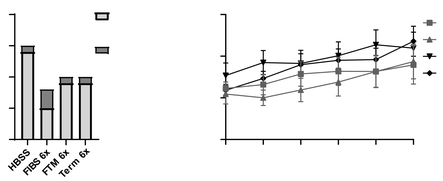

Figure 3. A. Mortality rates in each group (P=0.2, Chi-Square Test). B. **Animal Weight** (repeated injections only, not including sick animals that reached endpoint before 12M). While the weights of all groups showed a trend of increasing over 6 months, the increase in mean weight was only significant 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), Two-way ANOVA.

RESULTS

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

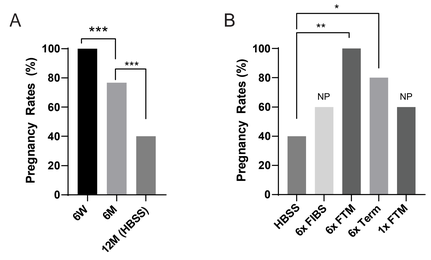

Figure 4. Pregnancy Rates in Young (6 weeks, 6months) and Aging (12 months) ICR females (**A**) and in Aging ICR females (12 months) treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC starting at 6 months, or a single injection of FTM at 11 months (**B**). Data was analyzed using a Chi-Square test and P values indicate significant differences with control group. NP = not powered for statistical analysis (due in part to mortalities in the fibroblast group).

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

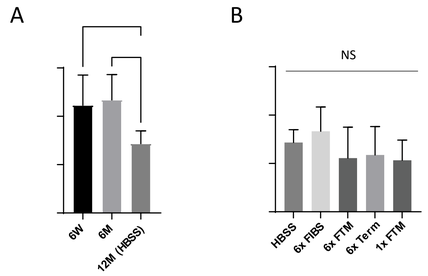

Figure 5. Litter Sizes in Young (6 weeks, 6months) and Aging (12 months) ICR females (**A**) and in Aging ICR females (12 months) treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC starting at 6 months, or a single injection of FTM at 11 months (**B**). Data was analyzed using One-Way Anova.

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

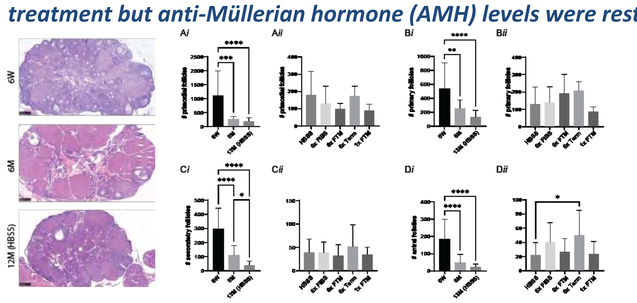

Figure 6. H&E staining of ovarian tissue in young and aging mice (6 weeks, 6 months and 12 months) and quantification of follicles at primordial (A), primary (B), secondary (pre-antral) (C) and antral stages (D) in ovaries of 6 week, 6months and aging (12 months) ICR females treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC (starting at 6 months, or a single injection of FTM at 11 months. Data was analyzed using One-Way Anova.

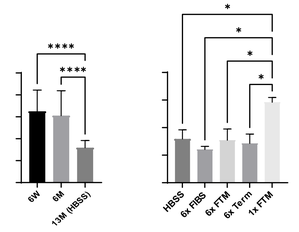

Figure 7. Anti-Müllerian Hormone (AMH) ELISA in in young (6 weeks, 6months) and aging (12 months) ICR females (**A**) and in Aging ICR females (12 months) treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC starting at 6 months, or a single injection of FTM at 11 months (B). Data was analyzed using One-Way Anova.

An age-associated increase in ovarian fibrosis was significantly reduced by HUCPVC treatment

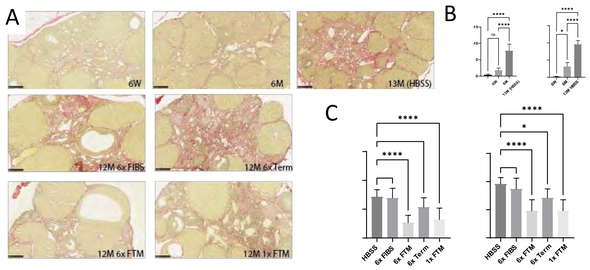

Figure 8. A. Analysis of ovarian fibrosis in young (6 weeks, 6 months) and aged (12 months) ICR females treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC starting at 6 months, or a single injection of FTM at 11 months. **A**) Representative images of each group **B-C**) Quantification of % of ovarian surface area (left panels) or stromal surface area (right panels) stained with picrosirius red in young controls and aging mice (B) and in cell-treated groups of aged mice compared to HBSS control at 12M(C). 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

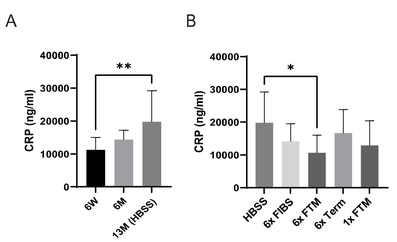

Figure 9. Assessment of Pro-Inflammatory C-Reactive Protein (CRP) by ELISA. Serum CRP levels in young (6 weeks, 6months) and aging (12 months) ICR females (**A**) and in aging ICR females (12 months) treated with HBSS (control), 6 injections at monthly intervals of fibroblast (cell control), FTM or TERM HUCPVC (RI) starting at 6 months, or a single injection of FTM at 11 months (**B**). Data was analyzed using One-Way Anova.

DISCUSSION and CONCLUSIONS

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 and AMH) or litter size.

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

Our data suggest that FTM HUCPVC have anti-inflammatory and anti-fibrotic effects and represent a promising source of MSC to reduce fertility decline in women of advanced reproductive age.

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Kif18a is essential for preventing chromosome segregation errors in oocyte meiosis-II

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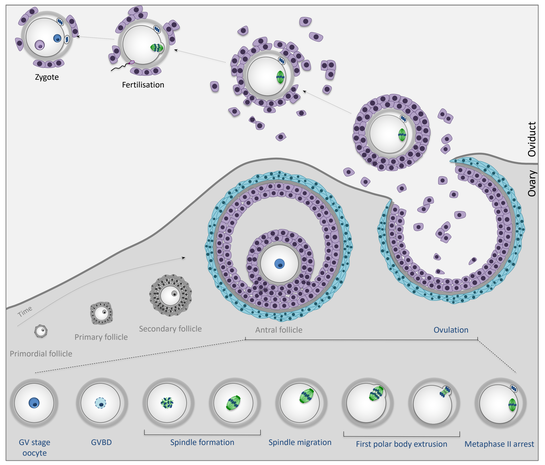
Introduction

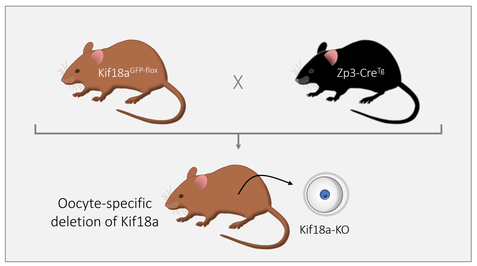
Female fertility significantly declines with advanced maternal age as a result of chromosome segregation errors in meiosis that cause egg aneuploidy and have devastating impact on embryo health. To ensure chromosome segregation fidelity during meiotic divisions, eggs heavily rely on molecular motor proteins to orchestrate spindle formation and regulate chromosome behaviour. One such motor involved in chromosome alignment in mitotic cells is Kif18a, but its role in mammalian oocyte meiosis is unknown.



Methods

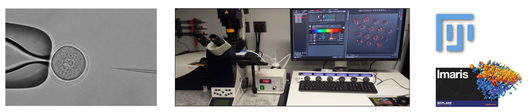
Here, we generated Kif18a$^{GFP\text{-}flox}$ mouse reporter line to investigate the expression and conditionally delete Kif18a (Kif18a-KO) in oocytes and investigate its role during female meiosis.

Animal model



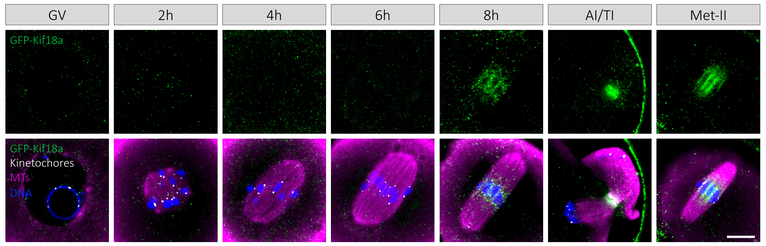
We combine micromanipulation with live and fixed high-resolution confocal imaging to study chromosome behaviour during female meiosis in Kif18a-KO and aged oocytes.

Microinjections | Confocal microscopy | Data analysis



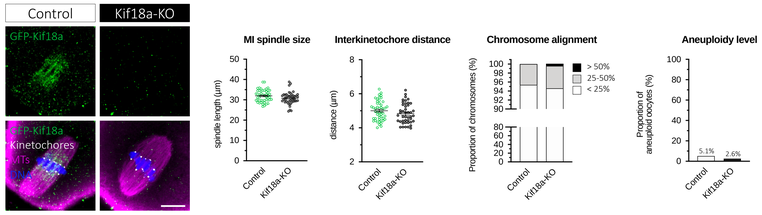
I am currently on a job market looking for a junior PI position!
Contact: aleksandar.mihajlovic00@gmail.com

1. Kif18a is present in mouse oocytes during both meiotic divisions



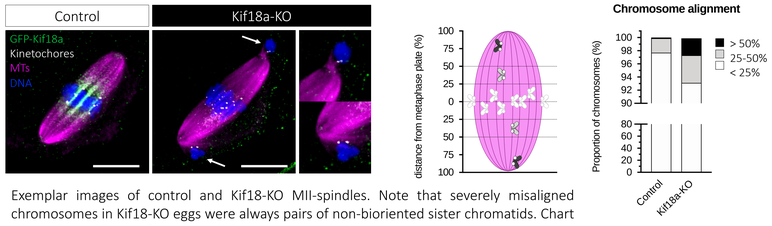
GFP-Kif18a expression in Kif18a$^{wt/GFP}$ oocytes during different stages of maturation (GV – germinal vesicle; h – hours post-IBMX release, AI/TI – anaphase-I/telophase-I; Met-II – metaphase-II) revealed Kif18a spindle midzone localization from late meiosis-I onwards.

2. Kif18a plays no role in meiosis-I (MI)



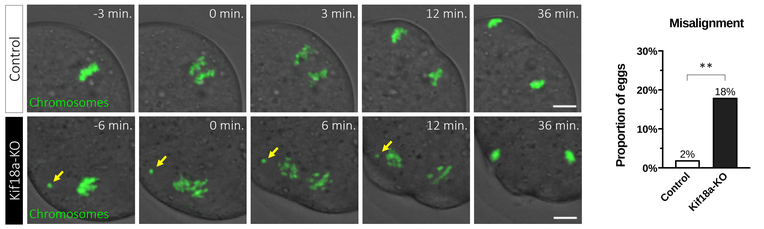
Confocal images show successful Kif18a removal. Charts show no difference in spindle length, interkinetochore distance, chromosome alignment (in late-MI) and aneuploidy level (in MII) between Kif18-KO and control group.

3. Kif18a is required for chromosome alignment in meiosis-II (MII)



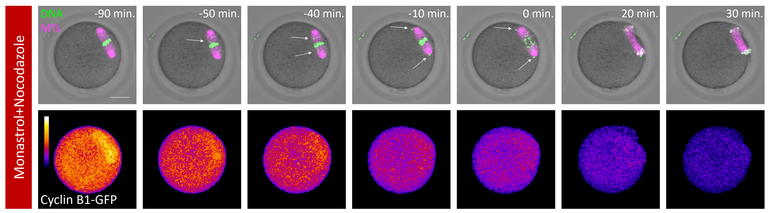
Exemplar images of control and Kif18-KO MII-spindles. Note that severely misaligned chromosomes in Kif18-KO eggs were always pairs of non-bioriented sister chromatids. Chart shows the incidence of misaligned chromosomes in both groups.

4. Misaligned chromosomes cause aneuploidy in MII



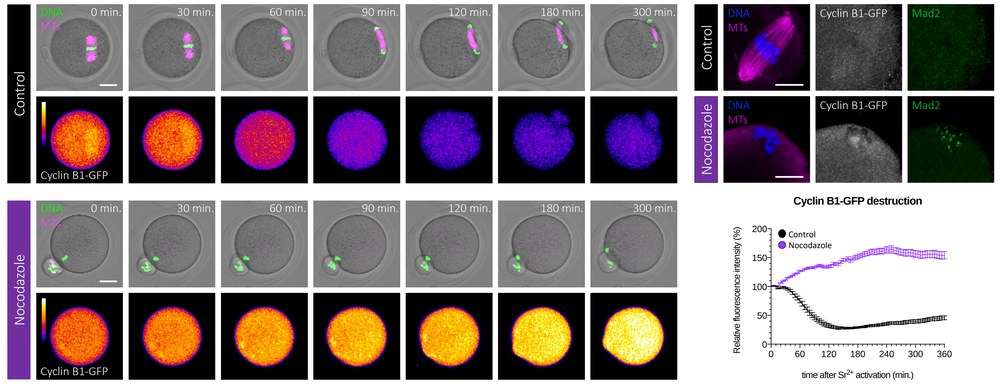
Time-lapse confocal images of parthenogenetically activated control and Kif18-KO eggs during anaphase-II. Note that, instead of normally splitting sister chromatids apart, severely misaligned chromosome (yellow arrow) travels intact towards the nearest spindle pole and missegregates. Chart shows significant increase in the proportion of eggs with misaligned chromosomes in Kif18-KO group.

5. Meiotic exit in the presence of misaligned chromosomes in CD1 eggs



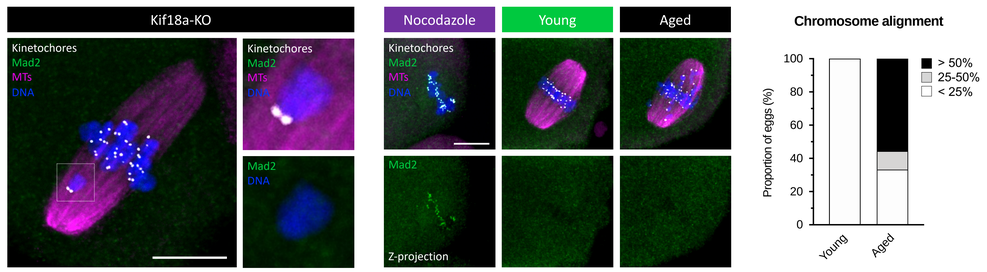
Time-lapse confocal images show that meiotic exit (Cyclin B1-GFP destruction and chromosome segregation) occurs despite the presence of misaligned chromosomes also in CD1 eggs (Kif18a wild-type).

6. Spindle assembly checkpoint (SAC) operates in mouse eggs



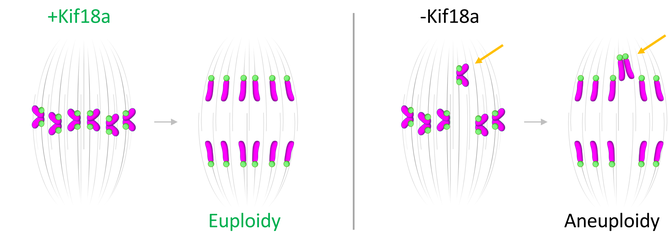
Time-lapse confocal images of parthenogenetically activated control and nocodazole-treated eggs (left panels). Note that nocodazole causes complete spindle disruption and prevents Cyclin B1-GFP destruction. Confocal images of fixed eggs show Mad2 recruitment at the kinetochores following nocodazole treatment (right panels). Chart shows changes in Cyclin B1-GFP fluorescence levels over time.

7. Misaligned chromosomes do not activate SAC in mouse eggs



Confocal images show that misaligned chromosomes do not recruit Mad2 in Kif18a-KO and aged eggs. Chart shows age-related increase in the proportion of eggs with misaligned chromosomes.

Conclusions



Kif18a is expressed during both meiotic divisions but is exclusively required for meiosis-II to ensure appropriate chromosome alignment. In the absence of Kif18a, chromosomes misalign from the metaphase plate but fail to activate spindle assembly checkpoint (SAC) and as a result missegregate and cause aneuploidy. Similarly, individual misaligned chromatids that increase in frequency with advanced maternal age are also undetected by the SAC and could lead to aneuploidy. Thus, this study identifies Kif18a as essential to preserve euploidy in mouse eggs and reveals previously unreported fallibility of the SAC at this developmental stage as a factor that can negatively impact future embryo health and contribute to female infertility.

Acknowledgment

Fondation Jean-Louis Lévesque

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

CRRD Centre for Research in Reproduction and Development / Centre de recherche en reproduction et développement

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CRRF Centre de recherche en reproduction et fertilité