The Effect of the Ontario Fertility Program on the Utilization of and Outcomes from In Vitro Fertilization in Women of Advanced Reproductive Age

Arielle Cantor, MD, FRCSC[1,2], Lynn Meng, MSc[3], Andrea Lanes, PhD[3], Ellen M Greenblatt, MD, FRCSC[1,2]

[1]Department of Obstetrics and Gynecology, University of Toronto, Toronto, Ontario, Canada

[2]Mount Sinai Fertility, Toronto, Ontario, Canada; [3]Better Outcomes Registry & Network (BORN) Ontario, Ottawa, Ontario, Canada

BACKGROUND

- Support for fertility services by public health programs varies widely
- Launch of **Ontario Fertility Program (OFP)** in December 2015
- Funding of one IVF cycle per eligible person per lifetime

Ontario resident | ***Valid Ontario health card*** | ***Under 43 years***

- Age threshold based on recommendation that those with cumulative live birth rate < 10% should not qualify
- Increasing demand for fertility services in Ontario since introduction of the OFP, especially in those of advanced reproductive age

OBJECTIVE

- To examine demographics and clinical outcomes of patients ≥ 40, but < 43 years, undergoing IVF in Ontario before and after OFP implementation to determine whether the age limit for OFP funded IVF access is appropriate

METHODS

- Retrospective database review from CARTR Plus and BORN Ontario
- Analysis of cycle outcomes from people undergoing IVF with autologous oocytes before and after OFP

INCLUSION CRITERIA

- Women aged ≥ 40 and < 43 years
- Had IVF prior to (Jan 2014 – Dec 2015) or after OFP (Jan 2016 – Dec 2017)
- Ontario resident with valid OHIP card
- Using autologous oocytes

EXCLUSION CRITERIA

- Women aged < 40 or > 43 years
- Non-Ontario resident
- Using donor oocytes

RESULTS

		ONTARIO PRE-OFP (2014-2015)	ONTARIO POST-OFP (2016-2017)	*P-VALUE*
IMPLANTATION RATE	%	17.4	19.0	0.10
CLINICAL PREGNANCY RATE	% CS	17.0	13.3	< 0.01
CUMULATIVE CLINICAL PREGNANCY RATE	% CS	20.5	16.8	< 0.01
LIVE BIRTH RATE	% CS	10.4	8.3	< 0.01
CUMULATIVE LIVE BIRTH RATE	**% CS**	**12.5**	**10.5**	**0.03**
PREGNANCY LOSS RATE	% CP	34.3	34.1	0.95

		ONTARIO PRE-OFP (2014-2015)	ONTARIO POST-OFP (2016-2017)
# STIMULATION CYCLES	**n**	**27 945**	**36 076**
≤ 34 Years	n	13 426 (48.0%)	15 174 (42.1%)
35-39 Years	n	11 284 (40.4%)	16 058 (44.5%)
40-42 Years	**n**	**1841 (6.6%)**	**3567 (9.9%)**
≥ 43 years	n	1394 (5.0%)	1277 (3.5%)

- 29% increase in total number of IVF stimulation cycles in all age groups after OFP
- **94%** increase in IVF cycles in patients aged 40-42

CONCLUSION

- Use of IVF in women over age 40 doubled in Ontario with access to OFP funding
- Cumulative live birth rate per cycle start lower after OFP in women aged 40-42
- At 10.5%, still exceeds threshold set by OFP advisory panel

CS = cycle start | CP = clinical pregnancy

The proportion of live births following in-vitro fertilization is higher in women with a history of recurrent pregnancy loss: a population study

Cho, Kristy[1]; Albert, Arianne[2]; Bedaiwy, Mohamed[1]

1) University of British Columbia, Division of Reproductive Endocrinology & Infertility, Vancouver, Canada 2) Women's Health Research Institute, Department of Biostatistics, Vancouver, Canada

Background

Recurrent pregnancy loss (RPL) is a frustrating and distressing condition as nearly 50% of patients have no medical explanation for the cause of pregnancy loss (1). Currently, patients will continue expectant management if no correctable factor is identified. In-vitro fertilization (IVF) with preimplantation genetic testing (PGT) has been utilized in the RPL population in attempt to improve pregnancy outcomes. It is postulated that the replacement of genetically normal embryos may reduce the incidence of pregnancy loss (2,3). Furthermore, infertility can occur concurrently with RPL.

This study aimed to determine if the proportion of live births resulting from IVF is different in patients with RPL and how the uptake has changed over time.

Methods

This is a retrospective cohort study utilizing the data from the British Columbia Perinatal Data Registry (BCPDR). All women with a live birth recorded in 2008-2018 were included. RPL is defined as 2 or more pregnancy losses before 20 weeks gestational age, occurring consecutively or nonconsecutively, and was entered as a time-varying factor in the model.

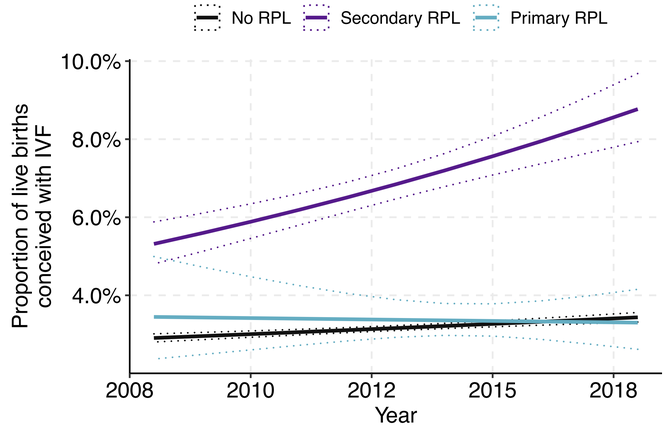
Methods (Continued)

For every livebirth, anonymized demographic variables, birth outcomes, and whether IVF was used were recorded within BCPDR database. The demographic information between the study groups were compared using ANOVA or Fisher's exact test for continuous and categorical data respectively. The proportion of livebirth after IVF in the group with RPL was compared with the proportion in those without RPL over time. To assess how changes in IVF use over time might be influenced by maternal age, we included age as a continuous variable to determine its interactions with both time and RPL status.

Results

There were 443,386 pregnancies in the BCPDR between January 1, 2008 and March 31, 2018. Of these, 406,191 pregnancies by 284,402 women had data regarding IVF utilization. There were 21,049 women (7.4%) with a history of RPL. From 2008 to 2018, a stronger increasing trend in the proportion of live births resulting from IVF was observed in those with secondary RPL compared to those with primary RPL or those without RPL (figure 1).

Figure 1. The percentage of births from IVF for those with RPL compared to those without from 2008 to 2018.

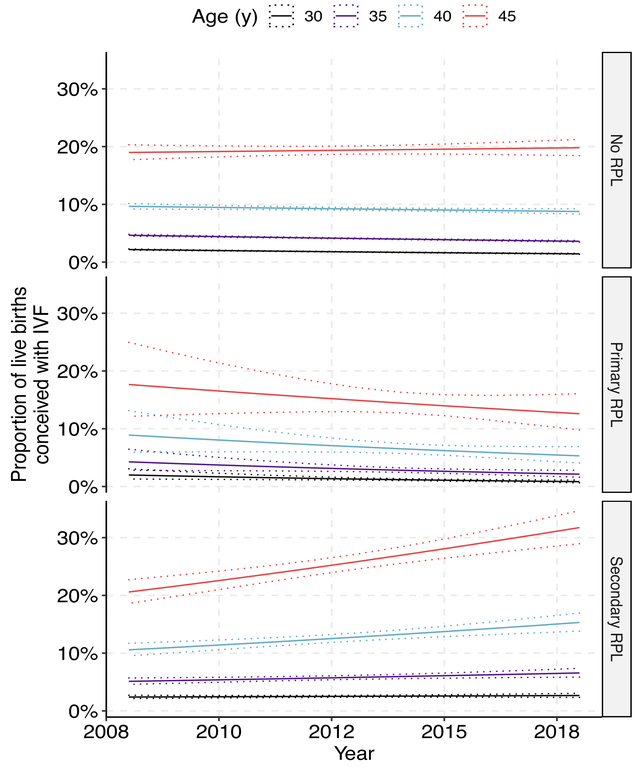


Results (Continued)

The patients with a history of RPL were older (32.6 vs 30.6), had higher gravidity (4.17 vs 1.88), and higher number of spontaneous miscarriages (2.02 vs 0.16) at first recorded livebirth compared to patients without (P all < 0.0001). A history of RPL was associated with preterm birth (12.3% vs 9.0%, P < 0.0001). Furthermore, the proportion of births following IVF was higher in the pregnancies associated with RPL (6.1% vs 3.2%, P < 0.0001).

Further subgroup analysis of the secondary RPL group showed that the increasing proportion of live births from the use of IVF is most pronounced in older maternal age groups (≥ 40-year-old) (figure 2).

Figure 2. The percentage of births from IVF by RPL, maternal age, and year of birth. Age was categorized for ease of representation. Dashed lines indicate 95%CI.



Discussion

- The proportion of live birth from IVF conception have largely remained the same overtime in non-RPL compared to increasing rates in those with secondary RPL.
- This may be explained by differences in fertility desires, infertility diagnosis, and treatment success rates. We suspect that the increasing proportion of live births from IVF in patients with secondary RPL may be the result of greater desire to conceive resulting in more IVF cycles and IVF conceived births, and/ or improved treatment success. A lower number of live births from spontaneous conception can also explain a higher proportion of LB from IVF, but this seems less likely.
- However, our data cannot determine if the increase is patient-driven or due to improved success with IVF (use of PGT, donor oocytes, planned social egg freezing, etc…).

Conclusion:

- **This provincial database showed that a higher proportion of live births conceived via IVF was noted in the RPL population. This has been increasing over time and this trend was most prominent in older patients with secondary RPL.**

Reference:

1. The Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: A committee opinion. Fertil Steril [Internet] 2012;98:1103–1111. American Society for Reproductive Medicine.
2. Murugappan G, Shahine LK, Perfetto CO, Hickok LR, Lathi RB. Intent to treat analysis of in vitro fertilization and preimplantation genetic screening versus expectant management in patients with recurrent pregnancy loss. Hum Reprod 2016;31:1668–1674.
3. Musters AM, Repping S, Korevaar JC, Mastenbroek S, Limpens J, Veen F Van Der, Goddijn M. Pregnancy outcome after preimplantation genetic screening or natural conception in couples with unexplained recurrent miscarriage: A systematic review of the best available evidence. Fertil Steril 2011;95:2153–2157.

Perinatal Services BC

WOMEN'S HEALTH RESEARCH INSTITUTE AT BC WOMEN'S

THE UNIVERSITY OF BRITISH COLUMBIA

Evaluation of Microfluidics-Based Sperm Separation Device to Isolate Spermatozoa for ART

Julia Louis[1], *Bill Yee*[1], *Sophia Rahimi*[1], *Sahib Shahani*[1], *Jacqueline Dynes*[1], *Sergey Moskovtsev*[1,2], *and Clifford Librach*[1,2,3,4]

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

INTRODUCTION

Sperm selection is a process that occurs naturally in the female reproductive tract; as sperm travel through, only the most highly motile and morphologically normal sperm will be able to reach the oocyte and successfully fertilize it.

In the lab, superior sperm are isolated from fresh semen samples primarily using one of two methods: density gradient centrifugation (DGC) and swim-up (SU) [Table 1].

The ZyMōt Sperm Separation Device is a newly developed tool that uses principles of microfluidics to isolate sperm for ART.

Method	Mechanism	Drawbacks
DGC	Centrifugation of semen over gradient media results in layers of seminal fluid, other cells, immotile sperm, and a pellet of motile sperm	Centrifugation tends to induce the production of reactive oxygen species → potentially damaging to sperm DNA integrity
SU	Within a test tube inclined at 45°, a layer of culture media is placed on top of semen, allowing sperm to swim upwards	Requires 1 hour of incubation → not time efficient
ZyMōt	Consists of two chambers separated by a porous polycarbonate filter, with two ports of access; semen is loaded into lower chamber and sperm are collected from upper chamber of culture media	To be determined…

Table 1: A comparison of sperm isolation methods.

OBJECTIVE & HYPOTHESIS

OBJECTIVE: To assess the performance of ZyMōt in isolating spermatozoa for ART compared to conventional semen preparation methods.

HYPOTHESIS: ZyMōt is able to isolate a subpopulation of spermatozoa with higher total motile count yield, enhanced kinetics, and lower DNA fragmentation than density gradient centrifugation and swim-up methods.

MATERIALS & METHODS

From May to August 2019, samples from 16 research-consented semen analysis patients were divided into three aliquots to allow for processing by three methods [Figure 1]. After processing, the following parameters were evaluated: concentration, motility, and morphology, assessed manually via light microscopy; kinetics, analyzed using a computer-assisted semen analysis (CASA) machine; and DNA damage, assessed by a standard acridine-orange flow cytometric-based assay. Statistics were performed using a paired t-test. A p-value less than 0.05 was considered to be significant.

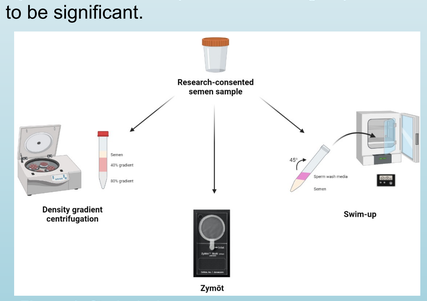


Figure 1: Study design.

RESULTS

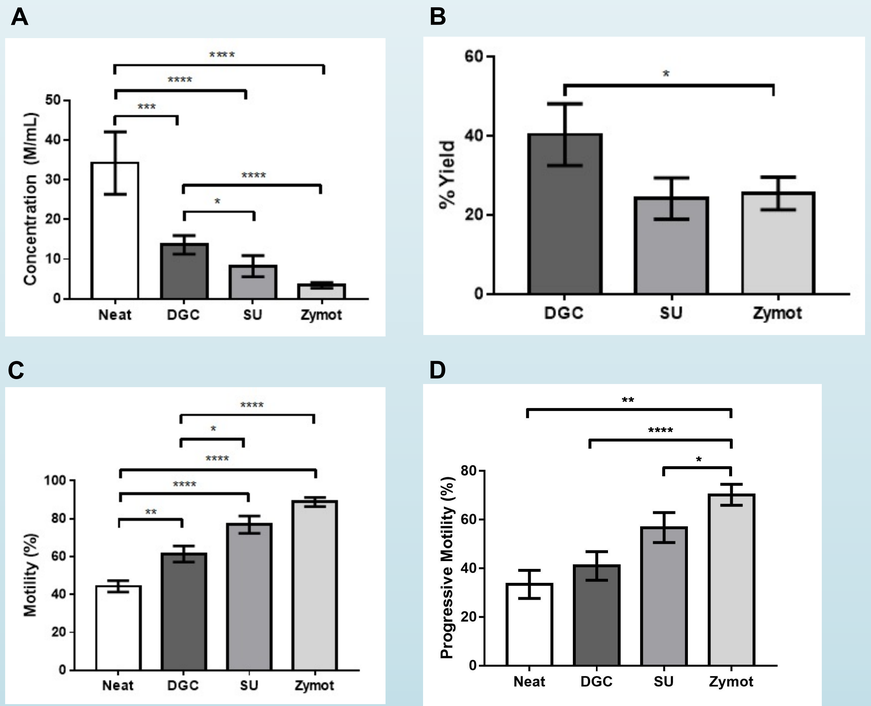


Figure 2: Sperm concentration, percent yield, and motility. Concentration (A) decreased significantly after all methods of processing. DGC had a significantly higher percent yield (B) compared to SU and ZyMōt, which had similar percent yield. Motility (C) increased significantly after all methods of processing, with ZyMōt having the greatest motility, which was statistically significant compared to DGC. Progressive motility (D) increased significantly only after ZyMōt, with a statistically significant difference compared to SU.

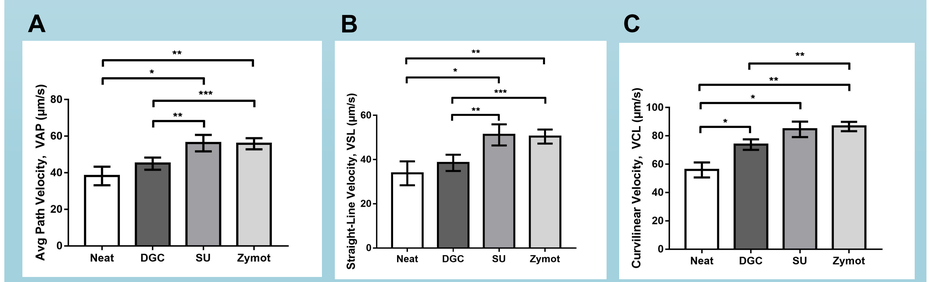


Figure 3: Sperm kinetics. Average path (A) and straight-line velocity (B) increased significantly after SU and ZyMōt. The differences between DGC and SU, and DGC and ZyMōt are statistically significant, but not between DGC and neat. Curvilinear velocity (C) increased significantly after all methods of processing, but the difference between SU and ZyMōt was not significant.

* P ≤ 0.05
** P ≤ 0.01
*** P ≤ 0.001
**** P ≤ 0.0001

RESULTS

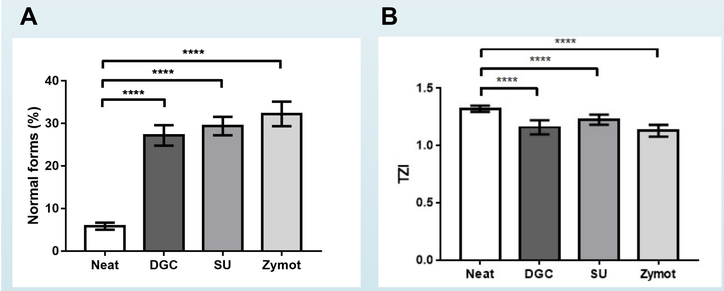


Figure 4: Sperm morphology. The percentage of morphologically normal sperm (A) increased significantly for all methods, while the teratozoospermia index (B) decreased significantly for all.

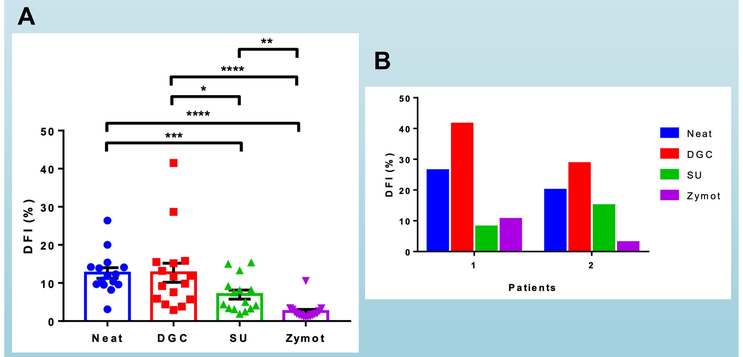


Figure 5: Sperm DNA fragmentation. The percentage of sperm with damaged DNA (A) decreased significantly after SU and ZyMōt, but not after DGC. The differences between DGC and SU, DGC and ZyMōt, and ZyMōt and SU were statistically significant. Two patients (B) had an increase in DFI only after DGC, but not after SU or ZyMōt.

CONCLUSION

ZyMōt is a user-friendly, time-efficient tool for isolating sperm with superior motility, kinetics, and DNA integrity, compared to conventional methods. Further investigations should focus on how it performs with frozen samples, as well as clinical outcomes, such as fertilization, pregnancy, and live birth rates.

ACKNOWLEDGEMENTS

Special thanks to all the Clinical Andrology Staff for their continued support.

Obstetrics & Gynaecology
UNIVERSITY OF TORONTO

P-225 CFAS 2021

The Fetal ANXA5 M2 Haplotype Contributes to Sporadic Miscarriage of Euploid Fetuses

CReATe Fertility Centre

Mitko Madjunkov[1,2], Maureen Wilkinson[1], Siwei Chen[1], Svetlana Madjunkova[1,3], Clifford Librach[1,2,4,5]

[1]CReATe Fertility Centre, Toronto, Canada; [2]Department of Obstetrics and Gynecology, [3]Department of Laboratory Medicine and Pathobiology, [4]Department of Physiology, [5]Institute of Medical Sciences, University of Toronto.

INTRODUCTION

Spontaneous abortion (SA) is a significant clinical problem with several different aetiologies. Certain thrombophilia and folate-related gene mutations have been associated with an increased risk of miscarriage which may be due to impaired coagulation homeostasis. The M2-haplotype of ANXA5-gene promotor encoding the anticoagulation protein Annexin-A5 has also been implicated in early and recurrent SA. ANXA5 M2 haplotype results in 60% decreased activity of ANXA5. Annexin A5 (ANXA5) is a protein that is abundantly expressed in normal placenta where it exerts an anticoagulant function on the surface of syncytiotrophoblasts, by setting up a bidimensional shield as a protective clotting function. ANXA5 also displays an essential role membrane repair that might be crucial for the integrity of a healthy placenta and in the fusion of villous trophoblasts. One important deficiency in the majority of the studies on this subject is a lack of information on the fetal/embryonic contribution to the thrombotic events during placentation and early embryo development.

OBJECTIVE

The objective of this study was to evaluate the prevalence of ANXA5 M2 and M1 haplotype and cumulative effect of parental and fetal M2 and M1 haplotype in women experiencing euploid first trimester miscarriage.

MATERIALS AND METHODS

This study had REB approval. This is a single centre retrospective cohort study at CReATe Fertility Centre. DNA from 109 mother-father-fetus trios were analyzed for presence of the M2/M1 ANXA5 haplotype. The fetal samples were from T1 miscarraiges, confirmed to be euploid by STR analysis and NGS. ANXA5 M2/M1 haplotype was analyzed by Sanger sequencing. ANXA5 M2/M1 allele and genotype frequencies were studies among fetuses and parents, as well as the cumulative effect of the M2 haplotype in mother-fetus pairs.

SSPS software was used for statistical analyses, and p<0.05 with CI 95% was considered significant.

RESULTS

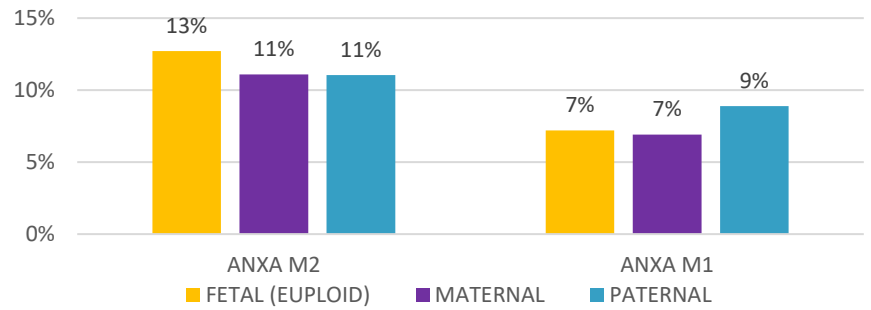


Figure 1: Allele frequency of ANXA5 M2 and M1 among parents and their fetuses. Allele frequency of ANXA5 M2 and M1 in the fetuses was not statistically different from the frequency in mothers and fathers.

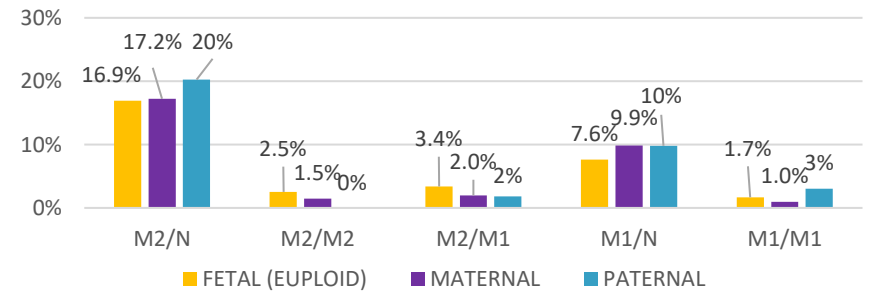


Figure 2: ANXA5 M2 and M1 genotype frequencies among parents and their fetuses. The frequency of the M2 ANXA5 haplotype was similar between the fetuses and their parents. None of the fathers was found to be homozygous for ANXA5 M2 haplotype.

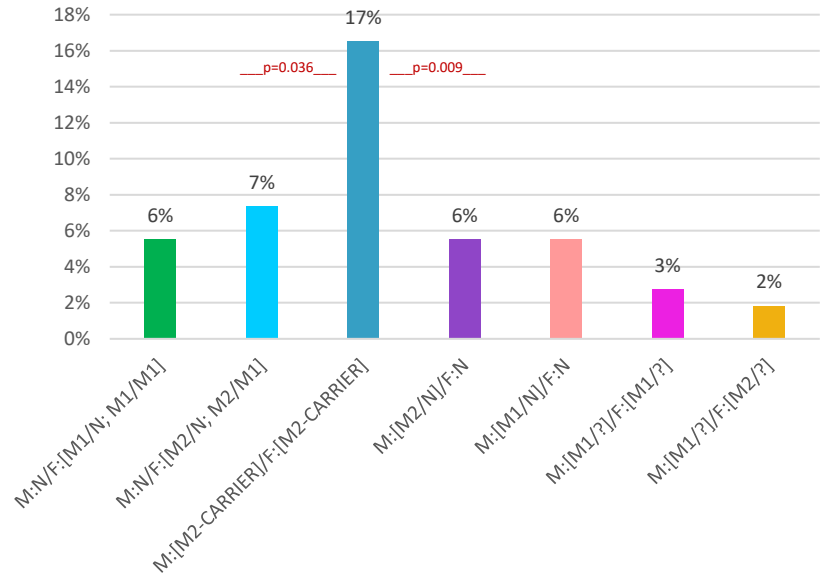


Figure 3: Frequency of shared ANXA5 M2 and M1 alleles between mother-fetus pairs to evaluate the cumulative effect of maternal and fetal alleles. We found a significantly higher number of mother –M2 carrier/fetus –M2-carrier pairs compared to: 1) mother-M2 carrier/fetus Normal (N) (17% vs 6%, p=0.009415, OR=3.4 (1.2-8.9)) and 2) mother-N/fetus M2 carrier pairs (17% vs 7%, p=0.036642, OR 2.4 (1.036-6.019). No difference in the frequency of M1 haplotype among mother-fetus pairs was detected (mother-M1 carrier/fetus-N, 6%; mother-N-fetus-M1 carrier, 6%; mother-M1/fetus-M1, 3%). The frequency of M2-negative mother-fetus pairs was 55%. N=normal allele; M=mother; F=fetus

CONCLUSION

Our study is one of the first to explore the fetal contribution of ANXA5 M2 haplotype in early euploid miscarriages and suggests that there is an accumulation of the effect of maternal and fetal M2 alleles to placental thrombophilia. These preliminary results provide indirect evidence that paternal M2 carrier status in addition to maternal M2 haplotype could increase the odds of euploid miscarriage. If these results can be corroborated with prospective studies involving increased numbers, and comparisons with normal live birth controls, preimplantation testing for the ANXA5 M2 haplotype in embryos and parents could be a new method for embryo selection to prevent early miscarriages.

ACKNOWLEDGEMENTS

This project was funded by the CReATe Fertility Centre.

Thanks to CReTAe Fertility Clinic Staff and patients .

The authors confirm that there are no conflicts of interest.

Microfluidic sperm sorting results in differences in progressive motility, hyperactivity and anti-sperm antibody binding compared to density gradient centrifugation

1,2 Megan Dufton, 3 Sabrina Floccari, 1 Frankie Slade, 3 Glenys Gibson;
1 Atlantic Assisted Reproductive and 2 Dept Obstetrics and Gynecology Dalhousie University,
Halifax Nova Scotia, Canada 3 Acadia University, Wolfville, Nova Scotia, Canada

Introduction

- 1 in 8 couples experience infertility, a third due to male factors.
- Optimizing semen processing can help to improve IVF success.
- Traditional semen processing uses density gradient centrifugation (DGC), increasing risk of DNA fragmentation.
- Microfluidic sorted sperm (MSS) has been shown to have reduce DNA fragmentation.
- Reduced DNA fragmentation has led to positive outcomes rates with ICSI and IUI (1,2).
- Limited real-world data exists for how MSS sperm performs with conventional IVF insemination.
- Hyperactivated motility and low levels of anti-sperm antibodies are necessary for successful fertilization with conventional insemination and serve as diagnostic predictors.

How do motility, hyperactivation and anti-sperm antibody binding, compare between microfluidic sorted sperm (MSS) and density gradient processed sperm?

Objectives

To determine if diagnostic factors that indicate the potential for fertilization when using conventional insemination vary between DGC processed semen and MSS processed semen. This will be analyzed by examining:

1) Sperm motility
2) Sperm hyperactivation
3) Anti-sperm antibodies (ASAB)

Methods

Study 1:

- 48 semen samples were collected from patients that presented at a single IVF center for infertility work-up.
- Following semen analysis, samples were divided into two, half processed with DGC, the other with Zymôt ™(850uL) MSS device, both in bicarbonate buffered conditions.
- After processing, Computer Aided Sperm Analysis (CASA) measured concentration, motility, and hyperactivation.
- Maximal hyperactivation was analyzed after agonist treatment (progesterone and pentoxifylline). Heavy and light chain anti-sperm antibodies were detected by direct Immunospheres assay.
- Wilcoxon tests were used to compare processing methods,.

Methods Continued

Study 1:

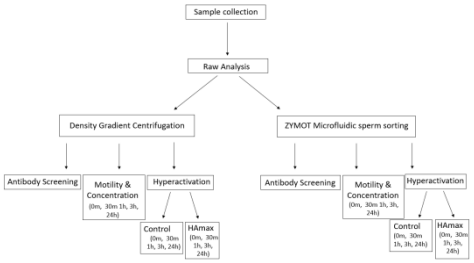


Figure 1: Sample processing and testing conducted for study 1.

Study 2:

- 24 semen samples were collected from patients that presented at a single IVF center for infertility work-up.
- Following semen analysis, each sample was divided into two, half processed with DGC, the other with Zymôt ™ (850uL) MSS device,.
- DGC was processed in bicarbonate buffered medium, MSS was processed in HEPES buffered medium. A 5 min, 500g washing step in bicarbonate buffered medium was added after MSS processing.
- After processing, CASA measured concentration, motility, and hyperactivation and maximal hyperactivation.
- Wilcoxon tests were used to compare processing methods, p<0.05 considered significant.

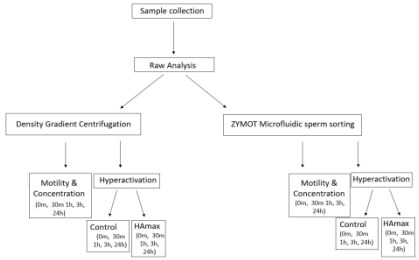


Figure 2. Sample processing and testing conducted for study 2.

Results

Study 1:

- MSS sperm had a significantly higher percentage of progressively motile sperm, immediately after and three hours after processing (p=0.01) compared to DGC.

Results Continued

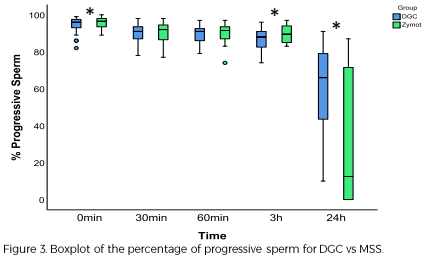


Figure 3. Boxplot of the percentage of progressive sperm for DGC vs MSS.

- CASA was used to compare the percentage of hyperactivated sperm cells present in samples after DGC vs MSS.
- Immediately after processing, the samples were not significantly different for percentage of hyperactivation (p=0.16). By 30, 60 and 90 minutes after processing DGC processed samples had a significantly higher percentage of hyperactivated cells (p<0.01, 24.0%±10.1, 12.8%±6.9; p<0.01, 25.8%±10.1, 14.1%±8.7; p<0.0, 34.4%±11.4, 17.9%±10.1.

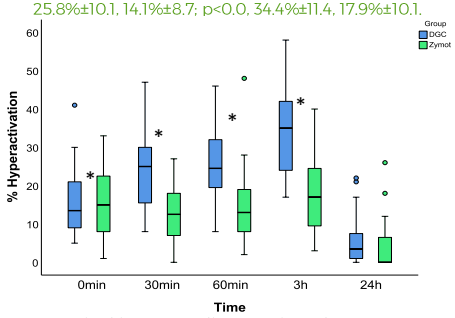


Figure 4. Boxplot of the percentage of hyperactivted sperm for DGC vs MSS.

- After treatment of hyperactivation agonist to induce maximal hyperactivation, DGC processed samples had a significantly higher percentage of hyperactivated cells at 30 and 60 minutes after agonist exposure (p<0.01, 37.8%±13.1, 28.2%±12.8; p<0.01, 42.5%±12.5, 31.5%±12.9 respectively).

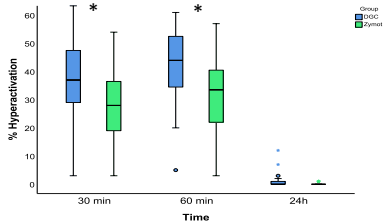


Figure 5. Boxplot of the percentage of agonized hyperactivted sperm for DGC vs MSS.

Results Continued

- 9 of the 48 samples were positive for anti-sperm antibodies. The percentage of sperm positive for anti-sperm antibodies did not differ between DGC and MSS (p=0.39; 5.8%±2.4, 3.6±2.7).
- DGC processed samples had significantly more tail-tip bound ASAB than MSS samples (p=0.02; 88.8%±16.1. 52.8%±43.1).

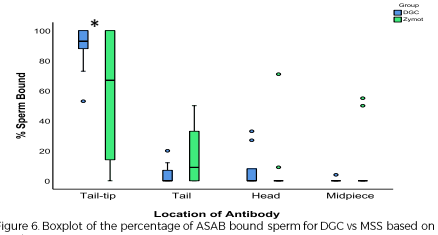


Figure 6. Boxplot of the percentage of ASAB bound sperm for DGC vs MSS based on location.

Study 2:

- MSS sperm had a significantly higher percentage of progressively motile sperm, at 24 hours after processing (p=0.01) compared to DGC, all other time points did not show a significant difference.
- The percentage of hyperactivated cells were not significantly different comparing MSS and DGC accept for 60 mins post processing (p=0.03).
- When a hyperactivation agonist was added to induce maximal hyperactivation, there were no significant differences in the percentage of hyperactivated cells between DGC and MSS processed samples.

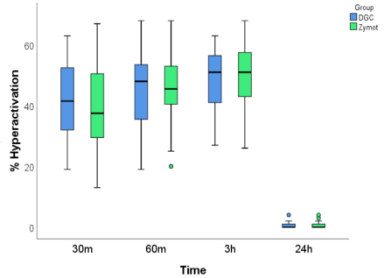


Figure 7. Boxplot of the percentage of agonized hyperactivted sperm for DGC vs MSS.

Conclusions

- Motility after MSS processing is superior to DGC.
- Hyperactivation in MSS processed samples was lower, however by adding a washing step for 5 min at 500 g after MSS processing hyperactivation was restored.
- MSS samples should be washed prior to use in conventional insemination.

References: 1) Gode F, Bodur T, Gunturkun F, Gurbuz AS,Tamer B, Pala I, Isik AZ (2019) Comparison of microfluid sperm sorting chip and density gradient methods for use in intrauterine insemination cycles. *Fertil Steri*, 112: 842-848. 2) Parrella A, Keating D, Cheung S, Xie P, Stewart JD, Rosenwaks Z, Palermo G (2019) A treatment approach for couples with disrupted sperm DNA integrity and recurrent ART failure. *J Assist Reprod Genet*, 36: 2057-2066.

Optimization of Whole Mitochondrial Genome Analysis of Trophectoderm Biopsy

Aashi Aggarwal[1,3], Ran Antes[1], Svetlana Madjunkova[1,5], Clifford Librach[1,2,3,4,]

[1]CReATe Fertility Centre, Toronto, Canada; [2]Department of Obstetrics and Gynecology, University of Toronto, Canada; [3]Department of Physiology, University of Toronto, Canada; [4]Institute of Medical Sciences, University of Toronto, Canada; [5]Department of Laboratory Medicine and Pathobiology, University of Toronto, Canada.

INTRODUCTION

- Mitochondria is the most abundant organelle in the oocyte and early embryo. Due to their role in ATP synthesis, mitochondrial function is crucial for fertilization and embryo development.
- Multiple copies of mitochondrial DNA (mtDNA) exist in a cell and the term heteroplasmy (Mt-Het) refers to the coexistence of wild type and mutated mtDNA.
- Around 30-40% of euploid embryos do not implant and this failure could at least partially be due to occurrence of low level heteroplasmy and de novo mtDNA variants.
- The knowledge on mtDNA variation is limited mainly due to restricted availability of human embryos for research and immaturity of techniques to allow low cell and DNA input for genomic analysis.
- Whole genome amplification (WGA) is integral to pre-implantation genetic testing (PGT) to acquire genomic DNA (gDNA) from a trophectoderm (TE) biopsy. SurePlex (Illumina) and RepliG (QIAGen) are two most commonly used WGA kits worldwide.
- WGA is known to introduce variant errors and uneven coverage bias leading to false detection of low level Mt-Het.

OBJECTIVE

We optimized the enrichment, sequencing and analysis of human embryonic mtDNA from TE biopsy and designed a criteria and threshold for accurate detection of Mt-Het in WGA samples.

MATERIALS AND METHODS

- The study had institutional REB approval. Two groups of samples were used for optimizations.
- **Group 1**: Mock granulosa cells (GC) (N=2) were split into two (i) gDNA was extracted and (ii) ~5 cell biopsy (CB) was taken to replicate a TE biopsy. Aliquots of gDNA and CB samples were WGA using RepliG and SurePlex each, resulting in 4 samples for each GC. All four WGA replicates along with unamplified gDNA was sequenced (5 X 2 samples; N=10).

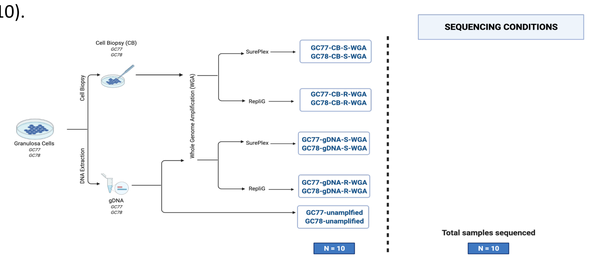


Figure 1: Experimental workflow for Group 1 control samples

- **Group 2**: gDNA from established cell lines (N=2) with known Mt-Het at specific positions was aliquot and whole genome amplified using RepliG and SurePlex. 2 WGA replicates and unamplified gDNA samples were sequenced for each sample in duplicates with altering temperature conditions to optimize library preparation conditions (N=12).

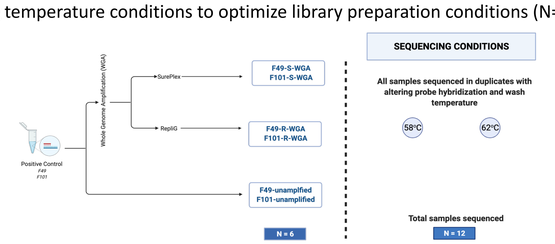


Figure 2: Experimental workflow for Group 2 control samples

- Library preparation was performed using Illumina DNA Prep with Enrichment and mtDNA was enriched using probe hybridization and capture using custom biotinylated probes. Enriched libraries were sequenced on Illumina Next seq 550 and analysis was performed using DRAGEN enrichment (Illumina) and Integrated Genomic Viewer (IGV).

RESULTS

1. Optimizing enrichment of whole Mt-Genome in WGA DNA

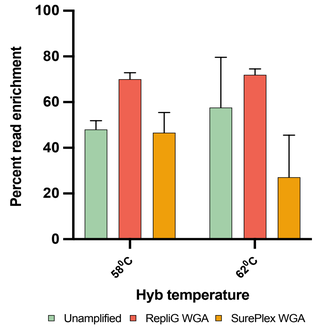


Figure 3: Percent enrichment of Group 2 control samples. Percent enrichment refers to the percent of reads enriched for target Mt-genome. A higher temperature was tested to optimize enrichment of WGA samples. Increase in temperature to 62^{0}C did not improve percent enrichment and a standard temperature of 58^{0}C was established for all samples.

2. Comparison of two WGA kits: RepliG and SurePlex

Comparison of WGA samples to unamplified samples from Group 1 showed that RepliG WGA resulted in a higher mean depth of mt-genome (9084x) in comparison to unamplified (6015x) and SurePlex WGA (3526x) samples.

Figure 4: IGV screenshots presenting the comparison of mt-genome coverage in unamplified (top), RepliG WGA (middle), and SurePlex WGA (bottom) samples. RepliG WGA resulted in even coverage of mt-genome analogous to the unamplified sample. SurePlex WGA showed uneven distribution of read depth with some over-represented and under-represented regions.

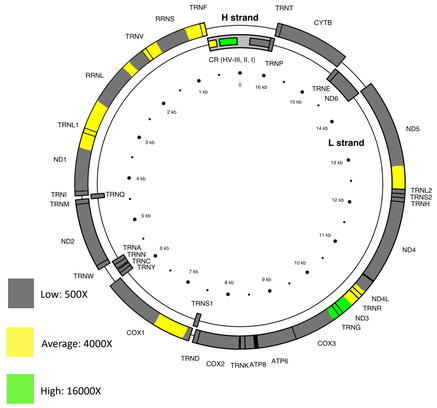


Figure 5: Mt-genome displaying the mean depth in SurePlex WGA sample. Mt-genome was categorized into regions based on coverage at each nucleotide position. 76.8% of the mt-genome had low coverage, 18% had average coverage and 4.8% had high coverage.

3. Addressing the bias introduced by WGA

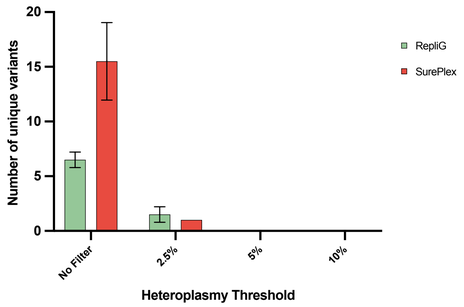


Figure 6: Low frequency false positive variants identified in SurePlex and RepliG WGA CB samples from Group 1. Variants detected in WGA samples and absent from respective unamplified samples were defined as false positives. SurePlex WGA introduced 2-fold more false positives compared to RepliG. All variants were present at low frequency level and were excluded with a heteroplasmy detection threshold of >5%.

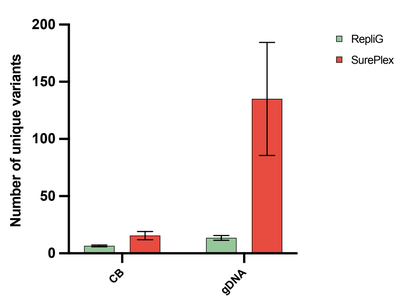


Figure 7: Comparison of false positives in WGA of CB and gDNA. Higher frequency of false positives were identified in WGA of gDNA in comparison to WGA of CB for both RepliG and SurePlex WGA. This increase is 10-fold higher in SurePlex WGA of gDNA compared to RepliG WGA of gDNA.

4. Defining the specificity of heteroplasmy level detection

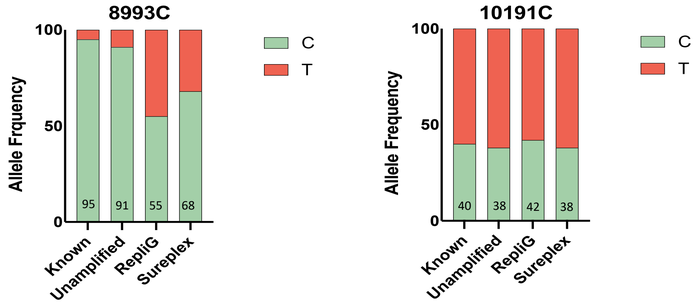


Figure 8: Detection of known heteroplasmy levels in unamplified, RepliG WGA, and SurePlex WGA samples from Group 2. Heteroplasmy levels detected by NGS after mtDNA enrichment in unamplified, RepliG WGA, and SurePlex WGA samples were compared to known heteroplasmy levels determined by qPCR (Dr. Sondheimer Lab). Heteroplasmy levels at mt.8993 in low coverage region deviated in RepliG WGA (by 40%) and SurePlex WGA (by 23%). Heteroplasmy levels in mt. 10191C in high coverage region were accurately detected across all samples.

CONCLUSION

- Probe hybridization temperature of 58^{0}C was established appropriate for WGA samples.
- RepliG WGA results in higher depth and even amplification of Mt-genome; SurePlex WGA results in lower depth and uneven coverage of Mt-genome.
- SurePlex WGA introduces 2-fold more false positives compared to RepliG WGA.
- All false positive variants are introduced at low Mt-Het and application of >5% heteroplasmy threshold omits false positives and allows detection of true mtDNA variants.
- WGA of gDNA compared to WGA of CB results in a higher frequency of false positives.
- WGA causes uneven amplification of mtDNA resulting in inaccurate detection of heteroplasmy levels in SurePlex annotated low coverage regions.

Impact

We optimized mtDNA enrichment and sequencing of WGA DNA from TE biopsy and addressed the technical challenges of WGA. The optimized pipeline can be applied to human embryonic DNA to accurately detect low Mt-Het variants.

The data adds to the formation of a framework for downstream analysis of infertile patients including mtDNA analysis of pre-implantation embryos – a potential biomarker for selection of euploid embryos.

Complete mitochondrial and nuclear genome analysis of human embryos from a single trophectoderm biopsy – an optimized approach

Aashi Aggarwal[1,3], Ran Antes[1], Svetlana Madjunkova[1,5], Clifford Librach[1,2,3,4],

[1]CReATe Fertility Centre, Toronto, Canada; [2]Department of Obstetrics and Gynecology, University of Toronto, Canada; [3]Department of Physiology, University of Toronto, Canada; [4]Institute of Medical Sciences, University of Toronto, Canada; [5]Department of Laboratory Medicine and Pathobiology, University of Toronto, Canada.

INTRODUCTION

- Mitochondria is the most abundant organelle in the oocyte and early embryo. Due to their role in ATP synthesis, mitochondrial function is crucial for fertilization and embryo development.
- Multiple copies of mitochondrial DNA (mtDNA) exist in a cell and the term heteroplasmy (Mt-Het) refers to the coexistence of wild type and mutated mtDNA.
- Around 30-40% of euploid embryos do not implant and this failure could at least partially be due to occurrence of low level heteroplasmy and de novo mtDNA variants.
- The knowledge on mtDNA variation is limited mainly due to restricted availability of human embryos for research and immaturity of techniques to allow low cell and DNA input for genomic analysis.
- Whole genome amplification (WGA) is integral to pre-implantation genetic testing (PGT) to acquire genomic DNA (gDNA) from a trophectoderm (TE) biopsy. SurePlex (Illumina) and RepliG (QIAGen) are two most commonly used WGA kits worldwide.
- WGA is known to introduce variant errors and uneven coverage bias leading to false detection of low level Mt-Het.

OBJECTIVE

We optimized the enrichment, sequencing and analysis of human embryonic mtDNA from TE biopsy and designed a criteria and threshold for accurate detection of Mt-Het in WGA samples.

MATERIALS AND METHODS

- The study had institutional REB approval. Two groups of samples were used for optimizations.
- **Group 1**: Mock granulosa cells (GC) (N=2) were split into two (i) gDNA was extracted and (ii) ~5 cell biopsy (CB) was taken to replicate a TE biopsy. Aliquots of gDNA and CB samples were WGA using RepliG and SurePlex each, resulting in 4 samples for each GC. All four WGA replicates along with unamplified gDNA was sequenced (5 X 2 samples; N=10).

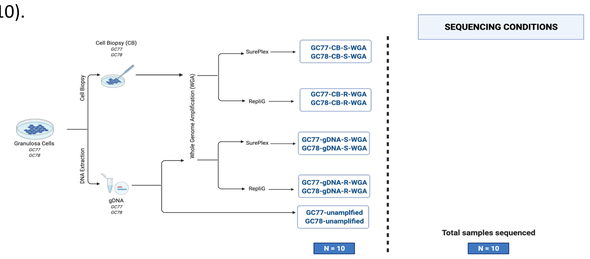


Figure 1: Experimental workflow for Group 1 control samples

- **Group 2**: gDNA from established cell lines (N=2) with known Mt-Het at specific positions was aliquot and whole genome amplified using RepliG and SurePlex. 2 WGA replicates and unamplified gDNA samples were sequenced for each sample in duplicates with altering temperature conditions to optimize library preparation conditions (N=12).

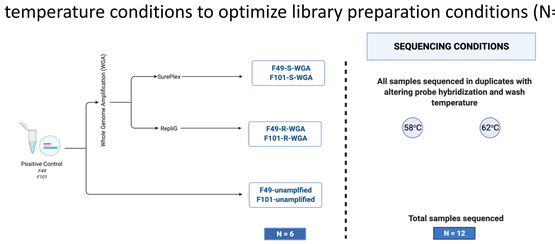


Figure 2: Experimental workflow for Group 2 control samples

- Library preparation was performed using Illumina DNA Prep with Enrichment and mtDNA was enriched using probe hybridization and capture using custom biotinylated probes. Enriched libraries were sequenced on Illumina Next seq 550 and analysis was performed using DRAGEN enrichment (Illumina) and Integrated Genomic Viewer (IGV).

RESULTS

1. Optimizing enrichment of whole Mt-Genome in WGA DNA

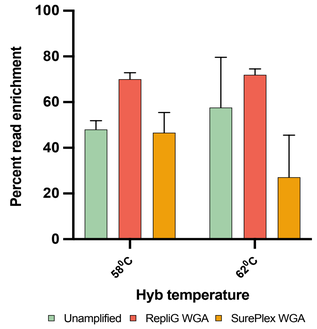


Figure 3: Percent enrichment of Group 2 control samples. Percent enrichment refers to the percent of reads enriched for target Mt-genome. A higher temperature was tested to optimize enrichment of WGA samples. Increase in temperature to 62^{0}C did not improve percent enrichment and a standard temperature of 58^{0}C was established for all samples.

2. Comparison of two WGA kits: RepliG and SurePlex

Comparison of WGA samples to unamplified samples from Group 1 showed that RepliG WGA resulted in a higher mean depth of mt-genome (9084x) in comparison to unamplified (6015x) and SurePlex WGA (3526x) samples.

Figure 4: IGV screenshots presenting the comparison of mt-genome coverage in unamplified (top), RepliG WGA (middle), and SurePlex WGA (bottom) samples. RepliG WGA resulted in even coverage of mt-genome analogous to the unamplified sample. SurePlex WGA showed uneven distribution of read depth with some over-represented and under-represented regions.

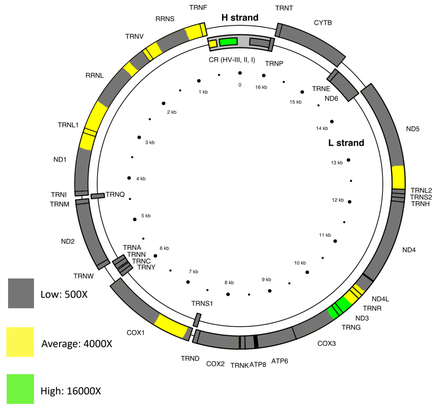


Figure 5: Mt-genome displaying the mean depth in SurePlex WGA sample. Mt-genome was categorized into regions based on coverage at each nucleotide position. 76.8% of the mt-genome had low coverage, 18% had average coverage and 4.8% had high coverage.

3. Addressing the bias introduced by WGA

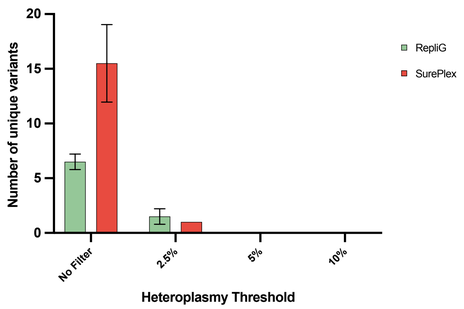


Figure 6: Low frequency false positive variants identified in SurePlex and RepliG WGA CB samples from Group 1. Variants detected in WGA samples and absent from respective unamplified samples were defined as false positives. SurePlex WGA introduced 2-fold more false positives compared to RepliG. All variants were present at low frequency level and were excluded with a heteroplasmy detection threshold of >5%.

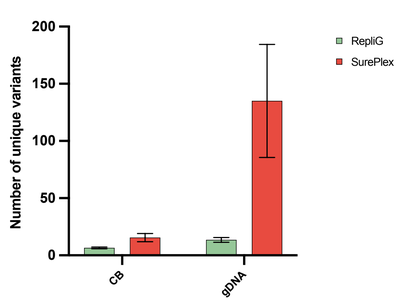


Figure 7: Comparison of false positives in WGA of CB and gDNA. Higher frequency of false positives were identified in WGA of gDNA in comparison to WGA of CB for both RepliG and SurePlex WGA. This increase is 10-fold higher in SurePlex WGA of gDNA compared to RepliG WGA of gDNA.

4. Defining the specificity of heteroplasmy level detection

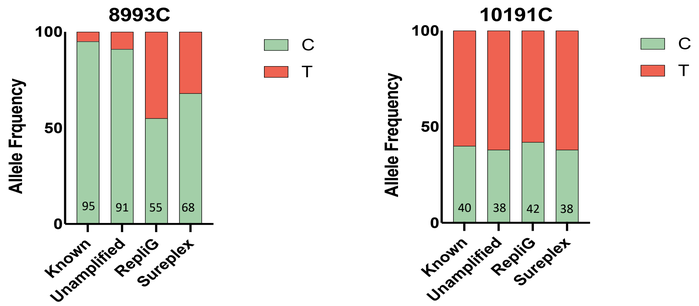


Figure 8: Detection of known heteroplasmy levels in unamplified, RepliG WGA, and SurePlex WGA samples from Group 2. Heteroplasmy levels detected by NGS after mtDNA enrichment in unamplified, RepliG WGA, and SurePlex WGA samples were compared to known heteroplasmy levels determined by qPCR (Dr. Sondheimer Lab). Heteroplasmy levels at mt.8993 in low coverage region deviated in RepliG WGA (by 40%) and SurePlex WGA (by 23%). Heteroplasmy levels in mt. 10191C in high coverage region were accurately detected across all samples.

CONCLUSION

- Probe hybridization temperature of 58^{0}C was established appropriate for WGA samples.
- RepliG WGA results in higher depth and even amplification of Mt-genome; SurePlex WGA results in lower depth and uneven coverage of Mt-genome.
- SurePlex WGA introduces 2-fold more false positives compared to RepliG WGA.
- All false positive variants are introduced at low Mt-Het and application of >5% heteroplasmy threshold omits false positives and allows detection of true mtDNA variants.
- WGA of gDNA compared to WGA of CB results in a higher frequency of false positives.
- WGA causes uneven amplification of mtDNA resulting in inaccurate detection of heteroplasmy levels in SurePlex annotated low coverage regions.

Impact

We optimized mtDNA enrichment and sequencing of WGA DNA from TE biopsy and addressed the technical challenges of WGA. The optimized pipeline can be applied to human embryonic DNA to accurately detect low Mt-Het variants.

The data adds to the formation of a framework for downstream analysis of infertile patients including mtDNA analysis of pre-implantation embryos – a potential biomarker for selection of euploid embryos.

Hyaluronan-rich supplemented medium (HRSM) does not improve pregnancy rate among frozen embryo transfer cycles

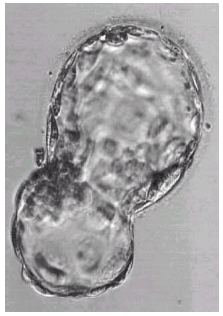
Charlie Rooney[1], Evan Taerk[2,3], Stacy Deniz[2,3], Mehrnoosh Faghih[2,3], Shilpa Amin[2,3], Megan Karnis[2,3], and Michael Neal[3]

[1]Faculty of Life Sciences; [2]Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, McMaster University, Hamilton, Ontario, Canada; [3]ONE Fertility, Burlington, Ontario, Canada.

Affiliated with McMaster University

INTRODUCTION:

- Hyaluronan-rich supplemented medium (HRSM) contains required nutrients for embryo implantation
- Placing embryos into HRSM media prior to transfer has been promoted to enhance implantation, pregnancy and live birth rates



OBJECTIVE:

To determine if there are any clinical benefits to HRSM (EmbryoGlue–VitroLife) for patients undergoing frozen embryo transfers (FETs).

MATERIALS AND METHODS:

- Study population: 206 patients underdoing FET organized into 3 groups:
  Group A: Exposed to HRSM 15-55 min prior to FET
  Group B: Embryos thawed with **no** HRSM
  Group C: Patients matched with Group A on 3:1 ratio
- All embryos thawed via fertility clinic's standard protocol
- Group C patients were selected via a computer-generated random calculator to ensure unbiased selection
- Matching was done for embryo age (age of oocyte at vitrification) and embryo quality (Gardner's blastocyst grading system - good embryo classified with a 2BB+ score)
- Descriptive statistics were used to compare groups and classical Chi-square calculations were done to determine significance ($P<0.05$) between groups

Table 1: Pregnancy rate stratified by age and embryo quality for three groups

	Group A – HRSM (n – 20)		Group B – No HRSM (n = 186)		Group C – No HRSM / matched (n = 60)	
Age	Good embryo	Poor embryo	Good embryo	Poor embryo	Good embryo	Poor embryo
< 38 y.o	50%	0%	64%	32%	65%	25%
> 38 y.o	57%	0%	58%	36%	56%	33%
All ages	53%	0%	62%	33%	62%	28%

- Pregnancy rates (+ve BhCG 12 days after FET) were assessed for all groups and stratified by embryo quality
- Age stratification: patients' younger and older than 38

RESULTS:

No statistical differences were seen in pregnancy rate between Group A and B (x= .440) or between A and C (x= .561). Pregnancy rates were noted to be clinically higher in the groups without HRSM.

CONCLUSION:

Our results do not show any clinical benefits of HRSM for FETs in our patient population. This can be used as a tool to inform patients about the clinical benefits of using HSRM.

MOVING FORWARD we postulate that competent embryos may be capable of producing adequate hyaluronan to promote implantation. Results from a larger HRSM cohort would be beneficial in supporting this theory.

We appreciate the support from all of the allied health professionals at ONE Fertility.

IS OOCYTE CRYOPRESERVATION A GOOD "INSURANCE POLICY"?

Angie Mace, LPN

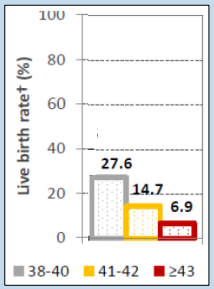
An observational study of patients under 38 years of age undergoing oocyte preservation using violet artificial intelligence live birth prediction tool vs actual live birth rates of patients 38 and over undergoing IVF with ET

INTRODUCTION:

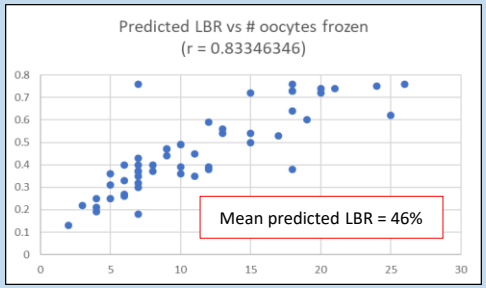
We present the results of a retrospective chart review analyzing 53 patients stimulated with a mixed protocol of follitropin delta (Rekovelle) and highly-purified human menopausal gonadotropin (HP-hMG; Menopur) for oocyte cryopreservation at Kelowna Regional Fertility Centre (KRFC) and Olive Fertility Centre.

METHODS:

Using the Violet artificial intelligence tool for oocyte assessment, we compared Violet predicted live birth rates (LBR) of patients <38 year old with known live birth rates of IVF patients for ages 38-40, 41-42 and 43+ using 2019 CARTR data (2018 IVF birth outcomes) (Graph 1).



Graph 1: CARTR IVF Live birth rates



Graph 2: KRFC predicted Live birth rates

RESULTS:

The mean (range) age, weight and serum AMH level measured within the last 12 months were 33.4 (25-37) years, 62.7 (42-98) kg, and 2.69 (0.08-7.95) ng/mL. The mean daily dose of Rekovelle and Menopur was 10.60 µg and 175.5 IU, and the mean total dose was 110.25 µg and 1645 IU, respectively. The mean (+/- SEM) number of dominant follicles (> 12 mm) at the end of stimulation was 11.1 (+/- 0.64). The mean (+/- SEM) number of retrieved, mature (MII) and frozen oocytes was 13.7 (+/- 0.94), 11.3 (+/- 0.86) and 11.2 (+/- 0.84), respectively. Mean (range) predicted LBR for 1 live birth at 46% (13-76%) correlated positively with the mean number of frozen oocytes (Graph 2), and was higher in all but 9 patients than LBR of IVF patients in 38-40 age group (27.6%), in all but 1 patient than in 41-42 age group (14.7%), and in all patients than in 43+ (6.9%) age group.

CONCLUSION:

The results of this retrospective study demonstrate that oocyte cryopreservation using a combination of follitropin delta and HP-hMG is a good "insurance policy" for patients under 38 years of age using Violet AI prediction tool in terms of higher live birth rates than those expected through IVF at an older age. The results of this study may help inform the design of a larger prospective randomized controlled trial comparing predicted live birth rates by Violet AI with mixed protocols using different follitropins. It would be also interesting to compare the predicted live birth rates from these cryopreservation cycles to an actual live birth rates utilizing frozen oocytes after 5 and 10 years.

#231

Elective oocyte preservation: An eight year single-centre experience.

Rahana Harjee, Jing Chen, Nadia Ouhibi, Sarah Edsall,
Jeff Caudle, Salah Abdelgadir, Gary Nakhuda

OLIVE fertility centre

INTRODUCTION

Over recent years, the interest in elective oocyte preservation (EOP) has increased. However, there are few reports that detail the outcomes of these cycles, with a paucity of information in regards to subsequent disposition and associated pregnancy outcomes. This observational study aimed to review an eight year experience with EOP at a single centre.

METHODS

A retrospective descriptive analysis of 655 egg freezing cycles between 2013 - 2021 at a private fertility centre was performed.

Table 1. Results of ovarian stimulation and oocyte maturation

Oocytes retrieved	12.7 (0-57, +/- 8.1)
M2 oocytes frozen	10.1 (0-43, +/- 6.8)

*results are displayed as mean (range +/- standard deviation)

RESULTS

Of the 655 egg freezing cycles, 574 cycles were completed in 483 patients for elective oocyte preservation. Most patients (414) underwent one cycle, 69 patients completed multiple cycles; 2(n=53), 3(n=12), 4 (n=2), 5 (n=2). The mean age was 36.6 years (19-46, +/- 3.6).

The oocyte retrieval and mature oocyte findings can be seen in Table 1. 51 patients (10.4%) have returned for disposition of embryos. The average time to disposition was 27 months (1-65, +/- 17) with an average age of 39.5 years (28-47, +/- 3.7). In patients who have not returned, the patients were significantly younger (38.3, +/- 4.0), $p<0.05$. The results of oocyte use are displayed in Table 2.

12 transfers occurred, with 9 achieving implantation (75%). 7 patients have an ongoing pregnancy or live birth, including one patient pregnant with her second child from 1 cycle of elective oocyte cryopreservation.

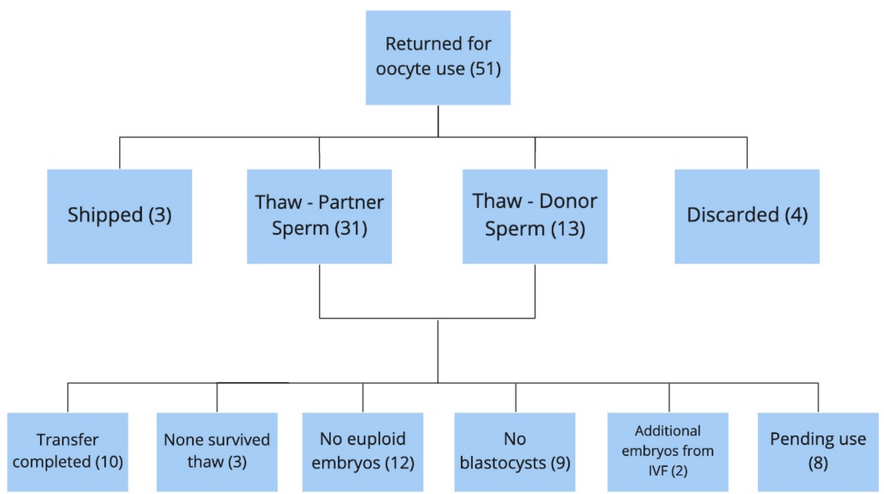


Figure 1. Oocyte disposition in patients who returned for use

Table 2. Results of oocyte use in returning patients

Oocytes thawed (per patient)	10.1 (2-22, +/- 5.4)
Oocyte survival rate (%)	64% (0-100, +/- 21.2)
Fertilization rate (%)	69.5% (0-100, +/- 27.5)
Age at retrieval in fertilized oocytes (years)	37.2 (25-44, +/- 3.3)
Blastulation rate (%)	34.5% (0-100, +/- 31.8)
Number of embryos per patient	1.8 (0-10, +/- 2.1)

*results are displayed as mean (range +/- standard deviation)

CONCLUSION

To date, this is the largest published experience with elective oocyte preservation in Canada, providing insights about the outcomes associated with oocyte cryopreservation. The results illustrate that EOP can be successful but cannot guarantee livebirth for all patients.