# CFAS 68TH
# ANNUAL MEETING
## Poster Presentation
## TV1 & TV2

The Canadian Fertility and Andrology Society

# Does Ovulation Trigger Choice in GnRH Antagonist IVF/ICSI Cycles Alter Oocyte Yield?

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## Introduction

While hCG has been the traditional method for achieving oocyte maturation (trigger) in IVF, it has been established that a GnRH agonist (GnRHa) trigger in GnRH antagonist IVF cycles decreases ovarian hyperstimulation syndrome (OHSS).[1] It is also well established that with fresh embryo transfer following GnRHa trigger alone, pregnancy rates are significantly reduced.[2,3] However, there exists a paucity of evidence surrounding laboratory outcomes of GnRHa trigger in GnRH antagonist IVF cycles compared to hCG trigger alone or GnRHa with hCG co-trigger for oocyte maturation. The purpose of this study is to determine if there are any differences in laboratory outcomes when these three methods of oocyte maturation are used.

## Methodology

- Retrospective cohort study
- Patients ≤38 years old with ten or more follicles ≥12 mm at PCRM from January 1, 2016 to December 31, 2020
- All patients must have undergone either their first or second GnRH antagonist IVF/ICSI cycles and were analyzed in three groups, patients triggered with:
  1. hCG alone (dose ≥5,000 IU) (n=714)
  2. GnRHa alone (n=198)
  3. GnRHa in combination with hCG (dose ≤2,500 IU) (co-trigger) (n=86)
- Primary outcome is number of oocytes retrieved per follicle
- Secondary outcomes:
  - Mature oocyte rate (MII/oocytes retrieved)
  - Fertilization rate
  - Blastulation rate
- Statistical analysis was performed with a zero-truncated poisson regression or logistic regression followed by calculation of rate ratios (RR) or Odds Ratios (OR) using 1200 bootstrap replicates and pairwise post-hoc tests where applicable.

## Results

Demographic information is depicted in table 1.

### Number of Oocytes Retrieved Per Follicle ≥12mm

- RR estimates suggest that there is a 7% greater number of oocytes received per follicle in GnRHa alone compared to hCG alone, while there is a 7% smaller number in co-trigger vs hCG alone (table 2)
- These relationships remained statistically significant when adjusted for age and gravida
- Predicted plot for 35 year old patient is depicted in figure 1

### Mature Oocyte Rate

- Table 2, there were no significant differences among the groups (p = 0.53)

### Fertilization Rates

- Table 2, hCG alone and co-trigger were statistically significantly different (p = 0.04)
- No difference between hCG alone and GnRHa alone (p = 0.97), or GnRHa alone and co-trigger (p = 0.053)

### Blastulation Rates

- Table 2, hCG alone and GnRHa alone were statistically significantly different (p = 0.02)
- No difference between hCG alone and co-trigger (p = 0.84), or GnRHa alone and co-trigger (p = 0.06)

| | Total | Group | | | |
|---|---|---|---|---|---|
| | | hCG only | Agonist only | Combined | P-value |
| | No. 998 | No. 714 | No. 198 | No. 86 | |
| **Age (years)** | | | | | |
| Mean (SD) | 33.4 (±3.1) | 33.7 (±3.0) | 33.0 (±3.2) | 32.5 (±3.2) | 0.0006 |
| **BMI** | | | | | |
| Mean (SD) | 24.7 (±5.4) | 24.5 (±5.1) | 25.0 (±6.1) | 25.6 (±6.2) | 0.12 |
| Missing | 12 (1.2%) | 11 (1.5%) | 1 (0.5%) | 0 (0%) | |
| **Gravida** | | | | | |
| 0 | 597 (59.8%) | 405 (56.7%) | 128 (64.6%) | 64 (74.4%) | 0.019 |
| 1 | 209 (20.9%) | 158 (22.1%) | 37 (18.7%) | 14 (16.3%) | |
| 2+ | 180 (18.0%) | 140 (19.6%) | 32 (16.2%) | 8 (9.3%) | |
| Missing | 12 (1.2%) | 11 (1.5%) | 1 (0.5%) | 0 (0.0%) | |
| **Parity** | | | | | |
| 0 | 808 (81.0%) | 572 (80.1%) | 162 (81.8%) | 74 (86.0%) | 0.68 |
| 1 | 165 (16.5%) | 122 (17.1%) | 31 (15.7%) | 12 (14.0%) | |
| 2+ | 13 (1.3%) | 9 (1.3%) | 4 (2.0%) | 0 (0.0%) | |
| Missing | 12 (1.2%) | 11 (1.5%) | 1 (0.5%) | 0 (0.0%) | |
| **AMH** | | | | | |
| Mean (SD) | 2.1 (±5.7) | 2.1 (±5.7) | 2.3 (±6.5) | 2.2 (±3.5) | 0.85 |
| Missing | 12 (1.2%) | 11 (1.5%) | 1 (0.5%) | 0 (0%) | |
| **FSH** | | | | | |
| Mean (SD) | 3.8 (±3.1) | 3.8 (±3.2) | 3.6 (±2.7) | 4.3 (±2.3) | 0.19 |
| Missing | 12 (1.2%) | 11 (1.5%) | 1 (0.5%) | 0 (0%) | |

*Table 1. Demographic information. AMH: Anti-Müllerian Hormone. BMI: Body Mass Index. FSH: Follicle-Stimulating Hormone.*

*Table 2. Primary and Secondary Outcomes. Rate ratios depicted by * and Odds ratios depicted by **, both are depicted with their 95% Confidence Intervals (CI).*

| Outcome | hCG alone | GnRHa alone RR* or OR** (95% CI) | Co-trigger RR* or OR** (95% CI) |
|---|---|---|---|
| Number of Oocytes Retrieved Per Follicle ≥12mm | Reference | 1.07 (1.01 to 1.15)* | 0.93 (0.87 to 0.99)* |
| Mature Oocyte Rate | Reference | 0.96 (0.82 to 1.12)** | 1.03 (0.85 to 1.27)** |
| Fertilization Rates | Reference | 1.01 (0.84 to 1.2)** | 0.86 (0.68 to 1.09)** |
| Blastulation Rates | Reference | 1.13 (1 to 1.27)** | 0.97 (0.78 to 1.15)** |

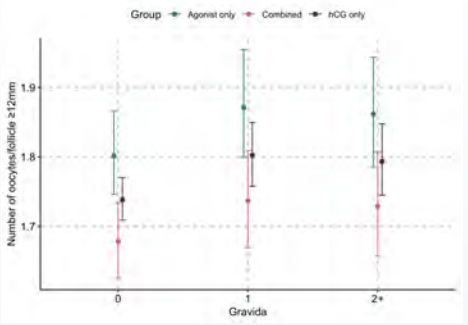


*Figure 1. Predicted Plot of Number of Oocytes Retrieved Per Follicle ≥12mm in a 35 year old patient when gravida is 0, 1, or ≥2*

## Conclusion

Amongst individuals with normal or high ovarian response, GnRHa trigger displayed a possible modest increase in oocytes retrieved per follicle when compared to hCG trigger alone. Oocyte maturity does not appear to be influenced by method of oocyte maturation trigger. GnRHa trigger is possibly associated with slightly improved blastulation rates when compared to hCG. While this is a retrospective study with inherent selection bias, the similar laboratory outcomes with the different trigger types provide reassurance that laboratory outcomes are unaffected by the type of medication used for oocyte maturation.

## Bibliography

1. Alexopoulou E, Stormlund S, Løssl K, Prætorius L, Sopa N, Bogstad JW et al. Embryo Morphokinetics and Blastocyst Development After GnRH Agonist versus hCG Triggering in Normo-ovulatory Women: a Secondary Analysis of a Multicenter Randomized Controlled Trial. Reproductive Sciences 2021
2. Beck-Fruchter R, Baram S, Geslevich Y, Weiss A. Gonadotropin Releasing Hormone Agonist Final Oocyte Maturation and Human Chorionic Gonadotropin as Exclusive Luteal Support in Normal Responders. Gynecologic and Obstetric Investigation 2019;84:27-34.
3. Castillo JC, Haahr T, Martínez-Moya M, Humaidan P. Gonadotropin-releasing hormone agonist for ovulation trigger–OHSS prevention and use of modified luteal phase support for fresh embryo transfer. Upsala Journal of Medical Sciences 2020;125:131-7.

# BPA affects DNA methyltransferases and related methylation genes in a miR-21 independent manner in bovine granulosa cells

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## INTRODUCTION

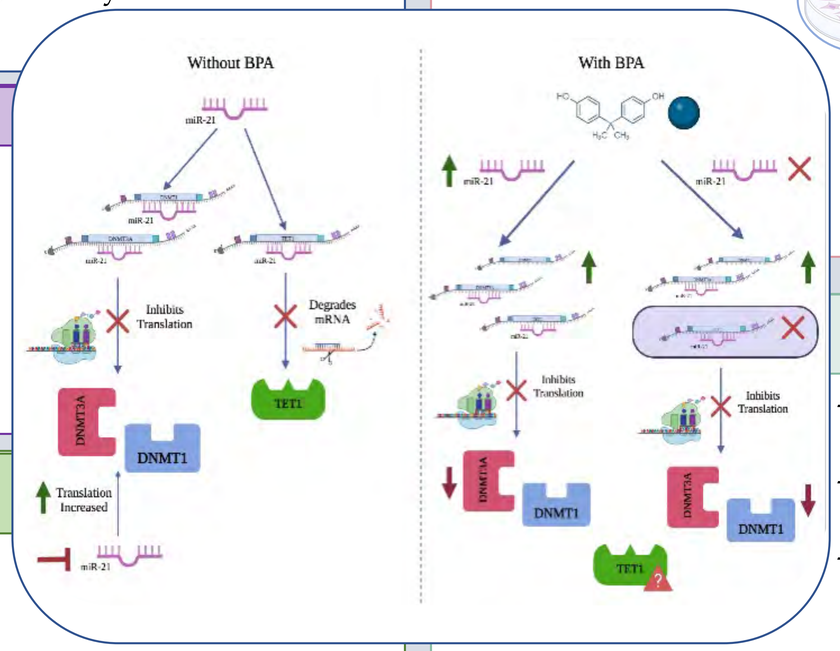
- Bisphenol A (BPA) is a common endocrine disruptor linked to poor fertility in humans and farm animals [1].
- The epigenetic mechanisms of action for BPA are currently being recognized as strong contributors to poor phenotypes [1].
- microRNAs and DNA methylation are two crucial epigenetic mechanisms during early development that are susceptible to BPA damage [1].
- miR-21 can regulate methylation by targeting DNMTs, thereby influencing overall fertility [2].

## HYPOTHESIS & OBJECTIVES

***BPA-induced increase in miR-21 expression negatively impacts downstream DNA Methylation in Granulosa Cells***

Objectives:

1. Quantify DNMTs and related methylation mRNA transcripts and proteins in granulosa cells treated with a miR-21 inhibitor and BPA
2. Characterize BPA effects on methylation patterns using global methylation analysis




## MATERIALS & METHODS

- Granulosa Cells cultured and transfected with miR-21 inhibitor
- Knocked-down cells also treated with BPA (0.05 mg/mL)
- Cells collected and frozen for RNA and protein extraction

Cell Culture & Transfections

qPCR

- DNMT1, 3A, 3B, TET1, and TDG mRNA quantified by qPCR

Global Methylation

- Global Methylation patterns will be assessed using Reduced Representation Bisulfite Sequencing.

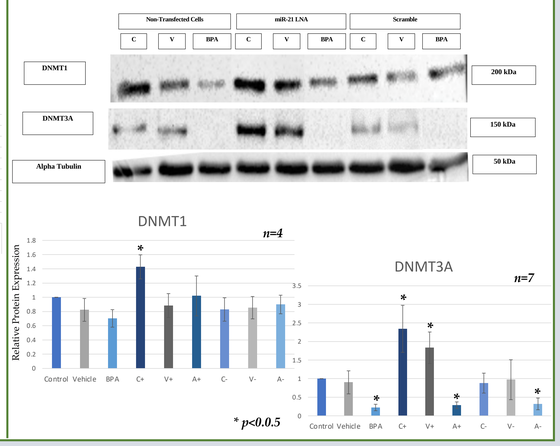
- DNMT1 & 3A proteins quantified by western blotting

Western Blotting

## RESULTS

**mRNA**


**Protein**




## CONCLUSIONS

- BPA increased all methylation transcripts.
- TET1 may be a downstream target of miR-21 regulation.
- DNMT1 and DNMT3A proteins significantly increased in miR-21 knocked-down cells, suggesting DNMT1 and DNMT3 are direct or indirect targets of miR-21 signaling.
- BPA decreased DNMT1 and DNMT3A protein independently of miR-21 inhibition, suggesting BPA utilizes an alternative pathway to decrease DNMTs.
- BPA-induced changes in methylation genes might disrupt global DNA methylation.

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## REFERENCES

1. Sabry, R.; Williams, M.; Werry, N.; LaMarre, J.; Favetta, L.A. BPA Decreases PDCD4 in Bovine Granulosa Cells Independently of miR-21 Inhibition. *Int. J. Mol. Sci.* **2022**, *23*, 8276. https://doi.org/10.3390/ijms23158276
2. Pan, W., Zhu, S., Yuan, M., Cui, H., Wang, L., Luo, X., Li, J., Zhou, H., Tang, Y., & Shen, N. (2010). MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4+ T cells by directly and indirectly targeting DNA methyltransferase 1. *Journal of immunology (Baltimore, Md. : 1950)*, *184*(12), 6773–6781. https://doi.org/10.4049/jimmunol.0904060

# Clinical Outcomes Following Endometrial Receptivity Analysis Testing: A Single Centre Experience

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## INTRODUCTION

The Endometrial Receptivity Analysis (ERA) has been proposed as a method to improve implantation rates by defining a "personalized embryo transfer" (PET) based on the endometrial gene expression profile. However, there is a paucity of data to support its use in the general IVF population.

## METHODS

A retrospective chart review of a subset of patients who received ERA testing at a private fertility clinic between January 1, 2014 and present (n=683). Patients with a receptive result received a standard protocol for endometrial synchronization prior to transfer; alternatively, the duration of progesterone prior to transfer was adjusted if indicated by ERA results. Results were analyzed for patients with documented pregnancy results, with Pearson's chi-square statistic calculated to compare receptive and PET outcomes.

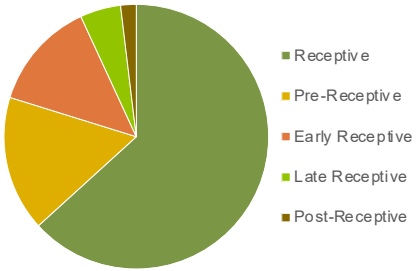


*Figure 1. Pie chart depicting 746 ERA test results*

## RESULTS

A total of 746 ERA tests were performed, for an average of 1.09 ERA tests per patient. The most common reason for repeat ERA testing was time passed since previous result affecting 26 patients resulting in 57 ERA tests, while repeat testing due to inadequate sample only occurred in 7 patients resulting in 14 ERA tests. The most common result was receptive (n=432), followed by pre-receptive (n=113), early receptive (n=91), late receptive (n=34), and post-receptive (n=13) (figure 1).

All outcomes are depicted in table 1.

**All patients**
- Live birth rate (LBR) in patients with a receptive result (n=313), was 41.7% compared to 40.3% for PET (n=196), (p=0.75)

**Euploid Embryos Transfers**
- Patients with a receptive result (n=200) had a LBR of 48.1% compared to 52.6% for PET (n=113), (p=0.44)

**Donor Egg Created Embryos**
- Patients with a receptive result (n=26) had a LBR of 27.5% compared to 34.1% for PET (n=31), (p=0.59)

**ERA Testing Prior to First Embryo Transfer**
- Patients with a receptive result (n=120) had a LBR of 54.6% compared to 44.1% for PET (n=71), (p=0.16)

**History of At Least One Implantation Failure**
- LBR was 39.8% in the receptive group (n=186) compared to 37.3% for those with PET (n=120), (p=0.66)

| Group | LBR in patients with receptive ERA result (n) | LBR in patients with PET (n) | p value |
|---|---|---|---|
| **All patients** | 41.7% (n=313) | 40.3% (n=196) | 0.75 |
| **Euploid Embryo Transfers** | 48.1% (n=200) | 52.6% (n=113) | 0.44 |
| **Donor Egg Created Embryos** | 27.5% (n=26) | 34.1% (n=31) | 0.59 |
| **ERA Testing Prior to First Embryo Transfer** | 54.6% (n=120) | 44.1% (n=71) | 0.16 |
| **History of At Least One Implantation Failure** | 39.8% (n=186) | 37.3% (n=120) | 0.66 |

*Table 1. Live Birth Rate in Patients with Receptive ERA Results Compared to Personalized Embryo Transfer. ERA: Endometrial Receptivity Analysis. LBR: Live Birth Rate. PET: Personalized Embryo Transfer.*

## CONCLUSION

- Outcomes appeared similar for patients with a receptive result and those who had embryo transfer time adjusted by ERA
- Although not statistically significant, patients undergoing ERA testing prior to first embryo transfer appeared to have a lower LBR after the protocol was adjusted for PET compared to those who were receptive after ERA and received conventional endometrial preparation
- Further analysis is required to determine if certain subgroups derive greater benefit
- Our results do not support the routine use of the ERA in the general IVF population

# CAN ARTIFICIAL INTELLIGENCE (AI) BE USED TO PREDICT EUPLOID EMBRYOS IN AN IVF SETTING?

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## INTRODUCTION

The use of morphokinetic analysis via time-lapse incubators enables clinical embryologists to observe the biological potential of embryo development in great detail. Recently emerging AI technology promised to allow embryologists to choose embryos with higher implantation potential and reduce time to pregnancy. The aim is to have a reliable, accurate, cost-effective method of determining the embryo's ploidy status and to select that is most likely to result in a healthy live birth. Objective: To investigate whether an AI system can be used as a prediction tool to select euploid embryos

## MATERIALS & METHODS

Our analysis is a retrospective observational analysis performed at a single centre for patients who underwent IVF with PGT-A treatment (n=122 cycles) between January and December 2021. The cohort for analysis includes 850 embryos that reached blastocyst stage. Women's mean age was 36.9±4.

The blastocysts were cultured in time-lapse incubators (Embryoscope®) and subsequently underwent PGT-A testing. IDAscore®® (Vitrolife) was applied and the two highest scored embryos selected by AI were compared against their PGT-A results. The participants in this study were couples who underwent IVF cycles using PGT-A with at least one euploid embryo reported. Two groups were created based on the IDAscore® (Vitrolife): group A had the embryos with the highest score, and group B had the second highest. These two groups were cross-checked with the PGT-A result report to confirm ploidy status. Statistical analysis using chi-square test was used with statistical significance to be confirmed at $p \leq 0.05$.

## RESULTS

In total, 850 blastocysts were subjected to trophectoderm biopsy, when 5-7 cells were taken, and the embryos were then vitrified. Group A had (86/121) euploid embryos, and the mean score reading for IDAscore® was 9±0.56. Group B had (59/121) euploid embryos with mean reading of 8.6±0.96. The results showed a statistically significant difference between the two groups, p= 0.0398.

The data were stratified based on women's age; Group A was for women <38 who had 74% (40/54) euploid embryos were given 9.10±0.56 by IDAscore®, Group B had 54% (29/54) euploid embryos scored 8.9±0.96. Women >38 for group A, 69% (47/68) euploid embryos scored 9±0.57. Group B had 46% (31/68) euploid embryos were scored 8.7±0.96. Statistical analysis indicated a significant difference between both groups across all ages.

## CONCLUSION

IDAscore® can give some indication of ploidy for the highest scored embryos and thus can assist embryologists in choosing embryos for transfer, thereby reducing time to pregnancy. However, AI by itself still cannot predict ploidy and thus when clinical indication for PGT-A is evident, biopsy of embryos and consequent analysis is necessary for such patient groups.

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London Women's Clinic

**Table 1. IDAscore®s and ploidy (all ages)**

| | Group A (all ages) | Group B (all ages) |
|---|---|---|
| Number of embryos | 121 | 121 |
| Number of euploid | 86 | 59 |
| Euploid percentage | 71% | 49% |
| Mean score | 9±0.56 | 8.6±0.96 |
| $p \leq 0.05$ | p= 0.0398 | |

**Table 2. IDAscore®s and ploidy (by age)**

| | Group A (<38) | Group B (<38) | Group A (>38) | Group B (>38) |
|---|---|---|---|---|
| Number of embryos | 54 | 54 | 68 | 68 |
| Number of euploid | 40 | 29 | 47 | 31 |
| Euploid percentage | 74% | 54% | 69% | 46% |
| Mean score | 9.10±0.56 | 8.9±0.96 | 9±0.57 | 8.7±0.96 |
| $p \leq 0.05$ | p= 0.0275 | | p=0.00425 | |

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Please contact balsam.alhashimi@londonwomensclinic.com if further information is required. With many thanks to all LWC staff past and present.

# Is it worth it trying controlled ovarian stimulation and Insemination after failing IVF? A retrospective study

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## OBJECTIVE

To evaluate the clinical pregnancy rate after COH IUI for patients who previously did not have a live birth following an IVF cycle.

## Materials and Methods

- Retrospective evaluation of data of patients treated at an academic fertility center between October 2008 and April 2018.
- Patients had a previous history of one or more IVF cycles that did not result in a live birth and subsequently underwent COH IUI with gonadotropins, letrozole or unstimulated.
- All subjects had at least one patent fallopian tube, did not have severe male factor infertility (less than 2 mil TMSC) and had a standard indication for IVF.
- Subjects received free IVF and when the government coverage for IVF ended, they elected to continue care with COH IUI which remained covered.
- Data is presented as mean ± SD. Chi squared tests or independent sample t tests were performed and multivariate logistic regression was used to model predictors of clinical pregnancy with COH IUI after failed IVF. Clinical pregnancy was defined as a pregnancy that has been confirmed by both high levels of human chorionic gonadotropin (HCG) and ultrasound visualization of an intrauterine gestational sac with a positive fetal cardiac activity in the first trimester. A failed IVF did not result in a live birth.

## Results

550 subjects who failed 1-3 fresh IVF cycles and any resultant embryo transfers subsequently underwent a total of 991 COH IUI cycles. The clinical pregnancy rate after the first IUI cycle was 5.81% (N=32, 3 multiple gestation). The cumulative clinical pregnancy rate for all IUI cycles was 9.45% per patient (N=52/550 pregnancies/patients, 3 multiples) and the clinical pregnancy rate per cycle was 5.24% (N=52/991 pregnancies/cycles)

The only discriminator using t-tests of clinical pregnancy was younger female age at treatment (36.25±3.73 vs. 37.65±4.3 years), P=0.025. When using multivariate logistic regression to find predictors of clinical pregnancy with IUI after failing IVF while controlling for confounding effects, none of the factors modeled were significant including: female age, male age, infertility diagnosis, number of previous pregnancies, number of failed IVF transfers, total motile sperm count pre and post processing, total FSH stimulation dose, and maximum endometrial thickness (p>0.05 all).

| | Number of Subjects | Rate of clinical pregnancy per patient | Rate of clinical pregnancy per cycle |
|---|---|---|---|
| Subjects that underwent one IUI cycle | 550 | 5.81% (N=32) | 5.81% (N=32) |
| Subjects that underwent 1-3 IUI cycles (total cycles 991) | 550 | 9.45% (N=52) | 5.24% (N=52) |

## Results

| | No clinical pregnancy (N =498) | Clinical pregnancy (N = 52) | P value | 95% CI of the difference | |
|---|---|---|---|---|---|
| | | | | Lower | upper |
| Gravidity | 1.27+/-1.48 | 1.34+/-1.23 | 0.746 | -0.48 | 0.34 |
| Parity | 0.355+/-0.61 | 0.36+/-0.56 | 0.911 | -0.18 | 0.16 |
| number of previous failed IVF | 2.11+/-1.62 | 2.15+/-1.37 | 0.852 | -0.50 | 0.41 |
| number of previous biochemical of pregnancies only | 0.18+/-0.48 | 0.21+/-0.53 | 0.677 | -0.17 | 0.10 |
| number of previous miscarriages and ectopic pregnancies | 0.20+/-0.49 | 0.23+/-0.58 | 0.766 | -0.16 | 0.12 |
| number of previous still births from IVF | 0.004+/-0.06 | 0.019+/-0.13 | 0.437 | -0.054 | 0.023 |
| number of previous live births from IVF | 0.16+/-0.38 | 0.11+/-0.32 | 0.376 | -0.05 | 0.15 |
| Number of IVF cycles before IUI | 2.67+/-1.94 | 2.75+/-1.97 | 0.791 | -0.63 | 0.48 |
| Age at Treatment (years) | 37.65+/-4.33 | 36.25+/-3.73 | 0.025 | 0.17 | 2.62 |
| Partner age at treatment (years) | 39.57+/-6.23 | 39.71+/-5.96 | 0.969 | -2.29 | 2.20 |
| Total motile sperm count pre-processing (millions) | 28.09+/-44.59 | 44.2+/-59.87 | 0.102 | -35.52 | 3.31 |
| Total motile sperm count post-processing (millions) | 18.55+/-17.89 | 20.58+/-18.12 | 0.493 | -7.84 | 3.31 |
| maximum endometrial thickness (mm) | 8.16+/-2.43 | 8.19+/-2.05 | 0.92 | -0.72 | 0.65 |
| Number of follicles >14mm | 1.56+/-1.29 | 1.57+/-0.84 | 0.97 | -0.37 | 0.36 |

## Comment

Our study population had unfavorable fertility prognosis given that they had already failed at least one IVF cycle. It was not surprising that their clinical pregnancy rate from COH IUI was lower than that of COH IUI for the general population seeking requiring fertility treatment.

## Conclusion

Clinical pregnancies with COH IUI after failed IVF occur but are not common. This could be considered, with proper counselling. Cost effectiveness will be modulated by price of COH IUI and medication used and should be studied in a location specific manner.

## Impact Statement

Patients should be counselled regarding the low clinical pregnancy rate with COH IUI if they have previously failed IVF.

# Scalable automation of PGT in a clinical setting

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## INTRODUCTION

Growing demand from patients and technical advancements in the ART Genetics field have led to steady growth and expansion of the portfolios and throughput of samples processed at the CReATe Reproductive Genetics Department. Under the PGT-A portfolio, we have seen an increase of 235%, 270%, and 365% in the number of patients, cycles, and samples. We previously demonstrated how we developed a centralized lab information management (LIMS) to create tailored workflows and allow streamlined processes and better data management. Here, we demonstrate that by automating most parts of the PGT-A workflow and eliminating manual processing, we are able to expand our throughput 16-fold (24 samples vs. 384 samples at the same time) while maintaining QC parameters eliminating manual pipetting, and significantly reducing cost and turn-around-time.

## MATERIALS AND METHODS

Library-prep protocol for low pass whole genome sequencing that utilizes Illumina kits was modified to process up to 384 samples simultaneously and sequence the libraries on the NextSeq 550 (Illumina). Optimized and validated reduced reaction volumes and workflow that we previously presented were incorporated in Illumina's original protocol.

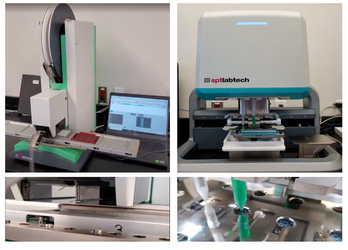
We automated DNA dilution, tagmentation, amplification, cleanup, and normalization using MosquitoHV®, while reagent distribution to the source plates, and adding beads to samples are performed on the Dragonfly® (SPT Labtech). Performance of manual vs. automated processing was evaluated using multiple QC parameters, including in 3 parallel runs: DNA concentration of the library, Q score, number of reads, DLR (noise), and cluster density.

## SUMMARY

An automation process was developed to allow high-throughput processing of PGT-A samples. This new process was shown to be highly efficient in terms of QC, turn-around time, and cost. Combined with our unique LIMS, we can successfully extend this workflow to process 192, 288 and 384 samples. Further adaptations to PGT-M, Product of Conception testing, and Thrombophilia genetic panel testing are being developed.

## RESULTS

**Mosquito® HV** **Dragonfly®**



SCAN ME SCAN ME

**Fig. 1: Liquid handlers used in the reproductive genetics department at CReATe Fertility Centre.**

Mosquito HV and Dragonfly are two liquid handlers that are being used for automating the Nextera NGS Library preparation process.
The Mosquito HV is a highly accurate and precise multi-channel positive displacement pipetting (handling volumes of 500 nL to 5 µL). It can be programmed to run a wide range of protocols with 96 and 384-well plates
The Dragonfly is low volume dispenser capable of dispensing volumes of 200nL to 4ml.

| Step | | Liquid Handler |
|---|---|---|
| Dilution | Prepare 2 water dilution plates | Dragonfly |
| | Dilute samples to 2ng/ul | Mosquito 9mm |
| | Dilute samples to 0.2ng/ul | Mosquito 4.5mm |
| Tagmentation + Amplification | Tagmentation + Amplification | Mosquito 4.5mm |
| | Prepare 98ul Qubit plates | Dragonfly |
| | Qubit Post-Amplification - 99ul plate | Mosquito 4.5mm |
| Cleanup with XP beads | Add XP beads to amplification plate | Dragonfly |
| | Cleanup with XP beads | Mosquito 4.5mm |
| | Add RSB clean libraries | Dragonfly |
| | Qubit Cleanup - 98ul plate | Mosquito 4.5mm |
| Normalization | Add Normalization beads to plate | Dragonfly |
| | Add LNW1 to samples (wash 1) | Dragonfly |
| | Remove LNW1 to waste plate (wash 1) | Mosquito 4.5mm |
| | Add LNW1 to samples (wash 2) | Dragonfly |
| | Remove LNW1 to waste plate (wash 2) | Mosquito 4.5mm |
| | Add NAOH to samples | Dragonfly |
| | Add LNS1 to new pool plate | Dragonfly |
| POOL | Transfer denatured libraries to the pool plate | Mosquito 4.5mm |
| | Pool all libraries in 1 column | Mosquito 4.5mm |
| | Pool column to 1.5ml tube | Manual |

**Table 1: Utilizing Liquid handlers for Nextera Library preparation – 192 samples.**
All the library preparation steps are being performed using both liquid handlers. This allows us to almost completely eliminate manual pipetting. The high accuracy and automation allows us to miniaturize the reaction volumes to ¼ of the original volumes indicated in Illumina's original protocol and to process 96-384 samples with the same basic steps.

**Key QC parameters are within acceptable range values by Illumina**

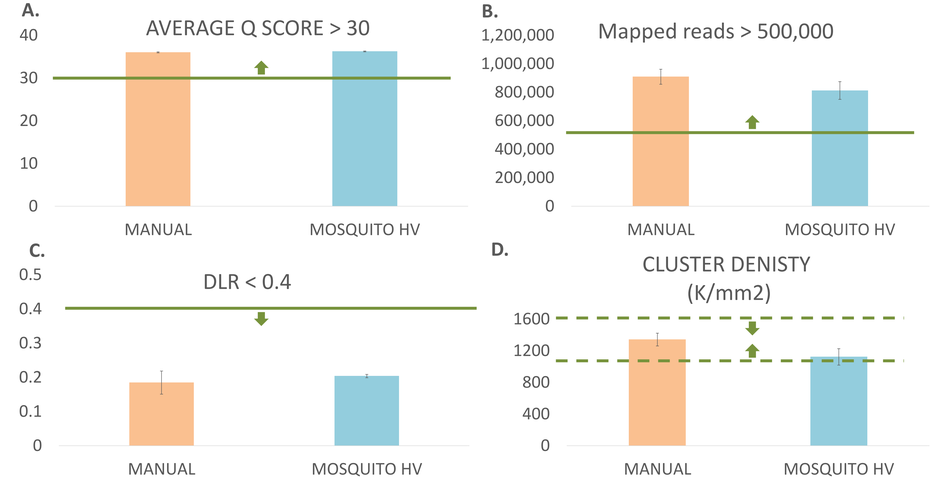



**Fig. 2: Key QC parameters.** QC values for manual vs automated, respectively were as follows: A. Average Q score = 36.0 vs. 36.2, (acceptable threshold = 30). B. Average mapped reads = 909,042.9 vs. 812,308.04 (acceptable threshold=500,000). C. Average DLR = 0.18 vs 0.20. Acceptable threshold <0.4. D. Average cluster density (K/mm2) = 1,342.0 vs. 1,122.6 (acceptable threshold =1,100-1,600). Green lines represent Illumina's thresholds for acceptable values.

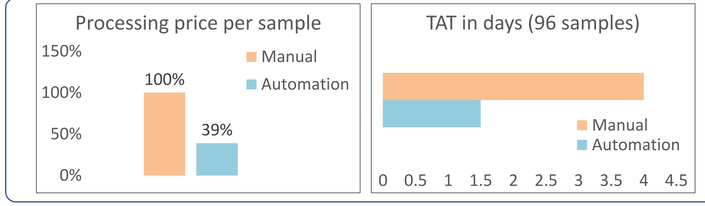



**Fig. 3: Processing parameters.** Cost per sample was reduced by 61%, turn-around-time for 96 samples was reduced to 1.5 days, and tech hands-on time was almost completely eliminated.

## ACKNOWLEDGEMENTS

The authors would like to thank the members of the Reproductive Genetics department at CReATe, and to the team at SPT Labtech for all their support.

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# A comparison between density gradient and straight wash methods in processing donor sperm for intrauterine insemination

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## BACKGROUND

Intrauterine insemination (IUI) is a well-established first line of treatment for sub-fertile patients. For same-sex female couples and single female patients who may not necessarily have fertility issues, IUI using donor sperm can be a cost-effective treatment to achieve pregnancy. The goal of our study is to compare the effectiveness of two methods in processing donor sperm samples for insemination.

## MATERIALS AND METHODS

A retrospective quality assurance study reviewed 258 IUI cycles with donor sperm samples from January 2020 to December 2021 at a Canadian fertility center. Donor sperm samples were processed with 40/80% density gradient for 132 cycles in 2020. For the 126 cycles in 2021, donor sperm samples were simply washed with buffer and then concentrated before insemination (straight wash). A comparison was drawn in Table 1 by examining key laboratory parameters from the insemination samples and pregnancy rates between the two methods.

## TABLE

| Year | 2020 | 2021 |
|---|---|---|
| Process Method | Density Gradient | Straight Wash |
| Number of cycles | 132 | 126 |
| Average age | 35.9 | 35.2 |
| Average total motile sperm at insemination (million) | 6.5 | 8.6 |
| Average motility at insemination (%) | 65 | 42 |
| Positive Beta Rate (%) | 19.7% | 17.5% |
| Clinical Pregnancy rate (%) | 16.7% | 15.1% |
| Ongoing Pregnancy rate (%) | 15.2% | 12.7% |

*Table 1. A Comparison of Laboratory Parameters and Clinical Outcomes between Two Donor Sperm Preparation Methods.*

## FIGURES

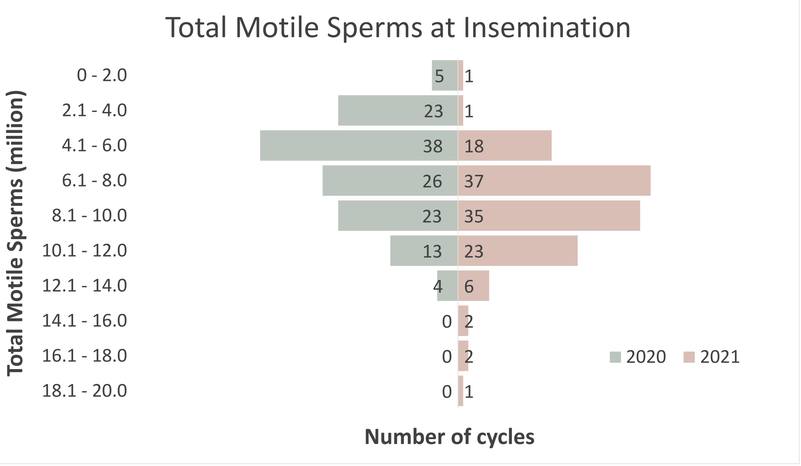


*Figure 1. Distribution of total motile sperm count per IUI cycle*

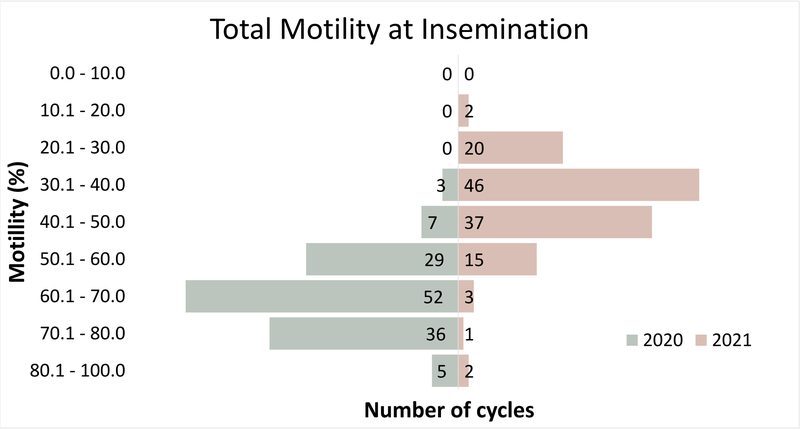


*Figure 2. Distribution of sperm motility of insemination samples*

## RESULTS

Both 2020 and 2021 groups of IUI cycles with donor sperm have similar cycle numbers (132 vs. 126) and average ages of the patients (35.9 vs. 35.2.) When examining the processed samples, the patients in 2021 received slightly more motile sperms back at insemination than the patients in 2020 (8.6 million vs. 6.5 million, $p$=0.54), but the insemination samples in 2021 had significantly worse motility (42% vs. 65%, $p$<0.01).

In terms of pregnancy rates (PR), both groups have comparable positive beta rates (19.7% vs. 17.5%, $p$=0.65) and clinical PR (16.7% vs. 15.1%, $p$=0.73). While the 2020 group achieved 2.5% higher in ongoing PR compared to the 2021 group, the difference is not statistically significant ($p$=0.57).

## CONCLUSION

By switching from the density gradient to straight wash, we streamlined the donor sperm preparation for IUI. The benefits of straight wash include shortened procedure time, reduced reagents/supplies usage, and a shortened learning curve for Andrologists. We were able to accomplish all of these without compromising pregnancy rates. While the motility was lower after the straight wash process, the total motile counts (TMC) was maintained at a similar level to the counts at thawing. Overall, these results have supported the decision to modify our donor sperm preparation for IUI.

# Simultaneous measurement of sperm oocyte activating factors, PLCζ and PAWP, and its implication in diagnosis of male infertility

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## INTRODUCTION

● At present, both PLCζ and PAWP are the main candidates as sperm oocyte activated factors (SOAFs) that are capable of activating oocytes by triggering the release of intracellular calcium during fertilization.

● Insufficient altered expression of PLCζ and PAWP may be related to male infertility, failed oocyte activation and poor embryo development. However, these two factors have been studied separately and their simultaneous expression levels have not been evaluated in the sperm of infertile men.

● **This study aimed to design a flow cytometry-based bioassay to simultaneously determine PLCζ and PAWP protein expression levels in the sperm of infertile patients and their potential relationship with sperm parameters and fertilization outcomes.**

## MATERIALS & METHODS

Independent Research Board (Veritas) approval was obtained.

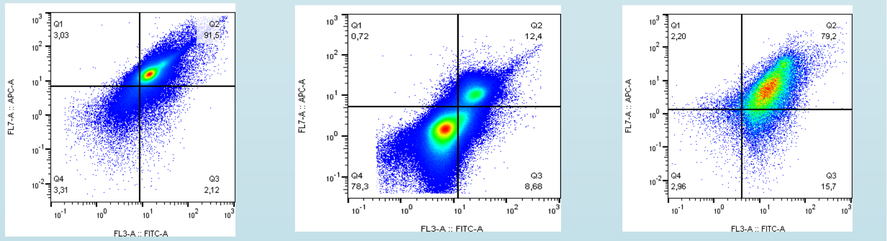
Excess sperm were collected after ICSI reproductive use.
All samples were prepared by density gradient centrifugation, fixed and incubated with antibodies specific for PLCζ and PAWP proteins.

A multicolor flow cytometry protocol was developed to simultaneously determine expression levels of PLCζ and PAWP protein in the sperm cells. At least 10,000 events were examined by flow cytometry and data was analysed using FlowJo™ software.

Sperm DNA fragmentation index (DFI) was assessed for all samples using MACSQuant 10 flow cytometer.
The Statistical Package for Social Sciences (SPSS) version 28 and Microsoft Excel 365 ProPlus spreadsheet were used for data entry and analysis.

## RESULTS

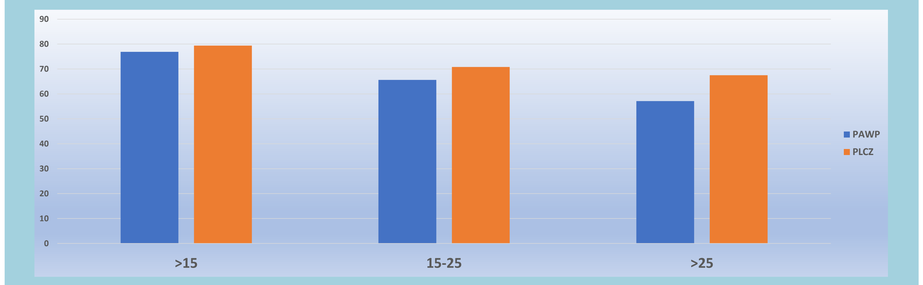
With flow cytometry assay we were able to successfully quantify PLCζ and PAWP expression levels in 139 samples. Mean expression levels of PLCζ and PAWP in sperm samples were 76.93±1.93% and 73.57±2.15%, respectively.
Approximately 67% of the sperm population contained both PLCζ and PAWP, while 10% lacked both factors.
PAWP and PLCζ levels were positively correlated with sperm motility ($P$=0.003) and negatively correlated with abnormal morphology ($P$=0.029).
Sperm PAWP levels were significantly lower in patients with a sperm concentration less than 20 million/ml ($P$ =0.001).
Levels of PAWP and PLCζ were not correlated with ICSI fertilization rate, DFI index or blastocyst development in this cohort of infertile men.




**PAWP expression evaluated by APC conjugated Ab (FL7-A: Y axis), PLCζ expression shown by FITC conjugated Ab (FL3-A: X axis). On the left: high expression of PAWP and PLCζ in the patient with DFI lower than 10 and good blastocyst development (#44), in the middle: low expression of PAWP and PLCζ in patient with DFI over 15% and 0 blastocyst (#19), on the right high expression in Globozoospermia patient (#42).**

| Sample | Expression PAWP | Expression PLCz | Age Male | DFI | ICSI Fert | Blast Development |
|---|---|---|---|---|---|---|
| # 44 | 94.5% | 93.6% | 39y | 9 | 2/2 Eggs 100% | 2 Blasts: Good & Average |
| # 19 | 12.5% | 21.1% | 47y | 20 | 3/3 Eggs 100% | 0 Blasts |
| # 42 | 79.2% | 95.0% | 36y | 22.7 | 2/4 Eggs 50% | 1 Blast: Poor |

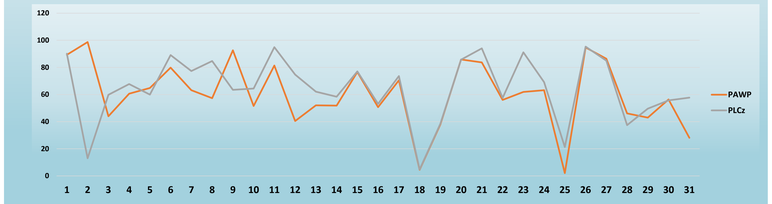
**Patient # 44; # 19 & # 42: Advanced Maternal Age/ MF , Patient # 42 Globozoospermia (High % of PAWP & PLCz)**




**PAWP and PLCZ expression in different DFI groups - Average: No correlation**

## RESULTS


**PAWP and PLCz expression in samples with motility over 60%**




**PAWP and PLCZ expression in motility lower than 40%**

## CONCLUSION

● We established a flow cytometry-based assay to simultaneously determine the expression levels of PLCζ and PAWP protein in sperm.
● This study demonstrated extremely high variability of PLCζ/PAWP within individual spermatozoa which may reflect their different capability for fertilization and oocyte activation.
● Expression of sperm factors and normal sperm parameters are highly dependent on proper spermatogenesis and spermiogenesis. We therefore hypothesize that lower levels of PLCζ and PAWP could be potential biomarkers of abnormal underlying mechanisms that lead to lower sperm concentration, lower motility and inability to trigger a strong $Ca^{2+}$ release during fertilization.
● The genetic reasons for the expression variability of PLCζ and PAWP are largely unknown. Further studies of sperm genetic variants (mutations) in exons of both factors may extend the spectrum of diagnostic markers for certain forms of male infertility.

## ACKNOWLEDGEMENTS

Special thanks to all the Clinical Embryology, Andrology and Biobank Staff for their continued assistance in data collection.

# Microfluidic Sperm Sorting Device versus Density Gradient Centrifugation: A Comparison of Laboratory and Clinical Outcomes for IVF Cycles Using Donor Sperm

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## BACKGROUND

The purpose of sperm selection techniques is to isolate the spermatozoa most capable of fertilization. Microfluidics offers an alternative to density gradient centrifugation to efficiently yield a high proportion of sperm with rapid motility and normal sperm morphology. The purpose of this study was to investigate whether frozen donor sperm preparation for conventional IVF insemination using Zymot™ Sperm Separation Device (SSD) would improve laboratory and/or clinical outcomes over conventional density gradient centrifugation (DGC).

## MATERIALS AND METHODS

We performed a quality assurance study on a retrospective cohort of 51 donor sperm in vitro fertilization (IVF) cycles from January 2021 to March 2022. Oocytes were fertilized using standard IVF insemination with 100,000 motile sperm per well containing up to 8 cumulus-oocyte complexes. Cycles from January to September 2021 were prepared with DGC, while cycles from October 2021 to March 2022 were with SSD. Embryos cultured up to day 6 were scored using the Gardner Blastocyst Scoring System[1]. Utilizable embryos were defined as blastocysts that were transferred or cryopreserved relative to the number of fertilized embryos. Post-processing motility, normal fertilization, utilizable embryo, and clinical pregnancy rates were observed between the two groups. Continuous variables were analyzed with the Mann-Whitney test and comparisons between proportions with the Fisher's Exact test.

## RESULTS

31 cycles had frozen donor sperm processed using DGC and 20 using SSD. SSD had a significantly higher post-processing motility (89%) compared to DGC (71%) ($p< 0.00001$). SSD preparation also had a higher normal fertilization rate than DGC (84% versus 72% $p< 0.0001$) and utilizable embryo rate (55% versus 46%, $p< 0.04$). There was no statistically significant difference in clinical pregnancy rates between DGC and SSD (47% and 50%, $p= 1$).

## TABLE 1

| | Density Gradient Centrifugation | Sperm Separation Device | P value |
|---|---|---|---|
| Number of Cycles | 31 | 20 | |
| Average Patient Age | 37.4 | 35.5 | 0.074 |
| Average Post-processing Motility | 71% | 89% | <0.00001 |
| Number of Oocytes Inseminated | 482 | 285 | |
| Percentage of MII Oocytes | 90% (435/482) | 80% (228/285) | <0.0001 |
| Normal Fertilization Rate | 72% (313/435) | 84% (192/228) | <0.0001 |
| Utilizable Embryos Rate | 46% (143/313) | 55% (107/192) | <0.04 |
| Number of Cycles with a Fresh ET | 17 | 14 | |
| Average Number of Embryos Transferred | 1.3 | 1.2 | 0.787 |
| Clinical Pregnancy Rate | 47% (8/17) | 50% (7/14) | 1 |

*Table 1. Comparison between Donor Sperm IVF Cycles prepared with DGC and SSD*

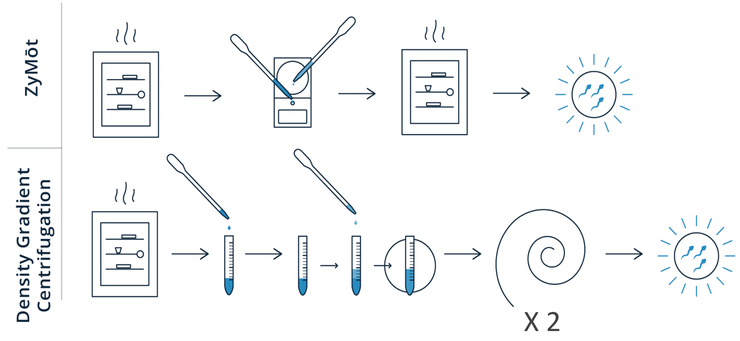


*Figure 1. Step-by-step comparison between DGC and SSD*

## CONCLUSION

In this analysis of 51 donor sperm IVF cycles, post-processing motility, rate of normal fertilization, proportion of utilizable blastocysts was higher using sperm separation device compared to density gradient centrifugation. Although clinical pregnancy rates were not statistically different between the two groups, patients who underwent IVF cycles using SSD as the sperm selection technique with frozen donor sperm resulted in higher number of utilizable blastocysts which might lead to a higher potential cumulative pregnancy rate or larger family. Limitations include the small sample size and retrospective nature of this study. Although not statistically significant, the average patient age was younger in the SSD group. Overall, the data collected so far supports use of Zymot™ SSD with at least equivalent or perhaps better laboratory and clinical outcomes compared to density gradient centrifugation.

## REFERENCES

[1] Gardner D.K, Schoolcraft W.B. In vitro culture of human blastocysts. in: Jansen R Mortimer D Towards reproductive certainty fertility and genetics beyond 1999. Parthenon Publishing, Carnforth, UK1999: 378-388.

[2] Revolutionizing the sperm separation process. ZyMt Fertility. (n.d.). Retrieved April 4, 2022, from https://www.zymotfertility.com/how-it-works/improves-lab-workflow

# Retrospective study of human embryo development in relation to different sperm selection methods: Microfluidic ZyMōt devices versus density gradient

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

## INTRODUCTION

Growing evidence suggests that sperm aneuploidy, sperm chromatin structural anomalies, DNA fragmentation, Y chromosome microdeletions or epigenetic defects have profound negative impact on embryo quality and IVF clinical outcomes. Therefore, selecting competent spermatozoa with the highest genomic integrity for ICSI is essential to achieve normal embryo development and a healthy live birth.

Several sperm preparation techniques have long been scrutinized with varied, rather unsatisfied success. The recent emerging adaptation of microfluidic-based technique, **novel ZyMōt device** appears promising for improving the quality of sperm used in Assisted Reproductive Technology. Preliminary tests have shown that ZyMōt may increase sperm motility and significantly decrease DNA fragmentation which could possibly enhance rates of euploid blastocysts.

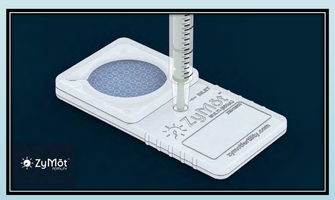
## OBJECTIVE & HYPOTHESIS

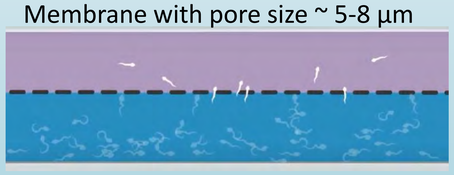
This study aimed to evaluate the effect of microfluidic sperm selection on fertilization rates, blastocyst rate, quality, and their ploidy in comparison with the alternative conventional density-gradient centrifugation technique.

## MATERIALS & METHODS

Our retrospective study consisted of fertility patients who had undergone ICSI and PGT-A treatment cycles from 2019-2021.

For sperm preparation microfluidic **ZyMōt device or gradient-density sperm selection methods** were used.




The blastocysts were quantified into 3 distinct groups according to their morphology before the trophectoderm biopsy: good (AA, AB), average (BB, BC) or poor (CB, CC, DD). Blastocyst ploidy was assessed by NGS analysis using BluGnome 24Sure Kit and VeriSeq (Illumina) kits.

## RESULTS

Patients' characteristics and laboratory data were collected for research using electronic medical records (e-IVF) and archived patient charts.

The study was approved by the Veritas Independent Review Board (IRB no. 16367).

### Table Displaying the Specific Parameters Being Studied

| | Zymot | Gradient | P-value |
|---|---|---|---|
| **# patients** | 1161 | 5407 | |
| **Fertilization Rate (%)** | 82 | 81 | |
| **# 2PN** | 6186 | 16401 | |
| **Total Blast #** | 4083 (66%) | 9677 (59%) | 0.00001 |
| **Usable Blast (%)** | 2421 | 4269 | 0.00001 |
| **PGT Blast** | 2766 | 2017 | |

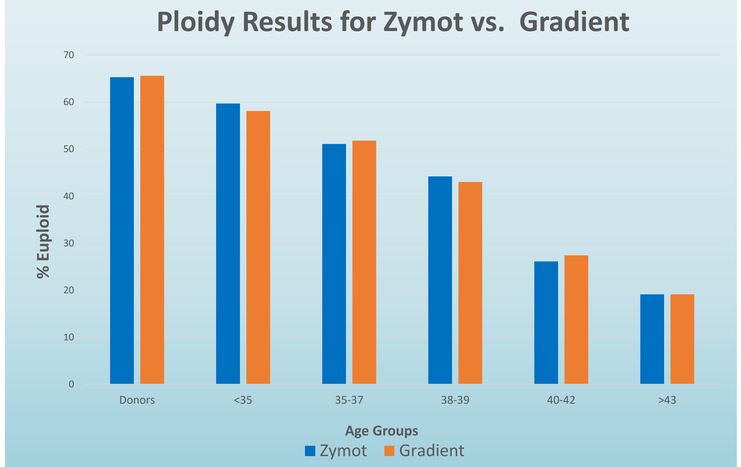
- ICSI Fertilization rate was similar in both sperm processing methods.
- The blast rate was significantly greater in Zymot as compared to Density Gradient.

### The Frequency of Euploid Blastocyst between Zymot and Density Gradient Groups

| | Zymot | | Gradient | | |
|---|---|---|---|---|---|
| | # Blasts | # Euploid | # Blasts | # Euploid | *P*-value |
| Donor Eggs <30 | 400 | 261 (65.3%) | 584 | 383 (65.6%) | 0.9607 |
| <35 | 638 | 381 (59.7%) | 358 | 208 (58.1%) | 0.8003 |
| 35-37 | 800 | 409 (51.1%) | 407 | 211 (51.8%) | 0.8937 |
| 38-39 | 403 | 178 (44.2%) | 272 | 117 (43.0%) | 0.8527 |
| 40-42 | 394 | 103 (26.1%) | 307 | 84 (27.4%) | 0.7830 |
| >43 | 131 | 25 (19.1%) | 89 | 17 (19.1%) | 0.9979 |
| Total | 2766 | 1357 (49.1%) | 2017 | 1020 (50.6%) | 0.5502 |

- Overall, euploidy is about 50% in Zymot and Density Gradient groups.

## RESULTS




As shown in the graph, there is no major difference between Zymot and Gradient techniques in the frequency of euploid blastocyst in different patient age cohorts. This suggests that Zymot has no positive effect on the ploidy.

## CONCLUSION

Compared to standard density gradient sperm processing, a microfluidic device, Zymot, significantly increased blastocyst rates and as well as the number of usable blastocysts that were frozen and/or transferred.

Sperm processing with a Zymot device did not improve euploidy rates of the blastocysts derived from patients of all ages: from young egg donors (≤ 30y of age) to patients with advanced maternal age (>37y to >43 y of age).

Our study also presented the strong correlation between maternal age and euploid blastocyst rates. The highest percentage of euploid embryos was observed between ages <30 and 35, and the lowest was observed after 40.

Further studies should be performed to fully determined the positive impact of ZyMōt sperm selection on embryo quality and clinical outcomes in different age and diagnosis groups of patients.

## ACKNOWLEDGEMENTS

We would like to thank the CReATe Fertility IVF lab for their assistance.

# Euploid pregnancies in recurrent pregnancy loss couples with parental karyotype abnormality

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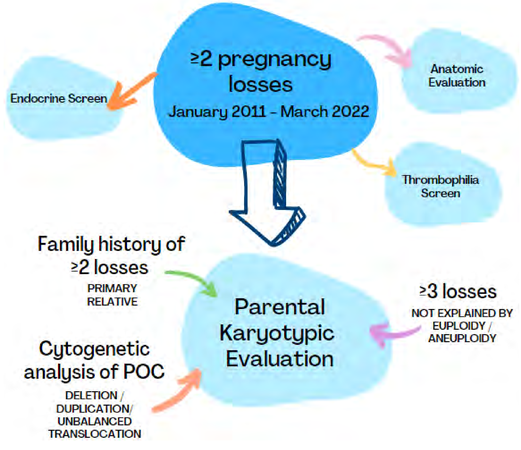
## Background

- Recurrent pregnancy loss impacts 1-5% of the reproductive population
- Approximately 2-4% of patients investigated for RPL are affected by abnormal parental karyotype
  - Translocation
  - Inversion
  - Duplication/Deletion
- Other known etiologies of RPL include: endocrinopathy, anatomic, thrombophilia

## Objectives

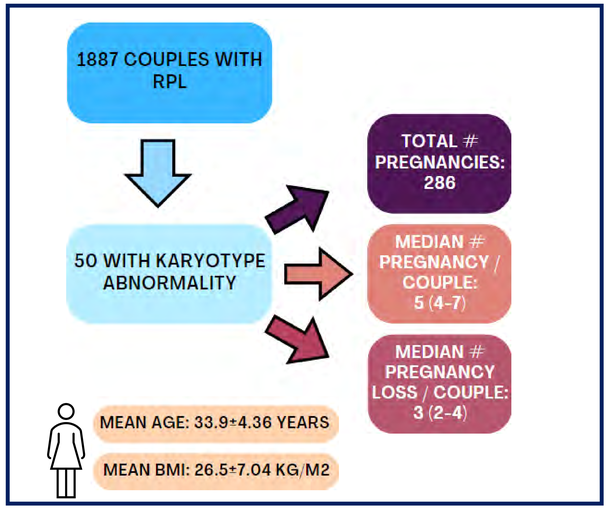
- Evaluate the prevalence of euploid pregnancy among couples with an affected partner(s) of a karyotype abnormality

## Methods

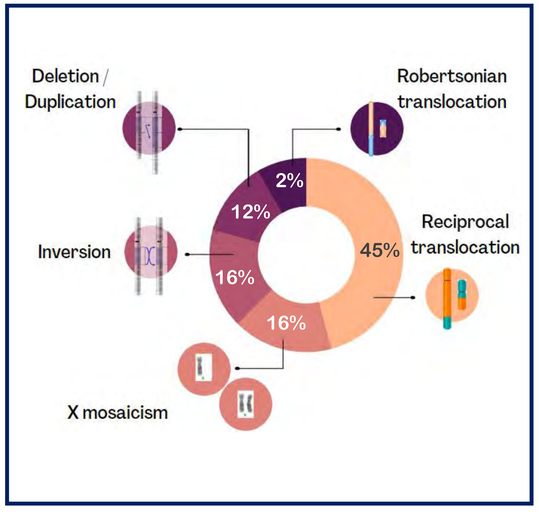



## Results

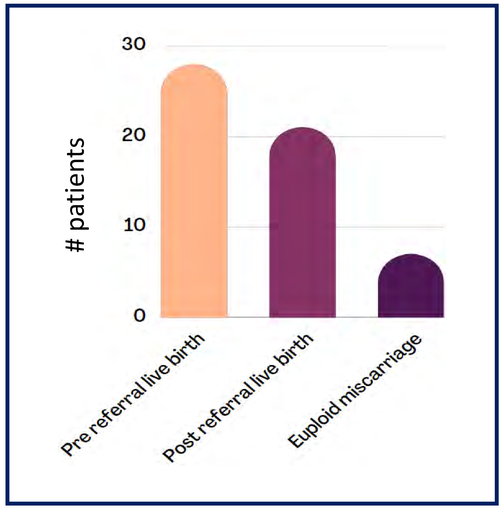
### Patient Demographics




### Parental Karyotype Distribution




### Pregnancy Outcomes




## Discussion

- Assuming all live birth were euploid, the prevalence of euploid conception among all pregnancy was approximately **20%**
- Prevalence of karyotypic abnormality is consistent with published demographic (ASRM 2012)
  - Distribution of abnormalities is similar to large longitudinal based studies (Park SJ. *Fertil Steril* 2022)
- Limitations of this study: Live birth and fertility treatment data (pre-implantation genetic testing for structural rearrangements) not available.

## Conclusion

- Probability of euploid pregnancy in patients with karyotypic abnormality with RPL is excellent
- Parental karyotyping is still warranted in couples with unexplained RPL despite euploid pregnancy loss, as 20% of pregnancies in this population are euploid.