CFAS 68TH
ANNUAL MEETING
Poster Presentation
TV3 & TV4

The Canadian Fertility and Andrology Society

"EMBRACE: THE FIRST CANADIAN CLINICAL EXPERIENCE"

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INTRODUCTION:

For more than 25 years, PGT-A has been the gold standard in assessing embryo ploidy[1]. At KARMA IVF, the clinical pregnancy rate in 2021 with and without PGT-A was 66.7% and 27.5%, respectively. Regardless of its advantages, the downsides of PGT-A include invasive technique, specialized technical skills, and the inability to represent the genetic status of an entire embryo[2]. To overcome these drawbacks, our clinic validated a novel non-invasive method known as EMBRACE by Igenomix to assess embryo ploidy. EMBRACE is a genetic test performed on spent blastocyst media (SBM) that analyses the cell-free DNA (cfDNA) released into the culture media, which offers a more comprehensive representation of the embryo ploidy status during in-vitro embryo development. The primary objective is to evaluate the concordance and reproducibility of cfDNA versus TE biopsy from a set of 11 Day 6 and 7 blastocysts.

EMBRACE: Embryo Analysis of Culture Environment SBM: spent blastocyst media cfDNA: cell free DNA

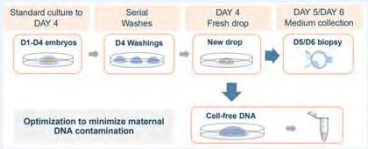
MATERIALS and METHODS:

This clinical validation using EMBRACE is based on 11 blastocyst samples that underwent TE biopsy for PGT-A testing, alongside non-invasive SBM analysis. The procedure and protocol for PGT-A and EMBRACE were discussed and consented with selected prospective patients at KARMA IVF.

- On Day 0, retrieved oocytes were denuded for microinjection (ICSI) and carefully washed in multiple microdroplets to avoid contamination with maternal DNA.
- On Day 1, presumptive zygotes were assessed for successful fertilization, washed in multiple microdroplets, and cultured using the standard laboratory protocol up to Day 4 of the development.
- On Day 4, embryos were washed in multiple microdroplets and moved to fresh 15µl media droplets, including negative droplet controls, until Days 6 & 7 without performing assisted hatching.
- All developed embryos were subjected to TE biopsy and vitrified. The culture medium of the 11 blastocysts was then individually collected under sterile conditions to avoid DNA contamination.
- TE biopsies and corresponding SBM samples were sent to Igenomix Canada, where analysis was performed using NGS, and data was analyzed using specific algorithms.

IGENOMIX METHODOLOGY:

The concordance results of TE biopsy and SBM from the same embryo were reported. The EMBRACE test is conducted using the Ion ReproSeqTM PGS Kit (Next Generation Sequencing) for 24 chromosomes aneuploidy screening (Thermo Fisher Scientific, Inc, MA USA). The kit/assay is performed on the Ion ChefTM and Ion System instruments (Thermo Fisher Scientific). Data is analyzed with the Ion Reporter software, which aligns the reads using the human genome build (hg19) (Thermo Fisher Scientific).



Rubio & Rienzi et al., Fertil Steril 2019

RESULTS:

A 100% informative rate was achieved for both SBM and TE samples.

The EMBRACE results for the SBM samples included 7 euploid embryos and 4 aneuploid embryos. The PGT-A results from TE biopsies matched with the SBM result in 10 out of the 11 samples tested which resulted in a concordance rate of 90.9% (Tabel 1). The presence of contamination with DNA from cumulus cells and/or the embryologist(s) could not be detected in any of the SBM samples using the negative controls (Table 2).

TABEL1

SBM Samples	*Informative Medium*	*Medium Result*	*TE Biopsy Results*	*Concordance*	*Contamination*	*ICSI or IVF*
SK 3 C E	Yes	Euploid	Euploid	Yes	No	ICSI
SK 4C E	Yes	Aneuploid, XXY	Aneuploid, XXY	Yes	No	ICSI
SK 10C E	Yes	Aneuploid, +15	Aneuploid, +15	Yes	No	ICSI
SK 11C E	Yes	Aneuploid, -14	Aneuploid, -14	Yes	No	ICSI
VJ 1 E	Yes	Euploid	Euploid	Yes	No	ICSI
VJ 3 E	Yes	Aneuploid, +9	Aneuploid, +9	Yes	No	ICSI
VJ 6 E	Yes	Euploid	Euploid	Yes	No	ICSI
VJ 7 E	Yes	Euploid	Euploid	Yes	No	ICSI
VJ 14 E	Yes	Euploid	Euploid	Yes	No	ICSI
VJ 15 E	Yes	Euploid	Aneuploid, -5	No	No	ICSI
VJ 18 E	Yes	Euploid	Euploid	Yes	No	ICSI
Total%	*100%*			*91%*	*0%*	

Tabel 2

Negative Controls	*NGS Result*	*Contamination*
Negative Control 1	No DNA detected	No
Negative Control 2	No DNA detected	No
Negative Control 3		
Negative Control 4		
Negative Control 5		
Total NC %		*0%*

CONCLUSIONS:

During embryo development, the number of cells increases at the blastocyst stage releasing higher amounts of cfDNA into the culture media. This non-invasive approach to assess the ploidy of the blastocyst from the SBM containing cfDNA, is analyzed using NGS. A 100% informativity rate was reported for both sample types and all negative controls were free of contamination. This signifies adequate cfDNA release from the developing embryo for DNA amplification and sequencing, with good embryology sterile techniques. EMBRACE testing established a high concordance rate (>90%) with the gold standard TE biopsy for PGT-A testing. SBM results were used in EMBRACE testing to generate a euploidy score ranking for embryo transfer.

This approach can be widely recommended for patients that choose to opt-out of biopsy or yield poor-quality blastocysts. Non-invasive testing can offer other benefits such as cost-efficiency and accessibility of testing to a wider patient population.

FUTURE DIRECTIONS:

Clinical pregnancy and pregnancy outcome data following the transfer of embryos analyzed using EMBRACE is ongoing globally and is also being collected at KARMA IVF. Studies on the origin of embryonic cfDNA and its mechanism of release should be studied further. A non-invasive solution to determine embryo ploidy status for patients could potentially lead to a safer, more efficient IVF treatment.

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1. Preimplantation genetic diagnosis: present and future. E Fragouli. J Assist Reprod Genet (2007) 24:201- 207.
2. Pregnancy outcomes following in vitro fertilization frozen embryo transfer (IVF- FET) with or without preimplantation genetic testing for aneuploidy (PGT- A) in women with recurrent pregnancy loss (RPL): a SART-CORS study (2021). S. J . Bhatt, N . M . Marchetto, J Roy, S.S . Morelli, P. G . McGovern. Hum Reprod (2021) 36(8):2339- 2344.
3. Multicenter prospective study of concordance between embryonic cell- free DNA and trophectoderm biopsies from 1301 human blastocysts. Rubio C, Navarro-Sánchez L, García Pascual CM, Ocali O, Cimadomo D, Venier W, Barroso G, Kopcow L, Bahçeci M, Kulmann MIR, López L, De la Fuente E, Navarro R, Valbuena D, Sakkas D, Rienzi L, Simón C. Am J Obstet Gynecol. (2020) 223(5):751.e 1 - 751.e13.
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Barriers to consultation and treatment in infertility patients and partners in Canada

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Objective

To understand the key drivers and barriers for infertile patients and partners to infertile patients in seeking consultation and undergoing treatment.

Design

Data from patients and partners in Canada and the United States (US) were collected as part of a global survey from 15th March – 17th May 2019.[1] Average time to treatment, patient and partner perspectives on the treatment journey, and drivers for, and barriers to, infertility treatment were assessed.

Materials and methods

- The global study used a quantitative questionnaire across 9 different countries (N=1944), comprising of a 30-minute online survey. Development of the survey was informed by a targeted literature review. To be eligible, respondents (<50 years old) needed to have received, or have a partner who had received, an infertility diagnosis.
- From this total population subgroup analyses were performed on respondents from Canada (N=200) and the US (N=275). A full overview of respondent characteristics is provided in Table 1. Patients/partners were not necessarily couples.
- Respondents were asked to record:
  - Time trying to achieve pregnancy before receiving an infertility diagnosis at a medical consultation.
  - Time trying to achieve pregnancy between receiving a medical infertility diagnosis and enrolment in treatment.
  - Time receiving fertility treatment before pregnancy.
  - Time for each stage was then averaged as mean ± standard deviation.
  - Information was captured from respondents on their reasons for seeking treatment, and on their experience of interactions with health care professionals (HCPs).
- Respondents were then asked to select barriers that they had experienced in seeking, and continuing with, treatment.
- When reporting drivers and barriers to treatment, respondents selected their responses from a list of prespecified answers.

Results

Table 1. Baseline characteristics of Canadian, US, and global respondents

Characteristic	Canada	US	Global
Total respondents, N	200	275	1944
Patients/partners, N	100/100	173/102	1037/907
Average age in years ± standard deviation	37.4 ± 10.4	36.5 ± 9.9	35.8 ± 9.7
Female, N (%)	115 (57.5%)	175 (63.6%)	1095 (56.3%)
Married, N (%)[a]	77 (56.6%)	85 (59.9%)	1119 (66.7%)
Heterosexual, N (%)	173 (86.5%)	249 (90.6%)	1773 (91.2%)

[a]Not all respondents were asked for their marital status; this sample comprised 136 respondents from Canada, 142 respondents from the US, and 1677 respondents from the global population.

Figure 1. Total delay for respondents achieving pregnancy

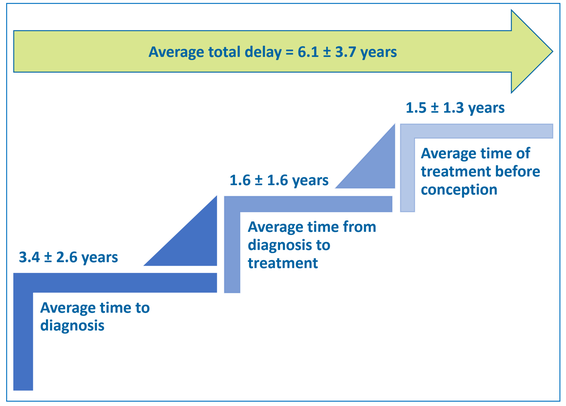

Figure shows pooled data for Canadian patients and partners. Times are mean ± standard deviation (N=200)

Figure 2. Proportion of patients in the Canadian, US, and global populations who sought treatment, stratified by whether they were offered "support services"

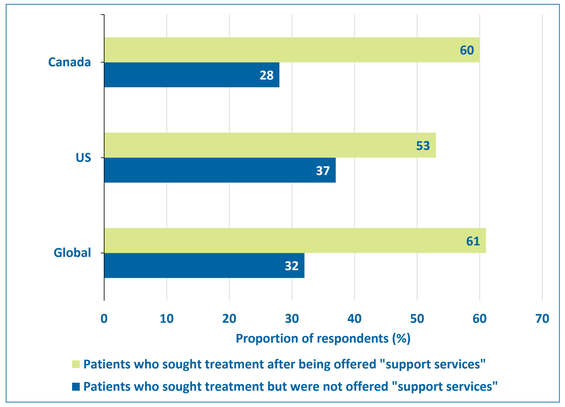

Figure shows data for Canadian (N=124), US (N=175), and global samples (N=1232).

Figure 3. For which of the following reasons did you decide to not explore a consultation to understand available treatment options to treat your infertility diagnosis?

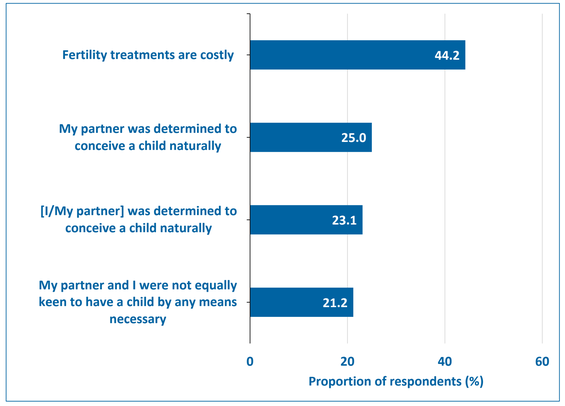

Figure shows pooled data for Canadian patients and partners that chose not to seek a treatment consultation. (N=52)

Figure 4. After you had your consultation about available fertility treatment options, for which of the following reasons did you decide not to enrol in fertility treatments?

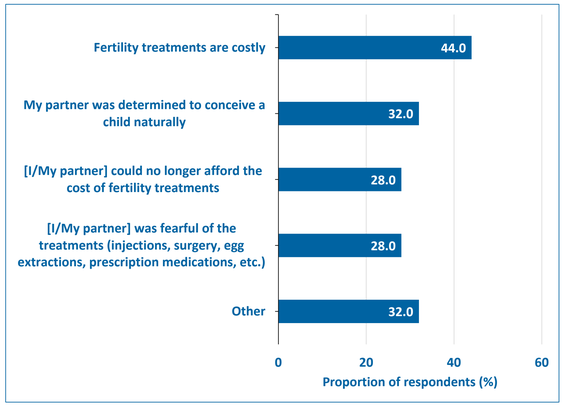

Figure shows pooled data for Canadian patients and partners that chose not to enrol in treatment. (N=25)

Conclusions

- Canadian individuals experiencing infertility took an average of **3.4 years** to get a diagnosis and another **1.6 years** to commence treatment.
- **Cost** was a major barrier to pursuing diagnosis and treatment.
- Canadian patients were **more likely to proceed to treatment** if offered **support services**, and this was more notable in the Canadian vs. the US sample.
- Canadian individuals affected by infertility need to be provided with **more information about the advantages of moving onto treatment earlier**, the issues of **cost** need to be addressed on a national level, and **support services** should be **offered to all patients**.

References

1. Domar, A et al. Barriers and factors associated with significant delays to initial consultation and treatment for infertile patients and partners of infertile patients. Reproductive BioMedicine Online, 2021; 43(6): 1126-1136

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FERRING PHARMACEUTICALS

Trends in Live Birth Rates Attributed to ART in British Columbia

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BACKGROUND

With the growing trend of delayed childbearing, increased access to and improvements in assisted reproductive technology (ART), more pregnancies have been conceived using ART. Although various countries and regions have published their live birth rates attributed to ART, British Columbia (BC) has yet to report on this. Analyzing the live birth rate in BC could serve as a benchmark for assessing future improvements and provide an impetus for health policy makers to adopt a model for publicly funded ART.

METHODS

The number of live births per year, method of conception, and maternal age data was collected from 2008-2018 using the BC Perinatal Data Registry. A logistic regression model was used to investigate the relationship between maternal age, year of delivery and the livebirths conceived using ART.

RESULTS

Of the 397,237 live births from 2008-2018 included in our analysis, 385,401 were conceived naturally and 11,836 were conceived using ART.

There was a wide range in maternal ages at delivery. The mean maternal age was 31 and the median (IQR) = 31 (28 – 35) years of age.

There has been an increase in the proportion of livebirths conceived by ART over time (Cochrane-Armitage test for trend, $p < 0.0001$).

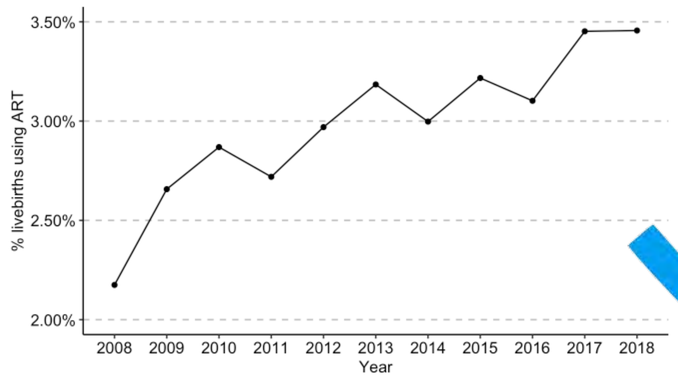

Figure 1. Proportion of livebirth conceived using ART by year

There was a significant relationship between the odds of the pregnancy being conceived by ART and maternal age (OR 1.21, CI 1.21-1.22).

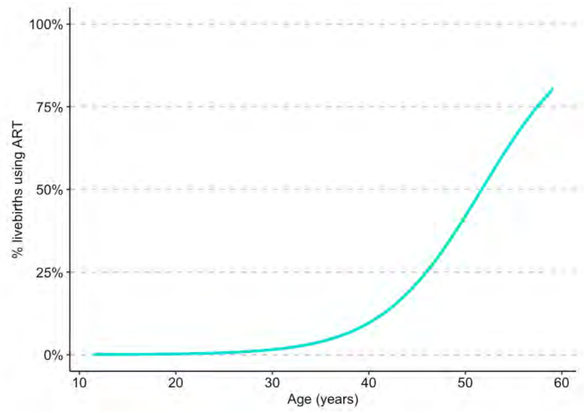

Figure 2. Proportion of livebirths conceived using ART by age

There was a significant interaction between age and year ($p<0.0001$). Among those <35 years of age, the proportion of live births conceived by ART has stayed constant over time. For older ages, there was an increase in the proportion of livebirths conceived by ART over time, with the largest increases in the older ages.

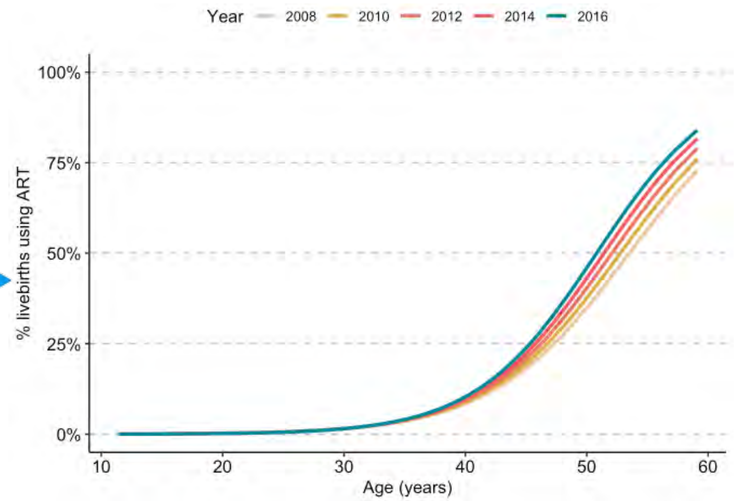

Figure 3. Proportion of livebirths conceived using ART by age and year

CONCLUSION

In BC there has been a significant increase in the proportion of live births attributed to ART amongst those of advanced reproductive age. The trends in live birth rates for multifetal pregnancies attributed to ART in BC requires further research.

Did COVID-19 Protocol Modifications Impact IUI Pregnancy Success Rates?

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Introduction

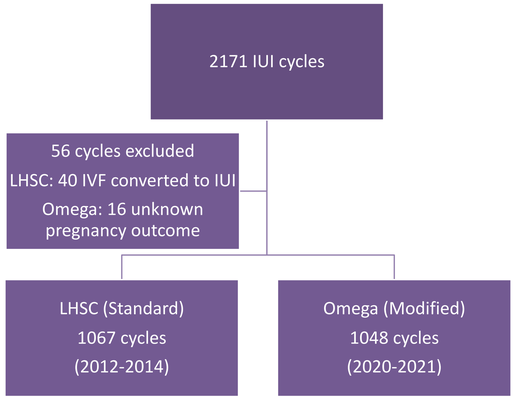
Intrauterine insemination (IUI) is often used as first-line treatment for couples with unexplained infertility or mild male-factor infertility as it is thought to improve pregnancy success rate while being relatively low cost and minimally invasive. Ovarian stimulation (OS) in combination with IUI is believed to improve cycle fecundity by increasing the number of oocytes that are available for fertilization while increasing the number of motile sperm in the uterus through IUI. OS can be achieved using oral medications or gonadotropin injections. In light of the COVID-19 pandemic, different international societies have released recommendations to emphasize the minimization of visits and prioritization of virtual care. As a result, a modified IUI protocol necessitating less monitoring compared to the standard gonadotropin-OS was developed to address the need of less in person visit.

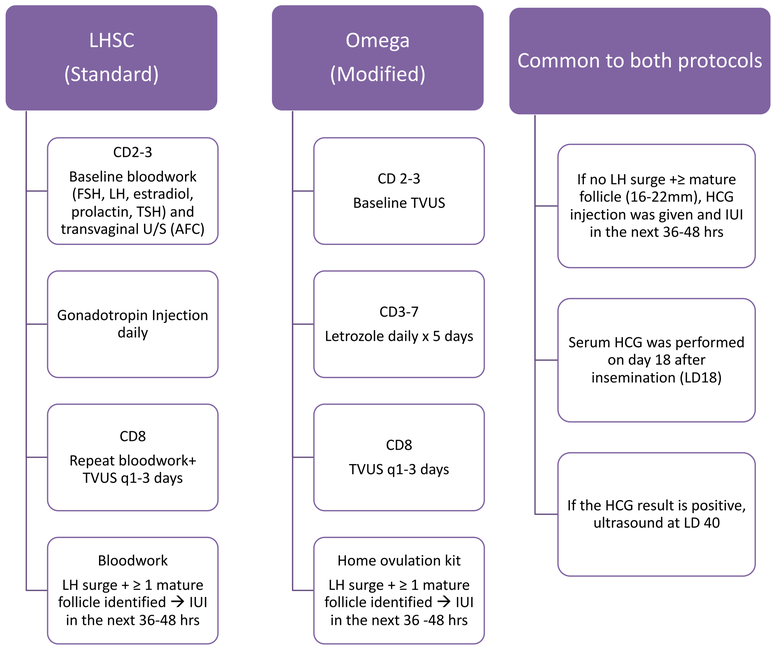
Objective

Our objective was to evaluate the effectiveness of a modified intrauterine insemination (IUI) protocol developed in response to the COVID-19 pandemic by comparing the pregnancy success rate to the standard protocol.

Methods

This was a retrospective cohort study comparing patients who received standard protocol (gonadotropins) and modified protocol (letrozole) developed subsequent to the COVID-19 pandemic at London Health Science Center (LHSC) and OMEGA Fertility Clinic. Data were obtained from a prospectively entered clinical database with information on patient fertility. A total of 2115 IUI cycles were included in this study (n=1067 LHSC, n=1048 OMEGA) occurring between 2012-2014 at LHSC and June 2020-June 2021 at OMEGA. The primary outcome was pregnancy success rate, and the secondary outcome was multiple gestation rate. Logistic regression models were used to examine group differences in terms of predicting pregnancy success, and a chi-square test was used to compare groups on the secondary outcome.





Results

Compared to the standard protocol, the pregnancy success rate was lower in the modified protocol group for both chemical (21.7 vs 14.1%, p<0.001) and clinical pregnancy success rate (18.2 vs 13.5%, p=0.003). However, there were higher rates of multiple gestations in the standard protocol group (10.3 vs 2.1%, p=0.004). The number of cycles and the final sperm count did not significantly predict pregnancy success rate (both chemical and clinical). Increasing BMI (OR=1.02, 95%CI=1.00-1.04, p=0.020) and number of mature follicles (OR=1.38, 95%CI=1.14-1.68, p<0.001) increased the likelihood of a successful pregnancy, whereas increasing age negatively affected the success rate (OR=0.96, 95%CI=0.93-0.98, p=0.002).

Table 1: Comparison of Baseline Characteristics between LHSC and Omega

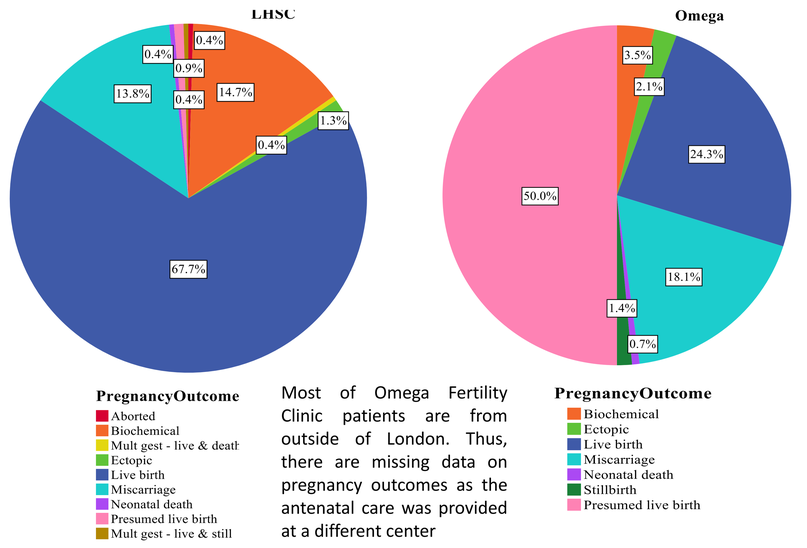
Variable (Mean value)	LHSC	Omega	P-value
Age (years)	33.7	34.2	0.007
Body Mass Index (BMI, kg/m^2)*	26.3	28.4*	<0.01
Number of mature follicles (≥16mm)	1.42	1.22	<0.01
Number of cycles ✢	2	2	0.015
Final Sperm Count (x 10^6)	44.4	37.9	<0.01

*Missing BMI value in 237 patients from Omega, ✢median value

Table 2: Comparison between Pregnancy Outcome at LHSC and Omega

% (frequency)	LHSC (Standard) Total (n=1071)	Omega (Modified) Total (n=1048)	P-value
Chemical pregnancy (β-HCG)	21.7 (n=232)	14.1 (n=148)	p<0.001
Clinical pregnancy (Ultrasound)	18.2 (n=195)	13.5 (n=141)	P=0.003
Rate of multiple gestation	10.3 (n=20)	2.1 (n=3)	P=0.004

Figure 1: Pregnancy Outcome at LHSC and Omega



Most of Omega Fertility Clinic patients are from outside of London. Thus, there are missing data on pregnancy outcomes as the antenatal care was provided at a different center

Table 3: Impact of Different Variables on Chemical Pregnancy Rate (Positive β-HCG)

Variable	Odds Ratio (OR)	95% CI	P-value
Age	0.63	0.49-0.81	<0.05
Body Mass Index	1.02	1.01-1.05	<0.01
Number of mature follicles (≥ 16mm)	1.39	1.14-1.68	<0.01
Number of cycles	0.96	0.89-1.04	0.34
Final Sperm Count	1.00	0.99-1.00	0.16

**Similar results were obtained when analyzing the impact of different variables on clinical pregnancy rate (as determined by ultrasound)

Conclusion

The COVID-19 modified protocol using letrozole negatively affected pregnancy success rates compared to the standard protocol using gonadotropins. However, the standard protocol increased the risk of multiple pregnancy rates, which are higher risk pregnancies.

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

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INTRODUCTION

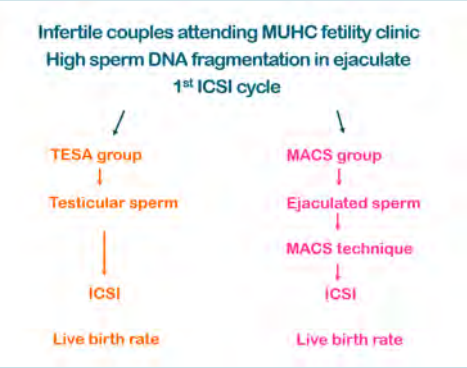
There has been a growing interest on the impact of sperm DNA fragmentation on reproductive outcomes, particularly in assisted reproduction. Even with advanced reproductive technologies such as ICSI, using sperm with high levels of DNA fragmentation may significantly impair the outcomes. Novel approaches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

OBJECTIVE

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

METHODS & EXPERIMENTAL DESIGN

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



RESULTS: Demographic characteristics

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

RESULTS: Embryological characteristics

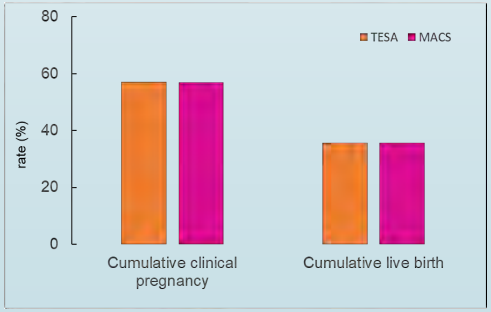
	TESA	MACS	p value
Number of matured oocytes (mean ± SD)	8.0 ± 4.7	7.5 ± 4.7	NS
Number of embryos transferred/fresh ET (mean ± SD)	1.3 ± 0.4	1.2 ± 0.4	NS
Number embryos transferred/frozen ET (mean ± SD)	1.1 ± 0.4	1.0 ± 0.2	NS
Number of embryos frozen (min-max #)	1.7 (0-6)	2.2 (0-8)	NS
Fertilization rate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

RESULTS: Sperm parameters

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

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

RESULTS: Reproductive outcomes



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

CONCLUSIONS

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

Up to 60% (n=128) of trans folks desire fertility; 78% (n=100) of whom have substantial barriers to achieving their reproductive goals.

Scan to receive a digital copy of the poster & expanded data sets

Background

- Trans: identity different than cultural expectation of sex assigned at birth
- Vulnerable, underemployed & growing population
- Medical/surgical transition may affect reproductive options
- Paucity of data regarding fertility interest & perceived barriers

Methods

Cohort, descriptive:

- 45 Q anonymous qualitative & quantitative survey

Volunteer sampling:

- Social media & email to LGBTQ+ groups

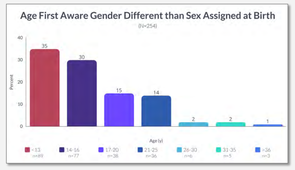
Inclusion criteria:

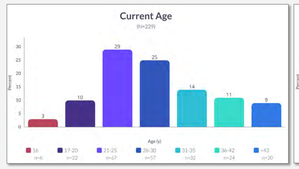
- Canadian
- English
- > 16yo
- Identify as trans

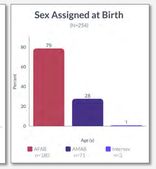
Descriptive statistics:

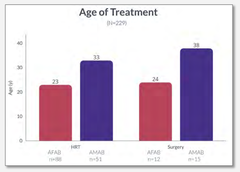
- Demographics
- Interest in fertility options
- Barriers to care

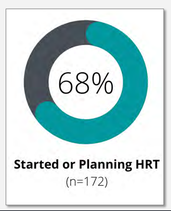
Results: Demographics



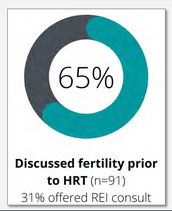




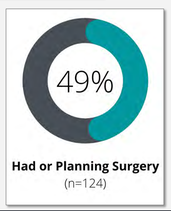




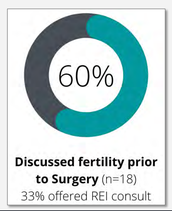
Started or Planning HRT (n=172)



Discussed fertility prior to HRT (n=91)
31% offered REI consult

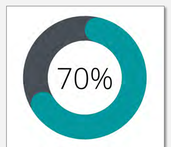


Had or Planning Surgery (n=124)

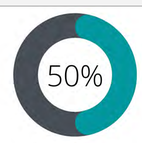


Discussed fertility prior to Surgery (n=18)
33% offered REI consult

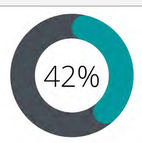
Results: Substantial Barriers



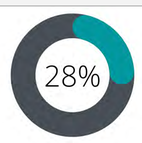
Cost (n=117)
AFAB 73% (n=81)
AMAB 62% (n=33)



Trans Safety (n=86)
AFAB 56% (n=64)
AMAB 35% (n=19)



Interrupting Transition (n=70)
AFAB 39% (n=43)
AMAB 49% (n=26)



Wait Time (n=47)
AFAB 33% (n=37)
AMAB 17% (n=9)

"I was in absolutely no position financially at 21 to make the fertility decision I wanted without enduring an unknown period of time without having a hysterectomy and oopherectomy which were imperative to my well being. I was given ample information about fertility options, but I think it is a mistake to call this a true choice that I made around my fertility as I did not and do not have the privilege of the thousands of dollars those options cost."

"I decided when I started HRT not to freeze any sperm. I couldn't afford it. ... I wish I'd frozen my sperm when I had the chance to without having to get off my HRT. Really really really dreading that. My Parents would have probably helped pay for it. But I was still scared from having just come out to them. Didn't know how to bring up my fertility. But I'd very much like to have children of my own, deeply so."

"I'm probably about a year out from bottom surgery .... I'd have to be off hormones for 3-6 months now to regain sperm production, and I suspect that would drive me close to suicidal."

"I have already frozen gametes, but it is prohibitively expensive to use them, especially in my province."

Trans Folk's Interest in Reproduction & Barriers to Care

Lara Des Roches MD PGY4 ObGyn McMaster
Bianca Ziegler CC3 McMaster
Stacy Deniz FRCSC McMaster, ONE Fertility

Discussion

- Young age of first awareness of gender reveals opportunity to address barriers before transition
- Not meeting WPATH recommendation of discussing fertility options
- Barriers experienced are compounded by the time-sensitive, competing interest to transition
- Impact of trans-specific barriers are entangled with inherent vulnerability of being trans

Conclusion

- Largest study of kind, assessing trans peoples' interest in accessing reproductive care
- Most trans people seek gender-affirming HRT or surgery before or during reproductive years
- Many barriers inhibit folks from meeting their reproductive goals; the impact of these barriers on QOL is significant
- Trans-specific barriers are unique and warrant further study & systematic changes

Should we use serum estradiol levels clinically during IVF cycles?

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Centre de la reproduction Reproductive Centre

Objective:

To assess how well serum estradiol levels on the day of trigger predict the total number of follicles stimulated ≥10mm in diameter and the number of MII oocytes obtained at IVF.

Materials and Methods:

Retrospective data, was obtained from 1175 unique subjects treated at a University IVF center, which fit our criteria for inclusion. The IVF cycles were performed between 2008 and 2020. A monoclonal chemiluminescent immunoassay, via the IMMULITE 2000 Immunoassay system (Siemens, Germany), was used in measurement of serum e2 levels. The assay had intra and inter assay coefficients of variation less than 7.5%. Data was analyzed using correlation coefficients and ROC curves. Data is mean ±SD.

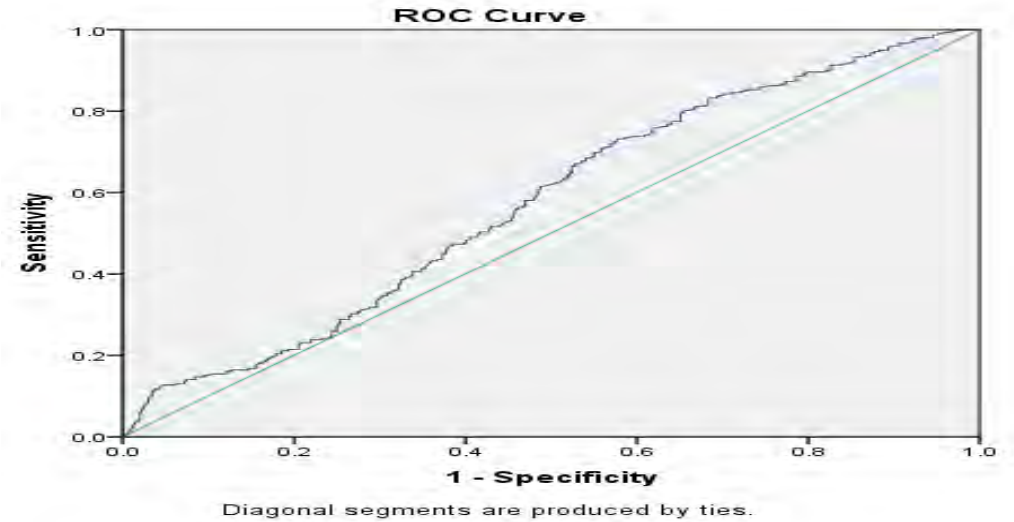
Results:

The correlation between pre-trigger serum E2 and number of follicles was r=0.11, p=0.0001. The correlation between peak e2 levels and number of MII oocytes collected was r=0.45, p=0.0001. This suggests that serum estradiol levels on the day of trigger accounted for only 1% of the variability in the number of follicles stimulated while it accounted for 20% of the variability in the number of MII oocytes collected. The receiver operator curve (ROC) was plotted for follicle yield ≥20 and <20 with area under the curve of 0.58 (95%CI 0.54-0.62), based on peak serum e2 levels. The ROC plotted for sub-group of patients with high risk of OHSS with at least 18 collected oocytes or not demonstrated AUC of 0.26 (95%CI 0.24-0.29), based on peak serum e2 levels. Neither being acceptable predictors of OHSS risk.

Table 1: Baseline characteristics of the study cohort

	Mean	Std. Deviation	N
Female age (years)	35.0	5.1762	1168
BMI (kg/m2)	25.6	5.74324	795
Number of Follicles	20.2	11.2817	795
Estradiol (E2) pmol/L	11759.7	7823.9499	1162
Number of Oocytes recovery	16.6	9.1277	1166
M2 Oocytes	13.015	7.3425	1169
Number of 2PN	9.797	6.1788	1170

Figure 1: ROC curve assessing association between estradiol level and group of patient with ≥ 20 follicles.



Conclusion:

Serum e2 levels on the day of trigger are weakly predictive of the number of stimulated follicles and the number of collected MII oocytes. ROC curves suggest that e2 levels poorly predict the likelihood of having a level of follicles or oocytes that increase the risk of OHSS with a relationship that is no better than flipping a coin or worse.

These results question the value of serum e2 levels in monitoring of most subjects doing IVF.

A Retrospective Quality Assurance Analysis Evaluating the Clinical Outcomes in Frozen Donor Egg Warming Cycles Following a Change of Luteal Phase Support (LPS) Protocol

Jason Au, Jenny Wu, May Nguyen, Jon Havelock, ***Pacific Centre for Reproductive Medicine***, *Burnaby, British Columbia*

PCRM Pacific Centre for Reproductive Medicine

BACKGROUND

The study by Devine et al provided strong evidence supporting the addition of intramuscular (IM) Progesterone (Prog) in oil to daily vaginal (PV) Prog would benefit clinical outcomes in frozen embryo transfer (FET) cycles compared to daily vaginal Prog alone.1 As a result, our clinic has implemented this new Luteal Phase Support (LPS) protocol for patients undergoing FET cycles in September 2019. This change was associated with an increase in clinical pregnancy rate of 7.9% ($p<0.002$) for patients undergoing FET cycles (data not shown). However, we did not observe the same increase for patients undergoing frozen donor egg (FDE) warming cycles. In fact, a decrease in clinical outcome was noted. As such, our clinic has reverted to the previous LPS protocol for FDE warming cycles. The purpose of this quality assurance (QA) assessment is to evaluate if this change of LPS protocol would restore the clinical outcomes for FDE cycles to the same level prior to the initial LPS protocol change in 2019.

MATERIALS AND METHODS

This is a retrospective data analysis comparing the laboratory and clinical data in FDE warming cycles in a Canadian clinic 9 months prior and 7 months following the change of the LPS protocol from Prog 200mg PV TID plus Prog in Oil 50mg IM every 3rd day to Prog 200mg PV TID only in September 2021. To reduce possible confounding factor on FDE quality, only cycles using FDE from one commercial donor egg bank, the single largest donor egg sources for our clinic, were considered. In this analysis, 33 and 36 cycles were evaluated in each group using different LPS protocols. There were no other changes in clinical and laboratory practices during this time. The laboratory and clinical data were compared. For continuous variables, Mann-Whitney test was used and for proportion, Fisher Exact test was used to determine statistical significance.

TABLE

LPS Protocol	Prog 200mg PV TID plus Prog in Oil 50mg IM every 3rd day	Prog 200mg PV TID	*p*-value
Sample Size	33	36	
Time Period	Jan 2021 to Sept 2021	Oct 2021 to Apr 2022	
Warming Survival Rate	91% (205/225)	94% (230/245)	0.293
Normal Fertilization Rate	87% (178/205)	84% (194/230)	0.497
Cleavage Rate	94% (168/178)	93% (181/194)	0.830
Utilization Rate[1]	48% (85/178)	66% (120/181)	<0.0005
Percentage of cases with surplus embryos for cryo (%)	78% (25/32)	76% (26/34)	1
Average number of blastocysts cryopreserved	2.1	3.1	<0.02
Proportion of cases had an ET	88% (29/33)	94% (34/36)	0.416
Average number of embryos transferred	1.1	1.2	0.624
Positive Pregnancy Rate (%)	59% (17/29)	74% (25/34)	0.285
Biochemical Loss Rate (%)	29% (5/17)	20% (5/25)	0.714
Clinical Pregnancy Rate (%)	41% (12/29)	59% (20/34)	0.210
Implantation Rate (%)	38% (12/32)	55% (22/40)	0.161
Miscarriage Rate (%)	8% (1/12)	0% (0/20)	0.375

Table 1. A Comparison of Laboratory and Clinical data before and after a Luteal Phase Support Protocol Change

RESULTS

The data comparison was presented in Table 1. There was no difference between the two groups in warming survival rate, normal fertilization rate, embryo cleavage rate, and percentage of patients with surplus embryos cryopreserved. However, there were a statistically significant difference in embryo utilization rate (48% versus 66%, $p<0.0005$) and average number of blastocysts cryopreserved (2.1 versus 3.1, $p<0.02$) between the two groups. The cause of this increase was unknown, as there was no change in laboratory practice or culture conditions during this period. It is reasonable to stipulate that the increase in utilizable embryos did not impact the quality of the embryos transferred but resulted in additional embryos cryopreserved after transfer or a potential increase in cumulative pregnancy rate. When comparing the clinical outcomes, although they were not statistical significance, the differences are highly clinically significant. Increases in positive pregnancy, clinical pregnancy, and on-going pregnancy rates were observed upon the change of LPS protocol, an increase of 15%, 18%, and 21% respectively.

CONCLUSION

Since the change of LPS protocol in September 2021, we observed a clinically significant improvement in clinical outcomes. The recent clinical outcomes were similar to clinical outcomes of FDE warming cycles prior to the initial change of LPS protocol in 2019. In this QA analysis, we have revealed that the addition of IM Prog might accelerate the endometrial development leading to a dyssynchrony of endometrial receptivity and embryo development. And this dyssynchrony may be exacerbated in patients undergoing FDE warming cycles. The result of this study suggests that clinics should assess FDE warming cycles independently from FET cycles when determining what LPS protocol to implement.

Due to the sample size of this analysis, statistically significant differences were not observed. It would be important to continue to monitor the differences in clinical outcomes. The data from this QA analysis supports the change of LPS protocol in improving clinical outcomes for FDE warming cycles.

Temporal Trends in Thyroid-Stimulating Hormone and Live Birth Rate in Subclinical Hypothyroid Patients in a Recurrent Pregnancy Loss Population

Sophie Jansen, Genevieve Leduc-Robert, Faten AbdelHafez, Arianne Albert, Ulrike Mayer, Mohamed Bedaiwy

Summary:

In a retrospective study of a recurrent pregnancy loss (RPL) population, thyroid stimulating hormone (TSH) levels were analyzed over time across patients with and without thyroperoxidase antibody (TPOAb) positivity. These levels were not found to change over time. A preliminary analysis of live birth rate for patients across levels of hypothyroidism was performed. Patients were categorized by severity of hypothyroidism into subclinical hypothyroidism (TSH 2.5-10 mIU/L), clinical hypothyroidism (TSH > 10 mIU/L) and euthyroid (TSH 0.1- 2.5 mIU/L). Patients with subclinical hypothyroidism treated with levothyroxine showed an increased live birth rate compared to untreated. This provides preliminary evidence towards how to best test and treat patient who present with RPL.

Introduction:

- Recurrent pregnancy loss (RPL) affects 2-5% of the fertile population.[1]
- In general, initial investigations look for potential genetic, endocrine, autoimmune, and anatomic causes.
- Thyroid autoimmunity is a known cause of hypothyroidism.[2]
- Overt hypothyroidism is an established cause of RPL that is effectively treated with levothyroxine.[2]
- It is unclear how TPOAb positivity impacts TSH levels during subsequent pregnancies.
- The primary objective of this study was to examine the TSH levels for RPL patients during pregnancies following the index visit.
- A preliminary analysis was performed to ascertain if subclinical hypothyroidism can be treated with levothyroxine to improve live birth rate.

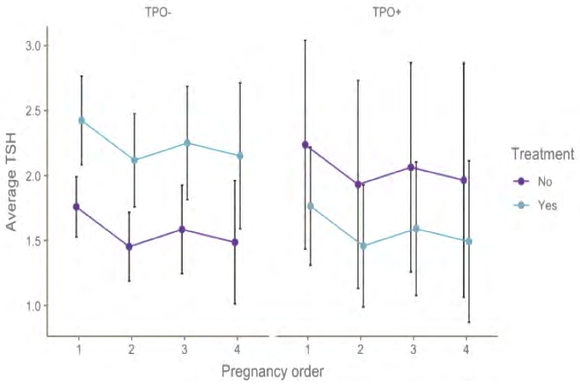


Figure 2. Average TSH across pregnancies for TPO antibody and levothyroxine treatment status. Patients were considered TPO antibody positive if a value above 35 IU/mL was identified. No significant interaction between pregnancy number and either TPO antibody status or levothyroxine treatment status was identified (p=0.24). (TSH= thyroid stimulating hormone; TPO= thyroperoxidase).

Results:

- There was no significant difference in patient demographics including age, body mass index, or num of previous live births or pregnancy losses between groups.
- Analysis of TSH showed no significant change across TPOAb or treatment status (p=0.24) for up to four subsequent pregnancies (Fig. 2).
- An increased live birth rate in subclinical hypothyroid when treated with levothyroxine relative to untreated subclinical (OR = 2.25, p<0.0001) was seen (Fig. 3).

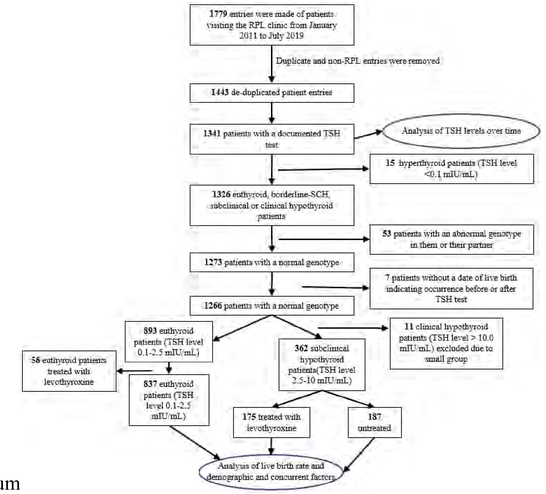


Figure 1. Flow chart of subject inclusion in the study. (RPL= recurrent pregnancy loss; TSH = thyroid stimulating hormone; borderline-SCH = borderline subclinical hypothyroidism).

- Of the 1779 entries made, 1443 unique patients were considered. 102 patients without a documented TSH test were excluded. 15 patients were excluded due to evidence of hyperthyroidism. 53 patients were excluded due to an abnormal genotype in them or their partner. 7 patients were excluded due to missing date information for their live births. 56 patients with TSH levels indicating euthyroidism were excluded due to having been treated with levothyroxine, suggesting a history of hypothyroidism. Due to the small number of patients with clinical hypothyroidism, this group was excluded. Thus, 1199 patients were included and categorized by thyroid status (Fig. 1).
- The baseline characteristics showed no significant difference in age, body mass index, or number of previous live births or pregnancy losses between euthyroid and subclinical hypothyroid groups.

Materials and Methods:

- This study is a retrospective chart review of 1443 patients was conducted. 837 euthyroid patients and 362 subclinical (175 treated, 187 untreated) were included.
- Patients were seen and recruited at the BC Women's Hospital Recurrent Pregnancy Loss Clinic.
- TSH in pregnancies after the initial visit across TPOAb status was analyzed using mixed-effects linear regression.
- Live birth rate in subclinical hypothyroidism (borderline-SCH) (TSH 2.5-10 mIU/L) patients with levothyroxine treatment was compared to those without.
- Across euthyroid and subclinical hypothyroid patients, live birth rate was analyzed using logistic regression.

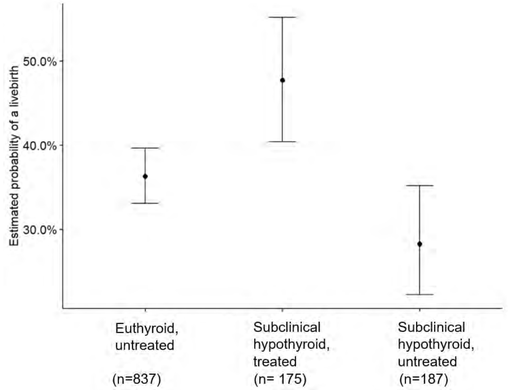


Figure 3. Predicted probability of a live birth across thyroid status categories with or without levothyroxine treatment. Error bars indicate 95% confidence intervals. (Borderline-SCH= borderline subclinical hypothyroidism).

Discussion and Conclusions:

In the RPL population, there was not significant demographic differences or differences in causes of RPL across the groups considered. It was found that TPOAb positivity was not correlated with change in TSH levels over time. This suggests that while TPOAb status should be considered, positive TPOAb is not necessarily predictive of hypothyroidism in the future.

A significant increase in live birth rate was found in patients with subclinical hypothyroidism when treated with levothyroxine. Due to the small number of patients in the clinical hypothyroid group in this study, analysis and thus conclusions could not be extended to that group. As well, the small number of patients with TPOAb positivity may have impeded the significance of the TPOAb levels over time between groups.

Overall, this study lends evidence to how hypothyroidism should be treated in RPL with consideration of both TPOAb status and TSH levels.

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The Efficacy of Implementing a Universal Warming Protocol for Externally Vitrified Blastocysts that used Dimethyl Sulfoxide (DMSO) Cryoprotectant

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OBJECTIVE

Shipping embryos between clinics has become a common practice amongst clinics worldwide. Each laboratory may use different vitrification protocols with recommendations to use a different commercial warming protocol. The objective of this poster is to recommend a universal warming protocol for all vitrified blastocysts that use dimethyl sulfoxide cryoprotectant. With the goal of eliminating the case-by-case preparations needed for each shipped in embryo.

BACKGROUND

It is common for patients to ship embryos between clinics or countries. These cryopreserved embryos have often been vitrified using a different protocol than the in-house method and come with recommendations to use a different commercial warming protocol and kit. To streamline the cycle setup process, the embryology laboratory can adopt a universal warming protocol. A universal warming protocol is the most cost effective and time efficient approach, as it eliminates the case-by-case sourcing and purchase of different commercial kits. A universal warming protocol also simplifies the training and proficiency analysis of embryologists dealing with externally vitrified embryos. The Kitazato warming kit was used as the universal warming kit at our centre for all shipped-in embryos vitrified using Dimethyl Sulfoxide (DMSO) cryoprotectant due to its high survival yield, accessibility, and DMSO compatibility. The aim of this quality control project was to evaluate the feasibility and efficacy of employing a single universal warming protocol for all externally created blastocysts, which were cryopreserved using a range of commercial vitrification protocols and kits.

MATERIALS AND METHODS

We performed a quality assurance study using a retrospective cohort of 50 frozen embryo transfers (FETs) between January 2019 and December 2021. The FET cycles included externally created, vitrified blastocysts that had been shipped to our centre. Based on the original vitrification method, one of two warming protocols was selected – Vitrolife RapidWarm for Blastocyst or Kitazato Warming kit (“Universal protocol”). Vitrolife was chosen if the vitrification solution had not included DMSO and Kitazato was chosen if vitrification solution contained DMSO cryoprotectant. 44 blastocysts for 33 patients were warmed using the Kitazato (“Universal”) warming protocol.

MATERIALS AND METHODS (cont…)

These blastocysts had been vitrified with vitrification kits from IrvineScientific (n = 40), CryoTech (n=2), COOK Sydney IVF (n=1), and CooperSurgical SAGE (n=1). The remaining 17 patients had 19 blastocysts warmed with Vitrolife Rapid Warm protocol. These blastocysts had also been vitrified with Vitrolife RapidVit protocol and solution. The primary objective was to compare blastocyst survival rates between embryos warmed with the same commercial kit as they had been vitrified with, versus those vitrified with a brand discordant to the “Universal” Kitazato protocol. The secondary objective was comparison of clinical pregnancy rates between externally created embryos warmed with concordant-brand and discordant-brand kits, versus contemporary FET cycles of embryos created in our own centre. Our centre uses the Vitrolife RapidVit Blast and RapidWarm Blast kits for cryopreservation and warming.

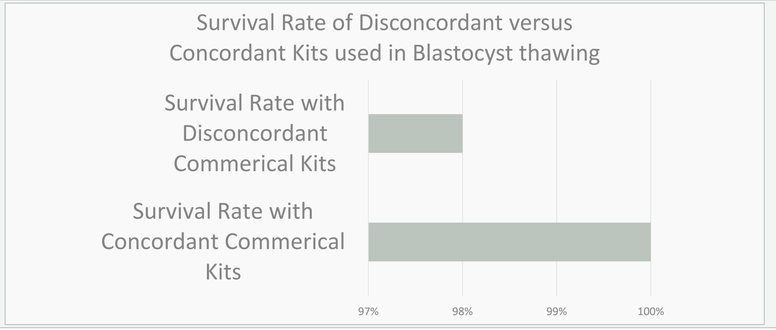
RESULTS

Overall, whether the embryos were warmed with concordant commercial kits or discordant commercial kits, blastocyst survival rates were similar: 100% vs 98%, respectively (p≥0.05). When assessed for positive serum pregnancy test per FET cycle, the two groups, warmed with the “Universal” Kitazato and RapidWarm protocols, yielded similar rates, 48% and 47%, respectively (p≥0.05). The “Universal protocol” (Kitazato kit) had a higher clinical pregnancy rate (CPR) per FET cycle (39%) than the Vitrolife RapidWarm protocol (29%), thou it is not statistically significant (p≥0.05). The internal CPR per FET cycle at our clinic for blastocyst transfers between 2019 to 2021 (including all ages) was 50%.

Table 1

	“Universal” Protocol (Kitazato)	“RapidWarm” Protocol (Vitrolfie)
Positive Blood Serum Pregnancy Test	48%	47%
Clinical Pregnancy Test (CPR)	39%	29%

FIGURE 1



DISCUSSION

We analyzed a group of 63 embryos that were externally created and vitrified then later shipped to our centre. Whether the embryos were vitrified and warmed using concordant Vitrolife kits or vitrified with a variety of other kits then warmed with our “Universal protocol” Kitazato kit, embryo survival rates and positive pregnancy test rates were similar. The differences in clinical pregnancy rates between the two warming protocols, and between the externally created embryos and our own pregnancy rates, can likely be attributed to egg and embryo characteristics, rather than the warming protocols.

CONCLUSION

This study supports that a universal warming protocol for shipped-in embryos does not compromise blastocyst potential. Our facility has improved efficiency and lowered costs using the “Universal” warming kit approach.

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Aneuploidy does not impact cytoplasmic movements that can be seen with non-invasive imaging in oocytes'

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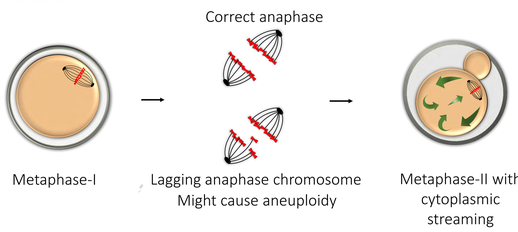
Introduction

Chromosomal segregation errors during early development lead to inheritance of incorrect number of chromosomes, known as aneuploidy, which causes infertility and birth defects. Aneuploidy has been estimated to affect about 25% of eggs from young females with the numbers increasing as females age. A known phenomena connected with aneuploidy is so-called lagging chromosome – a chromosome that lags behind the main mass of chromosomes being pulled toward the spindle pole during anaphase. But is there a way to predict which egg is aneuploid just by looking at it?

Actin-mediated cytoplasmic streaming in mammalian oocytes has been shown to help in directing spindle migration to the cell cortex during Meiosis-I and to keep the spindle at the cell cortex in Meiosis-II for prolonged periods of time while the egg is waiting to be fertilised.

Does this cytoplasmic streaming have an underlying pattern detectable with non-invasive microscopy imaging? More precisely, does aneuploidy further affect the cytoplasmic movement allowing for a selection tool of euploid eggs?

Mouse Meiosis-I:



Questions:

- Is actin-mediated cytoplasmic flow affected with the ploidy status of an egg?
- Can this cytoplasmic movement in Metaphase-II eggs be further used as a predictor tool for future embryo viability?

Methods

CD1

DIC confocal imaging

Human GV oocytes

Data analysis

Imaris · MATLAB · PIVlab

Results 1. There is no difference in actin-mediated cytoplasmic streaming between euploid and aneuploid mouse eggs.

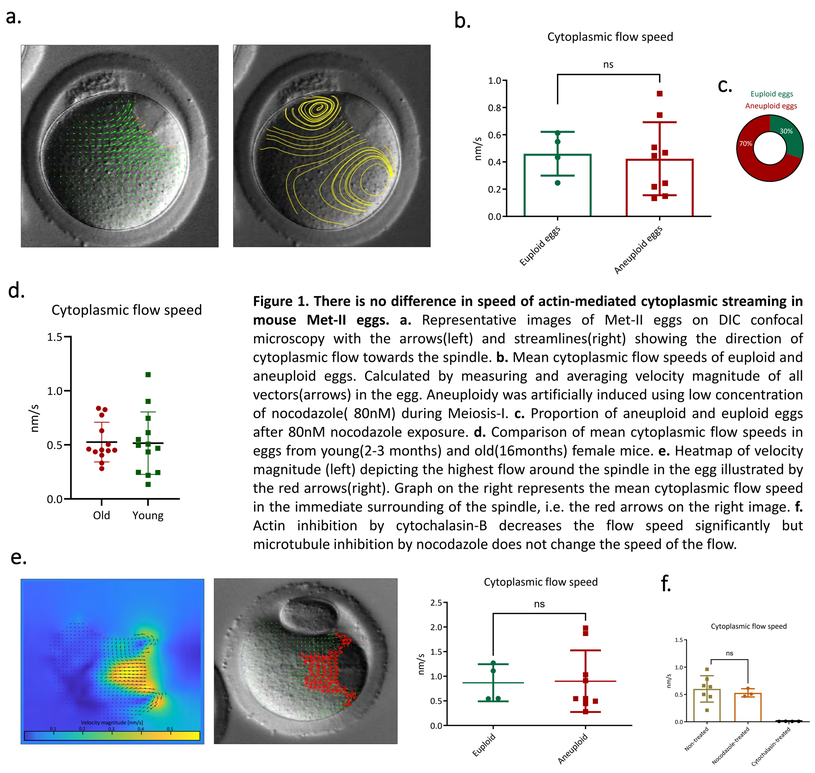


Figure 1. There is no difference in speed of actin-mediated cytoplasmic streaming in mouse Met-II eggs. a. Representative images of Met-II eggs on DIC confocal microscopy with the arrows(left) and streamlines(right) showing the direction of cytoplasmic flow towards the spindle. **b.** Mean cytoplasmic flow speeds of euploid and aneuploid eggs. Calculated by measuring and averaging velocity magnitude of all vectors(arrows) in the egg. Aneuploidy was artificially induced using low concentration of nocodazole(80nM) during Meiosis-I. **c.** Proportion of aneuploid and euploid eggs after 80nM nocodazole exposure. **d.** Comparison of mean cytoplasmic flow speeds in eggs from young(2-3 months) and old(16months) female mice. **e.** Heatmap of velocity magnitude (left) depicting the highest flow around the spindle in the egg illustrated by the red arrows(right). Graph on the right represents the mean cytoplasmic flow speed in the immediate surrounding of the spindle, i.e. the red arrows on the right image. **f.** Actin inhibition by cytochalasin-B decreases the flow speed significantly but microtubule inhibition by nocodazole does not change the speed of the flow.

Results 2. Microinjection of dragon beads shows no difference in cytoplasmic movements between euploid and aneuploid eggs.

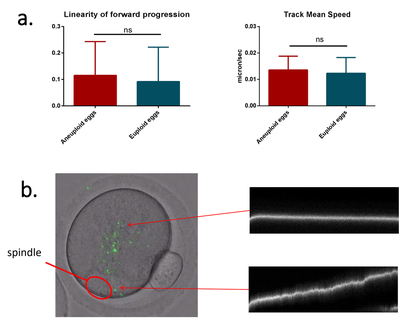


Figure 2. Time-lapse microscopy of fluorescently labelled dragon beads in cytoplasm of Met-II eggs. a. Analysis of dragon beads in cytoplasm did not reveal any differences in the movement of the bead in the direction of flow (linearity of forward progression) and average speed of all the dragon beads in an egg (track mean speed) between euploid and aneuploid eggs. **b.** Kymographs showing the movement of dragon beads close to the spindle (bottom kymograph) and further away from the spindle (upper kymograph). Dragon bead close to the spindle is moving forward with the flow, however the dragon bead further away from the spindle is not moving, showing that the flow is stronger around the spindle, as shown by DIC imaging in previous figure.

Results 3. Rotational cytoplasmic flow in human oocytes is not different between oocytes with normal and abnormal chromosomal segregation.

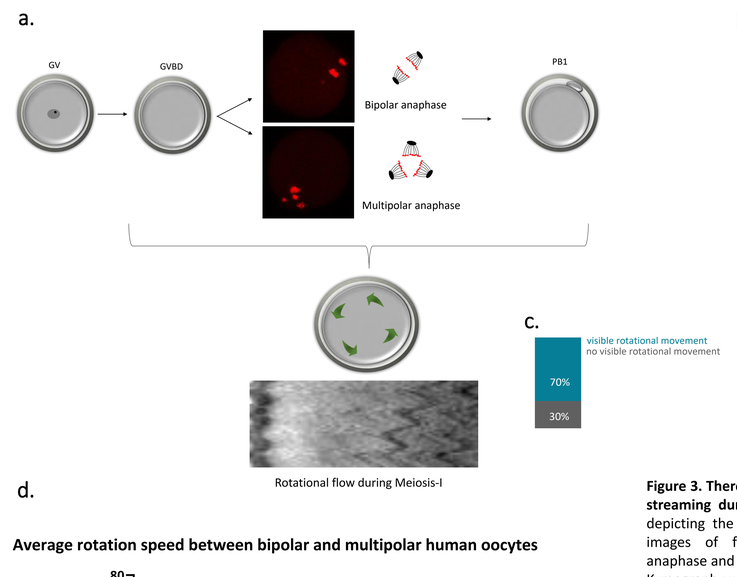


Figure 3. There is no difference in the rotational cytoplasmic streaming during Meiosis-I in human oocytes. a. Cartoon depicting the rotational streaming in human oocytes with images of fluorescently labelled chromosomes during anaphase and a kymograph showing the rotational streaming. Kymograph was generated by making straight line from the centre of the oocyte to the cortex, creating a wave like pattern every time the rotation passed through the line. **b.** Graphs showing rotational streaming duration of individual oocytes at each time point prior to Pb1 extrusion. Up- Individual oocytes and the duration of their rotational streaming at each detectable timepoint prior to Pb1 extrusion. Down- Different durations of rotational streaming detected at each time point prior to Pb1 extrusion. **c.** Proportion of oocytes with visible rotational movement. **d.** The duration of rotational streaming is similar between oocytes with bipolar anaphase and multipolar anaphase which could lead to aneuploidy. **e.** Proportion of oocytes with bipolar and multipolar anaphase.

Results 4. Rotational cytoplasmic flow in human Met-II eggs has similar duration as in oocytes in Met-I.

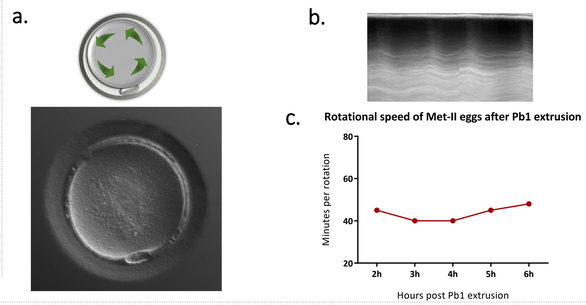


Figure 4. Rotational streaming in human Metaphase-II eggs has similar durations as during Meiosis-I. a. Cartoon showing the cytoplasmic rotational streaming. Image under is a DIC image of Metaphase-II human egg. **b.** Kymograph depicting the wave-like pattern of each rotation that took place during the DIC live-imaging microscopy. X-axis shows time progression with each wave symbolising one turn of rotational flow. **c.** Graphs showing the average duration of rotational streaming in Met-II.

Conclusions

Cytoplasmic flow analysis on mouse Met-II eggs revealed that:

- Cytoplasmic flow is the strongest around spindle, which keeps the spindle pushed towards and located at the cortex of the cell.
- Aneuploidy, which arises by incorrect chromosomal divisions, does not affect the velocity magnitude of the cytoplasmic flow as there is no significant difference in measured speed of flow between euploid and aneuploid eggs.
- Eggs from old female mice have similar cytoplasmic flow speeds as eggs from young female mice.
- Microinjection of dragon beads into oocytes, a more invasive technique for detection of cytoplasmic flow, revealed that the flow is stronger around the spindle area and weaker in the rest of the cytoplasm. Furthermore, it shows no difference in flow speed between euploid and aneuploid eggs, supporting the conclusion obtained in figure 1.

Cytoplasmic flow analysis on human oocytes and Met-II eggs revealed that:

- There is a different type of cytoplasmic flow found in human oocytes and eggs compared to mice. This rotational movement of the cytoplasm is found both in Meiosis-I and Met-II.
- Measurements of duration of rotational movements in both oocytes and eggs showed similar numbers, averaging at 55 minutes per rotation.
- Durations of rotations do not differ with time progression. In the measured time frame of 12 hours before polar body extrusion and up to six hours after polar body extrusion, average durations of rotations are not significantly different.
- Comparison of average rotational speeds between oocytes with bipolar (normal) anaphase and those with multipolar (abnormal) anaphase revealed no significant difference.

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A RETROSPECTIVE STUDY OF 350 PRODUCTS OF CONCEPTION (POC) TESTS ANALYZED by NEXT GENERATION SEQUENCING (NGS) in CANADIAN PATIENTS

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INTRODUCTION

A POC test is a genetic test which studies the fetal tissue from a pregnancy loss to determine if the loss was due to chromosomal aneuploidy. Most POCs are tested by conventional karyotyping and information about NGS-based POC testing in Canadian patients is lacking. NGS-based testing has many benefits compared to conventional karyotyping, including identification of fetal sample contamination with maternal cells through STR (short tandem repeat) analysis, thereby reducing misdiagnosis.

MATERIALS and METHODS

A retrospective study was performed on 350 NGS + STR POC test results obtained from spontaneous conception and IVF in Canadian patients, and on corresponding test requisition forms collected by Igenomix from June 2017 - June 2022. The majority of patients were referred by fertility clinics. Samples were excluded from analysis if gestational age was not indicated. Information regarding recurrent pregnancy loss (RPL) was voluntary and undefined, therefore for this study, it was assumed that RPL indicates that the tested sampled was not the patient's first loss. It was also assumed that there were RPL patient samples that were not identified as such, and therefore RPL data was underrepresented.

POC samples were received at Igenomix in sterile saline. Testing included a multiple dissection approach to identify fetal tissue in the sample received. The DNA from these extractions was analyzed by NGS using 24 chromosome aneuploidy screening (Thermo Fisher Scientific, Inc. MA USA). Data was analyzed using Ion Reporter software. If a 46XX (normal euploid female) result was obtained, DNA was extracted from the maternal sample provided (blood or buccal swab).

PCR-based highly polymorphic short tandem repeat (STR) fragment analysis was then performed on both the tested tissue DNA and on the maternal DNA. If there was a match, the original tissue sample was dissected again, and the process repeated. Dissection and subsequent testing was attempted up to 3 times. Subsequently, a 46XX result was reported as either fetal origin or maternal cell contamination (MCC).

RESULTS

The average gestational age of pregnancy loss in this study was 8 weeks (range 4.5-16). The earliest gestational age for which a fetal result was obtained, was 5 weeks. 348 of the 350 samples were 1st trimester losses (99.4%) while two were 2nd trimester losses (13+ weeks).
The average maternal age was 36.2 years old (range 22-52).

Table 1 Summary of results from analysis of 350 POC samples

Gestational Group	Total (%)	No result (%)	MCC (%)	Fetal Results(%)	Normal (%)	Aneuploid (%)	Trisomy (%)	Monosomy (%)	Polyploidy (%)	Del/Dup (%)	Mosaic(%)	Multiple Aneuploidies (%)
All patients	350 (100)	3(0.9)	75(21.6)	272(78.4)	96(35.3)	176(64.7)	120 (68.2)	11 (6.2)	25 (14.2)	4 (2.3)	1(0.6)	15 (8.5)
Maternal age												
AMA (35+)	228 (65.1)	3(1.3)	49(21.8)	176(78.2)	55(31.2)	121(68.8)	93(76.9)	3(2.5)	11(9.1)	1(0.8)	0	13(10.7)
Young patients (<35)	122 (34.9)	0	26(21.3)	96(78.7)	41(42.7)	55(57.3)	27(49.1)	8(14.5)	14(25.5)	3(5.5)	1(1.8)	2(3.6)
Collection method												
D&C	87 (24.9)	1(2.1)	9(10.5)	77(89.5)	31(40.3)	46(59.7)	29(63.0)	4(8.7)	7(15.2)	0	0	6(13.0)
At home collection	263 (75.1)	2(0.8)	66(25.3)	195(74.7)	65(33.3)	130(66.7)	91(70.0)	7(5.4)	18(13.8)	4(3.1)	1(0.8)	9(6.9)
Gestational age												
<8weeks	156 (44.6)	1(0.6)	42(27.1)	113(72.9)	46(40.7)	67(59.3)	50(74.6)	1(1.5)	6(9.0)	2(3.0)	1(1.5)	7(10.4)
8 - <10 weeks	172 (49.1)	2(1.2)	31(18.2)	139(81.8)	42(30.2)	97(69.8)	64(66.0)	8(8.2)	17(17.5)	1(1.0)	0	7(7.2)
10+ weeks	22(6.3)	0	2(9.1)	20(90.9)	8(40.0)	12(60.0)	6(50.0)	2(16.7)	2(16.7)	1(8.3)	0	1(8.3)
Other												
RPL	147 (42)	2(1.4)	30 (20.7)	115(79.3)	41 (35.7)	74 (64.3)	50 (67.6)	3 (4.1)	11 (14.9)	1 (1.3)	1 (1.3)	8 (10.8)

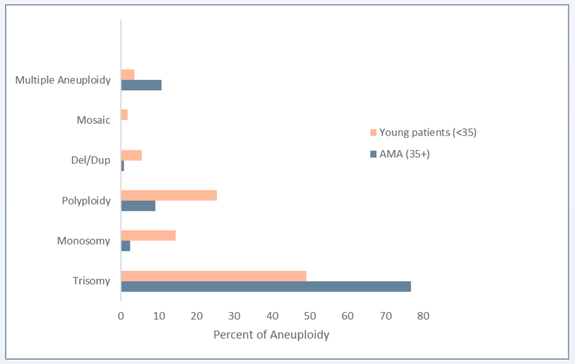

Figure 1 The impact of maternal age on the types of aneuploidies found in POC tests.

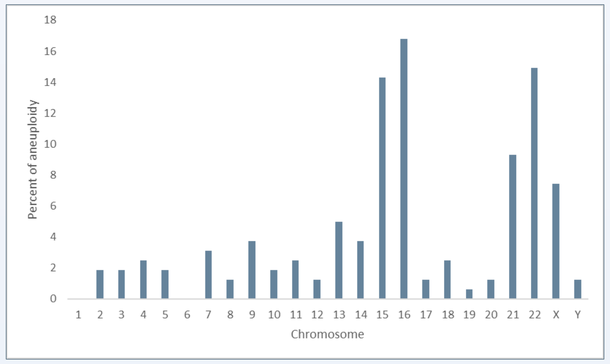

Figure 2 Frequency of aneuploidy per chromosome detected in POC tests.

DISCUSSION

The overall rate of aneuploidy was 64.7% which agrees well with the literature reporting average rates of aneuploidy higher than 60% in fertility patients.[1] As expected, the rate of aneuploidy was higher in AMA patients. It is interesting to note that considering gestational age of pregnancy loss, aneuploidy was the highest in 8-10 week losses, suggesting that this is the most common time for a chromosomal aneuploidy to cause a pregnancy loss.

The rate of aneuploidy in losses identified as RPL was not higher than the average rate of aneuploidy, suggesting that patients suffering from repeat losses are not necessarily predisposed to chromosomally aneuploid losses.

Considering maternal age, trisomy was the most common type of aneuploidy found in both young patients and in AMA patients, however, monosomy, polyploidy and deletions and duplications were more common in younger patients (Figure 1). These results agree with those of Al-Asmar et al., who reported that monosomy (especially Monosomy X) was more prevalent in young patients[2]. Of the 11 monosomy results, 10 were Monosomy X (XO) and one was an autosomal monosomy (-21). One mosaic result (mosaic trisomy 4) was found in a young patient. As expected, trisomy and multiple aneuploidy were more common in AMA patients.

When aneuploid POC results were examined at the chromosome level (Figure 2), it was determined that in this cohort of Canadian patients, the same chromosomes were commonly involved in pregnancy loss as per the literature; chromosomes 16, 22, 15, 21 and X[1,3,4]. No aneuploidies were found in chromosomes 1 or 6 (also rare in literature).

CONCLUSIONS

Pregnancy loss is a devasting event that affects thousands of Canadians every year. POC testing can help determine the cause of miscarriage, help provide answers to grieving patients, and aid in clinical decisions. Maternal cell contamination remains a problem in the determination of miscarriage cause and prevented chromosomal ploidy determination in approximately 20% of cases in this study, however, this varied depending on gestational age and method of sample collection. Despite this, the implementation of STR-analysis to NGS-based testing to identify maternal tissue, has improved the accuracy of POC testing by preventing misdiagnosis.

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