Understanding severe maternal morbidity in women pregnant by in vitro fertilization: a population-based cohort study of the Better Outcomes Registry & Network (BORN) Ontario

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ABSTRACT

Background: The use of in vitro fertilization (IVF) to achieve pregnancy increases the risk of severe maternal morbidity (SMM) – a composite outcome of severe "near miss" complications occurring at delivery – compared with unassisted pregnancy conception. Whether the elevated risk is due to infertility, maternal or paternal factors, or the treatment itself is unclear. It is plausible that the process of controlled ovarian stimulation (COS) used as part of a fresh embryo transfer (ET) cycle may contribute to this risk, mediated by high levels of estrogen and its possible impact on the endometrial lining and the vascular endothelium.

Objectives and hypotheses: The primary objective of this thesis was to evaluate the association between fresh ETs, as compared with frozen ETs, and risk of SMM during IVF pregnancy. The secondary objective was to assess whether one or more prior fresh cycles (i.e., cumulative dose of COS) is associated with SMM in women pregnant by IVF, compared with no prior fresh cycles.

Methods: Using data from the Better Outcome Registry & Network (BORN) Information System, we carried out an Ontario-wide population-based retrospective conception cohort study including 13 929 women aged 18-55 years pregnant via IVF between January 1, 2012, and March 5, 2018. The primary outcome for both objectives was a composite of SMM or mortality captured at the time of the index birth hospitalization. Secondary outcomes consisted of the most common subtypes of SMM in our cohort, which were (1) hemorrhagic events and (2) severe preeclampsia and/or cardiovascular events. We used multiple imputation to account for missing data, and univariable and multivariable log binomial regression models to estimate crude and adjusted risk ratios (RR) for primary and secondary outcomes, comparing women who had fresh ETs with those who had frozen ETs (primary objective), and comparing one or more prior fresh cycles with no prior fresh cycles (secondary objective). We performed three additional stratified analyses of the primary outcome by multi-fetal pregnancies (singletons or multiple gestation), by the presence or absence of polycystic ovary syndrome (PCOS; [i.e., PCOS or no PCOS]), and by year of conception (2013-2015 or 2015 to 2018).

Results: There were 14 812 births over the study period; after exclusions, our cohort included 13 929 IVF pregnancies (n=5660 fresh ET and n=8269 frozen-thawed ET; mean age 35.1±4.5 and 35.2 ±4.6, respectively). Women who received fresh ETs were less likely to be nulliparous, obese, or to have a diagnosis of PCOS, compared with women who received frozen-thawed ETs. Overall, 454 women had SMM (32.6 per 1000); among these, 174 conceived via fresh ETs (30.7 per 1000), and 280 conceived via frozen-thawed ETs (33.9 per 1000) (crude RR 0.91 [95% CI 0.75-1.09]). After adjustment for age at conception, nulliparity, pre-existing cardiometabolic diseases, PCOS, year of conception, and income quintile, the adjusted RR was 0.85 (95% CI 0.70-1.04). Fresh ET was associated with a significantly lower risk of hemorrhagic SMM events when compared with frozen-thawed ET (adjusted RR 0.63 [95% CI 0.48-0.82]). In contrast, fresh ET had a near-significant higher risk of severe preeclampsia when compared with frozen-thawed ET (adjusted RR 1.33 [95% CI 0.97-1.82]). In secondary analyses, there was no difference in the risk of a woman having SMM after having one cycle prior to index birth (adjusted RR 0.96 [95% CI 0.78-1.18]) or ≥2 cycles (adjusted RR 0.91 [95% CI 0.67-1.25]) when compared with no prior cycles. Our stratified analyses did not demonstrate any strong evidence indicating heterogeneity of effect of fresh versus frozen ET on SMM across strata of plurality, PCOS, and calendar year.

Conclusion: Results from this study suggest no difference in overall SMM between fresh ETs and frozen ETs. Furthermore, fresh cycles were associated with a lower risk of severe hemorrhage compared with frozen cycles while there was a trend towards increased severe preeclampsia or cardiovascular complications in fresh compared with frozen ETs. As infertility treatments become more available in Canada, better understanding associations between IVF and potential adverse maternal health outcomes can lead to improved care for individuals undergoing IVF. Given the preference for frozen ETs in recent years, it may be prudent to monitor for these complications among recipients of fresh and frozen IVF.

RÉSUMÉ

Contexte : Le recours à la fécondation in vitro (FIV) contribue à multiplier le risque de morbidité maternelle grave (MMS) - résultat composite de complications graves survenant à l'accouchement - par rapport à une grossesse non assistée. Ce n'est pas évident si ce risque élevé est dû à l'infertilité, à des facteurs maternels ou paternels, ou au traitement lui-même. Il est plausible que le processus de stimulation ovarienne contrôlée (SOC) utilisé dans le cadre des transferts d'embryons (TE) frais puisse contribuer à ce risque, en raison des niveaux élevés d'œstrogènes et de leur impact possible sur la muqueuse endométriale et l'endothélium vasculaire.

Objectifs et hypothèses : L'objectif principal de cette thèse était d'évaluer l'association entre les ET frais, par rapport aux ET congelés, et le MMS pendant la grossesse par FIV. L'objectif secondaire était d'évaluer si un ou plusieurs cycles frais antérieurs (c'est-à-dire la dose cumulative de SOC) est associé au MMS chez les femmes enceintes par FIV, par rapport à l'absence de cycles frais antérieurs.

Méthodes : À l'aide des données du système d'information du Better Outcome Registry and Network (BORN), nous avons réalisé une étude de cohorte de conception rétrospective à l'échelle de la population ontarienne incluant 13 929 femmes âgées de 18 à 55 ans enceintes par FIV entre le 1er janvier 2012 et le 5 mars 2018. Le résultat primaire pour les deux objectifs était un composite de SMM capturé au moment de l'hospitalisation de la naissance index. Les résultats secondaires étaient constitués des sous-types de MMS les plus courants dans notre cohorte, à savoir (1) les événements hémorragiques et (2) la prééclampsie sévère et/ou les événements cardiovasculaires. Nous avons utilisé l'imputation multiple pour tenir compte des données manquantes au hasard, et des modèles de régression log binomiale univariables et multivariables pour estimer les rapports de risque (RR) bruts et ajustés pour le résultat principal et les résultats secondaires, en comparant les femmes qui avaient des ET frais à celles qui avaient des ET congelés. En outre, à l'aide de modèles de régression logistique ordinale univariables et multivariables, nous avons estimé les RR bruts et ajustés pour les résultats primaires et secondaires en fonction d'un ou plusieurs cycles frais antérieurs, par rapport à l'absence de cycles frais antérieurs.

Résultats : Il y a eu 14 812 naissances au cours de la période d'étude ; après exclusions, notre cohorte comprenait 13 929 grossesses par FIV (n=5660 ET frais et n=8269 ET congelés-décongelés ; âge moyen 35,1±4,5 et 35,2 ±4,6, respectivement). Au total, 454 femmes ont présenté un MMS (32,6 pour 1000) ; parmi elles, 174 ont été conçues par des TE frais (30,7 pour 1000) et 280 par des TE congelés (33,9 pour 1000) (RR brut 0,91 [IC 95 % 0,75-1,09]). Après ajustement en fonction de l'âge au moment de la conception, de la nulliparité, des maladies cardiométaboliques préexistantes, du syndrome polycystic d'ovaire, de l'année de conception et du quintile de revenu, le RR de l'ET frais sur le SMM était de 0,85 (IC 95 % 0,70, 1,04). Le risque d'événements hémorragiques liés à la MMS était significativement plus faible avec des échantillons frais qu'avec des échantillons congelés et décongelés (RR ajusté de 0,63 [IC 95 % : 0,48, 0,82]). Les TEs frais présentaient un risque plus élevé de prééclampsie sévère par rapport aux ET décongelés (RR ajusté 1,33 [IC 95 % 0,97, 1,82]). Pour l'analyse secondaire, il n'y avait pas de différence dans le risque qu'une femme ait un MMS après avoir eu un cycle antérieur (RR ajusté 0,96 [IC 95% 0,78-1,18]) ou ≥2 cycles (RR ajusté 0,91 [IC 95% 0,67-1,25]) par rapport à l'absence de cycles antérieurs.

Conclusion : Les résultats de cette étude ne suggèrent aucune différence en termes de SMM globale entre les ET frais et les ET congelés. De plus, les cycles frais ont été associés à un risque plus faible d'hémorragie grave par rapport aux cycles congelés. Aucune différence n'a été observée en matière de prééclampsie sévère ou de complications cardiovasculaires entre les ET frais et congelés. À mesure que les traitements de l'infertilité deviennent plus accessibles au Canada, une meilleure compréhension des façons dont la FIV est associée aux problèmes de santé maternelle améliorera grandement les soins de santé maternels. Il se peut que les professionnels de la santé favorisent les cycles frais lorsqu'ils sont cliniquement indiqués, sauf dans les cas où la patiente présente un risque accru de décompensation cardiaque ou de prééclampsie grave.

ABBREVIATION LIST

Abbreviation	Meaning
BIS	BORN Information System
BMI	Body mass index
BORN	Better Outcomes Registry & Network
CARTR Plus	Canadian Assisted Reproductive Technologies Register
CI	Confidence interval
CIHI-DAD	Canadian Institute for Health Information – Discharge Abstract Database
COS	Controlled ovarian stimulation
ET	Embryo transfer
FSH	Follicle-stimulation hormone
GnRH-a	Gonadotropin-releasing hormone antagonist
hCG	Human chorionic gonadotropin
ICSI	Intracytoplasmic sperm injection
ICU	Intensive care uni
IUI	Intrauterine insemination
IVF	In vitro fertilization
OHSS	Ovarian hyperstimulation syndrome
OR	Odds ratio
PCOS	Polycystic ovarian syndrome
RR	Risk ratio
SMM	Severe maternal morbidity
WHO	World Health Organization

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PREFACE & CONTRIBUTIONS OF AUTHORS

This thesis was written following the manuscript-based thesis guidelines. I performed the literature review, established the methods and design, analyzed, and interpreted the data, and wrote the manuscript.

Drs. Natalie Dayan and Deshayne Fell assisted and supervised with the development of study design, statistical analyses, interpretation of data, and critical revision and editing of the manuscript. Dr. Olga Basso reviewed study protocol, provided statistical and content expertise throughout the study development and data analysis. Dr. Maria Velez provided expert advice on the theoretical aspects and is a co-author on the manuscript but not a committee member. Both Drs. Basso and Velez provided critical revisions of the manuscripts. Dr. Dayan acquired the funding for this study.

INTRODUCTION

Infertility is estimated to affect 10 to 15% of couples in high income countries.(1) In vitro fertilization (IVF) has contributed to the successful conception and birth of over eight million children worldwide and has experienced a continued average growth of half a million births per year.(2) In Canada, 1% to 4% of births each year are following IVF.(3, 4) The need for IVF is likely to increase alongside increases in mean maternal age, as maternal age ≥35 years is a well-established determinant of infertility.(5, 6) IVF is also an important treatment options for same sex couples or single individuals who desire children. And finally, the provincial governments of Ontario (December 2015-current)(7, 8) and Quebec (2010 – 2015 & 2020 – current)(9) implemented funding programs to facilitate access to infertility treatments. Consequently, it is likely that use of infertility treatments, particularly IVF, will continue to rise in Canada.

While IVF treatment has helped countless individuals achieve pregnancy and parenthood, it carries certain risks to the woman and her offspring. IVF has been associated with adverse obstetric and perinatal outcomes such as low birth weight, congenital abnormalities, negative metabolic outcomes, and neurodevelopmental disorders.(10-12) Maternal outcomes, on the other hand, have not been as well researched. Past studies (13-16) have found evidence to suggest that women undergoing IVF are at increased risk of severe maternal morbidity (SMM) or death - a composite indicator that measures several maternal life-threatening conditions, utilization of critical medical interventions, and maternal near-miss events (i.e., acute organ system dysfunction which, if not treated, may result in death).(17-20) In an Ontario cohort study, the risk of SMM or maternal death during pregnancy or within 42 days after birth in women who had conceived via any form of infertility treatment (i.e., IVF and related intracytoplasmic sperm injection [ICSI], intrauterine insemination [IUI], or ovulation induction alone) was 39% higher than among those with naturally-conceived pregnancies (95% confidence interval [CI] 1.23-1.56) after propensity score matching.(21) Furthermore, women who received invasive infertility treatment (i.e., IVF or ICSI) were 2 times (95% CI 1.56-3.33) more likely to have three or more severe morbidities included in the SMM composite, compared with women who received non-invasive infertility treatments (i.e., IUI or ovulation

induction alone).(21) Whether this finding is due to maternal/infertility characteristics or to treatment factors has not been determined.

Most measures of SMM using administrative health data include a variety of diagnostic and procedure codes indicating a maternal "near miss" event such as severe postpartum hemorrhage requiring transfusion or surgical intervention, need for mechanical ventilation, or severe organ dysfunction or failure.(18-20) Prior evidence suggests that patient-specific risk factors, such as advanced maternal age, obesity, hypertension, nulliparity, plurality, and type and severity of infertility have been associated with adverse outcomes in fertility-treated pregnancies.(22-25) It remains unclear, however, the extent to which treatment-specific risk factors may contribute to adverse outcomes.(26-29) For instance, fresh and frozen ETs are associated with differential risk of perinatal morbidity, likely due to controlled ovarian stimulation (COS) in the former, whereas frozen-thawed ETs only occur in subsequent cycles without ovarian stimulation. The high hormonal levels achieved during COS affect the endometrial lining as well as the vascular endothelium – factors associated with implantation, vascularization of the placenta, and potentially hypertension and proteinuria. Ovarian hyperstimulation syndrome (OHSS) can also ensue following COS, which is a potentially life threatening complication of IVF.(30-33) Fresh ETs commonly require superovulation protocols or, in cases of severe infertility, may require multiple rounds of ovarian stimulation.(29)

To conclude, SMM occurs more often in women treated for infertility compared with women who conceive spontaneously. Consequently, achieving greater insight into the ways IVF may lead to adverse maternal outcomes such as SMM can contribute important clinical knowledge for Canadian fertility care providers, allowing for the better identification and monitoring of at-risk patients. Furthermore, having in-depth knowledge on the potential determinants of SMM, such as embryo transfer type, would allow providers and patients to make better informed decisions on their infertility treatments based not only on likelihood of success but also potential complications. Thus, the overall objective of this thesis project is to

address the current knowledge gaps by investigating the associations between IVF treatment protocols and SMM in a population-based cohort within Canada.

BACKGROUND

Infertility is commonly defined as an inability to conceive following 12 months of regular unprotected sexual intercourse.(34) The reasons couples experience infertility vary, and are typically categorized as female factor (ovulatory dysfunction and anovulation, tubal infertility, endometriosis, diminished ovarian reserve, uterine and cervical factors), male factor (oligospermia), and other/unknown.(35)

There are two overarching methods to treat infertility: non-invasive and invasive infertility treatments. Non-invasive infertility treatments include ovulation induction and IUI, and are typically suited for women who are experiencing difficulties related to ovulating or cycle abnormalities.(35) In contrast, invasive treatments such as IVF, with or without ICSI, is a complex form of treatment for individuals and couples experiencing infertility and is characterized by oocyte and sperm retrieval, fertilization of sperm and oocyte outside the human body, with subsequent uterine implantation of an embryo. In instances of definitive diagnosis of infertility such as full fallopian tube blockage or severe male-factor infertility, IVF treatment is often the only recourse available to patients.(35)

The following sections define IVF procedure broadly and review COS protocols more specifically. I will subsequently discuss the potential risks of IVF and its association with SMM.

1. DEFINITION OF IN VITRO FERTILIZATION

Standard IVF procedure involves transvaginal retrieval of mature oocytes from the ovarian follicle and insemination in vitro by mobile sperm.(36) This process results in a zygote which, after a minimum of three days, divides into a multicellular embryo. Alternatively, in cases of severe male factor infertility, the fusion of oocyte and sperm can be accomplished by ICSI, an intervention that involves injecting the sperm

directly into the cytoplasm of an oocyte.(37, 38) Crucial elements for successful IVF include appropriate ovarian stimulation and endometrial receptivity.

1.1 OVARIAN STIMULATION PROTOCOLS

In preparation for IVF, there are multiple methods used to achieve oocyte maturation and subsequent retrieval. Some treatment plans rely on endogenous triggers to oocyte maturation - commonly known as ***natural cycle IVF***, while others employ exogenous hormones to trigger ovary stimulation – known as ***stimulated cycle IVF***.(38)

1.1.1 Natural Cycle IVF

The first successful IVF pregnancy and live birth was achieved using natural cycle IVF.(39, 40) This practice involves retrieving a patient's oocyte from the dominant follicle formed during a normal menstrual cycle.(40, 41) Specifically, oocyte maturation is achieved without the administration of exogenous stimulants, resulting in the production of a single oocyte per cycle. The retrieved oocyte is then fertilized and cultured in vitro.

Oocyte retrieval was initially achieved through laparoscopy, an invasive surgical procedure by which small incisions are made to the pelvis, allowing a surgeon to identify and retrieve the matured oocyte.(42, 43) Given that natural cycles relied on the successful maturation of a single oocyte, paired with the invasive nature of a laparoscopy, COS soon became the primary focus in clinical practice. This treatment allowed for production and retrieval of multiple oocytes within a single cycle, thus maximizing the success rates and minimizing the need for subsequent laparoscopies.

However, natural cycle IVF is still in use in some situations. For instance, this treatment is typically less costly and involves fewer injections than stimulated cycles.(44) Furthermore, stimulated IVF cycles may impose significant stress on patients, impacting their ability to conceive or complete the treatment.(45, 46) Natural IVF is often considered in instances where the patient is considered vulnerable to OHSS or when the infertility is attributed to male factor.(40) However, natural IVF typically has a lower success rate

per initiated cycle and a longer time-to-pregnancy when compared with stimulated cycles, particularly in situations of advanced maternal age (i.e, ≥ 35 year).(47) As such, COS remains the status quo in IVF treatments.(48, 49)

1.1.2 Stimulated Cycle IVF

The addition of COS to IVF protocols has increased the success rate of IVF treatment, particularly in those with reduced ovarian reserves. However, COS can take an emotional and financial toll on patients. Although the Ontario Fertility Program was launched to improve accessibility to infertility treatments, the program currently only funds one IVF cycle.(50) Given that remaining costs are largely out-of-pocket, the selection of an effective and appropriate stimulation protocol is critical.

There are two dominating COS protocols. The first more conventional IVF protocol, known as the ***long protocol***, begins with the downregulation of the pituitary gland by a gonadotropin-releasing hormone antagonist (GnRH-a) to achieve low levels of estrogen and, ultimately, halt follicular development. Following this cessation, the ovaries become stimulated by the exogenous administration of FSH, resulting in follicular development with a simultaneous estrogen surge. When opting for a stimulated IVF cycle, FSH provokes a surge of estrogen of between 5 000 - pmol L-1 and 20 000 pmol L^{-1}.(38) An ultrasound of the ovaries is then utilized to confirm the presence of suitable follicles, after which human chorionic gonadotropin (hCG) is administered for the final maturation of the oocytes and the subsequent oocyte extraction.(38, 51) In contrast, ***short protocols*** skip the downregulation phase and begin immediately with the administration of FSH for five to nine days before being paired with GnRH-a until an ovarian ultrasound confirms the presence of suitable follicles.(52) In the final step of either a long or short protocol, hCG is administered for the maturation of the oocytes and subsequent oocyte retrieval.(38)

Weighing the costs and benefits of short and long protocols can be challenging. The former often requires a shorter treatment phase and is more cost-effective while the latter is associated with higher pregnancy rates.(53, 54) For instance, it may be appropriate for someone of advanced maternal age to opt for the long protocol. Alternatively, a patient with a high likelihood of implantation success may consider

the short protocol for the reduced financial burden. As with all aspect of IVF, COS protocols must be carefully selected according to patient needs.

1.1.3 Short Term Health Risks of Controlled Ovarian Stimulation

COS protocols have evolved to accommodate patient needs. Although stimulated cycle IVF is believed to be safe and preferred by most patients, there may be unintended health risks in the short term that require close monitoring. For instance, there is a risk of a patient developing ovarian hyperstimulation syndrome (OHSS) during a stimulated cycle; this serious condition is characterized by ovarian enlargement and an accumulation of extravascular exudate, leading to hemoconcentration, decreased perfusion of vital organs, increased risk for thromboembolism, and organ failure.(55-57) OHSS can be mild, moderate, severe and, in very rare situations, fatal.(56) OHSS typically occurs a minimum of 10 days after the hCG injection and is believed to be related to the concomitant secretion of placental hCG.(57) The incidence of moderate to severe OHSS has been reported to range between 3% and 8% of the women undergoing COS in the general population and increases from 10% to 20% in high-risk populations, such as women with polycystic ovary syndrome (PCOS) – a population that, owing to ovulatory dysfunction, requires intensive COS to achieve oocyte maturation.(29, 58)

It has been suggested that the process of COS during a treatment cycle may cause structural and biochemical alterations to the endometrium.(59-61) This structure comprises tubular glands located in a cellular vascular stromal and varies in thickness in response to hormonal ovarian influence.(62) Studies comparing the endometrium following IVF cycles with COS, with natural IVF cycles, have demonstrated a greater proportion of glandular-stromal dyssynchrony among the former, potentially resulting in an endometrium that is at a more mature phase than in natural cycles.(63-65) That is, elevated estradiol and progesterone levels are associated with inappropriate development of the stroma relative to the glands in the periovulatory period.(32, 63, 66) As such, COS may paradoxically adversely affect endometrial receptivity in some patients, impacting on later development of perinatal and obstetric outcomes such as placental disorders (placenta previa syndromes) and preeclampsia.

1.2 FRESH AND FROZEN EMBRYO TRANSFER

The success of IVF relies on three main parameters: (i) embryo quality, (ii) endometrial receptivity, and a (iii) balanced embryo-endometrium interaction.(67) It is important to consider all three elements when deciding on embryo transfer type.

1.2.1 Embryo Transfer Type

There are two dominant types of embryos used in IVF: fresh and frozen. Fresh ETs refer to embryos transferred directly to the uterus after fertilization, within the same cycle as the COS.(33, 36) Alternatively, frozen ETs indicate the transfer of a frozen/thawed embryo to an individual, thus allowing for the stimulation and embryo transfer phases to be separate.(68) When multiple viable oocytes are retrieved within a single stimulation cycle, the remaining embryos can be cryopreserved to administer in subsequent cycles, in the instance of a failed cycle. Alternatively, in the freeze-all strategy, the entire cohort of embryos are cryopreserved, and the best quality embryos are transferred in a subsequent cycle once the endometrium has returned to baseline.(73, 74)

Originally indicated for individuals at risk of OHSS,(83) frozen ETs are now a viable alternative to fresh ETs for most patients of IVF and have been growing in popularity. In Canada, 33.3% of embryo transfers in 2013 were frozen. In 2018, this proportion increased to 48.0%.(84) This trend is not unique to Canada, as similar observations have been made in the United States,(85) in Europe,(86) and in Latin America.(87) Contributing factors to this increase in frozen ETs include the improvements in cryopreservation techniques, the increase in preimplantation genetic screening cycles, and the popularity of the “freeze-all” model.(88-91) The cryopreservation of embryos has become a common approach used by fertility clinics to circumvent the asynchrony between endometrium receptivity and time of embryo implantation.

1.2.2 Endometrial Preparation Prior to ET

Endometrial receptivity occurs when the endometrium has reached maturity and is ready to accept the implanting embryo.(62) A defective endometrium can result in foetal and maternal complications.(62, 69) As such, correct endometrial preparation is vital to a healthy pregnancy.

As previously discussed, the supraphysiological levels of hormones associated with COS are associated with the inappropriate development of the endometrium. Although frozen ETs occur in subsequent cycles to the COS, preparation of the endometrium is still necessary for frozen cycles to maximize endometrial receptivity. There are two main types of endometrial preparation in frozen ETs: ***natural cycles*** and ***artificial cycles***.

Natural cycle ET can be further divided into two subtypes: true natural cycle and modified natural cycle. In true natural cycles, the embryo can be transferred by timing the transfer based on the peak of luteinizing hormone, a hormone vital for ovarian trigger, or by ultrasounds to measure follicle size. Modified natural cycles, a hCG trigger is administered the facilitated embryo transfer scheduling.(70) However, these methods are only appropriate in individuals with regular menstrual cycles and ovulation may be challenging to detect.(71)

Stimulated cycles with estradiol and progesterone were introduced as a solution to improve control over frozen ET preparations.(70) Although the majority of frozen transfers used stimulated cycles, there is disagreement about which method of endometrium preparation is best.(72-74) Notably, no corpus luteum is created in stimulated ETs due to ovulation suppression. The corpus luteum is an endocrine gland formed from the remains of an ovarian follicle and is involved in the production of progesterone and oestrogen during pregnancy.(38) It has been suggested that the lack of a corpus luteum may drive the risk of preeclampsia and cardiovascular risks in pregnancy, given the lack of vascular growth factor and endogenous vasoactive agents, primordial for healthy vasoactivity.(70, 75, 76)

Given the intricacy of embryo-endometrium interactions and the complexity of endometrial preparation, careful consideration of the implications of natural and stimulated cycles is necessary. Specifically, a review of IVF procedures highlights a knowledge gap surrounding endometrial preparation and the contribution of the corpus luteum towards a healthy pregnancy.

1.2.3 Knowledge Gaps in Maternal Health After IVF

Over the years, success rates in IVF pregnancy has been extensively researched. However, the role of IVF on secondary maternal health outcomes has received less attention. Recent studies have confirmed an excess risk of serious maternal complications in pregnancy – globally termed severe maternal morbidity – in IVF as compared with unassisted pregnancies.(21, 77, 78) Whether this occur primarily in fresh or frozen embryo transfer cycles is less clear. In a 2018 systematic review and meta-analysis of 6 studies comparing outcomes of IVF cycles between fresh and frozen embryo transfers, Roque et al. underlined the ongoing uncertainty of which embryo implantation type is best for infant and maternal health.(64) With the exception of placenta previa, this study found frozen ETs to be associated with greater risk of all obstetric outcomes (i.e., pregnancy-induced hypertension, preeclampsia, and placenta accrete).(64) In contrast, an earlier systematic review of 11 studies by Maheshwari et al. sought to assess the risk of antepartum hemorrhage and emergency caesarean according to embryo type, concluding that pregnancies arising from frozen ETs appeared to have better obstetric outcomes when compared with fresh ETs.(79)

The link between IVF and SMM has recently been studied by several groups, including my supervisors.(21) However, considering the many subtypes of SMM and their differing pathophysiology, there remains a knowledge gap regarding the role of embryo transfer type and cumulative dose of COS on SMM risk.

2.0 EPIDEMIOLOGY AND PATHOGENESIS OF SEVERE MATERNAL MORBIDITY

Formal surveillance of SMM first began in 2004 by the World Health Organization (WHO) with the goal of monitoring progress towards worldwide improved maternal healthcare.(80) In high income

countries where maternal mortality is exceedingly rare, SMM has become an appropriate proxy by which to measure the quality of a country's maternal healthcare.

2.1 INCIDENCE AND ETIOLOGY

SMM comprises a range of diagnoses signifying a life-threatening pregnancy-related condition and contributes to several short- and long-term consequences for women and their families. The concept originated from case reviews of near-miss complications/sentinel hospital events and has evolved into an aggregate of diagnostic indicators that has garnered significant attention from researchers and policy makers internationally.(81-83) Through a delphi process, SMM became an alternative to maternal mortality for surveillance and identifying priorities in maternal health care.(84) Today, the definition includes validated scoring systems and diagnostic coding algorithms in administrative health datasets.(85) Definitions of SMM vary due to lack of international consensus with respect to which conditions or procedures should be included under the umbrella of SMM. Depending on the definition utilized, SMM is believed to impact up to 2% of births (86-88) and continues to increase in North America and Europe.(87, 89)

2.1.1 Standard Canadian Definition of SMM

In Canada, the Canadian Perinatal Surveillance System (CPSS) developed a comprehensive definition of SMM that comprises disease-based, procedure-specific, and organ failure codes classified into 44 subtypes of SMM.(88) This definition can be operationalized using validated perinatal data from the Canadian Institute for Health Information's (CIHI) Discharge Abstract Database (DAD), specifically 76 *International Classification of Diseases* Canadian implementation, version 10 (ICD-10-CA) diagnostic codes, 24 Classification of Health Interventions (CCI) intervention codes, and three CIHI-derived variables (Appendix Table S1). (90-92) These indicators were chosen based on their association with a high case fatality and prolonged hospitalization.(83) Using this definition, the CPSS reported that, between 2005 and 2014, the overall rate of SMM increased from 14.0 (95% CI 13.5-14.4) to 16.4 (95% CI 15.9-16.9) per 1,000 deliveries, then declined to 14.2 (95% CI 13.7-14.6) per 1,000 between 2014 and 2015. During this time (i.e., 2010 to 2015), the most common SMM subtypes were blood transfusion with or without comorbidity;

cardiac arrest, myocardial infarction, or pulmonary edema; embolization or ligation of pelvic vessels or suturing of uterus and postpartum hemorrhage, and hysterectomy.(93)

2.1.2 Patient Predictors of SMM

Advanced maternal age was the third most common reason for infertility treatment in 2019.(94) This observation is not surprising, given the societal trend of delayed childbearing in high-income countries.(95, 96) This patient characteristic is commonly associated with subfertility and negative obstetric and perinatal outcomes,(97, 98) and often coincides with other determinants of SMM. In particular, obesity and associated cardiovascular risk factors are both more common in older mothers and associated with neonatal morbidity and mortality and with SMM.(26, 27, 34) Given that the prevalence of cardiovascular risk factors among reproductive-aged persons has increased, with chronic hypertension affecting about 5% of pregnancies and pre-existing or congenital heart disease complicating 0.2 to 4% of pregnancies,(99, 100) it is not surprising that cardiac arrest or cardiovascular complications are one of the most common presentations of SMM in Canada. Risk stratification is especially vital in patients with higher risk of adverse outcomes, particularly with regards to those living with cardiometabolic or reproductive comorbidities. In an effort to synthesize this data and offer guidance to clinicians caring for women with cardiovascular disease seeking infertility treatment, I along with Drs. Dayan and Velez, performed a comprehensive narrative review of infertility treatments in people with cardiovascular disease, highlighting elements to consider when designing IVF protocols for these women. The manuscript was published online this year in the Canadian Journal of Cardiology (https://www.onlinecjc.ca/article/S0828-282X(21)00701-7/ppt; see Appendix 1 for full manuscript).

2.2 ASSOCIATIONS BETWEEN IVF AND SMM

Although infertility treatments such as IVF are great scientific advancements and allow for parenthood in couples with difficulty conceiving, they are not without risks to the woman. For instance, IVF pregnancy has been associated with a greater risk of caesarean delivery and preterm birth, preeclampsia and

other perinatal complications compared with unassisted pregnancy.(3) The association between IVF and SMM more broadly has been subject of considerable interest in recent years.

In a Canadian cohort study published in 2019, Drs. Dayan, Fell, and others evaluated the association between infertility treatments and SMM in pregnancy and the postpartum period.(21) Using a propensity score-matched cohort where pregnancies achieved through infertility treatment (n =11 546) were matched 1:4 with spontaneously-conceived pregnancies (n = 47 553), and followed until 42 days postpartum. The primary outcome (i.e., SMM or maternal death) occurred in 356 treated-pregnancies (30.8 per 1000 deliveries) compared with 1054 untreated pregnancies (22.2 per 1000 deliveries); adjusted RR 1.39 (95% CI 1.23–1.56) in the matched cohort. Moreover, the adjusted odds ratio (OR) for having 3 or more indicators of SMM was highest in women undergoing an invasive form of infertility treatment, adjusted OR 2.28 (95% CI 1.56-3.33), compared with spontaneous conceptions. By comparison, there was no association found for patients in the non-invasive treatment group when compared with spontaneous conceptions, adjusted OR 0.90 (95% CI 0.57-1.72).(21)

The mechanism for this increased risk of SMM and maternal death in women pregnant by IVF is not known. A principal challenge in identifying risks factors for SMM in IVF patients is the variability of IVF protocols across clinics and between countries. For instance, there has been a push in Canada towards single embryo transfers to reduce adverse outcomes.(4) In addition, protocols regarding the type of cycle used vary widely according to the individual patient's needs, available technologies, and protocols. As previously discussed, variability in practice regarding the type of embryo transfer in select patients (fresh or frozen-thawed), the number of repeated COS cycles, and the number of embryos transferred in a single cycle. For all these reasons, identifying specific risk factors can prove challenging.

Authors of a 2019 cohort study in the United States aimed to elucidate this question.(77) Through the analysis of 1,477,522 pregnancies spanning 8 states, researchers were able to evaluate the risk of SMM by maternal fertility status, oocyte source, and embryo state combinations. Fewer women undergoing fresh embryo transfer, compared with FET, had prior IVF cycles. Using spontaneous conceptions as a reference group and limiting to singleton vaginal births, researchers found that the likelihood of requiring blood

transfusion was greatest among recipients of frozen embryo transfers (adjusted OR 1.94 [95% CI 1.60-2.36], followed by fresh embryo transfers (adjusted OR 1.33 [95% CI 1.14-1.54]. (77) Overall, the investigators concluded that the risks of SMM were greater in subfertile and IVF births, particularly in donated, frozen embryo transfers. However, since this study depended on birth certificates to ascertain obstetric outcomes, the findings may be subject to non-differential misclassification, biasing the effect towards the null. The cohort was also limited to live vaginal births, possibly introducing selection bias. Furthermore, it is important to study this relationship within a Canadian context. Canada's universal healthcare system and the BORN information system (BIS) are uniquely positioned to capture perinatal data by providing accessible hospital care to all, in turn limiting this thesis' susceptibility to selection bias.

In summary, prior research investigating the relationship between the IVF and SMM indicators show a strong link between invasive forms of infertility treatment, such as IVF, and greater risk for at least one indicator of SMM. It remains unclear which specific components of IVF contribute to increased risk of SMM. Give the prior research on COS and its role in OHSS, it maybe possible that COS may also be related to increased risks of SMM.

SUMMARY AND RATIONALE FOR CURRENT STUDY

Invasive infertility treatments such as IVF are associated with a greater chance of conceiving but may confer some unintended risks to maternal health. Given the numerous protocols that exist for IVF, careful consideration is required to tailor each treatment to the individual according to their chance of success and risk for complications. Among this plethora of decisions include the consideration of employing a fresh or frozen-thawed embryo strategy, using COS processes, and to weigh the costs and benefits of single embryo transfers compared with multiple embryo transfers. Past hypotheses have posited that COS—used for fresh ET—may impact the intrauterine environment and the vascular endothelium and therefore affect maternal outcomes in IVF pregnancies. Furthermore, women with more severe infertility who are exposed to repeated rounds of COS may be at particular elevated risk of adverse outcomes in the short term due to cumulative dose of supraphysiologic estradiol. While it is known that IVF pregnancies are associated

with SMM, understanding treatment aspects that underlie this association will allow for more nuanced counseling and treatment decisions that consider both chances of success and risk of complications.

OBJECTIVES AND HYPOTHESES

Overall objective:

The overall objective is to assess for an association between fresh embryo transfers, as compared with frozen-thawed embryo transfers, with SMM in women pregnant by IVF.

Specific objectives:

Among women pregnancy by IVF,

1. To quantify the rate and relative risk of SMM and its most frequent components (hemorrhage and severe preeclampsia with or without cardiovascular complications) occurring at the index birth hospitalization comparing those who became pregnant following fresh embryo transfer with those who underwent frozen-thawed embryo transfer.

Given that COS is typically required in fresh and not frozen embryo transfers, with associated high estradiol levels and its downstream consequences, we hypothesize that the former will have a higher risk of hypertensive and cardiovascular subtypes of SMM.

2. To assess whether the rate and relative risk of primary (i.e., composite SMM) and secondary (i.e., hemorrhage or severe preeclampsia with or without cardiovascular complications) outcomes among women who successfully conceived after a single COS cycle differ from outcomes among women with 1 or more prior failed IVF treatments following COS cycles.

We hypothesize that women who require several rounds of IVF will have a higher risk, after adjustment for confounders, thus reflecting both more severe infertility, and a cumulative effect of repeated rounds of COS.

PREFACE TO MANUSCRIPT

An increasing number of Canadians seek assisted reproductive treatments to facilitate pregnancy. Prior research by Drs. Dayan, Fell, Basso and colleagues found an association between IVF, compared with spontaneous conceptions, and SMM.(21) This current manuscript aims to build upon these findings to assess whether fresh versus frozen embryo transfers, and number of fresh cycles, are associated with SMM in a similar population-based cohort in Ontario.

This manuscript has been drafted in accordance with the British Medical Journal (BMJ) publication guidelines and will be submitted for publication in late 2021 - early 2022. To our knowledge, this is the first Canadian cohort study to link the BORN Information System (BIS) with the Canadian Assisted Reproductive Technologies Register (CARTR-Plus) and the Canadian Institute for Health Information Discharge Abstract Database (CIHI-DAD) via unique patient identifiers with the goal of assessing risk of SMM between embryo transfer types and number of stimulation cycles.

MANUSCRIPT

FRESH VERSUS FROZEN EMBRYO TRANSFER AND RISK OF SEVERE MATERNAL MORBIDITY IN PREGNANCIES CONCEIVED BY IN VITRO FERTILIZATION

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ABSTRACT

Objective: Among pregnancies conceived by in vitro fertilization (IVF), to quantify the risk of severe maternal morbidity in fresh versus frozen-thawed embryo transfers.

Design: Retrospective cohort study using a province-wide assisted reproductive technology registry linked with a birth registry in Ontario, Canada.

Participants: 13 929 women aged 18 to 55 years pregnant via IVF between January 1, 2012, and March 5, 2018 and who delivered live or stillborn infants within an Ontario hospital at ≥20 weeks' gestation.

Main outcome measures: The primary outcome was a composite of severe maternal morbidity or maternal death, defined according to the Canadian Perinatal Surveillance System (CPSS).

Results: 174 IVF patients who conceived via fresh embryo transfers had severe maternal morbidity (30.7 per 1000), compared with 280 among IVF patients with frozen embryo transfers (33.9 per 1000); adjusted risk ratio (RR) 0.85 (95% confidence interval [CI] 0.70-1.04). Fresh cycles were associated with a lower risk of hemorrhagic events compared with frozen cycles; adjusted RR 0.63 (95% CI 0.48-0.82). In contrast, there was a trend towards a higher risk of severe preeclampsia and cardiovascular complications in fresh cycles compared with frozen cycles; adjusted RR 1.33 (95% CI 0.97-1.82).

Conclusion: We found no difference in overall severe maternal morbidity between fresh and frozen embryo transfers, and among individuals who conceived after first or previous cycles. Fresh cycles were associated with a lower risk of severe hemorrhage compared with frozen cycles. No difference was observed in severe preeclampsia or cardiovascular complications between fresh and frozen cycles. Although increasing in popularity, a freeze-all strategy may not be appropriate in all instances. Instead, an individualized approach based on chances of success and risk of specific complications may eventually guide IVF management.

INTRODUCTION

In high income countries, severe maternal morbidity has replaced maternal mortality as a key indicator of quality care in obstetrics.(1, 2) Severe maternal morbidity refers to a serious medical complication or near-fatal event occurring during, or within 42 days of, a pregnancy.(3) Although the definition varies somewhat across countries, severe maternal morbidity has been found to affect up to 2% of births and continues to increase in North America and Europe.(4-6) In Canada, where severe maternal morbidity indicators have been identified and validated using diagnostic and procedure codes in hospital administrative data, and the rate in 2015 was estimated to be 14.2 per 1,000 deliveries.(7)

Prior studies have reported that, compared with spontaneous conceptions pregnancies, pregnancies conceived via assisted reproductive technologies such as IVF have an approximate 2-fold elevated risk of severe maternal morbidity.(3, 8, 9) A proposed hypothesis is that this may be due to underlying patient-related determinants of health.(10-12) However, it remains uncertain whether treatment-related factors contribute to this association, such as the type of embryo transfer cycle (i.e., fresh or frozen).(13-15) Controlled ovarian stimulation and associated supraphysiological estradiol concentrations that occur immediately preceding fresh embryo transfer are suspected to contribute to a dysregulated endometrium.(9, 13, 16) Conversely, frozen embryo transfers have been linked to the absence of corpus luteum, an endocrine gland tied to the production of vasoactive hormones necessary for healthy vascularization of the placenta.(17, 18) Therefore, it is plausible that fresh embryo transfers increase the risk of some forms of maternal morbidity while frozen-thawed embryo transfers increase the risk of others.

While researchers have assessed the risks associated with fresh and frozen embryo transfers when compared with spontaneous conceptions,(9) an unresolved question is which type of embryo transfer is associated with greater risk of severe maternal morbidity within a subfertile population. Prior studies comparing fresh and frozen cycles have yielded diverse results, with some reporting better obstetric outcomes among recipients of frozen cycles when compared with those pregnant from fresh cycles.(19,

20) Nevertheless, these meta-analyses only investigated specific subtypes of severe maternal morbidity as secondary outcomes and may not fully capture the relationship between embryo type and severe maternal morbidity. In contrast, a recent large-scale cohort study by Luke et al. reported that frozen embryo transfers were associated with greater risk of postpartum hemorrhage.(9) However, this cohort only included vaginal live births, potentially introducing selection bias. Given these alternative narratives, it is unclear how fresh and frozen cycles are associated to severe maternal morbidity.

To address these remaining questions, we conducted a population-based retrospective cohort study of women pregnant by IVF in the province of Ontario, Canada, with the aim of assessing for an association between IVF treatment factors (e.g., type of embryo transfer and number of previous fresh cycles) and severe maternal morbidity.

METHODS

We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting this study.(21)

Study design and data sources

We performed a province-wide retrospective cohort study in Ontario, using data from the provincial birth registry, BORN Ontario (www.bornontario.ca). The BORN Information System (BIS) collects detailed demographic and clinical information for pregnancies resulting in a live or stillbirth at ≥20 weeks' gestation, capturing over 99% of all Ontario hospital deliveries.(22)The BIS data are considered to be of good quality, with moderate to high levels of agreement between the BIS and data collected through chart re-abstraction.(23) The BIS is linked, through unique patient identifiers, to the Canadian Assisted Reproductive Technologies Register Plus (CARTR Plus) from pregnancies conceived in 2013 onwards. CARTR Plus is the national ART registry, which receives and manages voluntarily reported data from 97% of IVF centres within Canada.(24) A data quality study revealed kappa

coefficients greater than 0.90 for most analyzed variables, whereas the number of embryos transferred in a given cycles was found to have moderate agreement.(25)

We additionally linked the BIS with the Canadian Institute for Health Information Discharge Abstract Database (CIHI-DAD), which includes information on diagnoses and procedures during hospital admissions, including obstetric delivery admissions.(26) Up to 25 diagnoses are coded using the *International Statistical Classification of Diseases* diagnoses and procedure codes, Canadian implementation, version 10 (ICD-10-CA) and procedures using the Canadian Classification of Health Interventions (CCI). Finally, Canadian Census data were obtained through linkage with the BIS.

Study population

We identified a conception cohort consisting of women who conceived between January 1, 2013 and March 5, 2018 following IVF and who delivered live or stillborn infants within an Ontario hospital at ≥20 weeks' gestation. This timeframe was chosen to reflect the availability of data from CARTR Plus, which began collecting treatment cycle information on January 1, 2013.(24) All study subjects were residents of Ontario, between 18 and 55 years of age at the start of the IVF cycle, and were registered with Ontario's universal health insurance program. We excluded pregnancies that resulted in ectopic or molar pregnancies, therapeutic abortions, and pregnancy losses.

Study outcomes

The primary study outcome was a composite of any severe maternal morbidity or maternal mortality occurring at the time of the index birth hospitalization. The outcome was determined based on a modified definition proposed by the Canadian Perinatal Surveillance System (CPSS) which includes a comprehensive list of 47 unique severe maternal morbidity indicators, identified by ICD-10-CA and CCI codes (Supplementary Table 1).(27, 28) Our definition was identical to that of the CPSS with the exception of maternal intensive care unit (ICU) admissions, information that was unavailable in our dataset. This composite outcome has previously been validated through assessments of fatality rates and

length of hospitalization for each indicator.(27) Women were coded as having had a severe maternal morbidity "event" when one or more conditions within the severe maternal morbidity composite were documented. Additionally, we assessed the two most frequently occurring severe maternal morbidity subtypes in this dataset: severe obstetric hemorrhage and severe preeclampsia with or without cardiovascular complications.(7) Severe maternal morbidity subtypes were categorized based on disease and treatment commonalities in adherence with similar recent Canadian studies (Supplementary Table 1).(6, 27, 29)

Study exposures

For the primary analysis, we considered patients as "exposed" if they conceived following a fresh embryo transfer and as "unexposed" if they received a frozen embryo transfer or frozen oocyte IVF. For the secondary analyses, the exposure was defined categorically, as none, 1, or 2 or more ovarian stimulation cycles prior to the index pregnancy with none as the reference. (25)

Covariates

Based on substantive knowledge, including evidence regarding fresh cycles and severe maternal morbidity,(10-12, 30) we decided *a priori* to assess the following potential confounders and effect modifiers in multivariable models: maternal age at conception (years), neighbourhood income (quintiles; lowest quintile as reference), calendar year of conception (2013 as reference), parity (nulliparous vs. multiparous), diagnosis of polycystic ovarian syndrome (PCOS; yes/no), and pre-existing cardiometabolic disorders (yes/no; see footnote to Table 1). Except for neighbourhood income quintile, obtained from Canadian Census data, all other covariates were directly abstract from patient charts and available in the BIS dataset. Cardiometabolic disorders was defined using a combination of BIS and CIHI-DAD (Supplemental Table 1).

Statistical analysis

We examined distributions of baseline demographic and clinical characteristics according to embryo transfer type (fresh vs. frozen). We tested continuous variables for normality using the Shapiro-Wilk test and log transformed if non-normal distributions were found.(31) Means and standard deviation (SD) or median and interquartile range (IQR) were calculated for continuous variables. We reported the overall number of severe maternal morbidity events and rates per 1000 deliveries. The number of composite outcome events and those in each subtype are similarly reported according to primary exposure status.

We used univariable and multivariable log-binomial regression models to estimate crude and adjusted RRs, and 95% CI of severe maternal morbidity or death at the index birth hospitalization, comparing women who had fresh embryo transfers with those who had frozen embryo transfers. Next, we used univariable and multivariable log-binomial regression models to estimate crude and adjusted RRs and their respective 95% CI, with prior stimulation cycles (i.e., 1 controlled ovarian stimulation cycle prior to the index pregnancy vs. none and ≥ 2 controlled ovarian stimulation cycle prior to the index pregnancy vs. none) as the exposure. As earlier studies have identified a higher risk of SMM in multiple pregnancies,(32, 33) we assessed effect modification by plurality by examining the heterogeneity of crude and adjusted RRs across two strata (singleton or multiple). In addition, we assessed effect modification for indication for treatment (polycystic ovarian syndrome [PCOS] vs. no PCOS) since individuals with PCOS are more likely to require intensive controlled ovarian stimulation.(34) Finally, given the temporal trend towards more frozen cycles in more recent years (Figure 2), we assessed effect modification over two periods of time: 2013-2015 or 2016-2018.

To address missing data, the baseline distributions of individuals with complete information on all variables were compared to those with incomplete information on one or more variables. Based on prior validation studies and BORN missing data reports,(25, 26) and patterns of missingness, we concluded that data were likely missing-at-random. The majority (82%) of subjects had complete information on all variables. Among subjects with incomplete data, the only variables with greater than 10% missingness

were BMI and pre-existing cardiometabolic disease with 14.7% and 14.5% missing, respectively. We performed a fully conditional specification (FCS) imputation model using PROC MI to generate 20 imputed datasets, including the same variables adjusted for in the main regression model.(35). The FCS method allows for unique imputation models for each variable. These 20 iterations were then pooled using PROC MIANALYZE to generate valid statistical inferences and subsequently analyzed. All statistical procedures were conducted using SAS software, version 9.4 (SAS Institute, Inc., Cary, NC).

Ethics approval

This study received approval from the Better Outcomes Registry & Network, and ethics approval from the Children's Hospital of Eastern Ontario Research Ethics Board, and the McGill University Research Ethics and Compliance Board. Since the study used secondary data, requirement for individual consent was waived.

RESULTS

From January 1, 2013 to March 5, 2018, there were 14 812 births in Ontario hospitals conceived by IVF (Figure 1). Following exclusions, the study population comprised 13 929 subjects who had conceived via IVF, with 5660 (40.6%) after fresh embryo transfers and 8269 (59.4%) after frozen embryo transfers (Figure 1, Table 1). The mean age of the study sample was (35.2 ± 4.6) and was similar between fresh and frozen groups. On average, recipients of fresh embryo transfers were more often nulliparous and less often diagnosed with unexplained infertility (Table 1). A greater proportion of patients with fresh embryo transfers received multiple embryo transfer and had a multiple pregnancy (Table 1). The proportion of individuals receiving frozen embryo transfers increased over time, from 42.8% in 2013 to 76% in 2018 (Figure 2).

Overall, there were 454 severe maternal morbidity events (32.6 per 1000); 174 severe maternal morbidity events among recipients of fresh embryo transfers (30.7 per 1000) and 280 events among recipients of frozen embryo transfers (33.9 per 1000; Table 2). The crude and adjusted RRs for a

composite of any severe maternal morbidity event comparing fresh embryo transfers with frozen embryo transfers were similar at 0.91 (95% CI 0.75-1.09) and 0.85 (95% CI 0.70-1.04), respectively (Table 3). The most common component indicators of severe maternal morbidity were postpartum hemorrhage (17.3 per 1000), severe preeclampsia or cardiovascular complications (11.0 per 1000), surgical complications (3.0 per 1000), puerperal sepsis (1.9 per 1000), acute renal failure (1.7 per 1000), assisted ventilation (1.6 per 1000), and other (3.9 per 1000; Table 2). There were no maternal deaths during the study period.

Among fresh embryo transfer pregnancies, 74 individuals experienced at least one severe hemorrhagic event (13.1 per 1000), compared with 167 among frozen embryo transfers (20.2 per 1000; Table 3), yielding an adjusted RR of 0.63 (95% CI 0.48-0.82; Table 3). In contrast, there were 76 cases of severe preeclampsia or cardiovascular complications among fresh embryo transfer pregnancies (13.4 per 1000), as compared with 77 among frozen embryo transfer recipients (9.3 per 1000), corresponding to an adjusted RR of 1.33 (95% CI 0.97-1.82; Table 3).

The risk of severe maternal morbidity at time of delivery did not differ according to number of controlled ovarian stimulation cycles prior to the index pregnancy. Within the cohort, 211 women with one prior cycle had at least one severe maternal morbidity event (31.2 per 1000), compared with 155 women with no prior fresh cycles (35.8 per 1000), yielding an adjusted risk ratio of 0.96 (95% CI 0.78-1.18; Table 4). Similarly, 88 women with 2 or more prior fresh cycles experienced at least one severe maternal morbidity event (32.1 per 1000), compared with no prior fresh cycles, resulting in an adjusted RR of 0.91 (95% CI 0.67-1.25).

The absolute risk of severe maternal morbidity in fresh and frozen groups was higher among multiple gestations (62.71 and 63.64 per 1000), compared with singletons (24.88 and 30.32 per 1000); however, no significant heterogeneity was observed in the effect of fresh versus frozen embryo transfers on severe maternal morbidity across strata of singletons and multiples (adjusted RR of 0.82 [95% CI 0.66-1.02] and 0.89 [95% CI 0.61-1.27], respectively) (Table 5). Furthermore, no significant heterogeneity was noted in the effect of fresh versus frozen embryo transfers on severe maternal morbidity among subjects

with or without pre-existing PCOS (adjusted RR 0.58 [95% CI 0.26-1.28] and 0.89 [95% CI 0.73-1.08], respectively). Between the years 2013-2015, the absolute risk of severe maternal morbidity for fresh and frozen embryo transfers was 31.92 and 32.56 per 1000, respectively. Between the years 2016-2018, the absolute risk decreased among recipients of fresh embryo transfers (28.86 per 1000) and increased in frozen embryo transfers (34.83 per 1000) when compared with years 2013-2015. However, no significant difference was found in the relative risk of fresh versus frozen embryo transfers on severe maternal morbidity across strata of calendar years 2013-2015 and 2016-2018 (adjusted RR 0.92 [95% CI 0.71-1.19] and 0.80 [95% CI 0.61-1.07], respectively).

DISCUSSION

In this population-based study of pregnant individuals who conceived following IVF, the risk of overall severe maternal morbidity was not different among those who conceived following fresh embryo transfers compared with frozen embryo transfers. However, fresh cycles were associated with a lower risk of severe hemorrhage compared with frozen cycles. Conversely, there was a trend towards higher risk of severe preeclampsia or cardiovascular complications in fresh compared with frozen cycles. We did not find any association between the number of controlled ovarian stimulation cycles prior to the index pregnancy and risk of severe maternal morbidity. The absolute risk suggested a higher incidence of severe maternal morbidity in those with no prior controlled ovarian stimulation cycles compared with those who had 1 or ≤2 controlled ovarian stimulation cycles prior to index the pregnancy. Overall, we did not observe strong evidence indicating heterogeneity of effects across strata for plurality, PCOS, and calendar year. Our results varied across strata of women with and without PCOS, in which severe maternal morbidity was 42% lower in fresh cycles among women with PCOS, but not different among fresh versus frozen cycles among women without PCOS.

Our rate of severe maternal morbidity among IVF pregnancies (32.6 per 1000) is similar to reports from the United States, France, and Sweden.(8, 36, 37) Subtypes were also similar to what has previously been reported, with most cases of severe maternal morbidity due to hypertensive disorders and

hemorrhaging requiring blood transfusions.(3, 22) While prior literature has typically compared risk of severe maternal morbidity between IVF pregnancies and spontaneous conceptions, (3, 8-9) the current study differs by considering subtypes of severe maternal morbidity among a cohort restricted to recipients of IVF.

Our finding that fresh embryo transfers were associated with lower risk of hemorrhage when compared with frozen embryos is consistent with previous studies, which have demonstrated that women who conceived following a frozen embryo transfer had an elevated risk of postpartum hemorrhage compared with those who conceived after fresh embryo transfer. (17, 38) A posited explanation is the initial endometrial preparation (i.e., natural or artificial) and resulting development of a corpus luteum among IVF patients. Contrary to spontaneous and fresh IVF cycle conceptions, most frozen IVF cycles occur without the development of a corpus luteum.(39) It has been hypothesized that absence of corpus luteum is associated with low levels of circulating vasoactive hormones that normally contribute to healthy vascularization of the placenta in early gestation. (17, 40, 41)

In their analysis of 477,522 pregnancies spanning 8 American states, Luke et al reported the likelihood of requiring blood transfusion, when compared to spontaneous conceptions, was greatest among autologous-frozen, followed by autologous oocytes-fresh.(9) They theorized that plurality and the association with of emergency caesarean section may drive the rate of peripartum hysterectomy and severe postpartum hemorrhage in IVF pregnancies.(9) Although our study observed a similar trend in hemorrhagic events, plurality was not found to modify the effect of fresh versus frozen embryo transfers on severe maternal morbidity. This observation may be explained by the lower number of multiple gestations in our dataset due to smaller sample size and a recent trend in elective single embryo transfer within Canada.(42) A larger sample may be needed to capture any effect modification of plurality on the relationship between embryo type and severe maternal morbidity.

We observed an increase in the proportion of frozen cycles between the years 2013 and 2018. Given this growing use of frozen embryo transfers, it is possible that the risk of hemorrhagic events was

driven by patient-related factors, with healthcare providers prioritizing the use of fresh embryos in subjects less susceptible to developing ovarian hyperstimulation syndrome.(43) While absolute rates of severe maternal morbidity were similar between fresh and frozen cycles between January 2013 – December 2015, a discrepancy can be observed between the groups between January 2016 – March 2018. However, we did not find strong evidence for heterogeneity of the effect by calendar year; the adjusted RR showed a non-significant reduction in risk of SMM during both calendar periods.

Contrary to our proposed hypothesis, the study found a somewhat greater risk of severe preeclampsia and cardiovascular complications in fresh compared with frozen embryo transfers and, while the result was not statistically significant, it may be clinically meaningful. A potential explanation is that the supraphysiological levels of gonadotropins or estrogen associated with fresh cycles may lead to endothelial injury.(44, 45) Indeed, inadequate vascularization of the placenta is an early harbinger of preeclampsia – a multisystem disorder characterized by placental inflammation, oxidative stress, and an antiangiogenic state.(46) The process of controlled ovarian stimulation has been observed to be linked with systemic inflammation and increased arteriolar stiffness,(47) potentially accelerating vascular damage in at-risk persons, and contributing to preeclampsia.

Our results are compatible with prior studies showing higher risk of severe preeclampsia in fresh compared with frozen cycles,(44, 47). Imudia et al demonstrated that, in women who conceived from fresh embryos, elevated peak serum estradiol levels (>90th percentile) on the day of hCG administration during COS increased the likelihood of preeclampsia of all severity (adjusted odds ratio 4.79 [95% CI 1.55-14.84]).(44) However, other studies have found that the risk of preeclampsia is more strongly associated with frozen ETs. (15, 48-50) The reason for these discrepant findings is unclear; it may be due to different definitions of preeclampsia across studies. For example, Sazonova et al. used ICD-10 codes O.14, O.15 to capture all forms of preeclampsia.(50) In comparison, we included ICD-10-CA codes to capture *severe* preeclampsia and included associated cardiac events (see footnote of Supplemental

Material 1). These indicators were combined to due to their overlapping pathophysiology and to maximize the power to detect a meaningful difference.

Strengths and limitations

The main strength of our study was the use of a relatively large and robust population-based dataset, allowing sufficient power to investigate a rare outcome. By linking province-wide databases for fertility treatment (CARTR Plus), pregnancy and birth (BIS) and hospitalizations (CIHI-DAD), patients' data, from conception to delivery, was captured by healthcare professionals and trained abstracters. Compared with population-based studies that use birth certificates to capture exposures and outcomes, this study was uniquely positioned for more accurate ascertainment of exposures and outcomes within a universal health care system in which there is partial coverage of IVF.

Among the limitations, we acknowledge the potential for non-differential outcome misclassification stemming from incomplete documentation in medical records and inability to ascertain all severe maternal morbidity events. This misclassification is likely minor since the specificity of the outcome measure is high. We acknowledge the possibility that some subjects could have experienced a severe maternal morbidity event after discharge from their birth hospitalization, which was not captured with our approach. The impact of this misclassification is likely minor, since most severe maternal morbidity events occur at the time of birth hospitalization.(3) Moreover, we did not have information pertaining to maternal ICU admissions, a subtype of composite severe maternal morbidity. It is unlikely that the addition of an ICU event would have identified additional cases of severe maternal morbidity, given that most conditions included typically require ICU admission.

It is possible that residual confounding by unmeasured factors impacted our results, especially with regards to the higher risk of severe preeclampsia and cardiac complications among recipients of fresh embryo transfers when compared with frozen transfers, an observation that was uncommon in prior studies. (15, 48-50) For example, given that certain race/ethnic groups have higher rates of severe

maternal morbidity,(51) additional demographic information regarding race and ethnicity may have reduced the likelihood of residual confounding; however, information race and ethnicity was not available within the BIS.

Finally, we were unable to assess the risk of all SMM subtypes among fresh and frozen cycles due to insufficient power. We also did not consider differences in IVF protocols beyond fresh versus frozen and number of IVF cycles due to limitations in data availability. For instance, differences between artificial and natural frozen cycles could modify the impact of severe maternal morbidity. Furthermore, information on endometrial thickness was not available, and could have reflected endometrial receptivity. These treatment elements are important discriminating elements and should be investigated in subsequent studies.

CONCLUSION

Results of this study suggest that women receiving fresh embryo transfers are not at increased risk of severe maternal morbidity when compared with recipients of frozen-thawed embryo transfers. In fact, the risk of hemorrhagic events was lower in fresh cycles compared with frozen embryo transfer cycles, while the opposite was true of severe preeclampsia or cardiovascular events. Future prospective studies that monitor differences in risk of severe maternal morbidity in individuals undergoing different types of IVF are needed to better discern subgroups at highest risk and contribute to personalized risk counseling and IVF protocol selection.

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Table 1. Baseline characteristics of all hospital births among women who underwent IVFaccording to embryo transfer type.

Characteristic		Whole cohort (n = 13 929)	No. (%)* of women with fresh ET (n = 5660)	No. (%)* of women with frozen ET (n = 8269)	Standardized difference
Year of conception					
	2013	2323 (16.7)	1329 (23.5)	994 (12.0)	0.30
	2014	2408 (17.3)	1201 (21.2)	1207 (14.6)	0.17
	2015	2278 (16.4)	947 (16.7)	1331 (16.1)	0.02
	2016	2965 (21.3)	1078 (19.1)	1887 (22.8)	0.09
	2017	3355 (24.1)	961 (17.0)	2394 (29.0)	0.29
	2018	600 (4.3)	144 (2.5)	456 (5.5)	0.15
Maternal age at conception (years)					
	Mean ± SD	35.2 ± 4.6	35.1 ± 4.5	35.2 ± 4.6	0.02
	18-24	59 (0.4)	26 (0.5)	33 (0.4)	0.01
	25-29	1250 (9.0)	524 (9.3)	726 (8.8)	0.02
	30-34	5165 (37.1)	2091 (36.9)	3074 (37.2)	0.01
	35-39	5167 (37.1)	2087 (36.9)	3080 (37.3)	0.01
	40-44	1826 (13.1)	763 (13.5)	1063 (12.9)	0.02
	≥ 45	462 (3.3)	169 (3.0)	293 (3.5)	0.03
Neighbourhood income level (quintiles)					
Lowest	1	1479 (10.6)	597 (10.6)	882 (10.7)	0.01
	2	1662 (11.9)	655 (11.6)	1007 (12.2)	0.02
	3	2361 (17.0)	966 (17.1)	1395 (16.9)	0.01
	4	3688 (26.5)	1504 (26.6)	2184 (26.4)	0.01
Highest	5	3642 (26.2)	1499 (26.5)	2143 (25.9)	0.01

	Missing	1097 (7.9)	439 (7.8)	658 (8.0)	0.01
Neighbourhood education level (quintiles)					
Lowest	1	915 (6.6)	415 (7.3)	500 (6.1)	0.05
	2	1653 (11.9)	697 (12.3)	956 (11.6)	0.02
	3	2534 (18.2)	990 (17.5)	1544 (18.7)	0.03
	4	3799 (27.3)	1514 (26.8)	2285 (27.6)	0.04
Highest	5	4015 (28.8)	1641 (29.0)	2374 (28.7)	0.01
	Missing	1013 (7.3)	403 (7.1)	610 (7.4)	0.01
Comorbidities					
BMI, kg/m^2	Median (IQR)	23.9 (6.5)	24.0 (6.6)	23.7 (6.4)	0.06
Obesity (BMI > $30kg/m^2$)		2002 (14.4)	842 (14.9)	1160 (14.0)	0.04
	Missing	2044 (14.7)	908 (16.0)	1136 (13.7)	0.07
Pre-existing cardiometabolic disease¶		492 (3.5)	198 (3.5)	294 (3.6)	0.01
	Missing	2014 (14.5)	776 (13.7)	1238 (15.0)	0.04
Tobacco Use		101 (0.7)	51 (0.9)	50 (0.6)	0.04
	Missing	1480 (10.6)	679 (12.0)	801 (9.7)	0.08
Any alcohol use		122 (0.9)	49 (0.9)	73 (0.9)	0.01
	Missing	1480 (10.6)	698 (12.3)	782 (9.7)	0.09
Drug Use		35 (0.3)	10 (0.2)	25 (0.3)	0.03
	Missing	1558 (11.2)	713 (12.6)	845 (10.2)	0.08
Parity					
	0	9031 (64.8)	4073 (72.0)	4958 (60.0)	0.26
	1	3712 (26.7)	1203 (21.3)	2509 (30.3)	0.21
	2	657 (4.7)	182 (3.2)	475 (5.7)	0.12
	≥3	211 (1.5)	66 (1.2)	145 (1.8)	0.05
	Missing	318 (2.3)	136 (2.4)	182 (2.2)	0.01

Number of embryos transferred					
	1	8816 (63.29)	2984 (52.7)	5832 (70.5)	0.37
	2	4755 (34.1)	2438 (43.1)	2317 (28.0)	0.32
	≥3	358 (2.6)	238 (4.2)	120 (1.5)	0.17
Reasons for fertility treatment**					
Female hormonal		5284 (37.9)	2222 (39.3)	3062 (37.0)	0.05
Female structural		2628 (18.9)	1218 (21.5)	1410 (17.1)	0.11
Male factor		4890 (35.1)	2140 (37.8)	2750 (33.3)	0.10
Unexplained		2718 (19.5)	1023 (18.1)	1695 (20.5)	0.06
Multiple reasons or other		2489 (17.9)	595 (10.5)	1894 (22.9)	0.29
Multiple Gestation		1757 (12.6)	877 (15.5)	880 (10.6)	0.15
Index pregnancy resulting in stillbirth		129 (0.9)	59 (1.0)	70 (0.8)	0.02
	Missing	318	136 (2.4)	182 (2.2)	0.01

Note: BMI = body mass index, IQR = interquartile range, SD = standard deviation.
*Data are presented as N (%) unless specified otherwise.
**Not mutually exclusive.
¶ Diabetes mellitus, cardiovascular disease, hypertension, renal disease.

Table 2. Number and rate per 1000 of any severe maternal morbidity according to IVF cycle type

	Whole cohort (n = 13 929)		Women with fresh embryo transfer (n = 5660)		Women with frozen embryo transfer (n = 8269)	
SMM type	**N**	**Rate per 1000**	**N**	**Rate per 1000**	**N**	**Rate per 1000**
Any SMM	454	32.6	174	30.7	280	33.9
Severe hemorrhage	241	17.3	74	13.1	167	20.2
Severe preeclampsia and cardiac conditions	153	11.0	76	13.4	77	9.3
Surgical complications	42	3	20	3.5	22	2.7
Puerperal sepsis	26	1.9	8	1.4	18	2.2
Acute renal failure	23	1.7	7	1.2	16	1.9
Assisted ventilation	22	1.6	10	1.8	12	1.5
Other*	54	3.9	20	3.5	34	4.1

Note: SMM = severe maternal morbidity

*Includes hysterectomy, embolism, shock, disseminated intravascular coagulation, severe uterine rupture, cerebrovascular accidents, and death.

Table 3. Unadjusted and adjusted risk ratios and 95% confidence interval of severe maternal morbidity in fresh as compared with frozen embryo transfer

Outcome	Unadjusted RR* (95% CI)	Adjusted RR* (95% CI)
Any SMM	0.91 (0.75-1.09)	0.85 (0.70-1.04)**
Severe hemorrhage	0.65 (0.49- 0.85)	0.63 (0.48-0.82)***
Severe preeclampsia and cardiovascular complications	1.44 (1.05-1.98)	1.33 (0.97-1.82)***

Note: CI = confidence interval, RR = risk ratio, ref. =reference, SMM = severe maternal morbidity.
*Risk ratios were calculated using log-binomial regression analysis.
**Adjusted for age at conception, nulliparity, pre-existing cardiometabolic diseases, polycystic ovarian syndrome, year of conception, and income quintile.
***Adjusted for age at conception, nulliparity, and pre-existing cardiometabolic diseases.

Table 4. Risk of severe maternal morbidity at index delivery by number of prior fresh embryo cycles with own oocytes in individuals pregnant by IVF.

Exposure¶	No. with SMM	Rate per 1000	Unadjusted RR* (95% CI)	Adjusted RR* (95% CI)
No prior fresh cycle	155	35.8	1.00 (ref.)	1.00 (ref.)
1 prior fresh cycle	211	31.2	0.87 (0.71-1.07)	0.96 (0.78-1.18)**
2 prior cycle or more	88	32.1	0.91 (0.67-1.12)	0.91 (0.67-1.25)**

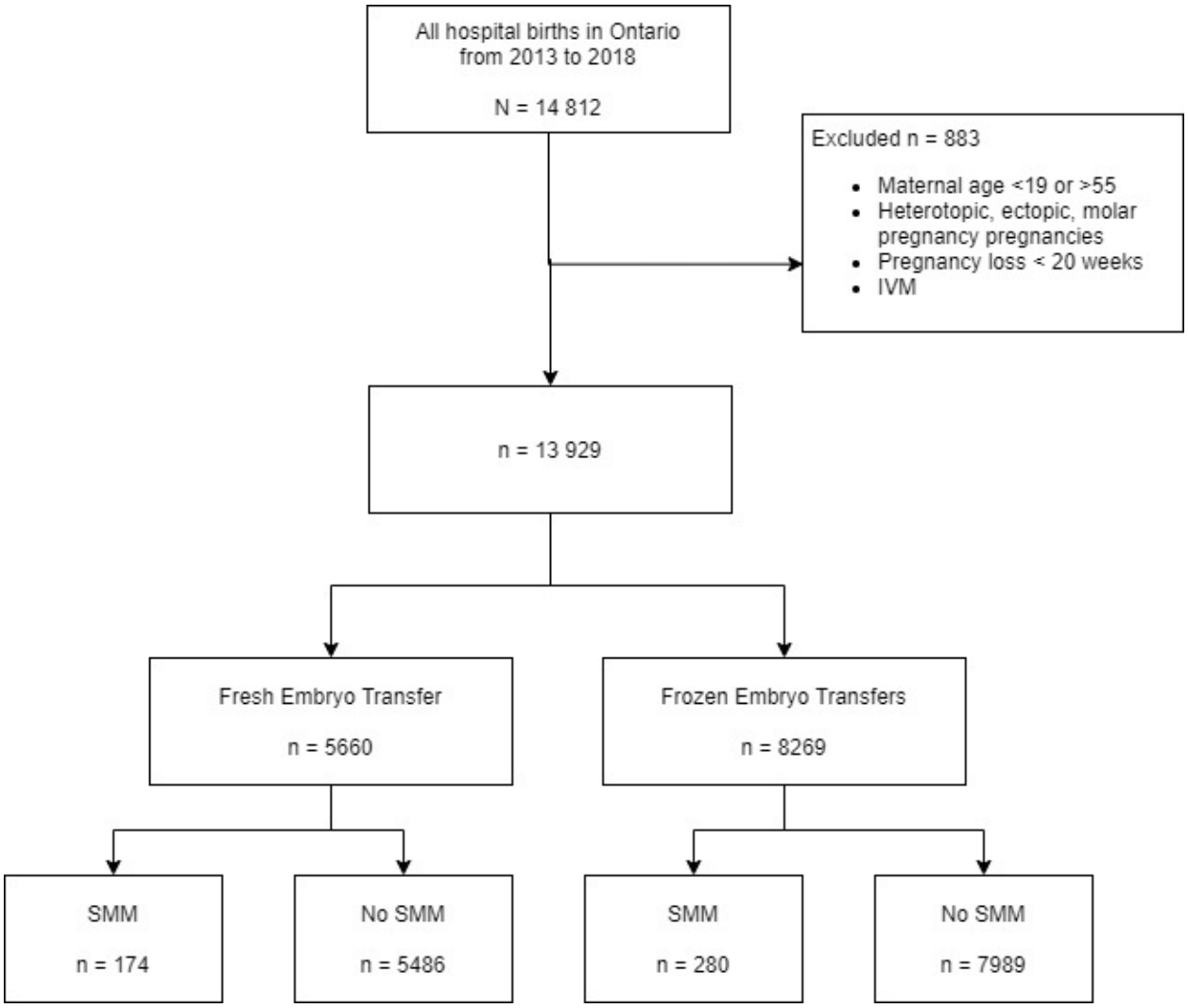
Note: CI = confidence interval, RR = risk ratio, ref. =reference, SMM = severe maternal morbidity.
¶ Ovarian stimulation cycle prior to the index pregnancy.
*Risk ratios were calculated using log-binomial regression analysis.
**Adjusted for age at conception, nulliparity, pre-existing cardiometabolic diseases, polycystic ovarian syndrome, year of conception, and income quintile.

Table 5. Risk ratios for type of embryo transfer and severe maternal morbidity at the index birth, stratified by multiple gestation, PCOS, and year of conception.

Stratification variable	Type of embryo transfer	No. with SMM/ No. at risk	Rate / 1000	Unadjusted RR (95% CI)	Adjusted RR* (95% CI)
Singleton	Fresh	119 / 4783	24.88	0.82 (0.66-1.02	0.82 (0.66-1.03)**
	Frozen	224 / 7388	30.32	1.00 (ref)	1.00 (ref)
Multiple Gestations	Fresh	55 / 877	62.71	0.99 (0.69-1.41)	0.89 (0.61-1.27)**
	Frozen	56 / 880	63.64	1.00 (ref)	1.00 (ref)
No PCOS	Fresh	166 / 5209	31.87	0.91 (0.76 – 1.11)	0.89 (0.73 – 1.08)***
	Frozen	251 / 7209	34.82	1.00 (ref)	1.00 (ref)
PCOS	Fresh	8 / 451	17.74	0.65 (0.30 – 1.41)	0.58 (0.26 – 1.28)***
	Frozen	29 / 1060	27.36	1.00 (ref)	1.00 (ref)
2013 - 2015	Fresh	111 / 3477	31.92	0.98 (0.76 – 1.27)	0.92 (0.71-1.19)****
	Frozen	115 / 3532	32.56	1.00 (ref)	1.00 (ref)
2016 - 2018	Fresh	63 / 2183	28.86	0.83 (0.62 – 1.10)	0.80 (0.61-1.07)****
	Frozen	165 / 4737	34.83	1.00 (ref)	1.00 (ref)

Note: PCOS = polycystic ovarian syndrome, SMM = severe maternal morbidity, RR = risk ratio, ref = reference
*Risk ratios were calculated using log-binomial regression analysis.
**Adjusted for age at conception, nulliparity, pre-existing cardiometabolic diseases, PCOS, year of conception, and income quintile.
*** Adjusted for age at conception, nulliparity, pre-existing cardiometabolic diseases, year of conception, and income quintile.
******** Adjusted for age at conception, nulliparity, pre-existing cardiometabolic diseases, PCOS, and income quintile.

Figure 1: **Study flow diagram**



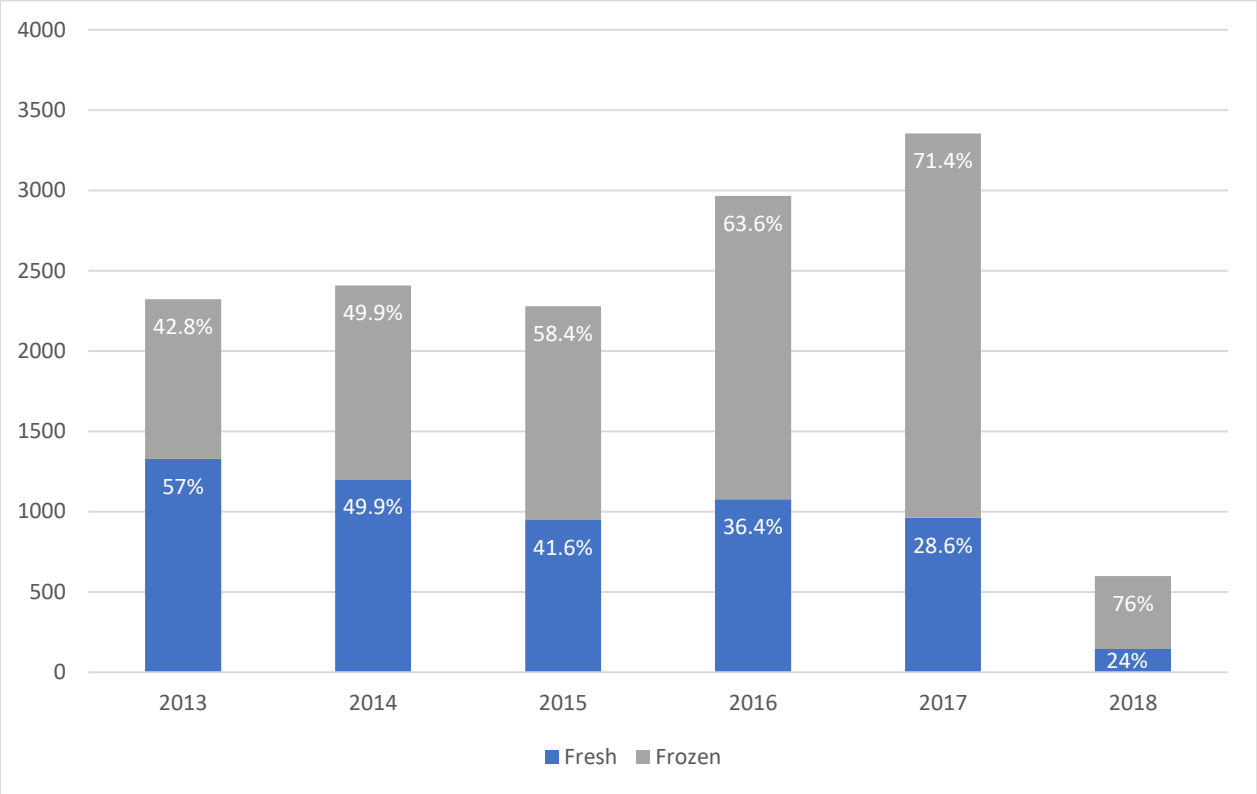


Figure 2. Comparison of embryo transfer type by year of conception, from January 1, 2013 to March 5, 2018
Note: The year 2018 is incomplete and is limited to January 1- March 5.

Supplemental Table 1: International Classification of Diseases (ICD-10CA) and Canadian Classification of Health Interventions (CCI) codes for each severe maternal morbidity (SMM) subtype.

SMM type	SMM subtype	ICD-10CA and CCI codes
Severe preeclampsia, HELLP syndrome, eclampsia	Severe preeclampsia	O14.1, O14.2
	HELLP syndrome	O15
	Eclampsia	
Severe haemorrhage	Placenta praevia with haemorrhage and red cell transfusion	O44.1 + RBCTRNSF = 'Y'
	Placental abruption with coagulation defect	O45.0
	Antepartum haemorrhage with coagulation defect	O46.0
	Intrapartum haemorrhage with coagulation defect	O67.0
	Intrapartum haemorrhage with red cell transfusion	O67 + RBCTRNSF = 'Y'
	Postpartum haemorrhage with red cell transfusion, procedures to the uterus, or hysterectomy	O72 + any of the following: RBCTRNSF = 'Y', or (1.RM.13,1.KT.51, PC.91.LA, or 5.PC.91.HV) + RBCTRNSF = 1, or (5.MD.60.RC, 5.MD.60.RD, 5.MD.60.KE, 5.MD.60.CB, or 1.RM.89. LA*), or 1.RM.87.LA-GX
	Curettage with red cell transfusion	(5.PC.91.GA, 5.PC.91.GC, or 5.PC.91.GD) + RBCTRNSF = 'Y'
Cardiac conditions	Cardiac complications of anaesthesia	O74.2, O89.1
	Cardiomyopathy	O90.3, I42, I43
	Cardiac arrest and resuscitation	I46, I49.0, 1.HZ.09, 1.HZ.30
	Myocardial infarction	I21, I22
	Pulmonary oedema and heart failure	I50, J81
Surgical complications	Complications of obstetric surgery and procedures	O75.4
	Evacuation of incisional haematoma with RBC transfusion	5.PC.73.JS + RBCTRNSF = 'Y'
	Repair of bladder, urethra, or intestine	5.PC.80.JR, 1.NK.80, or 1.NM.80
	Reclosure of caesarean wound with RBC transfusion	(5.PC.80.JM or 5.PC.80.JH) + RBCTRNSF = 'Y'

Hysterectomy	Caesarean hysterectomy	5.MD.60.RC, 5.MD.60.RD, 5.MD.60.KE, 5.MD.60.CB
	Hysterectomy using an open approach (without bladder neck suspension, suspension of vaginal vault, or pelvic floor repair)	1.RM.89.LA* (exclude if 1.PL.74, 1.RS.74, or 1.RS.80 code also present) or 1.RM.87.LA-GX
Sepsis	Puerperal sepsis	O85
	Septicaemia during labour	O75.3
Embolism, shock, DIC	Obstetric shock	O75.1, R57, T80.5, or T88.6
	Obstetric embolism	O88
	Disseminated intravascular coagulation	D65
Assisted ventilation	Assisted ventilation through endotracheal tube	1.GZ.31.CA-ND
	Assisted ventilation through tracheostomy	1.GZ.31.CR-ND
Acute renal failure	Acute renal failure	O90.4, N17, N19 or N99.0
	Dialysis	1.PZ.21
Severe uterine rupture	Rupture of the uterus with red cell transfusion, procedures to the uterus, or hysterectomy	(O71.0 or O71.1) + any of the following: RBCTRNSF='Y', or (1.RM.13, 1.KT.51, 5.PC.91.LA, or 5.PC.91.HV) + RBCTRNSF='Y', or (5.MD.60.RC, 5.MD.60.RD, 5.MD.60.KE, 5.MD.60.CB, or 1.RM.89. LA*), or 1.RM.87.LA-GX
Cerebrovascular accidents	Cerebral venous thrombosis in pregnancy	O22.5
	Cerebral venous thrombosis in the puerperium	O87.3
	Subarachnoid and intracranial haemorrhage, and cerebral infarction	I60, I61, I62, I63, or I64
Other	Acute fatty liver with red cell transfusion or plasma transfusion	O26.6 + (RBCTRNSF='Y' or PLSTRNSF='Y')
	Hepatic failure	K71 or K72
	Cerebral oedema or coma	G93.6 or R40.2
	Pulmonary, cardiac, and CNS complications of anaesthesia during pregnancy, labour, delivery, or the puerperium	O29.0, O29.1, O29.2, O89.0, O89.1, O89.2, O74.0, O74.1, O74.2, or O74.3

	Status asthmaticus	J45.01, J45.11, J45.81, or J45.91
	Adult respiratory distress syndrome	J80
	Acute abdomen	K35, K37, K65, N73.3, or N73.5
	Surgical or manual correction of inverted uterus for vaginal births only	5.PC.91.HQ or 5.PC.91.HP, restricted to vaginal births (ie absence of caesarean code 5.MD.60)
	Sickle cell anaemia with crisis	D57.0
	Acute psychosis	F53.1 or F23
	Status epilepticus	G41

Note on secondary outcome:

- Study outcome preeclampsia with or without cardiovascular events include any event of severe preeclampsia, HELLP syndrome, eclampsia (O14.1, O14.2, O15) and/or cardiac conditions (O74.2, O89.1, O90.3, I42, I43, I46, I49.0, 1.HZ.09, 1.HZ.30, I21, I22, I50, J81)

THESIS DISCUSSION

In this thesis, we investigated the association between fresh ETs as compared with frozen-thawed ETs and both overall SMM, and common SMM subtypes. Using Ontario registry data, we conducted a large, population-based retrospective cohort study of women who successfully conceived by IVF and delivered after 20 weeks' gestation. Furthermore, to account for missing data, we performed a fully conditional specification imputation model to generate 20 imputed datasets that were subsequently pooled to produce valid statistical inferences.

While we hypothesized that supraphysiological levels of hormones in fresh ETs would increase risk of SMM, our results suggested that overall SMM rates did not differ by cycle type. Among subtypes of SMM, the risk of severe hemorrhage was lower in fresh cycles compared with frozen cycles. In contrast, our results suggested a possible increase in risk of severe preeclampsia and cardiovascular complications in fresh cycles compared with frozen cycles. Moreover, we assessed the association between number of COS cycles prior to the index pregnancy (i.e., 1 prior COS versus none, and ≥2 COS versus none) and SMM at time of delivery, although no difference was found in the risk of SMM according to number of COS cycles prior to index pregnancy. Finally, we ran three stratified analyses to assess the effect modification of plurality, PCOS and calendar year on the main relationship of interest. However, these analyses did reveal any evidence indicating heterogeneity of effects across strata for these covariates.

Contributions to the literature

The work in this thesis contributed to our understanding of the nature of the association between IVF and SMM, specifically concerning the use of fresh versus frozen-thawed embryo transfers and the number of prior simulation cycles. In stimulated frozen cycles, it may be that the lack of a corpus luteum contributes to a suboptimal uterine environment via missing circulating vasoactive hormones, an important element for vascular health in early gestation.(75, 101) It remains unclear why this same trend isn't observed with regards to cases of severe preeclampsia and cardiovascular complications. Given that severe preeclampsia and cardiovascular complications have a different pathophysiology than hemorrhagic events,

it is possible that COS contributed to physiological and biochemical alterations that may only be associated with the former SMM subset. For instance, the supraphysiological levels of gonadotropins or estrogen associated with fresh cycles may lead to endothelial injury.(32, 102) COS involves systematic inflammatory activation and increased arteriolar stiffness which may contribute to vascular damage and the development of severe preeclampsia or other cardiovascular complications.(103) However, limited conclusions can be drawn regarding this relationship, given the results were not statistically significant.

Although a recent U.S. study also investigated the association between SMM and embryo transfer type,(77) we believe that our study contributes a unique interpretation centered on province-wide assisted reproductive technology registry of good quality data. For instance, the Luke et al. study was conducted based on birth certificate data which is known to be unreliable and involve a high rate of missing data. In contrast, the use of a Canadian birth registry which contains valid and reliable information abstracted directly from patient records, and subsequently linked deterministically with the CARTR-Plus and CIHI-DAD, permitted for a generalizable and high-quality study on the safety of IVF treatment and SMM. Furthermore, Luke et al. compared the risk of SMM in IVF subgroups to women who conceived spontaneously, without propensity score adjustment thus potentially introducing confounding by indication. Our study cohort consisted of only subjects pregnant by IVF, comparing the risk of SMM in fresh with frozen ETs. Finally, Luck and colleagues limited their cohort to pregnancies resulting in live births, potentially introducing selection bias. A strength of this manuscript is the inclusion of stillbirths, allowing this study's results to be generalizable to the Canadians receiving IVF treatment.

To summarize, this thesis contributes to the growing body of knowledge on the benefits and risks of varying IVF protocols and is based on high quality data and strong methodology. However, we recognize that our thesis had several limitations, outlined in our manuscript and in detail below.

Thesis Limitations

Administrative databases are a rich source of information for researchers. However, observational studies based on such sources must contend with certain types of limitations, such as a misclassification of exposures and outcomes, differences in coding criteria between institutions or across time, missing data, and limited control of collected variables.

Information Bias

Information bias is a distortion in the estimate of effect, caused by inaccurate measurements or misclassification of study variables, can impact exposures, outcomes, and confounders.(104) Non-differential outcome misclassification occurs when the degree of error in measurement of the outcome variable does not differ according to exposure status.(104) With regards to the ascertainment of our study outcome using diagnostic ICD codes, some non-differential misclassification is likely to be present due to issues such as incomplete documentation in medical records, and error in data coding and/or entry. However, this error is likely minimal given that the outcome is a validated definition and the conditions that comprise SMM are serious and likely well documented by healthcare professionals and the trained abstracters who code the data in the DAD. SMM events are typically ascertained from 20 weeks' gestation to 42 days postpartum,(21) however, our study's dataset was limited to events of SMM occurring at the time of the index birth hospitalization. As a result, some individuals may not have been identified as having an SMM event and, therefore, misclassified as having no SMM event. Since the majority of SMM events are captured at the index delivery hospitalization, the extent of this misclassification is expected to be minimal.(105-107) Since SMM is an uncommon event (i.e., low incidence) and is likely measured with high specificity, bias of the effect estimate from non-differential outcome misclassification would likely be minimal.(107)

Misclassification of exposure in the study dataset is highly unlikely given that it was directly captured in IVF clinics. Although there may be some misclassification of other covariates, a validation study of CARTR Plus found the database was of good quality, with kappa coefficients greater than 0.90 for most variables assessed, with the exception of FSH levels (ICC 0.68 [95% CI 0.64-0.72]), oocyte origin (Kappa 0.45 [95% CI 0.37-0.52]), and elective single or double embryo transfer (Kappa 0.55 [95% CI 0.49-0.61]),

which were found to have moderate agreement.(108) As such, while some data elements had only moderate agreement, the majority were of good quality, suggesting that it is unlikely that misclassification of study covariates would have introduced substantial bias.

Selection Bias

The cohort for this study was identified from a population-based provincial health registry which captures 99% of hospital deliveries in Ontario, therefore, minimizes selection bias. Study exclusion criteria, such as exclusion of pregnant women who were not residents of Ontario or registered with Ontario's universal health insurance program could potentially have introduced selection bias if it impacted the relative distribution of subjects by exposure and outcome; however, this is unlikely. Finally, this cohort was limited to women who delivered in an Ontario hospital. However, home births are uncommon in Canada (109) and women who conceive by IVF are very unlikely to give birth outside of a clinical setting, thus, this is unlikely to have contributed to any selection bias.

Selection bias can also be introduced by the inappropriate adjustment of colliders - variables that are independently caused by an exposure and an outcome.(110) In this study, a carefully constructed conceptual model was used *a priori* to identify the relationships between variables, and crude associations of all covariates were tested to assess for collinearity before developing our final models. Therefore, we believe that our efforts limited collider stratification bias.

Confounding Bias

As inherent with all observational studies, our study is subject to confounding bias, due to both measured and unmeasured potential confounders. Confounding by indication is possible; for example, women who are treated with multiple rounds of IVF have more severe underlying infertility, resulting in a higher risk of adverse pregnancy outcomes. Furthermore, confounding by indication could be introduced via the type of embryo transfer recommended by the treating physician. However, since the study population is limited to women who have successfully become pregnant following IVF, major baseline differences in

individual demographic and risk factors between the fresh ET and frozen-thawed ET groups were not expected and not found (Table 1). Finally, the subject's physician likely played a role in the selection of fresh versus frozen treatments based on their interpretation of the effectiveness and risks of each form of treatment. Although we were unable to control for healthcare practitioner patient selection, understanding how clinicians determine which IVF protocol to use may be an area of future research.

Measured confounders were identified using a conceptual model. The final regression model adjusted for all covariates found to confound the relationship between exposure and outcome. However, due to limited power and the retrospective nature of this study, some observed and unobserved confounders could not be adjusted for.

Missing Data

Observational studies are often prone to missing data. Based on findings from validation studies and BIS missing data reports,(108, 111) we assumed that missing data originating from BIS was likely missing-at-random. Moreover, most variables had missingness below 10%, except for the body mass index (BMI) and pre-existing cardiometabolic condition variables. Given limited power, we were unable to assess all subtypes of SMM. A country-wide cohort study could generate enough statistical power to fully capture the risk of all subtypes of SMM within an IVF population.

CONCLUSION

It has been previously reported that IVF pregnancy is at higher risk of SMM compared with spontaneous pregnancy but the reasons for this association have been not well described. Results of this thesis indicate that treatment-related factors, notably fresh or frozen embryo transfer may impact risk of some forms of SMM, such as hemorrhagic or hypertensive and cardiovascular. It is possible that unmeasured treatment-related predictors in frozen cycle IVF, such as the role of natural versus stimulated frozen ETs, may influence later maternal outcomes, resulting in a greater risk for severe hemorrhagic events. As demonstrated through this thesis' literature review and manuscript, the number of IVF clinics favoring

frozen over fresh ETs has increased. It is important that each individual seeking IVF receive personalized preconception counselling about their risks with each procedure, which perhaps may be mitigated through the use of enhanced surveillance and preventive strategies. Future large prospective studies that monitor differences in risk of SMM subtypes in women undergoing IVF will be able to better identify high-risk subgroups.

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Appendix 1: A 2021 review of infertility treatments and cardiovascular disease

INFERTILITY, INFERTILITY TREATMENT AND CARDIOVASCULAR DISEASE: AN OVERVIEW

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Abstract

The prevalence of maternal cardiovascular disease (CVD) has risen throughout the developed world, reflecting an increase in acquired cardiovascular risk factors, such as hypertension and diabetes, and the improved life expectancy of those living with congenital CVD due to advances in care. Because many cardiovascular risk factors or cardiovascular conditions are associated with infertility, reproductive-aged women with CVD may increasingly seek reproductive assistance. The worldwide use of assisted reproductive technologies (ART), such as in-vitro fertilization (IVF) with or without intracytoplasmic sperm injection, or intrauterine insemination following pharmacological ovulation induction have increased steadily over the last several decades. It is incumbent among providers who care for reproductive-aged women with pre-existing CVD or CVD risk factors to understand and appreciate the types of treatments offered and inherent risks related to infertility treatments, in order to guide their patients to making safe reproductive choices in line with their values and preferences. While infertility treatments increase the risk of complicated pregnancy, whether these risks are compounded among individuals with pre-existing CVD

is less well known. In this review, we summarize current available evidence regarding short-term and long-term cardiovascular implications of ART among individuals with and without CVD, as well as treatment considerations for these women. Existing knowledge gaps and priority areas for further study are presented.

Keywords: assisted reproductive technology, infertility treatment, in vitro fertilization, cardiovascular disease, congenital heart disease, acquired heart disease, hypertension, diabetes, venous thromboembolism

Brief summary

Many types of infertility are associated with cardiovascular disease, cardiovascular risk factors or congenital heart disease. Persons with infertility who desire pregnancy using assisted reproductive technologies require special preconception care and counseling focused not only on pregnancy-specific risks but potential added risks due to infertility treatment. This review article discusses short and long-term cardiovascular implications of infertility and its treatment, and provides a primer for clinicians caring for individuals planning assisted pregnancy about the safest approach to minimize maternal and offspring complications.

Abbreviations and Acronyms

ART = Assisted Reproductive Technologies

CARPREG = Cardiac Disease in Pregnancy

CARTR Plus = Canadian Assisted Reproductive Technologies Register Plus

CI = Confidence Interval

CVD = Cardiovascular disease

hCG = human chorionic gonadotropin

HDP = Hypertensive disorders of pregnancy

HR = Hazard ratio

IVF = In vitro fertilization

mWHO = modified World Health Organization

PCOS = Polycystic Ovarian Syndrome

VTE = venous thromboembolism

Introduction

In Canada, the prevalence of infertility among couples ranges from 12% to 16%,(1) and is similar to worldwide prevalence estimates. Increasingly, there has been a rise in availability of infertility treatments such as Assisted Reproductive Technologies (ART) – namely in vitro fertilization (IVF) to facilitate pregnancy. To meet this demand, the provincial governments of Ontario (December 2015 – current)(2, 3) and Quebec (2010 – 2015 & 2020 – current)(4) have developed publicly funded fertility programs to improve accessibility to ART. In Canada in 2013, 25,349 ART cycles were performed in 17,051 individuals, which increased to 35,347 ART cycles among 22,309 individuals in 2019.(5) After male factor infertility and unexplained infertility, "advanced female age" was the most common reason for treatment in 2019,(5) reflecting the societal trend of delayed childbearing in high-income countries.(1, 6, 7) Successful outcome (i.e., clinical pregnancy) ranges from 12% to 35% per cycle, depending on a variety of patient- and treatment-specific factors.(5)

In parallel with these fertility trends is an increase in the prevalence of cardiovascular risk factors among reproductive-aged individuals(8, 9) with chronic hypertension affecting approximately 5% of pregnancies,(10) and type 1 or type 2 diabetes affecting approximately 2% of pregnancies. Polycystic ovarian syndrome (PCOS), one the most common causes of female factor infertility affecting about 15% of individuals of reproductive age, is strongly linked with metabolic syndrome.(11, 12) Furthermore, recent evidence suggests that endometriosis, a gynecological condition that affects up to 11% of reproductive age individuals, and present in 30–50% of women with infertility, may also be associated with increased risk of CVD.(13-15)

Moreover, between 0.2% and 4% of pregnancies are complicated by pre-existing acquired or congenital heart disease.(16) Specifically, the prevalence of individuals with complex congenital heart disease who survive into childbearing years has dramatically increased over the last several decades, owing to advances in medical and surgical care.(17) The presence and severity of pre-existing cardiovascular risk factors, and of overt cardiovascular disease (CVD) strongly predict maternal and

neonatal morbidity and mortality.(16) In the latter case, validated algorithms exist (e.g., modified WHO [mWHO] and CARPREG I & II)(16, 18) which predict both maternal cardiovascular and perinatal outcome as a function of cardiovascular lesion and functional status to help guide reproductive planning.

It is widely accepted that pre-conception counseling and risk stratification are crucial to ensure optimal outcomes in individuals with CVD who are planning pregnancy.(19) However, despite pervasive societal trends in delayed childbearing and worldwide increased use of ART, there is a lack of general understanding among health care providers regarding how certain subtypes of infertility and their treatments may impact on pregnancy outcome in special populations with CVD. It is conceivable that use of ART poses additional cardiac and metabolic stress during pregnancy, owing to marked fluctuations in endogenous estrogen as well as inherent risks due to multiple gestation.(20-22) In **Figure 1**, a conceptual diagram outlines known and theoretical associations between infertility, its treatment, pregnancy outcome and cardiovascular and cerebrovascular disease. **Figure 2** depicts hormonal fluctuations during ART protocols and potential impacts on vasculature.

The importance of pre-ART counseling and health optimization must therefore be emphasized. While generally safe, use of infertility treatment, in particular IVF, has been associated with severe maternal complications, (8, 23) including preeclampsia and other hypertensive disorders of pregnancy (HDP), as well as obstetric hemorrhage and complications due to abnormal placentation.(24, 25) While it is unclear whether infertility treatments are independently associated with downstream CVD,(21, 26) many of these pregnancy-specific complications are also increasingly recognized as sex-specific cardiovascular risk factors.(27) An improved understanding of the potential additional effects of infertility treatments in at-risk women, both with respect to perinatal outcomes, and longer-term CVD, is needed to ensure safe reproduction and pregnancy. The Human Reproduction Act, a federal legislation aimed at regulating assisted human reproduction and related research,(28) does not address personal or medical factors that may pose substantial pregnancy health risks when treatment is undertaken, and there is a lack of clear formal guidance on how to approach medical risk assessment prior to ART.(29) In the absence of

abundant high quality data and guidelines, these are difficult, ethically complex decisions that must be individualized to each patient or couple based on their values and understanding of risk.

I. Infertility and infertility treatment considerations among individuals with congenital and acquired heart disease

The prevalence of infertility is likely to be similar among persons with heart disease and the general population.(16) However, there is an overlap of cardiometabolic conditions and certain subtypes of female infertility, reflecting some possible common pathways in reproductive and cardiovascular systems (Figure 1). While all-cause infertility has not been consistently associated with CVD in women,(30) most studies use self-reported history of infertility and did not completely adjust for other protective cardiovascular behaviours. However, severity of infertility has been evaluated as a potential marker of increased cardiovascular risk. A population-based study in Ontario showed that receipt of infertility treatment not resulting in pregnancy (i.e. unsuccessful treatment) was associated with a 19% increased risk of a composite cardiovascular outcome over 8 years compared with women who had a live birth after treatment.(27)

Infertility and adult congenital heart disease

Certain syndromes that include congenital cardiac defects are associated with infertility. For example, menstrual irregularities have been described in pregnant women after Fontan palliation.(31) Arguably the most common and well understood syndrome associated with infertility and congenital heart conditions is Turner's syndrome.(16) Because the majority of people with Turner's syndrome have premature ovarian insufficiency, it is highly unlikely for these individuals to conceive without reproductive assistance - specifically donor oocyte IVF.(32) Pregnancy in Turner's syndrome is considered high risk because of associated aortopathies and metabolic issues. As such, appropriate pre-IVF care in patients with Turner's includes cross-sectional imaging of the aortic root and aortic valve. Pregnancy, and therefore IVF, is contraindicated if the aortic size index exceeds 25 mm/m2, as the risk of dissection during pregnancy in

these cases approaches 10%.(16, 33, 34) Pre-pregnancy aortic root repair can be considered if the aortic diameter is 20-24 mm/m2.(35) Women with Turner's syndrome should also be counseled about the risk of gestational diabetes and require monitoring for the development or worsening of hypertension in pregnancy.(32)

Association between polycystic ovarian syndrome and cardiovascular disease

PCOS is a heterogeneous disorder characterized by oligo-anovulation, hyperandrogenism, and insulin resistance.(36) Because obesity and metabolic syndrome commonly co-exist with PCOS, it is typically recommended to screen all individuals with PCOS for type 2 diabetes mellitus, hypertension and dyslipidemia, which, if present, are typically managed with lifestyle approaches as well as insulin sensitizers and statins, while menstrual irregularities are managed with combined hormonal contraceptives.(37) Over the long-term, studies have indicated a 3-fold increase in the risk of type 2 diabetes and approximately 1.5-fold increased risk of chronic hypertension and cerebrovascular disease among individuals with PCOS compared with unaffected individuals.(38)

Individuals with PCOS attempting to conceive are initially treated with the aromatase inhibitor letrozole, which has shown superiority over clomiphene citrate in inducing ovulation and achieving live births.(39) If ovulation induction approaches are not successful, IVF is the next step along with intensive ovarian stimulation approaches, which puts patients at high risk of ovarian hyperstimulation syndrome – a potentially life-threatening complication of infertility treatment characterized by ovarian enlargement, widespread endothelial injury and third spacing.(40, 41) Once pregnant, persons with PCOS experience an increased risk of a variety of complications such as miscarriage, pre-term birth, gestational diabetes and hypertensive disorders of pregnancy (HDP).(42, 43)

Association between endometriosis and cardiovascular disease

Endometriosis is another condition that is strongly linked with infertility as well as poor cardiometabolic health. Endometriosis often presents with chronic pelvic pain and infertility and affects

up to 15% of reproductive-aged women.(43) Treatment is challenging, and pregnancy outcomes depend on the severity of disease.(44) Several observational studies have suggested an increased risk of CVD in women with endometriosis. The Nurses' Health Study II, a prospective cohort study from the United States, demonstrated that the risk of coronary disease and other cardiovascular conditions were 1.8-fold higher in a population with laparoscopically confirmed endometriosis compared with unaffected women, possibly explained by a greater proportion of individuals with endometriosis who require hysterectomy-oophorectomy.(45) Similarly, two recent population-based studies in Taiwan and the United Kingdom, also indicated higher risk of cardiovascular events among young people with endometriosis.(14, 15, 45)

In summary, various cardiac conditions and risk factors may exist in reproductive-aged individuals who present with infertility and desire pregnancy. Consideration of pregnancy-specific risks as well as potential risks related to infertility treatment are important components of pre-conception care in individuals at risk for, or with existing CVD.

II. An overview of infertility treatment protocols and their potential for cardiovascular decompensation

The first successful live birth following IVF occurred in 1978.(46) Over the subsequent 4 decades, more than ten million people have been born by IVF,(47) and in Canada, IVF accounts for up to 4% of births each year.(48) However, because the overall success rate of IVF is estimated at 22% per cycle across all ages,(21) many individuals are exposed to IVF but do not become pregnant, often with unknown consequences to their health. Within Canada, ART clinics report on reproductive outcomes of IVF cycles through the Canadian Fertility and Andrology Society,(49) while reporting of maternal health outcomes beyond reproductive success is not routine.

Assisted reproduction approaches

Pre-IVF and other infertility treatment protocols have advanced considerably since they were first used, and they are increasingly individualized to the needs of the couple or individual. Typically,

infertility treatments are categorized as either non-invasive (i.e., intrauterine insemination) or invasive (i.e., IVF-based treatment).(50) Non-invasive treatment consists of pharmacological ovulation induction, often accompanied by intrauterine insemination of sperm to facilitate fertilization. Non-invasive treatments are typically offered first to women and/or their partner when there is mild male factor infertility, oligo-anovulation or to same-sex female partners wishing to conceive. Invasive fertility treatments such as IVF are often used when noninvasive treatments are unsuccessful, and indicated in severe male factor infertility and tubal disease.(51, 52)

In vitro fertilization protocols

Standard IVF procedure involves retrieval of mature oocytes from the ovarian follicle and fertilization in vitro by freely mobile sperms (53) or through intracytoplasmic sperm injection.(54) It is likely that the pre-IVF strategies for oocyte maturation have important implications for women with CVD, as these often result in supraphysiologic levels of circulating estrogen, and dramatic fluid shifts, as outlined in detail below. Oocyte maturation may rely on endogenous triggers - so-called "natural cycle" IVF - although the success rate with this technique is relatively low given the low number of follicles that are produced in a given cycle.(55) More commonly, exogenous hormones are used to trigger oocyte maturation through controlled ovarian stimulation ("stimulated cycle" IVF).(20) In controlled ovarian stimulation, multiple follicles are stimulated, allowing for the retrieval of a greater number of oocytes, translating to a higher chance of successful fertilization and implantation.

There are two predominant controlled ovarian stimulation protocols that require careful consideration in a patient with pre-existing heart disease **(Figure 2)**. The first more conventional IVF protocol, known as the long protocol, begins with the downregulation of the pituitary gland using exogenous gonadotropin-releasing hormone agonists or antagonists.(20) Gonadotropin releasing hormone agonists improve the number of retrieved oocytes per cycle, thus contributing to increased clinical pregnancy rate compared with gonadotropin releasing antagonists.(42) Gonadotropin releasing hormone agonists have also been demonstrated to improve endometrial receptivity of IVF patients when compared

with gonadotropin releasing hormone antagonists.(56) However these advantages must be weighed against the risk of ovarian hyperstimulation syndrome. As such, the main rationale for choosing an antagonist protocol is to diminish the risk of ovarian hyperstimulation syndrome. The modestly lower clinical pregnancy rate associated with gonadotropin releasing hormone antagonists, however, may result in repeated implantation failure and thus may subject to patient to repeated rounds of controlled ovarian stimulation. The final step in the long protocol is to stimulate follicular growth and oocyte maturation using exogenous follicle-stimulating hormone (FSH), resulting in a surge in estradiol. Alternative short protocols skip the downregulation phase and begin immediately with the administration of FSH along with gonadotropin releasing hormone agonist.(57) In both long and short protocols, human chorionic gonadotropin is administered for the final maturation of the oocytes and oocyte extraction.(57)

Cardiovascular concerns of ovarian stimulation and in vitro fertilization

There are various reasons for concern about adverse cardiovascular effects of controlled ovarian stimulation in women with symptomatic CVD at high risk for decompensation. For instance, it has been suggested that the process of controlled ovarian stimulation may result in endothelial dysfunction and subsequent capillary leak syndrome **(Figure 2)**.(21, 58, 59) A small mechanistic study demonstrated marked activation of the renin-angiotensin system during periods of elevated estradiol in ovarian stimulation,(58) and another study suggested a direct association between the rise in pro-renin and number of follicles stimulated.(60) Ovarian hyperstimulation syndrome can be potentially devastating in women with cardiovascular conditions such as valvular disease or cardiomyopathy as they may be unable to tolerate the increased preload. The incidence of moderate to severe ovarian hyperstimulation syndrome has been reported to range between 3% and 8% of the women undergoing controlled ovarian stimulation in the general population and increases from 10% to 20% among women who require more intensive stimulation approaches, such as those with PCOS.(61, 62) While comparison of different protocols has not been studied in women with CVD, using approaches that minimize risk for ovarian hyperstimulation syndrome is likely to be safest. The European Society of Cardiology statement about pregnancy and heart

disease recommends consideration for natural cycle IVF, or controlled ovarian stimulation with careful monitoring, use of gonadotropin releasing hormone antagonists and low dose FSH.(16) Infertility treatment is contraindicated in patients with modified WHO (mWHO) class IV heart disease, since pregnancy is not advised in these individuals.(16)

Other considerations for individuals with pre-existing heart disease undergoing controlled ovarian stimulation and oocyte retrieval include peri-procedure anti-thrombotic and anticoagulation management for individuals with mechanical heart valves or atrial fibrillation. Moreover, there may be a role for anesthesia involvement to manage cardiovascular consequences of high parasympathetic tone during oocyte retrieval. Oocyte retrieval in such individuals, while typically an elective outpatient procedure, should ideally be planned by the reproductive specialist in tertiary monitored settings in conjunction with anesthesiologists, cardiologists, hematologists, reproductive specialists and obstetric medicine providers in keeping with the preferred multidisciplinary cardio-obstetrics program model that has been described by others.(63) Finally, the number of embryos transferred per cycle is an important consideration in individuals with heart disease planning ART. In most but not all jurisdictions, elective single embryo transfer is the preferred approach to avoid health risks due to multiple gestation.(64) Multiple gestation pregnancy is associated with increased metabolic and cardiovascular demands and should be avoided in most individuals with CVD.

III. Cardiovascular and pregnancy outcomes after infertility treatment

Pregnancy outcomes among women with CVD

Pregnancy is considered a cardiovascular stress test.(65, 66) Stroke volume and heart rate increase to achieve a plasma volume and cardiac output increase by approximately 50% by the third trimester.(67, 68) Cardiac output in twin pregnancies increases by another 20% when compared with singleton pregnancies.(69) These changes intensify during labour and delivery during uterine contractions, expulsive efforts, pain, stress, and blood loss.(16) Cardiac output increases by 15% during labour and up

to 80% in the early postpartum period(16) with persistent altered cardiovascular reserve for up to 6 months after delivery.(70)

These physiological demands of pregnancy are more difficult to tolerate among persons with pre-existing CVD, and may result in cardiovascular decompensation including arrythmias, congestive heart failure, systemic thromboembolism, stroke, endocarditis, and death.(71) Additionally, pregnant individuals with pre-existing CVD have higher rates of preeclampsia,(16, 72) postpartum hemorrhage(6, 16) and placental abruption(6, 16) when compared with the general population. Neonatal complications are also more common after obstetric delivery among individuals with pre-existing CVD, occurring in about a quarter of patients, with a risk of neonatal mortality approaching 4%.(16)

The mWHO classification of maternal cardiovascular risk classifies pregnant persons with CVD into 4 categories based on risk of decompensation during pregnancy (e.g., Class I, successfully repaired simple lesions, Class II, unrepaired atrial septal defect, Class III, Fontan circulation, and Class IV, pulmonary arterial hypertension or severe left ventricular dysfunction).(16) Age, comorbid conditions, functional class and use of medication predict poor outcomes in addition to the anatomic lesion.(16) Similarly, Siu, Silversides and colleagues developed and validated the maternal Cardiac Disease in Pregnancy (CARPREG I & II) risk scores to categorize the risk of adverse maternal outcomes among women with heart disease.(73, 74) In the CARPREG II score, use of infertility treatment was identified as an additional factor predicting cardiac decompensation.(73, 74)

Pregnancy outcomes of infertility treatment among individuals with CVD

Outcomes of infertility treatments in pregnant individuals with pre-existing CVD are poorly described. A 2014 case series reported outcomes of 22 pregnancies in 20 patients with CVD who used infertility treatment to conceive from the Pregnancy and Heart Disease Program in Toronto.(6) Among this sample, 73% of pregnancies resulted in at least 1 complication including cardiac outcomes (i.e., arrhythmia, congestive heart failure), obstetric outcomes (i.e., postpartum hemorrhage, antepartum

hemorrhage, preeclampsia, abruption), fetal and neonatal outcome (i.e., neonatal death, preterm birth, respiratory distress, small-for-gestational-age). A diagnosis of ovarian hyperstimulation syndrome was reported in 4 people (18%).(6)

Short- and long-term cardiovascular implications of infertility treatments

While it is known that individuals pregnant using infertility treatment are at greater risk of experiencing pregnancy complications such as preeclampsia and pre-term birth,(23) and that these pregnancy conditions are sex-specific risk factors for CVD,(22) it is unclear whether use of infertility treatment independently impacts on longer-term CVD. HDP has been consistently linked with ART. HDP comprises gestational hypertension, preeclampsia, or the hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome which together complicate up to 10% of pregnancies in the general population and are a leading cause of maternal morbidity and mortality worldwide.(75, 76) The risk of HDP appears to be increased among pregnant individuals who have conceived using ART, in particular among those with additional risk factors for HDP such as obesity.(8, 59, 77, 78) A recent systematic review of 85 cohort studies found that IVF increased the risk of HDP in both singleton pregnancies (OR: 1.70; 95% CI: 1.60 to 1.80) and multiple pregnancies (OR: 1.34; 95% CI: 1.20 to 1.50).This risk was particularly high among frozen embryo transfers (OR: 1.74; 95% CI: 1.58 to 1.92) and oocyte donation pregnancies (OR: 4.42; 95% CI: 3.00 to 6.51).(82) Given that many maternal risk factors for HDP are found in individuals who require ART or other infertility treatment (i.e. age, nulliparity, obesity),(79-81) it is challenging to disentangle the effect of these factors from the effect of treatment itself.

Pregnancy is a known risk factor for venous thromboembolism (VTE), with an estimated incidence of 1-2 per 1000 pregnancies.(82) Receipt of infertility treatment has been shown to further increase the risk of pregnancy-associated VTE.(83, 84) A systematic review of 21 studies revealed a two-fold increase in the risk of antepartum VTE in ART-exposed pregnant women compared with unexposed women, especially if ovarian hyperstimulation syndrome was present.(85) The risk of pulmonary embolism has also been reported to be higher following unsuccessful ART cycle as compared with

successful ART,(86) highlighting the importance of studying individuals who receive controlled ovarian stimulation, and not only those who become pregnant through ART. Long-term data on thromboembolic risk following exposure to ART are lacking, however one study demonstrated no increased risk of venous thromboembolism over a median follow-up of 9.7 years.(87)

A systematic review of long-term cardiovascular outcomes after ART including 6 studies reported no increased risk of cardiovascular morbidity or death among individuals previously exposed to infertility treatment than unexposed individuals when pooling data from 4 studies. There was insufficient data on the development of hypertension or diabetes mellitus following ART in this review. This same review showed a possible signal towards increased risk of stroke that warrants further study (pooled HR: 1.25; 95% CI: 0.96 to 1.63).(21) A factor common to all of these observational studies is the "healthy user effect" because ART has historically been out of pocket and mostly a treatment of socially advantaged populations.

IV. Antithrombotic therapy during ART pregnancy

Aspirin for the prevention of preeclampsia

The US Preventive Task Force(88) and other bodies(89, 90) recommend use of low dose acetylsalicylic acid (ASA) for the prevention of preeclampsia in pregnant individuals at high risk for this disorder, starting at 11 weeks' gestation. This recommendation is based on high-level evidence demonstrating a consistent reduction in overall rate of preeclampsia using ASA ranging from 50 – 150 mg nightly when initiated prior to 16 weeks.(91) A recent large randomized trial using a validated algorithm to identify pregnant persons at high risk for pre-term preeclampsia revealed 62% reduction in pre-term (i.e., with onset prior to 37 weeks' gestation) preeclampsia in those randomized to receive ASA 150 mg daily compared with placebo.(92) While clinical risk factors alone are known to be poorly predictive of preeclampsia,(85) risk factors prompting prescription of ASA noted in these guidelines include chronic

hypertension, obesity, chronic kidney disease, advanced female age, twin gestation, antiphospholipid syndrome, and a prior history of preeclampsia.

Clinical practice guidelines do not mention infertility treatment as a risk factor warranting prophylaxis with ASA to prevent preeclampsia. Therefore, at present, ASA is recommended in those who use ART and have additional risk factors for preeclampsia or using the validated algorithm that combines clinical risk factors with first trimester biomarkers, placental biomarkers and uterine artery dopplers.(85) Future clinical practice guidelines should consider including ART as a clinical risk factor for preeclampsia, warranting consideration for ASA if other risk factors co-exist.

ASA use is also not recommended for the purpose of improving clinical pregnancy live birth or reducing the rate of miscarriage since this approach has not been effective and may cause harm due to bleeding during oocyte retrieval.(93) Therefore, individuals with CVD planning IVF who are on secondary prevention of CVD with ASA 81 mg po daily may be advised to discontinue for a period of 5-7 days prior to oocyte retrieval and then resumed. Indications for ASA in pregnant persons with CVD do not differ with or without infertility treatment.

Anticoagulation

Despite the high thrombotic risk in persons who use ART, the absolute risk of VTE in all comers who use ART is estimated to be 1% or lower.(94) Prophylaxis with low molecular weight heparin is recommended when the estimated risk of pregnancy-associated VTE in pregnancy exceeds 1%, such as among women who undergo ART complicated by severe ovarian hyperstimulation syndrome, or among women with other risk factors for VTE.(94)

There is a lack of formal guidance on prophylactic anticoagulation in individuals with CVD undergoing ART. Individuals with a pre-existing indication for therapeutic anticoagulation should continue during infertility treatment as well as during pregnancy and should be followed by a multidisciplinary care team with expertise in thrombosis and cardiology regarding the choice and intensity

of anticoagulant therapy. Health care providers should withhold anticoagulation during oocyte retrieval and initiate peri-procedure bridging if required.(95)

Conclusion

The advancement and accessibility of infertility treatments in Canada and in other high-income countries has provided many infertile individuals and couples with the opportunity to reproduce. While generally safe, infertility treatments, particularly IVF, may pose cardiovascular challenges in patients due to supraphysiologic hormones and associated thrombosis and endothelial dysfunction. Individualized pre-conception counselling and antenatal care pathways are needed for women seeking reproductive assistance to estimate cardiovascular risk during pregnancy. Future research is needed to evaluate outcomes of different forms of ART in women with pre-existing heart disease. In the absence of high-quality data, using natural cycle IVF, low-dose controlled ovarian stimulation that avoids ovarian hyperstimulation syndrome, and single embryo transfer is likely the safest approach. A multidisciplinary care team should aid in preconception decision making about the cardiovascular safety of ART in keeping with the cardio-obstetrics program model of care.

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Competing interests

The authors declare no competing interest.

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Figure 1. Conceptual framework linking infertility subtypes, infertility treatment, pregnancy outcome and longer-term cardiovascular health

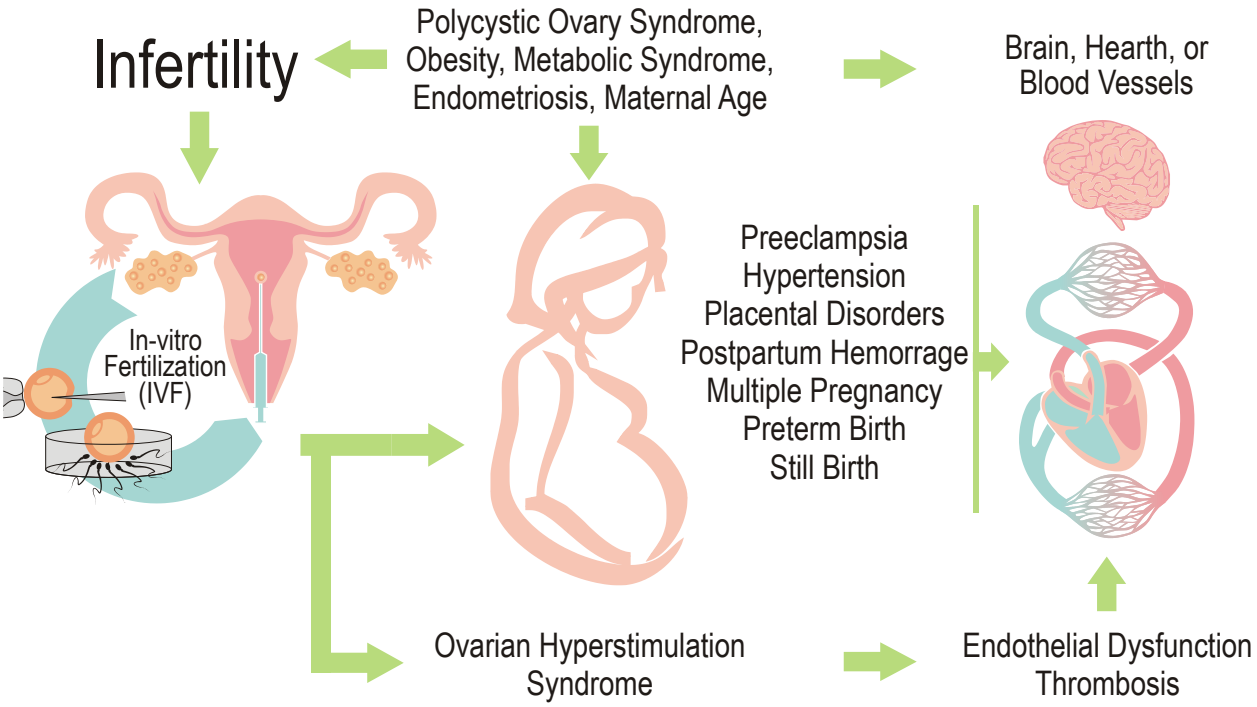

Figure 2. Hormonal changes throughout the reproductive cycle during in vitro fertilization protocols and associated vascular effects

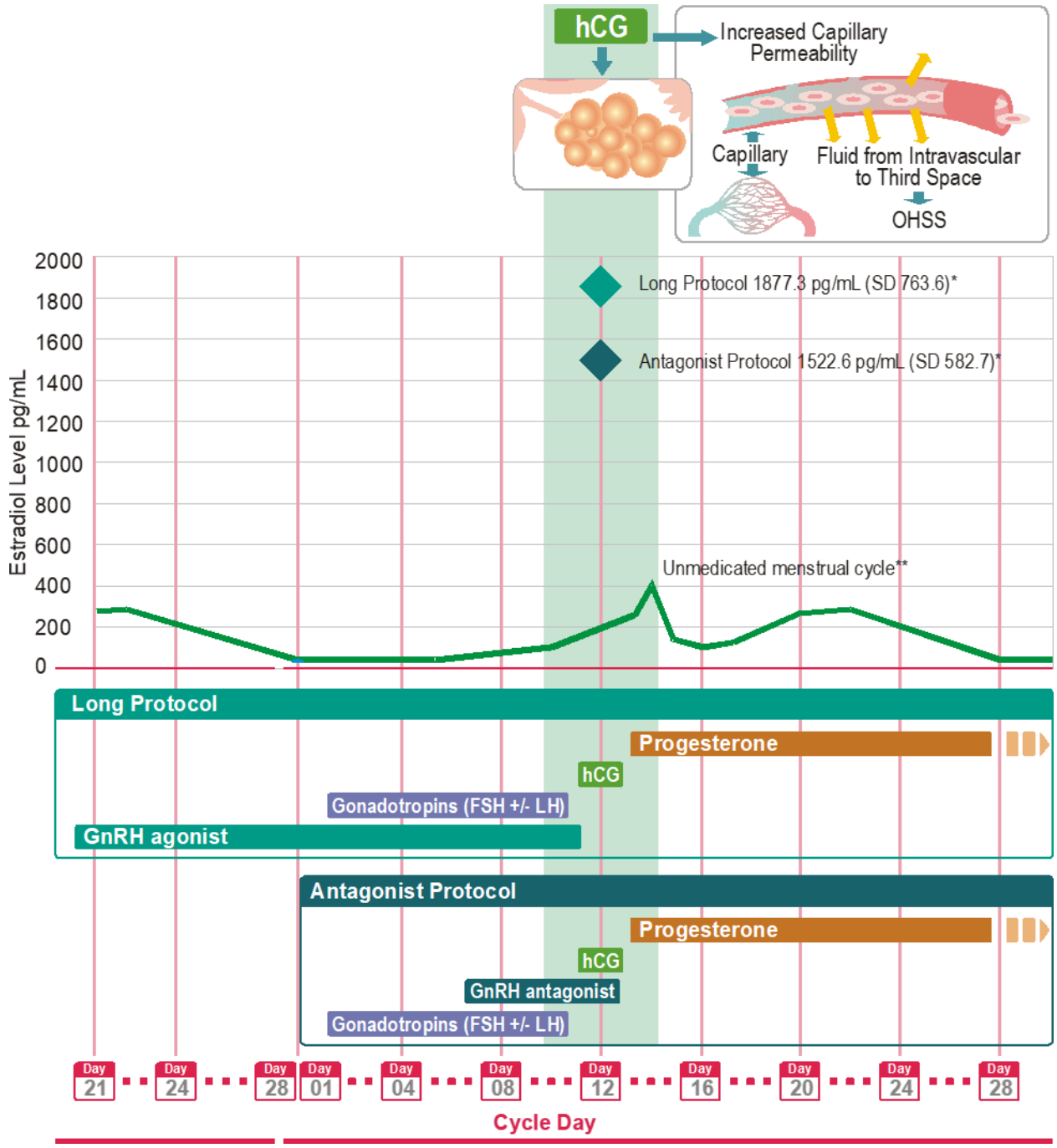

Figure 2 legend: Mean estradiol levels on the day of human chorionic gonadotrophin (HCG) administration based on * Wang R, Lin S, Wang Y, Qian W, Zhou L. Comparisons of GnRH antagonist protocol versus GnRH agonist long protocol in patients with normal ovarian reserve:

A systematic review and meta-analysis. PLoS One. 2017 Apr 24;12(4):e0175985. Estradiol levels in unmedicated menstrual cycle: ** Fritz MA, Speroff L. Clinical gynecologic endocrinology and infertility. 8th ed. ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2011.

Figure 2 Abbreviations: hcG human chorionic gonadotropin; OHSS ovarian hyperstimulation syndrome; GnRH gonadotropic releasing hormone; FSH follicle stimulating hormone; LH luteinizing hormone