

**IDENTIFYING RISKS OF AND MODIFIABLE FACTORS TO IMPROVE
PLACENTAL OUTCOMES IN ASSISTED CONCEPTION IN ONTARIO**

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List of Acronyms and Abbreviations

AHRA	Assisted Human Reproduction Act
aOR	Adjusted odds ratio
ART	Assisted reproductive technologies
ASD	Absolute standardized difference
BMI	Body mass index
BORN	Better Outcomes Registry & Network
CARTR	Canadian Assisted Reproductive Technologies Register
CFAS	Canadian Fertility & Andrology Society
DET	Double embryo transfer
eSET	Elective single embryo transfer
FGR	Fetal growth restriction
ICSI	Intracytoplasmic sperm injection
IPD	Ischemic placental disease
IPTW	Inverse probability of treatment weight
IUI	Intrauterine insemination
IUGR	Intrauterine growth restriction
IVF	In vitro fertilization
LGA	Large for gestational age
OFPP	Ontario Fertility Program
OR	Odds ratio
SET	Single embryo transfer
SGA	Small for gestational age

SOGC

Society of Obstetricians and Gynecologists of Canada

Abstract

The use of assisted reproductive technologies (ART) in Canada has increased rapidly over the past decade, with a 235% increase in reported ART cycle starts and a 190% increase in resultant clinical pregnancies from 2008-2018, outpacing our understanding of potential adverse perinatal outcomes. This dissertation project recognizes that the use of ART will continue to increase in both scale and scope, and that it will continue to be a valued means of overcoming infertility and involuntary childlessness. The balance of risks and benefits of these rapid technological innovations for human health is uncertain. Therefore, this project seeks to produce evidence about both the risks associated with ART as well as component treatment factors amenable to modification to make its use safer.

While available evidence indicates that medically assisted pregnancies are at greater risk of placental complications, data are scarce, and estimates of specific effects are varied and not reported in the context of service provision for Canada. Linking data from the Canadian Assisted Reproductive Technologies Register to the Better Outcomes Registry & Network Ontario data, this project commenced with a foundational retrospective cohort study to assess the relationship between ART (in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI)) and placental and placentally-mediated complications by comparing assisted conceptions from four years of ART cycle starts to non-assisted conceptions. Building on the findings from the foundational study, two additional studies were performed to investigate the contributions of two treatment factors, day or stage of embryo at transfer and cryopreservation, to the observed patterns of risk.

Compared to non-assisted pregnancies, we find that assisted pregnancies are associated with an increased risk of placental and placentally-mediated complications. Within assisted pregnancies, risk profiles are best achieved by stratifying the analysis by both treatment type (IVF or ICSI) and embryo transfer type (fresh or frozen), in keeping with clinical practice. Although we did not find the day or stage of embryo at transfer to be a primary independent contributor to the observed increase in risk of these complication, patterns observed in the relationship between method of cryopreservation and these outcomes warrant further investigation.

This thesis concludes by arguing for the pursuit of a hierarchy of risk approach to the use of ART, which is supported by the findings of this project, as it may be the most effective method to guide technological innovation and policy implementation to mitigate the risks associated with ART while not being overly restrictive of the options available to those seeking to build a family.

University of Adelaide Statement of Originality and Consent

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and the University of Ottawa.

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Name

Signature

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Chapter 1: Introduction

This dissertation work consists of a series of three interrelated articles (Chapters 2-4), which are standalone manuscripts that contribute to the collective understanding in this research and clinical area by addressing my research questions (described below). Chapter 1, the introduction, provides an overview of the main elements of the project (e.g., research question, objectives, and goals) and contextualizes the work in a population health approach and relevant history. The introduction ends with a summary of the dissertation.

This dissertation is supported by supplementary material, including a glossary of terms and appendices, as well as supplementary material for individual chapters as specifically noted therein.

1.1 Research Question

This dissertation research is situated within an ever-evolving conversation about the use of assisted reproductive technologies (ART) in response to infertility. ART, unquestionably, represents a valuable set of bio-technological innovations that provide people experiencing infertility with the opportunity to fulfil their desire for parenthood, whether that be through the use of their own gametes, or donated from another. However, it is now well established that ART increases the risks of various adverse obstetric, perinatal, and neonatal outcomes in assisted conceptions as compared with non-assisted conceptions. The use of ART and the expeditious implementation of constantly evolving technological innovations without comprehensive evidence as to their multifaceted outcomes, including safety, outpace our understanding of why these outcomes occur. Consequently, there are critical ethical, clinical, and policy arguments about how such technologies are and should be promulgated. This dissertation recognizes that

the use of ART will continue to increase in both scale and scope, and that it will continue to be a valued means of overcoming infertility and involuntary childlessness. Therefore, this project seeks to produce evidence about both the risks associated with ART as well as component factors amenable to modification to make its use safer.

A traditional approach to managing innovation adoption within public health is to identify and partition sources of risk, quantify risk factors, and identify opportunities for modifying risk. In the instance of ART, the course of pregnancy is distinctly different with regards to the risk of adverse outcomes. Some factors, such as the role of maternal pre-existing metabolic disease are being well-explored and managed. However, there are others, such as the transitory but critical role in pregnancy of placental development and function for maternal and child health in pregnancy, and beyond, which are less well understood. Placental function is both ephemeral and difficult to observe *in situ*, but with the benefit of routine large scale clinical data collections, we can map its relationship to mode of conception, maternal health, and pregnancy outcomes. In this context, this thesis has a focus on placental and placentally-mediated complications as major contributions to maternal and neonatal morbidity and mortality following assisted reproduction.

Accordingly, the project's overarching research question is "What modifiable factors exist to improve access to and perinatal outcomes of assisted conceptions?" Note that here "modifiable factors" are those factors contributing to adverse outcomes that are amenable to policy or technology interventions to improve and protect the health of the population.

1.2 Objectives and Goals

The objectives of my research are 1) to isolate the association between assisted reproduction and placental and placentally-mediated complications in pregnancy, and 2) to identify the

contributions of contemporary ART treatments and treatment factors to these outcomes. The ultimate goals of this research are twofold. The first is to help guide technological innovation in ART by identifying treatment factors that are amenable to modification through future research. The second is to inform the design of assisted reproduction regulatory and funding policy in Canada such that it reflects and incentivizes best practices that maximize the health outcomes of Canadian users and their children while limiting the potential harms. Both goals serve to improve health outcomes and reduce inequitable outcomes among the general population and those accessing ART to address infertility or involuntary childlessness.

1.3 Infertility and Assisted Reproduction Defined

Infertility, generally defined as the inability to achieve a pregnancy while exposed to the conditions required for conception, affects between 11.5% and 15.7% of Canadian couples of reproductive age (18-44).(1) Infertility can be grouped as biological or social in nature. The biological approach, reflected in numerous clinical and epidemiological definitions, identifies infertility as the result of disruptions to the reproductive process, including abnormalities in the production, function, or delivery of sperm; ovulatory disorders; tubal obstruction; anomalies of the uterus, as well as idiopathic infertility. Other sources of biological interruptions include senescence (biological age) and gonadotoxic therapy. Social infertility manifests where, for example, same-sex couple and individuals choosing to parent independently seek to have children. Social infertility due to voluntary contraception or sterilization is not broadly considered a handicap until it comes into conflict with the desire for parenthood and is outside the scope of this thesis.

Today, ART refers primarily to in vitro fertilization (IVF) and intracytoplasmic sperm injection

(ICSI) (and this is how we define ART in the included studies), but gamete intrafallopian transfer (GIFT), intrauterine insemination (IUI), ovulation induction only and the use of donor gametes/embryos also fall under the umbrella of assisted conception, representing a range of points and levels of intervention.

While IVF and ICSI share a number of clinical and laboratory steps in their process, they are distinct treatments where the risk of adverse pregnancy and perinatal outcomes have been reported. Therefore, this thesis stratifies by or adjusts analyses by treatment type to ensure that any differences in outcomes attributable to their differences are not obscured.

1.3.1 Day or Stage of Embryo at Transfer

One of the key decisions in assisted reproduction research and practice is that of when embryo transfer should occur after fertilization. In a non-assisted (natural) human conception, embryos reach the uterus two to three days after fertilization, allowing them to reach the morula or blastocyst stage prior to being exposed to the uterine environment. Historically, early human IVF involved the transfer of embryos at the two- to four-cell cell stage in a natural cycle, corresponding with 48 hours after oocyte retrieval as early blastocyst embryo transfer attempts by Edwards & Steptoe were unsuccessful in leading to viable pregnancies.(2) In spite of the biological asynchrony between the stage of embryo development and uterine environment, concerns about the potential for harm to the embryo in vitro due to prolonged culture exposure, as well as low embryo survival and in vitro development to the blastocyst stage promoted the continuation of this practice.(2) Further, early successes in cryopreservation were had with 8-cell embryos, which may also have influenced practice.(3) It was not until 13 years after the first successful IVF pregnancy that the first successful pregnancies following embryo transfer on Day

5 after oocyte retrieval occurred.(4) In addition to improved synchronicity between embryo developmental stage and the uterine environment, early arguments for the evidence-based expansion to Day 5 or blastocyst transfer included facilitating selective transfer of embryos that survive the gene activation selection point at the 4-8 cell stage, thus increasing the implantation rate and providing a better embryonic base for preimplantation genetic diagnosis (and sex-typing, at the time) thereby reducing the risk of damage to an embryo prior to implantation.(2) Accordingly, hypothesized barriers to successful blastocyst embryo transfer including culture media and ovarian stimulation became key areas of basic research to facilitate the viability of this option.(5)

Today, the debate about the transfer of Day 3 versus Day 5, or cleavage stage versus blastocyst transfer remains unresolved. As additional procedures were added to conventional IVF treatment, including ICSI and cryopreservation of embryos and gametes, the answer to the question became less clear as the embryo seemed to interact with these elements differently alone and combined, prompting further research. A 2005 systematic review (6) found that while there appeared to be no difference in clinical pregnancy and live birth rates between cleavage stage and blastocyst transfer, the latter was associated with a significant increase in failure to transfer any embryos; there was insufficient data to assess these rates in fresh and frozen transfers, respectively. In a departure from the historical and dominant focus in ART research on implantation, pregnancy, and live birth rates, there is an imperative to attend to adverse obstetric, perinatal, and neonatal outcomes with the maturation of the technology. This emergent perspective considers the transition of the patient population to more diverse infertility aetiologies and risk profiles for adverse outcomes, while considering the increasingly subtle contributions from laboratory exposures. It is this combination of scientific inertia and clinical

innovation that reinforces the need for targeted research.

1.3.2 Cryopreservation

Alongside refining the practice of transferring fresh embryos, the ability to preserve embryos and gametes became of interest to improve efficiency, reduce “wastage”, expand the market to cancer patients and the reproductively senescent, and address a wider range of infertility aetiologies, including improving understanding of infertility of unknown origin, which continues to represent one of the most common diagnostic categories.

Experimental cryopreservation of mammalian cells and tissues dates back to early 20th century animal science in the pursuit of improved methods of preserving tissue in frozen form.(7) In the 1960s and 1970s, there was a surge in interest in applying cryopreservation techniques to gametes and embryos following its successful application to human red blood cells.(8)

Numerous animal models (including mouse, rat, rabbit, sheep, and cow) were used to assess the viability of freezing oocytes, sperm, and embryos that could then be thawed and used to conceive a pregnancy. Efforts to freeze oocytes and embryos were less fruitful.

In 1971, Whittingham (9) outlined a new technique for improving the viability of embryos after cryopreservation upon discovering that adding an appropriate protective agent to the medium prior to freezing increased rates of embryo freeze-thaw survival and post-thaw cleavage to the blastocyst stage. In 1974, Leibo et al. (10) determined that rates of cooling and thawing were critical to successful cryopreservation using mouse models; three years later, Whittingham (11) reported success in thawing slow-frozen embryos, fertilizing them, and achieving term pregnancies and livebirths after embryo transfer in mice. In parallel, other scientists identified that temperature was more important than duration of storage in promoting embryo viability,

once again using a mouse model (12,13), and efforts to improve the cryopreservation of embryos - with applications for human reproduction in mind - continued.(14)

In 1983, Trounson & Mohr announced the first successful pregnancy after the transfer of a frozen-thawed 8-cell embryo that had been preserved in liquid nitrogen for four months.(3,15)

Although the pregnancy was ultimately terminated due to a severe complication, the pregnancy was a clear demonstration to the world that combining assisted reproduction and cryopreservation techniques was possible.

Though unbeknownst at the time, theirs was not, however, the world's first pregnancy from a frozen-thawed embryo. Within months of Louise Brown's mother becoming pregnant, Dr.

Subhas Mukerji successfully transferred a frozen-thawed eight-cell embryo into an anonymous mother who delivered a girl in October 1978 in West Bengal.(16,17) In response to the birth, the government established an inquiry committee of experts (though none had a background in reproductive technologies) to assess his claim, a claim they ultimately rejected.(17) In his report to the government and committee, Mukerji made the decision to omit detailed information about the exact slow-freezing and thaw method he used, how the coolant was removed after thaw, and the contents of the culture media until he could confirm that the work was reproducible and he had published in scientific journals. He also refused to prove the baby's existence as the family had requested confidentiality given the pervasive stigma associated with infertility at the time.

This decision did not please the committee, nor the government, and he was prevented from sharing his findings at any conference or in publication form without government permission.

Government actions, including banning him from leaving the country, combined with the maligning of his name and humiliation by his colleagues, ultimately took a significant and tragic toll on Mukerji's well-being.(17)

Although we may never know the exact procedures used, and although pregnancies from frozen-thawed embryos are now a routine component of ART treatment, it is remarkable that he managed to achieve what no one else could a full five years before anyone else.

In addition to Trounson & Mohr's success, 1983 was also the year of the first published successful use of cryopreserved semen in conventional IVF.(18) In the ensuing couple of years, other successful pregnancies from cryopreserved embryos or semen were announced around the world.(19–21) The first successful pregnancy following the transfer of an embryo developed from a frozen-thawed oocyte with insemination was reported in 1986.(22)

The 1980s end with an expansion of inquiry about the specific biologic and treatment factors affecting embryo survival and viability following cryopreservation. In their experiments, Fehilly et al. (23) examined the survival and implantation rates of cleavage stage embryos and expanded blastocysts, and while they achieved pregnancies after both, they identified that expanded blastocysts were more likely to survive cryopreservation than cleavage stage embryos, a finding which was also observed elsewhere.(24–26) Embryo viability was found to be related to cell stage and embryonic fragmentation at freezing (25), speed of growth post-thaw and morphology (particularly in blastocysts) (24), and survival was also identified as a function of the blastocyst's ability to protect itself from the cryoprotectant.(24)

In 1985, the field of human reproductive cryobiology was propelled forward when Rall & Fahy (27) developed the base of modern vitrification technology. At the time, low survival and viability rates with conventional cryopreservation were hypothesized to stem from extracellular ice formation damaging the intracellular structure. Conventional cryopreservation (slow-freezing) achieved intracellular vitrification, but it could still allow extracellular ice development. Their new method of cryopreservation prevented both intra- and extra-cellular ice

formation.

Amidst the growing interest and efforts to apply vitrification to embryos in assisted reproduction and a continued focus on implantation, pregnancy and live birth rates, caution was urged with respect to its application to human oocytes.(28) Kola et al. (28) presented the first documented findings of altered developmental potential of embryos and fetuses developed from vitrified mouse oocytes. They observed a threefold increase in the incidence of aneuploidy in zygotes regardless of whether vitrification or slow-freezing with dimethyl sulfoxide (DMSO) was used, and also observed malformed fetuses after both oocyte vitrification and exposure of oocytes to vitrification solutions. Given that they did not observe malformations following the use of slow-frozen or fresh oocytes, they concluded that chemicals used in vitrification had teratogenic potentially carcinogenic effects.(28)

The 1990s were a time of intense research activity and clinical application of vitrification and slow-freezing in combination with the suite of existing ART technologies and practices, starting with experimentation with ultrarapid freezing. By the late-1990s, relatively few pregnancies had resulted from cryopreserved oocytes, with injuries to the oocyte and low implantation rates impeding success (29,30), but scientists remained optimistic in their pursuits.(31) Membrane permeability, cryoprotectant use and ice formation continued to be the focus of basic science research (29) with the hope of overcoming the hurdle that had been cleared with respect to semen cryopreservation decades prior. The first documented birth following ICSI with a cryopreserved oocyte was achieved in 1997, confirming the possibility of pregnancy after oocyte cryopreservation and, more importantly, demonstrating the ability to use ICSI and oocyte cryopreservation together.(32) Similarly, the use of ICSI with frozen-thawed sperm was demonstrated to achieve comparable implantation, clinical pregnancy per embryo transfer, and

ongoing pregnancy rates to fresh sperm.(33)

Conversely, embryo cryopreservation was already an established, critical extension of IVF, particularly using slow-freezing methods (pregnancies following embryo vitrification did not appear until the turn of the century (34)). Although cryopreservation adversely affected embryo quality (35) and post-thaw developmental rates were significantly lower than survival rates in blastocysts at all developmental stages, particularly with increasing blastocyst age (36), no major impacts on the implantation or pregnancy potential of surviving high quality embryos were observed.(35) In a review of 10 years of thawed cycles in France from 1986-1995, Mandelbaum et al. found that the use of frozen-thawed embryos produced an average of 8% of additional births attributable to IVF.(30)

Improvements in successful application of cryopreservation followed the discovery that the exclusive transfer of embryos demonstrating cleavage post-thaw, rather than mixed transfer of embryos with and without further cleavage, resulted in higher implantation, delivery, and birth rates while, in contrast, the exclusive transfer of embryos with no further cleavage did not lead to livebirths.(37) Similarly, the fragility of embryos was demonstrated by biopsy prior to freezing resulting in significantly lower survivorship.(38)

The first study of obstetric outcomes following fresh and frozen embryo transfer in IVF and ICSI, which is a relatively recent area of research focus in ART, was published in 1999.(39) Biochemical pregnancy rates were found to be higher after frozen transfer in both IVF and ICSI cycles. Risks of preterm delivery, very low birth weight, intrauterine death and major malformations were similar between groups, though fresh IVF transfers had a higher rate of low birthweight compared to frozen transfers. While miscarriage rates were similar between fresh and frozen transfers, frozen ICSI cycles had a significantly higher miscarriage rate than frozen

IVF.(39)

In the early 2000's, research continued in pursuit of optimal freezing techniques (40–43) and refined pre-freeze embryo selection criteria.(44) The tenor of the literature shifted firmly from 'can we achieve successful cryopreservation and what can we use it with' to 'to what end are we incorporating cryopreservation in assisted reproduction'. The major benefit of cryopreservation for facilitating single embryo transfer to reduce multiple births without sacrificing cumulative live birth rates was not lost on scientists, clinicians, or policymakers.(45,46)

Today, vitrification has overtaken slow-freezing as the primary method of cryopreservation. Now that the viability of the method for producing ongoing pregnancies and livebirths has been established, much more research is needed to address the gap in our understanding of the effects of this technology on obstetric, perinatal, and neonatal outcomes.

1.4 The Innovation Disjuncture

Much of the history of assisted reproduction in clinical practice can be characterized by rapid adoption of technologies which have been shown to improve implantation, clinical pregnancy, ongoing pregnancy, and live birth rates, but with little other evidence as to their safety for mother or developing fetus.

In 2017, Davies et al. proposed an evidence-based hierarchy of risk for the use of assisted reproductive technologies, in which the likelihood of adverse outcomes increased with increasingly invasive treatment.(47) Many of the adverse outcomes associated with ART conceptions are reflective of clinical and patient decisions, and the current limits of technology, which could be addressed through policy or focused rapid technological development, respectively.(47) They propose that the safest, most minimally invasive treatment strategy should

be selected as the starting point for each patient. However, identifying this treatment option and changing practice accordingly is constrained by four critical knowledge gaps which exist even with the proliferation of research focused on obstetric, perinatal, and neonatal outcomes over the past twenty years. These gaps exist in the relative contributions of treatment and patient factors to observed outcomes, the independent and interactive contributions of contemporary treatments, the contribution of clinical and laboratory innovations, and changes in the patient population over time.(47) This inarguably creates an ethically ambiguous situation wherein services are provided without a full understanding the risks (even if, on the whole, the benefits are considered to outweigh known or hypothesized risks).

In keeping with the goal of using a hierarchy of risk approach to clinical practice, this thesis strives to fill some of the knowledge gaps that currently limit our ability to so orient clinical and laboratory practice.

1.5 Theoretical Background

1.5.1 Population Health Approach

Population health focuses on the distribution of health outcomes in a group of individuals, inclusive of any patterns of determinants and associated policies to improve the health and wellbeing of a population through policy.(48,49)

Although maternal and child health is a central concern in both population health, reproductive disorders and infertility is rather ignored within the field. I would argue that this is an oversight that risks disproportionately harming the population experiencing infertility and their offspring. Infertility both creates and perpetuates population-level patterns of disease, reactive service utilization, and health outcomes (50) which impact not only those living with infertility but also

the population at large. The experience of and response to infertility reflects interactions between underlying human biology, individual behaviour, the health care system, and the social context. In addition to the clear health risks posed by conditions contributing to biological infertility, particularly for women (such as polycystic ovary syndrome and endometriosis), the involuntary childlessness resulting from both biological and social infertility has been linked to poor mental health and psychosocial wellbeing of those affected.⁽⁵¹⁾ This is represented in normative claims about childlessness as undesirable and family building as integral to one's identity in our social climate. The resulting social stigma potentially contributes to suffering of those experiencing infertility, including a sense of bereavement, guilt, loss, and grief as a result.⁽⁵¹⁾ From this perspective, physiological and psychosocial harms are disproportionately distributed in the population, and as a result the need to address infertility with a population health approach is imperative.

Given the increasing use of ART in response to infertility or subfertility, and the anticipated increase in use following the introduction of public funding, the health outcomes of mothers undergoing treatment and of babies born should also be considered a pressing population health issue in maternal and child health. Perinatal outcomes, such as intrauterine growth restriction, preeclampsia, and abnormal placentation, for which assisted conceptions are at increased risk, are associated with acute and long-term health outcomes, costly care, and potential family distress. Ethical dilemmas exist in the provision of ART with little regulation and oversight, relegating users to increased risks and poorer pregnancy and childbirth outcomes and raising the question of whether alleviating the harms of infertility are creating another. The direct and indirect consequences of increasing the availability of ART in our health care system must be thoroughly understood so that risks can be mitigated while not being overly restrictive of the

options available to clinicians and patients. Accordingly, this thesis explores the health outcomes associated with ART in our current policy landscape (i.e., the arena in which past and current policy interact with system- and individual-level factors to shape the user population and determine the observed distribution of outcomes), while also providing evidence for strengthened public policy and regulation. It is important to note that specific investigations of outcome measures, as will be presented in this thesis as a jumping off point for future work in Ontario and Canada, are critical for population health research, but they are only part of the whole and must be situated in a larger body population health research on the issue at hand to develop an appropriate response.(49)

As an interdisciplinary field, population health research draws on a variety of methodological approaches to evidence generation. This thesis uses a “big data” epidemiological approach to query the distribution of our outcomes of interest across a series of ART-related exposures.

1.5.2 Brief History of Assisted Reproduction in Canada

Canada’s first baby conceived via in vitro fertilization (IVF) arrived 25 December 1983, just five years after the world’s first IVF baby was born in England.(52) Born nine weeks premature via caesarean section, the successful live birth to a woman without fallopian tubes represented the first success of the University of British Columbia’s (UBC) team, which had previously attempted 24 pregnancies in 16 other women.(52) Babies conceived in Ontario using IVF swiftly followed.(53) Dr. Victor Gomel, then director of UBC’s IVF clinic (only the second in Canada), predicted that IVF births would become commonplace; he was correct.(52)

The success at home and abroad led to rapidly increasing public demand from women and families struggling to conceive, but there were concerns among the public about the lack of

regulatory oversight and accountability for IVF. In response, the Government of Canada established the Royal Commission on New Reproductive Technologies in 1989, tasked with evaluating the health, social, ethical, legal, and economic implications of assisted reproduction through public and expert consultation. In 1993, the Commission issued 293 recommendations on how Canada should proceed with assisted reproduction such that its inarguable benefits were balanced with its known and unknown iatrogenic harms, both those inherent to the technologies themselves and those resulting from provider practices.⁽⁵⁴⁾ The Commission's key conclusions were that federal legislation was needed to regulate the use of assisted reproductive technologies, that a regulatory body was needed to oversee, monitor, and license ART, and that the country's response needed to be flexible enough to respond to evolving knowledge and technological development.⁽⁵⁴⁾ Subsequently, the Minister of Health proposed a voluntary moratorium on nine practices deemed ethically and socially problematic insofar as they were inconsistent with Canadian values, including sex-selection for non-medical purposes, commercialized surrogacy, gamete and embryo sourcing, egg donation to secure treatment, genetic alteration, human embryo cloning and the retrieval of gametes from fetuses and cadavers.^(55,56) Clinics did not adhere to the moratorium, unsurprisingly, as it was inconsistent with IVF as a commercial venture, and the Advisory Committee struck in 1996 to monitor compliance concluded that this would continue to be the case in the absence of stronger incentive or legislation.⁽⁵⁷⁾

There have been two primary attempts to legislate ART in Canada by the Canadian government. The first attempt to legislate restrictions on ART in Canada, Bill C-47: Human Reproductive and Genetic Technologies Act, was introduced in 1996, and passed its first and second readings before ultimately failing with the dissolution of government in 1997.⁽⁵⁸⁾ Bill C-47 proposed prohibiting 13 practices and applications of technology in the name of protecting the health and

safety of Canadians and the dignity of the women and children most affected by their application.⁽⁵⁸⁾ The Bill largely echoed the voluntary moratorium while also restricting the use of human reproductive materials in research, but fell short in establishing clear regulatory mechanisms for permitted activities in line with the 1993 report. In the next sitting of Parliament, concerns were expressed about Canada's lagging action relative to other countries, especially on human cloning activities, in the absence of legislation. An attempt to amend the Criminal Code to prohibit genetic manipulation was introduced (Bill C-247) but failed on the grounds that the government planned to reintroduce a bill specific to human reproduction.⁽⁵⁹⁾ With Canada still in a policy void, the Canadian Fertility and Andrology Society (CFAS) and the Society of Obstetricians and Gynecologists of Canada (SOGC) issued a joint statement policy statement on the ethics of numerous dimensions of ART treatment and practice.⁽⁶⁰⁾

In 2001, the House of Commons Standing Committee on Health tabled its report (Assisted Human Reproduction: Building Families) and recommendations on the government's Proposals for Legislation Governing Assisted Human Reproduction.⁽⁶¹⁾ In 2002, Bill C-56: An Act Respecting Assisted Human Reproduction and Bill C-13: Assisted Human Reproduction Act were proposed, eventually receiving royal assent to become the Assisted Human Reproduction Act (AHRA).⁽⁶²⁾ Amendments to the Act took place between 2005 and 2008, during which time Quebec initiated its constitutional challenge against the Government of Canada and the AHRA. In 2010, the Supreme Court of Canada unsurprisingly found that numerous sections of the Act pertaining to the created federal regulatory agency fell outside the scope of both criminal law and the federal government's authority and were thus unconstitutional.⁽⁶³⁾ The federal government revised the Act in 2012 to reflect this decision. Most recently, the Act was further strengthened in 2016 to provide better regulation over donor gametes, reimbursement, and administration and

enforcement of the Act but, overall, Canada has minimal regulations in place outlining the permissible use of ART in clinical and research settings.

As the federal government worked through legislation, Ontario was likewise engaged in a debate about funding for ART treatment. In the mid-1980s, Ontario introduced full funding for IVF, but severely restricted funding in 1994 with the release of the Royal Commission Report, *Proceed with Care*, in 1993.⁽⁶⁴⁾ From that point forward, Ontario would only fund IVF in cases of bilateral fallopian tube blockage (three rounds, excluding the cost of drugs), the only indication well supported by evidence at the time, and IUI (excluding the costs of drugs and sperm preparation).⁽⁶⁴⁾ In 2006, the Medical Advisory Secretariat (Ministry of Health and Long-Term Care) completed a health technology assessment of in vitro fertilization and multiple births to assess the clinical effectiveness and cost-effectiveness of IVF as an infertility treatment, and how to reduce the rate of multiple pregnancies associated with IVF.⁽⁶⁴⁾ The three key conclusions and recommendations stemmed from the assessment, namely that 1) that single embryo transfer (SET) in IVF was effective for reducing multiple births; 2) that the cost-savings associated with a reduction in multiple pregnancies from IVF-SET did not offset the cost of universal coverage of IVF-SET, which had been demonstrated to be a successful policy approach internationally (Europe), especially in light of the funding for IUI, and 3) that the government should consider extending access/coverage to ICSI for those with severe male factor infertility and IVF-SET for women in whom a multiple pregnancy is medically contraindicated.⁽⁶⁴⁾ The same year, the SOGC and CFAS released joint “Guidelines for the Number of Embryos to Transfer Following In Vitro Fertilization”, which recommended limiting the number of embryos transferred, but did not go so far as recommending SET as first choice practice.⁽⁶⁵⁾ The guidelines also explicitly called for a public funding scheme to support the implementation of guidelines for the number of

embryos transferred (N.B. clinical guidelines developed by medical groups are not legally binding in Canada and represent best practice suggestions only).(65)

In 2008, an Expert Panel on Infertility and Adoption was convened by the Ontario Cabinet to provide insight on potential adoption and assisted reproduction system reform in Ontario.(66)

With respect to assisted reproduction, the Panel concluded that expanding access to IVF by funding IVF for more forms of infertility in Ontario would be equitable, cost-effective and promote safer practices.(66) The high costs of IVF and other ART procedures, even for the few Ontarians who qualified for publicly-insured services in 2009, meant cost was the greatest barrier to conceiving with ART, limiting access to individuals or couples who were able to pay.(66) The prohibitively high cost of treatment was also found to drive the rate of multiple births up by transferring multiple embryos in a given cycle to increase the likelihood of success at a lower total cost, and by driving families to use IUI, which is associated with an increased chance of multiple births, even when it was not indicated.(66) Multiple births are riskier and costlier during pregnancy, delivery, and throughout life as compared to singleton births. Other jurisdictions with comparable health care systems, including Australia, Austria, Belgium, Finland, Israel, the Netherlands, Spain and Sweden were regulating and/or funding IVF at the time the Expert Panel compiled its report, with those jurisdictions often similarly motivated to reduce multiple births and the associated costs.(66,67) The Panel ultimately recommended that the government fund up to three cycles of IVF for women under 42, including ICSI and frozen embryo transfer where indicated, IUI, fertility preservation where indicated, a refundable tax credit to help address the cost of fertility medications, and the removal of barriers to access, including social and geographical barriers.(66)

Following the spirit of recommendations provided by the Expert Panel, the Ontario government

committed funds to implement a publicly funded fertility treatment program that would provide one cycle of IVF per patient per lifetime in the 2014 provincial budget.(68) The Advisory Process for Infertility Services was tasked with advising the government on clinical eligibility and quality measures and oversight for the program.(68) The Advisory Process provided ten key recommendations in its 2015 report related to the definition of a treatment cycle and core services, patient eligibility criteria, single and multiple embryo transfer, the use of donor materials or surrogates, prescription drug pricing, quality assurance and data collection, monitoring and reporting.(68) In late 2015, the Ontario Fertility Program (OFP) was introduced. The OFP expands funding for infertility treatment in Ontario to one round of IVF (one egg retrieval procedure and one-at-a-time transfer of all viable embryos) for all causes of infertility (medical and non-medical) for women up to 42 years of age (N.B. there is no age limit for men). The costs of required fertility drugs, genetic testing, and storage of gametes and/or embryos are not included.(69) The OFP remains in place today, with rules for single and double/multiple embryo transfer guiding treatment for women of different ages and treatment prognoses.

1.6 Ethical Considerations

The work presented in this thesis is based on the secondary analysis of health administrative data, with exact data sources discussed in the methods sections of each manuscript. I obtained ethics approval for the included analyses from the Children's Hospital of Eastern Ontario Research Ethics Board (CHEOREB# 19/04PE) (Appendix 1.1), the Ottawa Health Science Network Research Ethics Board (20190170-01H) (Appendix 1.2), and the University of Ottawa Research Ethics Board (H-04-19-3869) (Appendix 1.3). The University of Adelaide Human Research Ethics Committee did not require approval for this research. Support to work with the Better Outcomes Registry & Network (BORN) to access data was received from the registry.

1.7 Overview of the Dissertation

This dissertation is a series of three interrelated articles in which the candidate was the lead author. Each chapter has been prepared as a standalone manuscript addressing one of the project's central research questions. While each article can be read independently of the others and in any order, Chapter 2 establishes a baseline context for Ontario which readers may find to be a useful reference to accompany a reading of Chapters 3 and 4. Each manuscript has been prepared for submission to reputable journal and are set to be submitted in April 2021. An "Authors' Note" page has been included prior to each manuscript in Chapters 2-4 listing coauthors, their affiliations, contributions, and journal submission information where relevant. For ease of reading, all tables and figures associated with a particular chapter are situated after the main body of text.

Chapter 2 (Paper 1) seeks to understand the relationship, if any, between assisted reproduction and adverse placental outcomes. To do this, we assessed prevalence and risk of placental and placentally-mediated complications in both fresh and frozen embryo transfers following IVF and ICSI, respectively. We found that fresh and frozen transfers following IVF and ICSI led to an increased risk of all placental outcomes compared with non-assisted conceptions. This result establishes a basis for requiring detailed investigation of the independent and interactive contribution of maternal and treatment factors to the observed increase in risk. Given the severity of placental complications in pregnancy for both mother and fetus/offspring, this chapter suggests that detailed analyses of patient and treatment factors are required to better understand the mechanisms contributing to the observed increase in risk.

In Chapters 3 and 4 we turn our attention to the examination of two treatment factors to assess

their contribution to the increased risk of placental complications observed in Chapter 2. Chapter 3 (Paper 2) describes the results of our investigation of the association between embryo development and adverse placental outcomes. We examine embryo day (3 versus 5) at transfer in fresh cycles, and embryo stage (cleavage stage versus blastocyst) at transfer in frozen cycles. We found no association between the day or stage of embryo at transfer and our placental outcomes, which, pending replication, should offer some confidence that this treatment factor does not independently mediate the increased risk of placental complications.

Chapter 4 (Paper 3) compares the use of slow-freezing and vitrification to fresh embryo transfer in the development of placental complications. While we identified a decreased risk of placentally-mediated complications with the use of slow-freezing compared with vitrification, we did not observe any significant associations between placental and placentally-mediated complications and fresh and slow-frozen and cryopreserved transfers, respectively. Although this suggests that cryopreservation is not a leading treatment factor contributing to the increased risk of adverse placental outcomes in ART, clinical impacts of the difference in risk between slow-freezing and vitrification warrant further investigation.

This dissertation concludes in Chapter 5. The conclusion focuses on the major findings from the included studies, examining their implications in greater depth. I discuss the ways in which the findings contribute to establishing a hierarchy of risk for assisted reproduction that can be used to guide research and policy development. Lastly, I review the methodological contributions of the thesis, as well as its contributions to the fields of assisted reproduction, epidemiology, and population health.

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treatments

Chapter 2 (Article 1):

Placental and placentally-mediated complications following fresh and frozen embryo transfers in

IVF and ICSI: A retrospective population-based cohort study in Ontario, Canada

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All authors contributed to the conceptualization and design of the study, which was led by BO. BO and SH led the development of the data analysis plan with support from RD. BO led the data analysis, in consultation with SH and MD, and guidance from MW, KP and AA. BO wrote the first draft of the paper, facilitated discussions, and oversaw all edits.

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2.1 Abstract

Background: The extent to which abnormal placentation and placentally-mediated complications are associated with assisted reproduction is unclear. Our objective was to assess the association between conceptions from fresh and frozen embryo transfers in IVF and ICSI, respectively, and adverse placental outcomes.

Methods: We linked a national fertility treatment registry with a province-wide health administrative birth registry to conduct a retrospective cohort study in Ontario, Canada of clinical pregnancies from October 2013 to October 2017. We estimated adjusted odds ratios (aOR) for placental and placentally-mediated outcomes in pregnancies following fresh and frozen IVF and ICSI embryo transfers as compared to non-assisted pregnancies.

Results: Among 586,251 singleton pregnancies, 10,156 resulted from assisted conception, of which 8,499 had a recorded method of treatment. Disaggregation of exposure groups by treatment and embryo transfer types revealed clear patterns of significant increases in risk. Compared with non-assisted pregnancies, there was an increased risk of any placental complication in both fresh and frozen IVF (fresh: aOR 2.90; 95% confidence interval [CI] 1.98-4.25); frozen: aOR 2.77; 95% CI 1.73-4.44) and ICSI (fresh: aOR 2.45; 95% CI 2.03-2.96; frozen: aOR 2.29; 95% CI 1.92-2.73) cycles. Similarly, there was an increased risk of placentally-mediated complications in both fresh and frozen IVF (fresh: aOR 1.24; 95% CI 0.91-1.69; frozen: aOR 1.75; 95% CI 1.28-2.40) and ICSI (fresh: aOR 1.26; 95% CI 1.10-1.44; frozen: aOR 1.23; 95% CI 1.14-1.48) cycles.

Conclusions: Following both fresh or frozen embryo transfers in IVF and ICSI, there is an increased risk of placental and placentally-mediated complications.

2.2 Introduction

In Canada, the use of assisted reproductive technologies (ART) has increased rapidly over the past decade, with a 235% increase in reported ART cycle starts and a 190% increase in resultant clinical pregnancies from 2008-2018.^{1,2}

There is evidence that assisted reproduction is associated with an increased risk of pregnancy complications and adverse neonatal outcomes, including miscarriage, preterm birth, stillbirth, preeclampsia, gestational diabetes, low birth weight and congenital anomalies.³⁻¹⁶ A small but growing body of research points to an increased risk of placental-related complications in assisted pregnancies.¹⁷ Both in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are associated with an increased risk of abnormal placentation^{18,19} - namely placenta previa^{12,19-21}, placental abruption^{10,12,21,22}, and placenta accreta, percreta and increta²³⁻²⁶ – and placentally-mediated complications.²⁷ These adverse placental outcomes are associated with severe bleeding – intrapartum and postpartum hemorrhage – as well as high blood pressure (via preeclampsia), and are thereby leading causes of maternal morbidity and mortality.^{28,29} Similarly, placental dysfunction is associated with shortened gestation and low birth weight/restricted growth, which are leading causes of offspring morbidity and mortality.²⁹ Further, the association between maternal preeclampsia and low birthweight indicates a trans-generational transmission of parentally-mediated disease, including an increased risk of hypertensive disorders in general, and preeclampsia in daughters' subsequent pregnancy.^{30,31} Thus, identification of significant patient and treatment factors which contribute to placental-mediated adverse outcomes is critical for informed, timely and appropriate clinical decision-making, and directing clinical and etiologic research.

While a growing body of evidence points to an increased risk of placental complications with assisted conception, few studies have examined this exposure stratified by 1) fresh and frozen embryo transfer, and 2) IVF and ICSI conceptions. Lack of information about the distribution of risk impedes health care planning and targeted research on specific ART procedures.

In this study, we assess the association of placental and placentally-mediated complications during pregnancy and fresh and frozen IVF and ICSI conceptions.

2.3 Methods

2.3.1 Study Design, Data Sources, Study Population

Using a retrospective cohort study design, we examined the Better Outcomes Registry & Network (BORN) Ontario birth registry (<https://www.bornontario.ca/>) linked with the Canadian Assisted Reproductive Technologies Register (CARTR) Plus to identify the study population.

BORN is an Ontario-wide registry that contains maternal-newborn records for all documented clinical pregnancies, births and resulting infants.^{32,33} The CARTR Plus database is derived from CARTR, a registry developed by the Canadian Fertility and Andrology Society to track record-level information on fertility treatment (including treatment, pregnancy, and childbirth outcomes) for Canadian fertility clinic patients. CARTR Plus links records from all Ontario clinics with an embryology lab to BORN records.

We compared the outcomes of assisted conceptions to a random sample of 100,000 non-assisted clinical pregnancies in Ontario, Canada from 7 October 2013 to 6 October 2017. A random sample of 100,000 was selected because while there was no advantage in statistical power in including all non-assisted conceptions beyond a ratio of five to ten non-assisted for every

assisted conception in the database, there was a huge impact on computational requirements to run the modeling algorithms. Adolescent pregnancies (<20 years) were excluded from the analysis as they are separately associated with an increased risk of specific perinatal outcomes.^{34,35} While pregnancies to women over the age of 35 are also at increased risk of adverse perinatal and neonatal outcomes, they represent a significant portion of the ART population and are therefore included in this whole-of-population study of assisted conceptions.

2.3.2 *Exposure and Outcome Measurement*

The exposure of interest is the use of IVF or ICSI to conceive, determined by the presence of a record in the CARTR Plus dataset. Women were classified as an IVF pregnancy if they used conventional IVF on Day 0 after oocyte collection and were identified as ICSI pregnancy if they used ICSI on Days 0-3 or rescue ICSI on Day 1 after failed IVF. We classified women as non-assisted conceptions if there was no record in CARTR Plus of IVF or ICSI being used to conceive the observed pregnancy.

We identified two primary outcomes of interest *a priori*: any placental complication and any placentally-mediated complication. With respect to any placental complication, we defined a composite variable (placenta accrete, placenta increta, placenta percreta, placenta previa and placental abruption) and were also interested in the occurrence of each complication individually. The five placental complications were identified in the maternal labour, antenatal general and specialty antenatal records in BORN and recorded in an aggregate pregnancy complications variable and reasons for delivery type variables developed by the registry.

Similarly, we used a composite variable to measure any placentally-mediated complication, defined as preeclampsia, intrauterine growth restriction (IUGR), placental abruption and

stillbirth. These four outcomes were selected due to their severity and clinical importance, despite the heterogeneity in hypothesized etiologies (*N.B.* It has been hypothesized that in some cases, preeclampsia, IUGR and placental abruption collectively represent ischemic placental disease (IPD) due to similarities in underlying pathogenesis^{36,37}). Preeclampsia was identified in maternal antenatal, labour and birth records as a pregnancy complication. IUGR was ascertained by searching antenatal and birth records for pregnancy complications, indications for induction of labour and indications for caesarean section and aggregating the results per pregnancy across records. Stillbirth, ascertained by birth outcome recorded in birth records, was included as a surrogate measure of placental function because poor placentation dramatically increases the risk of stillbirth, of which IUGR can be a precursor.³⁸

2.3.3 Statistical Analyses

We described the characteristics of our study population through frequencies for categorical variables and means for continuous variables. To assess the balance of baseline covariates between our five exposure groups, we used standardized differences where an absolute standardized difference of less than 0.10 indicated a well-balanced covariate.^{39,40}

Prior to conducting our analysis, we multiply imputed missing values for covariates. We generated 20 imputed datasets using the R packages rms and Hmisc in R Version 4.0.2 (<https://www.r-project.org>).^{41,42} 17.93% of records had missing information for one or more covariates that had been preselected for inclusion in our propensity score. The percentage of missing data for any one of the covariates ranged from 0% for multiple covariates to 16.69% for maternal pre-pregnancy BMI.

We used propensity score weights to adjust for confounding. Using the R package twang^{43,44}, we

used a generalized boosted model to calculate a propensity score for each pregnancy, indicating the predicted probability of its method of conception. Twelve known or suspected confounding variables were selected *a priori* for inclusion in the propensity score models, developed in each of the 20 imputed datasets: maternal age, maternal pre-pregnancy BMI, parity, maternal smoking during pregnancy, preexisting maternal comorbidities (anaemia, asthma, diabetes, epilepsy, and hypertension), gestational hypertension, gestational diabetes, and history of previous caesarean section. The selected propensity score model was that which was best able to decrease both the maximum and mean absolute standardized difference (ASD) for each included covariate below 0.1, with the mean ASD used to develop the propensity score weights. To account for differences in exposure group size, we adjusted the propensity score in each imputation with sampling weights where the weight for each exposure group was equivalent to one over the number of pregnancies in each exposure group multiplied by the total sample population. We computed inverse probability of treatment weights (IPTWs) using the propensity score values leading to weights where each assisted conception exposure group was equivalent to the inverse of the propensity score and where the spontaneous conception exposure group was equivalent to the inverse of one minus the propensity score.⁴⁵

We used logistic regression models to estimate crude and adjusted odds ratios with 95% confidence intervals and robust standard errors in SAS Version 9.4 (<https://www.sas.com/>).

Crude odds ratios were estimated for each outcome in the original cohort with complete exposure and outcome data. Adjusted odds ratios were estimated from models for each outcome weighted by the IPTWs in each of the 20 multiple imputation datasets as well as maternal age. We pooled the resulting estimates, parameters, and standard errors across the 20 imputed datasets to construct a single adjusted estimate, 95% confidence interval, p-value and robust standard

error.⁴⁶

In sensitivity analyses, we assessed the presence and magnitude of any differences in the distribution of covariates (and outcomes) within unique women – that is, any difference between first and subsequent pregnancies – to determine whether adjustment was needed to account for first versus subsequent conceptions. Similarly, we assessed the need for adjustment for the clustering effect of conceptions within women. Finally, we repeated our analyses with a minimally adjusted model including only maternal age, maternal pre-pregnancy BMI and parity.

2.4 Results

2.4.1 Characteristics of Study Population

There were 49,836 IVF and ICSI cycle starts recorded in CARTR Plus during the study period. There were 586,251 clinical pregnancy records in the Ontario birth registry during the study period, of which 10,156 were the result of assisted conception. A total of 574,335 conceptions were included in the multiple imputation process. After imputation, 30,756 records were excluded for extreme maternal age, parity, or missing information on birth outcome in the original data, with some overlap between the groups of exclusions. Of the remaining 543,727 records, 148 (0.03%) were excluded for missing information on the type of gestation and number of fetuses, leaving 543,579 clinical pregnancy records eligible for inclusion in our final study population. 533,676 clinical pregnancies were non-assisted, of which we took a random sample of 100,000 for inclusion. 9,903 clinical pregnancies were assisted, of which 8,499 had a known method of conception (IVF or ICSI) and were included in the study. Our final study population thus consisted of 108,499 clinical pregnancies (Figure 2.1). In this paper, we report on the singleton conceptions, of which there were 105,927 in the final sample, with multiples

forthcoming.

In the original (unweighted) study population, Ontarians who conceived with IVF or ICSI were more likely to be older, nulliparous, and experience complications of pregnancy (gestational hypertension and gestational diabetes). Conversely, they were less likely to have a high BMI, to smoke, to have certain pre-existing conditions (anaemia, asthma, and epilepsy) and were less likely to have a history of prior caesarean section, with the exception for IVF frozen cycles (Tables 2.1a & 2.1b). Women who conceived through a fresh IVF cycle were more likely to be nulliparous, to smoke during pregnancy, experience gestational hypertension and have a spontaneous vaginal delivery as compared a frozen cycle. Women who conceived with fresh ICSI cycles were less likely to experience gestational hypertension and more likely to have a spontaneous vaginal delivery than in frozen ICSI cycles (N.B. Complimentary information on the distribution of neonatal characteristics is available in Supplemental Table 2.A.). After propensity score adjustment, all measured baseline covariates were well-balanced (ASD <0.10) except maternal age. While the balance between exposure groups on maternal age was drastically improved after adjustment, the difference remained in all four contrasts of assisted to non-assisted conceptions. Overall, adjustment improved the comparability of non-assisted and assisted pregnancies.

2.4.2 Incidence and Risks of Outcomes

Any placental complication occurred in a total of 1,849 (1.75%) conceptions, while 7,198 (6.80%) experienced a placentally-mediated complication (Table 2.2). Among our treatment groups of interest, the proportion of conceptions where any placental complication occurred was highest in fresh IVF conceptions (5.92%) and lowest in frozen ICSI conceptions (4.23%). The

incidences of placenta accreta/increta/percreta were too low to allow for analysis as individual outcome variables, thus we limited the analysis to placenta previa and placental abruption. The proportion of conceptions where a placental abruption occurred was highest in frozen IVF conceptions and lowest in frozen ICSI conceptions. The proportion of conceptions where placenta previa occurred was highest in fresh IVF conceptions (3.44%) and lowest in frozen IVF conceptions (1.52%). Conversely, the proportion of conceptions where a placentally-mediated complication occurred was highest in frozen IVF conceptions (1.52%) and lowest in fresh IVF cycles (0.65%).

We observed significant increases in risk among assisted conceptions for nearly all placental outcomes of interest as compared to non-assisted conceptions, both before and after adjustment (Table 2.3). IVF and ICSI conceptions are at significantly increased risk of experiencing any placental complication among both fresh (IVF: aOR 2.90, 95% CI 1.98-4.25; ICSI: aOR 2.45, 95% CI 2.03-2.96) and frozen transfers (IVF: aOR 2.77, 95% CI 1.73-4.44; ICSI: aOR 1.88, 95% CI 1.54-2.29) compared to non-assisted pregnancies. Both IVF and ICSI conceptions are at an increased risk of placenta previa following a fresh embryo transfer (IVF: aOR 4.26, 95% CI 2.57-7.04; ICSI: aOR 3.36, 95% CI 2.57-4.40), while only ICSI conceptions are at increased risk following a frozen transfer (aOR 2.31, 95% CI 1.76-3.02). Both IVF and ICSI frozen transfers are at an increased risk of placentally-mediated complications (IVF: aOR 1.75, 95% CI 1.28-2.40; ICSI: aOR 1.23, 95% CI 1.14-1.48), while only ICSI conceptions are at an increased risk following a fresh transfer (aOR 1.26, 95% CI 1.10-1.44).

Conceptions after IVF or ICSI, regardless of whether by fresh or frozen embryo transfer, are at increased risk of all outcomes of interest as compared to non-assisted conceptions, all of which remained significant after adjustment (Table 2.4). Given the non-significant association between

frozen IVF transfers and placenta previa, and fresh IVF transfers and placentally-mediated complications, this aggregated analysis masks important differences in risks for traditionally first and subsequent rounds of IVF/ICSI, and potential embryo transfers after freeze-all protocol.

Sensitivity analyses did not reveal any meaningful differences between first and subsequent pregnancies, nor did we identify a need to adjust for clustering within women, thus we did not adjust the primary analyses for these factors.

The minimally adjusted model produced a lower risk estimate than the propensity score adjusted model for all but three outcomes among fresh transfers (higher estimates for placental abruption (minimally) and placentally-mediated complications in ICSI conceptions, and an equal estimate for placentally-mediated complications in IVF conceptions) (Table 2.5). Consistent trends, with respect to our odds ratio estimates, were observed in both propensity score adjusted and minimally adjusted models for all but placentally-mediated complications in ICSI conceptions. Conversely, the minimally adjusted model produced a higher risk estimate than the propensity score adjusted models for frozen transfers for all but three outcomes (equal estimate for any placental complication in IVF transfers, and lower estimates for placental abruption in IVF and ICSI transfers) and produced the same trend in effect estimates as the propensity score adjusted models. Although our conclusions are not altered substantially, this pattern of lower risk estimates in fresh transfers and higher risk estimates in frozen transfers than the propensity score adjusted models suggests that these patterns of confounding are different across the different sub-analyses presented.

2.5 Discussion

In this large observational study of 8,499 assisted and 100,000 non-assisted conceptions, using

comprehensive Ontario databases with detailed information on infertility treatment, pregnancy and several potential confounders, we confirmed previous findings of an increased risk of placental and placentally-mediated complications among assisted conceptions compared to non-assisted conceptions. After propensity score adjustment, the association between IVF and ICSI in fresh and frozen transfers remained significant for all outcomes except for placentally mediated complications following fresh IVF and placenta previa following frozen IVF transfers.

When conceptions resulting from fresh and frozen transfers in IVF and ICSI conceptions were considered, we identified that, overall, the risk of any placental outcome or placenta previa was higher in fresh transfers than frozen transfers. While the risk of placental abruption and placentally mediated complications was higher in frozen IVF transfers compared to fresh transfers, we see the opposite in ICSI cycles whereby there was a greater increase in risk after fresh transfers rather than frozen. Differences in patterns of adverse placental outcomes between conceptions from fresh and frozen embryo transfers compared to non-assisted conceptions may be reflective of differential survival and selection pressure in fresh and frozen transfer processes. Unlike embryos transferred fresh, embryos that undergo cryopreservation are exposed to greater selection pressure in having to both survive until cryopreservation and survive cryopreservation itself, with survival possibly favouring more robust embryos.^{47,48} Alternatively, it could indicate altered placentation in the hyperstimulated environment of fresh transfers following ovarian stimulation. Supraphysiologic estradiol levels following ovarian stimulation have previously been linked to thinner endometrial thickness and subsequent preeclampsia⁴⁹ (which may share its pathogenesis with placental abruption), small for gestational age (SGA)^{49,50} and low birth weight^{51,52}, as well as altered endometrial gene expression⁵³, supporting a hypothesis that the hyperstimulated uterine environment (endometrial state) is less amenable to optimal implantation

and placentation. Further, Royster et al.⁵⁴ found supraphysiologic estradiol levels and ICSI to be both independent and compounding contributors to an increased risk of adverse placental events. Animal studies with baboons have demonstrated that elevated estradiol levels in early pregnancy increase maternal serum levels of sFlt-1 and decrease VEGF expressed from extravillous trophoblasts, significantly reducing uterine artery remodeling and uterine artery blood flow, while also increasing maternal blood pressure.^{55–58} Further to this point, murine models have demonstrated elevated inflammatory cytokine profiles in ICSI placenta.⁵⁹

Although these studies were not conducted in assisted reproduction models, the effects of elevated estradiol can reasonably be translated. In a frozen cycle, temporal separation from the hyperstimulated environment may allow for better synchronization between the embryo and endometrium, which is critical for normal trophoblastic invasion.⁶⁰

With respect to ICSI being independently associated with adverse placental events, there is insufficient evidence in this paper alone to sound a cautionary note about the increasingly routine use of ICSI, even in the absence of male factor infertility, particularly in explaining the reversed pattern of risk for fresh and frozen IVF and ICSI. The discordance in patterns of risk of adverse placental outcomes may reflect differences in interactions between IVF and ICSI protocols and other unmeasured treatment factors, such as in use of natural or programmed cycles for embryo transfer. Further work is required to clarify these clinically important, and from a public health perspective, increasingly urgent questions.

2.5.1 Strengths and Weaknesses of Study

The primary strength of the present study was the availability of a population-based validated birth registry and a comprehensive validated fertility database with near or complete coverage of

fertility clinics in Ontario that could be linked together, which provides statistical power and confidence in generalizability. However, while we observed a statistically robust pattern of increased risk for placental abruption in fresh and frozen IVF and ICSI conceptions, small event counts merit caution in the interpretation of the magnitude of the increased risk.

While CARTR Plus provides complete coverage of cycles initiated in Ontario, assisted pregnancies conceived through out-of-province clinics may be misclassified as non-assisted. We expect that the small number of pregnancies that would be misclassified would not alter the significance of odds ratios estimates but may alter the absolute prevalence of the outcomes in our exposure groups. Intrauterine insemination (IUI) conceptions are similarly misclassified, and while they are associated with an increased risk of select perinatal outcomes^{61–63}, we do not anticipate that this unavoidable misclassification alters the magnitude or significance of the results. In both cases, the misclassification bias is towards the much larger group of non-assisted pregnancies, where fluctuations in event counts or classification are less likely to have a significant impact.

As BORN does not routinely collect information on a woman's fertility history, we were unable to adjust for underlying infertility – which has been linked to adverse perinatal outcomes⁶⁴, and cannot be sure of the extent to which it affects the outcomes of interest in our non-assisted group. Preeclampsia is known to be under-captured in maternal labour and delivery records and is therefore underestimated in the BORN data. The prevalence of preeclampsia in the BORN data from all sources is 3.72%, while the prevalence of preeclampsia when Discharge Abstract Database (hospital administrative data) data is incorporated is 4.15%. Our determination of a previous caesarean section may be underestimated because we could only consider the pregnancies in the sample, not pregnancies to women before the inclusion date. Finally, in the

strictest understanding of the phenomenon, IUGR, or fetal growth restriction, is the failure of a fetus to reach its growth potential, which is difficult to establish. It has been suggested that using customized, as compared to population-based charts, improves the diagnostic accuracy of fetal growth restriction associated with abnormal placental pathology.⁶⁵ The use of customized growth charts to calculate individual IUGR was not feasible in our data.

While propensity scores have been demonstrated to be more effective in reducing bias in observational studies using large health administrative databases than traditional methods, namely multivariable adjustment methods⁶⁶, it is possible that there is residual confounding in our findings due to unmeasured or unknown confounders being omitted from the propensity scores. One such example may be closer monitoring of women with ART conceptions during infertility treatment and pregnancy, leading to a more accurate health history being recorded in the data.

All outcomes may be under-captured in the data, but we have no evidence to suggest that this would be different across exposure groups. Placenta abruption is most likely to be under-captured as a diagnosis of placental abruption is challenging to secure, and thus not all true placental abruptions are recorded. Our reported prevalence is likely underestimated as a result, as are the unadjusted and adjusted odds ratios. There is no evidence to suggest that there is a difference in the rate of diagnosis between assisted and spontaneous pregnancies, though it is possible that pregnancies from assisted conception are more closely monitored.

2.5.2 Similarities to Other Studies

With respect to increased risks of placental complications generally^{16,23,67,68}, placenta previa^{6,7,10,12,18–21,67–72} and placental abruption^{10,12,18,21,67,69,70,73} after IVF or ICSI, our results are

consistent with previously published evidence. But few of these studies examine placental complications specifically, especially in fresh and frozen transfers separately. Furthermore, multiple different control or comparison groups are used across the literature, increasing the heterogeneity in analyses when including various forms of sub-fertility and infertility diagnosis, and a variety of treatment methodologies.

To our knowledge, only one systematic review and meta-analysis focuses specifically on placental complications while also accounting for varied comparison populations.¹⁷ Differences in pooled odds ratios of placenta previa (4.40, 95% CI 3.42-5.67; 11 studies) and placental abruption (OR 2.12, 95% CI 1.82-2.47; 9 studies) between non-ART and ART pregnancies pointed to an increased risk associated with ART pregnancies, with which our results are consistent. Ginström et al.⁷⁴ assessed the risk of outcomes following frozen transfers specifically and identified an increased risk of placenta previa as compared to spontaneous pregnancies.

We identified two studies that examined the risk of ischemic placental disease and ART.^{73,75} Modest et al.⁷³ defined ischemic placental disease as preeclampsia, placental abruption, SGA or intrauterine fetal demise due to placental insufficiency, finding an increased risk in IVF versus non-IVF pregnancies in both autologous and donor transfers. Johnson et al.⁷⁵ use a definition similar to ours – preeclampsia, placental abruption, SGA or intrauterine fetal demise – and find that both preterm and term IPD are more likely in IVF pregnancies than non-IVF pregnancies. Although neither study stratified by fresh or frozen transfers, and both used SGA as a proxy for IUGR/FGR, nonetheless, our results were consistent with these previous findings. Within our placentally-mediated complications outcome variable, preeclampsia is the single-largest contributor to overall incidence across all five exposure groups. An increased risk of preeclampsia, or hypertensive disorders in pregnancy more generally, following assisted

conceptions has been well documented.^{7,12,27,67,72,73,76,77}

2.6 Conclusion

Our findings indicate that the use of IVF or ICSI to conceive a pregnancy is associated with an increased risk of any placental complication and placentally-mediated complications, as well as increased risks of placenta previa and placental abruption specifically.

Our findings are consistent with previous studies^{17,73,75} while also helping to address the limited research on adverse placental outcomes by embryo transfer type in IVF and ICSI. Clinical human biomarker and animal studies suggest that implantation and early placentation are altered in assisted conceptions^{54–58}, leading to increased risks of adverse placental outcomes which may be mediated by embryo transfer type or factors associated therewith. While further research is needed in this area, our findings have immediate relevance for clinical practice, essential patient information, the biology of pregnancy, and health care planning.

2.7 References

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2.8 Tables

Table 2.1a. Distribution of baseline characteristics of study population, overall and by assisted conception treatment type: IVF

Characteristic	Whole Sample (n=105,927) n(%)	Non-Assisted (n=98,489) n(%)	Assisted Conception Cycle Type (Unweighted)				Assisted Conception Treatment Type (IPTW weighted*)
			IVF Fresh (n=524) n(%)	Standardized Difference†	IVF Frozen (n=396) n(%)	Standardized Difference†	Standardized Difference‡
Maternal Age, years				0.9288		1.0220	0.1696
21-24	9,830 (9.28%)	9,816 (9.27%)	<6§		0 (0.00%)		
25-29	28,547 (26.95%)	28,052 (28.48%)	39 (7.44%)		27 (6.82%)		
30-34	39,874 (37.64%)	37,370 (37.94%)	189 (36.07%)		139 (35.10%)		
35-39	21,738 (20.52%)	18,748 (19.04%)	206 (39.31%)		167 (42.17%)		
>=40	5,303 (5.01%)	3,907 (3.97%)	85 (16.22%)		61 (15.40%)		
Missing	635 (0.60%)	596 (0.61%)	<6		<6		
Parity				0.6574		0.4769	0.0769
Nulliparous	45,228 (42.70%)	40,099 (40.71%)	354 (67.56%)		229 (57.83%)		
Primi/multi	59,446 (56.12%)	57,293 (58.17%)	162 (30.92%)		157 (39.65%)		
Missing	1,253 (1.18%)	1,097 (1.11%)	8 (1.53%)		10 (2.53%)		
Smoking¶				0.2643		0.4003	0.0499
Yes	8,717 (8.23%)	8,621 (8.75%)	14 (2.67%)		<6		
Pre-pregnancy BMI				0.0247		0.0412	0.0238
<=18.49	4,651 (4.39%)	4,436 (4.50%)	18 (3.44%)		6 (1.52%)		
18.50-24.99	47,691 (45.02%)	44,203 (44.88%)	235 (44.85%)		177 (44.70%)		
25.00-29.99	21,943 (20.72%)	20,500 (20.81%)	94 (17.94%)		75 (18.94%)		
>=30.00	16,564 (15.64%)	15,594 (15.83%)	76 (14.50%)		58 (14.65%)		
Missing	15,078 (14.23%)	13,756 (13.97%)	101 (19.27%)		80 (20.20%)		
Preexisting Conditions							
Preexisting Anaemia¶				0.0053		0.0670	0.01638
Yes	753 (0.71%)	707 (0.72%)	<6		<6		
Preexisting Asthma□				0.0273		0.0995	0.0146
Yes	3,461 (3.27%)	3,285 (3.34%)	15 (2.86%)		7 (1.77%)		
Preexisting Diabetesø				0.0213		0.0906	0.0121
Yes	999 (0.94%)	915 (0.93%)	6 (1.15%)		8 (2.02%)		
Preexisting Epilepsy'				0.0807		0.0135	0.0729
Yes	336 (0.32%)	320 (0.32%)	0 (0.00%)		<6		
Preexisting Hypertension^				0.0152		0.0933	0.0266
Yes	1,015 (0.96%)	888 (0.90%)	<6		8 (2.02%)		
Complications of Pregnancy							
Gestational Hypertension				0.0517		0.1562	0.0373
Yes	3,710 (3.50%)	3,339 (3.39%)	23 (4.39%)		27 (6.82%)		
Gestational Diabetes]				0.1340		0.2508	0.0835
Yes	7,458 (7.04%)	6,641 (6.74%)	55 (10.50%)		57 (14.39%)		
Birth Outcomes							
Live Birth				0.0324		0.0878	N/A
Yes	104,501 (98.65%)	97,188 (98.68%)	515 (98.28%)		386 (97.47%)		

Delivery Type				0.2456		0.5481	N/A
Spontaneous Vaginal	66,473 (62.75%)	63,105 (64.07%)	285 (54.39%)		285 (54.39%)		
Assisted Vaginal	9,314 (8.79%)	8,322 (8.45%)	52 (9.92%)		52 (9.92%)		
Induced or Spontaneous Labour							
Caesarean section	14,102 (13.31%)	12,532 (12.72%)	86 (16.41%)		86 (16.41%)		
No labour caesarean section	15,445 (14.58%)	13,972 (14.19%)	99 (18.89%)		99 (18.89%)		
Missing	593 (0.56%)	558 (0.57%)	<6		<6		
Previous Caesarean Section				0.1319		0.1550	0.0222
Yes	11,915 (11.25%)	11,329 (11.50%)	44 (8.40%)		51 (12.88%)		

*Inverse probability of treatment weighting (where IPTW=inverse probability of treatment weight).

†Absolute standardized difference where a value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

‡Mean absolute standardized difference where the value is an average of all standardized difference values over 20 imputation datasets. A value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

§Cells with fewer than six (6) records are suppressed (<6).

¥Smoking: Information on maternal smoking is collected for both smoking at first prenatal visit and at admission for birth. If a record indicated a woman smoked any amount at either of these time points they were classified as “Smoking”. Conversely, if a record indicated a woman was not smoking at both these time points they were classified as “Not Smoking”.

¶Anaemia includes chronic anaemia only.

⊞Asthma: Information for asthma is collected for both pre-existing asthma and asthma that occurred in the current pregnancy. If a record indicated that a woman had pre-existing asthma they were classified as “Yes”. If a record indicated that a woman had asthma that occurred in pregnancy but did not have recorded pre-existing asthma they were classified as “No”.

⌀Diabetes includes: diabetes, diabetes type I, diabetes type II with insulin, diabetes type II no insulin, diabetes type II no insulin with diet management only, diabetes type II no insulin with oral antihyperglycemic agents, and diabetes type unknown. If a record indicated the presence of any one of these, they were classified as “Yes”.

⌋Information for epilepsy is collected for both pre-existing epilepsy/seizures and for a seizure that occurs during the current pregnancy. If a record indicated that a woman had pre-existing epilepsy/seizures, they were classified as a “Yes”. If a record indicated that a woman had a seizure that occurred in pregnancy but did not have recorded pre-existing epilepsy, they were classified as “No”.

^Hypertension includes pre-existing hypertension only.

]Gestational diabetes includes: gestational diabetes; gestational diabetes with insulin; gestational diabetes with insulin/ACE inhibitors; gestational diabetes with insulin/statins; gestational diabetes with no ACE inhibitors or statins; gestational diabetes no insulin; gestational diabetes no insulin/no oral agents; gestational diabetes no insulin/oral antihyperglycemic agents; gestational diabetes insulin status unknown. If a record indicated the presence of any one of these, they were classified as “Yes”.

Table 2.1b. Distribution of baseline characteristics of study population, overall and by assisted conception treatment type: ICSI

Characteristic	Whole Sample (n=105,927) n(%)	Non-Assisted (n=98,489) n(%)	Assisted Conception Cycle Type (Unweighted)				Assisted Conception Treatment Type (IPTW weighted*) Standardized Difference‡
			ICSI Fresh (n=3,205) n(%)	Standardized Difference†	ICSI Frozen (n=3,313) n(%)	Standardized Difference†	
Maternal Age, years							
21-24	9,830 (9.28%)	9,816 (9.27%)	<6	1.0361	8 (0.24%)	1.0615	0.1696
25-29	28,547 (26.95%)	28,052 (28.48%)	227 (7.08%)		202 (6.10%)		
30-34	39,874 (37.64%)	37,370 (37.94%)	1,070 (33.39%)		1,106 (33.38%)		
35-39	21,738 (20.52%)	18,748 (19.04%)	1,302 (40.62%)		1,315 (39.69%)		
>=40	5,303 (5.01%)	3,907 (3.97%)	583 (18.19%)		667 (20.13%)		
Missing	635 (0.60%)	596 (0.61%)	20 (0.62%)		15 (0.45%)		
Parity				0.7383		0.6214	0.0769
Nulliparous	45,228 (42.70%)	40,099 (40.71%)	2,333 (72.79%)		2,213 (66.80%)		
Primi/multi	59,446 (56.12%)	57,293 (58.17%)	796 (24.84%)		1,038 (31.33%)		
Missing	1,253 (1.18%)	1,097 (1.11%)	76 (2.37%)		62 (1.87%)		
Smoking				0.3496		0.3522	0.0499
Yes	8,717 (8.23%)	8621 (8.75%)	40 (1.25%)		40 (1.21%)		
Pre-pregnancy BMI				0.0372		0.0979	0.0238
<=18.49	4,651 (4.39%)	4,436 (4.50%)	85 (2.65%)		106 (3.20%)		
18.50-24.99	47,691 (45.02%)	44,203 (44.88%)	1,481 (46.21%)		1,595 (48.14%)		
25.00-29.99	21,943 (20.72%)	20,500 (20.81%)	619 (19.31%)		655 (19.77%)		
>=30.00	16,564 (15.64%)	15,594 (15.83%)	427 (13.32%)		409 (12.35%)		
Missing	15,078 (14.23%)	13,756 (13.97%)	593 (18.50%)		548 (16.54%)		
Preexisting Conditions							
Preexisting Anaemia				0.0107		0.0348	0.01638
Yes	753 (0.71%)	707 (0.72%)	26 (0.81%)		15 (0.45%)		
Preexisting Asthma				0.0460		0.0711	0.0146
Yes	3,461 (3.27%)	3,285 (3.34%)	82 (2.56%)		72 (2.17%)		
Preexisting Diabetes				0.0025		0.0298	0.0121
Yes	999 (0.94%)	915 (0.93%)	29 (0.90%)		41 (1.24%)		
Preexisting Epilepsy				0.0205		0.0157	0.0729
Yes	336 (0.32%)	320 (0.32%)	7 (0.22%)		8 (0.24%)		
Preexisting Hypertension				0.0694		0.0809	0.0266
Yes	1,015 (0.96%)	888 (0.90%)	54 (1.68%)		61 (1.84%)		
Complications of Pregnancy							
Gestational Hypertension				0.0476		0.1035	0.0373
Yes	3,710 (3.50%)	3,339 (3.39%)	138 (4.31%)		183 (5.52%)		
Gestational Diabetes				0.1255		0.1620	0.0835
Yes	7,458 (7.04%)	6,641 (6.74%)	328 (10.23%)		377 (11.38%)		
Birth Outcomes							
Live Birth				0.0485		0.0006	N/A
Yes	104,501 (98.65%)	97,188 (98.68%)	3,143 (98.07%)		3,269 (98.67%)		
Delivery Type				0.3118		0.5163	N/A
Spontaneous Vaginal	66,473 (62.75%)	63,105 (64.07%)	1,582 (49.36%)		1,347 (40.66%)		
Assisted Vaginal	9,314 (8.79%)	8,322 (8.45%)	407 (12.70%)		491 (14.82%)		

Induced or Spontaneous Labour	14,102 (13.31%)	12,532 (12.72%)	578 (18.03%)		809 (24.42%)		
Caesarean section	15,445 (14.58%)	13,972 (14.19%)	620 (19.34%)		653 (19.71%)		
No labour caesarean section	593 (0.56%)	558 (0.57%)	18 (0.56%)		13 (0.39%)		
Missing							
Previous Caesarean Section				0.1517		0.0616	0.0222
Yes	11,915 (11.25%)	11,329 (11.50%)	204 (6.37%)		287 (8.66%)		

*Inverse probability of treatment weighting (where IPTW=inverse probability of treatment weight).

†Absolute standardized difference where a value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

‡Mean absolute standardized difference where the value is an average of all standardized difference values over 20 imputation datasets. A value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

§Cells with fewer than six (6) records are suppressed (<6).

¥Smoking: Information on maternal smoking is collected for both smoking at first prenatal visit and at admission for birth. If a record indicated a woman smoked any amount at either of these time points they were classified as “Smoking”. Conversely, if a record indicated a woman was not smoking at both these time points they were classified as “Not Smoking”.

¶Anaemia includes chronic anaemia only.

⊞Asthma: Information for asthma is collected for both pre-existing asthma and asthma that occurred in the current pregnancy. If a record indicated that a woman had pre-existing asthma they were classified as “Yes”. If a record indicated that a woman had asthma that occurred in pregnancy but did not have recorded pre-existing asthma they were classified as “No”.

øDiabetes includes: diabetes, diabetes type I, diabetes type II with insulin, diabetes type II no insulin, diabetes type II no insulin with diet management only, diabetes type II no insulin with oral antihyperglycemic agents, and diabetes type unknown. If a record indicated the presence of any one of these, they were classified as “Yes”.

!Information for epilepsy is collected for both pre-existing epilepsy/seizures and for a seizure that occurs during the current pregnancy. If a record indicated that a woman had pre-existing epilepsy/seizures, they were classified as a “Yes”. If a record indicated that a woman had a seizure that occurred in pregnancy but did not have recorded pre-existing epilepsy, they were classified as “No”.

^Hypertension includes pre-existing hypertension only.

]Gestational diabetes includes: gestational diabetes; gestational diabetes with insulin; gestational diabetes with insulin/ACE inhibitors; gestational diabetes with insulin/statins; gestational diabetes with no ACE inhibitors or statins; gestational diabetes no insulin; gestational diabetes no insulin/no oral agents; gestational diabetes no insulin/oral antihyperglycemic agents; gestational diabetes insulin status unknown. If a record indicated the presence of any one of these, they were classified as “Yes”.

Table 2.2. Number of events by assisted and non-assisted exposure groups

	Non-Assisted Conception (n=98,489) n(%)	IVF Fresh (n=524) n(%)	IVF Frozen (n=396) n(%)	ICSI Fresh (n=3,205) n(%)	ICSI Frozen (n=3,313) n(%)
Any Placental Complication*	1,863 (1.89%)	31 (5.92%)	21 (5.30%)	160 (4.99%)	140 (4.23%)
Placenta Previa	605 (0.61%)	18 (3.44%)	6 (1.52%)	87 (2.71%)	78 (2.35%)
Placental Abruptio	701 (0.71%)	9 (1.72%)	11 (2.78%)	43 (1.34 %)	30 (0.91%)
Placentally-Mediated Complications‡	6,740 (6.84%)	53 (10.11%)	52 (13.13%)	323 (10.08%)	350 (10.56%)
Preeclampsia	3,419 (3.47%)	24 (4.58%)	30 (7.58%)	166 (5.18%)	229 (6.91%)
IUGR	2,333 (2.37%)	15 (2.86%)	7 (1.77%)	101 (3.15%)	82 (2.48%)
Placental Abruptio	701 (0.71%)	9 (1.72%)	11 (2.78%)	43 (1.34 %)	30 (0.91%)
Stillbirth§	637 (0.65%)	8 (1.12%)	6 (1.52%)	36 (1.12%)	28 (0.85%)

*Any placental complication includes: placenta previa, placental abruptio, placenta accreta, placenta percreta, placenta increta. Sum of each individual condition does not equal the number of pregnancies with any placental complication as categories were not mutually exclusive. A pregnancy needed to have a record of any one of the listed conditions to be classified as a “Yes” for any placental complication.

‡Sum of each individual condition does not equal the number of pregnancies with a placentally-mediated complication as categories were not mutually exclusive. A pregnancy needed to have a record of preeclampsia, IUGR, placental abruptio or stillbirth to be classified as “Yes” for a placentally-mediated complication.

§Stillbirths include stillbirths at ≥ 20 weeks or ≥ 500 grams occurring during the antepartum or intrapartum period.

Table 2.3. Association between assisted conception and placental and placentally-mediated outcomes in fresh and frozen IVF and ICSI conceptions

Fresh Transfers												
	IVF vs. Non-Assisted				ICSI vs. Non-Assisted				IVF vs. ICSI			
	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)*	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)*	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)*	p value	Standard Error
Any Placental Complication	3.26 (2.26-4.70)	2.90 (1.98-4.25)	<0.0001	1.22	2.73 (2.31-3.22)	2.45 (2.03-2.96)	<0.0001	1.10	1.20 (0.81-1.78)	1.18 (0.78-1.79)	0.4253	1.23
Placenta Previa	5.76 (3.57-9.27)	4.26 (2.57-7.04)	<0.0001	1.29	4.51 (3.60-5.67)	3.36 (2.57-4.40)	<0.0001	1.15	1.27 (0.76-2.14)	1.27 (0.74-2.18)	0.3914	1.32
Placental Abruptio	2.43 (1.26-4.73)	2.51 (1.26-5.00)	0.0088	1.42	1.90 (1.39-2.59)	2.03 (1.44-2.88)	<0.0001	1.19	1.29 (0.62-2.65)	1.23 (0.58-2.62)	0.5842	1.47
Placentally-Mediated Complications	1.53 (1.15-2.04)	1.24 (0.91-1.69)	0.1816	1.17	1.53 (1.36-1.72)	1.26 (1.10-1.44)	0.0009	1.07	1.00 (0.74-1.36)	0.98 (0.70-1.37)	0.9114	1.18
Frozen Transfers												
	IVF vs. Non-Assisted				ICSI vs. Non-Assisted				IVF vs. ICSI			
	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)*	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)*	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)*	p value	Standard Error
Any Placental Complication	2.90 (1.86-4.52)	2.77 (1.73-4.44)	<0.0001	1.27	2.29 (1.92-2.73)	1.88 (1.54-2.29)	<0.0001	1.11	1.27 (0.79-2.03)	1.48 (0.90-2.43)	0.1224	1.29
Placenta Previa	2.49 (1.11-5.60)	1.67 (0.72-3.88)	0.2292	1.53	3.90 (3.07-4.95)	2.31 (1.76-3.02)	<0.0001	1.15	0.64 (0.28-1.47)	0.73 (0.31-1.72)	0.4661	1.55
Placental Abruptio	3.99 (2.18-7.29)	4.72 (2.50-8.92)	<0.0001	1.38	1.27 (0.88-1.84)	1.59 (1.05-2.40)	0.0283	1.23	3.13 (1.55-6.29)	2.97 (1.43-6.18)	0.0036	1.45
Placentally-Mediated Complications	2.05 (1.54-2.76)	1.75 (1.28-2.40)	0.0004	1.17	1.61 (1.44-1.80)	1.23 (1.14-1.48)	<0.0001	1.07	1.28 (0.94-1.75)	1.35 (0.97-1.88)	0.0731	1.18

*Adjusted using inverse probability of treatment weights.

Table 2.4. Association between assisted conception and placental and placentally-mediated outcomes in IVF and ICSI conceptions

	IVF vs. Non-Assisted				ICSI vs. Non-Assisted				IVF vs. ICSI			
	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)*	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)*	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)*	p value	Standard Error
Any Placental Complication	3.10 (2.34-4.13)	2.84 (2.09-3.85)	<0.0001	1.17	2.50 (2.21-2.83)	2.15 (1.86-2.50)	<0.0001	1.08	1.17 (0.92-1.68)	1.32 (0.96-1.81)	0.0924	1.18
Placenta Previa	4.33 (2.87-6.55)	2.93 (1.89-4.54)	<0.0001	1.25	4.20 (3.53-5.00)	2.82 (2.28-3.48)	<0.0001	1.11	1.03 (0.67-1.59)	1.04 (0.66-1.63)	0.8650	1.26
Placental Abruption	3.10 (1.98-4.86)	3.60 (2.22-5.84)	<0.0001	1.28	1.58 (1.24-2.01)	1.80 (1.36-2.39)	<0.0001	1.15	1.96 (1.19-3.23)	2.00 (1.18-3.38)	0.0100	1.31
Placentally-Mediated Complications	1.75 (1.43-2.15)	1.49 (1.19-1.87)	0.0005	1.12	1.57 (1.44-1.70)	1.28 (1.16-1.42)	<0.0001	1.05	1.12 (0.90-1.39)	1.17 (0.92-1.47)	0.1999	1.13

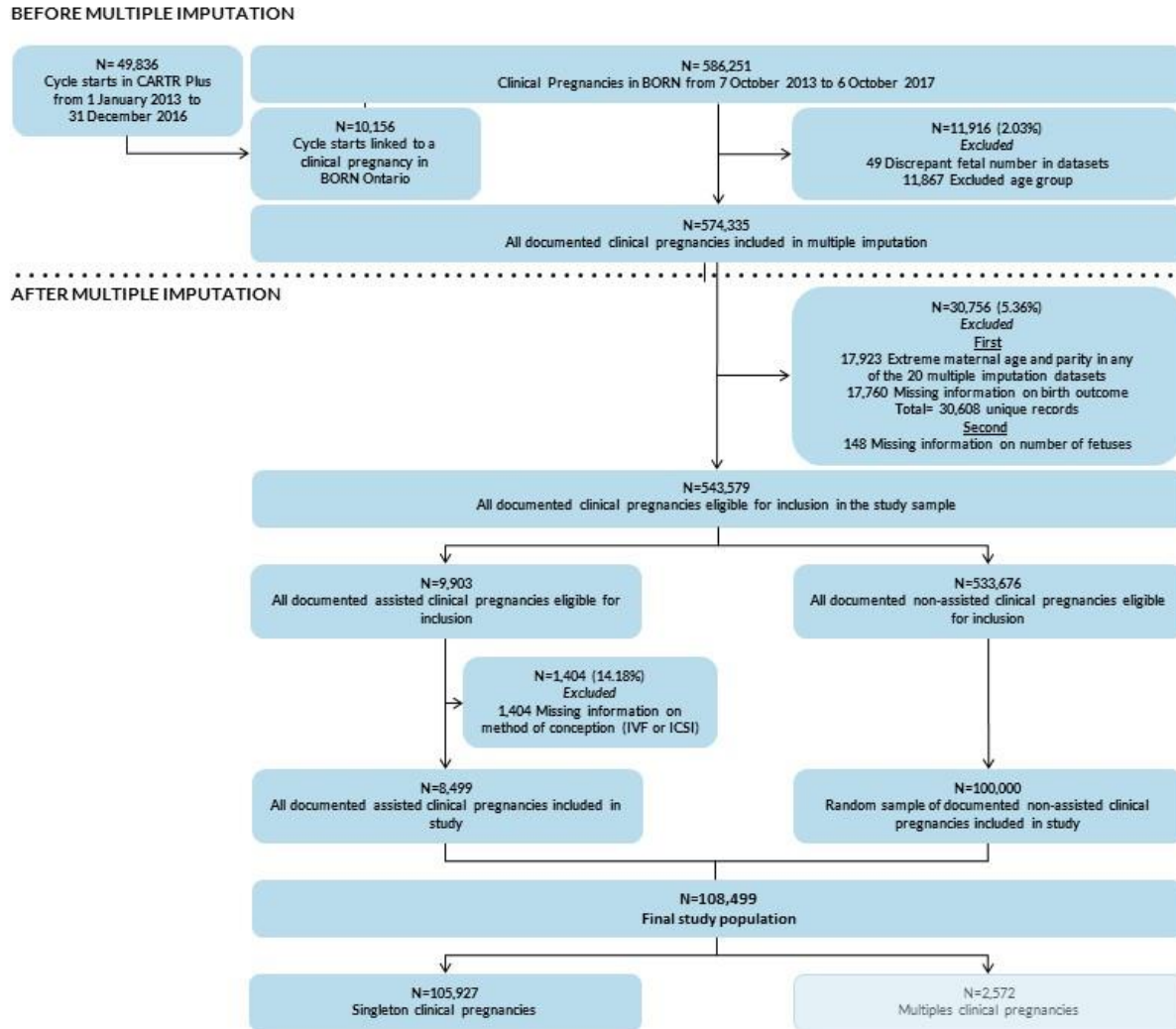
*Adjusted using inverse probability of treatment weights.

Table 2.5. Minimally adjusted model association between assisted conception and placental and placentally-mediated outcomes in fresh and frozen IVF and ICSI conceptions

Fresh Transfers												
	IVF vs. Non-Assisted				ICSI vs. Non-Assisted				IVF vs. ICSI			
	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	p value	Standard Error
Any Placental Complication	3.26 (2.26-4.70)	2.69 (1.76-4.13)	<0.0001	1.24	2.73 (2.31-3.22)	2.42 (2.00-2.94)	<0.0001	1.10	1.20 (0.81-1.78)	1.11 (0.70-1.75)	0.6506	1.26
Placenta Previa	5.76 (3.57-9.27)	3.83 (2.17-6.74)	<0.0001	1.34	4.51 (3.60-5.67)	3.16 (2.41-4.15)	<0.0001	1.15	1.27 (0.76-2.14)	1.21 (0.66-2.21)	0.5347	1.36
Placental Abruptio	2.43 (1.26-4.73)	2.26 (1.00-5.10)	0.0506	1.52	1.90 (1.39-2.59)	2.04 (1.41-2.94)	0.0001	1.21	1.29 (0.62-2.65)	1.11 (0.46-2.66)	0.8209	1.56
Placentally-Mediated Complications	1.53 (1.15-2.04)	1.24 (0.89-1.73)	0.2106	1.19	1.53 (1.36-1.72)	1.35 (1.18-1.55)	<0.0001	1.07	1.00 (0.74-1.36)	0.91 (0.64-1.30)	0.6167	1.20
Frozen Transfers												
	IVF vs. Non-Assisted				ICSI vs. Non-Assisted				IVF vs. ICSI			
	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	p value	Standard Error
Any Placental Complication	2.90 (1.86-4.52)	2.76 (1.70-4.47)	<0.0001	1.28	2.29 (1.92-2.73)	1.98 (1.62-2.43)	<0.0001	1.11	1.27 (0.79-2.03)	1.39 (0.83-2.32)	0.2065	1.30
Placenta Previa	2.49 (1.11-5.60)	1.83 (0.75-4.47)	0.1859	1.58	3.90 (3.07-4.95)	2.42 (1.81-3.23)	<0.0001	1.16	0.64 (0.28-1.47)	0.76 (0.30-1.90)	0.5511	1.60
Placental Abruptio	3.99 (2.18-7.29)	4.50 (2.29-8.84)	<0.0001	1.41	1.27 (0.88-1.84)	1.53 (1.02-2.28)	0.0382	1.23	3.13 (1.55-6.29)	2.95 (1.37-6.33)	0.0056	1.48
Placentally-Mediated Complications	2.05 (1.54-2.76)	1.98 (1.43-2.73)	<0.0001	1.18	1.61 (1.44-1.80)	1.45 (1.28-1.65)	<0.0001	1.07	1.28 (0.94-1.75)	1.36 (0.97-1.92)	0.0762	1.19

2.9 Figure

Figure 2.1 Flow diagram of study



2.10 Supplemental

Supplemental Table 2.A. Distribution of neonatal characteristics of study population, overall and by assisted conception treatment type

IVF Cycles						
Characteristic	Whole Sample (n=105,927) n(%)	Non-Assisted (n=98,489) n(%)	IVF Fresh (n=524) n(%)	Standardized Difference†	IVF Frozen (n=396) n(%)	Standardized Difference†
Neonatal Death				0.0214		0.0013
Yes	287 (0.24%)	256 (0.26%)	<6§		<6	
Missing	766 (0.72%)	712 (0.72%)	<6		<6	
Gestational Age, completed weeks				0.2746		0.2302
<28	821 (0.78%)	709 (0.72%)	10 (1.91)		7 (1.77%)	
28-31	598 (0.56%)	541 (0.55%)	<6		<6	
32-36	5,794 (5.47%)	5,174 (5.25%)	51 (9.73%)		47 (11.87%)	
37-39	58,666 (55.38%)	54,480 (55.32%)	314 (59.92%)		211 (53.28%)	
40-41	38,832 (36.66%)	36,446 (37.01%)	141 (26.91%)		124 (31.31%)	
>42	463 (0.44%)	439 (0.45%)	<6		<6	
Missing	753 (0.71%)	700 (0.71%)	<6		<6	
Mean Birth Weight (g) (+/-Std.Dev.)	3,350.10 (+/- 579.34)	3,355.24 (+/-574.27)	3166.87 (+/- 640.72)	0.3096	3291.86 (+/- 678.67)	0.1008
Missing	1,359 (1.28%)	1,217 (1.24%)	10 (1.91%)		6 (1.52%)	
Birth Weight Categories¶				0.1715		0.1086
VSGA (under 3 rd centile)	1,087 (1.03%)	1,018 (1.03%)	7 (1.34%)		0 (0.00%)	
SGA (under 10 th centile)	3,133 (2.96%)	2,883 (2.93%)	31 (5.92%)		6 (1.52%)	
LGA (90 th -96 th centile)	13,655 (12.89%)	12,745 (12.94%)	55 (10.50%)		49 (12.37%)	
VLGA (over 97 th centile)	8,765 (8.27%)	8,162 (8.29%)	29 (5.53%)		42 (10.61%)	
Unavailable (<24wks ga)	437 (0.41%)	373 (0.38%)	6 (1.15%)		<6	
Other (11-89 th centile)	77,542 (73.20%)	72,132 (73.24%)	390 (74.43%)		291 (73.48%)	
Missing	1,308 (1.23%)	1,176 (1.19%)	6 (1.15%)		6 (1.52%)	
ICSI Cycles						
Characteristic	Whole Sample (n=105,927) n(%)	Non-Assisted (n=98,489) n(%)	ICSI Fresh (n=3,205) n(%)	Standardized Difference†	ICSI Frozen (n=3,313) n(%)	Standardized Difference†
Neonatal Death				0.0020		0.0526
Yes	287 (0.24%)	256 (0.26%)	8 (0.25%)		20 (0.60%)	
Missing	766 (0.72%)	712 (0.72%)	27 (0.84%)		21 (0.63%)	
Gestational Age, completed weeks				0.2251		0.1221
<28	821 (0.78%)	709 (0.72%)	47 (1.47%)		48 (1.45%)	
28-31	598 (0.56%)	541 (0.55%)	28 (0.87%)		22 (0.66%)	
32-36	5,794 (5.47%)	5,174 (5.25%)	270 (8.42%)		252 (7.61%)	
37-39	58,666 (55.38%)	54,480 (55.32%)	1,880 (58.66%)		1,781 (53.76%)	
40-41	38,832 (36.66%)	36,446 (37.01%)	944 (29.45%)		1,177 (35.53%)	
>42	463 (0.44%)	439 (0.45%)	10 (0.31%)		12 (0.36%)	
Missing	753 (0.71%)	700 (0.71%)	26 (0.81%)		21 (0.63%)	
Mean Birth Weight (g) (+/-Std.Dev.)	3,350.10 (+/- 579.34)	3,355.24 (+/-574.27)	3,239.90 (+/- 640.24)	0.1896	3,338.99 (+/- 627.75)	0.0270
Missing	1,359 (1.28%)	1,217 (1.24%)	59 (1.84%)		67 (2.02%)	
Birth Weight Categories¶				0.0796		0.1489
VSGA (under 3 rd centile)	1,087 (1.03%)	1,018 (1.03%)	42 (1.31%)		20 (0.60%)	
SGA (under 10 th centile)	3,133 (2.96%)	2,883 (2.93%)	126 (3.93%)		87 (2.63%)	

LGA (90 th -96 th centile)	13,655 (12.89%)	12,745 (12.94%)	347 (10.83%)		459 (13.85%)	
VLGA (over 97 th centile)	8,765 (8.27%)	8,162 (8.29%)	242 (7.55%)		290 (8.75%)	
Unavailable (<24wks ga)	437 (0.41%)	373 (0.38%)	26 (0.81%)		30 (0.91%)	
Other (11-89 th centile)	77,542 (73.20%)	72,132 (73.24%)	2,365 (73.79%)		2,364 (71.36%)	
Missing	1,308 (1.23%)	1,176 (1.19%)	57 (1.78%)		63 (1.90%)	

†Absolute standardized difference where a value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

§Cells with fewer than six (6) records are suppressed (<6).

¶Birth weight categories are constructed in line with the INTERGROWTH-21 standards. VSGA – Very Small for Gestational Age. SGA – Small for Gestational Age. LGA – Large for Gestational Age. VLGA – Very Large for Gestational Age.

2.11 Bridge

Chapter 2 (Article 1) explored the association between assisted reproductive technologies (ART) and placental and placentally-mediated complications. We identified an increased risk of placental and placentally-mediated complications in conceptions following fresh and frozen IVF and ICSI. As the reason for this increase in risk is not immediately clear, the role of treatment and patient factors that may contribute to this risk warranted further investigation.

Chapter 3 (Article 2) examines the first of two treatment factors hypothesized to contribute the increased risk of placental and placentally-mediated outcomes: day or stage of embryo at transfer. Early placentation is highly dependent on a complex interplay of factors, including the hormonal and immunological environment, endometrial receptivity, and assorted cell-to-cell signaling (e.g., paracrine, endocrine). Early embryonic cell development and differentiation is paramount to facilitating this process. Thus, it is possible that the day or stage of embryo at transfer influences the trajectory of early placentation by leading to exposure to an asynchronous uterine environment or by prolonging exposure to the *in vivo* environment, for example.

Building on the findings from Chapter 2 (Article 1), we examine embryo transfer in fresh and frozen cycles, respectively, to identify risks along the lines of traditionally first (fresh) and subsequent (frozen) embryo transfer cycles.

Chapter 3 (Article 2):

No difference in risk of placental complications following cleavage stage or blastocyst transfer in fresh and frozen cycles: A retrospective population-based cohort study in Ontario

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Author's Note

All authors contributed to the conceptualization and design of the study, which was led by BO. BO and SH led the development of the data analysis plan with support from KP and RD. BO led the data analysis, in consultation with SH and MD, and guidance from MW, KP and AA. BO wrote the first draft of the paper, facilitated discussions, and oversaw all edits contributed by all authors.

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3.1 Abstract

Study question: Is the day or developmental stage at transfer in fresh and frozen embryo transfer cycles associated with increased risks of abnormal placentation and placentally-mediated complications?

Summary answer: In fresh embryo transfer cycles, the day of embryo at transfer (Day 3 versus Day 5) is not associated with an increase in risk of abnormal placentation or placentally-mediated outcomes. In frozen embryo transfer cycles, the stage of embryo at transfer (cleavage stage versus blastocyst) is not associated with an increase in risk of abnormal placentation or placentally-mediated outcomes.

What is known already: Blastocyst, or Day 5 embryos, have been found to have better or comparable clinical pregnancy and live birth rates compared with cleavage stage or Day 3 embryos, but have also been found to have an increased risk of adverse perinatal and obstetrical outcomes over cleavage stage transfer. Previous studies have yielded inconsistent results and are limited, particularly with respect to the safety of embryo stage at transfer following cryopreservation.

Study design, size, duration: This registry-based retrospective cohort study includes clinical pregnancies conceived after frozen-thawed and fresh transfers in Ontario, Canada between 1 January 2013 and 31 December 2016. The study includes 4199 pregnancies conceived after fresh embryo transfer, 1354 on Day 3 and 2845 on Day 5, as well as 3765 pregnancies conceived after frozen embryo transfer, 219 at cleavage stage and 3546 as blastocysts. Placental and placentally-mediated outcomes in pregnancies conceived from Day 3 and Day 5 embryos are compared to each other in fresh transfers, while the same outcomes are compared between cleavage stage and

blastocyst embryos in frozen transfers. Main outcomes included any placental outcome (placenta previa, placental abruption, placenta accreta/increta/percreta), placenta previa separately, and placentally-mediated complications (preeclampsia, IUGR, placental abruption, stillbirth).

Participants/materials, setting, methods: Data were obtained from CARTR (Canadian Assisted Reproductive Technologies Register) Plus. CARTR Plus links a Canadian national fertility treatment registry with BORN (Better Outcomes Registry & Network), a province-wide health administrative birth registry for Ontario. CARTR Plus includes all recorded clinical pregnancies conceived with ART treatment at all Ontario clinics with an embryology lab. Outcomes were examined using logistic regression, adjusting for confounders identified a priori through propensity score-derived inverse probability of treatment weights (IPTWs).

Main results and the role of chance: No statistically significant differences were observed for any placental complication, placenta previa, and placentally-mediated complications when comparing Day 3 and Day 5 fresh embryo transfers. No significant differences were observed for any placental complication, placenta previa, and placentally-mediated complications when cleavage stage and blastocyst frozen embryo transfers.

Limitations, reasons for caution: A small number of cleavage stage embryos in frozen transfers were available for analysis. CARTR Plus only dates from 2013, by which time the shift to vitrification as the cryopreservation method of choice was well underway, replacing the previous approach of slow-frozen cleavage stage transfer for frozen transfers. The possibility of residual confounding cannot be eliminated.

Wider implications of the findings: The findings of no significant association between the day or stage of embryo at transfer in fresh and frozen embryo transfers, respectively, offer confidence

that the increased risk of placental and placentally-mediated complications observed after the use of assisted reproductive technologies are not mediated through this particular component of the treatment process.

Study funding/competing interest(s): No funding or conflicts of interest to declare.

Trial registration number: Not applicable.

3.2 Introduction

Embryo selection and transfer during in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) is a critical step of the assisted reproduction treatment process. Typically, embryo transfer occurs at either the cleavage stage (days 2-3 post-fertilization) or the blastocyst stage (days 5-6 post fertilization), with a shift to blastocyst transfer as the more recent standard of care (Maheshwari *et al.*, 2013). In evaluating outcomes associated with day of embryo transfer, research has focused on implantation (Levi-Setti *et al.*, 2018), clinical pregnancy (Oron *et al.*, 2014; Kaye *et al.*, 2017; Martins *et al.*, 2017; Levi-Setti *et al.*, 2018; Bourdon *et al.*, 2019; Harlev *et al.*, 2020), cumulative (de Vos *et al.*, 2016) and live birth (Oron *et al.*, 2014; de Vos *et al.*, 2016; Martins *et al.*, 2017; Bourdon *et al.*, 2019; Harlev *et al.*, 2020) rates.

Prolonged embryo culture is thought to be advantageous in terms of a) allowing time for the attrition of nonviable embryos, thereby increasing the likelihood of identifying and transferring embryos with the greatest potential for continued development post-implantation, while also b) improving biological synchrony between embryo stage and the uterine environment, more accurately reflecting a natural cycle (Maheshwari *et al.*, 2013; Martins *et al.*, 2017). Blastocysts or Day 5 embryos have been found to have higher (Oron *et al.*, 2014; Harlev *et al.*, 2020) or comparable (Martins *et al.*, 2017; Levi-Setti *et al.*, 2018) clinical pregnancy rates than cleavage stage or Day 3 embryos. Similarly, blastocysts have been found to have higher (Oron *et al.*, 2014; de Vos *et al.*, 2016; Harlev *et al.*, 2020) or comparable (Martins *et al.*, 2017) live birth rates. It is against this background where similar implantation (Levi-Setti *et al.*, 2018) and cumulative birth (de Vos *et al.*, 2016) rates, and birth weights (Litzky *et al.*, 2018) have been observed.

Conversely, an increased risk of adverse perinatal and obstetrical outcomes has been observed to occur with blastocyst transfer, seemingly supporting the argument that prolonged embryo culture may be deleterious by providing greater opportunity for embryonic damage from exposures to artificial factors in the in vitro environment (Dar *et al.*, 2013; Martins *et al.*, 2017; Alviggi *et al.*, 2018). An increased risk of preterm and very preterm birth following fresh blastocyst transfer as compared to cleavage stage transfer (Dar *et al.*, 2013; Maheshwari *et al.*, 2013; Wang *et al.*, 2017; Alviggi *et al.*, 2018), and an increased risk of large for gestational age (LGA) with blastocyst transfer (Wang *et al.*, 2017; Alviggi *et al.*, 2018) have been identified (though evidence suggesting the contrary also exists (Marconi *et al.*, 2019)). In frozen transfers, there are comparable risks of preterm birth and small for gestational age (SGA) between cleavage stage and blastocysts (Wang *et al.*, 2017; Alviggi *et al.*, 2018), but an increased risk of LGA is associated with frozen blastocyst transfer (Alviggi *et al.*, 2018).

In summary, there are inconsistencies in outcomes between cleavage stage and blastocyst transfer (Martins *et al.*, 2016). There is limited evidence about the impacts of embryo age or stage at transfer, particularly for cryopreserved embryos, with respect to placental or placentally-mediated outcomes. Accordingly, in this study, we assess the association of placental and placentally mediated complications during pregnancy and the day or stage of embryo transfer in fresh and frozen cycles, respectively.

3.3 Methods

3.3.1 Study Design, Data Sources, Study Population

Using a retrospective cohort study design, we identified the study population using the Better Outcomes Registry & Network (BORN) Ontario birth registry (<https://www.bornontario.ca/>)

linked with the Canadian Assisted Reproductive Technologies Register (CARTR) Plus.

We compared the risk of severe placental complications among assisted clinical pregnancies in Ontario, Canada whose treatment cycles started between 1 January 2013 and 31 December 2016. CARTR Plus, an extension of the Canadian Fertility and Andrology Society's original registry for Canadian fertility clinics, contains record-level information on fertility treatment from all Ontario clinics with an embryology lab, which has been linked to BORN records. BORN, a province-wide registry, contains maternal-newborn records for all documented clinical pregnancies, births and resulting infants in Ontario (Dunn *et al.*, 2019; Miao *et al.*, 2019).

All assisted pregnancies from the study period that had the exposures of interest were included in the analysis.

3.3.2 *Exposure and Outcome Measurement*

The exposure of interest was the transfer of embryos at cleavage or blastocyst stage in IVF and ICSI cycles that led to a confirmed clinical pregnancy. In CARTR/CARTR Plus, detailed but slightly different information about embryos was available for fresh and frozen embryo transfer cycles. In fresh embryo transfer cycles, embryo development was recorded as age in days at transfer, from Day 0 to 7+. In frozen embryo transfer cycles, embryo development was recorded as embryo stage at freezing (i.e. the stage(s) the embryo(s) were at when they were cryopreserved) and at thaw (i.e. the stage(s) of the embryo(s) selected for thaw), where stage was 2PN, cleavage stage, morula, blastocyst or unknown. Due to similar but mismatched information for fresh and frozen transfers, we conducted two separate analyses, one comparing Day 3 and Day 5 embryo transfers in fresh cycles, and one comparing cleavage stage and blastocyst embryo transfers in frozen cycles (thereby excluding transfers at all other documented days or stages).

Where frozen transfers occurred in a cycle separate from embryo creation, we linked each cycle to its original fresh cycle to preserve information on treatment factors included as covariates in the analysis.

We identified two primary outcomes of interest *a priori*: any placental complication and any placentally-mediated complication. Using a composite variable of placenta accrete, placenta increta, placenta percreta, placenta previa and placental abruption, we identified any placental outcome. In addition, we assessed placenta previa separately. We used variables of pregnancy complications and reasons for delivery method developed by the registry to identify all five placental outcomes in BORN maternal labour, antenatal general and specialty antenatal records.

A second composite variable was used to examine placentally-mediated outcomes, defined herein as preeclampsia, intrauterine growth restriction (IUGR), placental abruption and stillbirth. All four outcomes are severe events of clinical importance, although there is heterogeneity in their hypothesized etiologies (*N.B.* Ischemic placental disease (IPD) has been proposed as a pathology to explain the development of preeclampsia, placental abruption and IUGR, in some cases, given similarities in the known or proposed pathogenesis (Ananth, 2014; Roberts, 2014)). Preeclampsia was identified as a pregnancy complication in maternal antenatal, labour and birth records. IUGR was identified by searching antenatal and birth records for pregnancy complications, indications for the induction of labour and indication for caesarean section, after which we aggregated the results by pregnancy across records. Given the relationship between poor placentation, IUGR and stillbirth (Bukowski *et al.*, 2014), we include stillbirth in our composite outcome as identified as a birth outcome in BORN.

3.3.3 Statistical Analyses

Characteristics of our fresh and frozen transfer study populations were described using frequencies for categorical variables and means and standard deviations for select continuous variables. We used standardized differences to assess the balance of our covariates at baseline between the two exposure groups in each analysis, where an absolute standardized difference of less than 0.10 indicated a well-balanced covariate (Austin, 2009a, 2009b).

Before conducting the analysis, we used multiple imputation using predictive mean matching to impute missing values for our *a priori* selected covariates. This process is described elsewhere (Olibris, 2021, unpublished [Chapter 2]). In brief, we generated 20 imputed datasets using packages *rms* and *Hmisc* (Harrell, 2020, 2021) to impute missing values for our *a priori* selected covariates in R Version 4.0.2 (<https://www.r-project.org>). Missingness ranged from 0% for multiple covariates to 19.65% for maternal pre-pregnancy BMI in fresh transfers. Missingness ranged from 0% for multiple covariates to 17.98% for maternal pre-pregnancy BMI in general pregnancy covariates among frozen transfers.

We adjusted for confounding using propensity scores based on inverse probability of treatment weights (IPTWs). Using the R package *twang* (McCaffrey *et al.*, 2013; Burgette *et al.*, 2020), we used a generalized boosted model to calculate a propensity score for each pregnancy, which indicated the predicted probability of the day (3 or 5) or stage (cleavage stage or blastocyst) of embryo at transfer in fresh and frozen transfer analyses, respectively. Propensity weights were derived using 18 covariates identified *a priori* in each of the 20 multiple imputation datasets. These covariates were: ART type, patient age at oocyte retrieval, type of infertility, stimulation protocol from harvest cycle, oocyte source, sperm source, number of embryos transferred,

maternal pre-pregnancy BMI, parity, maternal smoking during pregnancy, preexisting maternal comorbidities (anemia, asthma, diabetes, hypertension), complications of pregnancy (gestational hypertension and gestational diabetes) and history of previous caesarean section. The final propensity score model was derived based on the criteria of minimizing the mean absolute standardized difference across exposure categories. Propensity scores were then used to construct IPTWs. IPTWs for the fresh Day 5 and frozen blastocyst groups were equivalent to the inverse of the propensity score while the IPTWs for the fresh Day 3 and frozen cleavage stage groups were equivalent to the inverse of one minus the propensity score (Cole and Hernán, 2008).

We estimated unadjusted and adjusted odds ratios (OR) with 95% confidence intervals and robust standard errors using logistic regression models. The unadjusted estimates of risk were calculated in the original dataset, without imputation, as exposure and outcome variables were complete. We ran models weighted by the IPTWs calculated in each of the 20 imputed datasets to calculate the adjusted risk estimates for each outcome. Using Rubin's approach to pooling sources of variation within and among imputations (Rubin, 1987), we combined the resulting estimates, parameters, and standard errors into a single adjusted estimate, 95% confidence interval, p-value and robust standard error in SAS Version 9.4 (<https://www.sas.com/>).

We conducted sensitivity analyses to assess the impact of first versus subsequent conceptions on the distribution of covariates, and to assess the effect of clustering of conceptions within women.

3.4 Results

3.4.1 Characteristics of Study Population

A total of 49,836 IVF and ICSI cycle starts were recorded in CARTR during the study period, of which 10,156 (20.37%) were linked to a clinical pregnancy record in the Ontario birth registry. A

total of 10,155 assisted conceptions were initially eligible for inclusion. We excluded 1438 records (14.16%) where the treatment type (IVF or ICSI) was not specified in CARTR, leaving 8717 for inclusion in our multiple imputation. 8477 records remained eligible for inclusion after a further 240 (2.75%) records were excluded for the missing data on the type of gestation, birth outcome in the original data (as imputation of these variables is inappropriate for our analysis) or using mixed oocyte and sperm sources. For our analysis in fresh embryo transfer cycles, 4112 (48.51%) records were excluded for being frozen transfers and 166 (1.96%) records were excluded for being transferred on Days 0, 1, 2, 4, 6 or 7+, leaving 4199 records for inclusion. Of these, 1,354 were Day 3 and 2,845 were Day 5.

For our analysis in frozen embryo transfer cycles, 4365 (51.49%) records were excluded for being fresh transfers and 347 (4.09%) were excluded for being at the 2PN, morula, unknown stage at transfer or a mix of stages at transfer (as we are unable to determine which of the embryos transferred implanted), leaving 3765 records for inclusion (Figure 3.1).

In the fresh transfer study population, women who conceived following a Day 3 embryo transfer were older, and differed on parity, twin or higher order gestation, nulliparity, smoking, pre-pregnancy BMI, and multiplicity. Further, day 3 embryo transfer was more likely to follow autologous oocyte and sperm use, combined male and female factor infertility, and mild stimulation. Variations were also apparent for anemia, asthma, diabetes, pre-existing and pregnancy related hypertension, and gestational diabetes (Table 3.1). (N.B. Complimentary information on the distribution of neonatal characteristics following fresh embryo transfer is available in Supplemental Table 3.A.)

In contrast, women in the frozen transfer group who conceived following a cleavage stage embryo transfer were more likely to have a higher BMI but were comparable in age to women

receiving a blastocyst transfer (Table 3.2). Women who received a cleavage stage embryo transfer were more likely to have twin or higher order gestations, more likely to use donor sperm, more likely to have a multiple embryo transfer, and differed in infertility diagnoses and ovarian stimulation protocols (from the oocyte harvest in the embryo creation cycle) compared to the blastocyst transfer group. (N.B. Complimentary information on the distribution of neonatal characteristics following frozen embryo transfer is available in Supplemental Table 3.B.)

Our sensitivity analyses did not reveal a meaningful difference in covariate distribution between first and subsequent pregnancies, nor did we identify a clustering effect within women, and thus our analysis was not adjusted for either.

3.4.2 Risk of Placental and Placentally Mediated Complications with Day of Embryo Transfer in Fresh Cycles

Some 213 conceptions from fresh embryo transfers experienced a placental complication – 66 (4.87%) following a Day 3 transfer and 147 (5.17%) following a Day 5 transfer (Table 3.3). Of these conceptions, 36 (2.66%) and 75 (2.64%), respectively, experienced placenta previa.

Placentally-mediated complications occurred in 503 conceptions – 173 (12.78%) following a Day 3 transfer and 330 (11.60%) following a Day 5 transfer.

The risk of any placental complication (aOR 1.00, 95% CI 0.71-1.44), as well as the risk of placentally-mediated complications (aOR 0.91, 95% CI 0.72-1.16), was comparable between Day 3 and Day 5 transfers, with no significant association between day of transfer and the outcomes of interest (Table 3.4). There was also no significant association between day at transfer and placenta previa (aOR 0.91, 95% CI 0.57-1.46).

3.4.3 Risk of Placental and Placentally Mediated Complications with Stage of Embryo at Transfer in Frozen Cycles

A placental complication occurred in 176 (4.67%) conceptions from frozen embryo transfers – 12 (5.48%) following a cleavage stage transfer and 164 (4.62%) following a blastocyst transfer (Table 3.5). Some 445 (11.82%) conceptions experienced a placentally mediated complication – 32 (14.61%) following a cleavage stage transfer and 413 (11.65%) following a blastocyst transfer.

There were no significant associations between the stage of embryo at transfer and the risk of any placental complication (aOR 0.83, 95% CI 0.42-1.66), placenta previa (aOR 0.92, 95% CI 0.43-1.97), or placentally mediated complications (aOR 0.92, 95% CI 0.60-1.40) (Table 3.6).

3.5 Discussion

In this observational study of 7,964 assisted conceptions, using comprehensive Ontario databases containing detailed information on infertility treatment, pregnancy, and potential confounders, we found no significant difference in risk of placental and placentally-mediated complications as between Day 3 and Day 5 fresh embryo transfers, nor between cleavage stage and blastocyst cryopreserved embryo transfers, before and after propensity-score adjustment.

With regards to treatment strategy, there appears to be no advantage for selecting Day 5 or blastocyst transfer for the objective of reducing placental or placentally-mediated disease.

3.5.1 Strengths & Weaknesses of Study

The primary strength of this study was the availability of a population-based birth registry and a comprehensive fertility database with near or complete coverage of fertility clinics in Ontario

that could be linked together to track activity from pre-conception to delivery.

Placental complications are infrequent, and therefore our unadjusted and adjusted odds ratios are sensitive to small increases or decreases in incidence. Placental outcomes, particularly placental abruption, can be under-captured in health administrative data and, as such, it is possible that we have underestimated the risk of our outcomes, though equally as there is nothing to suggest that this would differ across study groups. In our placentally-mediated composite variable, we know that the incidence of preeclampsia is underestimated as the under-capture of preeclampsia in BORN's maternal, infant and birth datasets is known (prevalence of 3.72% in BORN across sources versus 4.15% in combination with separate hospital administrative data). Placental abruption is also likely under-captured due to difficulty in securing a diagnosis. IUGR, or fetal growth restriction, as recorded in BORN is predicated on population-based growth charts, effectively making it a proxy measure for true IUGR, which is the failure of a fetus to reach its individualized growth potential. This is difficult to establish. Melamed et al. (Melamed *et al.*, 2021) found that using customized, as compared to population-based charts, improves the diagnostic accuracy of fetal growth restriction associated with abnormal placental pathology, but the BORN data does not support this option at this time.

CARTR Plus contains detailed information about ART treatment cycles, including measurements of endometrial thickness in fresh and frozen transfer cycles. While missingness of 11.2% in fresh transfer cycles could have been managed with multiple imputation, missingness of 34.7% in frozen transfer cycles was deemed too high for use in this analysis and thus the measurements were not included for fresh or frozen embryo transfers. An increased risk of placenta previa with increased endometrial thickness, as well as very thick or thin endometrial thickness has previously been established (Rombauts *et al.*, 2014) and is a recognized unmeasured

confounder in this analysis.

While we cannot eliminate the possibility of residual confounding from other unmeasured or unknown confounders excluded from the propensity scores, we are confident in our estimation methods as propensity scores have been demonstrated to be more effective than traditional methods (such as multivariable adjustment methods) in reducing bias in observational studies using large health administrative databases (Austin and Stuart, 2015).

3.5.2 *Similarities to Other Studies*

We identified four studies that explored placental or placentally-mediated complications with respect to fresh embryo transfer. Using a composite variable of obstetric outcomes, including preeclampsia, placenta previa, placental abruption, IUGR and stillbirth, Harlev et al. (Harlev *et al.*, 2020) identified an increased risk of complications with increasing embryo age in days at transfer. While this study uses a broader composite variable than our study, a study which is also not specific to placental outcomes, the study's sample size is about one-eighth of this study. Two large observational studies (Ginström Erntstad *et al.*, 2016; Spangmose *et al.*, 2020) identified increased risks of placenta previa and placental abruption following fresh blastocyst transfer compared to cleavage stage transfer, while a fourth smaller study identified no difference in risk of placental abruption (Oron *et al.*, 2014). Similarly, studies have reported both an increase (Spangmose *et al.*, 2020) or no difference (Oron *et al.*, 2014) in risk of preeclampsia following fresh blastocyst transfer versus cleavage stage transfer.

We were unable to identify similar studies of placental or placentally-mediated outcomes in cryopreserved-warmed embryo transfers only. Fernando et al. (Fernando *et al.*, 2012) found no difference in risk of placenta previa, placental abruption, and preeclampsia in cleavage stage and

embryo transfers in an analysis that included but did not separate fresh and frozen embryos.

Our findings suggest an interesting future study of whether there may be significant differences when staging embryos not just temporally but based on morphology. Not only would this allow for the results of the fresh and frozen analyses to be compared with greater particularity, but fresh and frozen transfers themselves could be compared to assess the impact of cryopreservation at each stage. Subject to the ethical limits that exist for extended embryo culture, a controlled study would allow for holding culture media and in vitro protocols constant.

3.6 Conclusion

Day or stage of embryo at transfer is unrelated to placental disorders of pregnancy after assisted conception (IVF or ICSI). Subject to replication, this observation should offer confidence that the relationship of this technology to adverse events of pregnancy are not mediated through the observed pathways reported upon here. Day or stage of transfer does not influence the risk of placental or placentally-mediated pathology.

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3.8 Tables

Table 3.1. Distribution of baseline characteristics in fresh embryo transfer study population, overall and by day of transfer

Characteristic	Whole Population (unweighted) (n=4199) n(%)	Day of Transfer (unweighted)			Day of Transfer (IPTW weighted*) Mean Standardized Difference‡
		Day 3 (n=1354) n(%)	Day 5 (n=2845) n(%)	Standardized Difference†	
Oocyte Provider Age, years				0.2363	0.048
21-24	104 (2.48%)	35 (2.58%)	69 (2.43%)		
25-29	537 (12.79%)	131 (9.68%)	406 (14.27%)		
30-34	1642 (39.10%)	461 (34.05%)	1181 (41.51%)		
35-39	1517 (36.13%)	550 (40.62%)	967 (33.99%)		
>=40	399 (9.50%)	177 (13.07%)	222 (7.80%)		
Parity				0.0787	0.0235
0	3035 (72.28%)	952 (70.31%)	2083 (73.22%)		
1	894 (21.29%)	319 (23.56%)	575 (20.21%)		
2	137 (3.26%)	49 (3.62%)	88 (3.09%)		
3	33 (0.79%)	7 (0.52%)	26 (0.91%)		
>=4	12 (0.29%)	<6§	7 (0.25%)		
Missing	88 (2.10%)	22 (1.62%)	66 (2.32%)		
Nulliparous				0.0787	0.0235
Yes	3035 (72.28%)	952 (70.31%)	2083 (73.22%)		
Missing	88 (2.10%)	22 (1.62%)	66 (2.32%)		
Smoking				0.0446	0.005
Yes	59 (1.41%)	24 (1.77%)	35 (1.23%)		
Pre-pregnancy BMI				0.0274	0.004
<=18.49	111 (2.64%)	31 (2.29%)	80 (2.81%)		
18.50-24.99	1891 (45.03%)	633 (46.75%)	1258 (44.22%)		
25.00-29.99	801 (19.08%)	253 (18.69%)	548 (19.26%)		
>=30.00	571 (13.60%)	197 (14.55%)	374 (13.15%)		
Missing	825 (19.65%)	240 (17.73%)	585 (20.56%)		
Gestation Type				0.1393	0.0005
Singleton	3574 (85.12%)	1106 (81.68%)	2468 (86.75%)		
Multiples	625 (14.88%)	248 (18.32%)	377 (13.25%)		
Treatment Information					
ART Treatment Type				0.0254	0.005
IVF	598 (14.24%)	201 (14.84%)	397 (13.95%)		
ICSI	3601 (85.76%)	1153 (85.16%)	2448 (86.05%)		
Primary Infertility Category¶				0.1805	0.036
Female factor	1805 (42.99%)	602 (44.46%)	1203 (42.28%)		
Male factor	983 (23.41%)	297 (21.94%)	686 (24.11%)		
Female/Male	620 (14.77%)	248 (18.32%)	372 (13.08%)		
Unexplained	720 (17.15%)	181 (13.37%)	539 (18.95%)		
Other	71 (1.69%)	26 (1.92%)	45 (1.58%)		
Stimulation Protocol⁴				0.2203	0.021
Natural cycle	14 (0.33%)	10 (0.74%)	<6		

Modified natural cycle	20 (0.48%)	11 (0.81%)	9 (0.32%)		
Antagonist	2998 (71.40%)	940 (69.42%)	2058 (72.34%)		
Agonist	1122 (26.72%)	367 (27.10%)	755 (26.54%)		
Mild stimulation	32 (0.76%)	24 (1.77%)	8 (0.28%)		
IVM protocol/Other	13 (0.31%)	<6	11 (0.39%)		
Oocyte Source				0.1158	0.0115
Autologous	3886 (92.55%)	1280 (94.53%)	2606 (91.60%)		
Donor	313 (7.45%)	74 (5.47%)	239 (8.40%)		
Sperm Source				0.0539	0.02
Autologous	3936 (93.74%)	1257 (92.84%)	2679 (94.17%)		
Donor	263 (6.26%)	97 (7.16%)	166 (5.83%)		
Number of Embryos Transferred				0.8533	0.0825
1	1970 (46.92%)	284 (20.97%)	1686 (59.26%)		
2	2015 (47.99%)	929 (68.61%)	1086 (38.17%)		
>=3	214 (5.10%)	141 (10.41%)	73 (2.57%)		
Preexisting Conditions					
Preexisting Anaemia¶				0.0844	0.017
Yes	40 (0.95%)	21 (1.55%)	19 (0.67%)		
Preexisting Asthma¶				0.0102	0.0045
Yes	104 (2.48%)	35 (2.58%)	69 (2.43%)		
Preexisting Diabetes§				0.0369	0.0025
Yes	41 (0.98%)	10 (0.74%)	31 (1.09%)		
Preexisting Hypertension^				0.0414	0.029
Yes	74 (1.76%)	19 (1.40%)	55 (1.93%)		
Complications of Pregnancy					
Gestational Hypertension				0.0512	0.002
Yes	198 (4.72%)	74 (5.47%)	124 (4.36%)		
Gestational Diabetes‡				0.0072	0.0075
Yes	431 (10.26%)	141 (10.41%)	290 (10.19%)		
Birth Outcomes					
Live Birth				0.0427	
Yes	4121 (98.14%)	1334 (98.52%)	2787 (97.96%)		
Delivery Type				0.0998	
Spontaneous Vaginal	1956 (46.58%)	642 (47.42%)	1314 (46.19%)		
Assisted Vaginal	497 (11.84%)	151 (11.15%)	346 (12.16%)		
Induced or Spontaneous Labour Caesarean section	792 (18.86%)	230 (16.99%)	562 (19.75%)		
No labour caesarean section	935 (22.27%)	326 (24.08%)	609 (21.41%)		
Missing	19 (0.45%)	<6	14 (0.49%)		
Previous Caesarean Section				0.0146	0.0255
Yes	275 (6.55%)	92 (6.79%)	183 (6.43%)		

*Inverse probability of treatment weighting (where IPTW=inverse probability of treatment weight).

†Absolute standardized difference where a value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

‡Mean absolute standardized difference where the value is an average of all standardized difference values over 20 imputation datasets. A value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

§Cells with fewer than six (6) records are suppressed (<6).

¥Smoking: Information on maternal smoking is collected for both smoking at first prenatal visit and at admission for birth. If a record indicated a woman smoked any amount at either of these time points they were classified as “Smoking”. Conversely, if a record indicated a woman was not smoking at both these time points they were classified as “Not Smoking”.

⓪Primary Infertility Category: Detailed information on reasons for an IVF/ICSI cycle is collected in primary (embryo creation with potential for fresh transfer) cycles. Reasons for treatment were categorized as female factor, male factor, combined male and female factor, unexplained and other according to the following hierarchy: a) if only one reason for treatment is indicated, this is the primary infertility category; b) if multiple reasons for treatment are indicated, female and male factor infertility take precedence over unexplained, non-medical, and non-reproductive reasons; c) if both male and female factor infertility are indicated, they are recorded together as female/male. Female factor includes a record of any of the following on their own or in combination with other or unexplained infertility: advanced female age; diminished ovarian reserve; endometriosis; PCOS; peritoneal factor or severe adhesions; tubal factor; uterine factor; other ovulatory disorder; other female factor. Male factor includes: aspermia; asthenozoospermia; azoospermia; globozoospermia; necrozoospermia; oligozoospermia; teratozoospermia; erectile dysfunction; high sperm DNA fragmentation; Klinefelter Syndrome; retrograde ejaculation; sperm antibodies; other male; not specified. If a record identifies both a source of male factor and female factor infertility as a reason for treatment, they are classified as Female/Male. Unexplained includes unexplained infertility only. Other includes: embryo banking – cancer and other medical treatment; embryo banking – for non-cancer/non-medical treatment reasons; gonadotoxic therapy; social infertility (no male partner; no female partner); PGT-M/SR for known genetic factor; PGT-A for aneuploidy screening.

¼Stimulation Type: Stimulation protocols were categorized as follows based on information collected in a primary (embryo creation with potential for fresh transfer) cycle: natural cycle, modified natural cycle, antagonist, agonist (includes: flare agonist, microdose flare agonist, long agonist), mild stimulation, IVM protocol/other (includes: IVM protocol, other stimulation protocol).

¶Anaemia includes chronic anaemia only.

ⓇAsthma: Information for asthma is collected for both pre-existing asthma and asthma that occurred in the current pregnancy. If a record indicated that a woman had pre-existing asthma they were classified as “Yes”. If a record indicated that a woman had asthma that occurred in pregnancy but did not have recorded pre-existing asthma they were classified as “No”.

øDiabetes includes: diabetes, diabetes type I, diabetes type II with insulin, diabetes type II no insulin, diabetes type II no insulin with diet management only, diabetes type II no insulin with oral antihyperglycemic agents, and diabetes type unknown. If a record indicated the presence of any one of these, they were classified as “Yes”.

^Hypertension includes pre-existing hypertension only.

]Gestational diabetes includes: gestational diabetes; gestational diabetes with insulin; gestational diabetes with insulin/ACE inhibitors; gestational diabetes with insulin/statins; gestational diabetes with no ACE inhibitors or statins; gestational diabetes no insulin; gestational diabetes no insulin/no oral agents; gestational diabetes no insulin/oral antihyperglycemic agents; gestational diabetes insulin status unknown. If a record indicated the presence of any one of these, they were classified as “Yes”.

Table 3.2. Distribution of baseline characteristics in frozen embryo transfer study population, overall and by stage at transfer

Characteristic	Whole Population (unweighted) (n=3765) n(%)	Stage at Transfer (unweighted)			Stage at Transfer (IPTW weighted*)
		Cleavage Stage (n=219) n(%)	Blastocyst (n=3546) n(%)	Standardized Difference†	Mean Standardized Difference‡
Oocyte Provider Age, years				0.0181	0.007
21-24	116 (3.08%)	6 (2.74%)	110 (3.10%)		
25-29	547 (14.53%)	28 (12.79%)	519 (14.64%)		
30-34	1593 (42.31%)	96 (43.84%)	1497 (42.22%)		
35-39	1219 (32.38%)	74 (33.79%)	1145 (32.29%)		
>=40	263 (6.99%)	15 (6.85%)	248 (6.99%)		
Missing	27 (0.72%)	0 (0.00%)	27 (0.76%)		
Parity				0.0071	0.0345
0	2493 (66.22%)	147 (67.12%)	2346 (66.16%)		
1	965 (25.63%)	56 (25.57%)	909 (25.63%)		
2	167 (4.44%)	13 (5.94%)	154 (4.34%)		
3	37 (0.98%)	<6§	36 (1.02%)		
>=4	18 (0.48%)	<6	17 (0.48%)		
Missing	85 (2.26%)	<6	84 (2.37%)		
Nulliparous				0.0071	0.0345
Yes	2493 (66.22%)	71 (32.42%)	1116 (31.47%)		
Missing	85 (2.26%)	<6	84 (2.37%)		
Smoking¥				0.0893	0.0495
Yes	45 (1.20%)	<6	40 (1.13%)		
Pre-pregnancy BMI				0.1205	0.0305
<=18.49	109 (2.90%)	6 (2.74%)	103 (2.90%)		
18.50-24.99	1773 (47.09%)	95 (43.38%)	1678 (47.32%)		
25.00-29.99	728 (19.34%)	49 (22.37%)	679 (19.15%)		
>=30.00	478 (12.70%)	30 (13.70%)	448 (12.63%)		
Missing	677 (17.98%)	39 (17.81%)	638 (17.99%)		
Gestation Type				0.1332	0.008
Singleton	3400 (90.31%)	189 (86.30%)	3211 (90.55%)		
Multiples	365 (9.69%)	30 (13.70%)	335 (9.45%)		
Treatment Information					
ART Treatment Type				0.0998	0.0525
IVF	414 (11.00%)	18 (8.22%)	396 (11.17%)		
ICSI	3351 (89.00%)	201 (91.78%)	3150 (88.83%)		
Primary Infertility Category¶				0.2336	0.0235
Female factor	1611 (42.79%)	93 (42.47%)	1518 (42.81%)		
Male factor	842 (22.36%)	61 (27.85%)	781 (22.02%)		
Female/Male	432 (11.47%)	30 (13.70%)	402 (11.34%)		
Unexplained	880 (23.37%)	35 (15.98%)	845 (23.83%)		
Other	0	0	0		
Stimulation Protocol ¶				0.4092	0.1255
Natural cycle	225 (5.98%)	<6	224 (6.32%)		
Modified natural cycle	2634 (69.96%)	144 (65.75%)	2490 (70.22%)		
Antagonist	781 (20.74%)	65 (29.68%)	716 (20.19%)		
Agonist	32 (0.85%)	<6	30 (0.85%)		

Mild stimulation IVM protocol/Other Missing	0 0 93 (2.47%)	0 0 7 (3.20%)	0 0 86 (2.43%)		
Oocyte Source Autologous Donor Missing	3402 (90.36%) 341 (9.06%) 22 (0.58%)	193 (88.13%) 22 (10.05%) <6	3209 (90.50%) 319 (9.00%) 18 (0.51%)	0.0404	0.04
Sperm Source Autologous Donor Missing	3551 (94.32%) 192 (5.10%) 22 (0.58%)	192 (87.67%) 23 (10.50%) <6	3359 (94.73%) 169 (4.77%) 18 (0.51%)	0.2224	0.0655
Number of Embryos Transferred 1 2 ≥3	2533 (67.28%) 1170 (31.08%) 62 (1.65%)	64 (29.22%) 145 (66.21%) 10 (4.57%)	2469 (69.63%) 1025 (28.91%) 52 (1.47%)	0.9061	0.177
Preexisting Conditions					
Preexisting Anaemia¶ Yes	118 (0.48%)	<6	15 (0.42%)	0.1006	0.061
Preexisting Asthma¶ Yes	83 (2.20%)	7 (3.20%)	76 (2.14%)	0.0654	0.057
Preexisting Diabetes§ Yes	49 (1.30%)	<6	48 (1.35%)	0.0948	0.0635
Preexisting Hypertension^ Yes	75 (1.99%)	<6	70 (1.97%)	0.0214	0.0265
Complications of Pregnancy					
Gestational Hypertension Yes	215 (5.71%)	17 (7.76%)	198 (5.58%)	0.0874	0.021
Gestational Diabetes‡ Yes	446 (11.85%)	27 (12.33%)	419 (11.82%)	0.0157	0.0055
Birth Outcomes					
Live Birth Yes	3715 (98.67%)	213 (97.26%)	3502 (98.76%)	0.1075	
Delivery Type Spontaneous Vaginal Assisted Vaginal Induced or Spontaneous Labour Caesarean section No labour caesarean section Missing	1441 (38.27%) 535 (14.21%) 946 (25.13%) 831 (22.07%) 12 (0.32%)	81 (26.99%) 41 (18.72%) 43 (19.63%) 52 (23.74%) <6	1360 (38.25%) 494 (13.93%) 903 (25.47%) 779 (21.97%) 10 (0.28%)	0.2259	
Previous caesarean section Yes	330 (8.76%)	19 (8.68%)	311 (8.77%)	0.0034	0.018

*Inverse probability of treatment weighting (where IPTW=inverse probability of treatment weight).

†Absolute standardized difference where a value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

‡Mean absolute standardized difference where the value is an average of all standardized difference values over 20 imputation datasets. A value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

§Cells with fewer than six (6) records are suppressed (<6).

¥Smoking: Information on maternal smoking is collected for both smoking at first prenatal visit and at admission for birth. If a record indicated a woman smoked any amount at either of these time points they were classified as “Smoking”. Conversely, if a record indicated a woman was not smoking at both these time points they were classified as “Not Smoking”.

⓪Primary Infertility Category: Detailed information on reasons for an IVF/ICSI cycle is collected in primary (embryo creation with potential for fresh transfer) cycles. Reasons for treatment were categorized as female factor, male factor, combined male and female factor, unexplained and other according to the following hierarchy: a) if only one reason for treatment is indicated, this is the primary infertility category; b) if multiple reasons for treatment are indicated, female and male factor infertility take precedence over unexplained, non-medical, and non-reproductive reasons; c) if both male and female factor infertility are indicated, they are recorded together as female/male. Female factor includes a record of any of the following on their own or in combination with other or unexplained infertility: advanced female age; diminished ovarian reserve; endometriosis; PCOS; peritoneal factor or severe adhesions; tubal factor; uterine factor; other ovulatory disorder; other female factor. Male factor includes: aspermia; asthenozoospermia; azoospermia; globozoospermia; necrozoospermia; oligozoospermia; teratozoospermia; erectile dysfunction; high sperm DNA fragmentation; Klinefelter Syndrome; retrograde ejaculation; sperm antibodies; other male; not specified. If a record identifies both a source of male factor and female factor infertility as a reason for treatment, they are classified as Female/Male. Unexplained includes unexplained infertility only. Other includes: embryo banking – cancer and other medical treatment; embryo banking – for non-cancer/non-medical treatment reasons; gonadotoxic therapy; social infertility (no male partner; no female partner); PGT-M/SR for known genetic factor; PGT-A for aneuploidy screening.

¼Stimulation Type: Stimulation protocols were categorized as follows based on information collected in a primary (embryo creation with potential for fresh transfer) cycle: natural cycle, modified natural cycle, antagonist, agonist (includes: flare agonist, microdose flare agonist, long agonist), mild stimulation, IVM protocol/other (includes: IVM protocol, other stimulation protocol).

¶Anaemia includes chronic anaemia only.

ⓇAsthma: Information for asthma is collected for both pre-existing asthma and asthma that occurred in the current pregnancy. If a record indicated that a woman had pre-existing asthma they were classified as “Yes”. If a record indicated that a woman had asthma that occurred in pregnancy but did not have recorded pre-existing asthma they were classified as “No”.

øDiabetes includes: diabetes, diabetes type I, diabetes type II with insulin, diabetes type II no insulin, diabetes type II no insulin with diet management only, diabetes type II no insulin with oral antihyperglycemic agents, and diabetes type unknown. If a record indicated the presence of any one of these, they were classified as “Yes”.

^Hypertension includes pre-existing hypertension only.

]Gestational diabetes includes: gestational diabetes; gestational diabetes with insulin; gestational diabetes with insulin/ACE inhibitors; gestational diabetes with insulin/statins; gestational diabetes with no ACE inhibitors or statins; gestational diabetes no insulin; gestational diabetes no insulin/no oral agents; gestational diabetes no insulin/oral antihyperglycemic agents; gestational diabetes insulin status unknown. If a record indicated the presence of any one of these, they were classified as “Yes”.

Table 3.3. Number of events by day of transfer in fresh embryo transfers

	Day 3 (n=1354)	Day 5 (n=2845)
Any Placental Complication*	66 (4.87%)	147 (5.17%)
Placenta Previa	36 (2.66%)	75 (2.64%)
Placenta Mediated Complications‡	173 (12.78%)	330 (11.60%)
Preeclampsia	78 (5.76%)	161 (5.66%)
IUGR	71 (5.24%)	101 (3.55%)
Placental Abruptio	18 (1.33%)	43 (1.51%)
Stillbirth§	21 (1.55%)	44 (1.55%)

*Any placental complication includes: placenta previa, placental abruptio, placenta accreta, placenta percreta, placenta increta. Sum of each individual condition does not equal the number of pregnancies with any placental complication as categories were not mutually exclusive. A pregnancy needed to have a record of any one of the listed conditions to be classified as a “Yes” for any placental complication.

‡Sum of each individual condition does not equal the number of pregnancies with a placentally-mediated complication as categories were not mutually exclusive. A pregnancy needed to have a record of preeclampsia, IUGR, placental abruptio or stillbirth to be classified as “Yes” for a placentally-mediated complication.

§Stillbirths include stillbirths at ≥ 20 weeks or ≥ 500 grams occurring during the antepartum or intrapartum period.

Table 3.4. Association between day of transfer and placental and placentally-mediated outcomes in fresh embryo transfers

	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI) *	p value	Standard Error
Any Placental Complication	1.06 (0.79-1.43)	1.00 (0.71-1.44)	0.9615	1.20
Placenta Previa	0.99 (0.66-1.48)	0.92 (0.57-1.46)	0.7125	1.27
Placenta Mediated Complications	0.90 (0.74-1.09)	0.91 (0.72-1.16)	0.4601	1.13

*Adjusted using inverse probability of treatment weights.

Table 3.5. Number of events by stage at transfer in frozen embryo transfers

	Cleavage Stage (n=219) n(%)	Blastocyst (n=3546) n(%)
Any Placental Complication*	12 (5.48%)	164 (4.62%)
Placenta Previa	7 (3.20%)	83 (2.34%)
Placenta Mediated Complications‡	32 (14.61%)	413 (11.65%)
Preeclampsia	14 (6.39%)	260 (7.33%)
IUGR	11 (5.02%)	101 (2.85%)
Placental Abruptio	<6	41 (1.16%)
Stillbirth§	<6	32 (0.90%)

*Any placental complication includes: placenta previa, placental abruptio, placenta accreta, placenta percreta, placenta increta. Sum of each individual condition does not equal the number of pregnancies with any placental complication as categories were not mutually exclusive. A pregnancy needed to have a record of any one of the listed conditions to be classified as a “Yes” for any placental complication.

‡Sum of each individual condition does not equal the number of pregnancies with a placentally-mediated complication as categories were not mutually exclusive. A pregnancy needed to have a record of preeclampsia, IUGR, placental abruptio or stillbirth to be classified as “Yes” for a placentally-mediated complication.

§Stillbirths include stillbirths at ≥ 20 weeks or ≥ 500 grams occurring during the antepartum or intrapartum period.

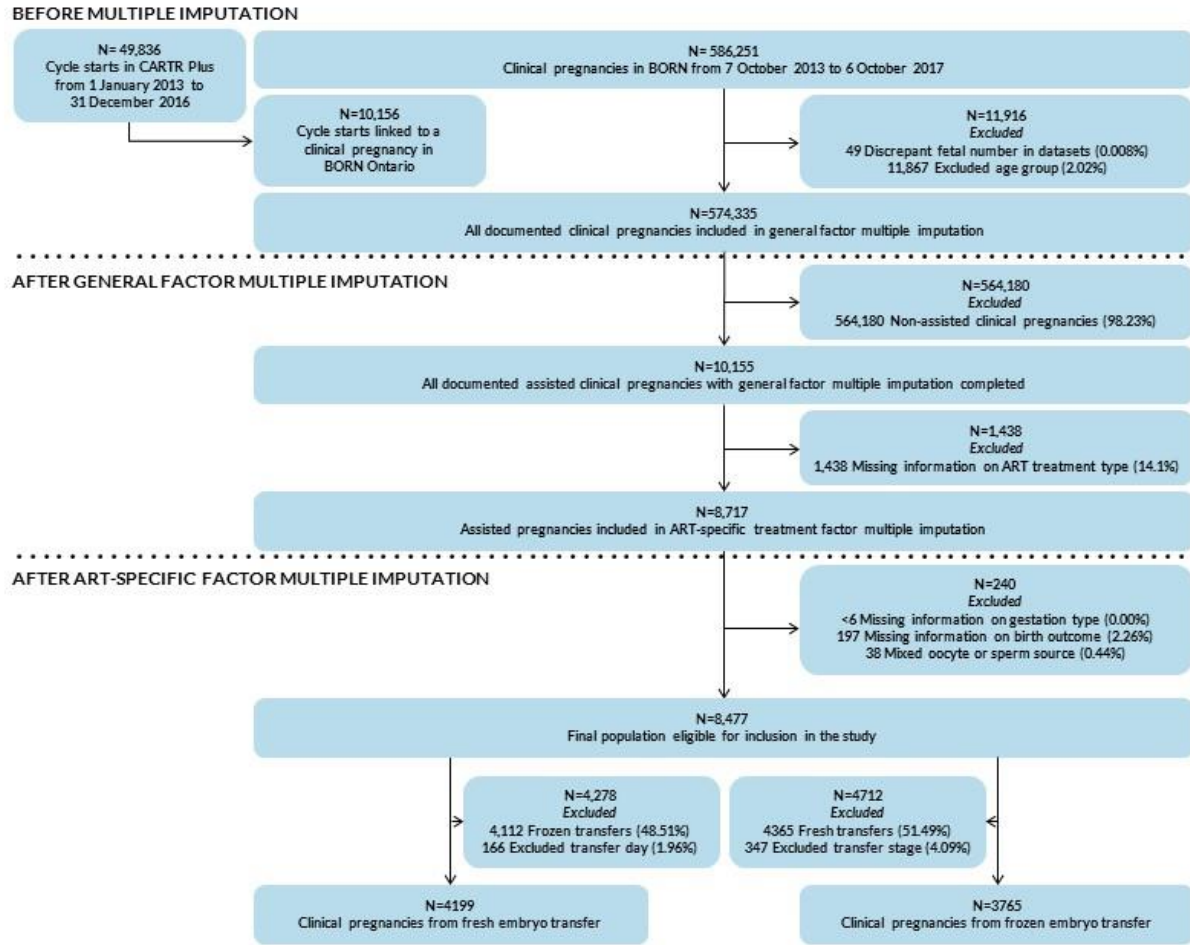
Table 3.6. Association between stage at transfer and placental and placentally-mediated outcomes in frozen embryo transfers

	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI) *	p value	Standard Error
Any Placental Complication	0.84 (0.46-1.53)	0.83 (0.42-1.66)	0.6080	1.42
Placenta Previa	0.73 (0.33-1.59)	0.92 (0.43-1.97)	0.8354	1.47
Placenta Mediated Complications	0.77 (0.52-1.14)	0.92 (0.60-1.40)	0.6903	1.24

*Adjusted using inverse probability of treatment weights.

3.9 Figure

Figure 3.1 Flow diagram of study



3.10 Supplemental

Supplemental Table 3.A: Distribution of neonatal characteristics in fresh embryo transfer study population

Characteristic	Whole Population (unweighted) (n=4795) n(%)	Day of Transfer (Unweighted)		Standardized Difference†
		Day 3 (n=1597) n(%)	Day 5 (n=3198) n(%)	
Neonatal Death				
Yes	37 (0.77%)	8 (0.50%)	29 (0.91%)	0.0486
Missing	<6§	0 (0.00%)	<6	
Gestational Age, completed weeks				
<28	121 (2.52%)	30 (1.88%)	91 (2.85%)	0.1141
28-31	112 (2.34%)	33 (2.07%)	79 (2.47%)	
32-36	904 (18.85%)	337 (21.10%)	567 (17.73%)	
37-39	2597 (54.16%)	872 (54.60%)	1725 (53.94%)	
40-41	1049 (21.88%)	320 (20.04%)	729 (22.80%)	
>42	11 (0.23%)	<6	6 (0.19%)	
Missing	<6	0 (0.00%)	<6	
Mean Birth Weight (g) (+/-Std.Dev.)	2998.71 (+/- 855.24)	2977.38 (+/- 746.39)	3009.41 (+/- 947.23)	0.0376
Missing	64 (1.33%)	16 (1.00%)	48 (1.50%)	
Birth Weight Categories¶				
VSGA (under 3 rd centile)	92 (1.92%)	40 (2.50%)	52 (1.63%)	0.2153
SGA (under 10 th centile)	282 (5.88%)	88 (5.51%)	194 (6.07%)	
LGA (90 th -96 th centile)	419 (8.74%)	144 (9.02%)	275 (8.60%)	
VLGA (over 97 th centile)	266 (5.55%)	99 (6.20%)	167 (5.22%)	
Unavailable (<24wks ga)	69 (1.44%)	13 (0.81%)	56 (1.75%)	
Other (11-89 th centiles)	3614 (75.37%)	1198 (75.02%)	2416 (75.55%)	
Missing	53 (1.11%)	15 (0.94%)	38 (1.75%)	

†Absolute standardized difference where a value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

§Cells with fewer than six (6) records are suppressed (<6).

¶Birth weight categories are constructed in line with the INTERGROWTH-21 standards. VSGA – Very Small for Gestational Age. SGA – Small for Gestational Age. LGA – Large for Gestational Age. VLGA – Very Large for Gestational Age.

Supplemental Table 3.B: Distribution of neonatal characteristics in frozen embryo transfer study population

Characteristic	Whole Population (unweighted) (n=4129) n(%)	Stage at Transfer (Unweighted)		
		Cleavage Stage (n=246) n(%)	Blastocyst (n=3861) n(%)	Standardized Difference†
Neonatal Death				
Yes	26 (0.63%)	<6§	23 (0.60%)	0.0658 (-)
Gestational Age, completed weeks				0.2029
<28	75 (1.83%)	7 (2.85%)	68 (1.76%)	
28-31	55 (1.34%)	<6	51 (1.32%)	
32-36	611 (14.88%)	42 (17.07%)	569 (14.74%)	
37-39	2135 (51.98%)	138 (56.10%)	1997 (51.72%)	
40-41	1218 (29.66%)	55 (22.36%)	1163 (30.12%)	
>42	13 (0.32%)	0 (0.00%)	13 (0.34%)	
Mean Birth Weight (g) (+/-Std.Dev.)	3178.63 (+/- 710.97)	3109.31 (+/- 765.17)	3183.06 (+/- 707.24)	0.1001
Missing	67 (1.63%)	<6	64 (1.66%)	
Birth Weight Categories¶				0.2115
VSGA (under 3 rd centile)	45 (1.10%)	<6	40 (1.04%)	
SGA (under 10 th centile)	155 (3.77%)	11 (4.47%)	144 (3.73%)	
LGA (90 th -96 th centile)	493 (12.00%)	18 (7.32%)	475 (12.30%)	
VLGA (over 97 th centile)	316 (7.69%)	25 (10.16%)	291 (7.54%)	
Unavailable (<24wks ga)	39 (0.95%)	<6	36 (0.93%)	
Other (11-89 th centiles)	2999 (73.02%)	183 (74.39%)	2816 (72.93%)	
Missing	60 (1.46%)	<6	59 (1.53%)	

†Absolute standardized difference where a value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

§Cells with fewer than six (6) records are suppressed (<6).

¶Birth weight categories are constructed in line with the INTERGROWTH-21 standards. VSGA – Very Small for Gestational Age. SGA – Small for Gestational Age. LGA – Large for Gestational Age. VLGA – Very Large for Gestational Age.

3.11 Bridge

Chapter 3 (Article 2) examined the association between day or stage of embryo at transfer and placental and placentally-mediated outcomes in fresh and frozen ART treatment cycles, respectively. We did not observe day or stage of embryo at transfer to have any bearing on abnormal placental development or function, though these results should be further investigated and replicated.

The second treatment factor hypothesized to influence the risk of adverse placental outcomes following assisted reproduction is cryopreservation, following the difference in risk in fresh and frozen treatment cycles observed in Chapter 2 (Article 1). As cryopreservation suspends intracellular function and has been found to adversely impact intracellular structure and function, it is possible that cryopreservation influences the trajectory of normal placentation on an initial molecular level. In Chapter 4 (Article 3), we compare the influence of slow-freezing and vitrification on the risk of placental and placentally-mediated outcomes as compared to fresh embryo transfers and to each other, respectively.

Chapter 4 (Article 3):

Method of cryopreservation is associated with an increased risk of placentally-mediated complications in assisted conceptions: A retrospective population-based cohort study in Ontario

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Author's Note

All authors contributed to the conceptualization and design of the study, which was led by BO. BO and SH led the development of the data analysis plan with support from RD. BO led the data analysis, in consultation with SH and MD, and guidance from MW, KP and AA. BO wrote the first draft of the paper, facilitated discussions, and oversaw all edits contributed by all authors.

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4.1 Abstract

Study question: Is the transfer of vitrified embryos associated with decreased risks of abnormal placentation and placentally-mediated complications compared to slow-frozen and fresh embryos?

Summary answer: Transfer of slow-freezing embryos was associated with a decreased risk of placentally-mediated complications as compared to vitrification, but neither vitrified nor slow-frozen embryo transfer are associated with an increase or decrease in risk of abnormal placentation or placentally-mediated outcomes compared with fresh transfers.

What is known already: The transfer of cryopreserved embryos is commonplace in contemporary fertility treatment, with vitrification most recently applied to embryos to increase post-thaw survival and pregnancy rates over the older slow-freezing technique. While there is a small body of evidence on the risks of abnormal placentation and placentally-mediated complications associated with frozen embryo transfer generally, studies on these outcomes following vitrification specifically/by method of cryopreservation are limited.

Study design, size, duration: This registry-based retrospective cohort study includes clinical pregnancies conceived after frozen-thawed and fresh transfers in Ontario, Canada between 1 January 2013 and 31 December 2016. The study includes 3741 pregnancies conceived after transfer of vitrified embryos, 318 pregnancies conceived after transfer of slow-frozen embryos, and 4354 pregnancies conceived after transfer of fresh embryos. Placental and placentally-mediated outcomes in pregnancies conceived from slow-frozen and vitrified embryos were compared to those from fresh transfer, as well as to each other. Main outcomes included any placental outcome (placenta previa, placental abruption, placenta accreta/increta/percreta),

placenta previa, placental abruption, and placentally-mediated complications (preeclampsia, IUGR, placental abruption, stillbirth).

Participants/materials, setting, methods: Data were obtained from CARTR (Canadian Assisted Reproductive Technologies Register) Plus. CARTR Plus links a Canadian national fertility treatment registry with BORN (Better Outcomes Registry & Network), a province-wide health administrative birth registry for Ontario. CARTR Plus includes all recorded clinical pregnancies conceived with ART treatment at all Ontario clinics with an embryology lab. Outcomes were examined using logistic regression, adjusting for confounders identified a priori through propensity score-derived inverse probability of treatment weights (IPTWs).

Main results and the role of chance: A decreased risk of placentally-mediated complications was identified in pregnancies after transfer of slow-frozen embryos compared with the transfer of vitrified embryos (adjusted odds ratio (aOR); 95%CI; 0.64; 0.41-0.98). A consistent pattern of lower, but non-significant risk estimates were observed for any placental complication, placenta previa and placental abruption when comparing slow-freezing to vitrification. A similar pattern was observed for all outcomes when comparing either slow-frozen or vitrified transfers to fresh transfers.

Limitations, reasons for caution: Since CARTR Plus only dates to 2013, by which time the standard of care shifted toward vitrification, a small sample of slow-frozen transfers were available. The possibility of residual confounding cannot be eliminated.

Wider implications of the findings: Vitrification of embryos is not protective of placentally-mediated outcomes compared with slow-frozen embryos or fresh transfers. The decreased risk of placentally-mediated complications following slow-frozen transfer may reflect differential

mediation of genetic and physiologic changes to the placenta linked to cryopreservation by method. Overall, cryopreservation does not present as a leading treatment factor contributing to the increased risk in adverse placental outcomes associated with assisted conceptions.

Study funding/competing interest(s): No funding or conflicts of interest to declare.

Trial registration number: Not applicable.

4.2 Introduction

The evolution of the use of assisted reproductive technologies (ART) for the treatment of infertility in humans has been characterized by the rapid adoption of new techniques with limited evidence when they offer desirable outcomes, particularly with respect to clinical pregnancy and live birth rates. Cryopreservation was initially a technique for improving the efficiency of storage and use of male gametes in bovine and equine species. Suitably adapted, it served the same role in humans, was subsequently expanded to embryos and, most recently, oocytes. Initial methods involved a “slow freeze” protocol following the administration of a cryoprotectant agent to reduce crystal formation within the cell structure. Losses from this process, expressed as the proportion of surviving embryos is substantial, in the order of 30% (van Landuyt *et al.*, 2013). This is a major efficiency loss, which begs the question as to whether there is a selection pressure for a cryo-durability developmental phenotype.

Vitrification is a contemporary technique developed to increase post-thaw survival and pregnancy rates that accelerates the cryopreservation process by plunging the oocyte/embryo contained within a small protective vessel directly into liquid nitrogen. Compared to both fresh and slow-frozen-thawed (henceforth slow-frozen) embryos, vitrified-warmed (henceforth vitrified) embryos have been shown to have higher embryo survival (Edgar and Gook, 2012; Rienzi *et al.*, 2017) and superior obstetric outcomes, including higher implantation (Abdelhafez *et al.*, 2010), clinical pregnancy ; Li *et al.*, 2014) and live birth rates (Abdelhafez *et al.*, 2010; Li *et al.*, 2014). Furthermore, compared to fresh embryos, vitrified embryos have improved perinatal outcomes (Roy *et al.*, 2014), including longer gestational age, higher live birth weight (Li *et al.*, 2014; Roy *et al.*, 2014), and lower risk of preterm birth (Li *et al.*, 2014). At a genetic level, vitrification is demonstrated to have a less detrimental effect on the integrity of embryo

DNA (Kopeika *et al.*, 2015) and alterations to gene expression (Larman *et al.*, 2011) than slow freezing in human and animal models, alternatively demonstrated to be greater than or no different to gene expression in fresh embryos (Larman *et al.*, 2011). Generally, cryopreserved embryos exhibit higher or at least as good obstetric (Pelkonen *et al.*, 2010; Maheshwari *et al.*, 2012), perinatal (Maheshwari *et al.*, 2012), and neonatal (Wennerholm *et al.*, 2009) outcomes as fresh embryos, including increased implantation rates (Ozgur *et al.*, 2015), higher birthweights (Wennerholm *et al.*, 2009; Ozgur *et al.*, 2015; Vidal *et al.*, 2017), and decreased risks of being small for gestational age (SGA) (Vidal *et al.*, 2017) and being born preterm (Wennerholm *et al.*, 2009).

There is, however, very little evidence on abnormal placentation and adverse placenta mediated complications following vitrified embryo transfer as compared to slow-frozen or fresh embryos. Further, current literature is characterized by mostly small sample sizes and disagreement in results (Barsky *et al.*, 2016; Ernstad *et al.*, 2019; Reljić and Porović, 2019). This is a significant matter as many diseases of pregnancy and anomalies of fetal development are mediated through placental factors. For example, cryopreservation research frequently compares fresh and frozen embryo transfers, without differentiating between slow-freezing and vitrification.

Cryopreservation has been associated with risks of abnormal placentation, namely an increased risk of placenta accrete (Ishihara *et al.*, 2014; Kaser *et al.*, 2015), and decreased risks of placenta previa (Sazonova *et al.*, 2012; Korosec *et al.*, 2014; Ginström Ernstad *et al.*, 2019) and placental abruption (Healy *et al.*, 2010; Sha *et al.*, 2018; Ginström Ernstad *et al.*, 2019; Conforti *et al.*, 2020). Similarly, an increased risk of adverse placenta mediated complications, such as preeclampsia (Sazonova *et al.*, 2012; Sites *et al.*, 2017; Roque *et al.*, 2018, 2019) and hypertensive disorders in pregnancy (Maheshwari *et al.*, 2018; Ginström Ernstad *et al.*, 2019;

Luke *et al.*, 2020), and a decreased risk as ischemic placental disease (Johnson *et al.*, 2019) have been observed with cryopreservation. Other studies report no difference in risk of these outcomes (Roque *et al.*, 2018; Johnson *et al.*, 2019). Given the relative contribution of placental complications as leading causes of maternal morbidity and mortality – namely haemorrhage and hypertensive disorders – and the move to vitrification as the cryopreservation method of choice, more research is needed to ensure the safest pathway to placental, fetal, and neonatal health.

In this study, we assessed the association of placental and placentally-mediated complications during pregnancy and method of cryopreservation.

4.3 Methods

4.3.1 Study Design, Data Sources, Study Population

Using a retrospective cohort study design, we identified the study population using the Better Outcomes Registry & Network (BORN) Ontario birth registry (<https://www.bornontario.ca/>) linked with the Canadian Assisted Reproductive Technologies Register (CARTR) Plus.

We compared the risk of clinically severe placental complications among assisted pregnancies in Ontario, Canada whose treatment cycles started between 1 January 2013 and 31 December 2016. The CARTR Plus database is an extension of CARTR, a voluntary registry developed by the Canadian Fertility and Andrology Society (CFAS). CARTR consists of record-level information on fertility treatment (including detailed treatment information, and pregnancy and childbirth outcomes) submitted by Canadian fertility clinics. CARTR Plus links records from all Ontario clinics with an embryology lab to records in BORN, a province-wide registry containing maternal-newborn records for all documented clinical pregnancies, births and resulting infants in Ontario (Dunn *et al.*, 2019; Miao *et al.*, 2019).

All assisted pregnancies from the study period were included with the exception of those conceived in treatment cycles where mixed methods of cryopreservation were used, which we excluded as we were unable to determine the method used for the embryo(s) transferred.

4.3.2 *Exposure and Outcome Measurement*

The exposure of interest was cryopreservation of embryos transferred in IVF and ICSI cycles that led to a documented confirmed clinical pregnancy. Cryopreservation techniques of interest were slow freezing and vitrification, with fresh transfers used as our comparison group. Embryo cryopreservation is recorded in the CARTR Plus database in primary cycles, specifically those in which embryo creation has occurred and a fresh transfer can occur. We linked frozen transfer cycles with their primary cycle to conserve all treatment factors, including cryopreservation, that impact upon gametes and embryos until transfer.

We identified two primary outcomes of interest *a priori*: any placental complication and any placentally-mediated complication. We examined the occurrence of any placental complication (placenta accreta, placenta increta, placenta percreta, placenta previa and placental abruption) in a composite variable, as well as that of placenta previa and placental abruption separately. All five placental complications were identified in the maternal labour, antenatal general and specialty antenatal records in BORN.

Placentally-mediated complications, defined as preeclampsia, intrauterine growth restriction (IUGR), placental abruption and stillbirth, were also measured using a composite variable. The selected outcomes are severe and are of clinical importance, leading to their aggregation despite the heterogeneity in their hypothesized etiologies (N.B. Preeclampsia, placental abruption, IUGR and some types of stillbirth have recently been hypothesized to have similar pathogenesis in

some cases (Ananth, 2014; Roberts, 2014), referred to as ischemic placental disease (IPD)). By searching maternal antenatal, labour and birth records, we were able to identify preeclampsia and IUGR in pre-identified data fields and aggregate the results per pregnancy. We identified stillbirth, included as a surrogate measure of placental function (Bukowski *et al.*, 2014), as a birth outcome in BORN maternal birth records.

4.3.3 Statistical Analyses

We described the characteristics of our study population using frequencies for categorical variables and means and standard deviations for select continuous variables. To assess the balance of our covariates at baseline between the three exposure groups, we used standardized differences where a standardized difference of less than 0.10 was interpreted as sufficient balance among groups (Austin, 2009a, 2009b).

Prior to analysis, we used multiple imputation using predictive mean matching to impute missing values for our *a priori* selected covariates. We created 20 imputed datasets using the R packages rms and Hmisc (Harrell, 2020, 2021) in R Version 4.0.2 (<https://www.r-project.org>). This process is described in detail elsewhere (Olibris, 2021, unpublished [Chapters 2 & 3]).

We used propensity score weights to adjust for confounding. We used the R package twang (McCaffrey *et al.*, 2013; Burgette *et al.*, 2020) to calculate a propensity score for each pregnancy using a generalized boosted model. The propensity score indicated the predicted probability of the method of cryopreservation (fresh, slow frozen or vitrification) of the embryo(s) used to conceive the pregnancy. Some 18 covariates were identified *a priori* for inclusion in the propensity score models, which were fitted in each of the 20 imputation datasets: ART type, patient age at oocyte retrieval, type of infertility, stimulation protocol from harvest cycle, oocyte

source, sperm source, number of embryos transferred, maternal pre-pregnancy BMI, parity, maternal smoking during pregnancy, preexisting maternal comorbidities (anaemia, asthma, diabetes, and hypertension), complications of pregnancy (gestational diabetes and gestational hypertension), gestation type (singleton vs. multiple) and a history of previous caesarean section. The final propensity score model was that which was best able to decrease both the maximum and mean absolute standardized difference for each included covariate below 0.1, with the mean absolute standardized difference being used to compute the propensity score weights. Given the differences in exposure group size, the propensity score model was adjusted with sampling weights where the value for each exposure group was equivalent to one over the number of conceptions in each exposure group multiplied by the total sample population. We used the propensity score values to develop inverse probability of treatment weights (IPTWs). IPTWs in both the slow freezing and vitrification exposure groups were equivalent to the inverse of the propensity score, and the IPTWs for the fresh exposure group were equivalent to the inverse of one minus the propensity score (Cole and Hernán, 2008).

We used logistic regression models to estimate unadjusted and adjusted odds ratios with 95% confidence intervals and robust standard errors. The unadjusted estimates were calculated without the use of multiple imputation as data for the exposure and outcomes were complete. To calculate the adjusted estimates, we ran weighted models for each outcome using the IPTWs in each of the 20 multiply imputed datasets. We combined the resulting estimates, parameters, and standard errors to compute a single adjusted estimate, 95% confidence interval, p-value and robust standard error using the MIANALYZE procedure in SAS (<https://www.sas.com/>), based on Rubin's approach to pooling within and among imputation sources of variation (Rubin, 1987).

As a sensitivity analysis, we compared first and subsequent pregnancies within individual

women in our sample to assess any differences in the distribution of covariates and outcomes between these pregnancies. Similarly, we assessed the need to adjust for the effect of clustering of conceptions within women by comparing models accounting for correlation within women via generalized estimating equations, to models that did not.

4.4 Results

4.4.1 Characteristics of Study Population

There were 49,836 IVF and ICSI cycle starts recorded in CARTR Plus during the study period, of which 10,156 (20.37%) linked to a clinical pregnancy record in the Ontario birth registry during the same period. Of the 10,155 conceptions originally eligible for inclusion in the study, 1438 records (14.16%) were excluded for missing information on the ART treatment type (IVF or ICSI) in the CARTR data, leaving a total of 8717 conceptions on which we based the multiple imputation for treatment covariates (Figure 4.1). 22.03% of records had missing information for one or more covariates to be included in the propensity score models. The percentage of missing values ranged from 0% for several covariates to 18.92% for maternal pre-pregnancy BMI. Following imputation, which excluded the main exposure variable, 240 (2.75%) records were excluded for missing birth outcome in the original data because stillbirth is an outcome of interest in our composite variable of placentally-mediated complications, and for missing information on gestation type, and mixed oocyte or sperm source in the original dataset because the use of imputed values for these two variables was deemed inappropriate. A final 64 records (0.75%) were excluded for using mixed methods of cryopreservation on a batch of embryos. Thus, our final study population consisted of 8413 clinical pregnancies.

Compared to women who conceived with a fresh embryo transfer, women who conceived with a

slow frozen or vitrified embryo transfer were younger, had a lower BMI, and were less likely to be nulliparous and have preexisting conditions (including anaemia, asthma and, for slow frozen conceptions, hypertension) (Table 4.1). Their pregnancies were less likely to be multiple gestations and involve transfer of more than one embryo. Slow frozen conceptions were more likely to involve a previous caesarean section, have different infertility diagnoses - as fewer had female factor infertility as their major infertility diagnosis while vitrified were more likely to have and unexplained infertility, use different ovarian stimulation protocols, and use own oocytes and partner sperm than both fresh and vitrified transfers. Vitrified conceptions were identified as more likely to be ICSI conceptions than both fresh and slow frozen transfers, were less likely to use agonists, and more likely to use modified natural cycles in ovarian stimulation than fresh transfers. (N.B. Complimentary information on the distribution of neonatal characteristics is available in Supplemental Table 4.A.)

In sensitivity analyses, we did not observe a meaningful difference in covariate distribution (as opposed to outcome) between first and subsequent pregnancies and thus did not adjust our analysis by birth order. Similarly, we did not identify a need to adjust our analysis for clustering within women.

4.4.2 Risk of Placental and Placental Mediated Outcomes with Cryopreservation

Any placental complication occurred in a total of 404 (4.80%) pregnancies – 220 (5.05%) in fresh cycles, 11 (3.46%) following slow freezing and 173 (4.62%) following vitrification (Table 4.2). Some 995 (11.82%) pregnancies experienced a placentally-mediated complication, with 518 (11.90%) in fresh cycles, 25 (7.86%) following slow freezing, and 452 (12.08%) following vitrification.

Adjusted odds ratios and 95% confidence intervals for the association between pregnancies resulting from either slow frozen or vitrified embryo transfers, respectively, and the following outcomes were suggestive of a lower risk as compared to fresh embryo transfers, but confidence intervals did not exclude 1 and thus did not reach the threshold for statistical significance before or after propensity score adjustment: any placental complication (aOR 0.65, 95% CI 0.34-1.23; aOR 0.83, 95% CI 0.67-1.04 respectively), placental previa (aOR 0.63, 95% CI 0.27-1.49; aOR 0.77, 95% CI 0.57-1.05 respectively), placental abruption (aOR 0.74, 95% CI 0.22-2.48; aOR 0.77, 95% CI 0.50-1.18 respectively), and our composite outcome of placentally-mediated complications (aOR 0.67, 95% CI 0.43-1.04; aOR 1.06, 95% CI 0.91-1.22 respectively) (Table 4.3). There is, nonetheless, a consistent, marked pattern of decreased risk of these outcomes following slow freezing and vitrification relative to fresh transfers, as well as in slow freezing when compared to vitrification. Conceptions from slow-frozen embryos were at a significantly reduced risk of placentally-mediated complications as compared to vitrified embryos (aOR 0.62, 95% CI 0.41-0.98).

4.5 Discussion

In this observational study of 8,413 assisted conceptions, using comprehensive Ontario databases containing detailed information on infertility treatment, pregnancy, and potential confounders, we identify a consistent pattern of estimates reflecting a lower risk of all outcomes associated with slow-freezing compared to both fresh and vitrified in turn, with the strongest association suggesting a lower risk of placentally-mediated complications in slow-freezing compared with vitrification in particular after propensity score adjustment.

Placental and placentally-mediated complications in pregnancy are rare, particularly those

complications of abnormal placentation (placenta previa, placental abruption, placenta accrete/increta/percreta). Although Ontario has a large annual birth volume (~140,000 births annually) and higher conception volume, and although our assisted cohort is not among the smallest in the literature, our outcome event counts are low. We therefore cannot rule out the possibility that the non-significant p values obtained in testing our hypothesis are the function of low event counts and limited power. It is nevertheless noteworthy that we consistently achieve estimates that suggest that using slow-frozen and vitrified embryos introduces a lower risk of all outcomes than using fresh embryos.

Although the 95% confidence intervals for our slow freezing estimates are wider than for our vitrification estimates (as is expected with our smaller slow freezing sample), both sets of confidence intervals reflect a substantial negative association with three of our outcomes, namely any placental complication, placenta previa, and placentally-mediated complications, and also with placental abruption in the vitrification group. This is reasonably consistent with our data, in which the incidence of our outcomes is lowest in the slow freezing groups and lower but more comparable in the vitrification group compared to the fresh group, and the assumptions of our framework underlying the models. Even with slightly wider confidence intervals than ideal, such a consistent pattern in our estimates suggests that a factor(s) other than error is at play.

Similarly, we consistently achieve estimates that suggest that slow-freezing presents a lesser risk of all outcomes than vitrification, particularly for placentally-mediated outcomes. While animal models have revealed increased alterations to gene expression and placental structure following both types of cryopreservation (Zander-Fox *et al.*, 2013; Gupta *et al.*, 2017), the changes appear to be more marked after vitrification. Studies with mouse models have demonstrated increased atrophy in maternal- and fetal-facing placentas following vitrification, suggesting impacted

decidualization or vascularization (Mocé *et al.*, 2010). Clinically, pregnancies conceived with vitrified embryos have been shown to be at significantly increased risk of major postpartum haemorrhage versus both fresh and slow-frozen transfers (Wikland *et al.*, 2010). Thus, while slow-freezing and vitrification may be comparable in risk in comparison to fresh cycles, they may not be equal in risk between themselves. These are important matters for further research.

We put forward three possible explanations for this outcome. First, there are multiple selection points for embryo survival from creation to implantation. Embryos cryopreserved at the morula or blastocyst stage have already demonstrated survivorship by robust development and developmental potential. However, attainment of a stage does not guarantee high quality embryo morphology. As vitrification has better rates of post-warming morula and blastocyst survival and development, it is also possible that a less robust, though developmentally advanced embryo may survive vitrification to be transferred where it would not survive slow-freezing, especially if embryos are cryopreserved prior to maturing to the blastocyst stage. Second, cryopreservation has been linked to greater abnormal placental physiology than observed after fresh embryo transfers, both of which are more likely to lead to abnormal pathology than natural embryos (Sacha *et al.*, 2020). Animal models have demonstrated that embryos derived from ART are also more likely to have altered placental transcriptomes, mRNA expression and vascular development (Grazul-Bilska *et al.*, 2014; Menelaou *et al.*, 2020). It is possible that these alterations are mediated by cryopreservation methods, explaining the results observed here. Third, there may be an unmeasured temporal effect in that the move to vitrification has happened at the same time as the rates of preeclampsia, placental abruption or IUGR have decreased in the population overall. However, given that the rates of placental abruption and preeclampsia were increasing until at least 2012 (Ananth *et al.*, 2015; Auger *et al.*, 2016), and including year of

cycle start date in our models did not alter the results, this is unlikely.

While these results suggest that cryopreservation is not a leading treatment factor contributing to the difference in placental complications observed in assisted conceptions as compared to spontaneous conceptions in our data, we observed no advantage from vitrification in placental or placentally mediated disorders of pregnancy; on the contrary, in fact. However, we advise caution in such an interpretation of the results due to the potential for residual confounding as the technologies are evolving very quickly, often with incomplete knowledge of the potential for residual confounding and effect modification by poorly measured or presently unknown treatment factors, as well as patient factors which may contribute to both infertility and placentally-mediated complications of pregnancy. Furthermore, recent research suggests that although vitrification impacts embryonic development, the genetic deviations introduced by vitrification are less than those introduced by the in vitro manipulation of the embryo prior to transfer (Garcia-Dominguez *et al.*, 2020) and therefore isolating the effect in an observational study may be challenging.

4.5.1 *Strengths and Weaknesses of Study*

The primary strength of the present study was the availability of a population-based birth registry and a comprehensive fertility database with near or complete coverage of fertility clinics in Ontario that could be linked together to track from pre-conception activity to delivery.

Propensity scores have been demonstrated to be more effective in reducing bias in observational studies using large health administrative databases as compared to more traditional methods such as multivariable adjustment methods (Austin and Stuart, 2015), but it is possible that residual confounding remains in our study from unmeasured or unknown confounders that were

excluded/left out of the propensity scores. For example, women with ART conceptions may be more closely monitored during infertility treatment and pregnancy, leading to a more accurate health history being recorded in the data with fewer missing variables.

Although CARTR contains a comprehensive record of a fresh embryo transfer cycle from ovulation stimulation through to delivery, less information is available for cryopreserved cycles. Notably, multiple studies have observed a difference in outcomes in natural versus programmed cryopreserved transfer cycles, specifically with respect presence or absence of the corpus luteum (Ginström Ernstad *et al.*, 2019; Singh *et al.*, 2020). We are currently unable to determine in which type of cycle a cryopreserved transfer occurs, which could be accomplished in a controlled study. Second, increasing endometrial thickness has been linked to an increased risk of placenta previa in assisted pregnancies. CARTR Plus includes data on endometrial thickness in fresh and frozen cycles respectively, however the high level of missing data, particularly for frozen cycles, led us to exclude it from our analysis (Rombauts *et al.*, 2014). Third, differences in placental histopathology have been identified by fetal sex (Volodarsky-Perel *et al.*, 2020). Given our sample size, having complete data on fetal sex to allow for a full case analysis including this variable may help further elucidate the mechanisms behind our finding. Fourth, the included measurement of IUGR, or fetal growth restriction, is based on population-based charts, not customized charts, which have been found to be more accurate for the identification of fetal growth restriction associated with abnormal placental pathology (Melamed *et al.*, 2021), given the data available for the study. Finally, cryopreservation protocols and kits may differ across clinics, affecting, which may impact our outcomes, though how much in either direction from the null is unknown.

4.5.2 Similarities to Other Studies

To date, few studies of cryopreservation have sought to assess an association with placental outcomes, with even fewer distinguishing between vitrification and slow-freezing. To our knowledge, only three studies have assessed the risk of placental or placentally-mediated outcomes with respect to vitrification specifically. Two assessed the risk of vitrification of blastocysts on preeclampsia, observing either an increased risk (Barsky *et al.*, 2016) or no significant difference in risk (Reljić and Porović, 2019) between vitrified warmed and fresh blastocyst transfers. A third larger study identified a significantly decreased risk of placenta previa and non-significant reduction in risk of placental abruption with vitrified warmed blastocyst transfer as compared to fresh blastocyst transfer (Ernstad *et al.*, 2019). With respect to IPD, Johnson *et al.* (Johnson *et al.*, 2019) found a lower risk of either IPD or intrauterine fetal demise in frozen embryo transfers compared to fresh, and comparable risks of preeclampsia and abruption independently between transfer types, expanding on another finding of an increased risk of term and preterm IPD in IVF pregnancies compared with non-IVF pregnancies (Johnson *et al.*, 2020).

4.6 Conclusion

In our population-based study of assisted conception in Ontario, while we found slow-freezing to be associated with a significantly reduced risk of placentally-mediated outcomes versus vitrification, we did not find any advantage for any outcome for slow-freezing compared with fresh transfers, nor did we find any advantage for any outcome for vitrification versus fresh or slow-freezing. While this suggests that cryopreservation is not a leading treatment factor contributing to the difference in placental complications observed between assisted and non-

assisted conceptions, we cannot rule out the possibility this null observation is a function of low event counts in our data, nor can we rule out the possibility that other treatment factors interact to contribute to this outcome. Future studies should focus on determining the differential mechanisms behind slow-freezing and vitrification and placental development to help better elucidate the relationship between cryopreservation and adverse placental outcomes.

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4.8 Tables

Table 4.1. Distribution of baseline characteristics of study population, overall and by embryo cryopreservation method

Characteristic	Whole Population (unweighted) (n=8413) n(%)	Fresh (unweighted) (n=4354) n(%)	Cryopreservation Method (unweighted)				Cryopreservation Method (IPTW weighted*)	
			Slow-Freezing (n=318) n(%)	Standardized Difference†	Vitrification (n=3741) n(%)	Standardized Difference†	Slow Freezing Mean Standardized Difference‡	Vitrification Mean Standardized Difference‡
Oocyte Provider Age, years				0.1194		0.1443	0.0165	0.005
21-24	240 (2.85%)	108 (2.48%)	6 (1.89%)		126 (3.37%)			
25-29	1144 (13.60%)	551 (12.66%)	45 (14.15%)		548 (14.65%)			
30-34	3394 (40.34%)	1690 (38.81%)	140 (44.03%)		1564 (41.81%)			
35-39	2893 (34.39%)	1583 (36.36%)	107 (33.65%)		1203 (32.16%)			
>=40	713 (8.47%)	422 (9.69%)	20 (6.29%)		271 (7.24%)			
Missing	29 (0.34%)	0 (0.00%)	0 (0.00%)		29 (0.78%)			
Parity				0.3456		0.1478	0.026	0.0195
0	5801 (68.95%)	3137 (72.05%)	185 (58.18%)		2479 (66.27%)			
1	1994 (23.70%)	934 (21.45%)	111 (34.91%)		949 (25.37%)			
2	332 (3.95%)	145 (3.33%)	14 (4.40%)		173 (4.62%)			
3	75 (0.89%)	33 (0.76%)	<6§		40 (1.07%)			
>=4	32 (0.38%)	12 (0.28%)	<6		19 (0.51%)			
Missing	179 (2.13%)	93 (2.14%)	<6		81 (2.17%)			
Smoking¥				0.0464		0.0263	0.0215	0.0015
Yes	109 (1.30%)	63 (1.45%)	<6		43 (1.15%)			
Pre-pregnancy BMI				0.0789		0.0623	0.017	0.008
<=18.49	234 (2.78%)	113 (2.60%)	6 (1.89%)		115 (3.07%)			
18.50-24.99	3864 (45.93%)	1952 (44.83%)	158 (49.69%)		1754 (46.89%)			
25.00-29.99	1622 (19.28%)	837 (19.22%)	70 (22.01%)		715 (19.11%)			
>=30.00	1101 (13.09%)	594 (13.64%)	37 (11.64%)		470 (12.56%)			
Missing	1592 (18.92%)	858 (19.71%)	47 (14.78%)		687 (18.36%)			
Gestation Type				0.2105		0.1416	0.049	0.0135
Singleton	7359 (87.47%)	3707 (85.14%)	292 (91.82%)		3360 (89.82%)			
Multiples	1054 (12.53%)	647 (14.86%)	26 (8.18%)		381 (10.18%)			
Treatment Information								
ART Treatment Type				0.0548		0.1073	0.0345	0.021
IVF	1059 (12.59%)	613 (14.08%)	51 (16.04%)		395 (10.56%)			
ICSI	7354 (87.41%)	3741 (85.92%)	267 (83.96%)		3346 (89.44%)			
Primary Infertility Category§				0.1496		0.1809	0.0085	0.0055
Female factor								
Male factor	3631 (43.16%)	1875 (43.06%)	125 (39.31%)		1631 (43.60%)			
Female/Male	1919 (22.81%)	1018 (23.38%)	79 (24.84%)		822 (21.97%)			
Unexplained	1118 (13.29%)	657 (15.09%)	60 (18.87%)		401 (10.72%)			
Other	1620 (19.26%)	733 (16.84%)	50 (15.72%)		837 (22.37%)			
	125 (1.49%)	71 (1.63%)	<6		50 (1.34%)			
Stimulation Protocol¶				0.2541		0.3738	0.0245	0.0395

Natural cycle	50 (0.59%)	13 (0.30%)	0 (0.00%)		37 (0.99%)			
Modified natural cycle	214 (2.54%)	21 (0.48%)	0 (0.00%)		193 (5.16%)			
Antagonist	5860 (69.65%)	3099 (71.18%)	185 (58.18%)		2576 (68.86%)			
Agonist	1984 (23.58%)	1172 (26.92%)	48 (15.09%)		764 (20.42%)			
Mild stimulation	62 (0.74%)	36 (0.83%)	0 (0.00%)		26 (0.70%)			
IVM protocol/Other	23 (0.27%)	13 (0.30%)	0 (0.00%)		10 (0.27%)			
Missing	220 (2.62%)	0 (0.00%)	85 (26.73%)		135 (3.61%)			
Oocyte Source				0.0420		0.0614	0.0185	0.0085
Autologous	7595 (90.28%)	4031 (92.58%)	221 (69.50%)		3343 (89.36%)			
Donor	673 (8.00%)	323 (7.42%)	15 (4.72%)		335 (8.95%)			
Missing	145 (1.72%)	0 (0.00)	82 (25.795)		63 (1.68%)			
Sperm Source				0.1815		0.0417	0.044	0.008
Autologous	7796 (92.67%)	4082 (93.75%)	230 (72.33%)		3484 (93.13%)			
Donor	472 (5.61%)	272 (6.25%)	6 (1.89%)		194 (5.19%)			
Missing	145 (1.72%)	0 (0.00%)	82 (25.79%)		63 (1.695)			
Number of Embryos Transferred				0.5236		0.3997	0.106	0.021
1	4733 (56.26%)	2045 (46.97%)	221 (69.50%)		2467 (65.94%)			
2	3387 (40.26%)	2087 (47.93%)	97 (30.50%)		1203 (32.16%)			
>=3	293 (3.48%)	222 (5.10%)	0 (0.00%)		71 (1.90%)			
Preexisting Conditions								
Preexisting Anaemia¶				0.1396		0.0571	0.06	0.0095
Yes	60 (0.71%)	42 (0.96%)	0 (0.00%)		18 (0.48%)			
Preexisting Asthma¶				0.1187		0.0173	0.07	0.001
Yes	194 (2.31%)	108 (2.48%)	<6		83 (2.22%)			
Preexisting Diabetes¶				0.0045		0.0302	0.011	0.011
Yes	95 (1.13%)	43 (0.99%)	3 (0.94%)		49 (1.31%)			
Preexisting Hypertension^				0.1421		0.0191	0.0765	0.0015
Yes	152 (1.81%)	76 (1.75%)	<6		75 (2.00%)			
Complications of Pregnancy								
Gestational Hypertension				0.0574		0.0455	0.0265	0.018
Yes	436 (5.18%)	204 (4.69%)	19 (5.97%)		213 (5.69%)			
Gestational Diabetes[				0.0255		0.0608	0.0065	0.013
Yes	934 (11.10%)	445 (10.22%)	35 (11.01%)		454 (12.14%)			
Birth Outcomes								
Live Birth				0.0520		0.0383		
Yes	8273 (98.34%)	4271 (98.09%)	314 (98.74%)		3688 (98.58%)			
Delivery Type				0.1721		0.1983		
Spontaneous Vaginal	3582 (42.58%)	2202 (46.44%)	156 (49.06%)		1404 (37.53%)			
Assisted Vaginal	1081 (12.85%)	514 (11.81%)	30 (9.43%)		537 (14.35%)			
Induced or Spontaneous								
Labour Caesarean section	1822 (21.66%)	820 (18.83%)	60 (18.87%)		942 (25.18%)			
No labour caesarean section	1892 (22.49%)	977 (22.44%)	70 (22.01%)		845 (22.59%)			
Missing	36 (0.43%)	21 (0.48%)	<6		13 (0.35%)			
Previous Caesarean Section				0.1680		0.0846	0.036	0.018
Yes	650 (7.73%)	285 (6.55%)	36 (11.32%)		329 (8.79%)			

*Inverse probability of treatment weighting (where IPTW=inverse probability of treatment weight).

†Absolute standardized difference where a value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

‡Mean absolute standardized difference where the value is an average of all standardized difference values over 20 imputation datasets. A value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

§Cells with fewer than six (6) records are suppressed (<6).

¥Smoking: Information on maternal smoking is collected for both smoking at first prenatal visit and at admission for birth. If a record indicated a woman smoked any amount at either of these time points they were classified as “Smoking”. Conversely, if a record indicated a woman was not smoking at both these time points they were classified as “Not Smoking”.

§Primary Infertility Category: Detailed information on reasons for an IVF/ICSI cycle is collected in primary (embryo creation with potential for fresh transfer) cycles. Reasons for treatment were categorized as female factor, male factor, combined male and female factor, unexplained and other according to the following hierarchy: a) if only one reason for treatment is indicated, this is the primary infertility category; b) if multiple reasons for treatment are indicated, female and male factor infertility take precedence over unexplained, non-medical, and non-reproductive reasons; c) if both male and female factor infertility are indicated, they are recorded together as female/male. Female factor includes a record of any of the following on their own or in combination with other or unexplained infertility: advanced female age; diminished ovarian reserve; endometriosis; PCOS; peritoneal factor or severe adhesions; tubal factor; uterine factor; other ovulatory disorder; other female factor. Male factor includes: aspermia; asthenozoospermia; azoospermia; globozoospermia; necrozoospermia; oligozoospermia; teratozoospermia; erectile dysfunction; high sperm DNA fragmentation; Klinefelter Syndrome; retrograde ejaculation; sperm antibodies; other male; not specified. If a record identifies both a source of male factor and female factor infertility as a reason for treatment, they are classified as Female/Male. Unexplained includes unexplained infertility only. Other includes: embryo banking – cancer and other medical treatment; embryo banking – for non-cancer/non-medical treatment reasons; gonadotoxic therapy; social infertility (no male partner; no female partner); PGT-M/SR for known genetic factor; PGT-A for aneuploidy screening.

§Stimulation Type: Stimulation protocols were categorized as follows based on information collected in a primary (embryo creation with potential for fresh transfer) cycle: natural cycle, modified natural cycle, antagonist, agonist (includes: flare agonist, microdose flare agonist, long agonist), mild stimulation, IVM protocol/other (includes: IVM protocol, other stimulation protocol).

¶Anaemia includes chronic anaemia only.

□Asthma: Information for asthma is collected for both pre-existing asthma and asthma that occurred in the current pregnancy. If a record indicated that a woman had pre-existing asthma they were classified as “Yes”. If a record indicated that a woman had asthma that occurred in pregnancy but did not have recorded pre-existing asthma they were classified as “No”.

∅Diabetes includes: diabetes, diabetes type I, diabetes type II with insulin, diabetes type II no insulin, diabetes type II no insulin with diet management only, diabetes type II no insulin with oral antihyperglycemic agents, and diabetes type unknown. If a record indicated the presence of any one of these, they were classified as “Yes”.

^Hypertension includes pre-existing hypertension only.

ℓGestational diabetes includes: gestational diabetes; gestational diabetes with insulin; gestational diabetes with insulin/ACE inhibitors; gestational diabetes with insulin/statins; gestational diabetes with no ACE inhibitors or statins; gestational diabetes no insulin; gestational diabetes no insulin/no oral agents; gestational diabetes no insulin/oral antihyperglycemic agents; gestational diabetes insulin status unknown. If a record indicated the presence of any one of these, they were classified as “Yes”.

Table 4.2. Number of events by cryopreservation method

	Fresh (n=4354) n(%)	Slow-Freezing (n=318) n(%)	Vitrification (n=3741) n(%)
Any Placental Complication*	220 (5.05%)	11 (3.46%)	173 (4.62%)
Placenta Previa	116 (2.66%)	6 (1.89%)	89 (2.38%)
Placental Abruption	63 (1.45%)	<6	43 (1.15%)
Placentally-Mediated Complications‡	518 (11.90%)	25 (7.86%)	452 (12.08%)
Preeclampsia	244 (5.60%)	17 (5.35%)	274 (7.32%)
IUGR	177 (4.07%)	6 (1.89%)	120 (3.21%)
Placental Abruption	63 (1.45%)	<6	43 (1.15%)
Stillbirth§	68 (1.56%)	<6	39 (1.04%)

*Any placental complication includes: placenta previa, placental abruption, placenta accreta, placenta percreta, placenta increta. Sum of each individual condition does not equal the number of pregnancies with any placental complication as categories were not mutually exclusive. A pregnancy needed to have a record of any one of the listed conditions to be classified as a “Yes” for any placental complication.

‡Sum of each individual condition does not equal the number of pregnancies with a placentally-mediated complication as categories were not mutually exclusive. A pregnancy needed to have a record of preeclampsia, IUGR, placental abruption or stillbirth to be classified as “Yes” for a placentally-mediated complication.

§Stillbirths include stillbirths at ≥ 20 weeks or ≥ 500 grams occurring during the antepartum or intrapartum period.

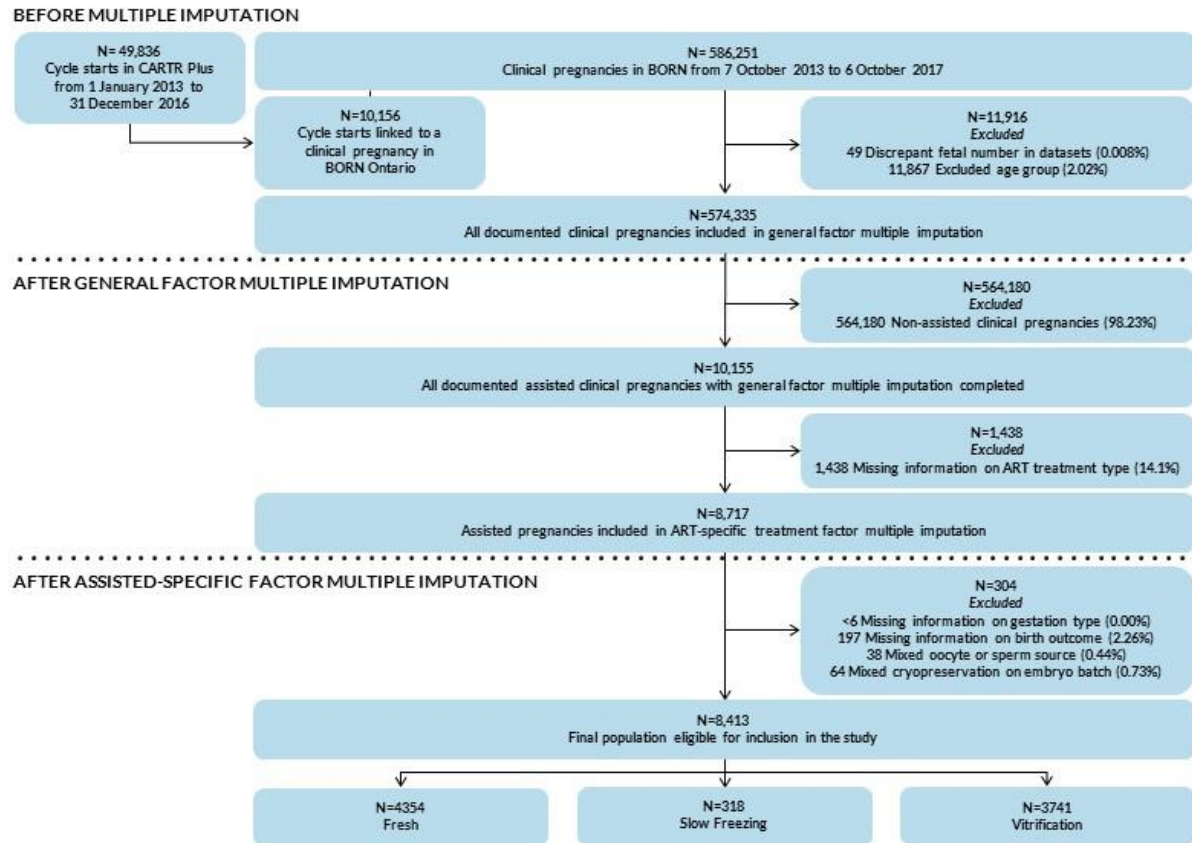
Table 4.3. Association between cryopreservation and placental and placentally-mediated outcomes in fresh, slow-freezing and vitrification exposure groups

	Slow-Freezing vs. Fresh				Vitrification vs. Fresh				Slow-Freezing vs. Vitrification			
	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)*	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)*	p value	Standard Error	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratios (95% CI)*	p value	Standard Error
Any Placental Complication	0.67 (0.36-1.25)	0.65 (0.34-1.23)	0.1874	1.39	0.91 (0.74-1.12)	0.83 (0.67-1.04)	0.0991	1.12	0.74 (0.40-1.37)	0.78 (0.41-1.49)	0.4525	1.39
Placenta Previa	0.70 (0.31-1.61)	0.63 (0.27-1.49)	0.2943	1.55	0.89 (0.67-1.18)	0.77 (0.57-1.05)	0.0952	1.17	0.79 (0.34-1.82)	0.81 (0.34-1.94)	0.6421	1.55
Placental Abruptio	0.65 (0.20-2.08)	0.74 (0.22-2.48)	0.6288	1.85	0.79 (0.54-1.17)	0.77 (0.50-1.18)	0.2240	1.25	0.82 (0.25-2.65)	0.97 (0.29-3.24)	0.9573	1.85
Placentally-Mediated Complications	0.63 (0.42-0.96)	0.67 (0.43-1.04)	0.0717	1.25	1.02 (0.89-1.16)	1.06 (0.91-1.22)	0.4700	1.08	0.62 (0.41-0.94)	0.64 (0.41-0.98)	0.0407	1.25

*Adjusted using inverse probability of treatment weights.

4.9 Figure

Figure 4.1 Flow diagram of study



4.10 Supplemental

Supplemental Table 4.A. Distribution of neonatal characteristics of study population, overall and by cryopreservation type*

Characteristic	Whole Population (n=9405) n(%)	Unweighted Population				
		Fresh (n=4969) n(%)	Slow-Freezing (n=340)	Standardized Difference†	Vitrification (n=4096)	Standardized Difference†
Neonatal Death						
Yes	69 (0.73%)	39 (0.78%)	<6	0.0671	29 (0.71%)	0.0090
Missing	<6§	<6	0 (0.00%)		0 (0.00%)	
Gestational Age, completed weeks				0.2159		0.2469
<28	214 (2.28%)	126 (2.54%)	<6		85 (2.08%)	
28-31	181 (1.92%)	115 (2.31%)	7 (2.06%)		59 (1.44%)	
32-36	1610 (17.12%)	942 (18.96%)	47 (13.82%)		621 (15.16%)	
37-39	5003 (53.20%)	2690 (54.14%)	192 (56.47%)		2121 (51.78%)	
40-41	2372 (25.22%)	1084 (21.825)	91 (26.76%)		1197 (29.22%)	
>42	24 (0.26%)	11 (0.22%)	0 (0.00%)		13 (0.32%)	
Missing	<6	<6	0 (0.00%)		0 (0.00%)	
Mean Birth Weight (g) (+/-Std.Dev.)	3077.31 (+/- 813.53)	2999.86 (+/- 880.09)	3213.81 (+/- 689.40)	0.2706	3160.03 (+/- 724.58)	0.1987
Median	3175.00	3100.00	3295.00		3259.50	
Missing	138 (1.47%)	70 (1.41%)	<6		64 (1.56%)	
Birth Weight Categories¶				0.2908		0.1724
VSGA (under 3 rd centile)	149 (1.58%)	95 (1.91%)	<6		52 (1.27%)	
SGA (under 10 th centile)	469 (4.99%)	293 (5.90%)	11 (3.24%)		165 (4.03%)	
LGA (90 th -96 th centile)	965 (10.26%)	436 (8.77%)	39 (11.47%)		490 (11.96%)	
VLGA (over 97 th centile)	610 (6.49%)	278 (5.59%)	29 (8.53%)		303 (7.40%)	
Unavailable (<24wks ga)	115 (1.22%)	22 (1.45%)	0 (0.00%)		43 (1.05%)	
Other (11-89 th centile)	6979 (74.21%)	3738 (75.23%)	255 (75.00%)		2986 (72.90%)	
Missing	118 (1.25%)	57 (1.15%)	<6		57 (1.39%)	

*Neonatal records are missing for 60 infants and are not included in the 9405 infants reported on here.

†Absolute standardized difference where a value >0.10 indicates an imbalance in the covariate distribution between assisted and non-assisted conceptions.

§Cells with fewer than six (6) records are suppressed (<6).

¶Birth weight categories are constructed in line with the INTERGROWTH-21 standards. VSGA – Very Small for Gestational Age. SGA – Small for Gestational Age. LGA – Large for Gestational Age. VLGA – Very Large for Gestational Age.

4.11 Bridge

Chapter 4 (Article 3) addressed the question of whether vitrification was associated with a decreased risk of adverse placental outcomes as compared to slow-freezing. We observed a pattern of decreased risk of placental and placentally-mediated complications with the use of slow-freezing as compared to vitrification. Although our risk estimates did not reach the level of statistical significance for all outcomes, that a consistent pattern of lower risk was observed with the use of slow-freezing compared with vitrification, and with the use of either method of cryopreservation as compared to fresh embryo transfer, suggests that factors other than error are at play.

In Chapter 5, I summarize and synthesize the key findings from the studies in Chapters 2-4 in the context of seeking to mitigate the current risks associated with assisted reproduction.

Chapter 5: Conclusion

In this dissertation, I have discussed the relationship between IVF and ICSI and adverse placental outcomes in pregnancy for Ontario, exploring the contributions of treatment factors, and, to a lesser extent, patient factors on the observed outcomes. To establish a baseline understanding of placental and placentally-mediated complications in Ontario, I report their respective incidence and risk in assisted and non-assisted conceptions (Chapter 2), treating IVF and ICSI as distinct treatment approaches. In Chapters 3 and 4, I build on Chapter 1 by isolating two critical treatment factors to begin unpacking their contribution to the observed increase in risk.

In this concluding chapter, I present the major findings for each study and their implications in an overarching context. I start by summarizing the significant conclusions of each article. I then discuss how the findings contribute to establishing a hierarchy of risk for the use of assisted reproductive technologies (ART) that can be used to guide research and policy development. Looking back on the historical context of ART in Canada, I address its impact on some of the trends we observed in this cohort that differ from other published cohorts and the opportunities this presents. Lastly, I consider the methodological contributions of this project and reflect on its contributions to the fields of assisted reproduction, epidemiology, and population health.

5.1 Summary of Major Findings

Overall, this dissertation research underscores the need for refinement in our understanding of the contribution of laboratory and clinical processes when assessing the impacts of assisted reproduction on perinatal outcomes, which here, are represented by placental and placentally-mediated complications.

Article 1, the foundational study, provides an understanding of the incidence and distribution of placental and placentally-mediated complications in Ontario conceptions overall and in assisted and non-assisted conceptions specifically, establishing a baseline understanding in this jurisdiction and setting a benchmark for future comparison. While we identified that both IVF and ICSI conceptions were at increased risk of all four outcomes of interest when compared with non-assisted conceptions, the most important finding is that conducting the analysis by treatment type (IVF or ICSI) exposures only was insufficient to adequately describe the risk profile of actual treatment practice, which is the use of IVF and ICSI followed by either a fresh or frozen embryo transfer. By disaggregating our IVF and ICSI exposure groups one step further by embryo transfer type, we were able to identify a clear pattern of significantly higher increased risks of any placental outcome and placenta previa specifically in fresh transfers. We were also able to identify that the observed increase in risk of placental abruption in ICSI cycles was primarily attributable to fresh cycles, while the observed increase in risk of placentally-mediated complications in IVF cycles was primarily attributable to frozen cycles. Such nuances are important in the weighing of risks associated with traditionally first (fresh) and subsequent (frozen) embryo transfer cycles.

The second most important finding is that while we observed increases in risk between IVF and ICSI transfers and non-assisted transfers, we did not observe significant differences in risk when comparing IVF and ICSI transfers, fresh or frozen, directly. This suggests that the factor(s) most responsible for the observed increase in risk are common to both treatment processes as the additional interventions in ICSI were insufficient to increase the risk significantly over IVF. Alternatively, these procedures may interact with other treatment and patient factors in such a manner as to balance out the increase in risk presented. With a shift in practice to ICSI as the

predominant treatment method, further investigation is warranted on treatment selection and pregnancy outcomes, which can now include placentally-mediated complications .

Consequently, this work demonstrates that a disaggregated analysis of perinatal outcomes in assisted conceptions is paramount not only to informing clinical practice but also to directing research and innovation at the underlying factors that may lead to the observed patterns of risk.

In the second article, we explore the first of two treatment factors hypothesized to contribute to the development of placental or placentally-mediated complications in assisted conceptions.

Building on the findings from the first article that disaggregating the analysis by fresh and frozen transfers provides valuable insight, we examined the impact of a Day 3 versus Day 5 embryo transfer in fresh cycles, and of a cleavage stage versus blastocyst transfer in frozen cycles.

Although we were limited by insufficient data to further disaggregate by treatment type, and we were unable to do a direct comparison of fresh to frozen cycles due to differences in data organization, we were able to identify that, at comparable stages of development in fresh and frozen transfers, the day or stage of embryo at transfer was not a major independent contributor to increased risks observed in the first article. While we cannot rule out the possibility that day or stage at transfer interacts with other patient or treatment factors in an unmeasured way to contribute to the increase in risk, this study raises no immediate concerns about embryo transfer practices in seeking to manage adverse placental outcomes.

The third article compares vitrification and slow-freezing as methods of cryopreservation to evaluate their respective contribution to the risk of adverse placental outcomes as compared to fresh transfers. Vitrification and slow-freezing are frequently examined under the umbrella of cryopreservation. Given the differences in increased risk observed for frozen IVF and ICSI cycles in our first article, cryopreservation warranted its own disaggregated examination. We

identified a consistent pattern of decreased risk of all outcomes associated with slow-frozen transfers as compared to both fresh and vitrified transfers. While our estimates were statistically non-significant, careful consideration of the nature of our data (low event counts for rare events) and the wide computed confidence intervals (generally indicative of a larger decreased risk of the outcomes than an increased risk) led us to conclude that while the risk estimates are reasonable, their statistical non-significance is likely the result of small numbers of events. Even so, the most salient finding from this study remains unchanged: despite the professed advantages of vitrified embryo transfer for assisted reproduction, we found that it offers no advantage with regards to placental complications of pregnancy. Further, the pattern of results and significantly increased risk of placentally-mediated complications as compared to slow-freezing create an imperative for larger scale studies to examine infrequent but potentially catastrophic outcomes for mother and baby. This is of rather urgent concern given the history of rapid adoption of technologies and procedures in ART combined with the increasing use of ART with vitrified embryos worldwide.

5.2 Promoting Innovation in Assisted Reproduction: Revisiting the Hierarchy of Risk

In Chapter 1, I introduced the argument for a hierarchy of risk and associated clinical treatment decision-making.⁽¹⁾ The ability to implement such an approach in clinical decision-making, as well as in research and policy planning, is undermined by four critical knowledge gaps, each of which this thesis strove to address.

5.2.1 Relative Contributions of Treatment and Patient Factors

In a treatment as complex as ART there are multiple potential sources of risk, which can be loosely grouped as stemming from treatment factors or patient factors. Assisted reproduction introduces a number of exogenous forces, potential iatrogenic harms, to an otherwise natural

process, and thus the impetus exists to minimize the risks introduced by these treatment factors. However, even non-assisted conceptions and pregnancies are not without complications. Patient characteristics, such as age, BMI, and preexisting conditions increase the risk of certain perinatal outcomes regardless of the mode of conception.(2–6) In assisted conceptions, we must also account for the contributions of causes and physiological states associated with infertility and subfertility.(7) Thus, while it can be tempting to ascribe risk to a single source or piece of the equation, it is significantly more likely that the increased risks of adverse outcomes associated with assisted reproduction are the result of independent contributions and interactions between treatment and patient factors.

ART treatment type and the type of embryo transfer cycle are two fundamental treatment factors whose potential to influence the risk of placental and placentally-mediated factors, among other perinatal outcomes, cannot be ignored. Both IVF and ICSI allow for natural selection barriers to be overridden and have been reported to induce epigenetic and physiological changes to gametes, embryos, and placentas.(8–21) Similarly, cryopreservation has been repeatedly found to do the same.(22–27) By using propensity scores to inform inverse probability of treatment weights (IPTWs) to adjust for confounding in our models, we have identified, with confidence, that both treatment type and embryo transfer type are prime contributors to the observed increase in risk.

The minimally adjusted model presented in Chapter 2/Article 1 allowed us to examine the potential impact of three key patient factors on the outcomes of interest: maternal age, maternal BMI, and parity. Despite this analysis deliberately employing an uncomplicated logistic regression model with administrative data, it suggests that maternal age, BMI, and parity may influence placental and placentally-mediated outcomes and warrant further investigation as to their independent contribution to the observed increases in risk.

Thus, this dissertation confirms that treatment factors contribute to the observed increased risks of adverse placental outcomes, identifies patient factors for further investigation, and determines that clusters of interactions of treatment factors require further investigation to help isolate the relative contributions to risk based on comprehensive treatment plans.

5.2.2 Independent and Interactive Contributions of Contemporary Treatments

Vitrification and Day 5/blastocyst embryo transfer (extended culture) are newer procedures characterizing contemporary IVF or ICSI treatment. As we identified prior to commencing our work, there is insufficient evidence available here to confidently identify the independent contributions of either procedure on perinatal outcomes. Rather than focusing on vitrification specifically, much of the research focuses on cryopreservation generally, grouping together the effects of slow-freezing and vitrification. As the day or stage of embryo at transfer has, in part, been a function of the innovation and success of other clinical and laboratory innovations, research is also limited in this area. In this thesis, I have investigated the independent contributions of vitrification and Day 5 embryo transfer after adjusting for a number of patient and treatment factors in order to set a baseline understanding before examining any interactive effects of these contemporary treatments.

Compared to slow-freezing, vitrification poses a greater risk of adverse placental outcomes in cryopreserved embryos and may more closely resemble the outcome of a fresh transfer. With the shift to vitrification as the cryopreservation method of choice, the mere possibility that it could be putting patients using cryopreserved embryos at increased risk of severe complications behooves scientists to assess its impacts in a larger population study to better elucidate the consistent but non-significant patterns of risk observed in Chapter 4 (Article 3).

Day or stage of embryo at transfer in fresh and frozen cycles, respectively, was not found to considerably contribute to the outcomes of interest in the Ontario data as an independent treatment factor. Further research is required to unpack any interactive contributions with other prominent treatment and patient factors.

5.2.3 Contribution of Clinical and Laboratory Innovations

The database used in this research, CARTR Plus, contains detailed information on assisted reproduction treatment from ovulation induction to confirmation of pregnancy and general pregnancy outcomes. This detailed record of fertility treatment allowed us to account for variation in clinical practices in our analysis, most notably the number of embryos transferred. The transfer of two or more embryos is associated with a higher rate of twinning or multiple gestations, which is in turn associated with an increased risk of perinatal (28–30) and placental complications specifically (31,32), even in non-assisted conceptions. In our baseline analysis, we examine singleton pregnancies on their own based on the number of fetal heartbeats confirmed on ultrasound. Our approach does not eliminate the possibility of placental development impacted by vanishing twin syndrome (VTS), as we were not able to identify affected pregnancies. Since the relationship between VTS and placental disorders is not clear, and an estimated 10% of assisted singleton pregnancies originated as twin gestations (33), we do not anticipate that the impact of this source of risk was large. In addition to the number of embryos transferred, we were also able to account for the type of ovulation induction protocol, though this may be more of a reflection of clinical preference and underlying infertility. Neither of these innovations were explored as exposures but being able to account for them in our analyses strengthened our examination of the selected treatment factors.

A significant drawback to CARTR Plus is that it is clinically oriented to monitor success rates, so much of the laboratory data is not included. Consequently, we were unable to account for different slow freezing and vitrification protocols and culture media composition and practices. However, as it is infeasible to account for all clinical and laboratory innovations in all analyses, it is reasonable for this not to have been covered by this thesis. Therefore, I will take this opportunity to echo calls for further research within the larger literature.

5.2.4 Changes in the Patient Population Over Time

The population of those using ART has changed drastically over time based on eligibility criteria, clinical practices, and the balance of public funding available and private funding required to cover the costs of treatment. The earliest data available in BORN dates to 2012, while the earliest CARTR Plus data available dates to 2013. Our study period includes one year during which the Ontario Fertility Program (OFP) was in place. Given the relatively short and recent study period, the studies included in this thesis do not capture major changes to the population over time, nor does the single year of cycle start data from 2016 allow us to identify changes to the patient population attributable to the OFP, especially in light of the implementation challenges faced in the first year of the program, such as determining how to set a waitlist.(34) However, the studies presented here do reflect the breadth of the contemporary patient population, with biological and social infertility reflected.

5.3 The Ontario Cohort

Leading up to and throughout most of the study period, access to assisted reproduction in Ontario was primarily limited to those who could afford to make the decision to pay for treatment out of pocket. From 1994 to late 2015, conventional IVF was publicly funded only in cases of bilateral

fallopian tube blockage, and IUI was funded save for the costs of drugs and sperm preparation. This funding practice stems from bilateral fallopian tube blockage being the only strongly evidence-based indication for IVF in the 1980s, and indeed, the basis for the invention of IVF by Edwards. Unfortunately, it represented approximately one-fifth of the need for infertility treatment (35), meaning that few women qualified for publicly funded treatment as the evidence-based indications for IVF grew. The high cost of treatment relative to pregnancy rates incentivized clinicians and patients to engage in double or multiple embryo transfer to increase their chances of success in fewer rounds.(36) Although recommendations and non-binding practice guidelines encouraged the use of single embryo transfer prior to the introduction of the OFP (37), and although the OFP mandates single embryo transfer in women of certain ages or prognosis, with some exceptions (38), we still see a high percentage of cycles using double or multiple embryo transfer when compared to published cohorts or whole-of-country reported data from other jurisdictions (primarily Australia/New Zealand and the Nordic countries) most of whom have regulated embryo transfer in some way.(39–43)

An internal review of elective single embryo transfer (eSET) at one hospital-based fertility clinic offering publicly funded cycles compared two years of cycle starts before and after the introduction of the OFP. They found that rates of both eSET and single embryo transfer (SET) increased significantly after the introduction of public funding and rates of double or multiple embryo transfer dropped significantly.(38) Furthermore, the rates of Day 3 embryo transfer dropped significantly, and the rates of Day 5 embryo transfer increased significantly as embryo stage at transfer was tied to rules around the number of embryos transferred.(38) There is no evidence to confirm whether or not the same trends have been observed across all clinics, but even so, the impact at a provincial level will depend on the proportion of cycles that are publicly

funded. However, an increase in SET over all four years of data underlying this dissertation was observed, revealing efforts to meet previously suggested policy and practice goals.(38)

In addition to transferring more embryos, the Ontario cohort consists of a greater proportion of ICSI cycles, and the patients seem to be slightly older than in other published cohorts (39,44), including those with samples drawn around the same time.(39,40,44,45) It is unclear why the relative proportions of conventional IVF and ICSI are more disproportionate in our sample, but there has been a trend towards the use of ICSI as treatment method of choice. As we conduct our analysis by treatment type or accounting for treatment type, this should not drastically impact the comparability of our findings to others.

Otherwise, the Ontario cohort appears to be healthier than others (based on a comparison of incidence of baseline characteristics, though no tests of significance were performed). The incidence of preexisting diabetes (45,46), preexisting hypertension (46), smoking (39,44), asthma (45), epilepsy (45), and anemia (45) are lower or, less often, comparable (47) in the Ontario cohort. Whether this is the result of a difference in our treatment-seeking population, a difference in practices and patterns in clinical decision-making, or a combination thereof is unclear. In a survey of waitlist management practices in 22/25 IVF clinics offering OFP-funded cycles in 2016, older age, being close to losing eligibility for funding, and longer duration of infertility were among the factors used to prioritize and manage waitlists for publicly funded cycles.(34) Two clinics, respectively, indicated that they also prioritized patients with greater chances of success or those without lifestyle factors that decrease the likelihood of success.(34) As this is self-report data, we cannot be sure that such practices were not in place at clinics that chose not to disclose this information, nor can we be sure about the extent to which physician selection of patients impacts privately-funded cycles. However, it is likely that our ART patient

population is influenced by exogenous factors as there are no clear clinical or epidemiological explanations for them appearing healthier than non-assisted pregnancies within the province, let alone as compared to cohorts from other jurisdictions.

5.4 Methodological Contributions

To date, this dissertation is one of a handful of population-based studies focused on placental and placentally-mediated complications in pregnancy following assisted reproduction. Historically, studies of assisted reproduction outcomes focused overwhelmingly on implantation, clinical pregnancy, ongoing pregnancy and live birth rates, or a suite of perinatal outcomes, which may among them include placental outcomes. While these studies are critical to the evidence base for ART, they are not designed to adjust for confounders of placental complications specifically. Targeted studies, such as those presented in this dissertation, form an important empirical basis for identifying factors amenable to modification to improve health outcomes at the population level through both innovation and the development of relevant policies and practices to minimize risks.

My dissertation research provides a comprehensive investigation of adverse placental outcomes that accounts for all assisted conceptions in Ontario during the study period through the linkage of health administrative databases. Specifically, the use of CARTR Plus in all three studies represents one of the first uses of the CFAS database linked to BORN's registry.

CARTR/CARTR Plus is one of few comprehensive databases focused specifically on assisted reproduction internationally, and work with it, such as this dissertation, demonstrates its validity in general comparability to other population registries. It is hoped that these studies promote the use of this relatively underused data source.

5.5 Contributions to the Field Thus Far

As of writing, the three articles included in this thesis had not yet been published but are prepared for submission to the journals identified on the cover pages (New England Journal of Medicine and Human Reproduction). In February 2020, I presented preliminary findings from Article 1 at the Canadian National Perinatal Research Meeting in Banff, Alberta. An abstract was also submitted for the Society for the Study of Reproduction 53rd Annual Meeting, planned for July 2020, but current world events altered the Meeting's structure and thus presenting was no longer an option.

5.6 Future Research and Actions

This thesis sets a comprehensive baseline for our understanding of placental complications in assisted reproduction in Ontario, while also contributing to the growing literature on placental and perinatal outcomes associated with contemporary ART treatments. In this section, I will outline some recommendations for moving forward in order to productively advance our understanding of the risks associated with ART while specifically focusing our attention on some of the more pressing knowledge gaps. These are described below and include a longer study period and strengthened data registry at the provincial/national level, as well as international standards to facilitate meta-analyses and internationally agreed upon standards of evidence to inform the uptake of new procedures.

5.6.1 Longer Study Period

This thesis has examined the outcomes of IVF and ICSI cycles initiated between 1 January 2013 and 31 December 2016, which was all the data available when the data request was first made to BORN. While Ontario has a high annual conception volume and accounts for a large portion of

Canada's ART treatment cycles, placental complications in pregnancy are rare and thus a much larger sample is needed to better study these outcomes, particularly as individual outcomes such as placental abruption and placenta accreta/increta/percreta. As previously discussed, the linkage between BORN and CARTR began in 2013 and is currently the only way in which provincial health administrative data is linked to ART treatment records. The relative newness of this linkage could also be offset by other provincial health administrative data registries being linked to the CARTR data for the clinics in their province, allowing us to take advantage of a greater number of our historical Canadian cycles.

5.6.2 Strengthened Data Registry

The CARTR and CARTR Plus registries present a valuable wealth of data on ART treatment from oocyte harvest through to embryo transfer and transfer outcomes. The data currently collected allows us to examine several treatment and patient factors, many of which were used in this thesis project. However, compared to data from other jurisdictions – most notably Australia, CARTR is limited with respect to the data shared by fertility clinics. Currently, it does not include information on ovulation stimulation drugs, oocyte and sperm retrieval procedures, culture media composition, slow-freezing and vitrification protocols, embryo staging for fresh embryo transfers, embryo morphology and quality, and embryo transfer method and placement. Some of these treatment factors have previously been linked with changes in embryo or pregnancy development and outcomes (48–52), but all require further investigation until we can identify the individual and interactive contributions of treatment factors to observed increases in risk.(53) In addition, given the impact of public funding and regulation on ART treatment profiles, it would be beneficial for publicly and privately funded cycles to be identified as such. Strengthening our existing registry would not only strengthen domestic research, but it would

improve comparability with and facilitate linkage with other well-developed fertility registries.

5.6.3 International Standards to Facilitate Meta-Analyses

One of the difficulties faced in comparing the results of this thesis project to the literature was navigating the variations in the definitions and reporting of treatment factors and adverse outcomes. This may limit the ability to perform precise meta-analyses, research that will be necessary to help confirm the risks of rare perinatal outcomes.

5.6.4 Internationally Agreed Upon Standards of Evidence to Inform the Uptake of New Technologies

Much of the history of assisted reproduction treatment can be characterized by the rapid adoption of new technologies with limited information as to their long-term effects and safety. As previously discussed, new technologies and procedures that are shown to improve implantation, clinical pregnancy, ongoing pregnancy, and live birth rates have historically been adopted rapidly with the hope of offering a better success rate to patients relying on ART. Be that as it may, the rapid adoption of technologies and procedures – or components thereof – without sufficient evidence has proved catastrophic in the past, as multiple embryo transfer comes to mind. Antenatal and postpartum hemorrhage, as well as hypertensive disorders in pregnancy, are unquestionably dangerous, and the adoption of techniques that may be putting a greater number of patients and offspring at risk is ethically dubious. If patients, scientists, clinicians, and other key stakeholders could agree to a series of stages of evidence required to introduce, scale-up and maintain use of ART innovations in humans, we could better balance innovation for treatment improvement and mitigating unknown risk. In combination with initiating treatment with the least invasive option, this might also allow us to manage the pace of innovation adoption within

ART with an emphasis on conservative evidence bases.

5.7 Reflections on Population Health

Coming into the Population Health PhD program, I knew that I wanted to challenge myself with respect to research methods without sacrificing interdisciplinarity as a basis for examining and taking action to ameliorate population health challenges. To further develop my quantitative research skills, I chose to undertake a heavily quantitative epidemiology research program, paired with a policy analysis approach to comprehensively examine outcomes associated with the use of assisted reproductive technologies in Ontario and the way in which policies shaped the observed outcomes. I had originally planned for this to come together in an evaluation of the impacts of the OFP on access to assisted reproduction and patient profiles after its introduction in late 2015. The goal was to assess the government's ability to provide this family-building option and health service in accordance with the Canada Health Act, the Canadian Charter of Rights and Freedoms, and the Ontario Human Rights Code, specifically its ability to address the documented inequitable distribution of barriers to access that existed prior to its introduction. Such an evaluation would have been a critical piece of approaching infertility from a population health perspective.

However, in attempting to undertake this analysis, a lack of data quickly posed a challenge. Income, geography and employment (i.e. benefit plan coverage, time off work) were identified as significant barriers to ART access in Ontario prior to the program's introduction.⁽³⁵⁾ Unlike other jurisdictions, such as Australia, which collect individual-level socioeconomic data for those who are pregnant, this information is not available in Ontario. Given that racialized Canadians are overrepresented at the lower end of the income distribution ⁽⁵⁴⁾ I had planned to assess the

racial and ethnic distribution of patients as well. However, we do not collect race-disaggregated data in health in Canada. Although census tract level data on average income, education level, and diversity were available to be used in conjunction with individual-level health administrative data, the risk of committing an ecological fallacy remained in interpreting the results of the evaluation.

Simply put, research and action in population health cannot move forward if we are not collecting the data requisite for understanding the issue at hand for all members of the population along dimensions of their social identities that may interact with and impact their experience of the issue. Similar to the limitations in the available fertility data, Canada is lagging behind in its collection of individual-level data in health, such as income, occupation and race-disaggregated data which allow for more nuanced analyses as a jumping off point for further population health research. We cannot in good conscience continue in this direction.

5.8 Overall Conclusion

This dissertation project has investigated the nature of the relationship between assisted reproduction and placental and placentally-mediated outcomes and the independent contributions of two treatment factors that are amenable to change through technological innovation and policy. The findings of the analyses contained herein indicate that ART treatment does increase the risk of placental and placentally-mediated outcomes relative to non-assisted conceptions, though the precise treatment factors or combinations thereof with patient factors creating that increased risk remain unclear. While this project has provided valuable insights to its central research questions, it reflects outcomes over a relatively short period of time and in a single jurisdiction. It is essential that these outcomes continue to be monitored as the use of assisted

reproduction increases and technology evolves. Continued sharing of fertility treatment data and linkage to health administrative data is critical to this effort. Future research stemming from this dissertation's research program will hopefully continue to build our knowledge of the relationship between assisted reproduction and adverse placental outcomes, specifically the independent and interactive contributions of treatment and patient factors, in order to improve outcomes, including safety, for those who rely on ART to build a family.

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Appendix 1

Appendix 1.1 Children's Hospital of Eastern Ontario Research Ethics Board Approval



CHEO Research Ethics Board Approval - Delegated Review

Principal Investigator: Dr. Mark Walker
REB Protocol No: 19/04PE
Romeo File No: 20190185
Project Title: CHEOREB# 19/04PE - Identifying Modifiable Factors to Improve Access to and Perinatal Outcomes of Assisted Conception in Ontario
Primary Affiliation: Clinical Research/Epidemiology
Protocol Status: Active
Approval Date*: March 26, 2019
Approval Expiry Date**: March 15, 2020

Documents Reviewed & Approved:

Document Name	Comments	Version Date
Protocol	Protocol	2019/03/13
Other Document	Required data fields and databases	2019/03/13

This is to notify you that the Children's Hospital of Eastern Ontario Research Ethics Board has granted approval to the above named research study on the date noted above. Your project was reviewed within the delegated stream, which is reserved for projects that involve no more than minimal risk to human participants.

Final approval is granted for the above noted study, with the understanding that the investigator agrees to comply with the following requirements:

1. The investigator must conduct the study in compliance with the protocol and any additional conditions set out by the Board.
2. The investigator is responsible for complying with all applicable guidelines and regulations regarding the ethical conduct of research with humans, as applicable to the research project.
3. Approval for studies that include an investigational device(s) is contingent upon the investigator securing an Investigational Testing Authorization notice from Health Canada.
4. Investigators must obtain annual renewal approval prior to the expiration date stated above.
5. The investigator must not implement any deviation from, or changes to, the protocol, consents or assents without the approval of the REB except where necessary to eliminate hazard to the research subject, or when the change involves only logistical or administrative aspects of the study (e.g., change of telephone number or research staff). As soon as possible, however, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted to the Board for review and approval.
6. The investigator must, prior to use, obtain approval from the Board for changes to the study documentation, e.g., changes to the informed consent letters, recruitment materials.
7. Investigators must obtain approval from the Board of French version(s) of the consent/assent form(s), unless a waiver has been granted. An interpreter should be offered to participants as required or at the request of the participant throughout the course of research.
8. The investigator must promptly report to the REB all unexpected and untoward occurrences (including the loss or theft of study data and other such privacy breaches).
9. Investigators must notify the REB of any study closures (closed to accrual, temporary, premature or permanent).
10. Investigators must submit a study closure event form at the conclusion of the study.

Should you have any questions or concerns, please do not hesitate to contact the Research Ethics Board Office at 613-737-7600 ext. 3350 or 2128.

Regards,

Richard Carpentier, PhD
Chair, Research Ethics Board
Président, Comité d'éthique de la recherche

* The final approval date for initial delegated study applications approved with or without modifications will be the date the REB has determined that the conditions of approval have been satisfied.

Appendix 1.2 Ottawa Health Science Network Research Ethics Board Approval



March 25, 2019

Dr. Steven Hawken

ICES uOttawa The Ottawa Hospital, Civic Campus 1053 Carling Ave., Box 684 Ottawa, ON K1Y 4E9

Re: OHRI Institutional Approval for Ottawa Health Science Network Research Ethics Board (OHSN-REB) Submission

20190170-01H;

Identifying Modifiable Factors to Improve Access to and Perinatal Outcomes of Assisted Conception in Ontario

Dear Dr. Steven Hawken,

This letter serves as **Ottawa Hospital Research Institute (OHRI)** Institutional Approval for the above-referenced study. Please maintain this documentation in your investigator study file.

Based on the information you provided about this study through the Clinical Research Registration Form, you have satisfied the requirements for institutional (OHRI) approval. This includes initial research ethics approval by OHSN-REB, appropriate departmental/service area notifications and execution (fully signed versions) of all agreement(s) required to begin the study locally. Please note there may be additional agreement(s) pending execution that are required to send funds, samples, or data to external sites, but are not required for you to begin your study locally.

Changes and/or additions to your study that may require additional agreement(s) or revisions to existing agreement(s) must be communicated to the OHRI Contracts Office. This should be undertaken simultaneously with any related OHSN-REB amendment submission.

Changes and/or additions to your study that affect various hospital/institution departments (e.g., pharmacy, Department of Medical Imaging, EORLA, EEG, etc.) must be communicated to the relevant departments.

As mentioned in the 'Response' tab of the Ethics application, you have 3 months from the date of initial OHSN-REB approval to submit French documents including the translation certificate to OHSN-REB through the Translated Documents section of the ethics application (if applicable).

Should you have any questions, please contact REBadministration@ohri.ca or 613-798-5555 extension 16719.

Nancy Camack, BScN RN MBA CCRP
Director, Clinical Research Administration
Ottawa Hospital Research Institute | Institut de recherche de l'Hôpital d'Ottawa

Civic Campus, Box 675, 725 Parkdale Avenue, Ottawa, Ontario, K1Y 4E9
613-798-5555 extension 16719 Fax : 613-761-4311 <http://www.ohri.ca/ohsn-reb>

Appendix 1.3 University of Ottawa Research Ethics Board Approval

25/04/2019

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

Lettre d'approbation administrative | Letter of administrative approval

Numéro de dossier / Ethics File Number
Titre du projet / Project Title

H-04-19-3869
Identifying Modifiable Factors to
Improve Access to and Perinatal
Outcomes of Assisted
Conception in Ontario

Type de projet / Project Type

Thèse de doctorat / Doctoral
thesis

CÉR primaire / Primary REB

Réseau de science de la santé
d'Ottawa (RSSO) / Ottawa
Health Science Network (OHSN)

Statut du projet / Project Status

Approuvé / Approved

Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)

25/04/2019

Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)

15/03/2020

Équipe de recherche / Research Team

Chercheur / Researcher	Affiliation	Role
Brianne OLIBRIS	École interdisciplinaire des sciences de la santé / Interdisciplinary School of Health Sciences	Chercheur Principal / Principal Investigator
Amir ATTARAN	Faculté de droit - Section de common law / Faculty of Law - Common Law Section	Superviseur / Supervisor
Steven HAWKEN	Département d'épidémiologie et santé publique / Department of Epidemiology and Public Health	Co-superviseur / Co-supervisor
Mark WALKER	TOH	Chercheur principal - site d'examen primaire / Primary review site PI
Karen PHILLIPS	École interdisciplinaire des sciences de la santé / Interdisciplinary School of Health Sciences	Collaborateur / Collaborator

Conditions spéciales ou commentaires / Special conditions or comments:

CHEO REB Protocol No: 19/04PE (Romeo File No: 20190185)

OHSN REB #: 20190170-01H

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