Pregnancy and neonatal outcomes associated with the use of assisted reproductive technologies

Andrea Lanes

A thesis submitted to the University of Ottawa in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Epidemiology

School of Epidemiology, Public Health and Preventive Medicine
Faculty of Medicine
University of Ottawa

Abstract

Assisted reproductive technologies have become a common method used to treat infertility. These techniques have advanced quickly since the first birth of an in vitro fertilization (IVF) baby in 1978, at the Royal Oldham Hospital in the United Kingdom. Currently, IVF with or without intracytoplasmic sperm injection, is used throughout the world to achieve oocyte retrieval, fertilization, implantation of an embryo, clinical pregnancy, ongoing clinical pregnancy, and a live-born infant. The rationale for selecting one type of fertility treatment over another is multifactorial: the confirmed or unconfirmed cause of infertility, the age of the gamete donor and the recipient, the availability of the type of treatment, and the cost associated with the treatment. The ultimate goal of any fertility treatment is to achieve a successful pregnancy that results in a healthy infant. However, the literature is equivocal on the effects of fertility treatment cycles on the health outcomes of infants and mothers.

Presently, there are thirty-six fertility treatment centres across Canada, eighteen of which reside in Ontario. A national, comprehensive database of assisted reproductive technology treatment cycles (Canadian Assisted Reproductive Technologies Register (CARTR) Plus) began collecting data in 2013, and has made the research objectives of this doctoral thesis feasible. Before this data collection system, population-wide studies involving fertility treatments were not possible in Canada. Two understudied issues associated with IVF are the impact of fertility treatments on the maternal serum screening markers used in prenatal screening programs to identify fetal aneuploidies; and the association between fertility treatments and adverse perinatal outcomes, such as preeclampsia and stillbirth. Given the increasing number of women who are using fertility treatments to conceive, it is imperative that studies investigating the association with adverse outcomes are conducted.

As the science supporting fertility treatment procedure has advanced, so has prenatal screening. One of the first screening tests that are performed for newly pregnant women, including women who conceived following IVF, is maternal serum screening. The first objective of this doctoral thesis was to systematically review the literature on the association between IVF treatment and maternal serum screening marker levels and nuchal translucency (NT) thickness. After the search and screening of the literature there were 40 studies that were included in this

systematic review. A decrease in pregnancy-associated plasma protein A (PAPP-A) and an increase in total human chorionic gonadotropin (hCG) was consistently reported for IVF pregnancies. However, since the levels of the other maternal serum screening markers reported also varied we were unable to generalize about the differences between prenatal screening results in the IVF population. These results led to investigating maternal serum screening marker levels among IVF patients in Ontario, Canada.

The second objective of this thesis was three-fold: 1) to investigate the accuracy of IVF identification on the Ontario prenatal screening record, relative to reference standard on the CARTR Plus database; 2) to compare the prenatal screening markers in IVF versus non-IVF pregnancies in the population of Ontario; and 3) to propose updated IVF adjustment factors for prenatal screening in the Ontario population, based on the more accurate coding for IVF status in the CARTR Plus database. Significant differences between IVF and non-IVF groups, based on both the prenatal screening requisition information and CARTR Plus information, were found among the ethnicity adjusted mean multiple of the median (MoM)s for several prenatal screening markers: alpha-fetoprotein (AFP), PAPP-A, unconjugated estriol (µE3), first trimester hCG, total hCG, and dimeric inhibin A (DIA). When we developed the proposed adjustment factors for all CARTR Plus identified pregnancies we found that for PAPP-A, total hCG, and µE3 the mean adjusted marker MoMs were significantly closer to 1.00, as compared to the prenatal screening adjusted or the unadjusted mean marker MoMs. Currently, there is no adjustment made to the other maternal serum screening markers and NT measurement.

The third objective was to examine the effect of type of infertility on placental-mediated adverse outcomes (preeclampsia, intrauterine growth restriction, placental abruption, and stillbirth). Type of infertility was classified as male factor (sperm count, poor sperm motility, and abnormal sperm morphology), female factor (ovulation disorders, tubal infertility, and uterine or cervical causes), and unexplained infertility. No significant associations were found between type of conception and the composite outcome, as well as each individual primary outcome. Similarly, the type of infertility was not associated with the composite outcome or any of the individual primary outcomes, except for female factor infertility, which was associated with increased probability of placental abruption.

Overall, the results from this doctoral thesis suggest that there are substantial differences seen in maternal serum screening marker MoMs among women who use IVF to conceive, suggesting that appropriate adjustment factors should be employed to ensure accurate results for determining the risk of Down syndrome and trisomy 18. Additionally, although the literature has shown an association between fertility treatment and placental-mediated adverse outcomes no significant associations were found in the population of Ontario. Further studies should be performed to confirm the results of these observational studies.

Acknowledgements

My doctoral training and research was made possible through the support of countless individuals. My PhD supervisor, Dr. Mark Walker, provided me with clinical guidance, financial support and continuous encouragement in my abilities. He provided me with multiple opportunities to present my research around the world, as well as furthering my education. My thesis advisory committee was indispensible to completing my dissertation. Drs. Ann Sprague, Art Leader and Beth Potter each provided me with essential insight. The complement of their expertise provided me with impeccable guidance.

I was fortunate to have the support of BORN Ontario and the OMNI Research Group throughout my degree. Thank you to Dr. Sandy Dunn for the opportunity to work on projects that were out of my scope and to Ruth Rennicks White for entrusting the OMNI Summer Research Program with me. I had the pleasure of sharing an office with Shelley Dougan. Her clinical knowledge of prenatal screening proved to be extremely helpful. However, it is our mutual love of sarcasm and coffee that sustains our friendship. The daily support from Natalie Rybak went far beyond working together. Thank you for always providing your perspective. I'm extremely grateful to call you a friend.

How fortuitous it was for me to assist with a systematic review for Dr. Deshayne Fell's PhD thesis, which led me to an amazing friendship and the most valuable mentorship. You have guided me throughout this degree. Thank you for always letting me talk through my thought process, whether it was in the office or in the car, and for always providing me with useful feedback. You are an honorary member of my thesis advisory committee. Your graduate students will greatly benefit from getting to work with and learn from you, as I have.

Anne Tsampalieros and I bonded in the first year of the PhD program through learning about managing large databases and SAS, but years later it is the compassion and comprehension about the daily process of completing this degree that I most appreciate.

Nicole Tishler and Jennifer Olechowski are two friends whose academic interests are vastly different, yet they were always there to support me, push me and celebrate every small achievement. The value of having friends that you can talk about anything with is irreplaceable.

Most importantly Mom and Dad, thank you for engraining the importance and enjoyment of education from an early age. You have always supported me unconditionally and guided me in the right direction. My work-ethic and determination can be attributed to the values that you instilled in me and have led me to the completion of this doctoral dissertation.

Contribution of Authors

Manuscript 1: Andrea Lanes, Tianhua Huang, Ann E. Sprague, Arthur Leader, Beth Potter, Mark Walker

Maternal serum screening markers and nuchal translucency measurements among in vitro fertilization pregnancies: a systematic review

Fertility and Sterility 2016; 106(Nov);6: 1463-1469.

I created the study protocol, developed the search strategy, conducted the search of the literature, did the primary and secondary screen of the articles, extracted data from the included articles, analyzed and interpreted these data, drafted and edited the manuscript. T. Huang performed the primary and secondary screen of the articles, extracted data from included articles and edited the manuscript. A.E. Sprague, A. Leader, and B. Potter assisted with interpreting the results and editing the manuscript. M. Walker assisted with the development of the study protocol, interpretation of results and editing of the manuscript.

Manuscript 2: Andrea Lanes, Shelley Dougan, Deshayne B. Fell, Tianhua Huang, Ann E. Sprague, Moya Johnson, Arthur Leader, Beth Potter, Nan Okun, Mark Walker

Comparing maternal serum screening markers among IVF and spontaneous conceptions in Ontario through registry data

Submitted to the Journal of Medical Screening

I created the study protocol and developed the analysis plan with consultation from S. Dougan and D.B. Fell. I conducted all analyses, interpreted the results and wrote the manuscript. S. Dougan, D.B. Fell, T. Huang, A.E. Sprague, A. Leader, B. Potter, and N. Okun assisted with interpreting the results and editing the manuscript. M. Walker assisted with the development of the study protocol, interpretation of results and editing of the manuscript.

Manuscript 3: Andrea Lanes, Ann E. Sprague, Arthur Leader, Beth Potter, Mark Walker
The effect of the type of infertility on placental-mediated adverse outcomes for patients that used IVF
Submitted to Fertility and Sterility

I created the study protocol and developed the analysis plan with consultation M. Walker. I conducted all analyses, interpreted the results and wrote the manuscript. A.E. Sprague, A. Leader, and B. Potter assisted with interpreting the results and editing the manuscript. M. Walker assisted with the development of the study protocol, interpretation of results and editing of the manuscript.

Statement of Originality

This statement stipulates that all of the work in this doctoral thesis provides additional original evidence regarding the role of in vitro fertilization treatment as a potential contributor to placental-mediated adverse outcomes, as well as the effect of in vitro fertilization use on the markers used in of maternal serum screening for predicting the risk of Down syndrome and trisomy 18.

The first manuscript was a systematic review that describes published studies examining the association between in vitro fertilization and maternal serum screening markers used for prenatal screening. The results from that study demonstrated trends among the different maternal serum screening markers; however the more impactful finding was that there is little consistency when reporting maternal serum marker multiples of the median (MoM) in the literature. Studies report either the mean or the median, which precluded the ability to meta-analyze these data.

Manuscript 2 quantitatively investigated the landscape of maternal serum screening among women who used in vitro fertilization to conceive in Ontario, Canada. Differences observed in Ontario aligned with the trends observed in Manuscript 1. Manuscript 2 also proposed new adjustment factors for each maternal serum screening marker when in vitro fertilization was used to conceive. These adjustments generate a more appropriate measurement of risk for Down syndrome or trisomy 18 among pregnant women in the population of Ontario who have received IVF.

The last manuscript adds to the current literature on the influence of in vitro fertilization and placental-mediated adverse outcomes. Again, specific to the Ontario population of conceptions and births, this study was carried out using all births conceived through in vitro fertilization or spontaneously.

I state that all of the work in this doctoral thesis, from commencement to completion, was my own. My PhD supervisor and thesis committee members provided superb guidance on the methodological aspects and clinical relevance of each study.

Table of Contents

List of Tables

List of Figures

List of Acronyms and Abbreviations

AFP	Alpha-fetoprotein
ART	Assisted reproductive technologies
BORN Ontario	Better Outcomes Registry & Network Ontario
CARTR Plus	Canadian Assisted Reproductive Technologies Register Plus
CI	Confidence interval
CIHI	Canadian Institute for Health Information
DIA	Dimeric inhibin A
FET	Frozen embryo transfer
FTS	First Trimester Combined Screening
free β-hCG	Free beta- human chorionic gonadotropin
GA	Gestational age
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilization
IPS	Integrated Prenatal Screening
MSS	Maternal serum screening
NICU	Neonatal intensive care unit
NT	Nuchal translucency
PAPP-A	Pregnancy-associate plasma protein A
PCOS	Polycystic ovarian syndrome
PGD	Preimplantation genetic diagnosis
PGS	Preimplantation genetic screening
RR	Relative risk
SIPS	Serum Integrated Prenatal Screening
Total hCG	Total human chorionic gonadotropin
μE^3	Unconjugated estriol

Chapter 1. Prologue

1.1 Background

The use of assisted reproductive technologies (ART) has been increasing globally and accounts for approximately five million births worldwide[1]. All of the technologies available in this field (e.g., in vitro fertilization, intrauterine insemination, donor gametes and frozen embryos) have progressed rapidly and this has resulted in remarkable advances in a relatively short timeframe. These developments have had positive impacts on subfertile couples with the caveat of more complex pregnancies. Babies that were conceived with ART may have increased risks for antenatal and postpartum complications and as a result may more often require greater medical attention.[2,3]

Research on the effects of these therapies has shown higher rates of placental-mediated adverse outcomes among ART pregnancies; however the etiology of the increased rate of adverse outcomes is uncertain.[4,5] This doctoral thesis investigated the effect of the type of infertility on placental-mediated adverse outcomes for patients that used fertility treatment and determined if and how maternal serum screening (MSS) markers are different in women that used in vitro fertilization (IVF) compared to women who conceived spontaneously, since changes in the serum screening markers may increase the rate of false-positive screens ascertained[6]. Given the increasing number of women who are using fertility treatments to conceive, it is imperative that studies investigating the use of maternal serum screening markers and the association with adverse outcomes are conducted. Until recently, population-wide studies involving fertility treatments were not possible in Canada.

1.2 Research objectives

The objective of this doctoral thesis was to investigate IVF use within Ontario with respect to its impact on measured maternal screening markers used in prenatal screening and its association with placental-mediated adverse outcomes. The three specific objectives were:

i. To perform a systematic review on maternal serum screening markers in women who used IVF to become pregnant (**Manuscript 1**).

ii. To investigate how maternal serum screening markers are different in women who used IVF with or without ICSI compared to women who conceived spontaneously in the population of Ontario (**Manuscript 2**).

iii. To determine the effect of the type of infertility on placental-mediated adverse outcomes for patients that used IVF with or without ICSI (**Manuscript 3**).

1.3 Rationale

Understanding differences that occur among potential adverse pregnancy and neonatal outcomes among the IVF population is important for assessing the risks and planning the appropriate path of clinical care. The first manuscript of this dissertation aimed to identify differences that were present in MSS markers and nuchal translucency thickness among the IVF population as compared to spontaneous conception. Acknowledging variations in MSS marker levels among IVF pregnancies, some of which have a substantive clinical hypothesis to support the differences and others which are consistently observed, but no causal link has been established, would provide a foundation for whether these differences persist in multiple populations. The results of Manuscript 1 directly lead into the rationale for Manuscript 2. Given that globally there were differences observed in MSS markers and nuchal translucency thickness, I aimed to investigate whether these differences were present in the population of Ontario. Additionally, I investigated if accurate identification of IVF conceptions were occurring with the current method of reporting (prenatal screening record), as compared to the new benchmark (Canadian Assisted Reproductive Technologies Register (CARTR) Plus). If the accuracy of identifying IVF conceptions was poor, MSS marker adjustments were inappropriately applied. This led to the rationale for the third objective of Manuscript 2, proposing new adjustment factors for MSS markers and nuchal translucency thickness that are appropriate for the Ontario population. The first two manuscripts involved MSS markers, which are produced by the fetus and the placenta and are used to determine the assessed risk for fetal aneuploidies. The aim of Manuscript 3 was to investigate placental-mediate adverse outcomes that may be associated with the underlying cause of infertility (male factor, female factor, unexplained).

1.4 Organization of dissertation

This dissertation is manuscript based and includes three original manuscripts. An overarching literature review is presented in Chapter 2. This is followed by the three manuscripts: 1) a systematic review on the differences in maternal serum screening marker levels in women who conceived using IVF (Chapter 3); 2) a study that used secondary analysis of population-based datasets to investigate the difference in maternal serum screening marker results among IVF patients in Ontario, Canada and applied adjustment factors to improve the accuracy of the results (Chapter 4); and 3) a study examining the effect of type of infertility on placental-mediated adverse outcomes among IVF patients in Ontario, Canada (Chapter 5). Preceding each manuscript is a preface that describes the purpose of the study and why its inclusion is appropriate for this dissertation. A final discussion is presented in Chapter 6 which summarizes and integrates the main findings, implications and final conclusions. The Reference List contains all publications used throughout this dissertation that were not only cited in a manuscript. Additionally, each manuscript contains an individual references lists at the end of the chapter.

Chapter 2. Literature Review

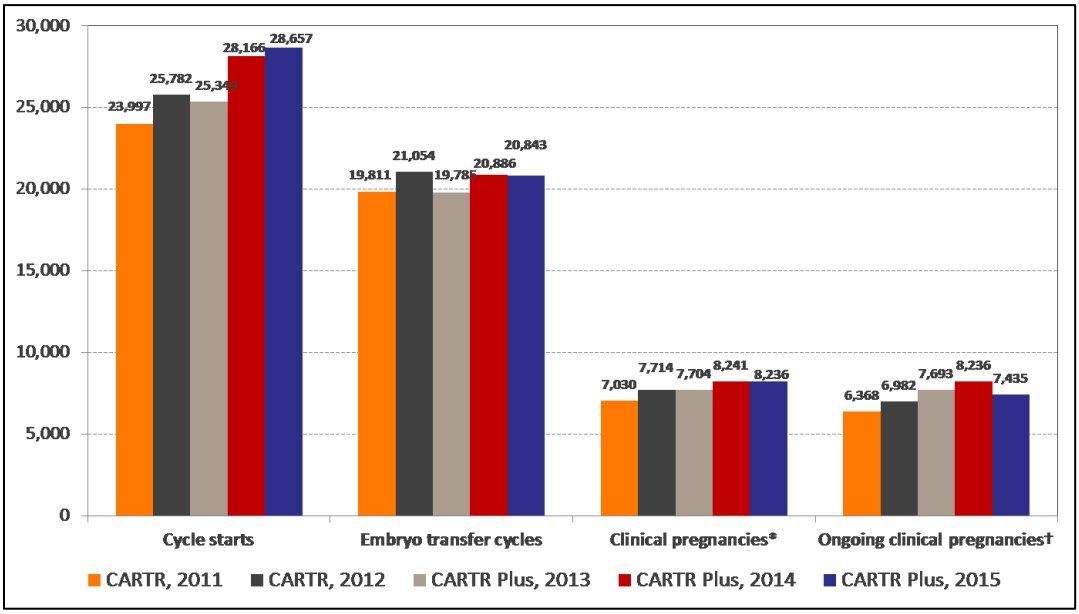
2.1 Infertility and fertility treatment

Infertility affects a growing number of individuals in Canada. Assisted reproductive technologies are used in the treatment of individuals who have difficulty conceiving naturally. The range of fertility treatments available has grown exponentially since the birth of Louise Brown, the first baby born though in vitro fertilization.[1] Multiple types of fertility treatments are currently available: medications for ovulation induction (e.g., clomiphene citrate, gonadotropins), surgical procedures (e.g., tubal surgical repair) and assisted conception (e.g., controlled ovarian hyperstimulation with intrauterine insemination or IVF).[7] ART includes all treatments where gametes are manipulated in an external environment (laboratory) in order to facilitate pregnancy and a healthy birth.[8,9] The rationale for selecting the type of fertility treatment is patient and clinical situation dependent. The confirmed or unconfirmed cause of infertility, the age of the gamete donor and the recipient, the availability of the type of treatment and the cost associated with the treatment are factors that influence both the physician and patient's decision of which treatment to follow.[10]

IVF is the most common form of ART in Canada.[11] It involves pharmacologically-controlled ovarian stimulation (a woman's ovaries are stimulated to produce multiple follicles and oocytes), transvaginal ultrasound-guided retrieval of oocytes (retrieval of oocytes from mature follicles), extracorporeal insemination or injection of the egg and fertilization (to combine genetic material from both gametes to produce a zygote), laboratory culture of the embryo in an incubator, and ends with the transfer of the developing embryo into a woman's uterus.[9] Insemination and fertilization can occur with or without intracytoplasmic sperm injection (ICSI), a method of insemination in which a single spermatozoon is directly injected into the oocyte cytoplasm.[9] This process is associated with an increased risk of chromosomal abnormalities in severe male factor infertility.[12,13] IVF treatment cycles can involve a fresh embryo transfer, which will be referred to as IVF throughout this dissertation, or a frozen embryo transfer (FET). The latter involves the previous cryopreservation of an embryo through vitrification, slow freezing or mixed methods. The frozen embryo is later thawed and transferred into a woman's uterus. For both IVF and FET treatment cycles, the transfer can occur at various points of embryonic

development. Most commonly in Canada, embryos are transferred at the cleavage stage (day-3), but better quality embryos are transferred at the blastocyst stage (day-5) of development.[11]

In 2013, there were 25,349 fertility treatment cycles started in Canadian fertility treatment centres, which resulted in 7,704 clinical pregnancies and 7,693 ongoing clinical pregnancies.[14] Over the past three years there has been an increase in the number of fertility treatment cycles and clinical pregnancies (Figure 2-1).[15] Unfortunately, there is also an increased risk of an adverse pregnancy outcome among women who use ART to conceive.[16] The type of infertility may influence the risk of adverse pregnancy outcomes. Maternal age at the time of oocyte retrieval, ovulation disorders, uterine structural malformations and sperm health will each affect the success of pregnancy.[17,18]



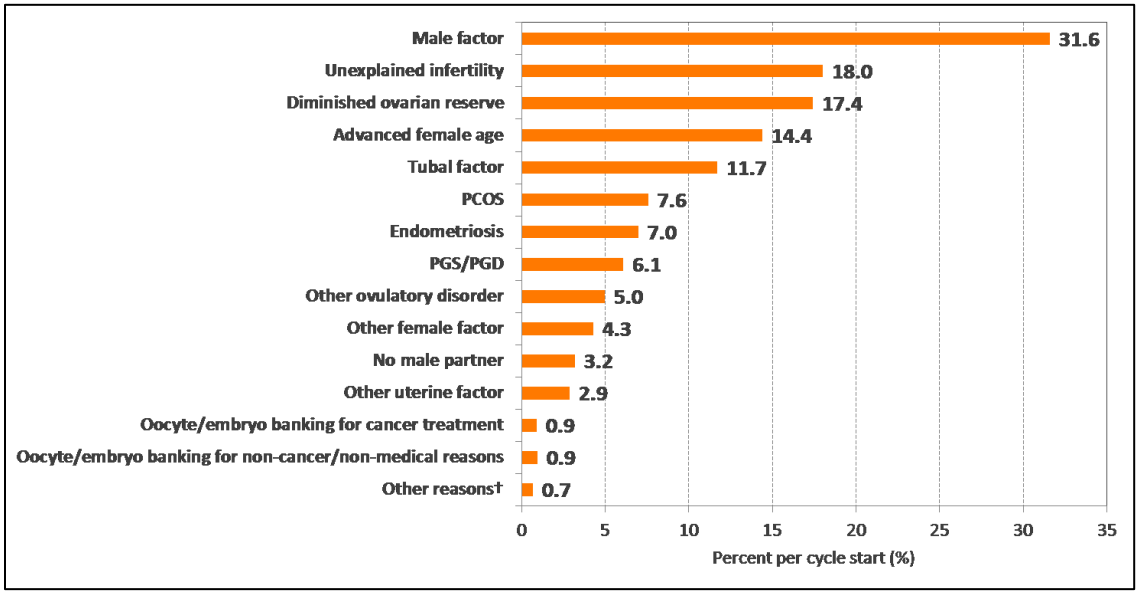
** Clinical pregnancy: clinical intrauterine, heterotopic, or ectopic pregnancy*
† Ongoing clinical pregnancy: clinical pregnancy with ≥1 fetal heart beat on ultrasound

Figure 2-1 Number of fertility treatment cycles and clinical pregnancies for all ART treatment cycle types in Canada[15]
Analyzed and presented at the Canadian Fertility and Andrology Society Annual Meeting (2016) by A. Lanes

2.2 Type of infertility

The World Health Organization defines infertility as: 1) the inability to become pregnant; 2) an inability to maintain a pregnancy; or 3) an inability to carry a pregnancy to a live birth.[19] The type

of infertility is classified into four categories: 1) male factor infertility; 2) female factor infertility; 3) combined infertility; and 4) unexplained infertility. Male factor infertility can result from a decreased sperm count, poor sperm motility or abnormal sperm morphology. Female factor infertility includes: 1) ovulation disorders (e.g., Polycystic Ovary Syndrome, hypothalamic dysfunction or premature ovarian insufficiency); 2) tubal infertility (e.g., blocked or damaged fallopian tubes, pelvic adhesive disease or endometriosis); and/or 3) uterine or cervical causes (e.g., benign uterine fibroids, congenital uterine malformations or unfavourable cervical mucus). Combined infertility results when both the female and male partners have demonstrable causes of infertility. An 'unexplained infertility' classification is given when the underlying etiology of the infertility cannot be determined. In 2015, male factor infertility was the most commonly cited reason for receiving fertility treatment (Figure 2-2).[15]



** Categories are not mutually exclusive*
† Other reasons include: gonadotoxic therapy, no female partner and peritoneal factor or severe adhesions

Figure 2-2 Distribution of reasons for receiving fertility treatment in Canada[15]
Analyzed and presented at the Canadian Fertility and Andrology Society Annual Meeting (2016) by A. Lanes

2.3 Maternal Serum Screening markers

Prenatal screening is performed to identify pregnancies at an increased risk of fetal aneuploidy. Screening for these abnormalities was first performed in the 1960s.[20] Currently, women are

offered prenatal screening options that include examination of a maternal blood sample with the addition of an ultrasound to identify markers that indicate an increased risk of fetal aneuploidy or other anomaly prior to any invasive testing.[20] Prenatal screening aims to detect Down syndrome, trisomy 18 and open neural tube defects.[21]

Children with Down syndrome have three copies of chromosome 21 and experience intellectual disabilities, which vary among affected children, and typically have specific facial characteristics and hypotonia.[20–22] Individuals with Down syndrome are also at increased risk of dementia, cardiac malformation, leukemia and immune system defects.[22] Trisomy 18 (Edwards syndrome) occurs due to a chromosomal imbalance that results in three copies of chromosome 18.[20] Most pregnancies affected by trisomy 18 end in miscarriage or stillbirth (95%), while death by 1 year of age is the expected outcome among live born infants with this syndrome.[21] Open neural tube defects occur when the neural tube fails to close during embryologic development.[23] Anencephaly and spina bifida are two of the most common types of open neural tube defects.[24] While anencephaly usually results in miscarriage or stillbirth, infants with spina bifida may survive with medical complications and long-term disabilities that vary in severity.[24]

The risk of these syndromes and conditions can be assessed during pregnancy using Integrated Prenatal Screening (IPS), Serum Integrated Prenatal Screening (SIPS), First Trimester Combined Screening (FTS) and Maternal Serum Triple and Quadruple Screening.[21] These prenatal screening tests differ in terms of the maternal serum screening markers that are examined, as well as whether nuchal translucency screening by ultrasound is included as part of the test. Levels of Pregnancy Associated Plasma Protein A (PAPP-A), alpha feto-protein (AFP), human chorionic gonadotrophin (hCG), unconjugated estriol (μE^3) and Dimeric Inhibin-A (DIA) are assessed during the prenatal screen (Table 2-1). Increases and/or decreases of these markers suggest an increased risk of Down syndrome, trisomy 18 or open neural tube defects (Table 2-2).

). These maternal serum markers are measured by the multiple of the median (MoM) as demonstrated by Equation 2-1. It is the median of the concentration of the marker being screened compared to the population reference value at a specific gestational age. An ultrasound is also conducted to assess the thickness of the nuchal translucency. If the screening test is positive, an amniocentesis for diagnostic confirmation may be performed. Ideally this takes place between 15 and 17 weeks, but it can be performed until 22 weeks'gestation.[21]

In 2013, non-invasive prenatal testing (NIPT) began to be offered in Canada.[25] It uses circulating cell free DNA in the maternal plasma to investigate chromosomal abnormalities in the fetus.[26] In Ontario, NIPT is available on a self-pay basis for a $800 to $1,000 fee.[25] However, in August 2016, the Ontario Ministry of Health and Long-term Care started funding NIPT for women who met eligibility criteria.[27] A physician can order a provincially-funded NIPT screen for a woman who has a singleton pregnancy and: 1) a positive result for aneuploidy on a MSS test; 2) the woman will be 40 years of age or older at the estimated date of delivery; 3) a fetal nuchal translucency ≥ 3.5mm; or 4) a past pregnancy aneuploidy or previous child with an aneuploidy.[27] A geneticist or maternal fetal medicine specialist can order this screen if: 1) a fetal congenital anomaly is identified through ultrasound and it suggested an increased risk for Down syndrome, trisomy 18 or trisomy 13; 2) the risk of an aneuploidy for Down syndrome, trisomy 18 or trisomy 13 is greater than that of a positive MSS marker screen; or 3) it is required for sex chromosome determination indicated for clinical management.[27] BORN Ontario (Supplemental Text C-1) is in the process of collecting all Ontario NIPT records to have test and outcome data linked together.

Table 2-1 Maternal Serum Screening markers tested among different prenatal screening tests

	First trimester 11-13+6/7 weeks	**Second trimester** 15-20+6/7 weeks	**Screen negative**
IPS	Nuchal Translucency PAPP-A	AFP µE3 hCG	Ultrasound (18-20weeks)
SIPS	PAPP-A	AFP µE[3] hCG DIA	Ultrasound (18-20weeks)
FTS	Nuchal Translucency PAPP-A βhCG		AFP (15-20 weeks) Ultrasound (18-20weeks)
Maternal Serum Triple Screening		AFP µE[3] hCG	Ultrasound (18-20weeks)
Maternal Serum Quadruple Screening		AFP µE[3] hCG DIA	Ultrasound (18-20weeks)

Table 2-2 Change of maternal serum markers present at the first trimester screen for Down syndrome, Trisomy 18 and neural tube defects

	Down Syndrome		Trisomy 18	Neural Tube Defect
	Screening at <14 weeks	Screening at >14 weeks		
AFP	Decreased	Decreased	Decreased	Increased
βhCG	Increased	Increased	Decreased	
μE^3	Decreased	Decreased	Decreased	
PAPP-A	Decreased		Decreased	
DIA	Increased	Increased	Decreased	

Equation 2-1 Multiple of the Median calculation

$$MoM(Patient) = \frac{Result(Patient)}{Median(Patient\ Population)}$$

2.3.1 Maternal Serum Screening and adjustment factors

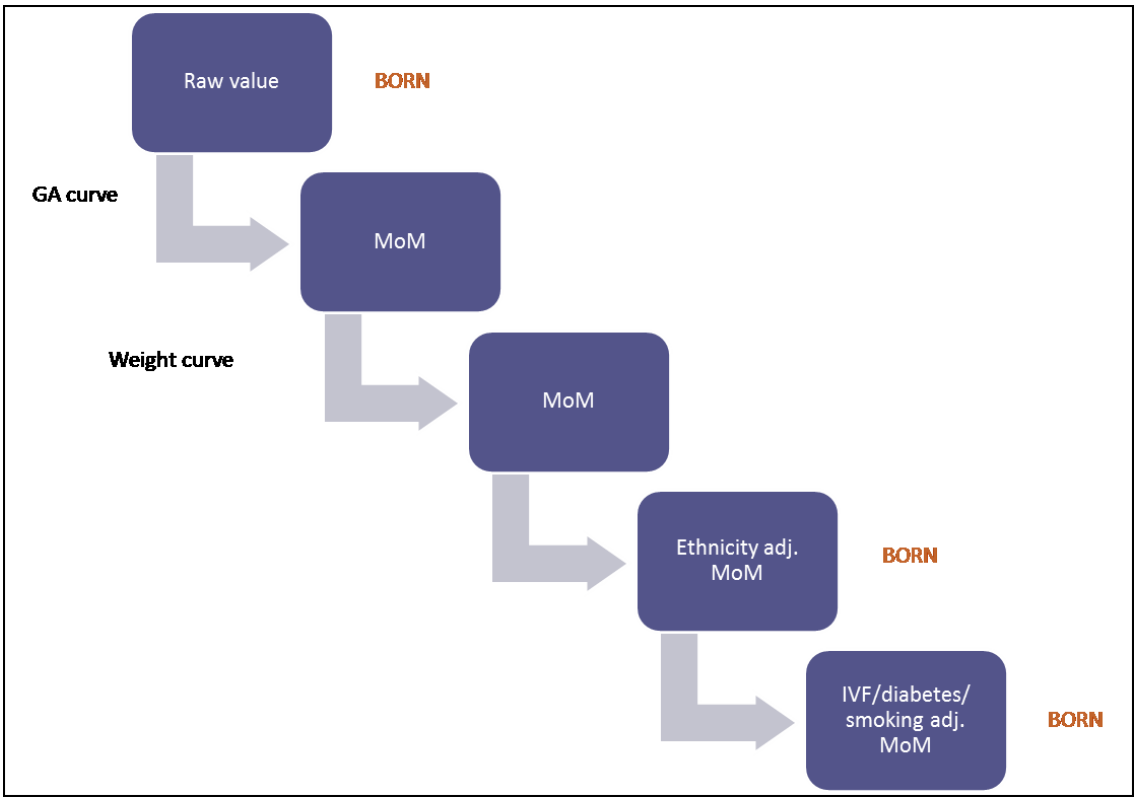
There are four prenatal screening labs in Ontario that perform these analyses and report on the risk of Down syndrome and trisomy 18. The adjustment factors are quantitatively the same at three of these prenatal screening labs and different at one lab (personal communication: S. Dougan October 24, 2016). Adjustment factors are required in specific clinical populations to bring the population mean MoM near 1.00. The raw serum screening markers are adjusted for maternal weight to account for the dilution of the MSS markers due to increased blood volume[28] and for gestational age, preferably estimated through ultrasound.[20] The amount of each MSS marker varies based on gestational age. Some MSS markers differ substantially from one day to the next; therefore an accurate gestation age is extremely important.

Currently in Ontario, there are four additional potential adjustment factors applied to the gestational age curve and weight curve modified MoM. In order to improve the accuracy of the screening test the MoMs are adjusted for ethnicity, insulin-dependent diabetes mellitus, smoking and IVF use where applicable.[20] There have been documented observed differences in MSS markers among ethnic groups. Spencer et al.,[29] found a 19% increase in free βhCG in Afro-Caribbean and Asian women as compared to Caucasian women, as well as a 48% and 35%

increase in PAPP-A among the same ethnic groups. An increased μE^3 has been found among Aboriginal women in Canada.[30] AFP and μE^3 have been found to be statistically lower among women with insulin-dependent diabetes mellitus as compared to women without insulin-dependent diabetes mellitus.[31] Although a meta-analysis by Bestwick et al.,[32] determined that the effect of adjusting for smoking was small since the application of the adjustment factor required minimal effort it should be applied when appropriate.

2.3.2 Maternal Serum Screening markers and an IVF adjustment factor

Currently in Ontario, at the time of the prenatal screen women are asked if they used IVF to assist with conception. This information is then used to determine if a correction factor should be applied during the analysis of the maternal serum markers. There is the potential for misclassification if a woman believes that she had IVF, but actually had another form of ART. The methods employed are contingent on women accurately disclosing the method of conception. Studies on small cohorts have shown that there are differences in the results seen among IVF pregnancies compared to non-IVF pregnancies.[33,34] There is an observed increase in total hCG which may result due to increased levels of progesterone produced by the multiple corpora lutea after ovarian stimulation.[35] Lower levels of μE^3 are also observed, however a causal link to this decrease has not been found.[35] In order to account for these differences, an adjustment factor is applied to each IVF pregnancy. This ensures that appropriate MSS marker MoMs are used in the calculation for the assessed risk of fetal aneuploidies. An incorrect interpretation of these results may cause unnecessary invasive testing (amniocentesis) to be performed; or conversely, a patient may be informed that her pregnancy does not have a high risk for a fetal aneuploidy when there actually is an increased assessed risk. This can lead to inappropriate decisions pathway for clinical care.



'BORN' identifies when those data are contained within BORN Ontario (Ontario maternal-child registry).

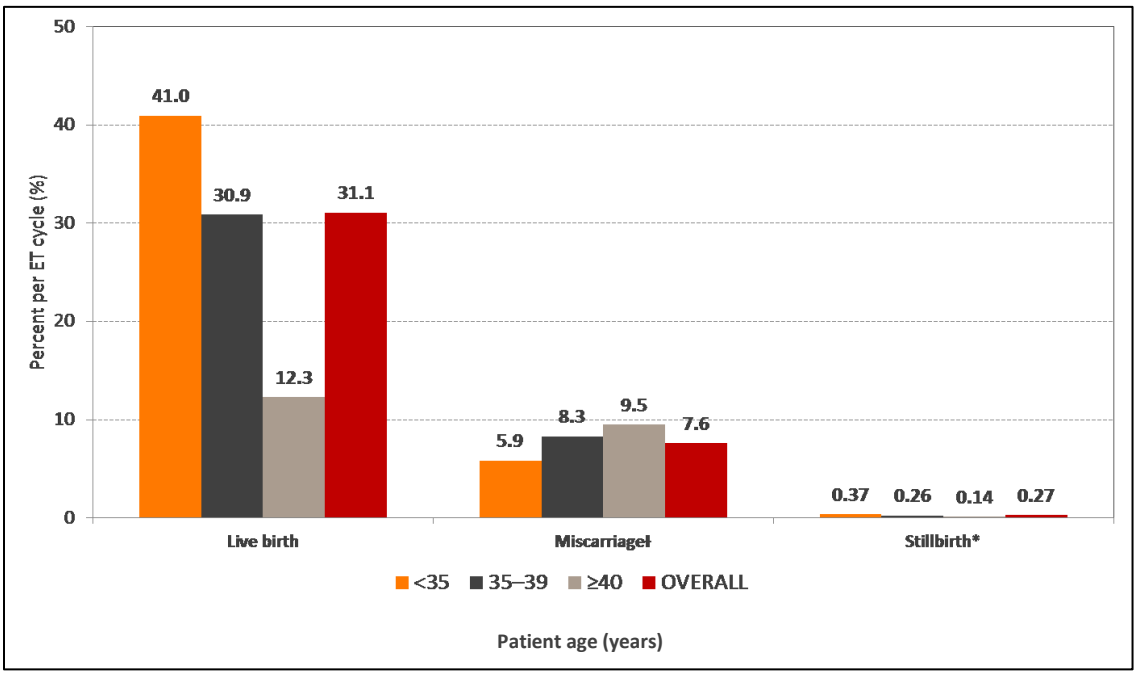
Figure 2-3 Flow diagram of current MSS marker adjustments in Ontario, Canada

2.4 Epidemiology of adverse perinatal outcomes

2.4.1 Stillbirth

The World Health Organization international definition for stillbirth is a death prior to birth and a weight of ≥1000g or if there is no information on weight a gestational age of ≥28 weeks, or if gestational age is not known a body length of ≥35cm.[36] However, the definition of stillbirth varies globally;[37] in Canada stillbirth is defined as fetal demise at ≥20 weeks' gestation and a birth weight of ≥500g.[38] Documenting stillbirths continues to be a challenge.[37] In 2015, sixty-five percent of worldwide stillbirths occurred in ten developing countries.[37] Stillbirths can be attributed to a variety of factors including socio-demographic, nutritional, maternal factors (e.g., diabetes, maternal age, and body mass index) and fetal factors (e.g., intrauterine growth restriction and congenital anomalies).[37,39,40]

Studies have previously found a significant difference in the rate of stillbirths among ART conceptions compared to spontaneous conception.[41,42] In Canada, the stillbirth rate per embryo transfer cycle was 0.27% for women who used IVF to conceive (Figure 2-4). The stillbirth rate was similar among FET cycles using autologous oocytes (0.27%).[14]



ART cycles using IVF – own oocytes, 2014
** Stillbirths: birth outcome where no fetus(es) was born alive and at least one stillbirth at ≥ 20 weeks' gestation*
† Miscarriage: birth outcome where all fetuses were a 'pregnancy loss' at <20 weeks' gestation

Figure 2-4 Proportion of miscarriage and stillbirth rates in Canada among IVF pregnancies[14]
Analyzed and presented at the Canadian Fertility and Andrology Society Annual Meeting (2016) by A. Lanes

2.4.2 Intrauterine growth restriction (IUGR)

Intrauterine growth restriction (IUGR) occurs when a fetus does not achieve its full growth potential and is smaller than average.[43–46] It is frequently due to genetic (chromosomal abnormalities) or environmental factors.[47,48] Most commonly small for gestational age is defined as an estimated fetal weight of less than the 10th percentile for gestational age.[45,48,49] IUGR is associated with an increased prevalence of adverse fetal, neonatal and adult outcomes.[43,45] However, IUGR is a challenge to diagnose and small for gestational age (SGA) is the most common surrogate.[43,48] A diagnosis of SGA is usually determined antenatally by fetal ultrasound, which may allow for an appropriate intervention to decrease morbidity and mortality.[45,49] It is

usually defined as less than the 10th percentile of estimated fetal weight;[49] however, SGA has also been defined as a birth weight or birth crown-heel length that is ≤2 SD from the mean for the specific age.[50]

Zhu et al.,[44] found that an increased risk for SGA among subfertile women remained after adjustments for maternal age, smoking and parity. Similar results were found in a meta-analysis by Jackson et al.,[16] among singleton pregnancies in women who conceived through IVF; although a few studies found no increased association.[51–55]

2.4.3 Preeclampsia

The prevalence of preeclampsia ranges from two percent to eight percent worldwide.[56] The Society of Obstetricians and Gynaecologists of Canada defines preeclampsia as gestational hypertension with one or more of the following: new proteinuria, or one or more adverse conditions, or one or more severe complications.[57] The placenta is thought to be the most significant organ associated with the development of preeclampsia.[58] It can be caused by flawed placentation, a lowered maternal threshold or excessive physiological placentation.[57] Risk factors for preeclampsia include: nulliparity, excess placental volume, and obesity.[56] Smoking has been shown to significantly decrease the risk of preeclampsia.[59] The removal of the placenta remains the most successful method of resolving the symptoms.[60] Preeclampsia is commonly linked to IUGR.[43,45,47,56,57] Studies have also shown that preeclampsia may be associated with negative long-term effects on the cardiovascular and renal systems.[61–63]

Previous studies have found a positive association between fertility treatment and preeclampsia.[12,64] Multiple pregnancies among women who used fertility treatments to conceive have been found to be associated with an increased risk of preeclampsia.[65] However, other studies suggested the opposite; that there was no significant association between fertility treatment and preeclampsia once maternal age and parity have been considered.[66,67]

2.4.4 Placental abruption

Placental abruption occurs when there is premature separation of the placenta prior to delivery after 20 weeks' gestation.[68,69] It is often linked with fetal or neonatal complications;[68] however it

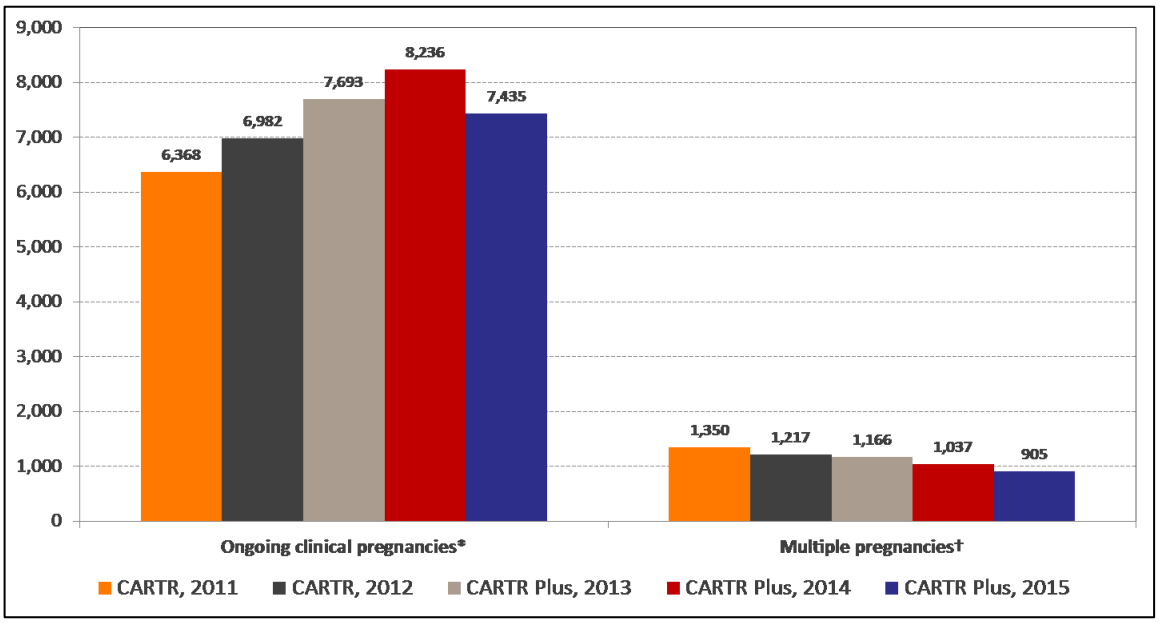
may also occur when a haematoma is diagnosed around the placenta and may not result in immediate treatment.[70] Preeclampsia, preterm pre-labour rupture of membranes (PPROM), and gestational hypertension have been associated with an increased risk of placental abruption.[71] Additionally, placental abruption has been found to be associated with preterm birth.[71,72]

Subfertile women have been found have an increased rate of placental abruption.[73,74] This is most likely due to the association between multiple gestation pregnancies and an increased risk for placental abruption.[75]

2.4.5 Preterm birth

Preterm birth is generally defined as a birth at less than 37 weeks' gestation, however this varies by country.[72] In Canada, a gestational age of less than 37 weeks is used to define a preterm birth, a gestational age of less than 32 weeks defines a very preterm birth, and a gestational age of less than 28 weeks defines an extremely preterm birth.[76] Globally preterm birth is the leading cause of perinatal morbidity and mortality.[72,77] In Ontario, the rate of preterm birth was 8.1% in 2011-2012, which was 0.3% higher than the national average.[76] The etiology of preterm birth is often grouped into three categories: 1) spontaneous labour with membranes intact; 2) PPROM; and 3) maternal or fetal indications without spontaneous labour.[72,77] Category number three is most relevant to the placental-mediated adverse outcomes discussed in this thesis. Several studies have found that preterm birth may be associated with IUGR, stillbirth or other placental-mediated adverse outcomes.[37,40,49,78]

The increased rate of preterm births is often attributed to multiple gestation pregnancies achieved through assisted reproductive technologies.[5,72,74] Policies in Canada have focused on performing single embryo transfers in order to reduce the multiple pregnancy rate.[74,79] Among women who used ART to conceive in Canada the multiple pregnancy rate per ongoing clinical pregnancy was reduced to 12.2% in 2015, down from 21.2% in 2011 (Figure 2-5). Preterm birth is associated with increased costs, as compared to term births, primarily due to increased time spent in the neonatal intensive care unit (NICU).[76] Therefore, the dramatic decrease in multiple gestation pregnancies achieved through ART may decrease the cost to the healthcare system.



** Ongoing clinical pregnancy: clinical pregnancy with ≥1 fetal heart beat on ultrasound*
† Multiple pregnancy: ongoing clinical pregnancy with >1 fetal heart beat on ultrasound

Figure 2-5 Multiple pregnancy rate in Canada among ART pregnancies[15]
Analyzed and presented at the Canadian Fertility and Andrology Society Annual Meeting (2016) by A. Lanes

Chapter 3. [Manuscript 1] Maternal serum screening markers and nuchal translucency measurements among in vitro fertilization pregnancies: a systematic review

3.1 Preface to Manuscript 1

Identifying evidence through a systematic review of the literature established the current state of our understanding about the association between fertility treatments and maternal serum screening markers (pregnancy-associated plasma protein A (PAPP-A), alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (μE^3), Dimeric Inhibin-A (DIA)) and nuchal translucency thickness. The search strategy was conservative to ensure capture of all appropriate studies. Ovid MEDLINE, Embase and COCHRANE databases were all extensively searched.

There have been a large number of studies conducted in this area; however most do not investigate all maternal serum screening markers, rather only a subset of maternal serum screening markers. Studies also did not always incorporate frozen embryo transfers or the method of insemination in their analyses and reporting. It was also important to investigate if the mean or median maternal serum screening marker MoM values were consistently reported, as this information is important when these values are used in algorithms for calculating a patient's risk of Down syndrome or trisomy 18.

Additionally, the majority of studies were conducted with smaller cohorts, as opposed to population-level data. However, eleven of the included forty studies each analyzed more than 10,000 pregnancies in their studies; thus allowing confidence in the summarized findings of this systematic review on the effects of fertility treatment on maternal serum screening markers and nuchal translucency thickness.

This manuscript was published in Fertility and Sterility in 2016 (Lanes A. et al. *Fertil Steril* 2016; 106:1463–1469).[80]

3.2 Title page

Title: Maternal serum screening markers and nuchal translucency measurements among in vitro fertilization pregnancies: a systematic review

Authors: Andrea Lanes, MSc[a,b], Tianhua Huang, PhD[b], Ann E. Sprague, PhD[b,f], Arthur Leader, MD, FRCSC[d,e], Beth Potter, PhD[a], Mark Walker, MD, FRCS(C)[b,c,d,f]

Affiliations:

[a]University of Ottawa School of Epidemiology, Public Health and Preventive Medicine

[b]BORN Ontario

[c]Ottawa Hospital Research Institute

[d]University of Ottawa Department of Obstetrics and Gynecology

[e]Ottawa Fertility Centre

[f]CHEO Research Institute

3.3 Abstract

Objective: To study the current literature on the association between in vitro fertilization treatment and maternal serum screening marker levels and nuchal translucency thickness.

Design: This was a systematic review.

Settings: There were no restrictions to where the included articles were published.

Patients: Eligible studies included those with an exposed group of pregnant women that used IVF with or without ICSI to conceive; and a control group of pregnant women that conceived spontaneously.

Intervention: In vitro fertilization treatment in order to conceive.

Main Outcome Measures: Outcomes evaluated included Maternal Serum Screening markers (pregnancy-associated plasma protein A (PAPP-A), alpha-fetoprotein, human chorionic gonadotropin (hCG), unconjugated estriol, Dimeric Inhibin-A) and nuchal translucency thickness.

Results: Database searches identified 4,118 titles and abstracts that were independently screened, which resulted in 76 articles that were assessed for eligibility. Additionally, one study was added for consideration based on expert knowledge. There were 29 cohort and 11 case-control studies in the descriptive review. The most commonly reported markers were PAPP-A and free β-hCG, reported in 28 and 26 studies respectively. The studies that reported effect sizes for PAPP-A and free β-hCG and were not statistically significant.

Conclusions: A decrease in PAPP-A and an increase in total hCG was consistently reported among the included studies. However, due to the variability in the levels of the other maternal serum screening markers reported and the inability to conduct a meta-analysis we were unable to generalize about the differences between prenatal screening results in the IVF population.

Keywords: In vitro fertilization, maternal serum screening markers, systematic review

3.4 Introduction

Prenatal screening is performed primarily to identify pregnancies at an increased risk for fetal anomalies, such as Down syndrome or trisomy 18, but can also detect risk of open neural tube defects. Aneuploidy screening was first performed in the 1960s[1] and has evolved since then. Currently in Ontario, women are offered prenatal screening options that include examination of maternal blood sample(s) with the addition of an ultrasound to estimate the risk of fetal aneuploidy prior to Cell-free fetal DNA or invasive testing (amniocentesis).[1] There are a variety of prenatal screening tests that can be used to assess risk including: First Trimester Combined Screening (FTS), Integrated Prenatal Screening (IPS), Serum Integrated Prenatal Screening (SIPS), Triple Screening, and Quadruple Screening.

Children with Down syndrome have three copies of chromosome 21 and experience intellectual disabilities, which vary among affected children, and typically have specific facial characteristics and hypotonia.[1–3] Individuals with Down syndrome are also at increased risk of dementia, cardiac malformation, leukemia and immune system defects.[3] Most pregnancies affected by trisomy 18 end in miscarriage or stillbirth (95%), while death by 1 year of age is the expected outcome among live born infants with this syndrome.[2] Open neural tube defects occur when the neural tube fails to close during embryologic development.[4] Anencephaly and variable degrees of spina bifida are two of the most common types of open neural tube defects.[5] While anencephaly usually results in miscarriage or stillbirth, infants with spina bifida may have minimal living restrictions or more significant problems that allow them to survive with medical complications and long-term disabilities that vary in severity.[5]

In vitro fertilization (IVF) is a form of assisted conception that includes extracorporeal fertilization and embryo culture.[6] It involves controlled ovarian stimulation, transvaginal ultrasound-guided retrieval of oocytes, insemination or injection of the egg to assist fertilization (to combine genetic material from both gametes resulting in a zygote), laboratory culture in an incubator over 4 to 6 days and transfer of the growing embryo.[6] Insemination and fertilization can occur with or without intracytoplasmic sperm injection (ICSI). ICSI is a method of insemination wherein a single spermatozoon is injected into the cytoplasm of the mature (MII) oocyte.[6] This process is associated with an increased risk of sex chromosomal abnormalities among offspring of men with severe male factor infertility.[7,8]

At the time of the prenatal screen, information about the method of conception is requested in order to determine if a correction factor should be applied to assay results of the maternal serum screening markers. Studies on small cohorts have shown that there are differences in the marker measurements seen among IVF pregnancies compared to non-IVF pregnancies.[9,10] An incorrect interpretation of these results may miss an affected pregnancy or cause unnecessary invasive testing (amniocentesis) to be performed. The objective of this study was to synthesize existing knowledge about the associations between IVF treatment and maternal serum screening marker levels and nuchal translucency thickness.

3.5 Methods

The protocol for this systematic review was registered with PROSPERO International prospective register of systematic reviews (registration number: CRD42015019545).[11] The manuscript was developed and reported in accordance with the preferred reporting items for systematic review and meta-analysis (PRISMA) 2015 statement.[12]

Search strategy

A search strategy was developed using medical subject headings and keywords related to the population, exposure and screen results. Ovid MEDLINE, Embase and COCHRANE databases were searched from inception until April 2015. Appendix A outlines the full search strategy. This search strategy included both observational (i.e. cross-sectional, cohort, case-control) and experimental (i.e. randomized clinical trial) study designs. Eligible studies included those with the exposure (IVF with or without ICSI) and a control group of pregnant women that conceived spontaneously. Outcomes evaluated included maternal serum screening markers (pregnancy-associated plasma protein A (PAPP-A), alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), free β-hCG, unconjugated estriol (μE3), Dimeric Inhibin-A (DIA)) and nuchal translucency (NT) thickness. There were no restrictions based on language of the study publication. Studies where the comparison group was not women that conceived spontaneously were excluded. Reference lists from the included studies were investigated for additional articles that met the inclusion criteria. All titles and abstracts were independently screened by two reviewers, an epidemiologist and a prenatal screening expert (A. Lanes and T.H.), and any discrepancies were resolved through discussion. A second full-text screen of the titles and abstracts that met the

study inclusion and exclusion criteria was performed. Studies were excluded if both reviewers determined that the articles were: irrelevant, inaccessible or adequate translations were not achievable. Data were then abstracted from the articles that were considered to meet all study inclusion and exclusion criteria after the second screen.

Data abstraction and assessment of quality

A data abstraction form was developed to systematically extract information from the included studies. This form was independently completed by both reviewers. Information captured included study design, type of publication, population size, study objective, definition of exposure, definition of outcome, outcomes included in each study, confounding variables adjusted for in the analysis, and unadjusted and adjusted measures of effect. Risk of bias was assessed by each reviewer using the Newcastle-Ottawa Scale.[13] This is a commonly used tool that investigates potential bias for cohort and case-control studies in three categories: selection, comparability and outcome.[13] A study can be given a minimum of zero stars and a maximum of nine stars. In order to estimate interrater reliability, congruence for a subset of six questions from the data abstraction form was evaluated. Questions were selected from different sections of the form including: data quality, definition of cohort and quantitative measures. Kappa statistics were calculated for four questions: three questions had perfect agreement (kappa statistic of 1.0) and one question had a kappa statistic of 0.58. We were unable to report kappa for two questions.

Data synthesis and analysis

Descriptive characteristics including type of study, year of publication, and study location were summarized. *A priori* the authors decided to abstract median multiples of the median (MoM) as the measure of effect for this systematic review. This was the appropriate descriptive statistic to account for the bimodal distribution of the maternal serum screening markers. Consequently, meta-analyses were not feasible since it is not possible to combine median MoMs across studies. We did not present forest plots without a pooled effect, because the median MoMs were reported using distinct measures of variance (i.e., standard deviation, 95% confidence interval, ranges). As a substitute we reported the magnitude of change for each maternal serum screening marker. When available, study data on the outcomes among frozen embryo transfer (FET) cycles and fertility treatment cycles that used donor oocytes were abstracted.

3.6 Results

Database searches identified 4,118 non-duplicate titles and abstracts that were independently screened. There were 4,042 records excluded from the primary screen, which resulted in 77 articles that were assessed for eligibility. Additionally, one study was added for consideration based on expert knowledge. Upon completion of the second screen 45 articles remained, of which 5 were excluded (Figure 3-1). One excluded article was determined to be irrelevant; two articles were inaccessible; and adequate translations were not attainable for two articles. In total 40 articles were included.

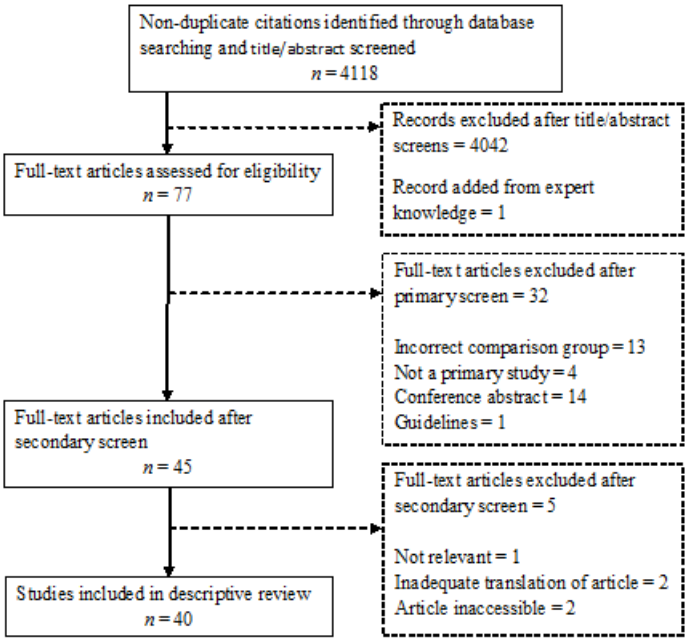


Figure 3-1 Flow diagram illustrating the selection of studies into the systematic review

The majority of studies included in this systematic review were published prior to 2010 (30 studies) with the remainder having a publication date between 2010 and 2015 (10 studies). Twenty-eight studies were from the United Kingdom or Europe; four were from North America; four from China; three from Israel; and one from Australia. There were 29 cohort and 11 case-control studies in the descriptive review. The Newcastle-Ottawa risk of bias assessment had a median score of 7 and a range between 4 and 9. For cohort studies the range of the scores was 2

to 4 with a median score of 4 for selection, 0 to 2 with a median score of 0 for comparability, and 2 to 3 with a median score of 3 for outcome. The scores varied slightly for case-control studies. The range for selection was 2 to 4 with a median score of 4, a range of 0 to 2 with a median score of 1 for comparability, and a range of 1 to 3 with a median score of 2 for outcome. Twenty-six of the included studies determined the presence of the exposure through record linkage or a secure record, whereas only five studies used self-report.

The most commonly reported maternal serum screening marker was PAPP-A in 28 studies. However, only fourteen studies reported a median MoM (Table 3-1). Almost all of these studies reported a reduction in PAPP-A among IVF patients compared to those that conceived spontaneously (13 studies). All seven studies that reported a median MoM for patients who used ICSI found a reduced estimate compared to a spontaneous conception group. A similar trend was seen for AFP. This serum marker was evaluated in 14 studies and the majority of studies that reported a median MoM saw a decrease (7 studies) compared to spontaneous conceptions. The three studies that measured the effect of ICSI on AFP saw a decrease or no change.

Table 3-1 Observed median MoM maternal marker serum levels and nuchal translucency thickness in IVF patients compared to patients who conceived spontaneously

Author and year	PAPP-A		AFP		free β-hCG		total hCG		µE3		DIA		NT	
	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI
Anckaert, 2008[14]	0.75*	0.94*			0.90	1.07								
Ball, 2013[15]														
Ball, 2012[16]														
Bar-Hava, 2001[17]														
Bellver, 2005[18]	1.06				0.83	1.13								
Bellver, 2013[19]	↓	↓			↑	↑							--	--
Bender, 2010[20]	0.86*	0.90*			1.10*	1.10*							1.03	1.02
Bersinger, 2001[21]														
Bersinger, 2004[22]														
Bersinger, 2005[23]														
Bredaki, 2011[24]														
Cowans, 2013[25]	1.01				1.03*									
Engels, 2010[9]														
Engels, 2013[26]														
Frishman, 1997[27]			0.95				1.22		0.90					
Ghisoni, 2003[28]														
Giorgetti, 2013[29]	0.74*	0.81*			0.96	1.09							1.02	1.06
Gjerris, 2009[30]														
Heinonen, 1996[31]														
Hui, 2003[32]			0.90*	0.86*			1.07	0.92						
Hui, 2005[33]													1.07*	1.09*
Hui, 2005[34]	0.83*	0.70*			0.87*	0.82								

Kagan, 2008[35]														
Lam, 1999[36]			0.88*	0.76*			1.15	0.88						
Lambert-Messerlian, 2006[10]	0.94		1.05		1.13		1.10*		0.94*		1.12*		1.03	
Lambert-Messerlian, 2009[37]	0.91*						1.04							
Liao, 2001[38]	1.00*	0.86*			1.21*	1.09							0.97	1.00
Matilainen, 2011[39]	0.82*				1.00								1.03	
Maymon, 2002[40]	0.96*		1.13*		1.16		1.12		0.94				1.16	
Maymon, 2004[41]	0.78*		1.02				1.10		0.99		0.98		1.14*	
Muller, 2003[42]			0.96	0.95	1.05	1.11	1.10	1.01	0.90	1.00				
Niemimaa, 2001[43]														
Orlandi, 2002[44]	0.79*	0.96			0.84	1.13							1.10	1.02
Raty, 2002[45]			0.95	1.00	1.19	1.07								
Ribbert, 1996[46]														
Rice, 2005[47]			0.99				1.12		1.12					
Tul, 2006[48]	0.94*	0.82*			1.04	0.91					1.11*	1.48*	1.00	0.99
Wald, 1999[49]			0.99		1.09*		1.14*		0.94*		0.89			
Wojdemann, 2001[50]	1.02				1.14								0.97	
Amor, 2009[51]														

Notes:

All values are median MoMs.

↑ Denotes an increase compared to spontaneous conceptions when exact data were not available.

↓ Denotes a decrease compared to spontaneous conceptions when exact data were not available.

-- Equivalent to spontaneous conception

** Statistically significant at α=0.05.*

If there was a separate group for ICSI in a study it was captured under the ICSI header.

This systematic review included studies that investigated free β-hCG in the first trimester or total hCG in the second trimester. Free β-hCG and total hCG were reported in 26 and 17 studies respectively. Amongst the studies that reported a median MoM for free β-hCG the results varied. Eight studies reported an increase among IVF users compared to spontaneous conception, while six studies reported a decrease. The results from the studies investigating total hCG were homogenous. All studies reported an increased estimate among IVF users.

A small number of studies investigated μE3 and DIA (8 studies and 4 studies respectively). An appropriate estimate was reported in eleven of the twelve studies. The majority of studies on μE3 reported a decrease among those using IVF, whereas the direction of change was evenly distributed for DIA. NT measurements were discussed in 19 studies and approximately fifty percent of these studies reported an appropriate estimate for this systematic review. Almost all of these studies found an increased NT median MoM in patients that used IVF to conceive compared to those who conceived spontaneously.

There were seven studies that reported median MoMs comparing maternal serum screening markers of patients who had a FET with individuals who conceived spontaneously (

Table 3-2). The direction of the estimate were lower among studies that reported PAPP-A and consistently higher among studies that reported AFP and NT. Studies that investigated free β-hCG reported estimates that varied in direction.

Two studies investigated the impact of donor oocytes on maternal serum marker levels and NT measurements.[10,19] Lambert-Messerlian et al.[10] found that the median MoM was increased for PAPP-A, AFP, total hCG, and DIA compared to those who conceived spontaneously, whereas a decrease was reported for free β-hCG, μE3, and NT. The study by Bellver et al.[19] investigated IVF and ICSI using donor oocytes separately. This study found and increase in both PAPP-A and free β-hCG for IVF conceptions with donor oocytes compared to spontaneous conception. When the authors investigated ICSI using donor oocytes compared to spontaneous conception they found a decrease in PAPP-A and a statistically significant increase in free β-hCG.

Table 3-2 Observed median MoM maternal serum marker serum levels and nuchal translucency thickness in women who had FET compared to women who conceived spontaneously

Author and year	FET						
	PAPP-A	**AFP**	**free β-hCG**	**total hCG**	**µE3**	**DIA**	**NT**
Anckaert, 2008[14]	1.05		1.12				
Bellver, 2013[19]	↓						
Hui, 2003[32]		1.10		1.24*			
Hui, 2005[33]							1.09*
Hui, 2005[34]	0.95*		1.21*				
Matilainen, 2011[39]	0.78		0.94				1.00
Raty, 2002[45]		1.15	1.33*				

Notes:

All values are median MoMs.

↓ Denotes a decrease compared to spontaneous conceptions when exact data were not available.

** Statistically significant at α=0.05.*

Table 3-3 Change of maternal serum screening markers present for Down syndrome, trisomy 18 and neural tube defects

	Down syndrome		Trisomy 18	Neural Tube Defect
	Screening at <14 weeks	Screening at >14 weeks		
AFP	Decreased	Decreased	Decreased	Increased
βhCG	Increased	Increased	Decreased	
μE^3		Decreased	Decreased	
PAPP-A	Decreased		Decreased	
DIA		Increased	Decreased	

3.7 Discussion

Main findings

Prenatal screening is primarily used to estimate the risk of aneuploidies. Different types of prenatal tests have varying sensitivity and specificity (i.e., First Trimester Combined Screening, Serum Integrated Prenatal Screening, Integrated Prenatal Screening, Triple Screening, and Quadruple Screening).[2] The current prevalence rate for infertility from countries around the world has been reported to range from 3.5% to 16.7%.[52] This systematic review summarized the variation of serum levels reported in 40 studies and over 700,000 pregnancies. The majority of studies included reported altered maternal serum screening marker levels among women who used IVF to conceive compared to women who conceived spontaneously.[10,14,18,20,25,27,29,32–34,36–42,44,47–50,53] This suggests that we should ensure that the results are being correctly interpreted.

Interpretation

The maternal serum markers in this systematic review were consistent, since they are universally defined. All of the studies had a cohort or case-control study design. However, the analytical approach varied across studies. Therefore there was substantial design heterogeneity present. The included studies used a variety of methods to reporting effect estimates. Only extracting data reported as a median MoM allowed for the inclusion of appropriate outcome measures. Including studies that only reported mean MoMs in the quantitative analysis of this

review would have provided biased results. The results would have been skewed in the direction of the outliers for each maternal serum screening marker.

Although we were not able to pool the results from these studies and interpret a magnitude of change, we were able to draw broad conclusions from the direction of change of the estimate of each maternal serum screening marker for IVF patients compared to women who conceived spontaneously (Table 3-1). The majority of studies found a decreased estimate of PAPP-A and an increased NT measurement in the IVF population; these markers were measured in the first trimester. In the second trimester, there was a decrease in AFP, an increase in total hCG and a decrease in the estimate of μE3 for IVF patients. This corresponds with an increased risk of Down syndrome (Table 3-3).[2] Either this is a true association or there may be an increased false positive rate for Down syndrome among IVF conceptions. This identified concerns regarding the robustness of this method of screening for IVF pregnancies. The presence of vanishing twins among singleton IVF pregnancies may have influenced the levels of the maternal serum screening markers that were observed in each study.

Non-invasive prenatal testing (NIPT) may be a more suitable first line method of testing for aneuploidy risks and should be investigated as an alternative screening method. NIPT uses circulating cell free maternal plasma DNA to investigate chromosomal abnormalities in the fetus and is usually a second-tier test for women who screen positive with conventional prenatal screening.[54] Additionally, the increased use of preimplantation genetic screening to screen for chromosomal aneuploidies among women that used IVF may modify the proportion of these women that use maternal serum screening to detect aneuploidies once an ongoing clinical pregnancy was achieved. The rate of positive screening results may differ among a self-selected population. This would bias the results found when IVF patients are compared to women who conceived spontaneously.

Strengths and Limitations

This study was strengthened by a rigorous and inclusive search strategy. The large number of studies included from a variety of countries around the world provided an opportunity to generalize interpretations. Unfortunately, the variations in reporting outcomes proved to be a substantial limitation. In order for the results to be comparable, only similar statistical estimates were abstracted (median MoMs). Due to this restriction, quantitative results were included from

23 of 40 studies. However, due to the estimate that was abstracted and the various ways in which variance was reported we could not meta-analyze these data. The majority of cohort studies (26) included in this review used record linkage or a secure record to ascertain the exposure. All of the case-control studies had controls selected from same study base as the cases. Therefore, the potential for selection bias was small.

3.8 Conclusions

A decrease in PAPP-A and an increase in total hCG was consistently reported among the included studies. However, due to the variability in the levels of the other maternal serum screening markers reported and the inability to conduct a meta-analysis we were unable to generalize about the differences between prenatal screening results in the IVF population. Due to the heterogeneity of these results, the authors cannot conclusively state whether maternal serum screening to be the most appropriate method of prenatal screening for women who use IVF to conceive. Nevertheless, this systematic review appropriately summarized the direction of change of multiple maternal serum screening markers among IVF conceptions.

Disclosure of interests

The authors have no conflicts of interest to declare.

Contribution to authorship

A. Lanes created the study protocol, developed the search strategy, conducted the search of the literature, did the primary and secondary screen of the articles, extracted data from the included articles, analyzed and interpreted these data, drafted and edited the manuscript. T.H. performed the primary and secondary screen of the articles, extracted data from included articles and edited the manuscript. A.S., A. Leader, and B.P. assisted with interpreting the results and editing the manuscript. M.W. assisted with the development of the study protocol, interpretation of results and editing of the manuscript.

Funding

A. Lanes was funded by the Ontario Graduate Scholarship (2013-2014) and the Queen Elizabeth II Scholarship in Science and Technology (2015-2016).

Acknowledgements

We acknowledge Risa Shorr, Medical Librarian at The Ottawa Hospital, for assisting with the search strategy.

3.9 References

1. Chitayat D, Langlois S, Wilson RD. Prenatal screening for fetal aneuploidy in singleton pregnancies. *J Obstet Gynaecol Canada*. 2011;33(7):736-750. http://www.ncbi.nlm.nih.gov/pubmed/21749752.

2. Carroll J, Allanson J, Epslen M, et al. *Reference Guide for Health Care Providers Prenatal Screening Tests for the Detection of : Down Syndrome, Trisomy 18 and Open Neural Tube Defects*.; 2007.

3. Korenberg J, Chen X, Schipper R, et al. Down syndrome phenotypes : The consequences of chromosomal imbalance. 1994;91(May):4997-5001.

4. Lemire RJ. Neural Tube Defects. *JAMA*. 1988;259(4):558-562.

5. Botto L, Moore C, Khoury M, Erickson J. Neural-Tube Defects. *N Engl J Med*. 1999;341(20):1509-1519.

6. Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. *Hum Reprod*. 2009;24(11):2683-2687. doi:10.1093/humrep/dep343.

7. Henningsen A-KA, Pinborg A. Birth and perinatal outcomes and complications for babies conceived following assisted reproductive techniques. *Semin Fetal Neonatal Med*. May 2014:1-5. doi:10.1016/j.siny.2014.04.001.

8. Bergh C, Wennerholm U-B. Obstetric outcome and long-term follow up of children conceived through assisted reproduction. *Best Pract Res Clin Obstet Gynaecol*. 2012;26(6):841-852. doi:10.1016/j.bpobgyn.2012.05.001.

9. Engels M a J, Kooij M, Schats R, et al. First-trimester serum marker distribution in singleton pregnancies conceived with assisted reproduction. *Prenat Diagn*. 2010;30(4):372-377. doi:10.1002/pd.2495.

10. Lambert-Messerlian G, Dugoff L, Vidaver J, et al. First- and second-trimester Down syndrome screening markers in pregnancies achieved through assisted reproductive technologies (ART): A FASTER trial study †. *Prenat Diagn*. 2006;(January):672-678. doi:10.1002/pd.

11. Stewart L, Moher D, Shekelle P. Why prospective registration of systematic reviews makes sense. *Syst Rev*. 2012;1(1):7. doi:10.1186/2046-4053-1-7.

12. Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev*. 2015;4(1):1-9. doi:10.1136/bmj.g7647.

13. Wells G, Shea B, O'Connell D, et al. Newcastle-Ottawa quality assessment scale cohort studies. Ottawa Hospital Research Institute. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Published 2014.

14. Anckaert E, Schiettecatte J, Sleurs E, Devroey P, Smitz J. First trimester screening for Down's syndrome after assisted reproductive technology: non-male factor infertility is associated with elevated free beta-human chorionic gonadotropin levels at 10-14 weeks of gestation. *Fertil Steril*. 2008;90(4):1206-1210. doi:10.1016/j.fertnstert.2007.08.050.

15. Ball S, Ekelund C, Wright D, et al. Temporal effects of maternal and pregnancy characteristics on serum pregnancy-associated plasma protein-A and free β-human chorionic gonadotropin at 7-14 weeks' gestation. *Ultrasound Obstet Gynecol*. 2013;41(1):33-39. doi:10.1002/uog.11209.

16. Ball S, Wright D, Sodre D, Lachmann R, Nicolaides K. Temporal effect of Afro-Caribbean race on serum pregnancy-associated plasma protein-a at 9-13 weeks' gestation in screening for aneuploidies. *Fetal Diagn Ther*. 2012;31(162-169).

17. Bar-Hava I, Yitzhak M, Krissi H, et al. Triple-Test Screening in In Vitro Fertilization Pregnancies. 2001;18(4):226-229.

18. Bellver J, Lara C, Soares SR, et al. First trimester biochemical screening for Down's syndrome in singleton pregnancies conceived by assisted reproduction. *Hum Reprod*. 2005;20(9):2623-2627. doi:10.1093/humrep/dei107.

19. Bellver J, Casanova C, Garrido N, et al. Additive effect of factors related to assisted conception on the reduction of maternal serum pregnancy-associated plasma protein A concentrations and the increased false-positive rates in first-trimester Down syndrome screening. *Fertil Steril*. 2013;100(5):1314-1320. doi:10.1016/j.fertnstert.2013.06.045.

20. Bender F, Hecken J, Reinsberg J, et al. Altered first-trimester screening markers after IVF/ICSI: no relationship with small-for-gestational-age and number of embryos transferred. *Reprod Biomed Online*. 2010;20(4):516-522. doi:10.1016/j.rbmo.2009.12.025.

21. Bersinger NA, Chanson A, Crazzolara S, et al. Serum levels of placenta protein markers: The relevance of differences between spontaneous and after in vitro fertilization pregnancies for fetal trisomy screening. *J fur Fertil und Reproduktion*. 2001;11(3):7-13.

22. Bersinger N a, Wunder D, Vanderlick F, et al. Maternal serum levels of placental proteins after in vitro fertilisation and their implications for prenatal screening. *Prenat Diagn*. 2004;24(6):471-477. doi:10.1002/pd.910.

23. Bersinger N a., Vanderlick F, Birkhäuser MH, Janecek P, Wunder D. First trimester serum concentrations of placental proteins in singleton and multiple IVF pregnancies: implications for Down syndrome screening. *Immuno-analyse Biol Spécialisée*. 2005;20(1):21-27. doi:10.1016/j.immbio.2004.11.006.

24. Bredaki FE, Wright D, Akolekar R, Cruz G, Nicolaides KH. Maternal serum alpha-fetoprotein in normal pregnancy at 11-13 weeks' gestation. *Fetal Diagn Ther*. 2011;30(4):274-279. doi:10.1159/000330200.

25. Cowans NJ, Spencer K. Effect of gestational age on first trimester maternal serum prenatal screening correction factors for ethnicity and IVF conception. *Prenat Diagn*.

2013;33(1):56-60. doi:10.1002/pd.4010.

26. Engels M a JM, Pajkrt E, Groot DDT, Schats R, Twisk JWR, van Vugt JJMG. Validation of correction factors for serum markers for first-trimester Down syndrome screening in singleton pregnancies conceived with assisted reproduction. *Fetal Diagn Ther*. 2013;34(4):217-224. doi:10.1159/000355527.

27. Frishman GN, Canick JA, Hogan JW, Kellner LH, Saller DN. Serum Triple-Marker Screening in In Vitro Fertilization and Naturally Conceived Pregnancies. 1997;7844(97):98-101.

28. Ghisoni L, Ferrazzi E, Castagna C, Setti PEL, Masini AC, Pigni A. Prenatal Diagnosis after ART Success : The Role of Early Combined. 2003;4004:99-103.

29. Giorgetti C, Vanden Meerschaut F, De Roo C, et al. Multivariate analysis identifies the estradiol level at ovulation triggering as an independent predictor of the first trimester pregnancy-associated plasma protein-A level in IVF/ICSI pregnancies. *Hum Reprod*. 2013;28(10):2636-2642. doi:10.1093/humrep/det295.

30. Gjerris AC, Loft A, Pinborg A, Christiansen M, Tabor A. First-trimester screening markers are altered in pregnancies conceived after IVF/ICSI. *Ultrasound Obstet Gynecol*. 2009;33(1):8-17. doi:10.1002/uog.6254.

31. Heinonen S, Hippelainen M, Ryynanen M, Saarikoski S, Kirkinen P. Effect of in vitro fertilization on human chorionic gonadotropin serum ceoncentrations and Down's syndrome screening. *Fertil Steril*. 1996;66(3):398-403.

32. Hui PW. Maternal serum hCG and alpha-fetoprotein levels in pregnancies conceived after IVF or ICSI with fresh and frozen-thawed embryos. *Hum Reprod*. 2003;18(3):572-575. doi:10.1093/humrep/deg153.

33. Hui PW, Tang MHY, Lam YH, Yeung WSB, Ng EHY, Ho PC. Nuchal translucency in pregnancies conceived after assisted reproduction technology. *Ultrasound Obstet Gynecol*. 2005;25(3):234-238. doi:10.1002/uog.1846.

34. Hui PW, Lam YH, Tang MHY, Ng EHY, Yeung WSB, Ho PC. Maternal serum pregnancy-associated plasma protein-A and free beta-human chorionic gonadotrophin in pregnancies conceived with fresh and frozen-thawed embryos from in vitro fertilization and intracytoplasmic sperm injection. *Prenat Diagn*. 2005;25(5):390-393. doi:10.1002/pd.1169.

35. Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound Obstet Gynecol*. 2008;31(5):493-502. doi:10.1002/uog.5332.

36. Lam YH, Shu W, Yeung B, et al. Maternal serum alpha-fetoprotein and human chorionic gonadotrophin in pregnancies conceived after intracytoplasmic sperm injection and conventional in-vitro fertilization. *Hum Reprod*. 1999;14(8):2120-2123.

37. Lambert-Messerlian G, Palomaki GE, Canick J a. Adjustment of serum markers in first trimester screening. *J Med Screen*. 2009;16(2):102-103. doi:10.1258/jms.2009.009028.

38. Liao a W, Heath V, Kametas N, Spencer K, Nicolaides KH. First-trimester screening for trisomy 21 in singleton pregnancies achieved by assisted reproduction. *Hum Reprod*. 2001;16(7):1501-1504. http://www.ncbi.nlm.nih.gov/pubmed/11425838.

39. Matilainen M, Peuhkurinen S, Laitinen P, Jarvela I, Morin-Papunen L, Ryynanen M. In combined first-trimester Down syndrome screening, the false-positive rate is not higher in pregnancies conceived after assisted reproduction compared with spontaneous pregnancies. *Fertil Steril*. 2011;95(1):378-381. doi:10.1016/j.fertnstert.2010.07.1048.

40. Maymon R, Shulman A. Serial first- and second-trimester Down's syndrome screening tests among IVF- versus naturally-conceived singletons. *Hum Reprod*. 2002;17(4):1081-1085.

41. Maymon R, Shulman A. Integrated first- and second-trimester Down syndrome screening test among unaffected IVF pregnancies. *Prenat Diagn*. 2004;24(2):125-129. doi:10.1002/pd.809.

42. Muller F, Dreux S, Lemeur A, et al. Medically assisted reproduction and second-trimester maternal serum marker screening for Down syndrome. *Prenat Diagn*. 2003;23(13):1073-1076. doi:10.1002/pd.756.

43. Niemimaa M, Heinonen S, Seppala M, Hippelainen M, Martikainen H, Ryynanen M. First-trimester screening for Down ' s syndrome in in vitro fertilization. *Fertil Steril*. 2001;76(6):1282-1283.

44. Orlandi F, Rossi C, Allegra A, et al. First trimester screening with free b -hCG , PAPP-A and nuchal translucency in pregnancies conceived with assisted reproduction. *Prenat Diagn*. 2002;22(March):718-721.

45. Raty R, Virtanen A, Koskinen P, et al. Serum free β -HCG and alpha-fetoprotein levels in IVF , ICSI and frozen embryo transfer pregnancies in maternal mid-trimester serum screening for Down ' s syndrome. *Hum Reprod*. 2002;17(2):481-484.

46. Ribbert L, Kornman L, De Wolf B, et al. Maternal serum screening for fetal Down syndrome in IVF pregnancies. *Prenat Diagn*. 1996;1:35-38.

47. Rice JD, McIntosh SF, Halstead AC. Second-trimester maternal serum screening for Down syndrome in in vitro fertilization pregnancies. *Prenat Diagn*. 2005;25(3):234-238. doi:10.1002/pd.1116.

48. Tul N, Novak-Antolic Z. Serum PAPP-A levels at 10 – 14 weeks of gestation are altered. *Prenat Diagn*. 2006;26(October):1206-1211. doi:10.1002/pd.

49. Wald N, White N, Morris J, Huttly W, Canick J. Serum markers for Down ' s syndrome in women who have had in vitro fertilisation : implications for antenatal screening. *Br J Obstet Gynaecol*. 1999;106(December):1304-1306.

50. Wøjdemann KR, Larsen SO, Shalmi A, Sundberg K, Christiansen M, Tabor A. First trimester screening for Down syndrome and assisted reproduction: no basis for concern. *Prenat Diagn*. 2001;21(March):563-565.

51. Amor DJ, Xu JX, Halliday JL, et al. Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for Down syndrome. *Hum Reprod*. 2009;24(6):1330-1338. doi:10.1093/humrep/dep046.

52. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Hum Reprod*. 2007;22(6):1506-1512. doi:10.1093/humrep/dem046.

53. Hui L. Non-invasive prenatal testing for fetal aneuploidy: charting the course from clinical validity to clinical utility. *Ultrasound Obstet Gynecol*. 2013;41(1):2-6. doi:10.1002/uog.12360.

54. Shi X, Zhang Z, Cram DS, Liu C. Feasibility of noninvasive prenatal testing for common fetal aneuploidies in an early gestational window. *Clin Chim Acta*. 2015;439:24-28. doi:10.1016/j.cca.2014.09.032.

The following supplemental information for Manuscript 1 can be found in Appendix B:

Supplemental Text B-1	Primary search strategy
Supplemental Table B-1	General characteristics of the 40 included studies
Supplemental Table B-2	Exposure and outcome information from the 40 included studies
Supplemental Text B-2	Reprint of published article

Chapter 4. [Manuscript 2] Comparing maternal serum screening markers among IVF and spontaneous conceptions in Ontario through registry data

4.1 Preface to Manuscript 2

The systematic review presented in Chapter 3 showed that there was consistently a decrease in PAPP-A and an increase in total hCG reported among IVF patients. We wanted to examine if the same patterns persisted among the IVF population in Ontario, Canada. This study used data from all pregnancies that had maternal serum screening marker screening performed in Ontario from March 19, 2013 to May 27, 2015, as well as all Ontario IVF conception data from the corresponding time period. The use of these comprehensive data allowed for a clear illustration of current results for the entire province.

Currently, the use of IVF is self-reported by the patient at the time of the maternal serum screen. We wanted to ensure that patients were appropriately identifying IVF use to assist with conception and not mistaking it with medication used to induce ovulation (e.g., clomiphene citrate). This study leveraged the CARTR Plus dataset and determined the concordance and discordance in the identification of IVF use for assisted conception in the Ontario population.

This study also proposed new adjustment factors for the use of IVF when maternal serum screening markers are used to determine the risk of Down syndrome and trisomy 18. Therefore, these proposed new adjustment factors may improve the accuracy of the algorithm used to obtain risk estimates for these aneuploidies by prenatal screening labs in Ontario; thereby presenting more appropriate results for clinicians and patients to use to determine whether or not to perform an invasive procedure.

This Manuscript 2 is currently submitted to Journal of Medical Screening.

4.2 Title page

Title: Comparing maternal serum screening markers among IVF and spontaneous conceptions in Ontario through registry data

Authors:

Andrea Lanes[a,b], Shelley Dougan[b,f], Deshayne B. Fell[a,f], Tianhua Huang[b], Ann Sprague[b,f], Moya Johnson[b], Arthur Leader[d,e], Beth Potter[a], Nan Okun[g], Mark Walker[b,c,d,f]

Affiliations:

[a]University of Ottawa School of Epidemiology, Public Health and Preventive Medicine

[b]BORN Ontario

[c]Ottawa Hospital Research Institute

[d]University of Ottawa Department of Obstetrics and Gynecology

[e]Ottawa Fertility Centre

[f]CHEO Research Institute

[g]Mt. Sinai Hospital

4.3 Abstract

Objective: The objectives of this study were: 1) to investigate the accuracy of IVF identification on the prenatal screening record from prenatal screening labs; 2) to compare the screening markers in IVF and non-IVF pregnancies in the population of Ontario; and 3) to propose more appropriate IVF adjustment factors for the Ontario population.

Methods: Two years of IVF treatment data from all fertility clinics in Ontario were merged with the corresponding prenatal screening data from all 5 prenatal screening centres. New adjustment factors for IVF were developed for each MSS marker and NT measurement. Means and standard deviations and linear regression models were reported for all prenatal screening records, as well as for those who had IVF identified through the prenatal screening requisition and those who were identified through the CARTR Plus database.

Results: Significant differences between IVF and non-IVF groups based on the prenatal screening requisition information and CARTR Plus information were found among the ethnicity adjusted mean MoMs for AFP, PAPP-A, µE3, first trimester hCG, total hCG, and DIA.

Conclusion: This study proposed alternate IVF adjustment factors that will produce more accurate screening results within the population of Ontario.

Keywords: in vitro fertilization; maternal serum screening; adjustment factors

4.4 Introduction

Multiple marker prenatal screening uses maternal serum markers and sonographically-determined fetal nuchal translucency (NT) to estimate a women's risk of having a fetus affected with Down syndrome, Trisomy 18 and open Neural Tube Defects. These maternal serum screening (MSS) markers are measured by the multiple of the median (MoM). It is the median of the concentration of the marker screened compared to the population at a specific gestational age. It is recognized that among some different clinical populations, the mean MoM does not remain close to 1.00 (personal communication: S. Dougan October 24, 2016). In order to obtain accurate risk assessments for a fetus, adjustment factors are applied to return the MoM to 1.00. In Ontario, MSS marker MoMs are modified with a combination of adjustment factors for ethnicity, diabetes, smoking and in vitro fertilization (IVF) use (personal communication: S. Dougan October 24, 2016).

Previous studies have shown that the first and second trimester serum markers used for Down syndrome screening are significantly altered in pregnancies achieved through IVF pregnancies compared to spontaneously conceived pregnancies.[1–6] In IVF pregnancies, first trimester pregnancy associated plasma protein A (PAPP-A) is lower and free β-human chorionic gonadotrophin (free β-hCG) may be higher.[1,3] The second trimester Alpha fetoprotein (AFP), total hCG and dimeric inhibin A (DIA) are also elevated.[2,3,7] In addition, the variations in the concentration of serum markers in IVF pregnancies may be associated with the specific type of assisted reproduction treatment used.[3,4,6] Studies have also suggested variations in NT measurement in IVF pregnancies.[4,8] The alterations in the serum markers in IVF pregnancies might reflect subtle metabolic changes or some underlying pathology unique to pregnancies in subfertile patients.[7] They might also be caused by one or more elements of assisted reproduction treatments, such as ovarian stimulation or in vitro culture.[3,6]

Information about the IVF treatment used is an important parameter in prenatal screening for Down syndrome. Given the known changes in the screening markers in IVF pregnancies described above, women with pregnancies conceived through IVF will be more likely to receive a false positive screening result.[9] Without adequate adjustments made to their screen interpretation, these women may undergo unnecessary follow-up testing, including invasive diagnostic tests, such as chorionic villus sampling or amniocentesis. In addition, because the risk of Down syndrome is associated with the maternal age[10] of the oocyte donor among IVF

pregnancies, missing or incorrect information on data surrounding fresh and frozen embryo transfers, will impact the accuracy of screening results.

Further contributing to challenges in accurately predicting risk based on prenatal screening for IVF pregnancies is the increased use of frozen embryo transfers (FET).[11] FET reduce the potential for treatment related complications, such as ovarian hyperstimulation syndrome; moreover, clinical pregnancy rates from FET have improved and are comparable to fresh embryo transfer.[12] Therefore, FET have become more common, but it is not standard practice in all centres.[13]

In prenatal screening labs in Ontario, the standard requisition contains a field for the ordering health care providers to identify an IVF pregnancy. Published adjustment factors for first trimester PAPP-A, second trimester unconjugated estriol (μE^3) and total hCG are applied to the MoM values of these serum makers for all pregnancies in which IVF treatment has been documented.

However, the completeness and accuracy of this information is unclear. Thus it is not certain whether the IVF adjustment factors are accurate for the Ontario population, if they are based on what is recorded on the prenatal screening requisitions. We carried out this study in order to: 1) investigate the accuracy of IVF identification on the prenatal screening record (sourced from the prenatal screening requisition from prenatal screening labs (PSL)); 2) compare the screening markers in IVF and non-IVF pregnancies in the population of Ontario; and 3) propose updated IVF adjustment factors for the Ontario population, based on more accurate coding for IVF status.

4.5 Methods

Data sources and study population:

The Better Outcomes Registry & Network (BORN) Ontario routinely collects comprehensive information in the BORN Information System (BIS) on prenatal screening from all 5 prenatal screening labs in the province, as well as information on the use of IVF from all 18 fertility clinics in Ontario through the Canadian Assisted Reproductive Technologies Register (CARTR) Plus database. Prenatal screening data and fertility data were merged creating a linked dataset. This study was based on a conception cohort of spontaneous pregnancies and pregnancies achieved through IVF and FET treatment cycles from January 1, 2013 through December 31, 2014. These

records were deterministically linked with their corresponding prenatal screening records (March 19, 2013 to May 27, 2015). This study included all prenatal screening records during this timeframe; both spontaneous conceptions and IVF conceptions documented on the prenatal screening record from PSL. Seventy percent of all pregnancies in Ontario have prenatal screening performed.[14] These analyses used all records for Ontario, Canada. There were 218,924 records from the prenatal screening labs included in this analysis and 21,140 records of IVF and FET conceptions from CARTR Plus (Figure 4-1).

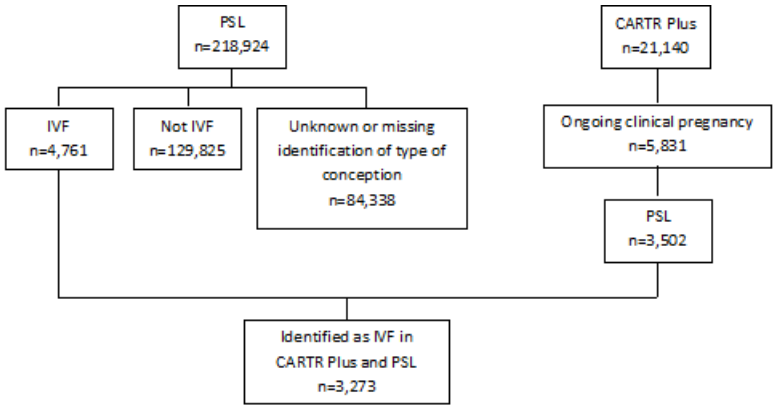


Figure 4-1 Study flow diagram

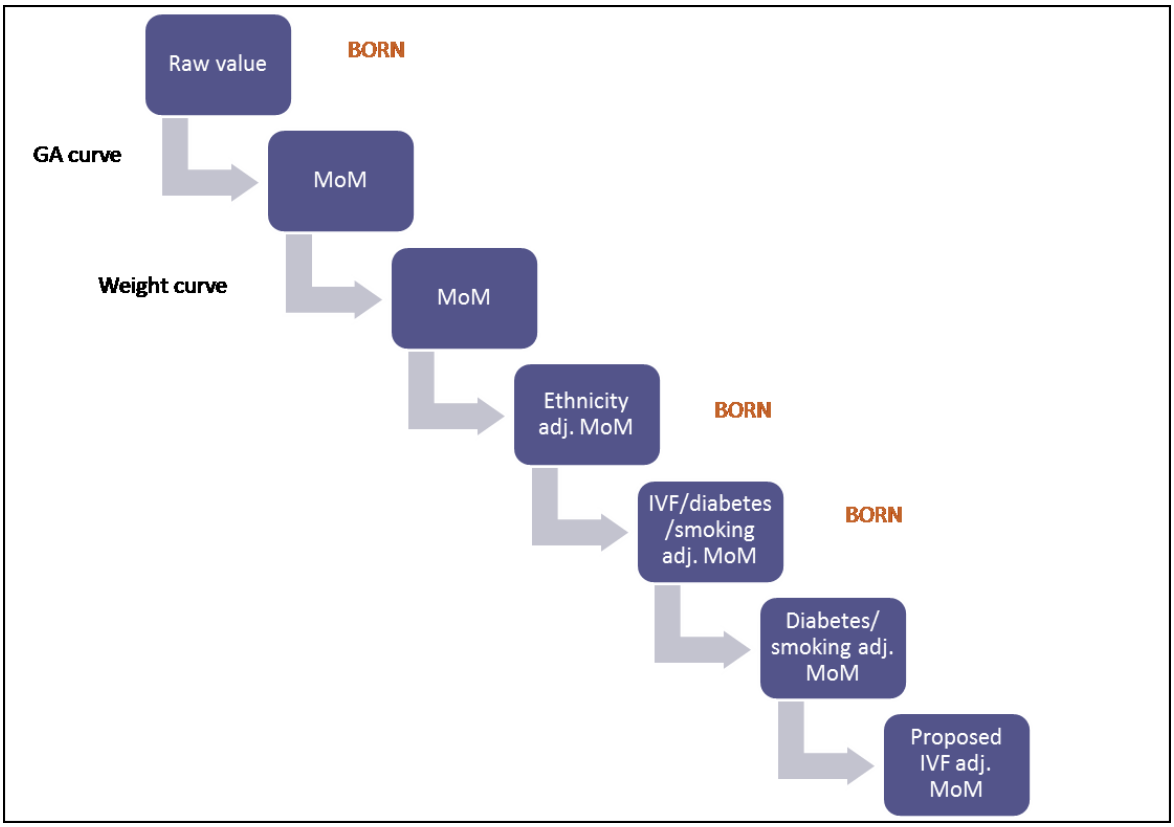


Figure 4-2 Flow diagram of MSS marker adjustments

Analysis:

Figure 4-2 illustrates the application of adjustments to MSS markers in Ontario. Currently, only first trimester PAPP-A, second trimester total hCG and μE^3 have an IVF adjustment factor applied. This is a multiplicative adjustment that is applied to each MSS marker. These adjustment factors are common among four prenatal screening labs and unique among one additional lab. For this study, we first removed the IVF adjustment factors for PAPP-A, total hCG, and μE^3 MoMs from the adjusted MoMs for all IVF records identified by the prenatal screening requisition (Equation 4-1). A new 'proposed' adjustment factor for IVF was then developed for each MSS marker and NT measurement that allowed the mean MoM to be as close to 1.00 as possible (Table 4-1). These proposed adjustment factors were then applied to the MSS marker MoMs for women who used IVF to conceive as identified by CARTR Plus.

Equation 4-1 Calculation of adjustment MSS marker MoMs

adjusted MoM $= MoM_{Eth} \times ADJ_{DBM} \times ADJ_{smk} \times ADJ_{IVF}$

ADJ=adjustment factor
DBM = Diabetes mellitus
Smk = smoker
IVF = in vitro fertilization used to conceive

Table 4-1 Proposed IVF adjustment factors for IVF identified pregnancies

MSS Marker	Proposed adjustment factor
PAPP-A	0.74
1st trimester β-hCG	0.69
FB-hCG	0.77
AFP	0.82
µE3	0.84
Total hCG	0.97
DIA	0.79
NT measurement	0.95

For each MSS marker and NT measurement, log10 transformations were performed in order to account for the skewed distributions. Means and standard deviations were determined for all prenatal screening records (model 1), as well as for those who had IVF identified through the prenatal screening requisition (model 2) and those who were identified through the CARTR Plus database (model 3). Linear regression models were produced for both of these scenarios with a variety of adjustments:

1. we modeled MSS marker MoMs that had an ethnicity adjustment with IVF status identified on the prenatal screening requisition by the PSL for all women who had a prenatal screening record;

2. we modeled MSS marker MoMs that had adjustments for ethnicity, diabetes and smoking with IVF status identified on the prenatal screening requisition by the PSL for all women who had a prenatal screening record;

3. we modeled MSS marker MoMs that had an ethnicity adjustment with IVF status identified in CARTR Plus for all women who had a prenatal screening record; and

4. we modeled MSS marker MoMs that had adjustments for ethnicity, diabetes and smoking with IVF status identified in CARTR Plus for all women who had a prenatal screening record.

Additional analyses were performed to compare the proposed IVF adjustment factors to the IVF adjustment factors used by the prenatal screening labs, and to a group with no IVF adjustment, for those women identified through CARTR Plus. Descriptive statistics were reported for these models and the Wilcoxon Sign Test was used to assess significance. Conceptions using IVF and FET were examined separately. Additional descriptive analyses examined the effect of intracytoplasmic sperm injection (ICSI) on MSS marker MoMs among IVF pregnancies identified in CARTR Plus.

Ethics approval for this study were obtained from the Children's Hospital of Eastern Ontario Research Ethics Board (CHEOREB#15/06PE), the Ottawa Health Science Network Research Ethics Board (20150644-01H) and the Mount Sinai Research Ethics Board (MSH REB 15-0282-C).

4.6 Results

We investigated the differences in documented mean maternal age at estimated date of delivery for the IVF and non-IVF groups as identified by both the prenatal screening requisition and CARTR Plus. We found that the mean maternal age was significantly different ($p<0.01$) between IVF and non-IVF pregnancies among both the PSL and CARTR Plus records. The mean maternal age was 35.6 years (SD±5.15) for the IVF group and 31.8 years (SD±5.03) for the non-IVF group, as identified on the prenatal screening requisition, and 35.7 years (SD±4.71) for the IVF group and 31.6 years (SD±5.07) for the non-IVF group as identified by the CARTR Plus record. There were 366 records identified in CARTR Plus where women used donor oocytes or embryos to conceive. The mean oocyte provider age was significantly different among women who

exclusively used their own oocytes or embryos (μ=34.1 years SD±3.98) as compared to women who used donor oocytes or embryos (μ=28.9 years SD±6.52).

Objective #1:

We first assessed the concordance and discordance of information about IVF conceptions as collected on the prenatal screening requisitions and uploaded to the BIS, compared to information collected in the CARTR Plus database, in order to determine if improvements were needed. When identification of the use of IVF as documented on the prenatal screening record was compared to the referenced standard (CARTR Plus database), the sensitivity was 95.8% and the specificity was 98.9%, the negative predictive value was 99.9%; however the positive predictive value was 68.8%.

Objective #2:

Significant differences between the identified IVF and non-IVF groups based on the prenatal screening requisition information (model 2) and CARTR Plus information (model 3) were found among the ethnicity adjusted mean MoMs for AFP, PAPP-A, μE3, first trimester hCG, total hCG, and DIA (Table 4-2**Error! Not a valid bookmark self-reference.**). Similarly, significant differences were seen among the ethnicity, smoking and diabetes adjusted mean MoMs for AFP, PAPP-A, μE3, total hCG and DIA for model 2 and AFP and DIA for model 3. NT measurement was significantly different in model 3. The largest mean differences between IVF and spontaneous conceptions were seen among AFP, PAPP-A, total hCG and DIA. NT measurement MoM was not influenced by the use of IVF to assist with conception; whether an IVF pregnancy was identified through the PSL record or CARTR Plus.

Objective #3:

When we developed the proposed adjustment factor to all CARTR Plus identified pregnancies we found that for PAPP-A, total hCG, and μE^3 the mean adjusted marker MoMs were significantly closer to 1.00, as compared to the prenatal screening adjusted or the unadjusted mean marker MoMs (Figure 4-3). Although there currently is no adjustment made to the other MSS markers and NT measurement, when we applied the proposed adjustment factors the mean MoMs were all closer to 1.00 as compared to the unadjusted mean MoMs (Figure 4-3).

Among the IVF cycles identified through CARTR Plus, the majority of embryos transferred resulting in conception cycles were at the blastocyst stage (63.9%) or at the cleavage stage (31.5%). The proportion of cycles that had one or two embryos transferred per embryo transfer cycle was similar (47.7% and 46.5% respectively) and 5.8% of embryo transfer cycles had three or more embryos transferred. Among FET cycles, 78.2 percent used vitrification to cryopreserve the embryos as opposed to the older slow freezing technique (21.8%). The method of insemination (conventional IVF or ICSI) was investigated for prenatal screening records that were in CARTR Plus. Only IVF cycles and FET cycles using own oocytes were included. Significant differences were found for the mean AFP, PAPP-A, total hCG, μE^3, DIA, and NT measurement mean MoMs with the proposed adjustment factor applied when we stratified by insemination method.

Table 4-2 Results of adjusted linear regression models for MSS markers and NT measurement

				95%CI	
	Mean	**±SD**	**Estimate**	**Lower**	**Upper**
PAPP-A					
Ethnicity adjusted MoM (log10)					
Model 1 (no IVF) n=191,763	-0.009	0.252			
Model 2 (PSO IVF status) n= 119,420					
No IVF	-0.011	0.248	0.0000	0.0000	0.0000
IVF	-0.040	0.269	0.0039	-0.0372	-0.0218
Model 3 (CARTR Plus IVF status) n=191,763					
No IVF	-0.008	0.251	0.0000	0.0000	0.0000
IVF	-0.041	0.269	0.0045	-0.0416	-0.0238
Ethnicity, smoking, diabetes adjusted MoM (log10)					
Model 1 (no IVF) n=153,227	0.040	0.253			
Model 2 (PSO IVF status) n=80,924					
No IVF	0.037	0.250	0.0000	0.0000	0.0000
IVF	0.050	0.269	0.0048	0.0034	0.0222
Model 3 (CARTR Plus IVF status) n=153,227					
No IVF	0.040	0.252	0.0000	0.0000	0.0000

IVF	0.050	0.267	0.0054	-0.0010	0.0203
1st trimester hCG					
Ethnicity adjusted MoM (log10)					
Model 1 (no IVF) n=7,736	-0.002	0.203			
Model 2 (PSO IVF status) n=4,993					
No IVF	-0.009	0.202	0.0000	0.0000	0.0000
IVF	0.042	0.203	0.0504	0.0251	0.0757
Model 3 (CARTR Plus IVF status) n=7,736					
No IVF	-0.003	0.202	0.0000	0.0000	0.0000
IVF	0.057	0.201	0.0604	0.0249	0.0959
Ethnicity, smoking, diabetes adjusted MoM (log10)					
Model 1 (no IVF) n=2,764	0.024	0.198			
Model 2 (PSO IVF status) n=24					
No IVF	0.021	-	0.0000	0.0000	0.0000
IVF	0.137	0.232	0.1159	-0.3280	0.5598
Model 3 (CARTR Plus IVF status) n=2,764					
No IVF	0.023	0.198	0.0000	0.0000	0.0000
IVF	0.106	0.227	0.0832	-0.0209	0.1873
free-β hCG					
Ethnicity adjusted MoM (log10)					
Model 1 (no IVF) n=21,987	0.009	0.273			
Model 2 (PSO IVF status) n=17,903					
No IVF	0.009	0.272	0.0000	0.0000	0.0000
IVF	0.006	0.266	-0.0033	-0.0249	0.0183
Model 3 (CARTR Plus IVF status) n=21,987					
No IVF	0.009	0.273	0.0000	0.0000	0.0000
IVF	0.007	0.276	-0.0024	-0.0264	0.0216
Ethnicity, smoking, diabetes adjusted MoM (log10)					

Model 1 (no IVF) n=12,205	0.014	0.277			
Model 2 (PSO IVF status) n=8137					
No IVF	0.015	0.276	0.0000	0.0000	0.0000
IVF	0.016	0.261	0.0014	-0.0435	0.0463
Model 3 (CARTR Plus IVF status) n=12,205					
No IVF	0.014	0.277	0.0000	0.0000	0.0000
IVF	0.023	0.274	0.0092	-0.0453	0.0637
AFP					
Ethnicity adjusted MoM (log10)					
Model 1 (no IVF) n=183,166	0.007	0.148			
Model 2 (PSO IVF status) n= 109,181					
No IVF	0.005	0.146	0.0000	0.0000	0.0000
IVF	0.053	0.169	0.0477	0.0428	0.0526
Model 3 (CARTR Plus IVF status) n=183,166					
No IVF	0.006	0.147	0.0000	0.0000	0.0000
IVF	0.048	0.173	0.0416	0.0360	0.0472
Ethnicity, smoking, diabetes adjusted MoM (log10)					
Model 1 (no IVF) n=159,295	0.006	0.148			
Model 2 (PSO IVF status) n= 84,147					
No IVF	0.005	0.147	0.0000	0.0000	0.0000
IVF	0.050	0.168	0.0454	0.0399	0.0509
Model 3 (CARTR Plus IVF status) n=159,295					
No IVF	0.005	0.148	0.0000	0.0000	0.0000
IVF	0.046	0.173	0.0031	0.0345	0.0467
uE3					
Ethnicity adjusted MoM (log10)					
Model 1 (no IVF) n=175,873	-0.002	0.118			
Model 2 (PSO IVF status) n= 103,372					

No IVF	-0.002	0.119	0.0000	0.0000	0.0000
IVF	0.009	0.123	0.0111	0.007	0.0151
Model 3 (CARTR Plus IVF status) n=175,873					
No IVF	-0.002	0.118	0.0000	0.0000	0.0000
IVF	0.004	0.124	0.0058	0.0012	0.0103
Ethnicity, smoking, diabetes adjusted MoM (log10)					
Model 1 (no IVF) n=152,002	0.027	0.118			
Model 2 (PSO IVF status) n= 79,522					
No IVF	0.026	0.120	0.0000	0.0000	0.0000
IVF	0.069	0.123	0.0428	0.0382	0.0473
Model 3 (CARTR Plus IVF status) n=152,002					
No IVF	0.026	0.118	0.0000	0.0000	0.0000
IVF	0.062	0.123	0.0097	-0.001	0.0203
Total hCG					
Ethnicity adjusted MoM (log10)					
Model 1 (no IVF) n=175,874	-0.004	0.228			
Model 2 (PSO IVF status) n= 103,371					
No IVF	-0.004	0.226	0.0000	0.0000	0.0000
IVF	0.046	0.240	0.0503	0.0426	0.058
Model 3 (CARTR Plus IVF status) n=175,874					
No IVF	-0.005	0.227	0.0000	0.0000	0.0000
IVF	0.041	0.243	0.046	0.0372	0.0547
Ethnicity, smoking, diabetes adjusted MoM (log10)					
Model 1 (no IVF) n=152,004	-0.056	0.227			
Model 2 (PSO IVF status) n= 79,522					
No IVF	-0.055	0.226	0.0000	0.0000	0.0000
IVF	-0.046	0.239	0.0088	0.0003	0.0174
Model 3 (CARTR Plus IVF status) n=152,004					

No IVF	-0.056	0.227	0.0000	0.0000	0.0000
IVF	-0.050	0.241	0.0065	-0.0031	0.0161
DIA					
Ethnicity adjusted MoM (log10)					
Model 1 (no IVF) n=19,952	0.014	0.194			
Model 2 (PSO IVF status) n=10,845					
No IVF	0.011	0.195	0.0000	0.0000	0.0000
IVF	0.075	0.214	0.0633	0.0383	0.0883
Model 3 (CARTR Plus IVF status) n=19,952					
No IVF	0.013	0.194	0.0000	0.0000	0.0000
IVF	0.056	0.207	0.0427	0.0139	0.0716
Ethnicity, smoking, diabetes adjusted MoM (log10)					
Model 1 (no IVF) n=19,254	-0.009	0.189			
Model 2 (PSO IVF status) n=10,148					
No IVF	-0.011	0.189	0.0000	0.0000	0.0000
IVF	0.069	0.216	0.0804	0.0539	0.1069
Model 3 (CARTR Plus IVF status) n=19,254					
No IVF	-0.010	0.189	0.0000	0.0000	0.0000
IVF	0.052	0.208	0.0614	0.031	0.0918
NT Measurement					
Unadjusted MoM (log10)					
Model 1 (no IVF) n=190,093	0.005	0.105			
Model 2 (PSO IVF status) n=134,342					
No IVF	0.008	0.105	0.0000	0.0000	0.0000
IVF	0.009	0.106	0.0008	-0.0024	0.0039
Model 3 (CARTR Plus IVF status) n=190,093					
No IVF	0.005	0.105	0.0000	0.0000	0.0000
IVF	0.009	0.106	0.0036	0.0000	0.0072

4.7 Discussion

This study clearly demonstrated that in the Ontario population the algorithm used for IVF adjustment of maternal serum screening results would benefit from the proposed IVF adjustment factors. Large differences in mean MoMs for the PSL and CARTR Plus models were seen for PAPP-A, total hCG and µE3, which was appropriate given that these three MSS markers were the ones that currently have adjustment factors for IVF applied (Table 4-2). However, large differences between the IVF group and non-IVF groups were also observed for AFP and DIA, suggesting that these two markers would also benefit from an IVF adjustment factor.

When we compared the prenatal screening results from all pregnant women who had MSS and were identified through CARTR Plus as having used IVF, we found that the proposed adjustment factor for IVF improved the mean MSS marker MoMs to a greater extent than the current PSL IVF adjustment factors (Figure 4-3: A,B,C). Slight differences were also observed between IVF and FET cycles for each marker. It was important that these mean MSS marker MoMs were extremely close to 1.00 in order for it to be representative of the Ontario population. Interestingly, the current PSL IVF adjustment factor for total hCG adjusted the mean MoM in the wrong direction; drawing it further away from 1.00. Wald et al.,[15] have postulated the increased total hCG MoM among IVF conceptions are due to increased level of progesterone after IVF treatment. As we were examining total hCG in isolation, decreasing the mean MoM in order for it to be closer to 1.00 would reduce the false-positive rates for Down syndrome.

Previously, the identification of IVF on the prenatal screening requisition through self-report was the most reliable source for the algorithm used to detect Down syndrome and Trisomy 18. Since there is a short time lag between receipt of fertility treatment and entering fertility treatment data into CARTR Plus, the prenatal screening labs in Ontario are not able to use this information to inform whether an IVF adjustment should be applied; however having a more appropriate IVF adjustment factor based on the entire IVF population for Ontario would strengthen the accuracy of the algorithm's results using the information on the prenatal screening requisition.

Additionally, there was a significant difference found between the use of conventional IVF and ICSI for the ethnicity, smoking and diabetes adjusted mean MoMs for AFP, PAPP-A, µE3, first total hCG, and DIA. Differences in PAPP-A MoM among ICSI pregnancies have previously been documented and are believed to be due to the use of hormones in ovarian stimulation and

imprinting events which lead to the interruption of the maturation of the placenta[7,16–20]. It may be prudent to account for the method of insemination in determining an appropriate IVF adjustment factor or provide an additional adjustment for method of insemination.

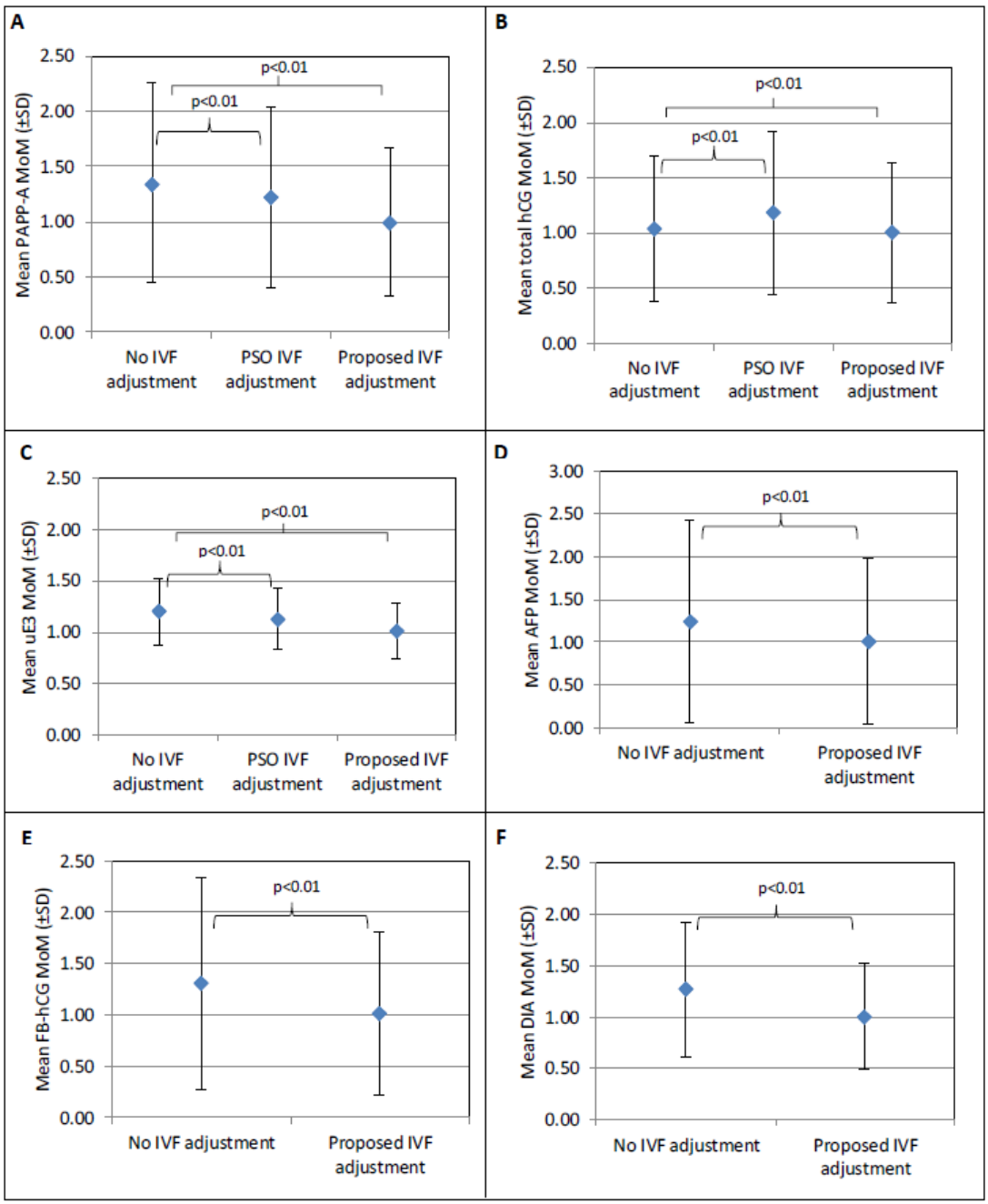


Figure 4-3 Comparing the MSS marker MoM for markers with and without a current PSL IVF adjustment factor

This study benefited from the use of comprehensive databases that included all records in Ontario for both prenatal screening and fertility treatment. This allowed the results to confidently be generalized for this population. The main limitation of this study was that we investigated each MSS marker in isolation and the algorithm that produces the calculated risk of Down syndrome and Trisomy 18 integrates the MSS marker MoMs from multiple markers. Further studies to assess the difference in false-positive and false-negative rates among the various IVF adjustment factors should be performed.

4.8 Conclusions

This study showed the high sensitivity and specificity of the PSL record for identifying IVF treatment use among women who have MSS performed, however of all PSL records identified as IVF only two thirds were confirmed IVF pregnancies by CARTR Plus. This study proposed alternate IVF adjustment factors that can be used to modify the current algorithm for detecting risk of aneuploidies among IVF pregnancies in Ontario. These adjustment factors will produce more accurate screening results within this population and will decrease the false positive and false negative rates.

Disclosure of interests

The authors have no conflicts of interest to declare.

Acknowledgements

A. Lanes was supported by the Queen Elizabeth II Graduate Scholarships in Science and Technology while this study was being conducted.

4.9 References

1. Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound Obstet Gynecol*. 2008;31(5):493-502. doi:10.1002/uog.5332.

2. Rice JD, McIntosh SF, Halstead AC. Second-trimester maternal serum screening for Down syndrome in in vitro fertilization pregnancies. *Prenat Diagn*. 2005;25(3):234-238. doi:10.1002/pd.1116.

3. Lambert-Messerlian G, Dugoff L, Vidaver J, et al. First- and second-trimester Down syndrome screening markers in pregnancies achieved through assisted reproductive technologies (ART): A FASTER trial study †. *Prenat Diagn*. 2006;(January):672-678. doi:10.1002/pd.

4. Gjerris AC, Loft A, Pinborg A, Christiansen M, Tabor A. First-trimester screening markers are altered in pregnancies conceived after IVF/ICSI. *Ultrasound Obstet Gynecol*. 2009;33(1):8-17. doi:10.1002/uog.6254.

5. Engels M a J, Kooij M, Schats R, et al. First-trimester serum marker distribution in singleton pregnancies conceived with assisted reproduction. *Prenat Diagn*. 2010;30(4):372-377. doi:10.1002/pd.2495.

6. Engels M a JM, Pajkrt E, Groot DDT, Schats R, Twisk JWR, van Vugt JJMG. Validation of correction factors for serum markers for first-trimester Down syndrome screening in singleton pregnancies conceived with assisted reproduction. *Fetal Diagn Ther*. 2013;34(4):217-224. doi:10.1159/000355527.

7. Tul N, Novak-Antolic Z. Serum PAPP-A levels at 10 – 14 weeks of gestation are altered. *Prenat Diagn*. 2006;26(October):1206-1211. doi:10.1002/pd.

8. Maymon R, Shulman A. Integrated first- and second-trimester Down syndrome screening test among unaffected IVF pregnancies. *Prenat Diagn*. 2004;24(2):125-129. doi:10.1002/pd.809.

9. Liao a W, Heath V, Kametas N, Spencer K, Nicolaides KH. First-trimester screening for trisomy 21 in singleton pregnancies achieved by assisted reproduction. *Hum Reprod*. 2001;16(7):1501-1504. http://www.ncbi.nlm.nih.gov/pubmed/11425838.

10. Carroll J, Allanson J, Epslen M, et al. *Reference Guide for Health Care Providers Prenatal Screening Tests for the Detection of : Down Syndrome, Trisomy 18 and Open Neural Tube Defects*.; 2007.

11. Chaudhuri AR, Chatterjee S. Frozen embryo transfer: the present practice and beyond. *J Basic Clin Physiol Pharmacol*. 2013;24(2):125-130. doi:10.1515/jbcpp-2012-0052.

12. Evans J, Hannan NJ, Edgell T a, et al. Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence. *Hum Reprod Update*. 2014;20(6):808-821. doi:10.1093/humupd/dmu027.

13. Better Outcomes Registry & Network Ontario. Canadian Reproductive Technologies Registry (CARTR) Plus. Treatment cycles for 2013.

14. BORN Ontario. *Better Outcomes Registry & Network (BORN) Ontario Annual Report 2012–2013 and 2013–2014*.; 2015.

15. Wald N, White N, Morris J, Huttly W, Canick J. Serum markers for Down ' s syndrome in women who have had in vitro fertilisation : implications for antenatal screening. *Br J Obstet Gynaecol*. 1999;106(December):1304-1306.

16. Hui PW, Lam YH, Tang MHY, Ng EHY, Yeung WSB, Ho PC. Maternal serum pregnancy-associated plasma protein-A and free beta-human chorionic gonadotrophin in pregnancies conceived with fresh and frozen-thawed embryos from in vitro fertilization and intracytoplasmic sperm injection. *Prenat Diagn*. 2005;25(5):390-393. doi:10.1002/pd.1169.

17. Hui PW. Maternal serum hCG and alpha-fetoprotein levels in pregnancies conceived after IVF or ICSI with fresh and frozen-thawed embryos. *Hum Reprod*. 2003;18(3):572-575. doi:10.1093/humrep/deg153.

18. Bersinger N a, Wunder D, Vanderlick F, et al. Maternal serum levels of placental proteins after in vitro fertilisation and their implications for prenatal screening. *Prenat Diagn*. 2004;24(6):471-477. doi:10.1002/pd.910.

19. Amor DJ, Xu JX, Halliday JL, et al. Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for Down syndrome. *Hum Reprod*. 2009;24(6):1330-1338. doi:10.1093/humrep/dep046.

20. Matilainen M, Peuhkurinen S, Laitinen P, Jarvela I, Morin-Papunen L, Ryynanen M. In combined first-trimester Down syndrome screening, the false-positive rate is not higher in pregnancies conceived after assisted reproduction compared with spontaneous pregnancies. *Fertil Steril*. 2011;95(1):378-381. doi:10.1016/j.fertnstert.2010.07.1048.

The following supplemental information for Manuscript 2 can be found in Appendix C:

Supplemental Text C-1	Description of data sources and record linkage methodology
Supplemental Figure C-1	Conception cohort of eligible prenatal screens
Supplemental Figure C-2	Documented IVF status by the prenatal screening labs compared to CARTR Plus
Supplemental Table C-1	Comparing the MSS marker MoM for markers with a current PSO IVF adjustment factor
Supplemental Table C-2	Comparing the MSS marker and NT MoM for measurements without a current PSO IVF adjustment factor
Supplemental Table C-3	Comparing MSS marker measurements with a current PSO IVF adjustment factor among IVF conceptions stratified by insemination method
Supplemental Table C-4	Comparing MSS marker measurements without a current PSO IVF adjustment factor among IVF conceptions stratified by insemination method
Supplemental Figures C-3	Histograms of maternal serum screening marker MoM
Supplemental Figure C-4	Histograms of proportion of PAPP-A MoM with different IVF adjustment factors
Supplemental Figure C-5	Histograms of proportion of total hCG MoM with different IVF adjustment factors
Supplemental Figure C-6	Histograms of proportion of unconjugated estriol MoM with different IVF adjustment factors

Chapter 5. [Manuscript 3] The effect of the type of infertility on placental-mediated adverse outcomes for patients that used IVF

5.1 Preface to Manuscript 3

In Chapter 4, we showed the value of using comprehensive population-level data to determine results that can be generalized. In Chapter 5, we used pregnancy and birth information from the BORN Information System (maternal-child health registry for Ontario) to capture all births in Ontario, Canada, as well as again using the CARTR Plus dataset to identify births conceived through in vitro fertilization, frozen embryo transfer or frozen oocyte IVF from May 21, 2013 through October 28, 2014.

Preeclampsia, placental abruption, intrauterine growth restriction, and stillbirth are placental-mediated adverse outcomes that are commonly investigated together. Differences in these placental-mediated adverse outcomes have previously been observed and reported among patients who used fertility treatment to conceive.[64,81,82] Manuscript 3 was designed to investigate whether these differences were found in the population of Ontario, Canada. The use of these previously described population-level data strengthened the study design, given that these exposures and outcomes are rare. Preterm birth (<37 weeks' gestation; and <32 weeks' gestation) was the secondary outcome investigated in this study. This adverse pregnancy outcome is often associated with extended stays in the neonatal intensive care unit and a greater number of medical interventions.[72,76]

Additionally, this study focused on investigating the associations between the underlying cause of infertility (male factor, female factor, and unexplained infertility) and the previously mentioned placental-mediated adverse outcomes. Studies with the ability to examine the cause of infertility and adverse pregnancy and birth outcomes are less common. The results from Manuscript 3 add to the current literature on the use of fertility treatment and placental-mediated adverse outcomes.

Manuscript 3 is currently submitted to Fertility and Sterility for publication.

5.2 Title page

Title: The effect of the type of infertility on placental-mediated adverse outcomes for patients that used IVF

Authors: Andrea Lanes, MSc[a,b], Ann E. Sprague, PhD[b,f], Arthur Leader, MD, FRCSC[d,e], Beth Potter, PhD[a], Mark Walker, MD, FRCS(C)[b,c,d,f]

Affiliations:

[a]University of Ottawa School of Epidemiology, Public Health and Preventive Medicine

[b]BORN Ontario

[c]Ottawa Hospital Research Institute

[d]University of Ottawa Department of Obstetrics and Gynecology

[e]Ottawa Fertility Centre

[f]CHEO Research Institute

5.3 Abstract

Objective: To investigate any association between type of infertility and placental-mediated adverse outcomes among women that used IVF to conceive compared to women who conceived spontaneously

Design: This was a population-level retrospective cohort study.

Settings: Births in Ontario, Canada, from May 31, 2013 through October 28, 2014.

Patients: An exposed group of pregnant women that used IVF with or without ICSI to conceive and a control group of pregnant women that conceived spontaneously.

Intervention: In vitro fertilization treatment and type of infertility.

Main Outcome Measures: Outcomes evaluated included a composite outcome for placental-mediated adverse outcomes, which included preeclampsia, small for gestational age, placental abruption and stillbirth. Preterm birth was also examined.

Results: There were 200,473 births from Ontario, Canada included in this retrospective cohort study. Only female factor infertility relative to spontaneous conception, was associated with a higher risk of placental abruption in the unadjusted model (RR: 2.39 95% CI: 1.19-4.76). IVF was associated with a higher risk of preterm birth (<37 weeks) and very preterm birth (<32 weeks), relative to spontaneous conception, adjusted RRs, 1.64 (95% CI: 1.40-1.93) and 1.75 (95% CI: 1.19-2.57), respectively.

Conclusions: No statistically significant differences were observed among the adjusted models for the primary outcomes. However, type of conception in association with preterm birth at less than 37 weeks' gestation was statistically significant.

Keywords: In vitro fertilization, placental-mediated adverse outcomes, preeclampsia, stillbirth, IUGR, placental abruption

5.4 Introduction

Infertility rates have been increasing globally,[1] as well as the number of patients seeking treatment.[2] All of the assisted reproductive technologies (ART) (e.g., in vitro fertilization (IVF), intrauterine insemination, donor gametes and frozen embryos) have progressed rapidly and have resulted in remarkable advances in a relatively short timeframe. These developments have had both positive and negative effects on the infants born, as well as their parents. Babies that were conceived with ART may have increased ante and postpartum complications and as a result may more often require greater medical attention.[3,4]

The World Health Organization defines infertility as an: 1) inability to become pregnant; 2) inability to maintain a pregnancy; or 3) inability to carry a pregnancy to a live birth.[5] Type of infertility can be classified into four categories: 1) male factor infertility; 2) female factor infertility; 3) combined infertility; and 4) unexplained infertility. Male factor infertility can be due to a decreased sperm count, poor sperm motility or abnormal sperm morphology. Female factor infertility is further classified into: 1) ovulation disorders (e.g., Polycystic Ovary Syndrome, hypothalamic dysfunction or premature ovarian insufficiency); 2) tubal infertility (e.g., blocked or damaged fallopian tubes, pelvic adhesive disease or endometriosis); and/or 3) uterine or cervical causes (e.g., benign uterine fibroids, congenital uterine malformations or hostile cervical mucus). Combined infertility results when both the female and male partners have demonstrable causes of infertility. An 'unexplained infertility' classification exists when the etiology of the infertility cannot be determined.

Adverse perinatal outcomes including hypertensive pregnancy disorders, preterm birth and a low birth weight have been associated with multiple births resulting from fertility treatment.[6,7] To reduce complications associated with multiple birth, care providers have decreased the number of embryos transferred in a single treatment cycle.[7] However, studies among singleton pregnancies have shown ART to be associated with a higher placental weight and abnormal umbilical cord insertions.[8,9] Associations with low birth weight and higher rates of pregnancy loss persist among singleton pregnancies that used ART to conceive.[10–12] Researchers have questioned whether it is the fertility treatment or the underlying conditions causing infertility that lead to the increased associations with adverse outcomes.[13,14]

Given the increasing number of women using fertility treatments to conceive, it is imperative that studies investigating the association with adverse outcomes are conducted. Previously in Canada, population-wide studies involving fertility treatments were not possible. The objective of this study was to investigate any association between type of infertility and placental-mediated adverse outcomes among women that used IVF to conceive compared to women who conceived spontaneously.

5.5 Methods

Data sources

This population-based cohort study used data from BORN (Better Outcomes Registry & Network) Ontario and CARTR (Canadian Assisted Reproductive Technologies Register) Plus. CARTR Plus is a dataset contained within the BORN Registry. BORN Ontario's database is an Internet-based maternal child information system that captures all births within the province of Ontario with a gestational age of greater or equal to 20 weeks.[15] These data were supplemented with data from Canadian Institute for Health Information (CIHI) to enhance specific data elements from the BORN Information System (BIS) for one large Ontario hospital, as their data were not complete within BORN. CARTR Plus contains detailed information on all fertility treatment cycles that occur in Canada. This cohort contained all IVF, FET and frozen oocyte IVF embryo transfer (ET) cycles in Ontario from January 1, 2013 to December 31, 2013 (Figure 5-2). The birth cohort contained all corresponding births (live births and stillbirths) from the fertility treatment cycles, as well as all births that occurred via spontaneous conception in the same time period in Ontario (May 21, 2103 to October 28, 2014). In order to avoid a fixed cohort bias, the birth cohort included all births up to 43 weeks after the last potential ART conception (Figure 5-2).[16]

Analysis

The composite outcome for placental-mediated adverse outcomes included preeclampsia (high blood pressure and proteinuria after 20 weeks' gestation[17]), small for gestational age (weight is less than the 10th percentile for the infants' gestational age based on sex and gestational age at birth) as a surrogate for Intrauterine growth restriction (IUGR), placental abruption (the lining of

the placenta has separated from the uterus), and stillbirth (fetal death at greater than 20 weeks' gestation). Preeclampsia, IUGR/small for gestational age, placental abruption and stillbirth were also assessed individually. In Canada, a gestational age of less than 37 weeks is used to define a preterm birth and a gestational age of less than 32 weeks defines a very preterm birth.[18] These definitions were used to define the secondary outcome of preterm birth. Multiple gestation pregnancies, pregnancies from donor oocytes, treatment cycles for combined type of infertility and deliveries that occurred outside of Ontario were excluded from the analysis.

Frequencies, proportions and chi squared statistics were produced for descriptive characteristics of this cohort. Frequencies and proportions were also reported for type of infertility and all primary and secondary outcomes, as well as the composite outcome. Unadjusted logistic regression models were carried out using generalized estimating equations for the composite outcome, as well as each individual primary outcome and the secondary outcomes with the spontaneous conception group as the reference group. Logistic regression models adjusted for maternal age, neighbourhood income level and smoking were generated for all outcomes. Sub-group analyses (unadjusted and adjusted logistic regression models) for the fertility treatment groups were also performed with female factor infertility as the reference group. All analyses were completed using Statistical Analysis System (SAS) software version 9.4.[19] Research Ethics Board approvals for this study were obtained from the Children's Hospital of Eastern Ontario (CHEOREB# 16/03PE) and the Ottawa Health Science Network (20160797-01H).

5.6 Results

There were 200,473 births from Ontario, Canada included in this retrospective cohort study (Figure 5-1). The majority of these births occurred via spontaneous conception (99.3%). Of the 1,359 live births and stillbirths conceived through IVF, FET or frozen oocyte IVF, the identified reasons for using fertility treatment varied: 49.5% were due to female factor infertility, 29.9% were due to male factor infertility and 20.5% were due to unexplained infertility. We found that among cases of female factor infertility (n= 673), 51.0% were ovulation disorders, 42.5% were tubal infertility, 7.6% were due to uterine or cervical causes and 12.6% could be attributed to other causes of female factor infertility (Table 5-1).

There were significant differences observed for maternal age, neighbourhood income level, parity, smoking at time of admission, hospital level of care, type of birth, type of caesarean section and maternal health conditions among the infertility groups and the spontaneous conception group (Table 5-2). When we investigated the descriptive characteristics among births that used IVF, FET or frozen oocyte IVF to conceive significant differences were found for maternal age, hospital level of care and pre-existing maternal health conditions (Table 5-2). There were no significant differences among the infertility groups with respect to characteristics of fertility treatment except for method of insemination, whereby births conceived among those who received ART due to male factor infertility were more likely to have used ICSI and less likely to have used conventional IVF relative to those where ART was initiated due to female factor infertility (p=0.01, Table 5-3).

The frequencies and proportions of the exposure and the primary and secondary outcomes are illustrated in Table 5-4. The placental-mediated adverse (composite) outcome was experienced amongst 10.74% of spontaneous conceptions; 9.58% of conceptions that occurred in patients with male factor infertility; 12.48% of conceptions that occurred in patients with female factor infertility; and 12.19% of patients that had unexplained infertility. Large differences were seen in preterm birth at less than 37 weeks' gestation among the different types of infertility. The lowest rate of preterm birth was found amongst spontaneous conceptions (6.39%) and the highest rate was among patients that had female factor infertility (12.33%).

Based on the logistic regression results, type of conception and type of infertility within the IVF group was not statistically significantly associated with the composite outcome, nor with each individual primary outcome, with the exception of female factor infertility relative to spontaneous conception, which was associated with a higher risk of placental abruption in the unadjusted model (RR: 2.39 95% CI: 1.19-4.76) (Table 5-5). This association was no longer statistically significant after adjusting for covariates (Table 5-5). Similarly, within the IVF cohort only, type of infertility (with female factor as the reference category) was not associated with the composite outcome or the individual primary outcomes, in either the unadjusted or adjusted models (Table 5-6).

Similar unadjusted and adjusted logistic regression models were performed for the secondary outcomes (preterm birth at less than 37 weeks' gestation and 32 weeks' gestation). Both the

unadjusted and adjusted models investigating type of conception in association with preterm birth at less than 37 weeks' gestation were statistically significant (Table 5-7). IVF was associated with a higher risk of preterm birth (<37 weeks) and very preterm birth (<32 weeks), relative to spontaneous conception, adjusted RRs, 1.64 (95% CI: 1.40-1.93) and 1.75 (95% CI: 1.19-2.57), respectively. When type of infertility was investigated we found IVF for female factor infertility was most strongly associated with a higher risk preterm birth: adjusted RRs, 1.77 (95% CI: 1.42-2.20) and 2.41 (95% CI: 1.52-3.81) for <37 weeks and <32 weeks, respectively, compared to spontaneous conceptions. No statistically significant differences were observed for type of infertility with female factor infertility as the reference group (male factor infertility adjusted RRs, 0.79 (95% CI: 0.54-1.17) and 0.37 (95% CI: 0.13-1.10) for <37 weeks and <32 weeks, respectively; and unexplained infertility adjusted RRs, 0.94 (95% CI: 0.62-1.43) and 0.56 (95% CI: 0.19-1.64) for <37 weeks and <32 weeks, respectively).

5.7 Discussion

Type of infertility is classified as female factor, male factor, combined or unexplained where each category represents the medical reasons for requiring fertility treatment[20]. The etiology of infertility varies considerably for each treatment group and many factors that are associated with type of infertility may be associated with adverse pregnancy outcomes. We had hypothesized that there would be an association between female factor infertility and the composite outcome due to the inference that underlying etiology of female factor infertility would lead to complications with the placenta; however no statistically significant results were found. A statistically significant result was only found for female factor infertility and placental abruption in the unadjusted analysis. That result did not remain significant after the regression model was adjusted for maternal age, neighbourhood income level and smoking status at time of admission (Table 5-5).

Previous studies have found associations between fertility treatment and stillbirth, [21] and preeclampsia [22]. Additionally, a recent meta-analysis found an association between fertility treatment and gestational hypertension and placental abruption.[23] Multiple pregnancies among women who used fertility treatments to conceive have been found to be associated with an increased risk of preeclampsia.[24] Since our study only included singleton births it may have

reduced the proportion of observed cases of preeclampsia. Conversely, there have been studies that found no significant association between fertility treatment and preeclampsia once they adjusted for maternal age and parity. [13,25]

Similar to previous studies, we found that the use of fertility treatment was associated with a statistically significant increased risk of preterm birth.[21,26,27] Relative to spontaneous conceptions, the largest increases in risk were found for female factor infertility and preterm birth at both less than 37 weeks' gestation and less than 32 week's gestation (RR: 1.77 95% CI: 1.42-2.20; RR: 2.41 95% CI: 1.52-3.81 respectively). Future studies should investigate gestational age by week at time of birth, as opposed to categorizing births into a preterm or term birth. This would help determine whether there were a higher proportion of births achieved through fertility treatment that occurred at a variety of gestational ages as there would be different clinical implications for births at each gestational age (i.e., 33 weeks' gestation compared to 36 weeks' gestation). Previous studies have documented that the clinical management of ART pregnancies differs compared to spontaneous conceptions, which may lead to preterm deliveries.[28]

The factors linking infertility to placental-mediated adverse outcomes have not been conclusively determined and reported in the literature. We had hypothesized that the hormonal and/or structural factors associated with female factor infertility may have affected the implantation and development of the placenta, leading to an increased risk of the primary outcome. The results of studies investigating differences among type of infertility and placental-mediated adverse outcomes have varied.[29] Some studies have found an increased association between unexplained infertility and IUGR, but no association with any other placental-mediated adverse outcome [29]. Palomba et al.,[30] found that women with polycystic ovary syndrome have an increased risk of placental-mediated adverse outcomes. However, this study found no differences between the different types of infertility and the primary and secondary outcomes (Table 5-5 and Table 5-7).

Strengths and Limitations

This study was strengthened by the large number of patients in these databases along with the detailed variables which enabled the investigation of this question. Secondly, the comprehensive CARTR Plus database allowed for definitive classification of women who used

IVF, FET or frozen oocyte IVF to assist with conception. Additionally, these results can be generalized for the population of Ontario, Canada, as all available births from Ontario were included in this cohort. Selection bias was not present in this study, due to the inclusion of all births due to spontaneous conception and IVF treatment in Ontario. Nevertheless, this study was potentially affected by misclassification of the exposure and/or outcome variables, a data quality issue that plagues studies that use administrative data. This study used comprehensive administrative data; however it was the first time that these outcomes were investigated. The prevalence rate of the primary outcomes when compared to the literature suggested that there may be underreporting. If misclassification bias existed in this study it was most likely non-differential, but it may underestimate the effect of the exposure. However, this study did supplement CIHI data for one Ontario hospital. A future study will investigate these outcomes using a combination of BORN and CIHI outcome information, in order to better capture the desired information. Additionally, this study may have benefited from adjusting for parity in addition to maternal age, neighbourhood income level and smoking in the regression models.

5.8 Conclusions

This study found no statistically significant association between type of conception or type of infertility and preeclampsia, stillbirth, placental abruption or SGA after adjusting for confounders; yet an increased associated persisted for preterm birth. Although this was a large population-based cohort study, it would benefit from the births associated with a greater number of years of fertility data.

Disclosure of interests

The authors have no conflicts of interest to declare.

Contribution to authorship

A. Lanes created the study protocol and developed the analysis plan with consultation M. Walker. A. Lanes conducted all analyses, interpreted the results and wrote the manuscript. A.E. Sprague, A. Leader, and B. Potter assisted with interpreting the results and editing the

manuscript. M. Walker assisted with the development of the study protocol, interpretation of results and editing of the manuscript.

Funding

A. Lanes was supported by the Queen Elizabeth II Graduate Scholarships in Science and Technology while this study was being conducted.

5.9 References

1. Buzadzic B, Vucetic M, Jankovic A, et al. New insights into male (in)fertility: The importance of NO. *Br J Pharmacol*. 2014;(1):1-42. doi:10.1111/bph.12675.

2. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Hum Reprod*. 2007;22(6):1506-1512. doi:10.1093/humrep/dem046.

3. Shevell T, Malone FD, Vidaver J, et al. Assisted reproductive technology and pregnancy outcome. *Obstet Gynecol*. 2005;106(5 Pt 1):1039-1045. doi:10.1097/01.AOG.0000183593.24583.7c.

4. Allen C, Bowdin S, Harrison RF, et al. Pregnancy and perinatal outcomes after assisted reproduction: a comparative study. *Ir J Med Sci*. 2008;177(3):233-241. doi:10.1007/s11845-008-0172-9.

5. Infertility. World Health Organization. http://www.who.int/topics/infertility/en/.

6. Bissonnette F, Cohen J, Collins J, et al. Canadian Fertility and Andrology Society Incidence and complications of multiple gestation in Canada: proceedings of an expert meeting. *Reprod Biomed Online*. 2007;14(6).

7. Tanbo T, Abyholm T. Obsetric and perinatal outcome in pregnancy after assisted reproduction. *Obstet Gynecol*. 1996;8:193-198.

8. Daniel Y, Schreiber L, Geva E, et al. Do placentae of term singleton pregnancies obtained by assisted reproductive technologies differ from those of spontaneously conceived pregnancies? *Hum Reprod*. 1999;14(4):1107-1110. http://www.ncbi.nlm.nih.gov/pubmed/10221249.

9. Jauniaux E, Englert Y, Vanesse M, Hiden M, Wilkin P. Pathologic features of placentas from singleton pregnancies obtained by in vitro fertilization and embryo transfer. *Obstet Gynecol*. 1990;76(1):61-64.

10. Caserta D, Marci R, Tatone C, et al. IVF pregnancies: neonatal outcomes after the new Italian law on assisted reproduction technology (law 40/2004). *Acta Obstet Gynecol Scand*. 2008;87(9):935-939. doi:10.1080/00016340802283681.

11. Reddy U, Wapner R, Rebar R, Tasca R. Infertility, Assisted Reproductive Technology, and Adverse Pregnancy Outcomes. *Obstet Gynecol*. 2007;109(4):967-977.

12. Cetin I, Cozzi V, Antonazzo P. Fetal development after assisted reproduction--a review. *Placenta*. 2003;24 Suppl B:S104-13. http://www.ncbi.nlm.nih.gov/pubmed/14559039.

13. Li TC, Macleod I, Singhal V, Duncan SLB. The obstetric and neonatal outcome of pregnancy in women with a previous history of infertility: a prospective study. 1991;98(November):1087-1092.

14. Thomson F, Shanbhag S, Templeton A, Bhattacharya S. Obstetric outcome in women with subfertility. 2005;112(May):632-637.

15. About BORN. Better Outcomes Registry & Network (BORN) Ontario. https://www.bornontario.ca/en/about-born/. Published 2014.

16. Barnett AG. Time-Dependent Exposures and the Fixed-Cohort Bias. *Environ Health Perspect*. 2011;119(10):A422-3. doi:10.1289/ehp.1103956.

17. Magee L, Pels A, Helewa M, et al. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: executive summary. *J Obs Gynaecol Can*. 2014;36(5):416-438. doi:10.1016/S1701-2163(15)30588-0.

18. Canadian Premature Babies Foundation. *Premature Birth in Canada: An Environmental Scan*.; 2014.

19. SAS. Analytics software & solutions. http://www.sas.com/en_ca/home.html. Published 2016.

20. Vikström J, Hammar M, Josefsson A, Bladh M, Sydsjö G. Birth characteristics in a clinical sample of women seeking infertility treatment: a case-control study. *BMJ Open*. 2014;4(3):e004197. doi:10.1136/bmjopen-2013-004197.

21. Marino JL, Moore VM, Willson KJ, et al. Perinatal outcomes by mode of assisted conception and sub-fertility in an Australian data linkage cohort. *PLoS One*. 2014;9(1):e80398. doi:10.1371/journal.pone.0080398.

22. Sibai B. Subfertility/infertility and assisted reproductive conception are independent risk factors for pre-eclampsia. *BJOG an Int J Obstet Gynaecol*. 2014:1-2. doi:10.1111/1471-0528.13090.

23. Qin J, Liu X, Sheng X, Wang H, Gao S. Assisted reproductive technology and the risk of pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies: A meta-analysis of cohort studies. *Fertil Steril*. 2016;105(1):73-85e6. doi:10.1016/j.fertnstert.2015.09.007.

24. FridstrÖM M, Nisell H, SjÖBlom P, Hillensjo T. Are Women with Polycystic Ovary Syndrome at an Increased Risk of Pregnancy-Induced Hypertension and/or Preeclampsia? *Hypertens Pregnancy*. 1999;18(1):73-80. doi:10.3109/10641959909009612.

25. Watanabe N, Fujiwara T, Suzuki T, et al. Is in vitro fertilization associated with preeclampsia? A propensity score matched study. *BMC Pregnancy Childbirth*. 2014;14(1):69. doi:10.1186/1471-2393-14-69.

26. Perri T, Chen R, Yoeli R, et al. Are singleton assisted reproductive technology pregnancies at risk of prematurity? *J Assist Reprod Genet*. 2001;18(5):245-249. doi:10.1023/A:1016614217411.

27. Schieve LA, Ferre C, Peterson HB, Macaluso M, Reynolds MA, Wright VC. Perinatal outcome among singleton infants conceived through assisted reproductive technology in the United States. *Obstet Gynecol*. 2004;103(6):1144-1153. doi:10.1097/01.AOG.0000127037.12652.76.

28. Hayashi M, Nakai A, Satoh S, Matsuda Y. Adverse obstetric and perinatal outcomes of singleton pregnancies may be related to maternal factors associated with infertility rather than the type of assisted reproductive technology procedure used. *Fertil Steril*. 2012;98(4):922-928. doi:10.1016/j.fertnstert.2012.05.049.

29. Isaksson R, Gissler M, Tiitinen A. Obstetric outcome among women with unexplained infertility after IVF: a matched case – control study. *Hum Reprod*. 2002;17(7):1755-1761.

30. Palomba S, de Wilde M a, Falbo A, Koster MPH, La Sala GB, Fauser BCJM. Pregnancy complications in women with polycystic ovary syndrome. *Hum Reprod Update*. 2015;21(5):575-592. doi:10.1093/humupd/dmv029.

5.10 Figures and tables

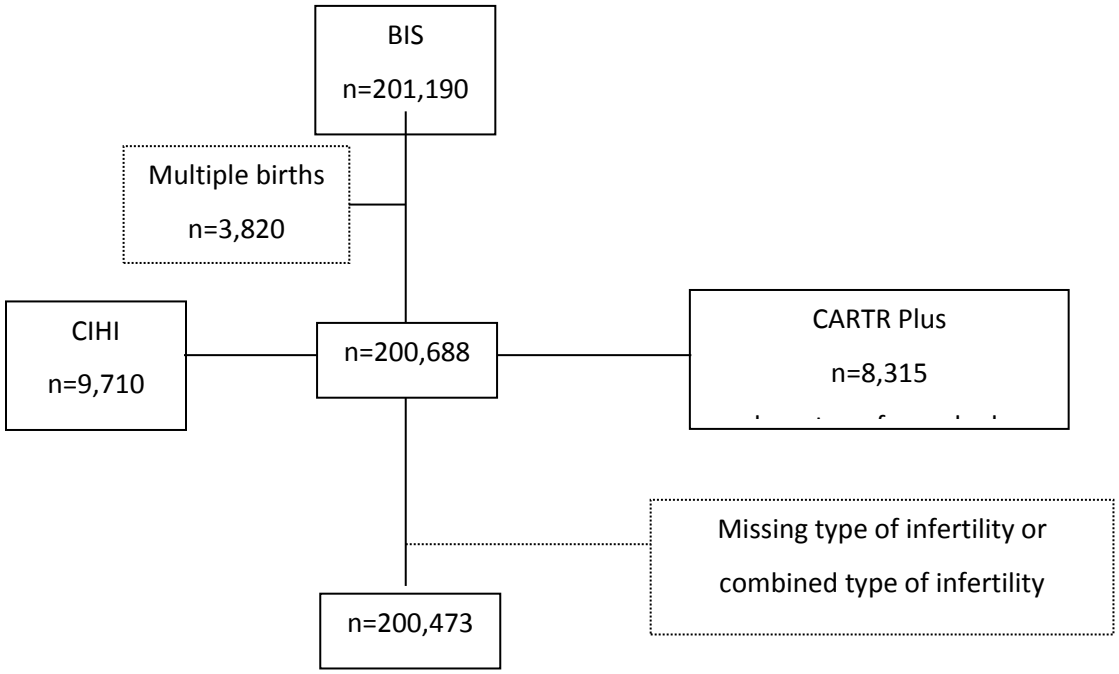


Figure 5-1 Flow diagram of study cohort

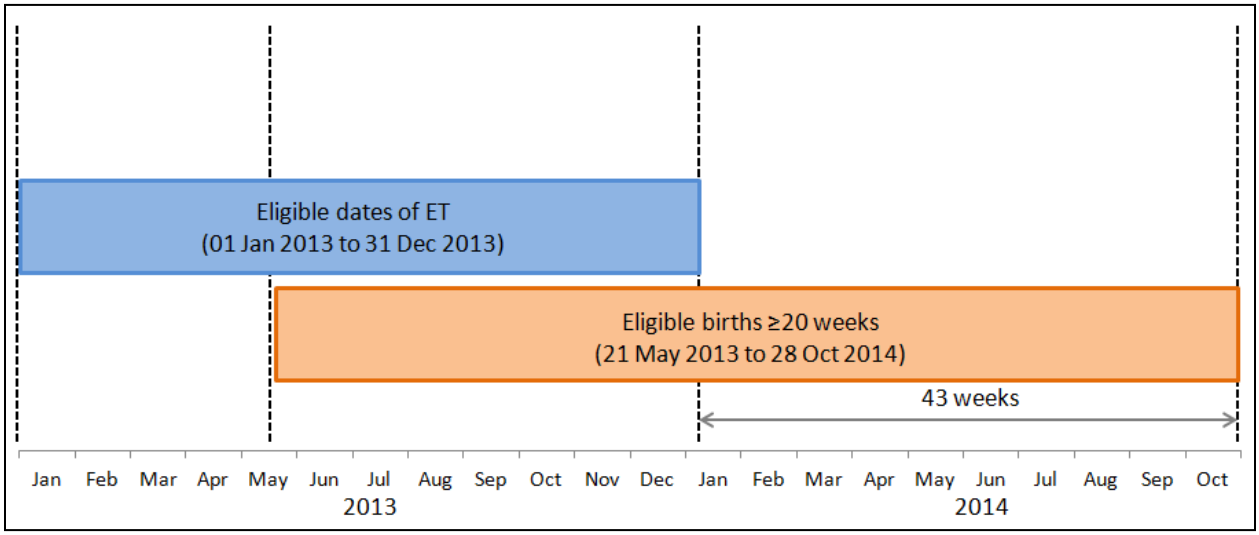


Figure 5-2 Development of birth cohort

Table 5-1 Frequencies and proportions of type of infertility for the study cohort

Type of infertility	Frequency	Percent
Spontaneous conception	199114	99.32
Male factor	407	0.20
Female factor*	673	0.34
Ovulation disorders	343	50.97
Tubal infertility	286	42.50
Uterine or cervical causes	51	7.58
Other female factor	85	12.63
Unexplained	279	0.14

Notes:

**Different types of female factor infertility are not mutually exclusive*

Table 5-2 Descriptive characteristics for the study cohort

	Type of infertility						
	Male factor	**Female factor**	**Unexplained**	**p-value**	**Spontaneous conception**	**Total**	**p-value**
Maternal age at birth							
<35	210 (51.60)	274 (40.71)	108 (38.71)	<0.01	155394 (78.06)	155394	<0.01
35-39	166 (40.79)	273 (40.56)	140 (50.18)		35780 (17.97)	35780	
40+	31 (7.62)	126 (18.72)	31 (11.11)		7908 (3.97)	7908	
Smoking at time of admission							
None	385 (99.23)	610 (99.03)	250 (99.60)	0.67	173067 (91.39)	173067	<0.01
Smoked	S	6 (0.97)	S		16313 (8.61)	16323	
Neighbourhood income level							
Lowest quintile	23 (5.65)	47 (7.01)	11 (3.99)	0.57	28566 (14.44)	28647	<0.01
Medium-low quintile	34 (8.35)	55 (8.21)	28 (10.14)		29193 (14.76)	29193	
Middle quintile	60 (14.74)	86 (12.84)	28 (10.14)		35080 (17.74)	35080	
Medium-high quintile	81 (19.9)	146 (21.79)	64 (23.19)		37442 (18.93)	37442	
Highest quintile	129 (31.7)	198 (29.55)	91 (32.97)		37686 (19.05)	37686	
Missing	80 (19.66)	138 (20.6)	54 (19.57)		29830 (15.08)	29830	
Parity							
Nulliparous	276 (68.15)	445 (66.52)	184 (65.95)	0.99	84178 (42.3)	84178	<0.01
Multiparous	125 (30.86)	203 (30.34)	85 (30.47)		111308 (55.93)	111308	
Hospital LOC							
Neonatal Level I	26 (6.39)	27 (4.01)	11 (3.94)	<0.05	22761 (11.43)	22761	<0.01
Neonatal Level IIa	42 (10.32)	33 (4.9)	14 (5.02)		21936 (11.02)	21936	
Neonatal Level IIb	84 (20.64)	158 (23.48)	70 (25.09)		49496 (24.86)	49496	
Neonatal Level IIc	143 (35.14)	236 (35.07)	95 (34.05)		66357 (33.33)	66357	
Neonatal Level IIIa/IIIb	105 (25.8)	209 (31.05)	82 (29.39)		32830 (16.49)	32830	
Type of birth							

Assisted Vaginal	48 (11.79)	98 (14.56)	36 (12.9)	0.18	17649 (8.86)	17831	<0.01
Induced or Spontaneous Labour Cesarean Section	78 (19.16)	136 (20.21)	60 (21.51)		25038 (12.58)	25312	
No Labour - Cesarean Section	72 (17.69)	145 (21.55)	46 (16.49)		27480 (13.8)	27480	
Spontaneous Vaginal	209 (51.35)	294 (43.68)	137 (49.1)		128922 (64.76)	128922	
Type of Cesarean section							
Unplanned	78 (52.7)	142 (50.71)	56 (53.33)	0.87	23920 (45.56)	23920	<0.05
Planned	70 (47.3)	138 (49.29)	49 (46.67)		28578 (54.44)	28578	
Maternal health condition							
None	275 (72.75)	383 (63.20)	185 (74.00)	<0.01	151576 (81.19)	151576	<0.01
Previous health condition	103 (27.25)	223 (36.80)	65 (26.00)		35125 (18.81)	35125	
Notes:							
S Suppressed cells when cell count <6							

Table 5-3 Descriptive characteristics for women in the study cohort that used IVF, FET or frozen oocyte IVF to conceive

	Type of infertility				
	Male factor	**Female factor**	**Unexplained**	**Total**	**p-value**
Ovarian stimulation					
Agonist	88 (38.6)	129 (36.44)	63 (38.41)	280	0.84
Antagonist	140 (61.4)	225 (63.56)	101 (61.59)	466	
Type of fertility treatment					
IVF	228 (56.02)	361 (53.64)	164 (58.78)	753	0.59
Transfer day					
Day-3	74 (32.89)	138 (40.12)	53 (32.92)	265	0.13
Day-5	151 (67.11)	206 (59.88)	108 (67.08)	108	
FET	178 (43.73)	308 (45.77)	114 (40.86)	600	
Embryo stage at thaw					
Cleavage stage	27 (15.17)	57 (18.51)	14 (12.28)	98	0.27
Blastocyst stage	150 (84.27)	249 (80.84)	100 (87.72)	499	0.22
Frozen oocyte IVF	S	S	S	6	0.32
Embryo cryopreservation method					
Slow freezing	48 (29.63)	77 (31.82)	36 (25.53)	161	0.43
Vitrification	114 (70.37)	165 (68.18)	105 (74.47)	384	
Method of insemination					
Conventional IVF only	8 (2.48)	141 (26.06)	58 (26.48)	207	<0.01
ICSI only	298 (92.55)	343 (63.40)	127 (57.99)	768	
Conventional IVF and ICSI	16 (4.97)	57 (10.54)	34 (15.53)	107	

Notes:

S Suppressed cells when cell count <6

Table 5-4 Frequency and proportions of the primary and secondary outcomes with type of infertility

	Primary outcomes					Secondary outcomes	
Type of infertility	**Composite**	**Preeclampsia**	**Placental abruption**	**Stillbirth**	**SGA**	**Birth at <37 weeks**	**Birth at <32 weeks**
Spontaneous conception n=199,114	21391 (10.74)	1436 (0.72)	992 (0.50)	1147 (0.58)	18552 (9.37)	12713 (6.39)	2352 (1.18)
Male factor n=407	39 (9.58)	4 (0.98)	2 (0.49)	4 (0.98)	33 (8.15)	38 (9.34)	5 (1.23)
Female factor* n=673	84 (12.48)	9 (1.34)	8 (1.19)	4 (0.59)	66 (9.88)	83 (12.33)	21 (3.12)
Ovulation disorders n=343	35 (10.20)	4 (1.17)	4 (1.17)	2 (0.58)	26 (7.69)	42 (12.24)	11 (3.21)
Tubal infertility n=286	40 (13.99)	4 (1.40)	4 (1.40)	3 (1.05)	30 (10.56)	36 (12.59)	10 (3.50)
Uterine or cervical causes n=51	8 (15.69)	1 (1.96)	0 (0)	0 (0)	8 (15.69)	9 (17.65)	2 (3.92)
Other female factor n=85	13 (15.29)	1 (1.18)	1 (1.18)	0 (0)	12 (14.12)	9 (10.59)	0 (0)
Unexplained n=279	34 (12.19)	1 (0.36)	1 (0.36)	1 (0.36)	32 (11.47)	30 (10.75)	4 (1.43)

Notes:

**Different types of female factor infertility are not mutually exclusive*

Table 5-5 Unadjusted and adjusted logistic regression models for type of conception and primary outcomes

	Composite		Preeclampsia		Placental abruption		Stillbirth		SGA	
Type of conception	RR (95% CI)	adj. RR (95% CI)	RR (95% CI)	adj. RR (95% CI)	RR (95% CI)	adj. RR (95% CI)	RR (95% CI)	adj. RR (95% CI)	RR (95% CI)	adj. RR (95% CI)
Spontaneous	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
IVF, FET or Frozen oocyte IVF	1.07 (0.92-1.24)	1.11 (0.95-1.29)	1.40 (0.83-2.37)	1.24 (0.70-2.18)	1.74 (0.99-3.06)	1.25 (0.62-2.50)	1.13 (0.59-2.17)	1.22 (0.64-2.36)	1.02 (0.87-1.20)	1.10 (0.93-1.31)
Spontaneous	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Male factor	0.89 (0.66-1.20)	0.99 (0.73-1.35)	1.36 (0.51-3.62)	1.41 (0.53-3.75)	0.99 (0.25-3.94)	0.53 (0.08-3.77)	1.71 (0.64-4.53)	1.93 (0.73-5.13)	0.87 (0.63-1.21)	1.00 (0.72-1.39)
Female factor	1.16 (0.95-1.42)	1.18 (0.95-1.46)	1.85 (0.97-3.56)	1.45 (0.69-3.04)	2.39 (1.19-4.76)*	1.89 (0.85-4.21)	1.03 (0.39-2.75)	1.06 (0.40-2.83)	1.06 (0.84-1.33)	1.12 (0.88-1.43)
Unexplained	1.13 (0.83-1.55)	1.15 (0.81-1.63)	0.50 (0.07-3.52)	0.53 (0.08-3.76)	0.72 (0.10-5.09)	0.80 (0.11-5.67)	0.62 (0.09-4.40)	0.72 (0.10-5.10)	1.22 (0.88-1.70)	1.25 (0.87-1.79)

Notes:

** Significant at alpha of <0.05.*

Adjusted for maternal age, neighbourhood income level and smoking status.

Table 5-6 Unadjusted and adjusted logistic regression models for type of infertility and primary outcomes

	Composite*		Preeclampsia**		Placental abruption**		Stillbirth***		SGA*	
Type of infertility	RR (95% CI)	Adj. RR (95% CI)	RR (95% CI)	Adj. RR (95% CI)	RR (95% CI)	Adj. RR (95% CI)	RR (95% CI)	Adj. RR (95% CI)	RR (95% CI)	Adj. RR (95% CI)
Female factor	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Male factor	0.77 (0.54-1.10)	0.81 (0.56-1.17)	0.73 (0.23-2.37)	0.76 (0.23-2.49)	0.41 (0.09-1.94)	0.45 (0.09-2.15)	1.65 (0.42-6.58)	1.91 (0.47-7.77)	0.82 (0.55-1.23)	0.85 (0.56-1.28)
Unexplained	0.98 (0.67-1.42)	0.99 (0.65-1.49)	0.27 (0.03-2.11)	0.29 (0.04-2.31)	0.30 (0.04-2.40)	0.33 (0.04-2.68)	0.60 (0.07-5.37)	0.61 (0.07-5.51)	1.16 (0.78-1.73)	1.13 (0.73-1.75)

Notes:

**Adjusted for maternal age, neighbourhood income level and smoking status.*

***Adjusted for maternal age and neighbourhood income level.*

****Adjusted for maternal age.*

Table 5-7 Unadjusted and adjusted logistic regression models for type of conception and type of infertility and secondary outcomes

	Birth at <37 weeks		Birth at <32 weeks	
	Unadjusted RR (95% CI)	**Adjusted RR (95%CI)**	**Unadjusted RR (95% CI)**	**Adjusted RR (95%CI)**
Type of conception				
Spontaneous	ref	ref	ref	ref
IVF, FET or Frozen oocyte IVF	1.73 (1.49-2.01)	1.64 (1.40-1.93)	1.83 (1.28-2.62)	1.75 (1.19-2.57)
Type of conception				
Spontaneous	ref	ref	ref	ref
Male factor	1.46 (1.08-1.98)	1.45 (1.06-1.99)	1.04 (0.43-2.49)	0.94 (0.35-2.45)
Female factor	1.93 (1.58-2.36)	1.77 (1.42-2.20)	2.64 (1.73-4.03)	2.41 (1.52-3.81)
Unexplained	1.68 (1.20-2.36)	1.69 (1.18-2.41)	1.21 (0.46-3.21)	1.39 (0.52-3.68)
Type of infertility				
Female factor	ref	ref	ref	ref
Male factor	0.76 (0.53-1.09)	0.79 (0.54-1.17)	0.39 (0.45-1.04)	0.37 (0.13-1.10)
Unexplained	0.87 (0.59-1.29)	0.94 (0.62-1.43)	0.46 (0.16-1.33)	0.56 (0.19-1.64)

Notes:

Adjusted for maternal age, neighbourhood income level and smoking status.

The following supplemental information for Manuscript 3 can be found in Appendix D:

Supplemental Text D-1	Data sources and preparation
Supplemental Table D-1	Summary of procedures used to code study variables from the Canadian implementation of the International Classification of Diseases
Supplemental Table D-2	Unadjusted logistic regression models for type of infertility and primary outcomes when type of insemination was only IVF without ICSI
Supplemental Table D-3	Unadjusted logistic regression models for type of infertility and primary outcomes when type of insemination was only IVF with ICSI
Supplemental Table D-4	Unadjusted logistic regression models for type of conception and type of infertility and secondary outcomes when type of insemination was only IVF without ICSI
Supplemental Table D-5	Unadjusted logistic regression models for type of conception and type of infertility and secondary outcomes when type of insemination was only IVF with ICSI

Chapter 6. Discussion

Assisted reproductive technologies, especially in vitro fertilization, have become the predominant method of achieving conception when infertility has been confirmed. The rates of the use of fertility treatments vary worldwide;[83] however in Canada there were 28,657 fertility treatment cycles performed in 2015.[15] These treatment cycles resulted in a clinical pregnancy rate of 28.7% per cycle start, where clinical pregnancy was defined as clinical intrauterine, heterotopic, or ectopic pregnancy.[15] The literature suggested that there were statistically significant differences in maternal serum screening marker levels among pregnancies that used IVF to conceive,[6,34,84–97,35] however studies using population-wide data were rare. Similarly, the literature suggested that process of using IVF to conceive may increase the risk of a placental-mediated adverse outcome compared to women who conceived spontaneously.[64,81,82] However, few studies investigated the effect that the underlying type of infertility has on these placental-mediated adverse outcomes.

The overall aim of this dissertation was to evaluate the influence that the use of IVF had on important pregnancy and neonatal outcomes with an emphasis on the population of Ontario, Canada. In order to accomplish this, I systematically reviewed the existing literature on maternal serum screening markers in women using IVF to become pregnant and then conducted two quantitative studies that involved analysis of population-based secondary data and focused on: 1) differences in maternal serum screening markers among IVF pregnancies for all pregnancies in Ontario; and 2) the effect of IVF, and the type of infertility among those using IVF, on placental-mediated adverse outcomes.

6.1 Summary of research findings

The first study, titled “Maternal serum screening markers and nuchal translucency measurements among in vitro fertilization pregnancies: a systematic review”, published in Fertility & Sterility, summarized the existing literature on associations between IVF treatment and maternal serum screening marker levels and nuchal translucency thickness up to April 2015. Forty cohort and case-control studies, primarily published in the United Kingdom or Europe, were included in the synthesis. Six maternal serum screening markers (PAPP-A, AFP, free β-hCG,

total hCG, μE3, and DIA) were assessed. The most commonly reported maternal serum screening markers were PAPP-A and free β-hCG, reported in 28 and 26 studies respectively. A decrease in PAPP-A and an increase in total hCG was consistently reported among pregnancies that were conceived through IVF relative to those conceived spontaneously. Amongst the studies that investigated FET as a method of fertility treatment (seven studies) the direction of the effect estimates were lower among studies that reported PAPP-A and consistently higher among studies that reported AFP, as well as higher for nuchal translucency.

Although we intended to meta-analyze the median MoMs for each maternal serum screening marker and nuchal translucency, we were unable to pool effect sizes due to the presence of design heterogeneity. We had decided *a priori* to abstract median maternal serum screening marker MoMs from each study. However, studies did not consistently report medians, rather there was mixed reporting of results as median MoMs and mean MoMs, without enough information available to calculate the median MoM for each maternal serum screening marker. Including studies that only reported mean MoMs in a meta-analysis would have led to biased results.

The second study of this dissertation, titled "Comparing maternal serum screening markers among IVF and spontaneous conceptions in Ontario through registry data" was designed to investigate the accuracy of IVF identification on the prenatal screening record, to compare the screening markers in IVF and non-IVF pregnancies in the population of Ontario, and to propose updated IVF adjustment factors for the Ontario population. We had information on prenatal screening from all prenatal screening labs in the province, as well as comprehensive information on the use of IVF from all fertility treatment clinics in Ontario. The sensitivity and specificity was 95.8% and 98.9% respectively when we compare IVF status on the prenatal screening record to CARTR Plus.

As expected, the mean maternal age was significantly higher among the IVF group compared to the non-IVF group. Additionally, the oocyte provider's mean age was significantly different among women who exclusively used autologous oocytes or embryos (μ=34.1 years SD±3.98) as compared to donor oocytes or embryos (μ=28.9 years SD±6.52). Throughout the process of creating the dataset for this study, documentation of maternal age on the prenatal screening record when donor oocytes or embryos were used was investigated. Documentation of donor

maternal age often replaced the patient age in order for the algorithm calculating the risk of Down syndrome and trisomy 18 to be accurately attained. It would be clearer if the donor maternal age was captured separately and the algorithm would use this information if it was present and patient maternal age if it was not present.

Overall, significant differences were found between the identified IVF and non-IVF groups based on the prenatal screening requisition information and CARTR Plus information for the ethnicity adjusted mean MoMs for AFP, PAPP-A, µE3, first trimester hCG, total hCG, and DIA. Additionally, when we applied the proposed adjustment factor to all IVF pregnancies that were identified though CARTR Plus we found that the mean adjusted marker MoMs for PAPP-A, total hCG, and μE^3 were significantly closer to 1.00.

The third study of this dissertation, titled "The effect of the type of infertility on placental-mediated adverse outcomes for patients that used IVF" investigated whether there was a significant increase in the risk of having preeclampsia, placental abruption, stillbirth or small for gestational age babies dependent on the use of IVF and the underlying type of infertility of the patient. This was a population-based cohort study that used the CARTR Plus database. The analyses performed investigating type of conception and the composite outcome (preeclampsia, placental abruption, stillbirth and small for gestational age), as well as each individual primary outcome found no statistically significant results. The literature suggested that a significant difference would be observed. [64,81,82]

When we investigated the underlying type of infertility with the placental-mediated adverse outcomes we only found a significant difference between female factor infertility and placental abruption and this was no longer statistically significant after adjustment for confounders. However, relative to spontaneous conception, we found that IVF, particularly in response to female infertility, was associated with a significantly increased risk of preterm birth (gestational age <37 weeks) and very preterm birth (gestational age <32 weeks).

6.2 Strengths and limitations

Each manuscript contained a strengths and limitation section in Chapters 3 to 5. The section below provides information that reflects the dissertation as a whole.

The main limitations that were present in Manuscript 1 resulted from an inability to meta-analyze the results of the studies included in the systematic review, due to inconsistency in how studies reported results. Quantitative results were included from 23 of 40 studies, however we were unable to group the results and interpret a magnitude of change. Studies varied in the metric used to report maternal serum screening marker MoMs (means versus medians). Including mean MoMs would have produced results that would be skewed in the direction of outliers.

The major strength of the studies in Manuscript 2 and 3 stem from the use of comprehensive databases, which captures IVF records, prenatal screening records, and information on placental-mediated adverse outcomes. Therefore these studies were not affected by selection bias. Selection bias can result from flawed study design and participant recruitment and can occur as result of an unmeasured variable that is associated with both the exposure and the outcome[98]. Selection bias can affect a study's internal validity (validity of the conclusions drawn as they pertain to members of the source population),[99] as well as its external validity (the validity of inferences as they relate to the general population).[99] Therefore, the results from Manuscripts 2 and 3 can confidently be generalized for the population of Ontario, Canada. However, selection bias may exist in Manuscript 2, since women are self-selecting to have MSS performed. Sixty percent of IVF pregnancies in Ontario had MSS, as compared to 70% in the general obstetrical population of Ontario.[100] Patients that conceived through IVF may be using NIPT more often for prenatal screening. Future studies investigating NIPT use among IVF pregnancies will be conducted.

Additionally, since CARTR Plus and the BORN Information System have been deterministically linked through a proprietary algorithm which takes into account a number of variables (e.g., OHIP number, date of birth, and maternal postal code); it is likely that the exposures and outcomes in Manuscript 2 and 3 are appropriately assigned to the correct woman. Furthermore, lost to follow-up bias (differential loss to follow-up)[101] was not present, due to the complete capture of these databases. Most studies that assess adverse pregnancy outcomes do not involve study follow-up for extended periods of time. Since the investigated outcomes were identified and occurred within a finite period of time (gestation, delivery, and birth), attrition due to loss to follow-up was not present in the studies included in Manuscripts 2 and 3.

Population-based cohort study designs have limitations that influence the validity of the analyses conducted. In perinatal outcomes databases observations can be collected for each birth or each pregnancy. However, Manuscript 2 clearly identified the pregnancies that were used in the analysis (Supplemental Figure C-1). Similarly, Manuscript 3 clearly defined the births that were eligible and included in the study (Figure 5-2). Additionally, it is important to address and account for multiple live births or stillbirths from a single pregnancy from the same mother, as well as multiple pregnancies recorded in a database from the same mother. Due to difference in observed maternal serum screening marker levels in multiple gestation pregnancies, only singleton pregnancies were included in the study design for Manuscript 2. Likewise, due to an increased prevalence of specific placental-mediated adverse outcomes in multiple gestation pregnancies and births[102,103] only singleton births were included in the study for Manuscript 3.

It has been shown that in Canada the preterm birth rate among live births is significantly higher among IVF conceptions with autologous oocytes (28.4%) compared to all live births in the general obstetrical population (7.7%);[104] and this difference remains among singleton live births (14.5% and 6.2% respectively).[104] Although there has been a dramatic decrease in the proportion of multiple pregnancies resulting from ART in Canada[15] it would be beneficial to investigate the associated risk of placental-mediated adverse outcomes among multiple gestation pregnancies, since the rate of multiple gestation pregnancies remains higher in this population compared to pregnancies achieved through spontaneous conception.[105]

Although comprehensive population-level databases were used for the studies in Manuscripts 2 and 3, they are administrative databases. These databases contain variables that were collected for other purposes rather than for study design, inclusion or analysis. In Manuscript 2, misclassification of IVF status on the prenatal screening record can lead to false-positives or false-negatives. MSS markers are used in prenatal screening to assess the risk of fetal aneuploidies. Due to inherent difference among pregnancies that used IVF to assist with conception an adjustment factor is applied. However, if there were flaws in identifying whether IVF was used, adjustment factors may be inappropriately applied or not applied. A high false-positive rate will result in the application of the IVF adjustment, which will incorrectly modify a patient's assessed risk for a fetal aneuploidy. These patients may not have the appropriate test performed to investigate an increased assessed. Conversely, patients that are documented as spontaneous conceptions, but are actually IVF conceptions (false-negatives) may unnecessarily

have invasive testing (amniocentesis or Chorionic Villus Sampling) performed. These invasive tests put the fetus at an increased risk for an adverse pregnancy outcome (miscarriage rate: amniocentesis 0.01-0.5%; Chorionic Villus Sampling 1%).[21]

When the pregnancies and births were selected based on measurements or responses in CARTR Plus or the BORN Information System, selection bias may occur due to misclassification and information bias that exist due to how these data were collected.[106] Since the information on these placental-mediated adverse outcomes were documented for reasons other than clinical research there was the possibility of misclassification bias. Misclassification may occur during the process of entering and coding data in large databases, as well as through the presence of fully or partially duplicated data. Manuscript 3 suggested that data quality issues persisted with the documentation of placental-mediated adverse outcomes, since the prevalence rates were lower than expected (e.g., preeclampsia).

Moreover, there were competing risks for earlier adverse outcomes for some of the placental-mediated outcomes used in the primary analysis, as well as the secondary outcome (preterm birth). For example, an infant cannot be considered small for gestational age if stillbirth has occurred. Nevertheless, this study adds to the current literature on IVF use and the risk for placental-mediated adverse outcomes and questions the positive associations that were previously found.

Additionally, although attempts were made to define the placental-mediated adverse outcomes analyzed in Manuscript 3 in the same manner, variations may persist between outcomes identified through the BORN Information System compared to those identified through the Canadian Institute for Health Information Discharge Abstract Database. Preeclampsia was defined as gestational (pregnancy-induced) hypertension with significant proteinuria or eclampsia (ensuring that all preeclampsia cases were captured). Placental abruption was defined as premature separation of placenta (abruption placentae)). Live birth and stillbirth information was ascertained by linking the Discharge Abstract Database (DAD) and the maternal abstracts (99.1% of records were linked). These records were then linked to BORN-CIHI mapping file. This file was generated using a complicated probabilistic linkage.[107]

A final limitation that plagues studies in perinatal epidemiology is the missing information regarding pregnancy loss prior to 20 weeks' gestation in perinatal databases. Although the

CARTR Plus database did capture early fetal loss, the completeness of capturing this information has never been evaluated. The issue with not having comprehensive information on early fetal loss is that these cases are more likely to have been later documented as an adverse perinatal outcome.[108] If this occurs we are underestimating the prevalence of these adverse perinatal outcomes.

6.3 Implications for future research and public health

As the use of IVF has increased among countries around the world so too has the scientific and medical research on its risks and benefits. Although randomized clinical trials are the gold standard of research they are rare when investigating maternal serum screening markers or placental-mediated adverse outcomes among an IVF population. Rather, observational studies are most commonly conducted. Given the rare nature of the exposure (IVF) and outcomes discussed in this dissertation, the use of administrative databases to conduct population-level retrospective cohort studies was appropriate. Previously in Canada population-based studies of IVF conceptions in relation to perinatal outcomes were not possible. The studies presented in Chapters 4 and 5 continue to inform on associations of risk of these adverse outcomes, as well as describing the completeness and appropriateness of these data. Specifically the studies that were performed using the CARTR Plus database were among the first using these data for research.

Currently, prenatal screening labs are using population multiple of the median from other populations to assess a woman's risk of having Down syndrome or trisomy 18. Using metrics from a different population may affect the screening results. Different regions use varied adjustment factors for a variety of characteristics for specific maternal serum screening markers. Given these variations, the population multiple of the median that is currently used in practice may not be appropriate. Once the results from Manuscript 2 are published, prenatal screening labs in Ontario can use population multiple of the median from an Ontario population.

Additionally, modifications of the adjustment factors for specific maternal serum screening markers for IVF used in the algorithm will also generate more appropriate results for the IVF population in Ontario; thereby decreasing the false positive and false negative rate for this

population. Since approximately two-thirds of pregnant women in Ontario undergo prenatal screening this would have a substantial effect.[100] The use of NIPT among IVF patients in this same population is the next logical analysis. Currently, I have commenced analyses to investigate differences in NIPT results among IVF patients and spontaneous conceptions for all NIPT users in Ontario. The results of this study will further the knowledge on prenatal screening among IVF patients in Canada.

Since the prevalence of placental-mediated adverse outcomes was lower than expected, a future study that confirms the results from the study presented in Manuscript 3 should ideally be performed. This study would be prospective and would also examine the placenta of women who used IVF to conceive or conceived spontaneously. The clinical analysis of the placenta would allow further investigation into the role of the placenta among women who use IVF and have placental-mediated adverse outcomes. Unfortunately, the cost associated with conducting this study at a population level would be substantial, since the exposures and outcomes are rare and a large sample would be required.

Manuscript 3 found statistically significant differences in maternal age and neighbourhood income level. Women who used fertility treatment to conceive had a higher maternal age and a higher neighbourhood income level. Over the next few years the disparities in income may be reduced. In December 2015, Ontario began publically funding a portion of IVF cycles performed.[109] This was to assist with the large financial burden that patients have when using IVF to conceive. In Ontario, the cost of an uninsured IVF treatment cycle ranges from $7,000 to $11,000.[109] Changes to the socio-demographic characteristics of patients conceiving through IVF will modify the population that are used in these studies.

Finally, it important that the results from the manuscripts contained within this PhD dissertation are disseminated to the appropriate people and groups in Ontario. This is essential in order encourage policy change and to facilitate clinical practice change. Once Manuscript 2 is published in a peer-reviewed journal the results will be discussed with the prenatal screening chemistry group which is supported by BORN Ontario. This group consists of prenatal screening lab directors and charge technicians at the serum screening labs in Ontario. Additionally, once the results are published there will need to be coordination with the directors of the biochemistry labs to approach software vendors about updating the IVF adjustment factors used

in the algorithm to determine the risk for Down syndrome and trisomy 18. These results will also be presented at various genetics education rounds, many of which are broadcast to multiple centres via the Ontario Telemedicine Network.

Similarly, it is important that the results from Manuscript 3 are distributed to the fertility treatment community (e.g., reproductive endocrinology and infertility specialists, lab directors, maternal fetal medicine specialists). Once the study results are published in a peer-reviewed journal the findings can be presented at the Canadian Fertility and Andrology Society meeting, where there are attendees from all of the Canadian fertility treatment centres. These results will also be distributed through obstetrics and gynecology grand rounds.

6.4 Final conclusions

This doctoral dissertation adds strong studies to the current literature investigating the use of IVF to conceive and its effect on: 1) maternal serum screening markers; and 2) placental-mediated adverse outcomes. Through a systematic review of the literature and two population-based analyses of secondary data, I have demonstrated that using IVF to conceive has an effect on pregnancy and birth outcomes, but it may not have significant effect on all outcomes. The results of the systematic review showed that using IVF to assist with conception produced substantial increased effect estimate in total hCG and a decreased effect estimate in PAPP-A as compared to women who conceived spontaneously. Moreover, the inconsistency of reporting median maternal serum marker MoMs throughout the literature was a cause for concern and did permit pooling of the effect estimates. Nevertheless, since maternal serum screening marker MoMs reflect the population from which they were obtained it was pertinent to perform the analyses contained in Manuscript 2. Investigating the maternal serum screening markers in Ontario, Canada, using population-level data generated results that can be used for future analyses within this population. These results can be used to better estimate the population median for each maternal screening marker for Ontario. As well, the application of the proposed adjustment factors would produce more reliable results for patients who used IVF to conceive. These two changes may create more accurate risk estimates for Down syndrome and trisomy 18 among the IVF population in Ontario.

The etiology for the type of infertility varies for male factor infertility, female factor infertility and unexplained infertility and may be multifactorial for each type of infertility. Among the primary outcomes, Manuscript 3 only found significant associations between female factor infertility and placental abruption in the unadjusted model. Therefore, our results demonstrated that there were not significant negative effects associated with IVF use for assisted conception. Although we found significant associations between all types of infertility and preterm birth (secondary outcome) this may be due to different management of clinical care for patients who used IVF.

These results are extremely meaningful, as the frequency with which patients are using IVF to conceive in Canada is on the rise. It is important that well-planned studies using Canada's own data are used to inform clinical health decisions and health policy decisions in Canada. The studies in this doctoral dissertation are among the first studies to be conducted using the CARTR Plus database.

Reference List

1. Kamel RM. Assisted Reproductive Technology after the Birth of Louise Brown. *J Reprod Infertil*. 2013;14(3):96-109. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3799275&tool=pmcentrez&rendertype=abstract.
2. Shevell T, Malone FD, Vidaver J, et al. Assisted reproductive technology and pregnancy outcome. *Obstet Gynecol*. 2005;106(5 Pt 1):1039-1045. doi:10.1097/01.AOG.0000183593.24583.7c.
3. Allen C, Bowdin S, Harrison RF, et al. Pregnancy and perinatal outcomes after assisted reproduction: a comparative study. *Ir J Med Sci*. 2008;177(3):233-241. doi:10.1007/s11845-008-0172-9.
4. Cetin I, Cozzi V, Antonazzo P. Fetal development after assisted reproduction--a review. *Placenta*. 2003;24 Suppl B:S104-13. http://www.ncbi.nlm.nih.gov/pubmed/14559039.
5. Schieve LA, Ferre C, Peterson HB, Macaluso M, Reynolds MA, Wright VC. Perinatal outcome among singleton infants conceived through assisted reproductive technology in the United States. *Obstet Gynecol*. 2004;103(6):1144-1153. doi:10.1097/01.AOG.0000127037.12652.76.
6. Tul N, Novak-Antolic Z. Serum PAPP-A levels at 10 – 14 weeks of gestation are altered. *Prenat Diagn*. 2006;26(October):1206-1211. doi:10.1002/pd.
7. Duwe K, Reefhuis J, Honein M, Schieve L, Rasmussen S, Study the NaBDP. Epidemiology of Fertility Treatment Use Among U.S. Women with Liveborn Infants, 1997–2004. 2010;19(3).
8. Talaulikar VS, Arulkumaran S. Maternal, perinatal and long-term outcomes after assisted reproductive techniques (ART): implications for clinical practice. *Eur J Obstet Gynecol Reprod Biol*. 2013;170(1):13-19. doi:10.1016/j.ejogrb.2013.04.014.
9. Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. *Hum Reprod*. 2009;24(11):2683-2687. doi:10.1093/humrep/dep343.
10. Karande V, Morris R, Chapman C, Rinehart J, Gleicher N. Impact of the "physician factor" on pregnancy rates in a large assisted reproductive technology program: do too many cooks spoil the broth? *Fertil Steril*. 1999;71(6):1001-1009.
11. Better Outcomes Registry & Network Ontario. Canadian Reproductive Technologies Registry (CARTR) Plus. Treatment cycles for 2013.
12. Henningsen A-KA, Pinborg A. Birth and perinatal outcomes and complications for babies conceived following assisted reproductive techniques. *Semin Fetal Neonatal Med*. May 2014:1-5. doi:10.1016/j.siny.2014.04.001.
13. Bergh C, Wennerholm U-B. Obstetric outcome and long-term follow up of children conceived through assisted reproduction. *Best Pract Res Clin Obstet Gynaecol*. 2012;26(6):841-852. doi:10.1016/j.bpobgyn.2012.05.001.

14. Canadian Assisted Reproductive Technologies Registry (CARTR) Plus. *Final Treatment Cycle and Pregnancy Outcome Data for 2014*. Ottawa, Canada; 2016.

15. Canadian Assisted Reproductive Technologies Registry (CARTR) Plus. *Preliminary Treatment Cycle Data for 2015. Better Outcomes Registry & Network Ontario*. Ottawa, Canada; 2016.

16. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol*. 2004;103(3):551-563. doi:10.1097/01.AOG.0000114989.84822.51.

17. Holoch KJ, Lessey BA. Endometriosis and Infertility. *Clin Obstet Gynecol*. 2010;53(2):429-438.

18. Sokol R. The diagnosis and treatment of male factor infertility. *Obstet Gynecol*. 1995;7:177-181.

19. Infertility. World Health Organization. http://www.who.int/topics/infertility/en/.

20. Chitayat D, Langlois S, Wilson RD. Prenatal screening for fetal aneuploidy in singleton pregnancies. *J Obstet Gynaecol Canada*. 2011;33(7):736-750. http://www.ncbi.nlm.nih.gov/pubmed/21749752.

21. Carroll J, Allanson J, Epslen M, et al. *Reference Guide for Health Care Providers Prenatal Screening Tests for the Detection of : Down Syndrome, Trisomy 18 and Open Neural Tube Defects*.; 2007.

22. Korenberg J, Chen X, Schipper R, et al. Down syndrome phenotypes : The consequences of chromosomal imbalance. 1994;91(May):4997-5001.

23. Lemire RJ. Neural Tube Defects. *JAMA*. 1988;259(4):558-562.

24. Botto L, Moore C, Khoury M, Erickson J. Neural-Tube Defects. *N Engl J Med*. 1999;341(20):1509-1519.

25. Petch J, Caulfield T, Okun N. Non-invasive prenatal testing and chromosomal microarray: changing the landscape of prenatal genetic testing. healthydebate. http://healthydebate.ca/2014/05/topic/non-invasive-prenatal-testing. Published 2014.

26. Shi X, Zhang Z, Cram DS, Liu C. Feasibility of noninvasive prenatal testing for common fetal aneuploidies in an early gestational window. *Clin Chim Acta*. 2015;439:24-28. doi:10.1016/j.cca.2014.09.032.

27. Organization GECK. NIPT in Ontario. http://geneticseducation.ca/educational-resources/gec-ko-on-the-run/non-invasive-prenatal-testing/#ON. Published 2016.

28. Neveux LM, Palomaki GE, Larrivee DA, Knight GJ, Haddow JE. Refinements in managing maternal weight adjustment for interpreting prenatal screening results. *Prenat Diagn*. 1996;16(12):1115-1119. doi:10.1002/(SICI)1097-0223(199612)16:12<1115::AID-PD3>3.0.CO;2-6.

29. Spencer K, Ong CYT, Liao AWJ, Nicolaides KH. The influence of ethnic origin on first trimester biochemical markers of chromosomal abnormalities. *Prenat Diagn*. 2000;20(6):491-494. doi:10.1002/1097-0223(200006)20:6<491::AID-PD844>3.0.CO;2-3.

30. Huang T, Summers AM, Wyatt PR, Meier C, Côté GB. Maternal serum marker medians in

Aboriginal Canadian women. *Prenat Diagn*. 2003;23(2):98-100. doi:10.1002/pd.537.

31. Huttly W, Rudnicka A, Wald NJ. Second-trimester prenatal screening markers for Down syndrome in women with insulin-dependent diabetes mellitus. *Prenat Diagn*. 2004;24(10):804-807. doi:10.1002/pd.994.

32. Bestwick JP, Huttly WJ, Wald NJ. First trimester Down's syndrome screening marker values and cigarette smoking: new data and a meta-analysis on free beta human chorionic gonadotophin, pregnancy-associated plasma protein-A and nuchal translucency. *J Med Screen*. 2008;15(4):204-206. doi:10.1258/jms.2008.008049.

33. Engels M a J, Kooij M, Schats R, et al. First-trimester serum marker distribution in singleton pregnancies conceived with assisted reproduction. *Prenat Diagn*. 2010;30(4):372-377. doi:10.1002/pd.2495.

34. Lambert-Messerlian G, Dugoff L, Vidaver J, et al. First- and second-trimester Down syndrome screening markers in pregnancies achieved through assisted reproductive technologies (ART): A FASTER trial study †. *Prenat Diagn*. 2006;(January):672-678. doi:10.1002/pd.

35. Wald N, White N, Morris J, Huttly W, Canick J. Serum markers for Down ' s syndrome in women who have had in vitro fertilisation : implications for antenatal screening. *Br J Obstet Gynaecol*. 1999;106(December):1304-1306.

36. World Health Organisation (WHO). *Making Every Baby Count : Audit and Review of Stillbirths and Neonatal Deaths*.; 2016.

37. Lawn JE, Blencowe H, Waiswa P, et al. Stillbirths: Rates, risk factors, and acceleration towards 2030. *Lancet*. 2016;387(10018):587-603. doi:10.1016/S0140-6736(15)00837-5.

38. Statistics Canada. Data quality, concepts and methodology: Definitions. Governtment of Canada. http://www.statcan.gc.ca/pub/84f0210x/2008000/technote-notetech1-eng.htm. Published 2012.

39. Fretts RC. Etiology and prevention of stillbirth. *Am J Obstet Gynecol*. 2005;193(6):1923-1935. doi:10.1016/j.ajog.2005.03.074.

40. Liu LC, Wang YC, Yu MH, Su HY. Major risk factors for stillbirth in different trimesters of pregnancy-A systematic review. *Taiwan J Obstet Gynecol*. 2014;53(2):141-145. doi:10.1016/j.tjog.2014.04.003.

41. Dayan N, Lanes A, Walker MC, Spitzer KA, Laskin CA. Effect of chronic hypertension on assisted pregnancy outcomes: A population-based study in Ontario, Canada. *Fertil Steril*. 2016;105(4):1003-1009. doi:10.1016/j.fertnstert.2015.11.039.

42. Henningsen A, Wennerholm U, Gissler M, et al. Risk of stillbirth and infant deaths after assisted reproductive technology: a Nordic study from the CoNARTaS group. *Hum Reprod*. 2014;29(5):1090-1096. doi:10.1093/humrep/deu031.

43. Hay W, Catz C, Grave G, Yaffe S. Workshop Summary: Fetal Growth: Its Regulation and Disorders. *Pediatrics*. 1997;99(4):585-591.

44. Zhu J, Obel C, Beck B, Olsen J, Basso O. Infertility, infertility treatment and fetal growth restriction. *Obstet Gynecol*. 2008;110(6):1326-1334.

45. Suhag A, Berghella V. Intrauterine Growth Restriction (IUGR): Etiology and Diagnosis. *Curr*

Obstet Gynecol Rep. 2013;2(2):102-111. doi:10.1007/s13669-013-0041-z.

46. İskender-Mazman D, Akçören Z, Yiğit Ş, et al. Placental findings of IUGR and non-IUGR. *Turk J Pediatr*. 2014;56(4):368-373.

47. Hasmasanu MG, Bolboaca SD, Drugan TC, Matyas M, Zaharie GC. Parental Factors Associated with Intrauterine Growth Restriction. *Srp Arh Celok Lek*. 2015;143(11-12):701-706. doi:10.2298/SARH1512701H.

48. Resnik R. Intrauterine Growth Restriction. *Am Coll Obstet Gynecol*. 2002;99(3):490-496.

49. Lausman A, On T, Kingdom J, et al. Intrauterine Growth Restriction: Screening, Diagnosis, and Management. *J Obs Gynaecol Can*. 2013;74135(2958):741-748.

50. Lee P, Chernausek S, Hokken-Kpelega A, Czernichow P. International Small for Gestational Age Advisory Board Consensus Development Conference Statement: Management of Short Children Born Small for Gestational Age, April 24-October 1, 2001. *Pediatrics*. 2003;111(6):1253-1261.

51. Maman E, Lunenfeld E, Levy a, Vardi H, Potashnik G. Obstetric outcome of singleton pregnancies conceived by in vitro fertilization and ovulation induction compared with those conceived spontaneously. *Fertil Steril*. 1998;70(2):240-245. http://www.ncbi.nlm.nih.gov/pubmed/9696214.

52. Reubinoff BE, Friedler S, Samueloff A, Schenker JG, Ben-Haim M, Lewin A. Is the obstetric outcome of in vitro fertilized singleton gestations different from natural ones? A controlled study. *Fertil Steril*. 1997;67(6):1077-1083. doi:10.1016/S0015-0282(97)81442-2.

53. Dhont M, De Neubourg F, Van der Elst J, De Sutter P. Perinatal outcome of pregnancies after assisted reproduction: a case-control study. *J Assist Reprod Genet*. 1997;14(10):575-580. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3454731&tool=pmcentrez&rendertype=abstract.

54. Howe R, RA S, Durinzi K, Tureck R. Perinatal outcome of singleton pregnancies conceived by in vitro fertilization: a controlled study. *Jounral Perinatol*. 1990;10(3):261-266.

55. Tallo CP, Vohr B, Oh W, Rubin LP, Seifer DB, Haning R V. Maternal and neonatal morbidity associated with in vitro fertilization. *J Pediatr*. 1995;127(5):794-800. doi:10.1016/S0022-3476(95)70175-3.

56. Jeyabalan A. Epidemiology of preeclampsia: Impact of obesity. *Nutr Rev*. 2013;71(SUPPL1):1-14. doi:10.1111/nure.12055.

57. Magee L, Pels A, Helewa M, et al. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: executive summary. *J Obs Gynaecol Can*. 2014;36(5):416-438. doi:10.1016/S1701-2163(15)30588-0.

58. Roberts J, Hubel C. The two stage model of preeclampsia: Variations on the theme. *Placenta*. 2009;30:S32–S37. doi:10.1016/j.placenta.2008.11.009.The.

59. Wei J, Liu CX, Gong TT, Wu QJ WL. Cigarette smoking during pregnancy and preeclampsia risk: a systematic review and meta-analysis of prospective studies. *Oncotarget*. 2015;6(41):43667-43678. doi:10.18632.

60. Phipps E, Prasanna D, Brima W, Jim B. Preeclampsia: Updates in Pathogenesis, Definitions, and Guidelines. *Clin J Am Soc Nephrol*. 2016;(6):1-12. doi:10.2215/CJN.12081115.

61. Mongraw-Chaffin ML, Cirillo PM, Cohn BA. Prospective Evidence From the Child Heatlh and. *Hypertension*. 2010;56(1):166-171. doi:10.1161/HYPERTENSIONAHA.110.150078.PREECLAMPSIA.

62. Paruk F, Moodley J. Maternal and neonatal outcome in early- and late-onset pre-eclampsia. *Semin Neonatol*. 2000;5(3):197-207. doi:10.1053/siny.2000.0023.

63. Vikse BE, Irgens LM, Leivestad T, Skjaerven R, Iversen BM. Preeclampsia and the risk of end-stage renal disease. *N Engl J Med*. 2008;359(8):800-809. doi:10.1097/01.ogx.0000340761.87862.dc.

64. Sibai B. Subfertility/infertility and assisted reproductive conception are independent risk factors for pre-eclampsia. *BJOG an Int J Obstet Gynaecol*. 2014:1-2. doi:10.1111/1471-0528.13090.

65. FridstrÖM M, Nisell H, SjÖBlom P, Hillensjo T. Are Women with Polycystic Ovary Syndrome at an Increased Risk of Pregnancy-Induced Hypertension and/or Preeclampsia? *Hypertens Pregnancy*. 1999;18(1):73-80. doi:10.3109/10641959909009612.

66. Watanabe N, Fujiwara T, Suzuki T, et al. Is in vitro fertilization associated with preeclampsia? A propensity score matched study. *BMC Pregnancy Childbirth*. 2014;14(1):69. doi:10.1186/1471-2393-14-69.

67. Li TC, Macleod I, Singhal V, Duncan SLB. The obstetric and neonatal outcome of pregnancy in women with a previous history of infertility: a prospective study. 1991;98(November):1087-1092.

68. Ananth C V., Lavery JA, Vintzileos AM, et al. Severe placental abruption: Clinical definition and associations with maternal complications. *Am J Obstet Gynecol*. 2016;214(2):272e1-272e9. doi:10.1016/j.ajog.2015.09.069.

69. Ghaheh HS, Feizi A, Mousavi M, Sohrabi D, Mesghari L, Hosseini Z. Risk factors of placental abruption. *J Res Med Sci*. 2013;18(May):422-426. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3810579&tool=pmcentrez&rendertype=abstract.

70. Hasegawa J, Nakamura M, Hamada S, et al. Capable of identifying risk factors for placental abruption. *J Matern Neonatal Med*. 2014;27(271):1476-7058. doi:10.3109/14767058.2013.799659.

71. Boisramé T, Sananès N, Fritz G, et al. Placental abruption: Risk factors, management and maternal-fetal prognosis. Cohort study over 10 years. *Eur J Obstet Gynecol Reprod Biol*. 2014;179:100-104. doi:10.1016/j.ejogrb.2014.05.026.

72. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008;371(9606):75-84. doi:10.1016/S0140-6736(08)60074-4.

73. Thomson F, Shanbhag S, Templeton A, Bhattacharya S. Obstetric outcome in women with subfertility. 2005;112(May):632-637.

74. Okun N, Sierra S, Wilson R, et al. Pregnancy Outcomes After Assisted Human

Reproduction. *JOGC*. 2014;2014(302).

75. Allen VM, Wilson RD, Cheung A. Pregnancy outcomes after assisted reproductive technology. *J Obstet Gynaecol Canada JOGC*. 2006;28(173):220-250. http://www.ncbi.nlm.nih.gov/pubmed/24484993.

76. Canadian Premature Babies Foundation. *Premature Birth in Canada: An Environmental Scan*.; 2014.

77. Henderson JJ, McWilliam O a, Newnham JP, Pennell CE. Preterm birth aetiology 2004-2008. Maternal factors associated with three phenotypes: spontaneous preterm labour, preterm pre-labour rupture of membranes and medically indicated preterm birth. *J Matern Fetal Neonatal Med*. 2012;25(6):642-647. doi:10.3109/14767058.2011.597899.

78. Bukowski R, Hansen NI, Willinger M, et al. Fetal Growth and Risk of Stillbirth: A Population-Based Case-Control Study. *PLoS Med*. 2014;11(4). doi:10.1371/journal.pmed.1001633.

79. Canadian Fertility and Andrology Society. *Reduction of Multiple Pregnancy Risk Associated with IVF/ICSI IVF Medical Directors of Canada Position Statement 2012*.; 2012.

80. Lanes A, Huang T, Sprague AE, Leader A, Potter B, Walker M. Maternal serum screening markers and nuchal translucency measurements in in vitro fertilization pregnancies: a systematic review. *Fertil Steril*. 2016;106(6):1463-1469.e2. doi:10.1016/j.fertnstert.2016.07.1120.

81. Qin J, Liu X, Sheng X, Wang H, Gao S. Assisted reproductive technology and the risk of pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies: A meta-analysis of cohort studies. *Fertil Steril*. 2016;105(1):73-85e6. doi:10.1016/j.fertnstert.2015.09.007.

82. Marino JL, Moore VM, Willson KJ, et al. Perinatal outcomes by mode of assisted conception and sub-fertility in an Australian data linkage cohort. *PLoS One*. 2014;9(1):e80398. doi:10.1371/journal.pone.0080398.

83. Sullivan E, Zegers-Hochschild F, Mansour R, et al. International Committee for Monitoring Assisted Reproductive Technologies (ICMART) world report: Assisted reproductive technology 2004. *Hum Reprod*. 2013;28(5):1375-1390. doi:10.1093/humrep/det036.

84. Anckaert E, Schiettecatte J, Sleurs E, Devroey P, Smitz J. First trimester screening for Down's syndrome after assisted reproductive technology: non-male factor infertility is associated with elevated free beta-human chorionic gonadotropin levels at 10-14 weeks of gestation. *Fertil Steril*. 2008;90(4):1206-1210. doi:10.1016/j.fertnstert.2007.08.050.

85. Bender F, Hecken J, Reinsberg J, et al. Altered first-trimester screening markers after IVF/ICSI: no relationship with small-for-gestational-age and number of embryos transferred. *Reprod Biomed Online*. 2010;20(4):516-522. doi:10.1016/j.rbmo.2009.12.025.

86. Cowans NJ, Spencer K. Effect of gestational age on first trimester maternal serum prenatal screening correction factors for ethnicity and IVF conception. *Prenat Diagn*. 2013;33(1):56-60. doi:10.1002/pd.4010.

87. Giorgetti C, Vanden Meerschaut F, De Roo C, et al. Multivariate analysis identifies the estradiol level at ovulation triggering as an independent predictor of the first trimester

pregnancy-associated plasma protein-A level in IVF/ICSI pregnancies. *Hum Reprod*. 2013;28(10):2636-2642. doi:10.1093/humrep/det295.

88. Hui PW. Maternal serum hCG and alpha-fetoprotein levels in pregnancies conceived after IVF or ICSI with fresh and frozen-thawed embryos. *Hum Reprod*. 2003;18(3):572-575. doi:10.1093/humrep/deg153.

89. Hui PW, Lam YH, Tang MHY, Ng EHY, Yeung WSB, Ho PC. Maternal serum pregnancy-associated plasma protein-A and free beta-human chorionic gonadotrophin in pregnancies conceived with fresh and frozen-thawed embryos from in vitro fertilization and intracytoplasmic sperm injection. *Prenat Diagn*. 2005;25(5):390-393. doi:10.1002/pd.1169.

90. Hui PW, Tang MHY, Lam YH, Yeung WSB, Ng EHY, Ho PC. Nuchal translucency in pregnancies conceived after assisted reproduction technology. *Ultrasound Obstet Gynecol*. 2005;25(3):234-238. doi:10.1002/uog.1846.

91. Lam YH, Shu W, Yeung B, et al. Maternal serum alpha-fetoprotein and human chorionic gonadotrophin in pregnancies conceived after intracytoplasmic sperm injection and conventional in-vitro fertilization. *Hum Reprod*. 1999;14(8):2120-2123.

92. Lambert-Messerlian G, Palomaki GE, Canick J a. Adjustment of serum markers in first trimester screening. *J Med Screen*. 2009;16(2):102-103. doi:10.1258/jms.2009.009028.

93. Liao a W, Heath V, Kametas N, Spencer K, Nicolaides KH. First-trimester screening for trisomy 21 in singleton pregnancies achieved by assisted reproduction. *Hum Reprod*. 2001;16(7):1501-1504. http://www.ncbi.nlm.nih.gov/pubmed/11425838.

94. Matilainen M, Peuhkurinen S, Laitinen P, Jarvela I, Morin-Papunen L, Ryynanen M. In combined first-trimester Down syndrome screening, the false-positive rate is not higher in pregnancies conceived after assisted reproduction compared with spontaneous pregnancies. *Fertil Steril*. 2011;95(1):378-381. doi:10.1016/j.fertnstert.2010.07.1048.

95. Maymon R, Shulman A. Serial first- and second-trimester Down's syndrome screening tests among IVF- versus naturally-conceived singletons. *Hum Reprod*. 2002;17(4):1081-1085.

96. Maymon R, Shulman A. Integrated first- and second-trimester Down syndrome screening test among unaffected IVF pregnancies. *Prenat Diagn*. 2004;24(2):125-129. doi:10.1002/pd.809.

97. Orlandi F, Rossi C, Allegra A, et al. First trimester screening with free b -hCG , PAPP-A and nuchal translucency in pregnancies conceived with assisted reproduction. *Prenat Diagn*. 2002;22(March):718-721.

98. Rothman KJ. *Epidemiology An Introduction*. 2nd ed. New York: Oxford University Press; 2012.

99. Rothman JR, Greenland S, Lash TL. *Modern Epidemiology*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2008.

100. BORN Ontario. *Better Outcomes Registry & Network (BORN) Ontario Annual Report 2012–2013 and 2013–2014*.; 2015.

101. Szklo Nieto MFJ. *Epidemiology Beyond the Basics*. Third Edit. Burlington, MA: Jones &

Bartlett Learning; 2014.

102. Bissonnette F, Cohen J, Collins J, et al. Canadian Fertility and Andrology Society Incidence and complications of multiple gestation in Canada: proceedings of an expert meeting. *Reprod Biomed Online*. 2007;14(6).

103. Tanbo T, Abyholm T. Obsetric and perinatal outcome in pregnancy after assisted reproduction. *Obstet Gynecol*. 1996;8:193-198.

104. Better Outcomes Registry & Network Ontario. *Canadian Assisted Reproductive Technologies Registry (CARTR) Plus. Final Treatment Cycle and Pregnancy Outcome Data for 2013*.; 2015.

105. Murray SR, Norman JE. Multiple pregnancies following assisted reproductive technologies--a happy consequence or double trouble? *Semin Fetal Neonatal Med*. 2014;19(4):222-227. doi:10.1016/j.siny.2014.03.001.

106. Lash TL, Abrams B, Bodnar LM. Comparison of bias analysis strategies applied to a large data set. *Epidemiology*. 2014;25(4):576-582. doi:10.1097/EDE.0000000000000102.

107. Miao G. *BIS and CIHI-DAD FY 2012-2015 Data Linkage Flow Chart*.; 2016.

108. Kramer MS, Zhang X, Platt RW. Analyzing risks of adverse pregnancy outcomes. *Am J Epidemiol*. 2014;179(3):361-367. doi:10.1093/aje/kwt285.

109. Greenblatt E. *Advisory Process for Infertility Services Key Recommendations Report*.; 2015. http://www.health.gov.on.ca/en/public/programs/ivf/docs/ivf_report.pdf.

110. About BORN. Better Outcomes Registry & Network (BORN) Ontario. https://www.bornontario.ca/en/about-born/. Published 2014.

111. Statistics Canada. *Postal Code Conversion File Plus (PCCF +) Version 6A, Reference Guide*.; 2014. doi:Minister of Industry. Statistics Canada Catalogue no. 82-E0086-XDB.

112. Health PC for M and C. *Standardized Maternal and Newborn Levels of Care Definitions*.; 2013.

APPENDIX A: Certificates of ethical approval

Manuscript 1: Maternal serum screening markers and nuchal translucency measurements among in vitro fertilization pregnancies: a systematic review

- Ethics approval was not required for this systematic review

Manuscript 2: Comparing maternal serum screening markers among IVF and spontaneous conceptions in Ontario through registry data

- Children's Hospital of Eastern Ontario Research Ethics Board (CHEOREB#15/06PE)
- Ottawa Health Science Network Research Ethics Board (20150644-01H)
- Mount Sinai Research Ethics Board (MSH REB 15-0282-C)

Manuscript 3: The effect of the type of infertility on placental-mediated adverse outcomes for patients that used IVF

- Children's Hospital of Eastern Ontario Research Ethics Board (CHEOREB# 16/03PE)
- Ottawa Health Science Network (20160797-01H).

CHEO Research Ethics Board
Approval - Delegated Review

Principal Investigator: Dr. Mark Walker
REB Protocol No: 15/06PE
Romeo File No: 20150311
Project Title: CHEOREB#15/06PE - Investigating maternal serum marker screening in the Ontario IVF population
Primary Affiliation: Clinical Research\Epidemiology
Protocol Status: Active
Approval Date: July 28, 2015
Valid Until: July 15, 2016
Annual Renewal Submission Deadline: June 15, 2016

Documents Reviewed & Approved:

Document Name	Comments	Version Date
Protocol	Protocol	2015/07/16
Other Document	List of variables	2015/07/16

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 under the delegated review stream, which is reserved for projects that involve no more than minimal risk to human subjects.

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 must not implement any deviation from, or changes to, the protocol without the approval of the REB, or when the change involves only logistical or administrative aspects of the study (e.g., change of telephone number or research staff).
3. The investigator must, prior to use, submit to the Board changes to the study documentation, e.g., changes to the informed consent letters, recruitment materials.
4. For all other research studies, investigators must promptly report to the REB all unexpected and untoward occurrences (including the loss or theft of study data and other such privacy breaches).
5. Investigators must submit an annual renewal report to the REB 30 days prior to the expiration date stated above.
6. Investigators must submit a final report at the conclusion of the study.
7. Investigators must provide the Board with French versions of the consent form, unless a waiver has been granted.

Ottawa Hospital Research Institute
Institut de recherche de l'Hôpital d'Ottawa

uOttawa

University of Ottawa Heart Institute
Institut de cardiologie de l'Université d'Ottawa

Ottawa Health Science Network Research Ethics Board/ Conseil d'éthique de la recherche du Réseau de science de la santé d'Ottawa

August 29, 2016

Dear Dr. Walker:

RE: Protocol# - 20150644-01H **Investigating maternal serum marker screening in the Ontario IVF population**

Renewal Expiry Date - August 31, 2017

I am pleased to inform you that your Annual Renewal Request was reviewed by the Ottawa Health Science Network Research Ethics Board (OHSN-REB) and is approved. No changes, amendments or addenda may be made in the protocol without the OHSN-REB's review and approval.

The projected date of study completion has been extended to December 31, 2017.

Renewal is valid for a period of one year. Approximately one month prior to that time, a single renewal form should be sent to the REB office.

The OHSN-REB complies with the membership requirements and operates in compliance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans; the International Conference on Harmonization - Good Clinical Practice: Consolidated Guideline and the provisions of the Personal Health Information Protection Act 2004.

Yours sincerely,

Raphael Saginur, M.D.
Chairperson
Ottawa Health Science Network Research Ethics Board

/kd

MOUNT SINAI HOSPITAL

Research Ethics Board

Notification of REB Continued Approval

Date: November 24, 2016

To: Dr. Nan Okun

Re: 15-0282-C
Investigating Maternal Serum Marker Screening in the Ontario IVF Population

REB Review Type:	Delegated
REB Initial Approval Date:	30 November 2015
REB Expiry Date:	30 November 2017
Consent Form(s) Currently Approved for Use:	N/A

The above-named study has received continued approval from the Mount Sinai Hospital Research Ethics Board until the expiry date noted above. Additionally, the REB has approved the above mentioned consent form. If the study is expected to continue beyond the expiry date, you are responsible for ensuring the study receives re-approval. The REB must also be notified of the completion or termination of this study and a final report provided.

If, during the course of the research, there are any serious adverse events, confidentiality concerns, changes in the approved project, or any new information that must be considered with respect to the project, these should be brought to the immediate attention of the REB. In the event of a privacy breach, you are responsible for reporting the breach to the MSH REB and the MSH Corporate Privacy Office (in accordance with Ontario health privacy legislation – Personal Health Information Protection Act, 2004). Additionally, the MSH REB requires reports of inappropriate/unauthorized use of the information. As the Principal Investigator, you are responsible for the ethical conduct of this study.

The MSH Research Ethics Board operates in compliance with the Tri-Council Policy Statement, ICH/GCP Guidelines and Part C, Division 5 of the Food and Drug Regulations of Health Canada.

Sincerely,

Amanda Hindle, B.Sc.
Ethics Coordinator, Research Ethics Board

For: Ronald Heslegrave, Ph.D.
Chair, Mount Sinai Hospital Research Ethics Board

CHEO Research Ethics Board
Approval - Delegated Review

Principal Investigator: Dr. Mark Walker
REB Protocol No: 16/03PE
Romeo File No: 20160258
Project Title: CHEOREB# 16/03PE - The effect of the type of infertility on placental-mediated adverse outcomes for patients that used IVF with or without ICSI
Primary Affiliation: Clinical Research\Epidemiology
Protocol Status: Active
Approval Date*: June 08, 2016
Valid Until:** May 15, 2017
Annual Renewal Submission Deadline: April 15, 2017

Documents Reviewed & Approved:

Document Name	Comments	Version Date
Other Document	List of variables	2016/05/17
Protocol	Protocol	2016/05/18

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 under the delegated review stream, which is reserved for projects that involve no more than minimal risk to human subjects.

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. Investigators must submit an annual renewal report to the REB 30 days prior to the expiration date stated above.
3. The investigator must not implement any deviation from, or changes to, the protocol, consents or assents without the approval of the REB.
4. The investigator must, prior to use, submit to the Board changes to the study documentation, e.g., changes to the informed consent letters, recruitment materials.
5. Investigators must provide the Board with French versions of the consent form, 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.

6. 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).
7. Investigators must notify the REB of any study closures (closed to accrual, temporary, premature or permanent).
8. Investigators must submit a final report at the conclusion of the study.

Should you have any questions or concerns, please do not hesitate to contact the Research Ethics Board Office

Regards,

Dr. Carole Gentile
Chair, Research Ethics Board
Présidente, 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.

**The expiry date of REB approval for initial study application that required no modifications will be as follows:

- If the date of review and approval was **on or before** the 15th of the month, the expiry date will be the 15th of the month prior to the date of review and approval by the Chair and/or delegate *in the following year*;
- If the date of review and approval was **after** the 15th the expiry date will be the 15th of the month in which the date of review and approval by the REB *in the following year*.

The expiry date of REB approval for initial study applications that **require modifications** will be as follows:

- If the initial feedback was sent **on or before** the 15th of the month, the expiry date will be the 15th of the month prior to the date the letter of REB feedback is issued to the investigator(s) *in the following year*;
- If the initial feedback was sent **after** the 15th the expiry date will be the 15th of the month in which the feedback was sent *in the following year*.

Ottawa Hospital Research Institute
Institut de recherche de l'Hôpital d'Ottawa

uOttawa

University of Ottawa Heart Institute
Institut de cardiologie de l'Université d'Ottawa

Ottawa Health Science Network Research Ethics Board/ Conseil d'éthique de la recherche du Réseau de science de la santé d'Ottawa

October 28, 2

Dr. Mark Walker
Attn: Ruth Rennicks White

Re: Protocol # 20160797-01H **The effect of the type of infertility on placental-mediated adverse outcomes for patients that used IVF with or without ICSI**

Protocol approval valid until - October 27, 2017

Thank you for the e-mail of October 27, 2016 from Andrea Lanes. I am pleased to inform you that your Application for Chart Review underwent expedited review by the Ottawa Health Science Network Research Ethics Board (OHSN-REB), and is approved. No changes, amendments or addenda may be made to the protocol without the OHSN-REB's review and approval.

Approval is for the following:
- Protocol (version 1) dated October 6, 2016

If the study is to continue beyond the expiry date noted above, a Renewal Form should be submitted to the OHSN-REB approximately six weeks prior to the current expiry date. If the study has been completed by this date, a Termination Report should be submitted.

The Ottawa Health Science Network Research Ethics Board (OHSN-REB) was created by the merger of both the Ottawa Hospital Research Ethics Board (OHREB) and the Human Research Ethics Board (HREB) for meetings held at the University of Ottawa Heart Institute.

OHSN-REB complies with the membership requirements and operates in compliance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans; the International Conference on Harmonization - Good Clinical Practice: Consolidated Guideline and the provisions of the Personal Health Information Protection Act 2004.

Yours sincerely,

Raphael Saginur, M.D.
Chairperson
Ottawa Health Science Network Research Ethics Board

/kd

APPENDIX B: Supplemental information for Manuscript 1

Supplemental Text B-1 Primary search strategy

The primary search strategy was developed using Ovid MEDLINE, Embase and COCHRANE databases. Appropriate articles were chosen using medical subject headings (MeSH) and text words (tw).

Database: Embase Classic+Embase <1947 to 2015 April 08>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present>

Search Strategy: April 9, 2015

---

1 maternal serum screening tests/ (209)

2 ((maternal or prenatal) adj3 serum adj5 screen$).tw. (2426)

3 ((maternal or prenatal) adj3 blood adj5 screen$).tw. (144)

4 alpha-Fetoproteins/bl, an (7917)

5 Pregnancy-Associated Plasma Protein-A/ (3363)

6 (papp a or papp alpha or pregnancy associated plasma protein a or pappalysin 1).tw. (3630)

7 (alphafetoprotein$ or alpha-Fetoprotein$ or afp).tw. (42310)

8 Chorionic Gonadotropin, beta Subunit, Human/bl, an (1820)

9 (b-hcg or bhcg).tw. (846)

10 Chorionic Gonadotropin/bl, an (7084)

11 (human chorionic gonadotropin or hcg).tw. (59567)

12 exp estriol/ (16774)

13 (uE3 or free estriol or free oestriol or unconjugated estriol or unconjugated oestriol).tw. (1540)

14 Nuchal Translucency Measurement/ (2845)

15 Nuchal Translucen$.tw. (4213)

16 inhibins/ (9739)

17 inhibin$ a.tw. (2324)

18 or/1-17 (134980)

19 exp fertilization in vitro/ or sperm injections, intracytoplasmic/ (75624)

20 (in vitro fertil$ or ivf).tw. (62842)

21 sperm injection$.tw. (13078)

22 or/19-21 (94404)

23 18 and 22 (8619)

24 Pregnancy/ (1323036)

25 prenatal diagnosis/ (80819)

26 pregnancy trimester, first/ or pregnancy trimester, second/ (62377)

27 Fetus/ (247561)

28 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).tw. (1372125)

29 or/24-28 (1976885)

30 23 and 29 (6071)

31 30 use prmz (2525)

32 *maternal serum screening test/ (105)

33 ((maternal or prenatal) adj3 serum adj5 screen$).tw. (2426)

34 ((maternal or prenatal) adj3 blood adj5 screen$).tw. (144)

35 *alpha fetoprotein blood level/ or *alpha fetoprotein/ (17041)

36 (alphafetoprotein$ or alpha-Fetoprotein$ or afp).tw. (42310)

37 *pregnancy associated plasma protein A/ (1723)

38 (papp a or papp alpha or pregnancy associated plasma protein a or pappalysin 1).tw. (3630)

39 *chorionic gonadotropin beta subunit/ (3113)

40 (b-hcg or bhcg).tw. (846)

41 *chorionic gonadotropin/ (34598)

42 (human chorionic gonadotropin or hcg).tw. (59567)

43 estriol/ (16752)

44 (uE3 or free estriol or free oestriol or unconjugated estriol or unconjugated oestriol).tw. (1540)

45 *nuchal translucency measurement/ (978)

46 nuchal translucen$.tw. (4213)

47 inhibin A/ (1576)

48 inhibin$ a.tw. (2324)

49 or/32-48 (138154)

50 *fertilization in vitro/ (34764)

51 *intracytoplasmic sperm injection/ (7064)

52 (in vitro fertil$ or ivf).tw. (62842)

53 sperm injection$.tw. (13078)

54 50 or 51 or 52 or 53 (77604)

55 49 and 54 (8291)

56 *pregnancy/ (204266)

57 *prenatal diagnosis/ (43792)

58 *first trimester pregnancy/ (8632)

59 *second trimester pregnancy/ (3778)

60 *prenatal screening/ (21565)

61 *fetus/ (63951)

62 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).tw. (1372125)

63 or/56-62 (1429236)

64 55 and 63 (5440)

65 64 use emczd (3458)

66 31 or 65 (5983)

67 remove duplicates from 66 (4269)

68 67 use prmz (2508)

69 67 use emczd (1761)

Database: EBM Reviews - Cochrane Central Register of Controlled Trials <March 2015>

Search Strategy: April 9, 2015

---

1 maternal serum screening tests/ (0)

2 ((maternal or prenatal) adj3 serum adj5 screen$).tw,hw. (15)

3 ((maternal or prenatal) adj3 blood adj5 screen$).tw,hw. (3)

4 alpha-Fetoproteins/bl, an (3)

5 Pregnancy-Associated Plasma Protein-A/ (15)

6 (papp a or papp alpha or pregnancy associated plasma protein a or pappalysin 1).tw,hw. (42)

7 (alphafetoprotein$ or alpha-Fetoprotein$ or afp).tw,hw. (354)

8 Chorionic Gonadotropin, beta Subunit, Human/bl, an (0)

9 (b-hcg or bhcg).tw,hw. (18)

10 Chorionic Gonadotropin/bl, an (7)

11 (human chorionic gonadotropin or hcg).tw,hw. (1403)

12 exp estriol/ (195)

13 (uE3 or free estriol or free oestriol or unconjugated estriol or unconjugated oestriol).tw,hw. (16)

14 Nuchal Translucency Measurement/ (9)

15 Nuchal Translucen$.tw,hw. (17)

16 inhibins/ (85)

17 inhibin$ a.tw,hw. (174)

18 or/1-17 (2123)

19 exp fertilization in vitro/ or sperm injections, intracytoplasmic/ (1618)

20 (in vitro fertil$ or ivf).tw,hw. (3116)

21 sperm injection$.tw,hw. (918)

22 or/19-21 (3539)

23 18 and 22 (637)

24 Pregnancy/ (15093)

25 prenatal diagnosis/ (140)

26 pregnancy trimester, first/ or pregnancy trimester, second/ (923)

27 Fetus/ (281)

28 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).tw,hw. (25968)

29 or/24-28 (25968)

30 23 and 29 (491)

Supplemental Table B-1 General characteristics of the 40 included studies

First author and year of publication	Study design	Location of study	Number of subjects	Newcastle-Ottawa Scale*
Anckaert, 2008[14]	Cohort	Beligium	4,341	6
Ball, 2013[15]	Cohort	UK and Denmark	153,369	7
Ball, 2012[16]	Cohort	UK	26,213	8
Bar-Hava, 2001[17]	Case-control	Israel	140	7
Bellver, 2005[18]	Cohort	Spain	1,054	7
Bellver, 2013[19]	Cohort	Spain	4,053	8
Bender, 2010[20]	Cohort	Germany	1782	7
Bersinger, 2001[21]	Cohort	Switzerland	93	7
Bersinger, 2004[22]	Case-control	Switzerland	176	7
Bersinger, 2005[23]	Case-control	Switzerland	252	7
Bredaki, 2011[24]	Cohort	London, UK	1,500	8
Cowans, 2013[25]	Cohort	England	205,341	7
Engels, 2010[9]	Case-control	Amsterdam	1,559	9
Engels, 2013[26]	Cohort	Amsterdam	20,190	8
Frishman, 1997[27]	Cohort	Providence, USA	69	7
Ghisoni, 2003[28]	Case-control	Milan, Italy	571	8
Giorgetti, 2013[29]	Cohort	Marseille	1,848	7
Gjerris, 2009[30]	Cohort	Denmark	3,543	7
Heinonen, 1996[31]	Cohort	Kuopio, Finland	4,976	7
Hui, 2003[32]	Cohort	China	17,446	4

Hui, 2005[33]	Cohort	China	16,935	6
Hui, 2005[34]	Case-control	China	550	7
Kagan, 2008[35]	Cohort	England	97,294	6
Lam, 1999[36]	Cohort	England	2,865	7
Lambert-Messerlian, 2006[10]	Cohort	China	38,032	7
Lambert-Messerlian, 2009[37]	Cohort	US and Canada	15,363	9
Liao, 2001[38]	Cohort	US	1,453	5
Matilainen, 2011[39]	Case-control	UK	24,959	8
Maymon, 2002[40]	Cohort	Finland	356	7
Maymon, 2004[41]	Cohort	Israel	1,880	7
Muller, 2003[42]	Cohort	Israel	22,529	6
Niemimaa, 2001[43]	Cohort	France	4,314	6
Orlandi, 2002[44]	Case-control	Italy	444	4
Raty, 2002[45]	Cohort	Finland	6,664	7
Ribbert, 1996[46]	Cohort	Netherlands	4,799	8
Rice, 2005[47]	Case-control	British Columbia, Canada	684	7
Tul, 2006[48]	Case-control	Slovenia	1,098	4
Wald, 1999[49]	Case-control	London, UK	906	5
Wojdemann, 2001[50]	Cohort	Copenhagen	3,073	6
Amor, 2009[51]	Cohort	Victoria, Australia	51,992	7

* Newcastle Ottawa Scale: maximum possible score = 9.

Supplemental Table B-2 Exposure and outcome information from the 40 included studies

	Exposure	Outcomes	MSS Markers						
First author and year of publication	**How was use of IVF achieved?**	**How were the MSS markers and/or nuchal translucency thickness ascertained?**	**PAPP-A**	**AFP**	**free beta-hCG**	**total hCG**	**µE3**	**DIA**	**NT thickness**
Anckaert, 2008[14]	Medical record review	Other electronic database	Yes	No	Yes	Yes	No	No	No
Ball, 2013[15]	Other	Other electronic database	Yes	No	Yes	No	No	No	No
Ball, 2012[16]	Self-report	Other electronic database	Yes	No	Yes	Yes	No	No	No
Bar-Hava, 2001[17]	Medical record review	Other electronic database	No	Yes	No	Yes	Yes	No	No
Bellver, 2005[18]	Medical record review	Other electronic database	Yes	No	Yes	No	No	No	Yes
Bellver, 2013[19]	Medical record review	Other electronic database	Yes	No	Yes	No	No	No	Yes
Bender, 2010[20]	Other electronic database	Other electronic database	Yes	No	Yes	No	No	No	Yes
Bersinger, 2001[21]	Medical record review	Other	Yes	No	No	No	No	No	No
Bersinger, 2004[22]	Medical record	Other	Yes	No	No	Yes	No	No	No

	review								
Bersinger, 2005[23]	Medical record review	Other	Yes	No	No	Yes	No	No	No
Bredaki, 2011[24]	Other electronic database	Other electronic database	No	Yes	No	No	No	No	No
Cowans, 2013[25]	Other electronic database	Other electronic database	Yes	No	Yes	No	No	No	No
Engels, 2010[9]	Other electronic database	Other electronic database	Yes	No	Yes	No	No	No	Yes
Engels, 2013[26]	Medical record review	Other electronic database	Yes	No	Yes	No	No	No	Yes
Frishman, 1997[27]	Other	Other electronic database	No	Yes	No	Yes	Yes	No	No
Ghisoni, 2003[28]	Medical record review	Other electronic database	Yes	No	Yes	No	No	No	Yes
Giorgetti, 2013[29]	Medical record review	Other electronic database	Yes	No	Yes	No	No	No	Yes
Gjerris, 2009[30]	Registry	Registry	Yes	No	Yes	No	No	No	Yes
Heinonen, 1996[31]	Other electronic database	Other electronic database	No	Yes	No	Yes	No	No	No
Hui, 2003[32]	Other	Other electronic database	No	Yes	No	Yes	No	No	No
Hui, 2005[33]	Other electronic	Other electronic	No	No	No	No	No	No	Yes

	database	database							
Hui, 2005[34]	Other electronic database	Other electronic database	Yes	No	Yes	No	No	No	No
Kagan, 2008[35]	Other	Other electronic database	Yes	No	Yes	No	No	No	No
Lam, 1999[36]	Medical record review	Other electronic database	No	Yes	No	Yes	No	No	No
Lambert-Messerlian, 2006[10]	Other electronic database	Other electronic database	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lambert-Messerlian, 2009[37]	Other electronic database	Other electronic database	Yes	No	No	Yes	No	No	No
Liao, 2001[38]	Other electronic database	Other electronic database	Yes	No	Yes	No	No	No	Yes
Matilainen, 2011[39]	Medical record review	Other electronic database	Yes	No	Yes	No	No	No	Yes
Maymon, 2002[40]	Medical record review	Medical record review	Yes	Yes	Yes	Yes	Yes	No	Yes
Maymon, 2004[41]	Medical record review	Other electronic database	Yes	Yes	No	Yes	Yes	Yes	Yes
Muller, 2003[42]	Other electronic database	Other electronic database	No	Yes	Yes	Yes	Yes	No	No
Niemimaa, 2001[43]	Other electronic database	Other electronic database	Yes	No	Yes	No	No	No	No

Orlandi, 2002[44]	Other electronic database	Other electronic database	Yes	No	Yes	No	No	No	Yes
Raty, 2002[45]	Registry	Registry	No	Yes	Yes	No	No	No	No
Ribbert, 1996[46]	Other electronic database	Other electronic database	No	Yes	No	Yes	No	No	No
Rice, 2005[47]	Other electronic database	Other electronic database	No	Yes	No	Yes	Yes	No	No
Tul, 2006[48]	Other	Other electronic database	Yes	No	Yes	No	No	Yes	Yes
Wald, 1999[49]	Other	Other electronic database	No	Yes	Yes	Yes	Yes	Yes	No
Wojdemann, 2001[50]	Other	Other electronic database	Yes	No	Yes	No	No	No	Yes
Amor, 2009[51]	Registry	Registry	Yes	No	Yes	No	No	No	Yes

Supplemental Text B-2 Reprint of published article

Maternal serum screening markers and nuchal translucency measurements in in vitro fertilization pregnancies: a systematic review

Andrea Lanes, M.Sc.,[a,b] Tianhua Huang, Ph.D.,[b] Ann E. Sprague, Ph.D.,[b,f] Arthur Leader, M.D., F.R.C.S.C.,[d,e] Beth Potter, Ph.D.,[a] and Mark Walker, M.D., F.R.C.S.(C.)[b,c,d,f]

[a] School of Epidemiology, Public Health and Preventive Medicine, University of Ottawa; [b] BORN Ontario, CHEO Research Institute; [c] Ottawa Hospital Research Institute, Center for Practice-Changing Research; [d] Department of Obstetrics and Gynecology, University of Ottawa; [e] Ottawa Fertility Centre; and [f] CHEO Research Institute, Centre for Practice-Changing Research, Ottawa, Ontario, Canada

Objective: To study the current literature on the association between IVF treatment and maternal serum screening marker levels and nuchal translucency thickness.
Design: Systematic review.
Settings: Not applicable.
Patient(s): Eligible studies included those with an exposed group of pregnant women that used IVF with or without intracytoplasmic sperm injection to conceive and a control group of pregnant women who conceived spontaneously.
Intervention(s): IVF treatment to conceive.
Main Outcome Measure(s): Outcomes evaluated included maternal serum screening markers (pregnancy-associated plasma protein A [PAPP-A], alpha-fetoprotein, hCG, unconjugated estriol, dimeric inhibin-A) and nuchal translucency thickness.
Result(s): Database searches identified 4,118 titles and abstracts that were independently screened, which resulted in 76 articles that were assessed for eligibility. Additionally, one study was added for consideration based on expert knowledge. There were 29 cohort and 11 case-control studies in the descriptive review. The most commonly reported markers were PAPP-A and free β-hCG, which were reported in 28 and 26 studies, respectively. The studies that reported effect sizes for PAPP-A and free β-hCG were not statistically significant.
Conclusion(s): A decrease in PAPP-A and an increase in total hCG was consistently reported among the included studies. However, owing to the variability in the levels of the other maternal serum screening markers reported and the inability to conduct a meta-analysis, we were unable to generalize about the differences between prenatal screening results in the IVF population. (Fertil Steril® 2016;106:1463–9. ©2016 by American Society for Reproductive Medicine.)
Key Words: In vitro fertilization, maternal serum screening markers, systematic review

Discuss: You can discuss this article with its authors and with other ASRM members at https://www.fertstertdialog.com/posts/11309-maternal-serum-screening-markers-and-nuchal-translucency-measurements-in-in-vitro-fertilization-pregnancies-a-systematic-review

Prenatal screening is performed primarily to identify pregnancies at an increased risk for fetal anomalies, such as Down syndrome or trisomy 18, but can also detect risk of open neural tube defects. Aneuploidy screening was first performed in the 1960s (1) and has evolved since then. Currently, in Ontario, women are offered prenatal screening options that include examination of maternal blood sample(s) with the addition of an ultrasound to estimate the risk of fetal aneuploidy before cell-free fetal DNA or invasive testing (amniocentesis) (1). There are a variety of prenatal screening tests that can be used to assess risk including first trimester combined screening, integrated prenatal screening, serum integrated prenatal screening, triple screening, and quadruple screening.

Children with Down syndrome have three copies of chromosome 21, experience intellectual disabilities, which vary among affected children, and typically have specific facial characteristics and hypotonia (1–3). Individuals with

Received March 30, 2016; revised July 26, 2016; accepted July 28, 2016; published online August 13, 2016.
A. Lanes has nothing to disclose. T.H. has nothing to disclose. A.E.S. has nothing to disclose. A. Leader has nothing to disclose. B.P. has nothing to disclose. M.W. has nothing to disclose.
Supported by the Ontario Graduate Scholarship (2013–14; to A. Lanes) and the Queen Elizabeth II Scholarship in Science and Technology (2015–16; to A. Lanes).
Reprint requests: Andrea Lanes, M.Sc., CHEO Research Institute, Centre for Practice-Changing Research Building, 401 Smyth Road, Room L1151, Ottawa, Ontario, Canada K1H 8L1 (E-mail: alane098@uottawa.ca).

Fertility and Sterility® Vol. 106, No. 6, November 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.07.1120

Down syndrome are also at increased risk of dementia, cardiac malformation, leukemia, and immune system defects (3). Most pregnancies affected by trisomy 18 end in miscarriage or stillbirth (95%), while death by 1 year of age is the expected outcome among live-born infants with this syndrome (2). Open neural tube defects occur when the neural tube fails to close during embryologic development (4). Anencephaly and variable degrees of spina bifida are two of the most common types of open neural tube defects (5). While anencephaly usually results in miscarriage or stillbirth, infants with spina bifida may have minimal living restrictions or more significant problems that allow them to survive with medical complications and long-term disabilities that vary in severity (5).

IVF is a form of assisted conception that includes extracorporeal fertilization and embryo culture (6). It involves controlled ovarian stimulation, transvaginal ultrasound-guided retrieval of oocytes, insemination or injection of the egg to assist fertilization (to combine genetic material from both gametes resulting in a zygote), laboratory culture in an incubator over 4–6 days, and transfer of the growing embryo (6). Insemination and fertilization can occur with or without intracytoplasmic sperm injection (ICSI). ICSI is a method of insemination wherein a single spermatozoon is injected into the cytoplasm of the mature (metaphase II) oocyte (6). This process is associated with an increased risk of sex chromosomal abnormalities among offspring of men with severe male factor infertility (7, 8).

At the time of the prenatal screen, information about the method of conception is requested to determine whether a correction factor should be applied to assay results of the maternal serum screening markers. Studies on small cohorts have shown that there are differences in the marker measurements seen among IVF pregnancies compared with non-IVF pregnancies (9, 10). An incorrect interpretation of these results may miss an affected pregnancy or cause unnecessary invasive testing (amniocentesis) to be performed. The objective of this study was to synthesize existing knowledge about the associations between IVF treatment and maternal serum screening marker levels and nuchal translucency (NT) thickness.

MATERIALS AND METHODS

The protocol for this systematic review was registered with the PROSPERO International prospective register of systematic reviews (registration no. CRD42015019545) (11). The manuscript was developed and reported in accordance with the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement (12).

Search Strategy

A search strategy was developed using medical subject headings and key words related to the population, exposure, and screen results. The Ovid MEDLINE, Embase, and COCHRANE databases were searched from inception until April 2015. The Supplemental Appendix outlines the full search strategy. This search strategy included both observational (i.e., cross-sectional, cohort, case-control) and experimental (i.e., randomized clinical trial) study designs. Eligible studies included those with the exposure (IVF with or without ICSI) and a control group of pregnant women who conceived spontaneously. Outcomes evaluated included maternal serum screening markers (pregnancy-associated plasma protein A [PAPP-A], alpha-fetoprotein [AFP], hCG, free β-hCG, unconjugated estriol [μE3], dimeric inhibin-A [DIA]) and NT thickness. There were no restrictions based on language of the study publication. Studies where the comparison group was not women who conceived spontaneously were excluded. Reference lists from the included studies were investigated for additional articles that met the inclusion criteria. All titles and abstracts were independently screened by two reviewers, an epidemiologist and a prenatal screening expert (A. Lanes and T.H.), and any discrepancies were resolved through discussion. A second full-text screen of the titles and abstracts that met the study inclusion and exclusion criteria was performed. Studies were excluded if both reviewers determined that the articles were irrelevant or inaccessible or that adequate translations were not achievable. Data were then abstracted from the articles that were considered to meet all study inclusion and exclusion criteria after the second screen.

Data Abstraction and Assessment of Quality

A data abstraction form was developed to systematically extract information from the included studies. This form was independently completed by both reviewers. Information captured included study design, type of publication, population size, study objective, definition of exposure, definition of outcome, outcomes included in each study, confounding variables adjusted for in the analysis, and unadjusted and adjusted measures of effect. Risk of bias was assessed by each reviewer using the Newcastle-Ottawa Scale (13). This is a commonly used tool that investigates potential bias for cohort and case-control studies in three categories: selection, comparability, and outcome (13). A study can be given a minimum of zero stars and a maximum of nine stars. To estimate interrater reliability, congruence for a subset of six questions from the data abstraction form was evaluated. Questions were selected from different sections of the form including data quality, definition of cohort, and quantitative measures. Kappa statistics were calculated for four questions: three questions had perfect agreement (kappa statistic of 1.0), and one question had a kappa statistic of 0.58. We were unable to report kappa for two questions.

Data Synthesis and Analysis

Descriptive characteristics including type of study, year of publication, and study location were summarized. A priori the authors decided to abstract median multiples of the median (MoM) as the measure of effect for this systematic review. This was the appropriate descriptive statistic to account for the bimodal distribution of the maternal serum screening markers. Consequently, meta-analyses were not feasible since it is not possible to combine median MoMs across studies. We did not present forest plots without a pooled effect, because the median MoMs were reported using

distinct measures of variance (i.e., standard deviation, 95% confidence interval, ranges). As a substitute we reported the magnitude of change for each maternal serum screening marker. When available, study data on the outcomes among frozen embryo transfer (FET) cycles and fertility treatment cycles that used donor oocytes were abstracted.

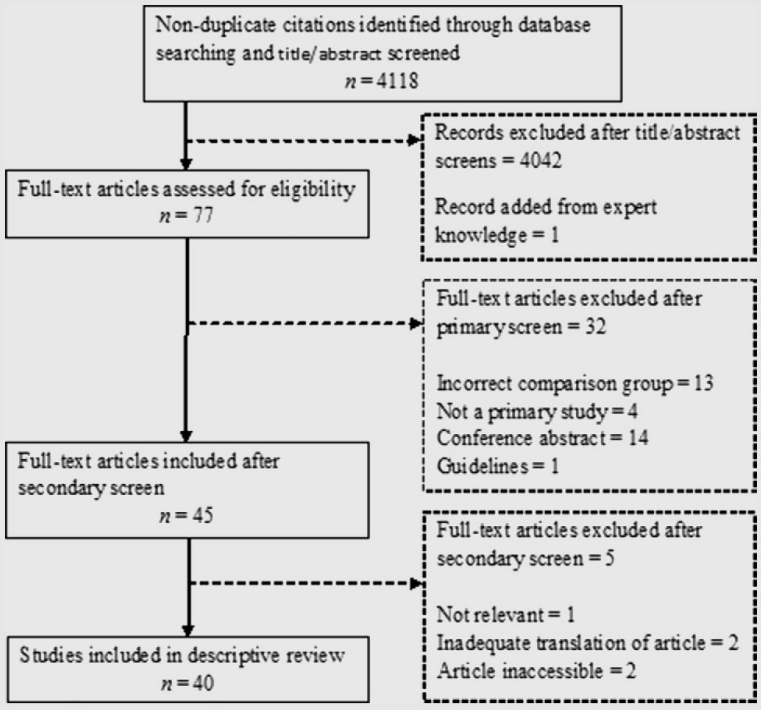
RESULTS

Database searches identified 4,118 nonduplicate titles and abstracts that were independently screened. There were 4,042 records excluded from the primary screen, which resulted in 77 articles that were assessed for eligibility. Additionally, one study was added for consideration based on expert knowledge. Upon completion of the second screen, 45 articles remained, of which five were excluded (Fig. 1). One excluded article was determined to be irrelevant; two articles were inaccessible; and adequate translations were not attainable for two articles. In total 40 articles were included.

The majority of studies included in this systematic review were published before 2010 (30 studies), with the remainder having a publication date between 2010 and 2015 (10 studies). Twenty-eight studies were from the United Kingdom or Europe, four from North America, four from China, three from Israel, and one from Australia. There were 29 cohort and 11 case-control studies in the descriptive review. The Newcastle-Ottawa risk of bias assessment had a median score of 7 and a range between 4 and 9. For cohort studies, the range of the scores was 2–4 with a median score of 4 for selection, 0–2 with a median score of 0 for comparability, and 2–3 with a median score of 3 for outcome. The scores varied slightly for case-control studies. The range for selection was 2–4 with a median score of 4, a range of 0–2 with a median score of 1 for comparability, and a range of 1–3 with a median score of 2 for outcome. Twenty-six of the included studies determined the presence of the exposure through record linkage or a secure record, whereas only five studies used self-report.

The most commonly reported maternal serum screening marker was PAPP-A in 28 studies. However, only 14 studies reported a median MoM (Table 1). Almost all of these studies reported a reduction in PAPP-A among IVF patients compared with those who conceived spontaneously (13 studies). All seven studies that reported a median MoM for patients who used ICSI found a reduced estimate compared with a spontaneous conception group. A similar trend was seen for AFP. This serum marker was evaluated in 14 studies, and the majority of studies that reported a median MoM saw a

FIGURE 1



Flow diagram illustrating the selection of studies into the systematic review.

Lanes. Maternal serum screening markers in IVF. Fertil Steril 2016.

TABLE 1

Observed median MoM maternal marker serum levels and NT thickness in IVF patients compared with patients who conceived spontaneously.

	PAPP-A		AFP		Free β-hCG		Total hCG		μE3		DIA		NT	
Reference	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI
Anckaert et al. 2008 (14)	0.75[a]	0.94[a]			0.90	1.07								
Ball et al. 2013 (15)														
Ball et al. 2012 (16)														
Bar-Hava et al. 2001 (17)														
Bellver et al. 2005 (18)	1.06				0.83	1.13								
Bellver et al. 2013 (19)	↓	↓			↑	↑							—	—
Bender et al. 2010 (20)	0.86[a]	0.90[a]			1.10[a]	1.10[a]							1.03	1.02
Bersinger et al. 2001 (21)														
Bersinger et al. 2004 (22)														
Bersinger et al. 2005 (23)														
Bredaki et al. 2011 (24)														
Cowans and Spencer 2013 (25)	1.01				1.03[a]									
Engels et al. 2010 (9)														
Engels et al. 2013 (26)														
Frishman et al. 1997 (27)			0.95				1.22		0.90					
Ghisoni et al. 2003 (28)														
Giorgetti et al. 2013 (29)	0.74[a]	0.81[a]			0.96	1.09							1.02	1.06
Gjerris et al. 2009 (30)														
Heinonen et al. 1996 (31)														
Hui 2003 (32)			0.90[a]	0.86[a]			1.07	0.92						
Hui et al. 2005 (33)													1.07[a]	1.09[a]
Hui et al. 2005 (34)	0.83[a]	0.70[a]			0.87[a]	0.82								
Kagan et al. 2008 (35)														
Lam et al. 1999 (36)			0.88[a]	0.76[a]			1.15	0.88						
Lambert-Messerlian et al. 2006 (10)	0.94		1.05		1.13		1.10[a]		0.94[a]		1.12[a]		1.03	
Lambert-Messerlian et al. 2009 (37)	0.91[a]						1.04							
Liao et al. 2001 (38)	1.00[a]	0.86[a]			1.21[a]	1.09							0.97	1.00
Matilainen et al. 2011 (39)	0.82[a]				1.00								1.03	
Maymon and Shulman 2002 (40)	0.96[a]		1.13[a]		1.16		1.12		0.94				1.16	
Maymon and Shulman 2004 (41)	0.78[a]		1.02				1.10		0.99		0.98		1.14[a]	
Muller et al. 2003 (42)			0.96	0.95	1.05	1.11	1.10	1.01	0.90	1.00				
Niemimaa et al. 2001 (43)														
Orlandi et al. 2002 (44)	0.79[a]	0.96			0.84	1.13							1.10	1.02
Raty et al. 2002 (45)			0.95	1.00	1.19	1.07								
Ribbert et al. 1996 (46)														
Rice et al. 2005 (47)			0.99				1.12		1.12					
Tul et al. 2006 (48)	0.94[a]	0.82[a]			1.04	0.91					1.11[a]	1.48[a]	1.00	0.99
Wald et al. 1999 (49)			0.99		1.09[a]		1.14[a]		0.94[a]		0.89			
Wojdemann et al. 2001 (50)	1.02				1.14								0.97	
Amor et al. 2009 (51)														

Note: All values are median MoMs. The upward arrow (↑) denotes an increase compared with spontaneous conceptions when exact data were not available. The downward arrow (↓) denotes a decrease compared with spontaneous conceptions when exact data were not available. The dash (—) indicates equivalent to spontaneous conception. If there was a separate group for ICSI in a study, it was captured under the ICSI header.

[a] Statistically significant at $\alpha = .05$.

Lanes. Maternal serum screening markers in IVF. Fertil Steril 2016.

decrease (seven studies) compared with spontaneous conceptions. The three studies that measured the effect of ICSI on AFP saw a decrease or no change.

This systematic review included studies that investigated free β-hCG in the first trimester or total hCG in the second trimester. Free β-hCG and total hCG were reported in 26 and 17 studies, respectively. Among the studies that reported a median MoM for free β-hCG, the results varied. Eight studies reported an increase among IVF users compared with spontaneous conception, while six studies reported a decrease. The results from the studies investigating total hCG were homogenous. All studies reported an increased estimate among IVF users.

A small number of studies investigated μE3 and DIA (eight and four studies, respectively). An appropriate estimate was reported in 11 of the 12 studies. The majority of studies on μE3 reported a decrease among those using IVF, whereas the direction of change was evenly distributed for DIA. NT measurements were discussed in 19 studies, and approximately 50% of these studies reported an appropriate estimate for this systematic review. Almost all of these studies found an increased NT median MoM in patients

who used IVF to conceive compared with those who conceived spontaneously.

There were seven studies that reported median MoMs comparing maternal serum screening markers of patients who had an FET with individuals who conceived spontaneously (Table 2). The direction of the estimate was lower among studies that reported PAPP-A and consistently higher among studies that reported AFP and NT. Studies that investigated free β-hCG reported estimates that varied in direction.

Two studies investigated the impact of donor oocytes on maternal serum marker levels and NT measurements (10, 19). Lambert-Messerlian et al. (10) found that the median MoM was increased for PAPP-A, AFP, total hCG, and DIA compared with those who conceived spontaneously, whereas a decrease was reported for free β-hCG, μE3, and NT. The study by Bellver et al. (19) investigated IVF and ICSI using donor oocytes separately. This study found an increase in both PAPP-A and free β-hCG for IVF conceptions with donor oocytes compared with spontaneous conception. When the authors investigated ICSI using donor oocytes compared with spontaneous conception they found a decrease in PAPP-A and a statistically significant increase in free β-hCG.

DISCUSSION

Main Findings

Prenatal screening is primarily used to estimate the risk of aneuploidies. Different types of prenatal tests have varying sensitivity and specificity (i.e., first trimester combined screening, serum integrated prenatal screening, integrated prenatal screening, triple screening, and quadruple screening) (2). The current prevalence rate for infertility from countries around the world has been reported to range from 3.5% to 16.7% (52). This systematic review summarized the variation of serum levels reported in 40 studies and over 700,000 pregnancies. The majority of studies included reported altered maternal serum screening marker levels among women who used IVF to conceive compared with women who conceived spontaneously (10, 14, 18, 20, 25, 27, 29, 32–34, 36–42, 44, 47–50, 53). This suggests that we should ensure that the results are being correctly interpreted.

Interpretation

The maternal serum markers in this systematic review were consistent, since they are universally defined. All of the studies had a cohort or case-control study design. However, the analytical approach varied across studies. Therefore there was substantial design heterogeneity present. The included studies used a variety of methods to report effect estimates. Only extracting data reported as a median MoM allowed for the inclusion of appropriate outcome measures. Including studies that only reported mean MoMs in the quantitative analysis of this review would have provided biased results. The results would have been skewed in the direction of the outliers for each maternal serum screening marker.

Although we were not able to pool the results from these studies and interpret a magnitude of change, we were able to draw broad conclusions from the direction of change of the estimate of each maternal serum screening marker for IVF patients compared with women who conceived spontaneously (Table 1). The majority of studies found a decreased estimate of PAPP-A and an increased NT measurement in the IVF population; these markers were measured in the first trimester. In the second trimester, there was a decrease in AFP, an increase in total hCG, and a decrease in the estimate of μE3 for IVF patients. This corresponds with an increased risk of Down syndrome (Table 3) (2). Either this is a true association or there may be an increased false positive rate for Down syndrome among IVF conceptions. This identified concerns regarding the robustness of this method of screening for IVF pregnancies. The presence of vanishing twins among singleton IVF pregnancies may have influenced the levels of the maternal serum screening markers that were observed in each study.

Noninvasive prenatal testing (NIPT) may be a more suitable first-line method of testing for aneuploidy risks and should be investigated as an alternative screening method. NIPT uses circulating cell free maternal plasma DNA to investigate chromosomal abnormalities in the fetus and is usually a second-tier test for women who screen positive with

TABLE 2

Observed median MoM maternal serum marker serum levels and NT thickness in women who had FET compared with women who conceived spontaneously.

			FET				
Reference	PAPP-A	AFP	Free β-hCG	Total hCG	μE3	DIA	NT
Anckaert et al. 2008 (14)	1.05		1.12				
Bellver et al. 2013 (19)	↓						
Hui 2003 (32)		1.10		1.24[a]			
Hui et al. 2005 (33)							1.09[a]
Hui et al. 2005 (34)	0.95[a]		1.21[a]				
Matilainen et al. 2011 (39)	0.78		0.94				1.00
Raty et al. 2002 (45)		1.15	1.33[a]				

Note: All values are median MoMs. A downward arrow (↓) denotes a decrease compared with spontaneous conceptions when exact data were not available.
[a] Statistically significant at $\alpha = .05$.

Lanes. Maternal serum screening markers in IVF. Fertil Steril 2016.

TABLE 3

Change of maternal serum screening markers present for Down syndrome, trisomy 18 and neural tube defects.

	Down syndrome			
Marker	Screening at < 14 wk	Screening at > 14 wk	Trisomy 18	Neural tube defect
AFP	Decreased	Decreased	Decreased	Increased
B-hCG	Increased	Increased	Decreased	
μE3		Decreased	Decreased	
PAPP-A	Decreased		Decreased	
DIA		Increased	Decreased	

Lanes. Maternal serum screening markers in IVF. Fertil Steril 2016.

conventional prenatal screening (54). Additionally, the increased use of preimplantation genetic screening to screen for chromosomal aneuploidies among women who used IVF may modify the proportion of these women who use maternal serum screening to detect aneuploidies once an ongoing clinical pregnancy is achieved. The rate of positive screening results may differ among a self-selected population. This would bias the results that are found when IVF patients are compared with women who conceived spontaneously.

Strengths and Limitations

This study was strengthened by a rigorous and inclusive search strategy. The large number of studies included from a variety of countries around the world provided an opportunity to generalize interpretations. Unfortunately, the variations in reporting outcomes proved to be a substantial limitation. In order for the results to be comparable, only similar statistical estimates were abstracted (median MoMs). Due to this restriction, quantitative results were included from 23 of 40 studies. However, due to the estimate that was abstracted and the various ways in which variance was reported, we could not meta-analyze these data. The majority of cohort studies (26 studies) included in this review used record linkage or a secure record to ascertain the exposure. All of the case-control studies had controls selected from the same study base as the cases. Therefore, the potential for selection bias was small.

Conclusions

A decrease in PAPP-A and an increase in total hCG was consistently reported among the included studies. However, due to the variability in the levels of the other maternal serum screening markers reported and the inability to conduct a meta-analysis, we were unable to generalize about the differences between prenatal screening results in the IVF population. Due to the heterogeneity of these results, the authors cannot conclusively state whether maternal serum screening is the most appropriate method of prenatal screening for women who use IVF to conceive. Nevertheless, this systematic review appropriately summarized the direction of change of multiple maternal serum screening markers among IVF conceptions.

Acknowledgments: The authors thank Risa Shorr, medical librarian at the Ottawa Hospital, for assisting with the search strategy.

REFERENCES

1. Chitayat D, Langlois S, Wilson RD. Prenatal screening for fetal aneuploidy in singleton pregnancies. J Obstet Gynaecol Can 2011;33:736–50.
2. Carroll J, Allanson J, Epslen M, Meschino W, Miyazaki J, Spooner L, et al. Reference guide for health care providers. Prenatal screening tests for the detection of: Down syndrome, trisomy 18 and open neural tube defects; 2007. Available at: http://www.cheo.on.ca/documents/3/genetics-provider-guide-e.pdf. Last accessed September 7, 2016.
3. Korenberg J, Chen X, Schipper R, Sun Z, Gonsky R, Gerwehr N, et al. Down syndrome phenotypes: the consequences of chromosomal imbalance. Proc Nati Acad Sci USA 1994;91:4997–5001.
4. Lemire RJ. Neural tube defects. JAMA 1988;259:558–62.
5. Botto L, Moore C, Khoury M, Erickson J. Neural-tube defects. N Engl J Med 1999;341:1509–19.
6. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. Hum Reprod 2009;24:2683–7.
7. Henningsen A-KA, Pinborg A. Birth and perinatal outcomes and complications for babies conceived following assisted reproductive techniques. Semin Fetal Neonatal Med 2014;19:234–8.
8. Bergh C, Wennerholm U-B. Obstetric outcome and long-term follow up of children conceived through assisted reproduction. Best Pract Res Clin Obstet Gynaecol 2012;26:841–52.
9. Engels MAJ, Kooij M, Schats R, Twisk JWR, Blankenstein MA, van Vugt JMG, et al. First-trimester serum marker distribution in singleton pregnancies conceived with assisted reproduction. Prenat Diagn 2010;30:372–7.
10. Lambert-Messerlian G, Dugoff L, Vidaver J, Canick J, Malone F, Ball R, et al. First- and second-trimester Down syndrome screening markers in pregnancies achieved through assisted reproductive technologies (ART): A FASTER trial study. Prenat Diagn 2006;26:672–8.
11. Stewart L, Moher D, Shekelle P. Why prospective registration of systematic reviews makes sense. Syst Rev 2012;1:7.
12. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev 2015;4:1–9.
13. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. Newcastle-Ottawa quality assessment scale cohort studies. Ottawa Hosp Res Inst 2014. Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Last accessed September 7, 2016.
14. Anckaert E, Schiettecatte J, Sleurs E, Devroey P, Smitz J. First trimester screening for Down's syndrome after assisted reproductive technology: non-male factor infertility is associated with elevated free beta-human chorionic gonadotropin levels at 10–14 weeks of gestation. Fertil Steril 2008;90:1206–10.
15. Ball S, Ekelund C, Wright D, Kirkegaard I, Nørgaard P, Petersen OB, et al. Temporal effects of maternal and pregnancy characteristics on serum pregnancy-associated plasma protein-A and free β-human chorionic gonadotropin at 714 weeks' gestation. Ultrasound Obstet Gynecol 2013;41:33–9.
16. Ball S, Wright D, Sodre D, Lachmann R, Nicolaides K. Temporal effect of Afro-Caribbean race on serum pregnancy-associated plasma protein-A at 9–13 weeks' gestation in screening for aneuploidies. Fetal Diagn Ther 2012;31:162–9.
17. Bar-Hava I, Yitzhak M, Krissi H, Shohat M, Shalev J, Czitron B, et al. Triple-test screening in in vitro fertilization pregnancies. J Assist Reprod Genet 2001;18:226–9.
18. Bellver J, Lara C, Soares SR, Ramírez A, Pellicer A, Remohí J, et al. First trimester biochemical screening for Down's syndrome in singleton pregnancies conceived by assisted reproduction. Hum Reprod 2005;20:2623–7.
19. Bellver J, Casanova C, Garrido N, Lara C, Remohí J, Pellicer A, et al. Additive effect of factors related to assisted conception on the reduction of maternal serum pregnancy-associated plasma protein A concentrations and the increased false-positive rates in first-trimester Down syndrome screening. Fertil Steril 2013;100:1314–20.
20. Bender F, Hecken J, Reinsberg J, Berg C, van der Ven H, Gembruch U, et al. Altered first-trimester screening markers after IVF/ICSI: no relationship with small-for-gestational-age and number of embryos transferred. Reprod Biomed Online 2010;20:516–22.
21. Bersinger NA, Chanson A, Crazzolara S, Hanggi W, Pescia G, Scheier M, et al. Serum levels of placenta protein markers: the relevance of differences between spontaneous and after in vitro fertilization pregnancies for fetal trisomy screening. J Fertil Reprod 2001;11:7–13.
22. Bersinger NA, Wunder D, Vanderlick F, Chanson A, Pescia G, Janecek P, et al. Maternal serum levels of placental proteins after in vitro fertilisation and their implications for prenatal screening. Prenat Diagn 2004;24:471–7.
23. Bersinger NA, Vanderlick F, Birkhäuser MH, Janecek P, Wunder D. First trimester serum concentrations of placental proteins in singleton and multiple IVF pregnancies: implications for Down syndrome screening. Immunoanalyse Biol Spécialisée 2005;20:21–7.

24. Bredaki FE, Wright D, Akolekar R, Cruz G, Nicolaides KH. Maternal serum alpha-fetoprotein in normal pregnancy at 11–13 weeks' gestation. Fetal Diagn Ther 2011;30:274–9.
25. Cowans NJ, Spencer K. Effect of gestational age on first trimester maternal serum prenatal screening correction factors for ethnicity and IVF conception. Prenat Diagn 2013;33:56–60.
26. Engels MA, Pajkrt E, Groot DDT, Schats R, Twisk JWR, van Vugt JJMG. Validation of correction factors for serum markers for first-trimester Down syndrome screening in singleton pregnancies conceived with assisted reproduction. Fetal Diagn Ther 2013;34:217–24.
27. Frishman GN, Canick JA, Hogan JW, Kellner LH, Saller DN. Serum triple-marker screening in in vitro fertilization and naturally conceived pregnancies. Obstet Gynecol 1997;7844:98–101.
28. Ghisoni L, Ferrazzi E, Castagna C, Setti PEL, Masini AC, Pigni A. Prenatal diagnosis after ART success: the role of early combined screening tests in counselling pregnant patients. Placenta 2003;4004:99–103.
29. Giorgetti C, Vanden Meerschaut F, De Roo C, Saunier O, Quarello E, Hairion D, et al. Multivariate analysis identifies the estradiol level at ovulation triggering as an independent predictor of the first trimester pregnancy-associated plasma protein-A level in IVF/ICSI pregnancies. Hum Reprod 2013;28:2636–42.
30. Gjerris AC, Loft A, Pinborg A, Christiansen M, Tabor A. First-trimester screening markers are altered in pregnancies conceived after IVF/ICSI. Ultrasound Obstet Gynecol 2009;33:8–17.
31. Heinonen S, Hippelainen M, Ryynanen M, Saarikoski S, Kirkinen P. Effect of in vitro fertilization on human chorionic gonadotropin serum ceoncentrations and Down's syndrome screening. Fertil Steril 1996;66:398–403.
32. Hui PW. Maternal serum hCG and alpha-fetoprotein levels in pregnancies conceived after IVF or ICSI with fresh and frozen-thawed embryos. Hum Reprod 2003;18:572–5.
33. Hui PW, Tang MHY, Lam YH, Yeung WSB, Ng EHY, Ho PC. Nuchal translucency in pregnancies conceived after assisted reproduction technology. Ultrasound Obstet Gynecol 2005;25:234–8.
34. Hui PW, Lam YH, Tang MHY, Ng EHY, Yeung WSB, Ho PC. Maternal serum pregnancy-associated plasma protein-A and free beta-human chorionic gonadotrophin in pregnancies conceived with fresh and frozen-thawed embryos from in vitro fertilization and intracytoplasmic sperm injection. Prenat Diagn 2005;25:390–3.
35. Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. Ultrasound Obstet Gynecol 2008;31:493–502.
36. Lam YH, Shu W, Yeung B, Hoi M, Tang Y, Hung E, et al. Maternal serum alpha-fetoprotein and human chorionic gonadotrophin in pregnancies conceived after intracytoplasmic sperm injection and conventional in-vitro fertilization. Hum Reprod 1999;14:2120–3.
37. Lambert-Messerlian G, Palomaki GE, Canick JA. Adjustment of serum markers in first trimester screening. J Med Screen 2009;16:102–3.
38. Liao AW, Heath V, Kametas N, Spencer K, Nicolaides KH. First-trimester screening for trisomy 21 in singleton pregnancies achieved by assisted reproduction. Hum Reprod 2001;16:1501–4.
39. Matilainen M, Peuhkurinen S, Laitinen P, Jarvela I, Morin-Papunen L, Ryynanen M. In combined first-trimester Down syndrome screening, the false-positive rate is not higher in pregnancies conceived after assisted reproduction compared with spontaneous pregnancies. Fertil Steril 2011;95:378–81.
40. Maymon R, Shulman A. Serial first- and second-trimester Down's syndrome screening tests among IVF- versus naturally-conceived singletons. Hum Reprod 2002;17:1081–5.
41. Maymon R, Shulman A. Integrated first- and second-trimester Down syndrome screening test among unaffected IVF pregnancies. Prenat Diagn 2004;24:125–9.
42. Muller F, Dreux S, Lemeur A, Sault C, Desgrès J, Bernard M-A, et al. Medically assisted reproduction and second-trimester maternal serum marker screening for Down syndrome. Prenat Diagn 2003;23:1073–6.
43. Niemimaa M, Heinonen S, Seppala M, Hippelainen M, Martikainen H, Ryynanen M. First-trimester screening for Down's syndrome in in vitro fertilization. Fertil Steril 2001;76:1282–3.
44. Orlandi F, Rossi C, Allegra A, Krantz D, Hallahan T, Orlandi E, et al. First trimester screening with free b-hCG, PAPP-A and nuchal translucency in pregnancies conceived with assisted reproduction. Prenat Diagn 2002;22:718–21.
45. Raty R, Virtanen A, Koskinen P, Anttila L, Forsstro J, Laitinen P, et al. Serum free β-HCG and alpha-fetoprotein levels in IVF, ICSI and frozen embryo transfer pregnancies in maternal mid-trimester serum screening for Down's syndrome. Hum Reprod 2002;17:481–4.
46. Ribbert L, Komman L, De Wolf B, Simons A, Jansen C, Beekhuis J, et al. Maternal serum screening for fetal Down syndrome in IVF pregnancies. Prenat Diagn 1996;1:35–8.
47. Rice JD, McIntosh SF, Halstead AC. Second-trimester maternal serum screening for Down syndrome in in vitro fertilization pregnancies. Prenat Diagn 2005;25:234–8.
48. Tul N, Novak-Antolic Z. Serum PAPP-A levels at 10–14 weeks of gestation are altered. Prenat Diagn 2006;26:1206–11.
49. Wald NJ, Researcher NW, Lecturer JKMS, Researcher WJH, Canick JA. Serum markers for Down's syndrome in women who have had in vitro fertilisation: implications for antenatal screening. Br J Obstet Gynaecol 1999;106:1304–6.
50. Wøjdemann KR, Larsen SO, Shalmi A, Sundberg K, Christiansen M, Tabor A. First trimester screening for Down syndrome and assisted reproduction: no basis for concern. Prenat Diagn 2001;21:563–5.
51. Amor DJ, Xu JX, Halliday JL, Francis I, Healy DL, Breheny S, et al. Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for Down syndrome. Hum Reprod 2009;24:1330–8.
52. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod 2007;22:1506–12.
53. Hui L. Non-invasive prenatal testing for fetal aneuploidy: charting the course from clinical validity to clinical utility. Ultrasound Obstet Gynecol 2013;41:2–6.
54. Shi X, Zhang Z, Cram DS, Liu C. Feasibility of noninvasive prenatal testing for common fetal aneuploidies in an early gestational window. Clin Chim Acta 2015;439:24–8.

SUPPLEMENTAL APPENDIX

Database: Embase Classic+Embase <1947 to 2015 April 08>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present>

Search Strategy: April 9, 2015

1 maternal serum screening tests/ (209)
2 ((maternal or prenatal) adj3 serum adj5 screen$).tw. (2426)
3 ((maternal or prenatal) adj3 blood adj5 screen$).tw. (144)
4 alpha-Fetoproteins/bl, an (7917)
5 Pregnancy-Associated Plasma Protein-A/ (3363)
6 (papp a or papp alpha or pregnancy associated plasma protein a or pappalysin 1).tw. (3630)
7 (alphafetoprotein$ or alpha-Fetoprotein$ or afp).tw. (42310)
8 Chorionic Gonadotropin, beta Subunit, Human/bl, an (1820)
9 (b-hcg or bhcg).tw. (846)
10 Chorionic Gonadotropin/bl, an (7084)
11 (human chorionic gonadotropin or hcg).tw. (59567)
12 exp estriol/ (16774)
13 (uE3 or free estriol or free oestriol or unconjugated estriol or unconjugated oestriol).tw. (1540)
14 Nuchal Translucency Measurement/ (2845)
15 Nuchal Translucen$.tw. (4213)
16 inhibins/ (9739)
17 inhibin$ a.tw. (2324)
18 or/1–17 (134980)
19 exp fertilization in vitro/ or sperm injections, intracytoplasmic/ (75624)
20 (in vitro fertil$ or ivf).tw. (62842)
21 sperm injection$.tw. (13078)
22 or/19–21 (94404)
23 18 and 22 (8619)
24 Pregnancy/ (1323036)
25 prenatal diagnosis/ (80819)
26 pregnancy trimester, first/ or pregnancy trimester, second/ (62377)
27 Fetus/ (247561)
28 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).tw. (1372125)
29 or/24–28 (1976885)
30 23 and 29 (6071)
31 30 use prmz (2525)
32 *maternal serum screening test/ (105)
33 ((maternal or prenatal) adj3 serum adj5 screen$).tw. (2426)
34 ((maternal or prenatal) adj3 blood adj5 screen$).tw. (144)
35 *alpha fetoprotein blood level/ or *alpha fetoprotein/ (17041)
36 (alphafetoprotein$ or alpha-Fetoprotein$ or afp).tw. (42310)
37 *pregnancy associated plasma protein A/ (1723)
38 (papp a or papp alpha or pregnancy associated plasma protein a or pappalysin 1).tw. (3630)
39 *chorionic gonadotropin beta subunit/ (3113)
40 (b-hcg or bhcg).tw. (846)
41 *chorionic gonadotropin/ (34598)
42 (human chorionic gonadotropin or hcg).tw. (59567)
43 estriol/ (16752)
44 (uE3 or free estriol or free oestriol or unconjugated estriol or unconjugated oestriol).tw. (1540)
45 *nuchal translucency measurement/ (978)
46 nuchal translucen$.tw. (4213)
47 inhibin A/ (1576)
48 inhibin$ a.tw. (2324)
49 or/32–48 (138154)
50 *fertilization in vitro/ (34764)
51 *intracytoplasmic sperm injection/ (7064)
52 (in vitro fertil$ or ivf).tw. (62842)
53 sperm injection$.tw. (13078)
54 50 or 51 or 52 or 53 (77604)
55 49 and 54 (8291)
56 *pregnancy/ (204266)
57 *prenatal diagnosis/ (43792)
58 *first trimester pregnancy/ (8632)
59 *second trimester pregnancy/ (3778)
60 *prenatal screening/ (21565)
61 *fetus/ (63951)
62 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).tw. (1372125)
63 or/56–62 (1429236)
64 55 and 63 (5440)
65 64 use emczd (3458)
66 31 or 65 (5983)
67 remove duplicates from 66 (4269)
68 67 use prmz (2508)
69 67 use emczd (1761)

Lanes. Maternal serum screening markers in IVF. Fertil Steril 2016.

Database: EBM Reviews - Cochrane Central Register of Controlled Trials <March 2015>
Search Strategy: April 9, 2015

1 maternal serum screening tests/ (0)
2 ((maternal or prenatal) adj3 serum adj5 screen$).tw,hw. (15)
3 ((maternal or prenatal) adj3 blood adj5 screen$).tw,hw. (3)
4 alpha-Fetoproteins/bl, an (3)
5 Pregnancy-Associated Plasma Protein-A/ (15)
6 (papp a or papp alpha or pregnancy associated plasma protein a or pappalysin 1).tw,hw. (42)
7 (alphafetoprotein$ or alpha-Fetoprotein$ or afp).tw,hw. (354)
8 Chorionic Gonadotropin, beta Subunit, Human/bl, an (0)
9 (b-hcg or bhcg).tw,hw. (18)
10 Chorionic Gonadotropin/bl, an (7)
11 (human chorionic gonadotropin or hcg).tw,hw. (1403)
12 exp estriol/ (195)
13 (uE3 or free estriol or free oestriol or unconjugated estriol or unconjugated oestriol).tw,hw. (16)
14 Nuchal Translucency Measurement/ (9)
15 Nuchal Translucen$.tw,hw. (17)
16 inhibins/ (85)
17 inhibin$ a.tw,hw. (174)
18 or/1–17 (2123)
19 exp fertilization in vitro/ or sperm injections, intracytoplasmic/ (1618)
20 (in vitro fertil$ or ivf).tw,hw. (3116)
21 sperm injection$.tw,hw. (918)
22 or/19–21 (3539)
23 18 and 22 (637)
24 Pregnancy/ (15093)
25 prenatal diagnosis/ (140)
26 pregnancy trimester, first/ or pregnancy trimester, second/ (923)
27 Fetus/ (281)
28 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).tw,hw. (25968)
29 or/24–28 (25968)
30 23 and 29 (491)>>

Lanes. Maternal serum screening markers in IVF. Fertil Steril 2016.

APPENDIX C: Supplemental information for Manuscript 2

Supplemental Text C-1 Description of data sources and record linkage methodology

Better Outcomes Registry & Network (BORN) Ontario

BORN Ontario's database is a web-based maternal child information system that captures all births within the province of Ontario.[110] This headquarters for the BORN registry is at the Children's Hospital of Eastern Ontario (CHEO) and they also host the BORN Information System (BIS).[110] The BIS contains information on prenatal screening, labour, birth, NICU care and congenital anomalies and started collecting data in 2012. This registry captures all hospital, home and birth centre births with a gestational age of greater or equal to 20 weeks.

Canadian Assisted Reproductive Technologies Register (CARTR) Plus

Data collection for CARTR Plus commenced on January 1, 2013. It includes data from 35 Canadian fertility clinics, of which 18 are located in Ontario. Unique to CARTR Plus is the ability to link data from multiple treatment cycles for the same woman. CARTR Plus contains detailed information on fertility treatment including treatment cycles, reason for treatment, types of stimulation and treatment outcome. CARTR Plus is a dataset contained within the BORN Registry.

Record linkage

Records from the BORN Information System were deterministically linked to CARTR Plus data using a unique identifier.

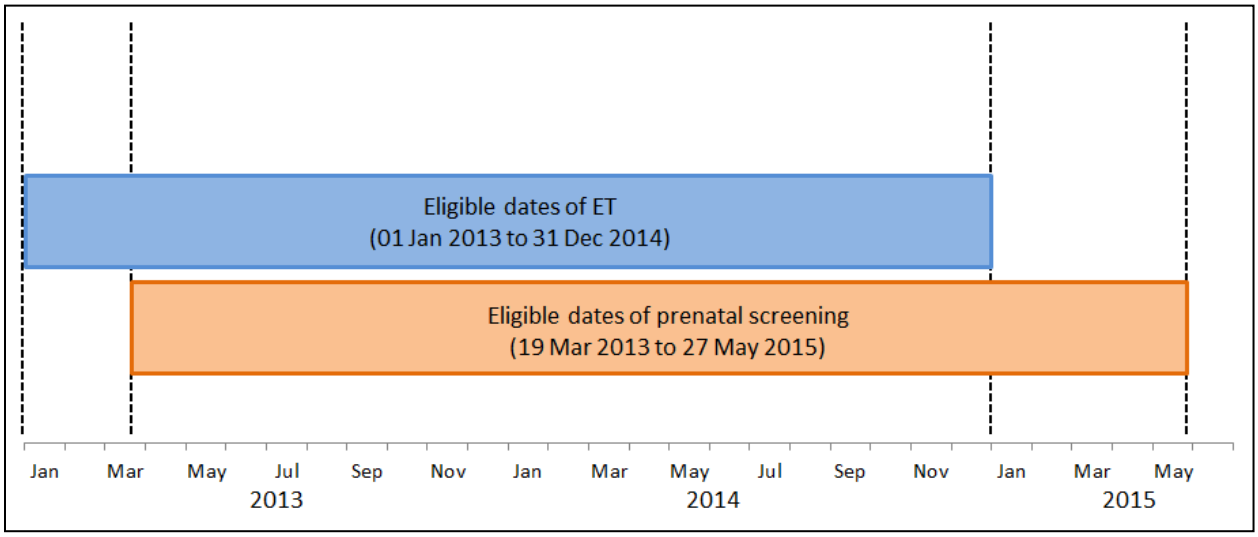
Cohort description

Prenatal screening records, captured in the BORN Information System, for samples collected between March 13, 2013 and May 27, 2015, which had a valid screening test result were included. Multiple pregnancies were removed. An IVF pregnancy flag, based on the prenatal screening record, was added. These data were deterministically linked using the Ontario subset of CARTR Plus data using a pregnancy ID. A CARTR Plus IVF flag was created. Cycles that used donor oocytes were investigated in order to ensure that appropriate maternal age was used in the calculation for the risk assessment for fetal

aneuploidies. From the CARTR Plus records, it was determined that there were 18 cases where maternal age was ≥45 years when donor cycles were used. Through examination, it was determined that patient age was used to determine maternal age when age at time of oocyte retrieval was not available. It was also determined that donor age was not as well documented on the prenatal screening record.

In Ontario, three prenatal screening labs used the same adjustment factors for IVF and one lab uses a different adjustment factor in order to correct three MSS markers (PAPP-A, total hCG and μE^3). In order to develop the proposed adjustment factors for IVF, the appropriate values for the adjustment factors were removed. The mean MoM for each MSS marker was determined for all records that were identified as an IVF or FET conception using the CARTR Plus dataset. The proposed adjustment factors were determined in order to achieved a mean MSS marker MoM as close to 1.00 as possible. A similar approach was used proposed adjustment factors for the other MSS markers.

Supplemental Figure C-1 Conception cohort of eligible prenatal screens



Supplemental Figure C-2 Documented IVF status by the prenatal screening labs compared to CARTR Plus

		Canadian Assisted Reproductive Technologies Register Plus				
		IVF	No IVF	**Total**		
Prenatal screening record	IVF	3273	1488	4761	**PPV**	68.7%
	No IVF	143	129682	129825	**NPV**	99.9%
	Total	3416	131170	134586		
		Sensitivity	**Specificity**			
		95.8%	98.9%			

Supplemental Table C-1 Comparing the MSS marker MoM for markers with a current PSO IVF adjustment factor

		No IVF adjustment				PSO IVF adjustment						Proposed IVF adjustment					
		Mean	±SD	Min	Max		Mean	±SD	Min	Max	p-value*		Mean	±SD	Min	Max	p-value**
PAPP-A																	
Ethnicity, smoking, diabetes adjusted MoM n=2,205	**IVF and FET**	1.348	0.901	0.066	13.626	**IVF and FET**	1.226	0.820	0.060	12.400	<.0001	**IVF and FET**	0.997	0.667	0.049	10.084	<.0001
	IVF	1.246	0.769	0.143	5.868	IVF	1.134	0.700	0.130	5.340	<.0001	IVF	0.922	0.569	0.106	4.342	<.0001
	FET	1.385	0.898	0.143	10.033	FET	1.260	0.817	0.130	9.130	<.0001	FET	1.025	0.664	0.106	7.424	<.0001
Total hCG																	
Ethnicity, smoking, diabetes adjusted MoM n=2,199	**IVF and FET**	1.039	0.650	0.035	11.132	**IVF and FET**	1.185	0.741	0.040	12.690	<.0001	**IVF and FET**	1.008	0.630	0.034	10.798	<.0001
	IVF	0.996	0.578	0.158	4.912	IVF	1.135	0.658	0.180	5.600	<.0001	IVF	0.966	0.560	0.153	4.765	<.0001
	FET	1.056	0.646	0.035	8.193	FET	1.204	0.736	0.040	9.340	<.0001	FET	1.025	0.626	0.034	7.947	<.0001
uE3																	
Ethnicity, smoking, diabetes adjusted MoM n=2,199	**IVF and FET**	1.196	0.323	0.043	2.819	**IVF and FET**	1.124	0.304	0.040	2.650	<.0001	**IVF and FET**	1.005	0.271	0.036	2.368	<.0001
	IVF	1.117	0.289	0.043	2.287	IVF	1.050	0.272	0.040	2.150	<.0001	IVF	1.005	0.271	0.036	2.368	<.0001
	FET	1.262	0.337	0.096	2.755	FET	1.186	0.317	0.090	2.590	<.0001	FET	1.060	0.283	0.080	2.314	<.0001

Notes:

IVF and FET with own oocytes

*compares PSO IVF adjusted MoM and no IVF adjusted MoM

**compares proposed IVF adjusted MoM and no IVF adjusted MoM

Supplemental Table C-2 Comparing the MSS marker and NT MoM for measurements without a current PSO IVF adjustment factor

		No IVF adjustment				Proposed IVF adjustment					
		Mean	**±SD**	**Min**	**Max**		**Mean**	**±SD**	**Min**	**Max**	**p-value***
AFP											
Ethnicity, smoking, diabetes adjusted MoM n=2,286	**IVF and FET**	1.232	1.188	0.210	45.06	**IVF and FET**	1.010	0.974	0.172	36.949	<.0001
	IVF - own oocytes	1.126	1.566	0.210	45.06	IVF - own oocytes	0.923	1.284	0.172	36.949	<.0001
	FET - own oocytes	1.311	0.754	0.410	14.54	FET - own oocytes	1.075	0.619	0.336	11.923	<.0001
FB-hCG											
Ethnicity, smoking, diabetes adjusted MoM n=100	**IVF and FET**	1.305	1.033	0.230	7.160	**IVF and FET**	1.005	0.795	0.177	5.513	<.0001
	IVF - own oocytes	1.063	0.546	0.400	2.900	IVF - own oocytes	0.818	0.420	0.308	2.233	<.0001
	FET - own oocytes	1.496	1.282	0.230	7.160	FET - own oocytes	1.152	0.987	0.177	5.513	<.0001
hCG - 1st trimester											
Ethnicity, smoking, diabetes adjusted MoM n=14	**IVF and FET**	1.454	0.807	0.510	3.530	**IVF and FET**	1.003	0.557	0.352	2.436	0.0001
	IVF - own oocytes	1.537	0.833	0.830	3.530	IVF - own oocytes	1.060	0.575	0.573	2.436	0.0039
	FET - own oocytes	1.304	0.827	0.510	2.350	FET - own oocytes	0.900	0.571	0.352	1.622	0.0625
DIA											
Ethnicity, smoking, diabetes adjusted MoM n=149	**IVF and FET**	1.263	0.655	0.230	4.230	**IVF and FET**	0.998	0.517	0.182	3.342	<.0001
	IVF - own oocytes	1.207	0.631	0.380	4.230	IVF - own oocytes	0.953	0.499	0.300	3.342	<.0001
	FET - own oocytes	1.247	0.638	0.230	3.810	FET - own oocytes	0.985	0.504	0.182	3.010	<.0001
NT measurement											
Ethnicity adjusted MoM n=3,365	**IVF and FET**	1.057	0.393	0.490	9.290	**IVF and FET**	1.005	0.373	0.466	8.826	<.0001
	IVF - own oocytes	1.051	0.346	0.490	7.990	IVF - own oocytes	0.998	0.329	0.466	7.591	<.0001

	FET - own oocytes	1.060	0.427	0.540	9.290	FET - own oocytes	1.007	0.405	0.513	8.826	<.0001

Notes:

*compares proposed IVF adjusted MoM and no IVF adjusted MoM

Supplemental Table C-3 Comparing MSS marker measurements with a current PSO IVF adjustment factor among IVF conceptions stratified by insemination method

		No IVF adjustment				PSO IVF adjustment					Proposed IVF adjustment					
		Mean	±SD	Min	Max		Mean	±SD	Min	Max		Mean	±SD	Min	Max	p value*
PAPP-A																
Ethnicity, smoking, diabetes adjusted MoM n=1,512	**Conventional IVF**	1.390	0.955	0.165	7.945	**Conventional IVF**	1.265	0.869	0.150	7.230	**Conventional IVF**	1.028	0.707	0.122	5.879	<.0001
	ICSI	1.286	0.824	0.143	10.033	**ICSI**	1.170	0.750	0.130	9.130	**ICSI**	0.951	0.610	0.106	7.424	
Total hCG																
Ethnicity, smoking, diabetes adjusted MoM n=1,503	**Conventional IVF**	1.001	0.709	0.061	8.193	**Conventional IVF**	1.141	0.808	0.070	9.340	**Conventional IVF**	0.971	0.688	0.060	7.947	<.0001
	ICSI	1.029	0.594	0.158	4.535	**ICSI**	1.173	0.677	0.180	5.170	**ICSI**	0.998	0.576	0.153	4.399	
uE3																
Ethnicity, smoking, diabetes adjusted MoM n=1,503	**Conventional IVF**	1.179	0.327	0.138	2.755	**Conventional IVF**	1.108	0.308	0.130	2.590	**Conventional IVF**	0.990	0.275	0.116	2.314	0.0022
	ICSI	1.191	0.326	0.043	2.638	**ICSI**	1.119	0.307	0.040	2.480	**ICSI**	1.000	0.274	0.036	2.216	

Notes:

IVF and FET with own oocytes

* comparing IVF and ICSI of proposed IVF adjustment model

Supplemental Table C-4 Comparing MSS marker measurements without a current PSO IVF adjustment factor among IVF conceptions stratified by insemination method

		No IVF adjustment				Proposed IVF adjustment					
		Mean	±SD	Min	Max		Mean	±SD	Min	Max	p value*
AFP											
Ethnicity, smoking, diabetes adjusted MoM n=1,569	**Conventional IVF**	1.276	2.464	0.210	45.06	**Conventional IVF**	1.047	2.021	0.172	36.949	<.0001
	ICSI	1.206	0.897	0.360	21.10	**ICSI**	1.206	0.897	0.360	21.100	
FB-hCG											
Ethnicity, smoking, diabetes adjusted MoM n=66	**Conventional IVF**	1.514	0.707	0.540	2.900	**Conventional IVF**	1.166	0.544	0.416	2.233	0.0869
	ICSI	1.278	0.866	0.380	5.130	**ICSI**	0.984	0.667	0.293	3.950	
hCG - 1st trimester											
Ethnicity, smoking, diabetes adjusted MoM n=13	**Conventional IVF**	1.120	0.796	0.510	2.020	**Conventional IVF**	0.773	0.549	0.352	1.394	1.000
	ICSI	1.587	0.854	0.700	3.530	**ICSI**	1.095	0.590	0.483	2.436	
DIA											
Ethnicity, smoking, diabetes adjusted MoM n=101	**Conventional IVF**	1.176	0.570	0.340	2.520	**Conventional IVF**	0.929	0.450	0.269	1.991	0.0352

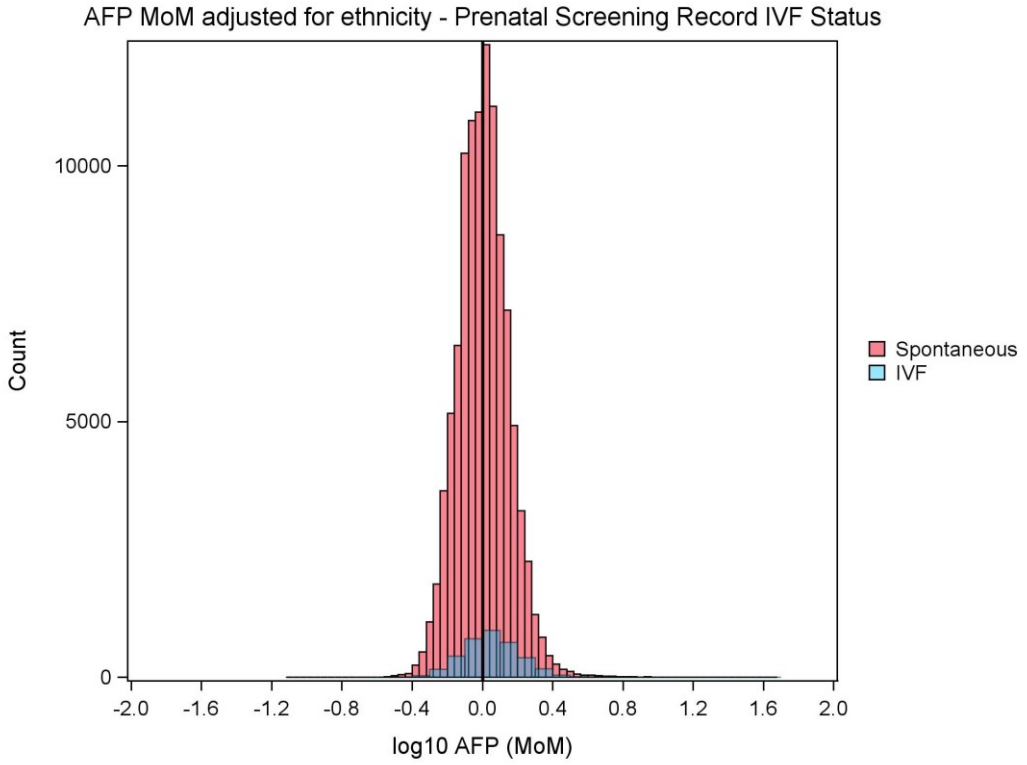
	ICSI	1.245	0.642	0.500	4.230	**ICSI**	0.983	0.507	0.395	3.342	
NT measurement											
Ethnicity adjusted MoM n=2,289	**Conventional IVF**	1.024	0.265	0.570	3.810	**Conventional IVF**	0.972	0.251	0.542	3.620	<.0001
	ICSI	1.066	0.414	0.490	9.290	**ICSI**	1.012	0.394	0.466	8.826	

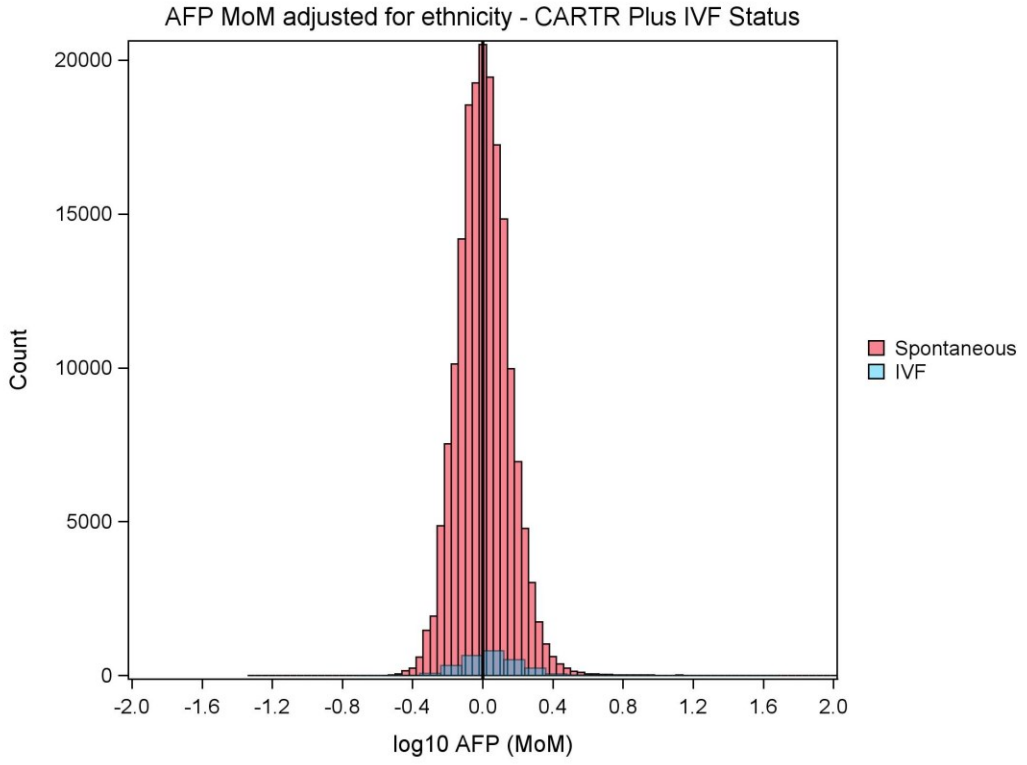
Notes:

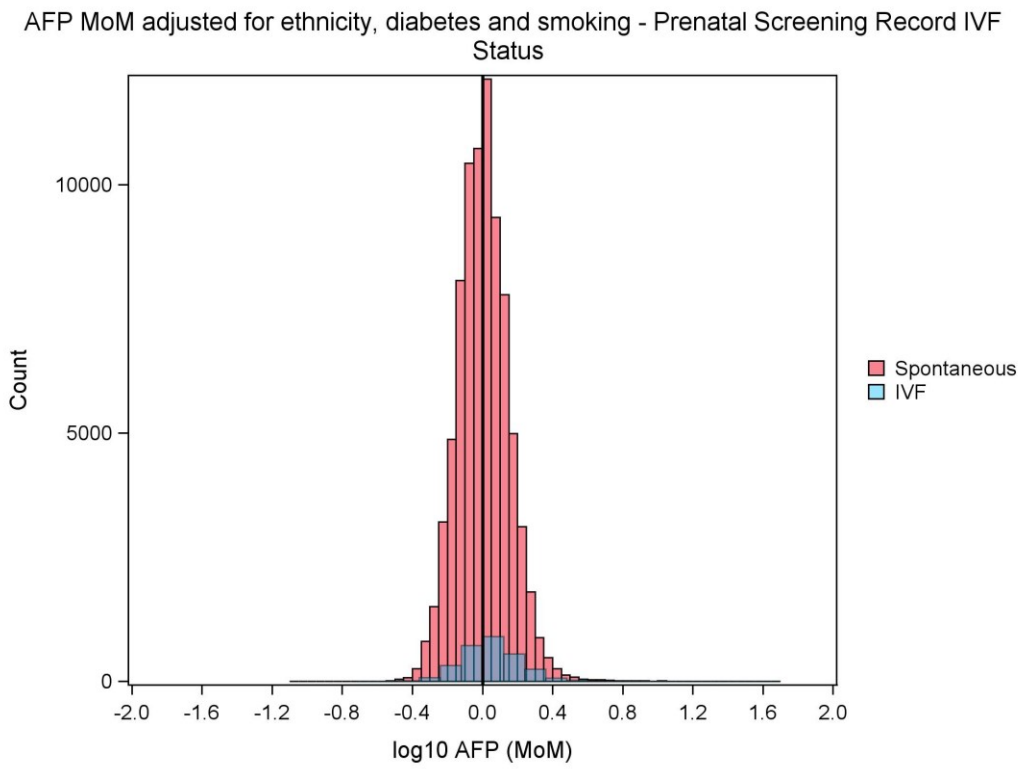
IVF and FET with own oocytes

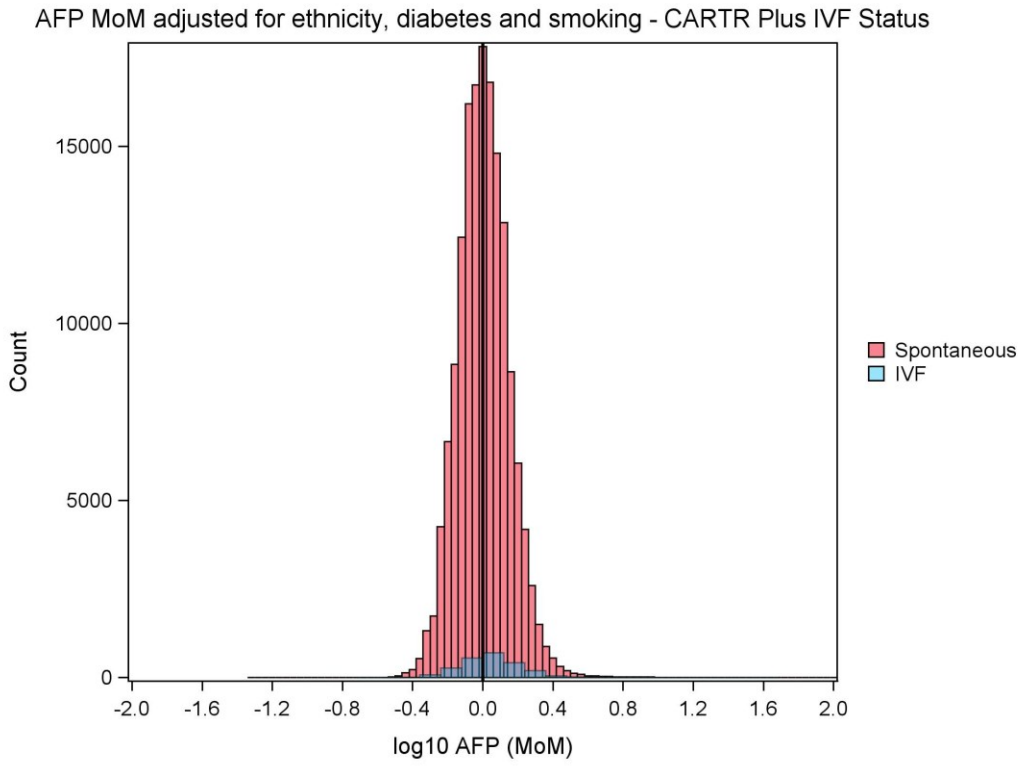
* comparing IVF and ICSI of proposed IVF adjustment model

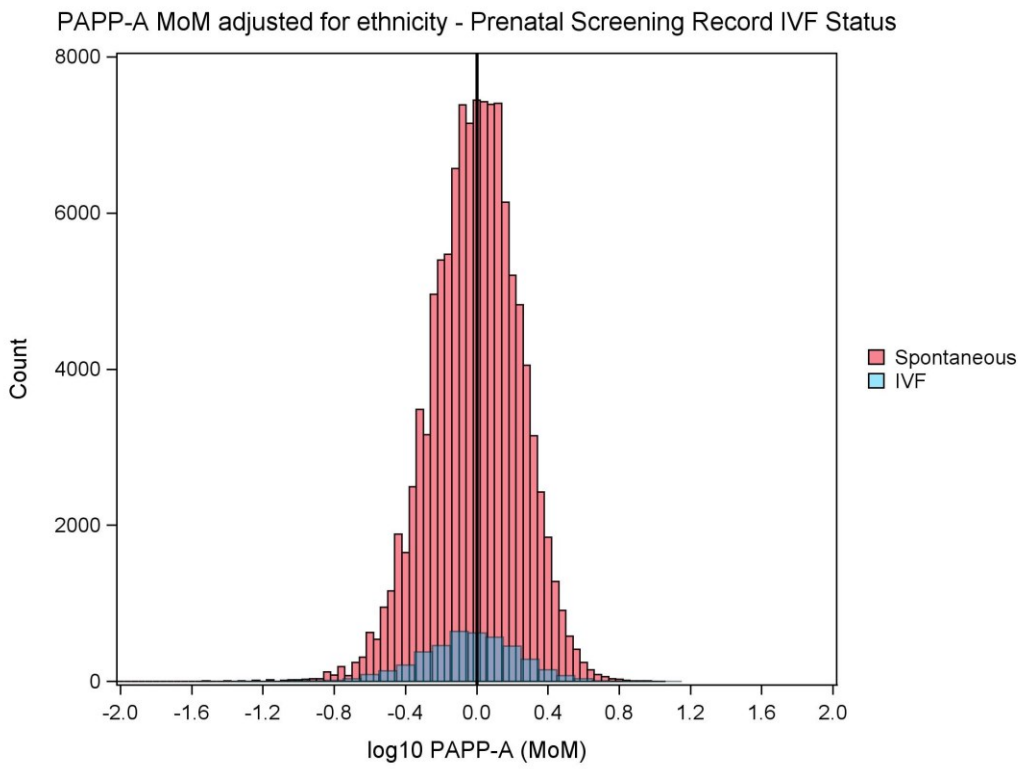
Supplemental Figures C-3 Histograms of maternal serum screening marker MoM

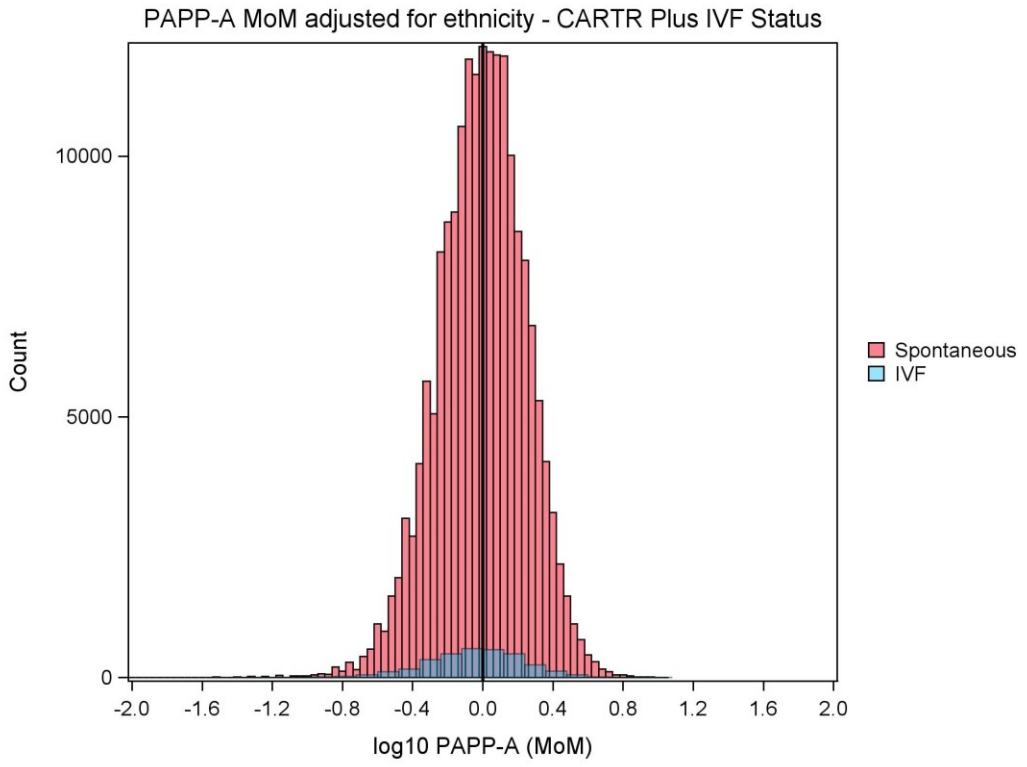


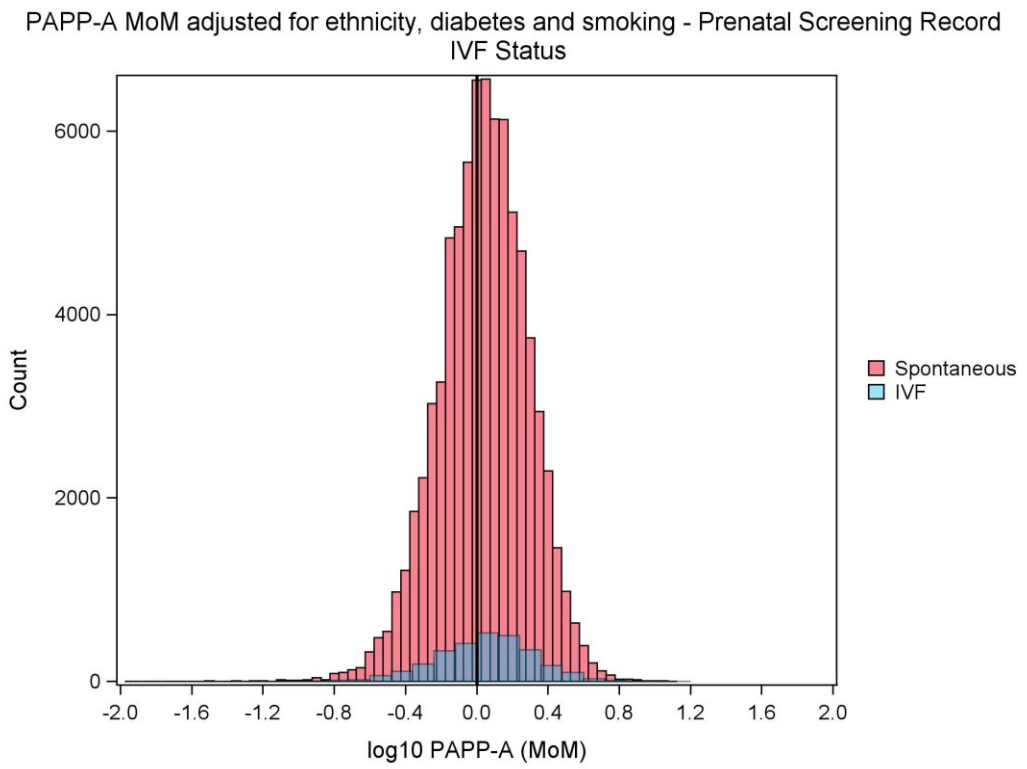


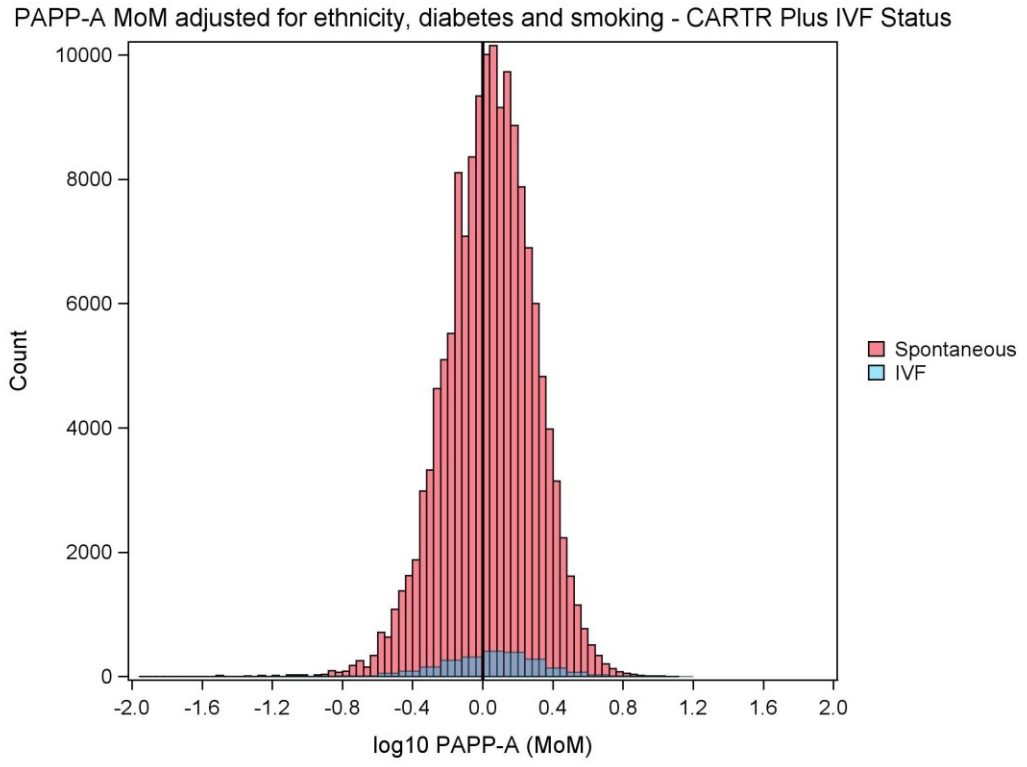

AFP MoM adjusted for ethnicity, diabetes and smoking - Prenatal Screening Record IVF Status
Count
10000
5000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 AFP (MoM)
Spontaneous
IVF

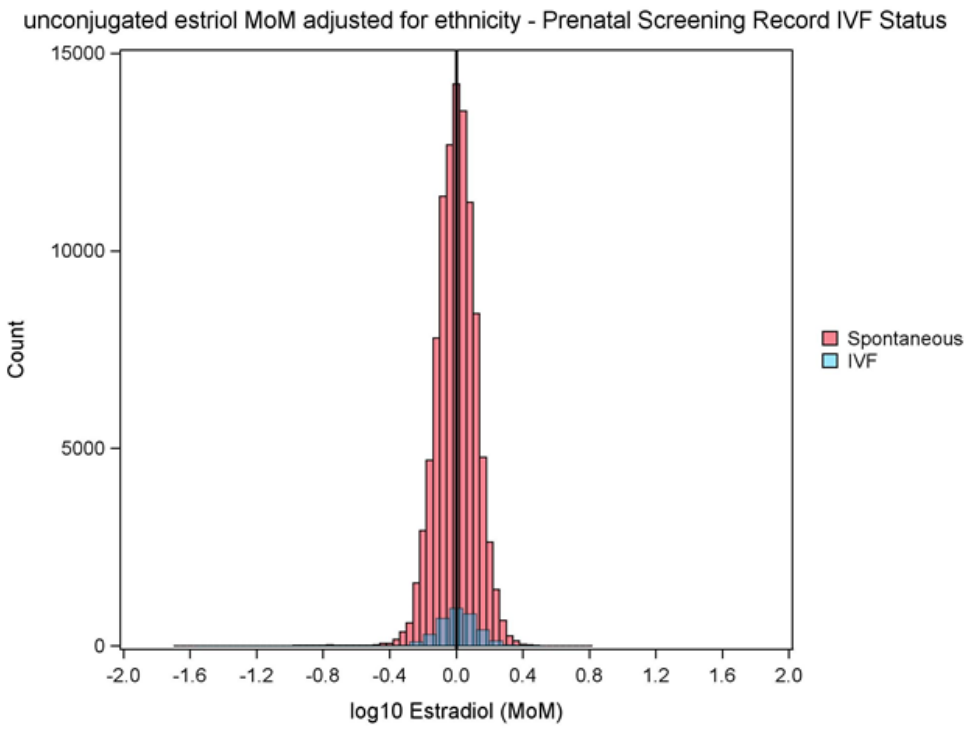

AFP MoM adjusted for ethnicity, diabetes and smoking - CARTR Plus IVF Status
Count
15000
10000
5000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 AFP (MoM)
Spontaneous
IVF

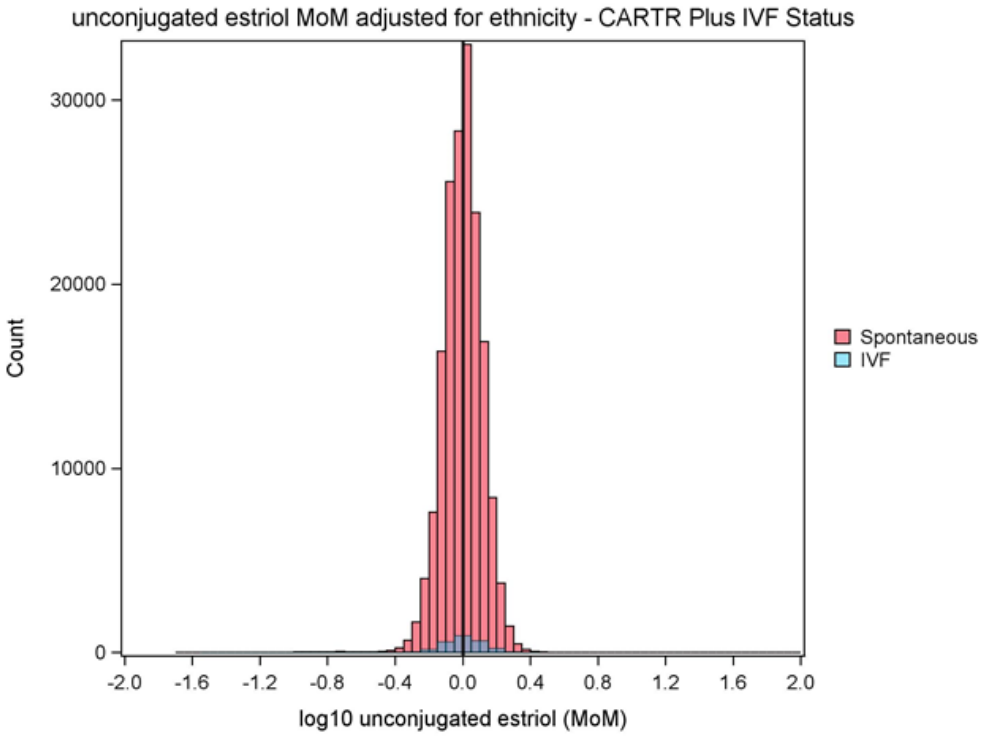

PAPP-A MoM adjusted for ethnicity - Prenatal Screening Record IVF Status
Count
8000
6000
4000
2000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 PAPP-A (MoM)
Spontaneous
IVF

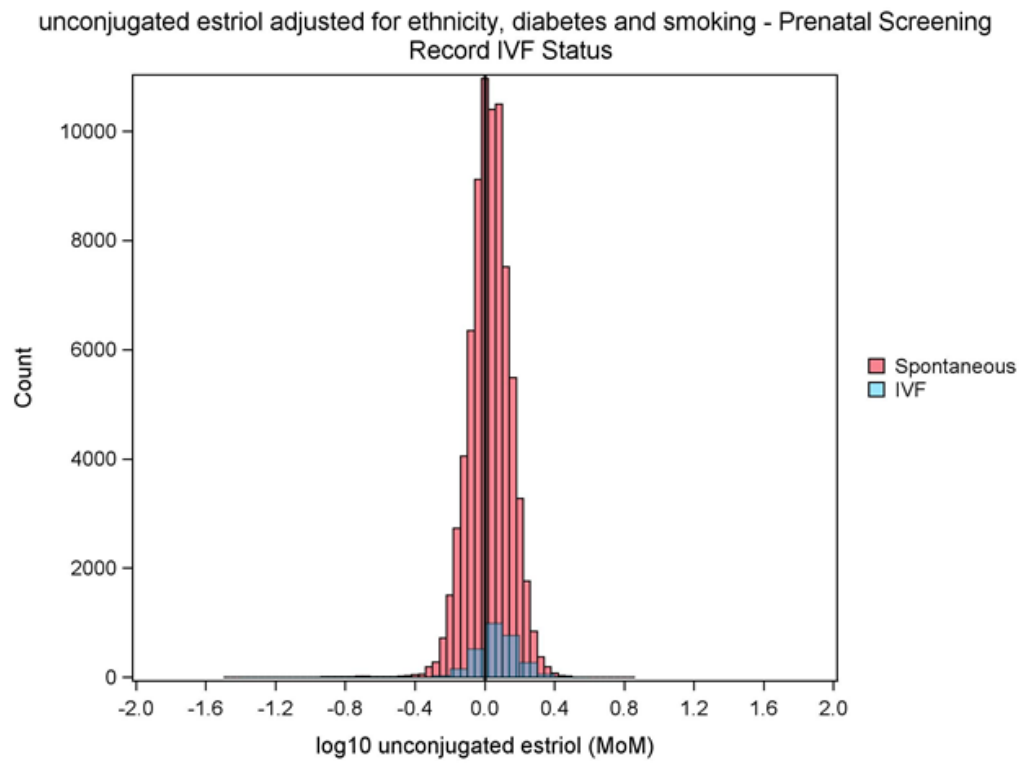

PAPP-A MoM adjusted for ethnicity - CARTR Plus IVF Status
Count
10000
5000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 PAPP-A (MoM)
Spontaneous
IVF

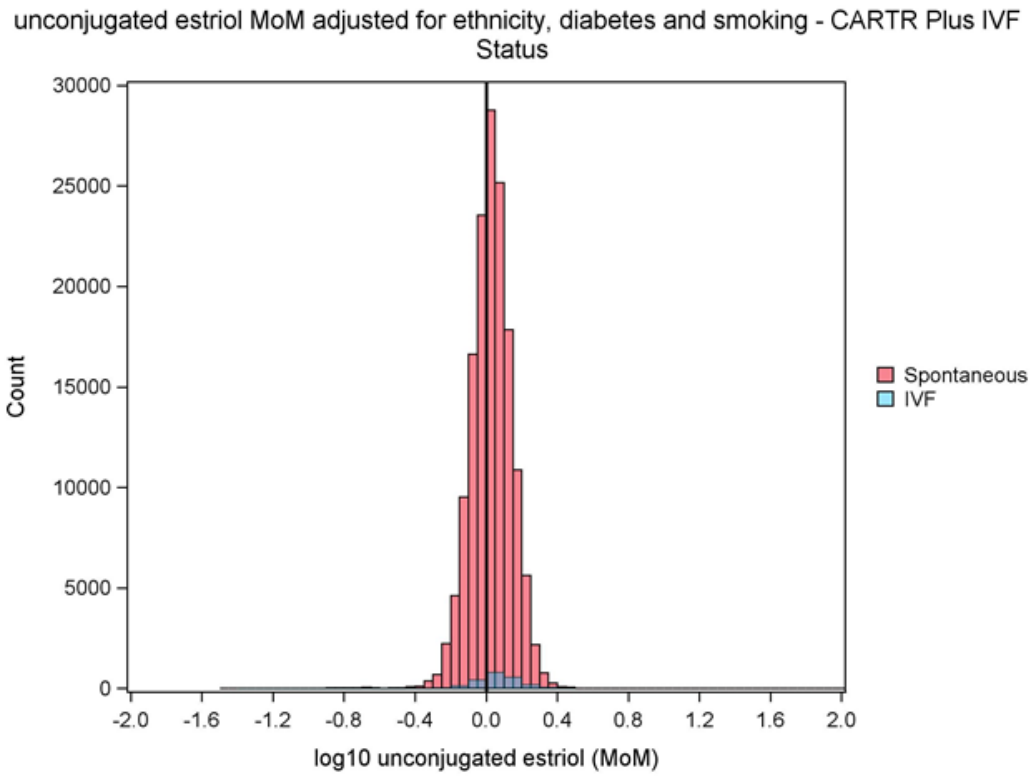

PAPP-A MoM adjusted for ethnicity, diabetes and smoking - Prenatal Screening Record
IVF Status
Count
6000
4000
2000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 PAPP-A (MoM)
Spontaneous
IVF

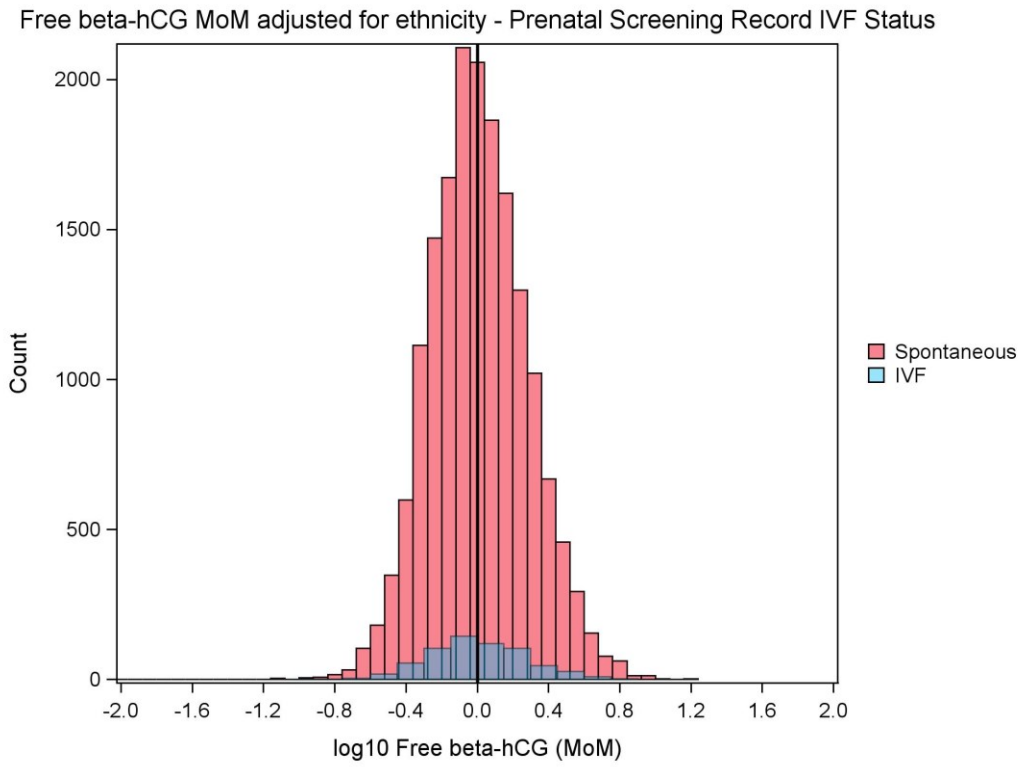

PAPP-A MoM adjusted for ethnicity, diabetes and smoking - CARTR Plus IVF Status
Count
10000
8000
6000
4000
2000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 PAPP-A (MoM)
Spontaneous
IVF

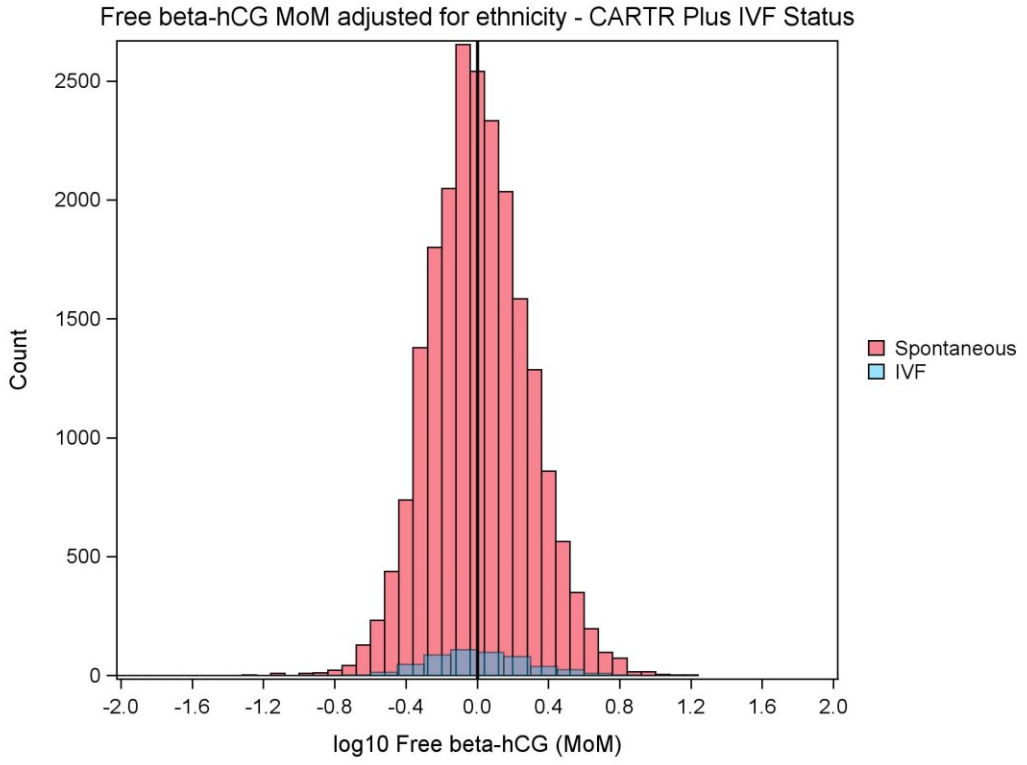

unconjugated estriol MoM adjusted for ethnicity - Prenatal Screening Record IVF Status
15000
10000
5000
0
Count
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 Estradiol (MoM)
Spontaneous
IVF

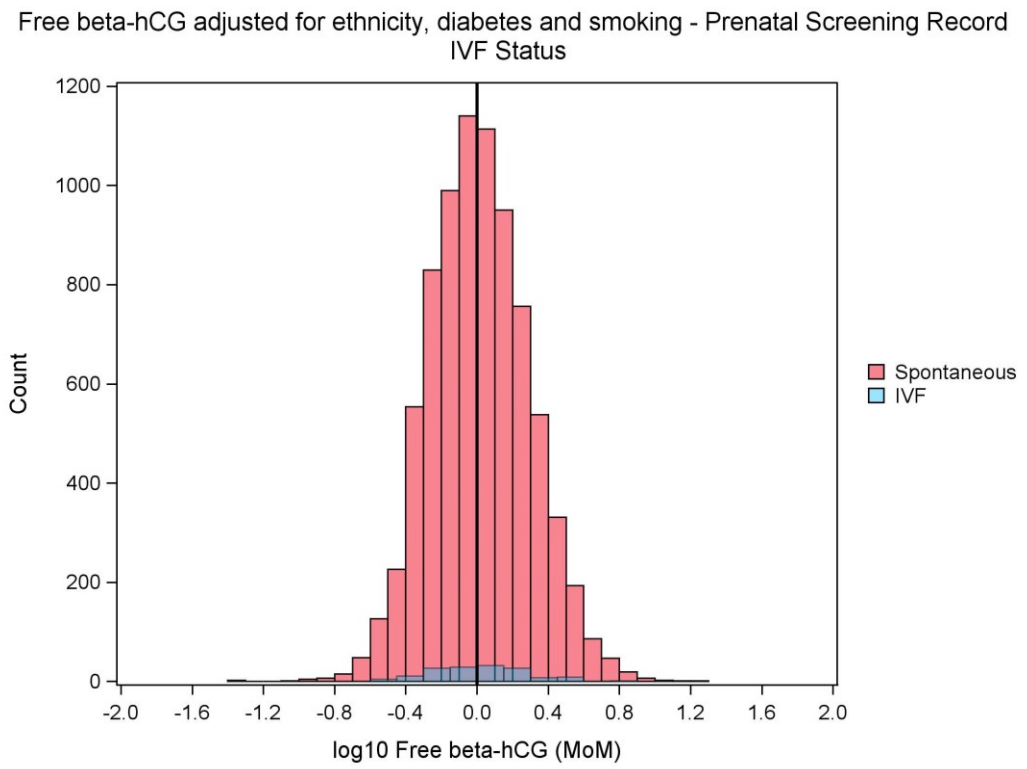

unconjugated estriol MoM adjusted for ethnicity - CARTR Plus IVF Status
30000
20000
10000
0
Count
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 unconjugated estriol (MoM)
Spontaneous
IVF

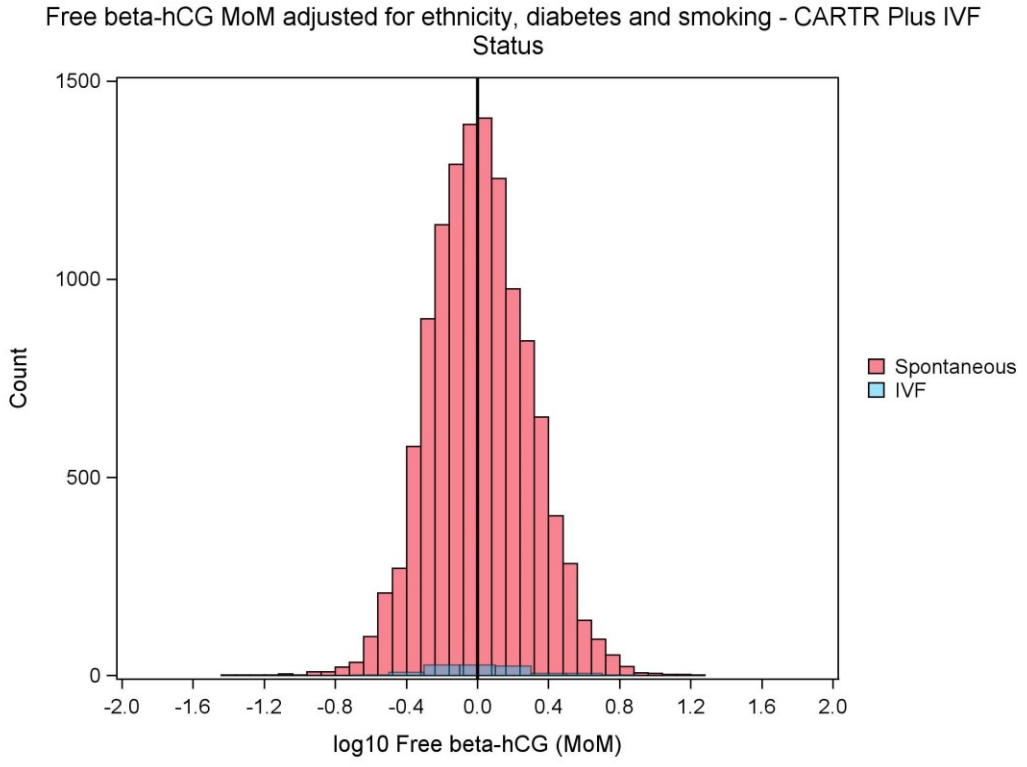

unconjugated estriol adjusted for ethnicity, diabetes and smoking - Prenatal Screening
Record IVF Status
Count
10000
8000
6000
4000
2000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 unconjugated estriol (MoM)
Spontaneous
IVF

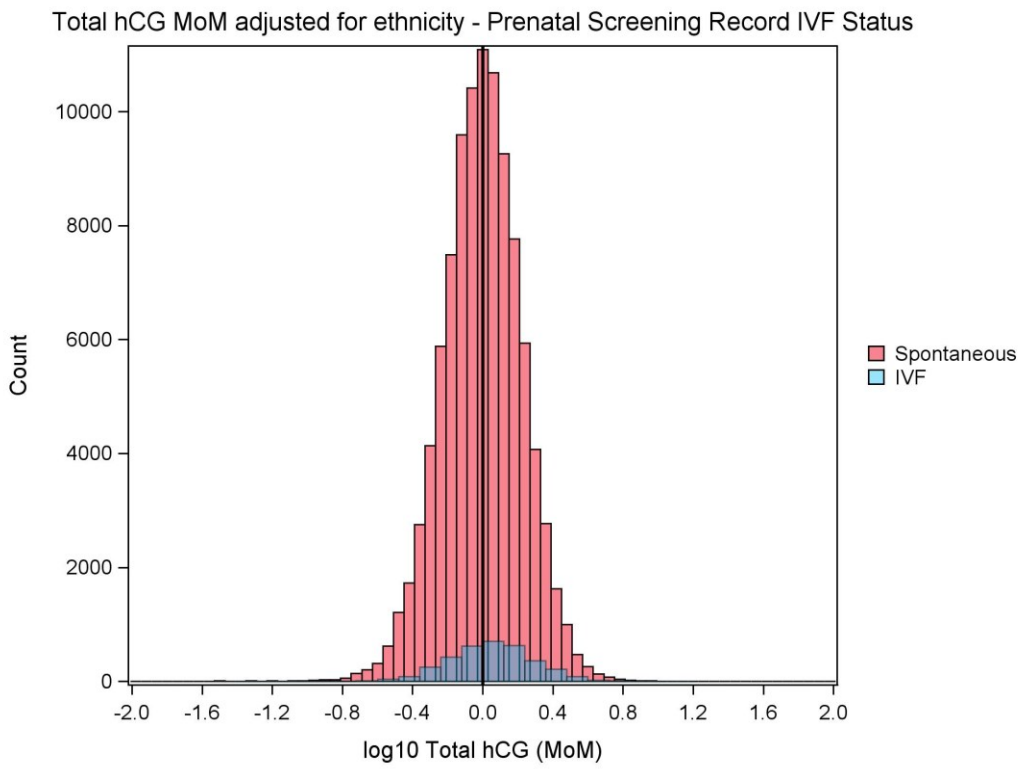

unconjugated estriol MoM adjusted for ethnicity, diabetes and smoking - CARTR Plus IVF
Status
Count
30000
25000
20000
15000
10000
5000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 unconjugated estriol (MoM)
Spontaneous
IVF

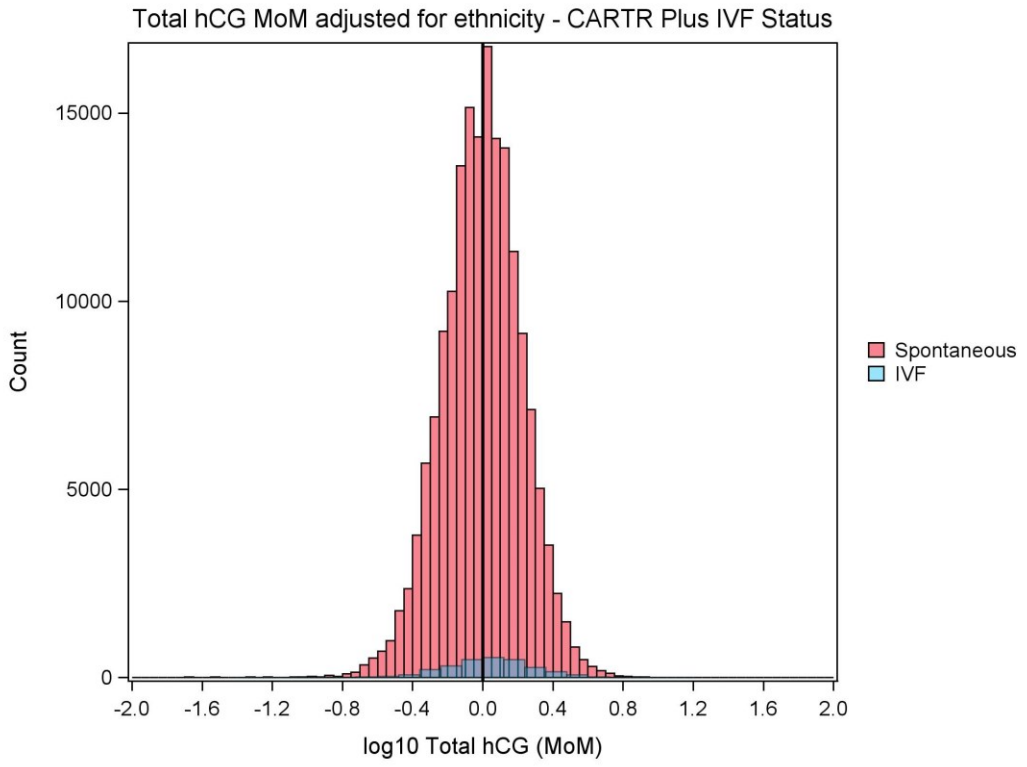

Free beta-hCG MoM adjusted for ethnicity - Prenatal Screening Record IVF Status
Count
2000
1500
1000
500
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 Free beta-hCG (MoM)
Spontaneous
IVF

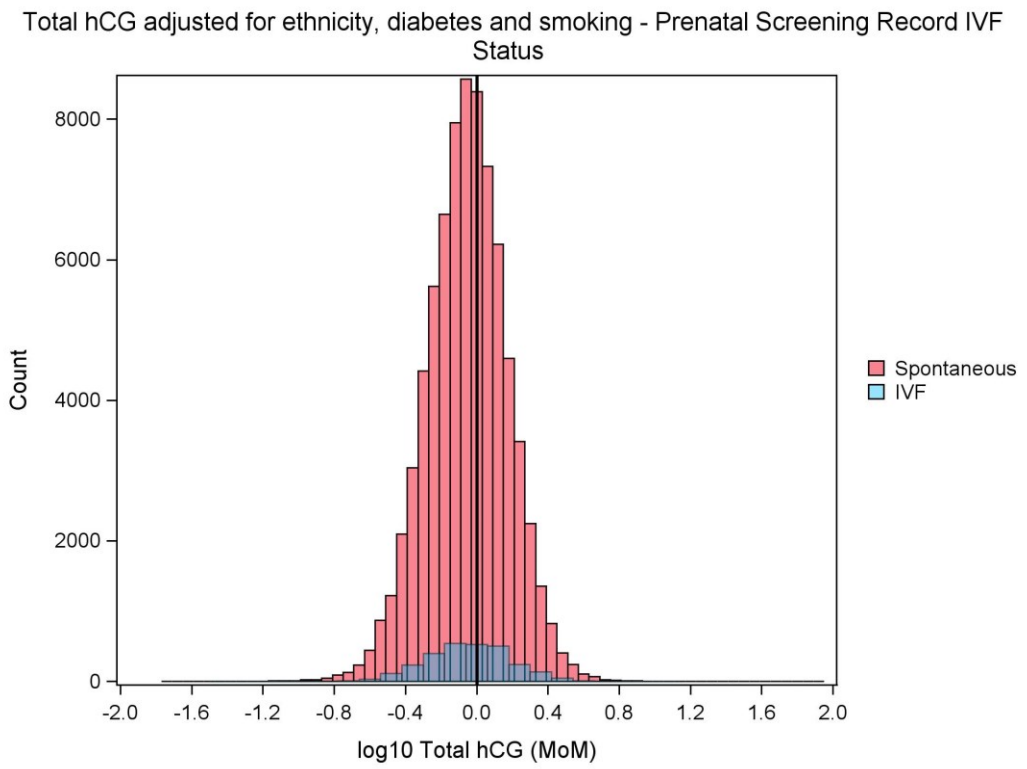

Free beta-hCG MoM adjusted for ethnicity - CARTR Plus IVF Status
Count
2500
2000
1500
1000
500
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 Free beta-hCG (MoM)
Spontaneous
IVF

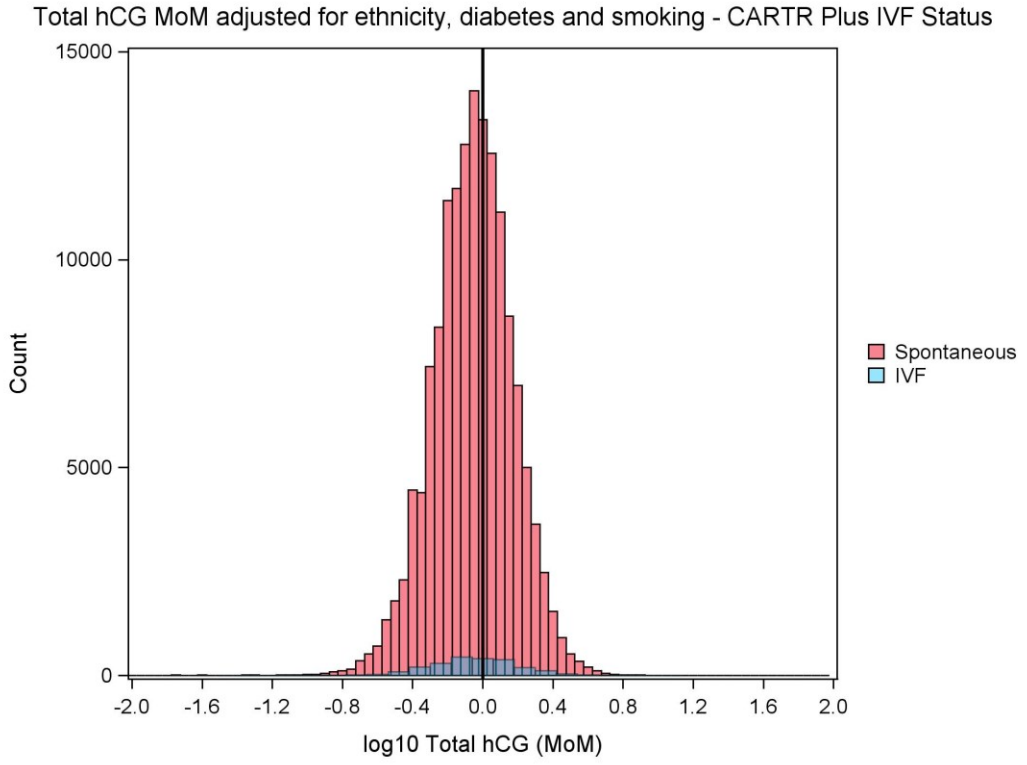

Free beta-hCG adjusted for ethnicity, diabetes and smoking - Prenatal Screening Record
IVF Status
Count
1200
1000
800
600
400
200
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 Free beta-hCG (MoM)
Spontaneous
IVF

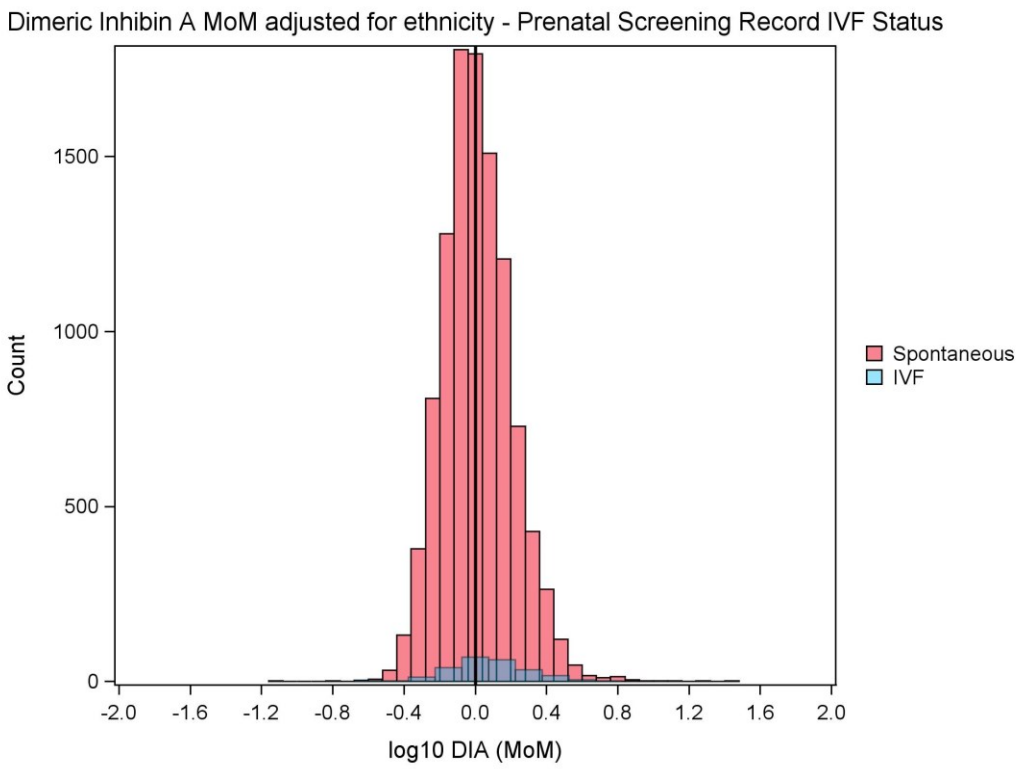

Free beta-hCG MoM adjusted for ethnicity, diabetes and smoking - CARTR Plus IVF
Status
Count
1500
1000
500
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 Free beta-hCG (MoM)
Spontaneous
IVF

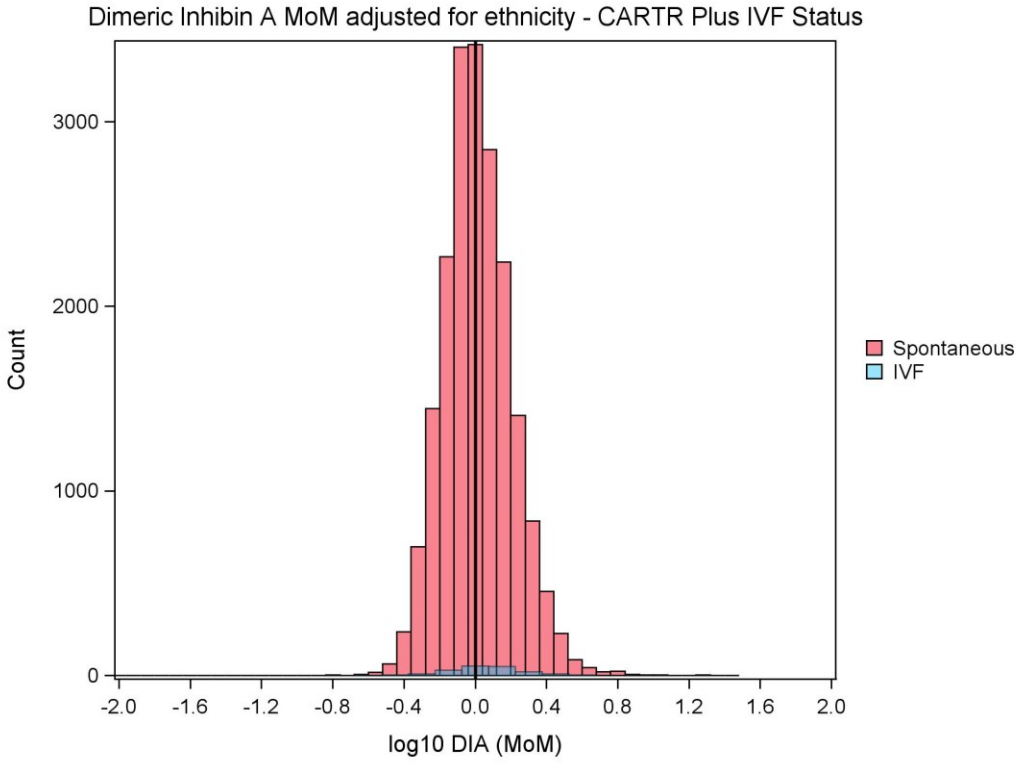

Total hCG MoM adjusted for ethnicity - Prenatal Screening Record IVF Status
Count
10000
8000
6000
4000
2000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 Total hCG (MoM)
Spontaneous
IVF

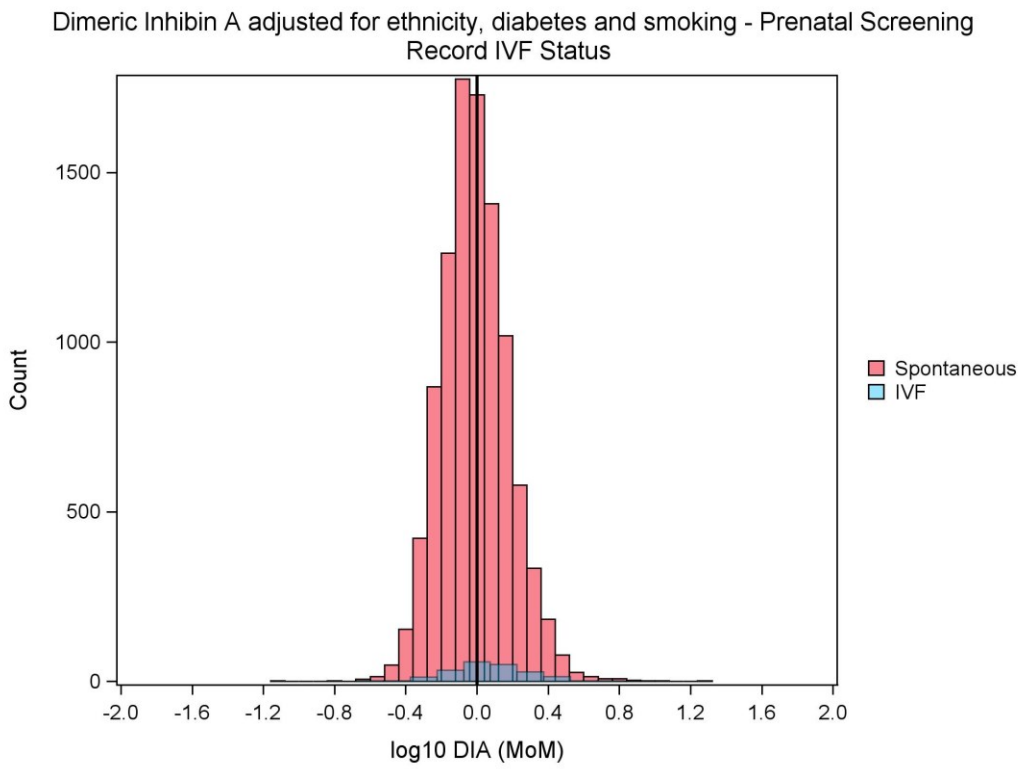

Total hCG MoM adjusted for ethnicity - CARTR Plus IVF Status
Count
15000
10000
5000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 Total hCG (MoM)
Spontaneous
IVF

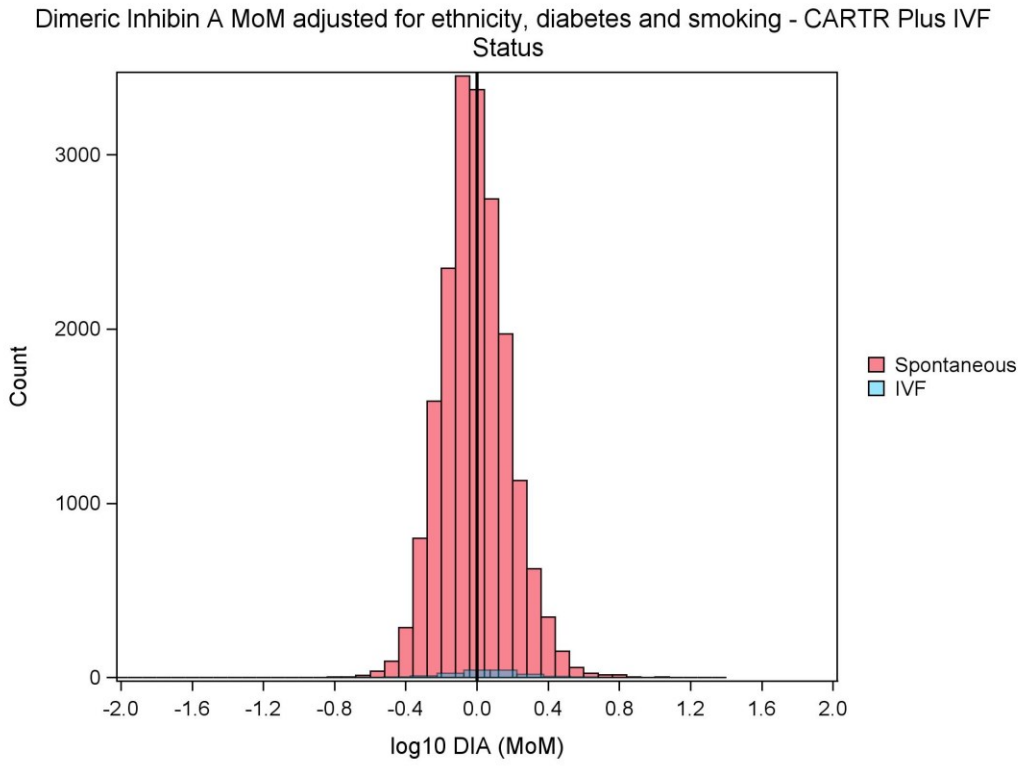

Total hCG adjusted for ethnicity, diabetes and smoking - Prenatal Screening Record IVF Status
Count
8000
6000
4000
2000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 Total hCG (MoM)
Spontaneous
IVF

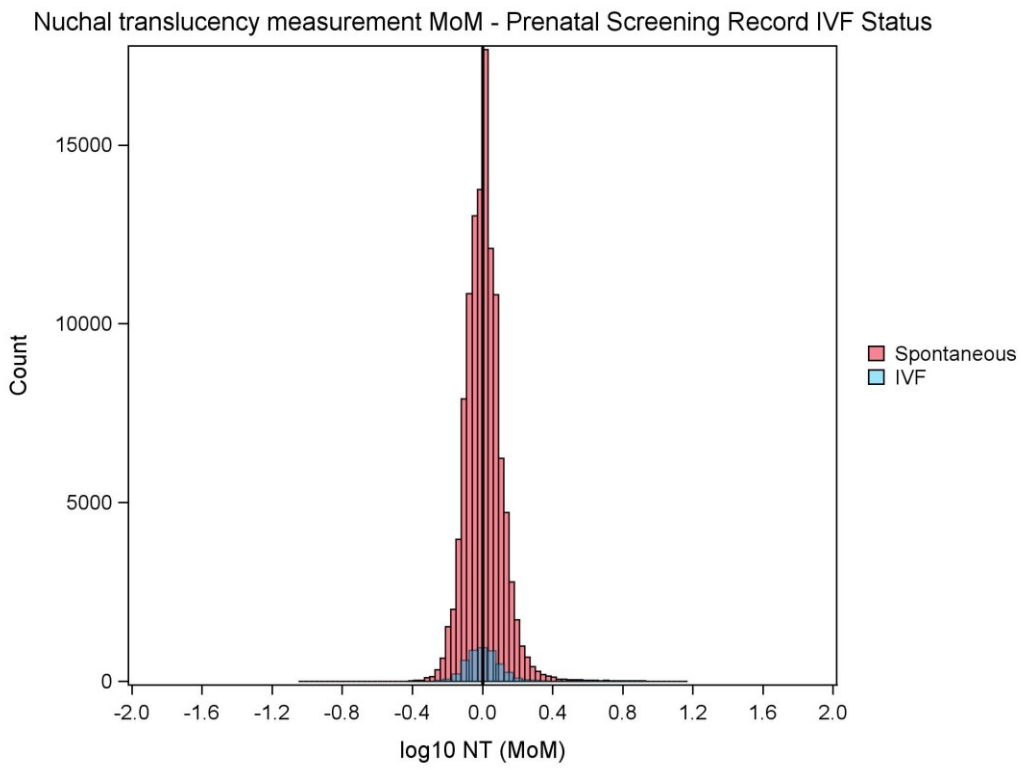

Total hCG MoM adjusted for ethnicity, diabetes and smoking - CARTR Plus IVF Status
Count
15000
10000
5000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 Total hCG (MoM)
Spontaneous
IVF

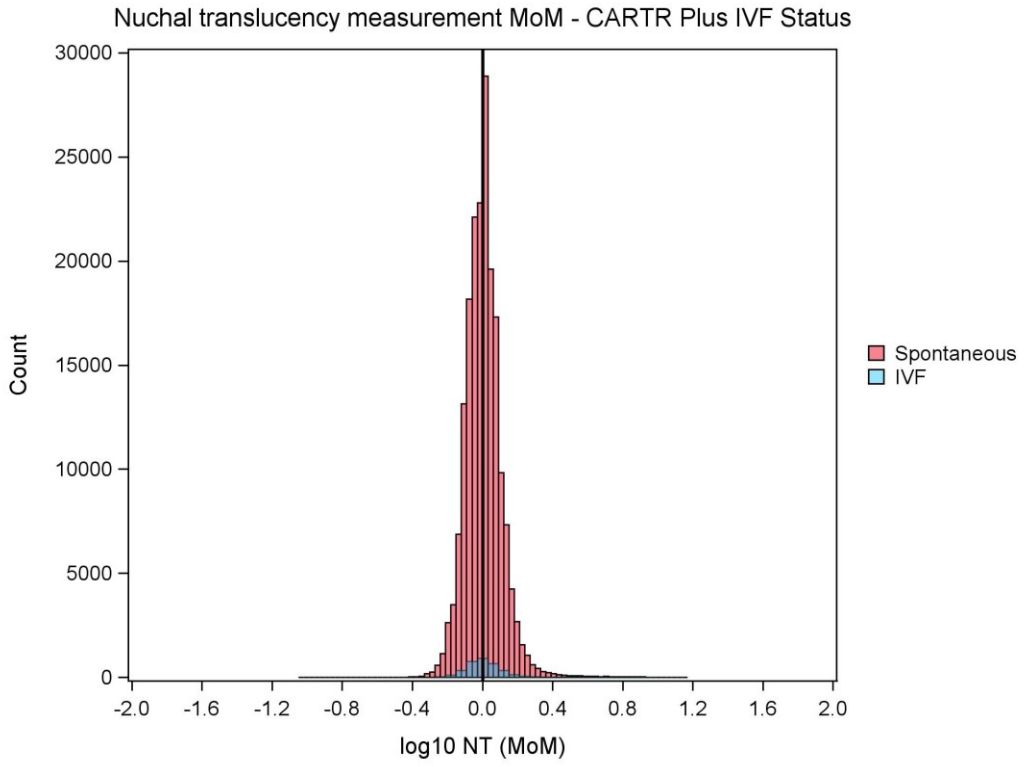

Dimeric Inhibin A MoM adjusted for ethnicity - Prenatal Screening Record IVF Status
Count
1500
1000
500
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 DIA (MoM)
Spontaneous
IVF


Dimeric Inhibin A MoM adjusted for ethnicity - CARTR Plus IVF Status
Count
3000
2000
1000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 DIA (MoM)
Spontaneous
IVF

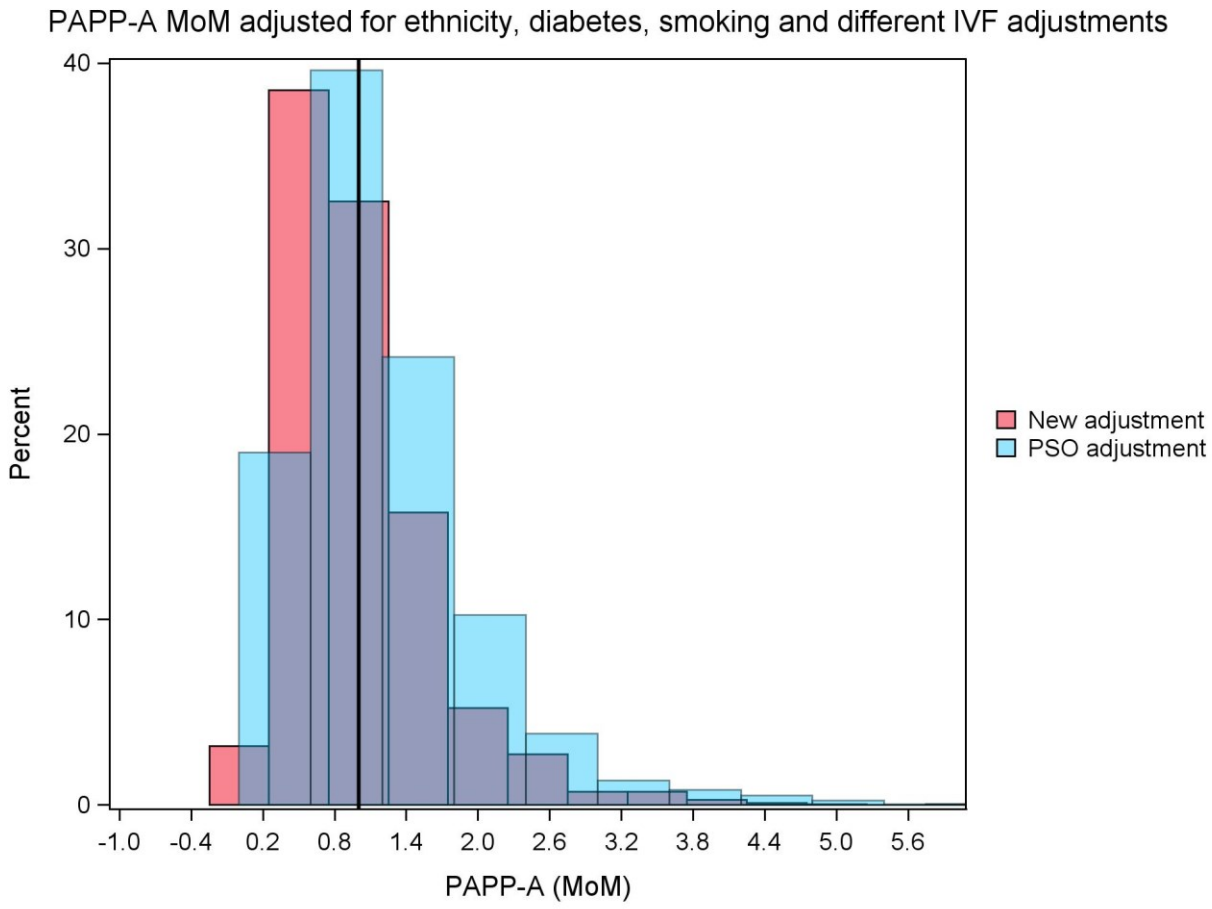

Dimeric Inhibin A adjusted for ethnicity, diabetes and smoking - Prenatal Screening Record IVF Status
Count
1500
1000
500
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 DIA (MoM)
Spontaneous
IVF


Dimeric Inhibin A MoM adjusted for ethnicity, diabetes and smoking - CARTR Plus IVF Status
Count
3000
2000
1000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 DIA (MoM)
Spontaneous
IVF

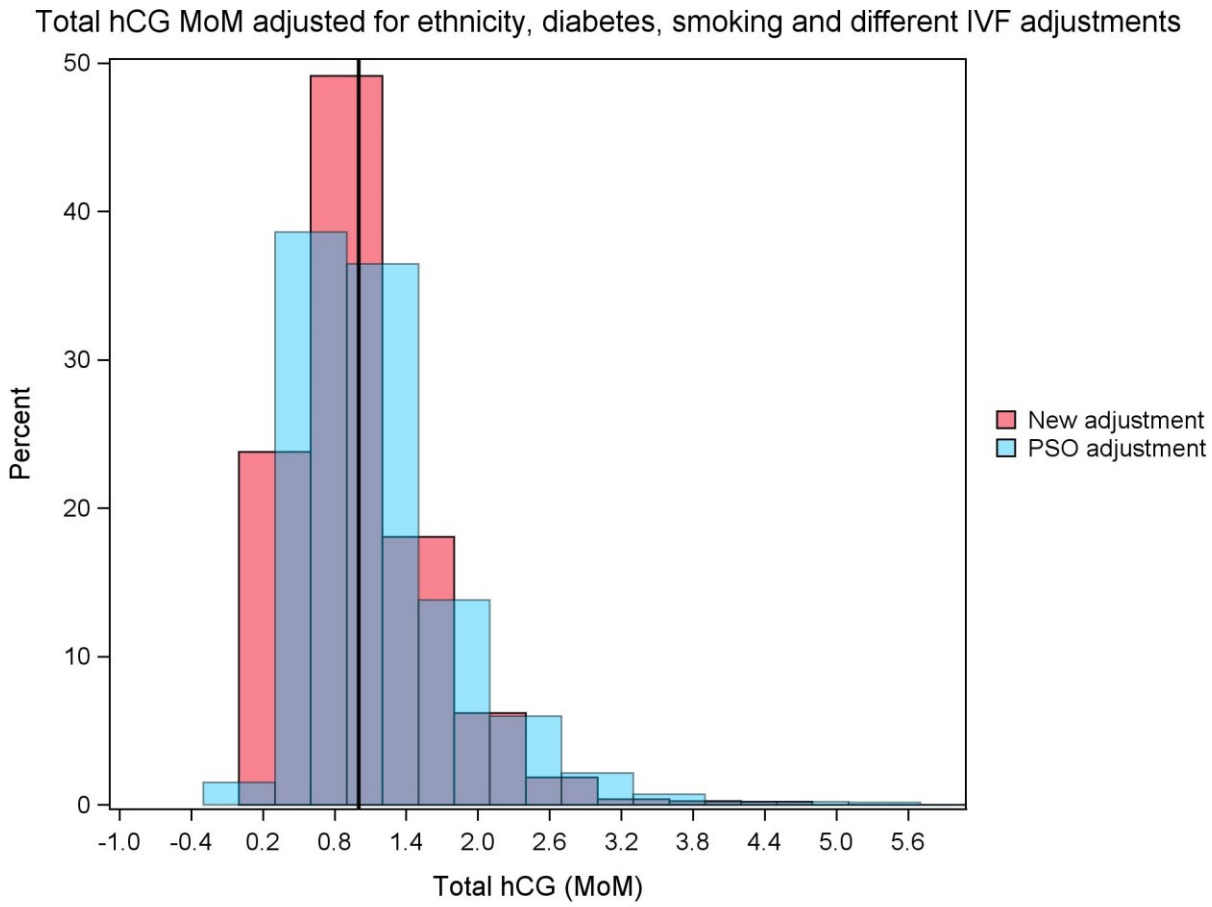

Nuchal translucency measurement MoM - Prenatal Screening Record IVF Status
Count
15000
10000
5000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 NT (MoM)
Spontaneous
IVF


Nuchal translucency measurement MoM - CARTR Plus IVF Status
Count
30000
25000
20000
15000
10000
5000
0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
log10 NT (MoM)
Spontaneous
IVF

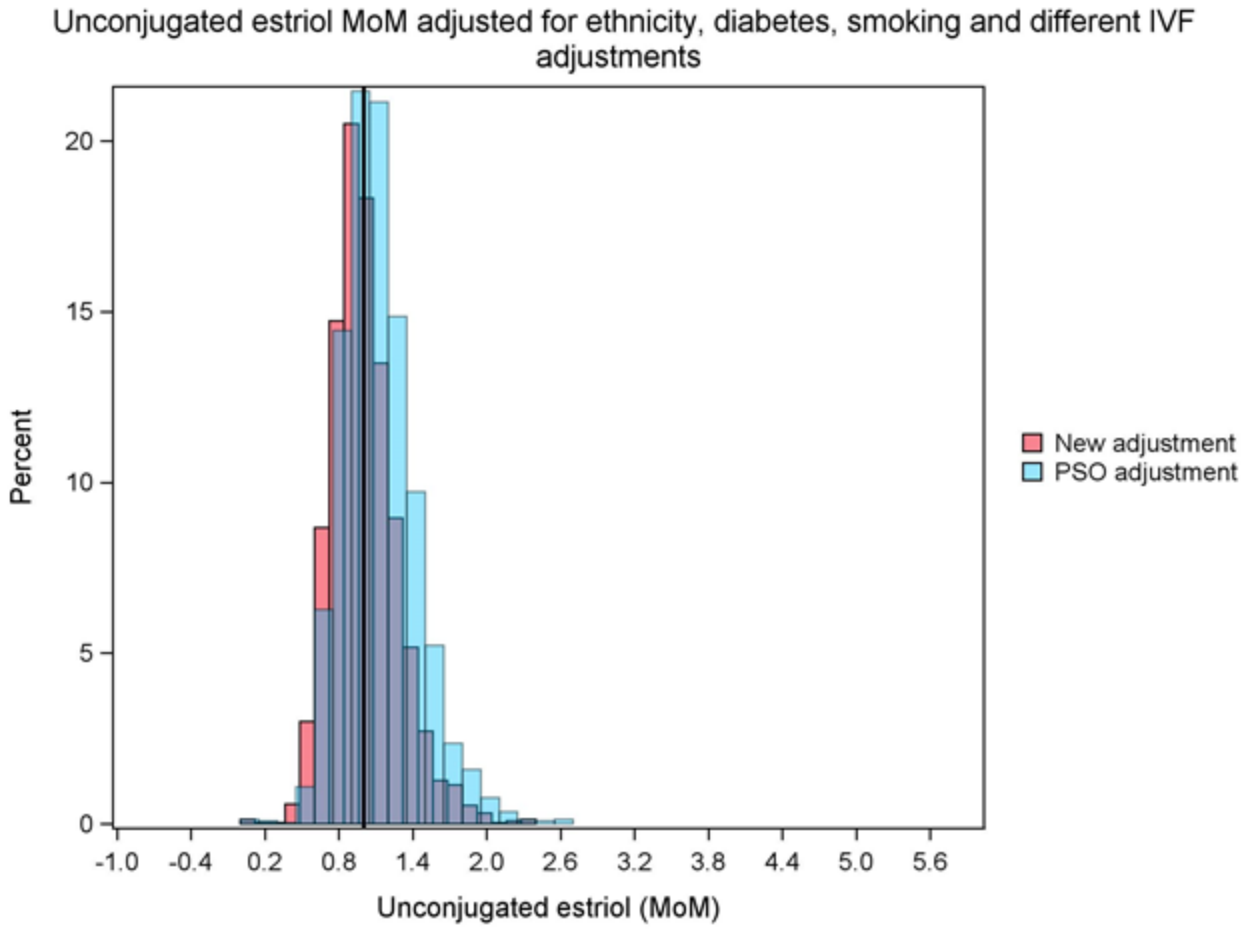
Supplemental Figure C-4 Histograms of proportion of PAPP-A MoM with different IVF adjustment factors



Supplemental Figure C-5 Histograms of proportion of total hCG MoM with different IVF adjustment factors



Supplemental Figure C-6 Histograms of proportion of unconjugated estriol MoM with different IVF adjustment factors



APPENDIX D: Supplemental information for Manuscript 3

Supplemental Text D-1 Data sources and preparation

Better Outcomes Registry & Network (BORN) Ontario

BORN Ontario's database is a web-based maternal child information system that captures all births within the province of Ontario.[110] This headquarters for the BORN registry is at the Children's Hospital of Eastern Ontario (CHEO) and they also host the BORN Information System (BIS).[110] The BIS contains information on prenatal screening, labour, birth, NICU care and congenital anomalies. This registry captures all hospital, home and birth centre births with a gestational age of greater or equal to 20 weeks.

Canadian Assisted Reproductive Technologies Register (CARTR) Plus

Data collection for CARTR Plus commenced on January 1, 2013. It includes data from 35 Canadian fertility clinics, of which 18 are located in Ontario. Unique to CARTR Plus is the ability to link data from multiple treatment cycles for the same woman. CARTR Plus contains detailed information on fertility treatment including treatment cycles, reason for treatment, types of stimulation and success of embryo transfer cycles, implantation success, and pregnancy outcome. CARTR Plus is a dataset contained within the BORN Registry.

Postal Code Conversion File Plus (PCCF+)[111]

The PCCF+ file provides a link between a Canadian postal code and the 2011 census geographic areas. In urban areas there is a distinct match between postal code and census geographic areas; however, in rural areas a single postal code can overlap with more than one census geographic area. The PCCF+ uses random weight assignment to assign a postal code to one of the census geographic areas. This linkage then allows for the use of socio-demographic information that can be provided at the neighbourhood-level from the Canadian Census for specific geographic areas.

Canadian Institute for Health Information (CIHI)

Canadian Institute for Health Information (CIHI) dataset contains information about the health of Canadians. An annual dataset of all Ontario maternal and newborn records is sent to BORN Ontario. These data include all hospital live births, stillbirths, hospital admissions and deliveries. The maternal and newborn records were deterministically linked by maternal-newborn chart number. This linkage was performed by BORN Ontario. These data were then used to enhance specific data elements from the BORN Information System.

Record linkage

The CIHI records from 2012 through 2015 were probabilistically linked to records in the BORN Information System for at BORN Ontario.[107] Ontario Health Insurance (OHIP) number was the first identifier used to link these records. Next, infant chart number and hospital code was used; followed by maternal OHIP number combined with infant date of birth; and finally maternal hospital chart record, hospital code and infant date of birth. The majority of CIHI records linked to records in the BORN Information System (98.2%).

This crosswalk file was then used to link the appropriate CIHI records for one Ontario hospital to the appropriate data from the BORN Information System.

These data were then deterministically linked to the CARTR Plus dataset.

Cohort description

Fertility treatment records in the CARTR Plus database for IVF, FET and frozen oocyte IVF for embryo transfer cycles from January 1, 2013 to December 31, 2013, were deterministically linked to birth records (live births and stillbirths) in the BORN Information System (corresponding birth dates: May 21, 2013 to October 28 2014). Multiple gestation pregnancies, pregnancies from donor oocytes, treatment cycles for combined type of infertility and deliveries that occurred outside of Ontario were excluded. Additional information was obtained through linking to the CIHI-DAD for one large hospital that was missing outcome information in the BORN Information System.

Level of Care (LOC)

A hospital's level of maternal and newborn care in Ontario is determined by the Ministry of Health and Long-Term Care. The levels pertain to the medical services/capacities available on site. A level I site can handle low maternal and neonatal risk (no significant maternal risk factors) and a delivery of a baby at a gestational age of ≥36 weeks.[112] Additionally, a Level Ia site does not have the capacity to provide caesarean delivery 24 hours and day, 365 days a year, whereas a Level Ib site does.[112] A level II site has the capacity for deliveries at a gestational age of ≥32 weeks and can care for uncomplicated dichorionic twin pregnancies.[112] A level IIc site can handle deliveries at a gestational age of ≥30 weeks that have a moderate level of maternal or neonatal risk.[112] Finally, a level III hospital can deliver any gestational age and manage high risk maternal and neonatal situations and conditions.[112]

Supplemental Table D-1 Summary of procedures used to code study variables from the Canadian implementation of the International Classification of Diseases

Variable	Purpose of variable	Method of coding
Preeclampsia	Required to identify cases of preeclampsia	All ICD-10-CA codes starting with “O14” and “O15”.
Placental abruption	Required to identify cases of placental abruption	All ICD-10-CA codes starting with “O45”.

Notes:

ICD-10-CA, Canadian implementation of the International Classification of Diseases, 10th Revision

Supplemental Table D-2 Unadjusted logistic regression models for type of infertility and primary outcomes when type of insemination was only IVF without ICSI

Type of infertility	Composite	Preeclampsia	Placental abruption	Stillbirth	SGA
	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)
Female factor	ref	ref	ref	ref	ref
Male factor	--	--	--	--	--
Unexplained	0.85 (0.40-1.78)	1.22 (0.11-13.15)	--	--	1.02 (0.47-2.19)

Supplemental Table D-3 Unadjusted logistic regression models for type of infertility and primary outcomes when type of insemination was only IVF with ICSI

Type of infertility	Composite	Preeclampsia	Placental abruption	Stillbirth	SGA
	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)
Female factor	ref	ref	ref	ref	ref
Male factor	0.85 (0.53-1.35)	0.58 (0.11-3.12)	0.38 (0.04-3.67)	4.60 (0.52-40.97)	0.89 (0.53-1.48)
Unexplained	1.14 (0.66-1.97)	--	0.90 (0.09-8.58)	2.70 (0.17-42.86)	1.21 (0.67-2.20)

Supplemental Table D-4 Unadjusted logistic regression models for type of conception and type of infertility and secondary outcomes when type of insemination was only IVF without ICSI

	Birth at <37 weeks	Birth at <32 weeks
	RR (95% CI)	RR (95% CI)
Type of infertility		
Female factor	ref	ref
Male factor	1.04 (0.16-6.84)	--
Unexplained	0.72 (0.28-1.85)	--

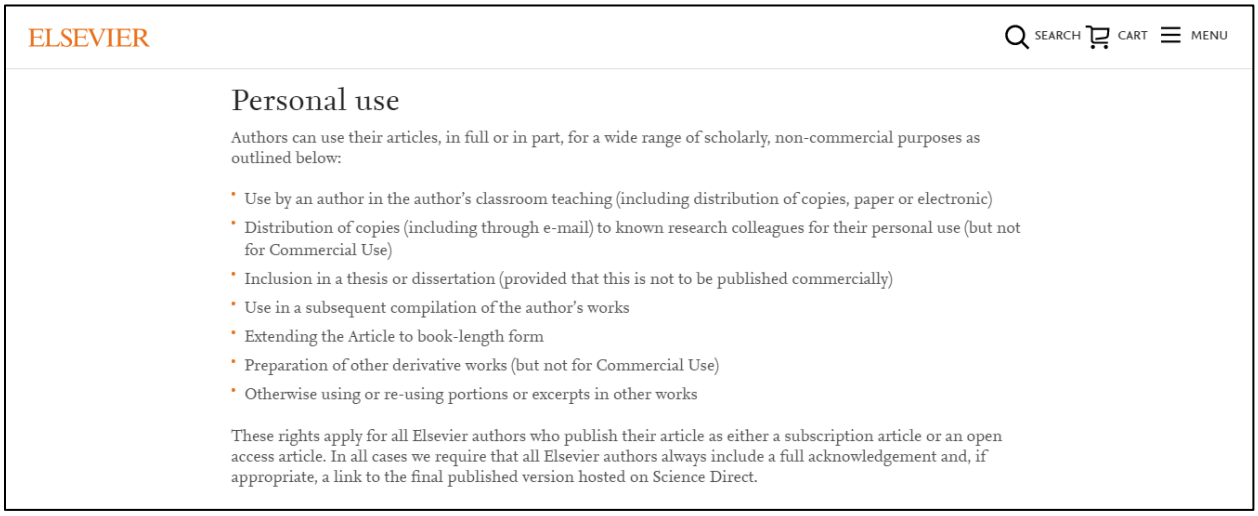
Supplemental Table D-5 Unadjusted logistic regression models for type of conception and type of infertility and secondary outcomes when type of insemination was only IVF with ICSI

	Birth at <37 weeks	Birth at <32 weeks
	RR (95% CI)	RR (95% CI)
Type of infertility		
Female factor	ref	ref
Male factor	0.68 (0.43-1.06)	0.64 (0.22-1.89)
Unexplained	0.88 (0.51-1.52)	0.90 (0.25-3.27)

APPENDIX E: Copyright permission

Manuscript 1: Maternal serum screening markers and nuchal translucency measurements among in vitro fertilization pregnancies: a systematic review

https://www.elsevier.com/about/company-information/policies/copyright/personal-use



ELSEVIER

SEARCH CART MENU

Personal use

Authors can use their articles, in full or in part, for a wide range of scholarly, non-commercial purposes as outlined below:

- Use by an author in the author's classroom teaching (including distribution of copies, paper or electronic)
- Distribution of copies (including through e-mail) to known research colleagues for their personal use (but not for Commercial Use)
- Inclusion in a thesis or dissertation (provided that this is not to be published commercially)
- Use in a subsequent compilation of the author's works
- Extending the Article to book-length form
- Preparation of other derivative works (but not for Commercial Use)
- Otherwise using or re-using portions or excerpts in other works

These rights apply for all Elsevier authors who publish their article as either a subscription article or an open access article. In all cases we require that all Elsevier authors always include a full acknowledgement and, if appropriate, a link to the final published version hosted on Science Direct.

APPENDIX F: Status of manuscript submission

Manuscript 2: Comparing maternal serum screening markers among IVF and spontaneous conceptions in Ontario through registry data

Dear Ms. Lanes:

Thank you for sending us your manuscript entitled "Comparing maternal serum screening markers among IVF and spontaneous conceptions in Ontario through registry data" which is being considered for publication in the Journal of Medical Screening.

Your manuscript ID is JMS-17-009.

We shall contact you again when the manuscript has been reviewed.

Yours sincerely
Janette Mackie
Journal of Medical Screening Editorial Office

Manuscript 3: The effect of the type of infertility on placental-mediated adverse outcomes for patients that used IVF

Dear Ms. Lanes,

Your submission entitled "The effect of the type of infertility on placental-mediated adverse outcomes for patients that used IVF" has been received by Fertility and Sterility.

You will be able to check on the progress of your paper by logging on to the Elsevier Editorial System of Fertility and Sterility as an author. The URL is https://ees.elsevier.com/fns/.

Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to this journal.

Sincerely,

Fertility and Sterility Editorial Office