IN VITRO FERTILIZATION (IVF) AND TWIN PREGNANCY OUTCOMES

by

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Abstract

Background

IVF treatment has been used to transfer multiple embryos leading to multiple pregnancies which are known to be associated with increased maternal and perinatal complications. One question is whether IVF itself contributes to these risks.

We compare the outcomes (maternal and perinatal) of IVF twin pregnancies with those of non-IVF twin pregnancies in the general population, in the province of British Columbia, Canada. We hypothesized twin pregnancies conceived by IVF are associated with a greater risk of maternal complications, abnormal labour and delivery events, and worse perinatal outcomes, compared with their non-IVF counterparts.

Methods

An IVF twin pregnancy group (n = 161) was identified, in the former IVF Program of the University of British Columbia Centre for Reproductive Health (UBCCRH). We obtained these cases from the UBCCRH database between April 1, 1998, and March 31, 2010. A non-IVF twin pregnancy group was obtained by linking to the BC Perinatal Database to serve as a comparison group (n = 5525).

Pregnancy outcomes and labour and delivery events were compared between IVF and non-IVF pregnancies using logistic and linear regression after adjustment for mothers' age, body mass index (BMI), and parity.

Results

After adjustment for age, BMI, and parity, IVF twin pregnancies had higher rates of gestational hypertension, antepartum hemorrhage, gestational diabetes Mellitus, higher risks of C-section as the method of delivery and steroid use for lung maturation in IVF mothers. Also there were higher risks of IUGR and congenital anomalies in IVF babies, and IVF births had a lower birth weight, birth length, head circumference, and gestational age at birth.

Conclusion

IVF twin pregnancies have an increased risk of some maternal complications, abnormal labour and delivery characteristics, and poorer perinatal outcomes, than naturally conceived twin pregnancies, even after adjustment for age, BMI, and parity. Further research is needed to clarify whether it is the infertility, maternal characteristics and co-morbidity associated with infertility, the IVF procedure or the potentially differential care of IVF patients that account for this increase in risk.

Lay summary

Infertility is a global health issue. Until recently, IVF treatment had been used to transfer of more than one embryo leading to multiple pregnancies. However, multiple pregnancies are known to be associated with increased preterm birth, low birthweight (LBW), and increased perinatal morbidity and mortality. One question is whether IVF treatment itself contributes additional maternal and perinatal risks, after controlling for multiple pregnancies.

In this study, we hypothesized that, in line with related literature, maternal and perinatal complications are more common in twin pregnancies conceived by IVF in comparison with their non-IVF counterparts. Our results, after adjusting for the mother's age, body mass index, and parity, show that IVF twin pregnancies are at an increased risk of maternal complications, abnormal labour and delivery events, and poorer perinatal outcomes when compared with natural twin pregnancies.

Preface

This dissertation is original, unpublished, independent work by the author, P. Khan Mohammad Beigi. The research project, of which this thesis is a part, received research certificate and ethics approval from the Perinatal Service of British Columbia, project name "Assisted Reproductive Technology (ART) and Pregnancy Outcomes", No. R2009001, 20 Jan 2016.

This project and the associated methods were approved by the University of British Columbia's Research Ethics Board, project name "Assisted Reproductive Technology (ART) and Pregnancy Outcomes", certificate number H08-01224, 29 Jan 2016.

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List of Abbreviations

APH: Antepartum hemorrhage

BMI: Body Mass Index

GDM: Gestational Diabetes Mellitus

GH: Gestational Hypertension

IUGR: Intra-Uterine Growth Restriction

Glossary

Definitions of terms used in data extracted from the BC Perinatal Database:

Antepartum hemorrhage: mother had bleeding before childbirth

Antihypertensive drugs in pregnancy: mother received antihypertensive medications during her pregnancy (antepartum period only)

Gestational age at first ultrasound (weeks): gestational age determined at the time of the first ultrasound performed under 20 weeks of gestation

Gestational age from the maternal record (weeks): gestational age documented by the clinician before delivery, determined by maternal last menstrual period and/or ultrasound

Gravida: total number of prior pregnancies plus the current pregnancy; twins and other multiples counted as one pregnancy

Hypertension > (140/90 mm Hg): mother had a blood-pressure reading of greater than or equal to 140/90 mm Hg on two consecutive readings during the pregnancy, prior to labour

IUGR: Intrauterine growth restriction

Number of antenatal visits: total number of primary care antenatal visits (does not include consultations or specialized clinic visits, e.g., to a diabetic clinic)

Number of previous preterm pregnancies: total number of previous pregnancies delivered before 37 completed weeks of gestation

Number of previous spontaneous abortions: total number of previous pregnancies spontaneously ending prior to 20 completed weeks of gestation

Number of previous term pregnancies: total number of previous pregnancies delivered at greater than or equal to 37 completed weeks of gestation

Parity: number of previous pregnancies delivered at equal to or greater than 20 completed weeks (140 days) gestation or 500 grams birth weight, regardless of outcome

Prior major congenital anomalies: mother had at least one previous pregnancy in which the baby was diagnosed with a major congenital anomaly.

Prior neonatal death: mother had at least one prior live-born infant who died within the first 28 days after life

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I officially dedicate this thesis to my parents, Mohammad Khanmohammad Beigi and Parvin Mojabi and my wife, Fatemeh Jalalian.

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Dedication

To my real hero

Mohammad Khan Mohammad Beigi

who inspired me to achieve and succeed by focusing only ever on the goal and never on the obstacles

and

To my real teacher

Parvin Mojabi

to whom I owe my life and all that I have accomplished and become

and

To my real companion

Fatemeh Jalalian

to whom I owe my achievements when I felt tired

Introduction

Literature review

Infertility continues to be a significant global health problem, affecting up to 16% of couples worldwide (Sharlip et al., 2002; Secretariat, 2006; Inhorn et al., 2015; Chu et al., 2019). Infertility is the failure to achieve pregnancy after one year of regular, unprotected sexual intercourse (Vander Borght et al., 2018) There are many forms and causes of either male or female infertility. Researchers continue to develop solutions to provide infertile couples with the opportunity of parenthood through assisted reproductive technology (ART). One such ART is in vitro fertilization (IVF), a common treatment for infertility, yet there remain adverse effects on both the mother and the fetus, which are found to be more prevalent among twin gestations following IVF.

Twin gestations are more common in IVF pregnancies due to the techniques involving the transfer of more than one embryo to increase the chances of success. However, it remains unclear whether the outcomes of twin IVF pregnancies are related to the IVF procedure or the twin gestation itself. The process of embryo implantation is a very complex one that requires replication of many steps that occur during human reproduction (Szamatowicz et al., 2016). In IVF, the transfer of multiple fertilized eggs increases chances of pregnancy but also multiple gestation. (Secretariat, 2006).

Female and male infertility

As mentioned, infertility is a medical condition characterized by an inability to achieve pregnancy after one year of regular, unprotected sexual activity (Secretariat, 2006; Smith et al., 2003; Szamatowicz, 2016; Walker et al., 2021). Current estimates show that infertility may affect 8–16% of couples worldwide (Secretariat, 2006; Sharlip et al., 2002; Chu et al., 2019). The World Health Organization (WHO) considers infertility to be a social disease due to its high prevalence (Szamatowicz, 2016).

Infertility is a unique health problem because it usually involves, and personally affects, two individuals (Szamatowicz, 2016; Walker et al., 2021). However, among couples suffering from

infertility, the woman is often stigmatized, despite both partners' potential contribution (Turner et al., 2020). Couples dealing with infertility can experience psychological stress, depression, and anxiety (Cunningham, 2017). The burden of treatment, and providing explanations for infertility, falls primarily on women, due to societal perception: males produce sperm, whereas women provide eggs, undergo the fertilization treatment process, and deliver the pregnancy (Turner et al., 2020).

Infertility may be defined as primary or secondary infertility (Inhorn et al., 2015; Smith et al., 2003; Okun et al., 2014). Primary infertility is the inability to achieve pregnancy with no previous pregnancies, and secondary infertility is the inability to achieve pregnancy following a prior pregnancy (Inhorn et al., 2015). In most places around the world, especially in developing countries, secondary infertility is much more prevalent than primary infertility (Inhorn et al., 2015). Secondary infertility can be the result of reproductive-tract infections (RTIs), which, if left untreated, can lead to irreversible tubal blockages (Inhorn et al., 2015; Walker et al., 2021).

The number of people affected by infertility worldwide is estimated at 186 million (Inhorn et al., 2015). A study by Cunningham (2017) found that infertility affects 6% of married women of reproductive age in the United States and 11.5–15.7% of women in Canada (Cunningham, 2017). Another study estimates 10% of women who are of reproductive age are infertile (Szamatowicz, 2016). In developing countries, it is estimated that 37% of infertility cases are attributed to female factors, while only 8% to male factors (Barbieri, 2019; Cunningham, 2017). Male and female factors combined comprise around 35% of infertility cases, and about 20% of infertility cases are unexplained (Cunningham, 2017). Other research suggests that male factors account for 20% to 30% and even up to 70% of cases of infertility (Agarwal et al, 2015). The exact epidemiology of infertility may be difficult to determine, because precise numbers are difficult to estimate, and definitions of infertility differ around the globe (Inhorn et al., 2015). Another contributing factor is that there is very little data available on male infertility (Inhorn et al., 2015).

Recent advances in procedural conception have significantly improved outcomes for affected couples. Despite this improvement, the highest infertility rates are always found in regions with limited access to ARTs (Inhorn et al., 2015): generally developing countries, which, therefore,

have a higher prevalence of infertility. Indeed, infertility may affect 30% of women of reproductive age in developing countries, with one study showing that one-quarter of married women in developing countries struggled with infertility (Inhorn et al., 2015). Issues at social and government levels may have an additional impact. Unsafe abortions in areas where abortions are prohibited may increase the prevalence of infertility in women (Inhorn et al., 2015).

Table 1 summarizes the causes of infertility in males and females. These causes can occur at any stage of the reproductive process (Secretariat, 2006). Causes may vary depending on male-factor infertility, female-factor infertility, or a combination, and may include environmental factors (Secretariat, 2006; Smith et al., 2003; Walker et al., 2021). Women are more likely to seek medical care and follow progenitive health evaluation at an earlier age (Chu et al., 2019). Given the importance of the male factor, however, it is essential to conduct appropriate investigations on both partners to address the often-reversible causes of infertility, and to improve non-IVF fertility treatments (Chu et al., 2019). Moreover, male infertility has the potential to be a surrogate marker for adverse health outcomes, and early identification of the problem could improve the patient's lifestyle (Chu et al., 2019). The success of infertility treatments may have affected the development of diagnostic methods and treatments for male infertility. (Aitken et al., 2018).

Female factors leading to infertility	
Ovarian	
Ovulation disorders (Smith et al., 2003; Walker et al., 2021)	Includes decreased ovarian reserve related to maternal age and premature ovarian insufficiency, as well as anovulation in which there is the absence of oocyte release per month. Common examples include pituitary adenoma, polycystic ovarian syndrome (PCOS), and hypothalamic amenorrhea (Walker et al., 2021; Smith et al., 2003)

Tubal Obstruction of fallopian tubes / tubal blockage (Secretariat, 2006; Smith et al., 2003; Walker et al., 2021)	Preventing fertilization (Walker et al., 2021)
Uterine Endometriosis (Cunningham, 2017; Secretariat, 2006; Smith et al., 2003; Walker et al., 2021)	Presence of endometrial tissue outside of the uterine cavity, most commonly in the pelvis (Walker et al., 2021)
Fibroids (Secretariat, 2006; Walker et al., 2021)	Irregular growth in the walls (myometrium) of the uterus leading to difficulty with implantation of the zygote
Hostile cervical mucus (Secretariat, 2006)	Irregular cervical mucus because of hormonal imbalance
Agenesis	*Müllerian agenesis*, is a congenital malformation which causes the vagina and uterus to be underdeveloped or absent
Male factors leading to infertility	
Testicular Failure of sperm production (Secretariat, 2006) due to genetic causes, failure of testes to descend,	Inability to produce sperm within the seminiferous tubules

infection, or torsion, Production of antibodies to spermatozoids (Secretariat, 2006) Low sperm motility (Secretariat, 2006)	 Production of antibodies attacking the developing sperm and preventing development within the male reproductive tract Inability of the sperm to move effectively towards the egg to complete fertilization
Erection, Ejaculation Problem Sexual dysfunction	
Obstruction Prostate-related problem Vasectomy Absence of vas deferens	

Table 1 Female and male factors that lead to infertility. While the factors outlined can be defined and studied, infertility may also be commonly unexplained (Cunningham, 2017; Secretariat, 2006; Smith et al., 2003; Walker et al., 2021).

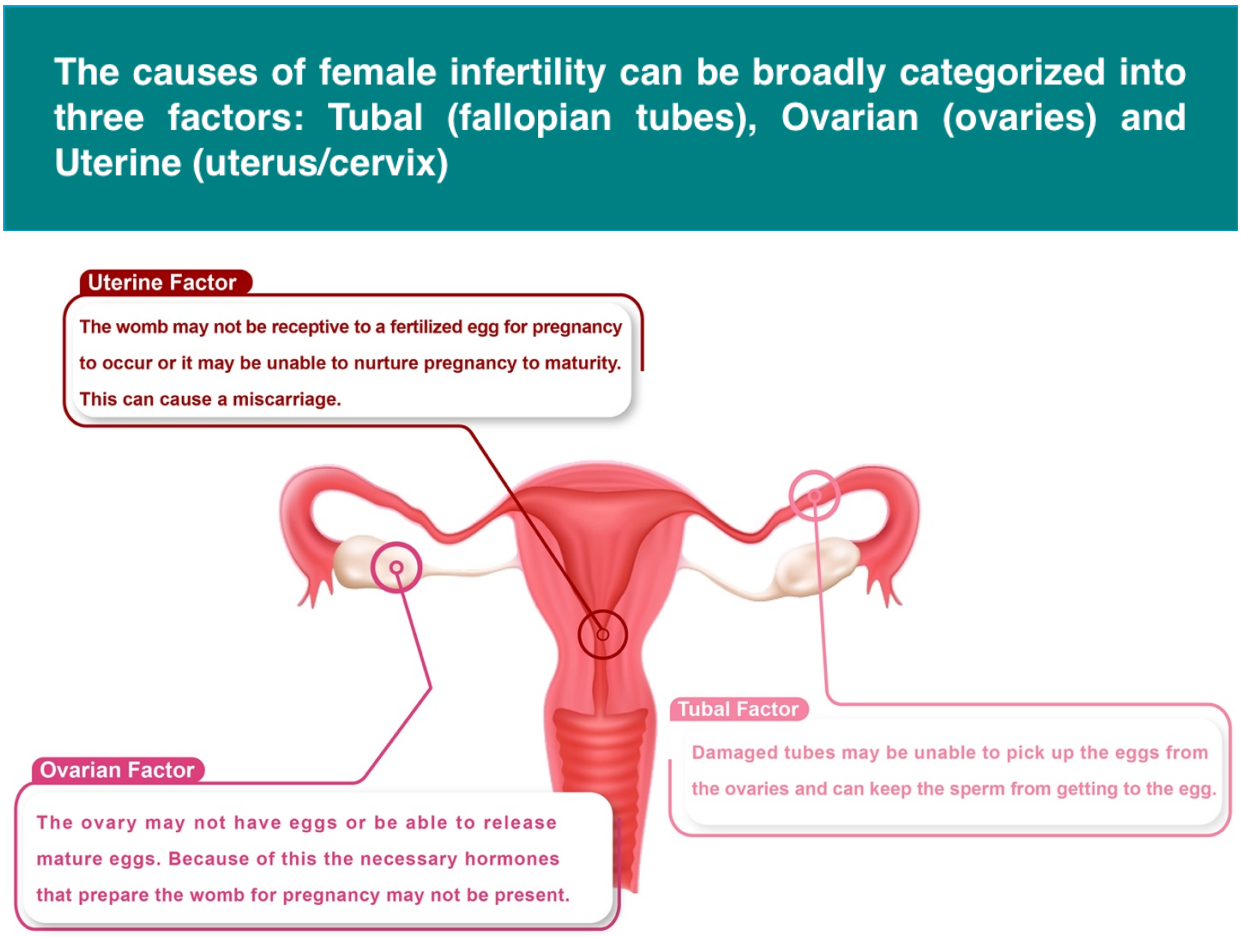

Figure 1. Female factors that lead to infertility. (with permission from the website source: "Factors in infertility – mariref.com", 2022)

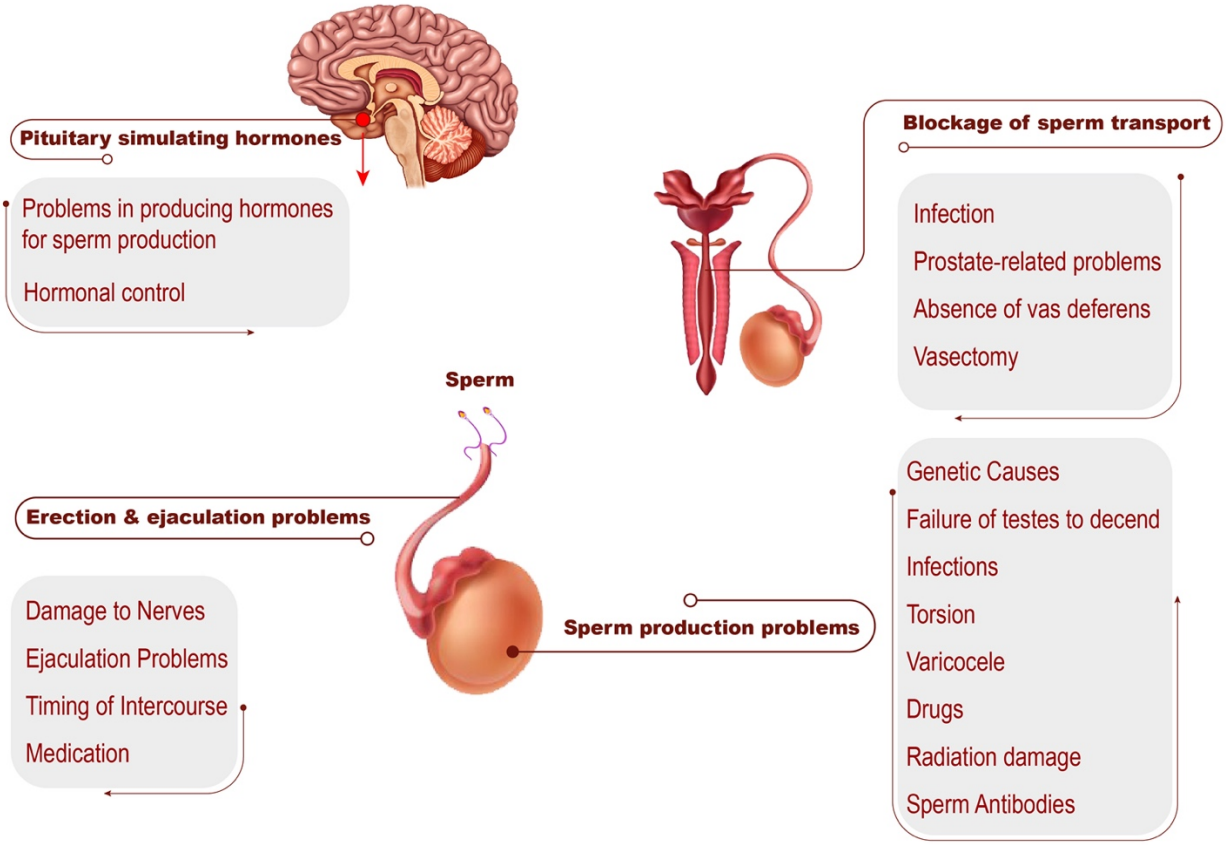

Figure 2. Male factors that lead to infertility. (with permission from the website source: "Factors in infertility – mariref.com", 2022)

Female infertility

(Female factors leading to infertility are also shown in Table 1 and Figure 1).

Maternal age is of primary importance in determining fertility. As women age, the quality and quantity of oocytes changes (Walker et al., 2021). While women are born with roughly one million follicles, by the time they go through puberty the follicle count drops to 700,000. With each menstrual cycle, the number of follicles decreases and some ova undergo apoptosis. Overall, only 400 follicles will undergo full maturation by menopause (Macklon et al., 2006; Walker et al., 2021). With age, the length of the menstrual cycle also decreases, due to the shortening of the follicular phase (Macklon et al., 2006). These characteristics of ovarian aging are dependent on the aging of the follicles themselves, the development of the granulosa cells, as well as a hormonal contribution from the follicle-stimulating hormone (FSH), the anti-Mullerian hormone (AMH), and inhibin B (Macklon et al., 2006).

External (or environmental) and genetic factors can increase the rate of follicle loss, in tandem with aging. Smoking can accelerate early menopause (defined as menopause before the age of 40 years) (Walker et al., 2021). Genetic factors involving aneuploid pregnancies due to meiotic nondisjunction events also increase with age (Szamatowicz, 2016; Walker et al., 2021). Altogether, however, the success rates for women using ART as a solution for infertility decline after 35 years of age, primarily due to the natural follicle-number decline mentioned above (Secretariat, 2006).

Diminished ovarian reserve can also result from other causes of accelerated loss of the ovarian follicular pool (Gurtcheff & Klein, 2011). This loss may have a genetic component, such as Fragile X syndrome, Turner syndrome, or X mosaicism, or can result from chemotherapy, endometriosis, pelvic infection, or ovarian surgery (Gurtcheff & Klein, 2011). It can result in oocyte abnormalities, leading to infertility, decreased implantation rate, or increased rate of pregnancy loss by spontaneous abortion (Gurtcheff & Klein, 2011). Ovarian-reserve testing involves measuring serum levels of FSH and estradiol, along with inhibin and anti-Mullerian hormone (AMH) (Gurtcheff & Klein, 2011).

Fertility is also affected by the obstruction of the fallopian tubes or tubal blockage, due to mucus debris, occlusions, or uterotubal junction spasms (Barbieri, 2019; Secretariat, 2006; Smith et al., 2003; Walker et al., 2021), or as the result of the blockage of physiological fluid movement, resulting in fluid accumulation (Walker et al., 2021). Tubal blockage can impair the release and movement of the oocyte (Bulletti et al., 2010). Tubal patency can be detected via a hysterosalpingogram (Barbieri, 2019); known treatments involve recanalization or surgical reanastomosis of the fallopian tubes (Barbieri, 2019).

Endometriosis, another cause of female infertility, is the result of the presence of endometrial tissue outside the uterine cavity (ectopic endometrial tissue), most commonly in the pelvis (Walker et al., 2021). Endometriosis occurs in approximately 6–10% of females and results in pain and/or infertility in approximately 35–50% (Bulletti et al., 2010). The chronic inflammatory reaction accompanying endometriosis can alter the pelvic anatomy and peritoneal function, and can affect the endometrium hormone stimulation and balance, and cellular processes (Bulletti et al., 2010). Approximately 40–50% of patients note chronic pelvic pain and 60–80% have dysmenorrhea; these can be associated with infertility (Bulletti et al., 2010). While there is an association between female infertility and endometriosis, research is still required to understand the mechanism (Bulletti et al., 2010).

Anovulation, a monthly absence of oocyte release, can contribute to infertility (Barbieri, 2019; Li et al., 2012). Detailed histories are taken to determine the cause of anovulation, and include the cycle length, and the regularity of the menstrual period (Li et al., 2012). Recorded causes of anovulation include polycystic ovarian syndrome (PCOS), thyroid dysfunction, hyperprolactinemia, hypogonadism, hyperandrogenic symptoms, Sheehan syndrome, and Cushing's syndrome; incomplete genital development is also a possibility (Barbieri, 2019; Li et al., 2012). Anovulation is also commonly associated with amenorrhea or oligomenorrhea (Barbieri, 2019; Li et al., 2012). Patients are tested for follicle-stimulating hormone (FSH), thyroid stimulating hormone (TSH), prolactin, and progesterone, and are given a general androgen profile (Barbieri, 2019; Li et al., 2012). Some of the offered methods for ovarian induction involve drug therapies using clomiphene citrate, aromatase inhibitors, dopamine agonists, tamoxifen, letrozole, or insulin-sensitizing agents (Barbieri, 2019; Li et al., 2012). However, further research is required

to better understand the outcomes of prescribing varying doses, which require close monitoring to prevent additional complications (Li et al., 2012). Other methods for treating PCOS include ovarian drilling (Li et al., 2012). While needing more research, weight modulation among women trying to achieve pregnancy is considered important in reducing complications (Barbieri, 2019).

Fibroids are tumors or irregular growths in the walls (myometrium) of the uterus that lead to difficulty with implantation of the zygote (Guo & Segars, 2012; Secretariat, 2006; Walker et al., 2021). They can result in fallopian tubal obstruction or affect endometrial development, depending on their size and location (Guo & Segars, 2012; Purohit & Vigneswaran, 2016). They are found to affect myometrial contractility and are associated with changes in macrophage and uterine natural killer cell count, which can hinder implantation (Purohit & Vigneswaran, 2016). Their diagnosis and removal, through procedures such as hysteroscopy and myomectomy can influence infertility (Guo & Segars, 2012; Purohit & Vigneswaran, 2016).

There may also be a role for body weight in female infertility. Findings from Cunningham (2017) show that maintaining a BMI between approximately 19 and 30 kg/m^2 as preventative measures for infertility. With a BMI below 17 kg/m^2, there are higher chances of developing hypogonadotropic hypogonadism (ovulatory disorder; Walker et al., 2021). Additional findings suggest that, with high BMI, obesity may alter the hypothalamic-pituitary-gonadal axis, resulting in a hyperestrogenic, hypogonadotropic response (Kahn, 2017; Chu et al., 2019). There is increasing evidence that, in addition to affecting hormonal levels (Kahn, 2017), obesity in women may also contribute to adverse events after IVF treatment (Sermondade et al., 2019).

Irregular cervical mucus because of hormonal imbalance also affects infertility (Nakano et al., 2015; Secretariat, 2006). Changes in the vaginal pH can affect the cervical mucus; a pH below 6.0 can damage the sperm and the buffering capacity of the semen, and this can contribute to infertility (Nakano et al., 2015). Conditions such as acute inflammatory conditions and cystic fibrosis can affect the consistency and amount of mucus produced, which, if too thick, can act as a barrier to the sperm (Nakano et al., 2015). There are also certain drugs and nicotine that can affect the consistency of mucus.

Male infertility

(Male factors leading to infertility are also shown in Table 1 and Figure 2.)

To equalize the burden of diagnosis and treatment for men and women, more research is needed focusing primarily on male infertility, to improve the options for treatment, and to increase non-IVF conception chances (Turner et al., 2020). Pandruvada et al. (2021) note that the diagnostic tools for male infertility are limited to sperm analysis; even the available tools for such analysis are incomplete and insufficient for a detailed understanding of sperm defects, e.g., number, shape, motility, and viability, and of underlying diagnoses. Idiopathic male infertility indicates that male infertility causes are unknown: there may be unexplained infertility with normal semen, with failure to conceive being of unknown cause (Pandruvada et al., 2021).

The inability or decreased ability to produce sperm within the seminiferous tubules has long been researched as a cause of infertility (Sharpe, 2012). The production of sperm is reliant on the function and number of sertoli cells within the testes and the time of the last ejaculation (Sharpe, 2012). This time can give information about rate and frequency of sperm release and about the sperm count (Sharpe, 2012). A low count or inability to produce sperm can affect male fertility; decreased production can be a result of genetic, lifestyle, or environmental causes (Sharpe, 2012).

Antisperm antibodies (ASA) are a rare cause of infertility and can damage and/or prevent development of sperm within the male reproductive tract (Bach & Schlegel, 2019; Vickram et al., 2019). ASA form from trauma or disruption of the blood-testis-barrier (BTB) (Bach & Schlegel, 2019; Vickram et al., 2019). While ASA have been documented as present in fertile males and females, there remain associated risks with their presence, including prostatitis, testicular cancer, microbial infections, seminal infections, varicocele, and erectile dysfunction (Vickram et al., 2019). Infertility associated with the presence of ASA may affect implantation or alter sperm motility, progression, and fertilizing capacity. However, further investigation is required to fully understand the role of ASA in infertility (Vickram et al., 2019).

Ejaculation duct obstruction (EDO) may present in males with oligospermia, azoospermia, low semen pH, negative semen fructose, or palpable vas deferens, and can contribute to male infertility

(Bach & Schlegel, 2019). EDO can be treated with transurethral dilation of the ejaculatory ducts (TURED), but it comes with risks and may result in retrograde ejaculation, urine reflex, or epididymitis (Bach & Schlegel, 2019).

Assisted reproductive technology (ART)

Different treatment options are available for infertility. The three main therapeutic strategies include pharmacological therapy or medications, surgical intervention (commonly endoscopic), and assisted reproductive technology (ART) (Secretariat, 2006; Szamatowicz, 2016, Cunningham, 2017).

ART consists of procedures that manipulate eggs and sperm outside the body. ART includes several different techniques, including intrauterine insemination (IUI) and the focus of this study, IVF which may also involve intracytoplasmic sperm injection (ICSI), followed by embryo transfer (ET) (Allen et al., 2006; De Geyter, 2019; Lu et al., 2013; Secretariat, 2006; Sunderam et al., 2015; Szamatowicz, 2016). Recent years have seen significant progress in ART in the treatment of previously incurable cases using multiple modalities, including fertility preservation, uterine transplantation, preimplantation screening for aneuploidy, and mitochondrial replacement therapy (Szamatowicz, 2016). ART is considered an integral part of modern medicine and plays a critical role in family planning (De Geyter, 2019).

ART techniques may be followed by progesterone supplementation (Choe et al., 2021). Progesterone is widely understood to promote the development of the endometrium during the luteal phase of the menstrual cycle. In ART, the progesterone levels are typically considered insufficient, which may affect the chance of successful implantation. In a meta-analysis, van der Linden et al. (2011) studied the effects of progesterone supplementation in ART pregnancies and found a significant increase in the number of live births.

In vitro fertilization (IVF)

IVF exposes a sperm to an egg to enable fertilization within a petri dish. The embryo is cultured and then transferred into the female uterus for implantation. This procedure was originally developed as a solution for fallopian-tube obstruction and has since become an alternative treatment for infertility (Secretariat, 2006). To increase the success rate of live births, more than one embryo is transferred, but this has led to increases in multi-fetal pregnancies (Secretariat, 2016; Zollner & Dietl, 2013). For intracytoplasmic sperm injection (ICSI), there is an additional, microscopic laboratory procedure, with a single sperm injected directly into a mature egg (Allen et al., 2006; Secretariat, 2006; Walker et al., 2021). This procedure is common when there are abnormal sperm (Secretariat, 2006; Walker et al., 2021).

Ovarian stimulation is the first step of IVF treatment, with the objective of promoting the development of dominant follicles. The number of mature oocytes recovered is crucial (Drakopoulos et al., 2016; Macklon et al., 2006; Secretariat, 2006; Sunkara et al., 2011). Ovarian stimulation begins with erasing the chance of luteinizing hormone (LH) surge by providing women with a gonadotropin hormone-releasing hormone (GnRH) analog, followed by human chorionic gonadotropin (hCG) injection (Choe et al., 2021). The GnRH analogs usually have an increased half-life and efficiacy compared with natural hormones, are designed to interact with the GnRH receptors, and are usually decapeptides (Shrestha et al., 2015). Modifications in the analogs involve an altered amino-acid sequence compared with the natural GnRH (Macklon et al., 2006; Shrestha et al., 2015). Such modifications include replacing D-amino acids for glycine (Macklon et al., 2006). The use of GnRH analogs in IVF may prevent an endogenous LH surge which can help to induce folliculogenesis (Shrestha et al., 2015). However, with prolonged administration of the GnRH agonists, there is a possibility for desensitization of the pituitary GnRH receptors after short successive gonadal function (Macklon et al., 2006). Use of the GnRH agonist results in sustained gonadotropin secretion (Shrestha et al., 2015). GnRH agonists include triptorelin, leuprorelin, deslorelin, goserelin, and nafarelin, and the protocols can be characterized as ultra-short, short, and long (Shrestha et al., 2015). An alternative to GnRH agonists are GnRH antagonists (Macklon et al., 2006; Shrestha et al., 2015).

Through this step (ovarian stimualtion), the physician is then able to extract or retrieve multiple oocytes (Choe et al., 2021). Three or fewer recovered oocytes are associated with a low live-birth rate, but fifteen or more are considered optimal for increasing post-IVF live birth rates (Biljan et al., 2000, Sunkara et al., 2011). However, multiple-gestation pregnancies can result due to the transfer of multiple embryos to increase the chances of pregnancy (Secretariat, 2006). Natural-cycle IVF, without any ovarian stimulation, results in a lower pregnancy rate of 7–9% per initiated cycle (Gordon et al., 2013, Pelinck et al., 2002).

Oocyte retrieval occurs 34–36 hours after hCG administration (Choe et al., 2021). Retrieval can be performed using technologies such as transvaginal aspiration guided by ultrasound (Choe et al., 2021). An ultrasound probe with a needle guide helps the practitioner direct the needle into the follicles (Choe et al., 2021). Once the needle has entered the follicle, both the follicular fluid and the oocyte are drawn out (Choe et al., 2021). This procedure is performed with sedation (Choe et al., 2021).

Fertilization then follows: a semen sample is prepared by using density centrifugation to isolate the sperm, which is then washed with a high-protein concentration to promote capacitation, and allows the sperm to become fertilizable (Choe et al., 2021). Once the sperm are capacitated and fertilizable, they are incubated with an oocyte for 12-18 hours at a 50000:1 ratio (Choe et al., 2021). If the cause of infertility is paternal, practitioners may decide to directly inject an immobilized sperm into the oocyte using ICSI (direct injection), which bypasses the natural step of conception (Choe et al., 2021).

Embryo transfer is the next step (Choe et al., 2021). An embryo is fertilized in vitro and allowed to grow and survive in culture. Transfer to the uterine environment occurs when the embryo reaches the cleavage stage, which occurs three days after fertilization, or the blastocyst stage, which occurs five days after fertilization (Choe et al., 2021). Usually, transfer at the blastocyst stage results in a higher percentage of live births and may be preferred to transfer at the cleavage stage (Choe et al., 2021). However, it may be harder for embryos in culture to survive until day five (Choe et al., 2021). Practitioners use a catheter inserted into the cervix, which is guided by

transabdominal ultrasound, to transfer the embryo (Choe et al., 2021). The ideal location for transfer is 1–2 centimeters from the fundus (Choe et al., 2021).

Current guidelines recommend that two blastocysts be transferred to women aged 37 or younger, and three blastocysts to women aged 38–42 (Choe et al., 2021). The exact number may depend on factors such as embryo stage and quality, patient preference, and age (Secretariat, 2016; Zollner & Dietl, 2013). If the technique involves transferring embryos at the cleavage stage, two can be transferred for women aged 35 or younger, three for women aged 35–37, four for women aged 38–40, and five or fewer for women aged 41–42 (Choe et al., 2021). Single embryo transfer (SET) is an option available for women who are unable to sustain, or who do not wish for, multiple pregnancies (Min et al., 2010; Walker et al., 2021). This thesis will not discuss SET further.

Multiple pregnancies – background

Multiple pregnancies occur when two or more embryos grow simultaneously and are birthed from the same mother (Bricker et al., 2016; Health (UK), 2011). The incidence of multiple pregnancies has increased rapidly over the past 30 years. A significant factor in this increase has been infertility solutions such as ART (Bricker et al., 2016; Health (UK), 2011; Okun et al., 2014; Santana et al., 2018; Secretariat, 2006; Walker et al., 2021). Non-IVF twin pregnancies result from one in 80 pregnancies (Bricker et al., 2016).

Multiple pregnancies (here, we refer specifically to twin births) differ in terms of zygosity, chorionicity, and amnionicity (Bricker et al., 2016; Tocino et al., 2015). Monozygotic twins result from the fertilization of a single oocyte which later splits into two genetically identical embryos, while dizygotic twins are a product of fertilization of two separate oocytes (Bricker et al., 2016; Tocino et al., 2015). Among non-IVF twin births, monozygotic pregnancies constitute roughly 30% of twin pregnancies (Santana et al., 2018) and dizygotic twins make up approximately 70% of twin pregnancies (Gill et al., 2021).

Twin births can be distinguished by the sharing of the chorion/placenta (mono- or dichorionic) and the amniotic sac (mono- or diamniotic) (Song et al., 2017; Santana et al., 2018; Tocino et al., 2015). Chorionicity refers to the number of chorions/placentas for the pregnancy, assessed using

ultrasonography near the end of the first trimester (Santana et al., 2018). Dizygotic twins are dichorionic/diamniotic, where there are two individual placentas, separate or fused, and two individual amniotic sacs (Machin, 2001). Monozygotic twins can be dichorionic/diamniotic or monochorionic/diamniotic (Machin, 2001). If the division of the zygote occurs within the first three days after fertilization, then it results in dichorionic/diamniotic twins, in which each fetus has a separate amniotic sac and separate placenta/chorion (Santana, 2018; Gibson & Cameron, 2008). Division occurring between four and eight days after fertilization results in monochorionic/diamniotic twins (Santana, 2018). Division occurring between nine and 14 days also results in monoamniotic/monochorionic twins, because it is beyond the stage of amniotic differentiation; division after 14 days results in conjoined monochorionic/monoamniotic twins (Gibson & Cameron, 2008).

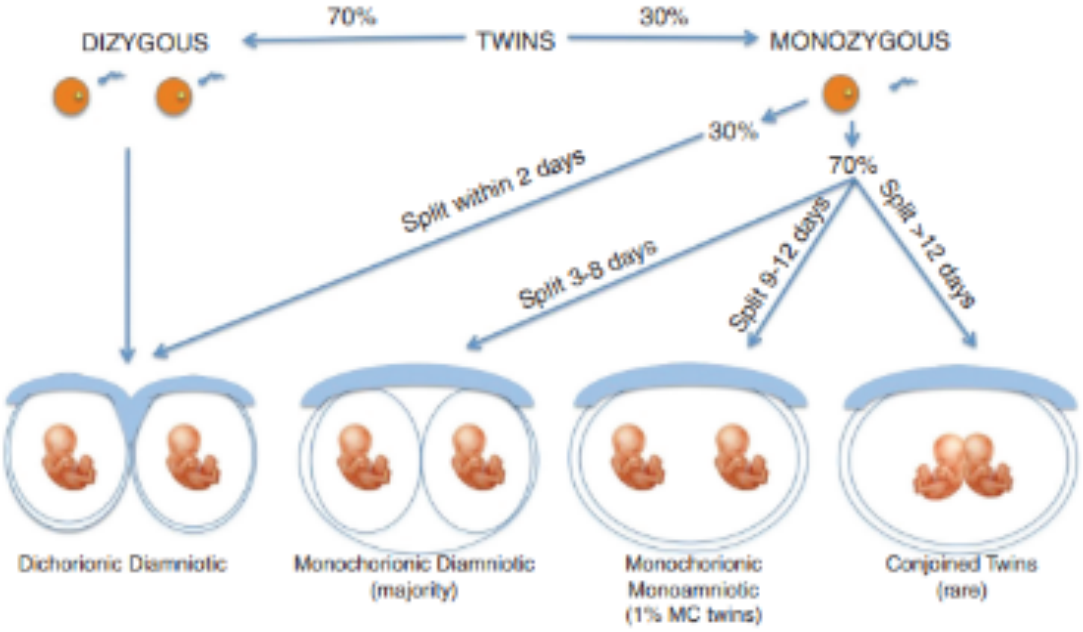

Figure 3 Zygosity, chorionicity and amnionicity (Bricker et al., 2016)

Non-IVF twin births are caused by several factors. Genetic characteristics within maternal lineages most commonly result in dizygotic, fraternal twins (Bricker et al., 2016). Among twin births, the rate of monozygotic twinning remains relatively constant at 3–5 per 1000 births, whereas the rate

of dizygotic twinning can vary (Gill et al., 2012). Other factors that may contribute to non-IVF twin pregnancy rates include population variation, parity, oral contraceptive use, along with maternal age and lifestyle, and socioeconomic status (Bricker et al., 2016; Zhu et al., 2016). Older women may experience multiple-birth pregnancies due to rising levels of gonadotropins, especially in women aged 35–39 years (Bricker et al., 2016). Higher parity may result in multiple pregnancies due to a woman being more fertile, but this finding is coexistent with maternal age (Bricker et al., 2016; Zhu et al., 2016). It is unclear whether factors such as social class, dietary habits, maternal height, and obesity, result in higher rates of twins (Bricker et al., 2016).

According to new research, there is a noted increase in monozygotic twins with the use of IVF, but the cause is not clear (Bos-Mikich, 2018). The chance of monozygotic twins with IVF is correlated with the timeframe in which the embryo is transferred and implanted into the uterine wall of the woman (Bricker et al., 2016). Dizygotic twins in IVF result from transfer of more than one embryo, followed by successful double implantation. Previous research explains how the IVF procedure may be adjusted for mothers of increased age to promote conception. Choe et al. (2021) mention that if the technique involves transferring embryos at the cleavage stage, two embryos can be transferred for women aged 35 years or lower, three for women aged 35–37 years, four for women aged 38–40 years, and five or fewer for women aged 41–42 years.

Kozinszky et al. estimated that 25% of cases involving assisted reproduction result in multiple pregnancies, which may lead to an increased rate of preterm births, intrauterine growth restriction, and low birthweight, all contributing to the significantly higher morbidity and mortality rates for the children born through IVF (Zollner & Dietl, 2013). Furthermore, the risks of birth defects and perinatal morbidity and mortality associated with IVF monozygotic twin pregnancies (MZT) are higher than those associated with IVF singleton or IVF dizygotic pregnancies (Bricker et al., 2016; Santana et al., 2018; Vaughan et al., 2016).

Pregnancy complications

Various physiological changes occur within the maternal various organ systems during pregnancy. The process of adaptation and restructuring of maternal physiology for a singleton pregnancy is even more drastic when more than one oocyte is fertilized. Pregnancy-related complications are

increased in multi-fetal pregnancy and can be divided into three categories: (1) maternal complications; (2) labour and delivery-related outcomes; and (3) perinatal and baby-related outcomes (Table 2).

Maternal complications and outcomes
Gestational hypertension (GH) • Hypertension > (140/90): Mother had a blood-pressure reading of greater than or equal to 140/90 mm Hg on two consecutive occasions during the pregnancy, prior to labour. There are three levels of gestational hypertension: (1) mild: SBP 140-149, DBP 90-99 mm Hg; (2) moderate: SBP 150-159, DBP 100-109 mm Hg; and (3) severe: SBP ≥160, DBP ≥110 mm Hg (Visintin et al., 2010). • Need for antihypertensive drugs Mother receives antihypertensive drugs during her pregnancy (antepartum period only) • Preeclampsia: High blood pressure and signs of damage to another organ system in particular liver (elevated liver enzymes and hepatic failure) and kidney (renal failure leading to proteinuria) (Rana et al., 2019) • Eclampsia: Eclampsia is a severe complication of preeclampsia and can include seizures or coma (Rana et al., 2019) • Eclampsia, hemolysis, elevated liver enzymes, low platelet count [HELLP] syndrome A severe type of preeclampsia leading to life-threatening pregnancy complications (Santana et al., 2018).

Gestational diabetes mellitus (GDM): • Onset or first recognition of glucose intolerance during pregnancy
Antepartum hemorrhage • Mother has bleeding in pregnancy before childbirth
Number of antenatal visits • Total number of primary-care antenatal visits (does not include consultations or specialized clinic visits, e.g., diabetic clinic)
Fatty liver of pregnancy (Santana et al., 2018)
Labour and delivery outcomes
Change in the length of time between Rupture of Membranes (ROM) and delivery
Change in the length of first stage of labour
Change in the length of second stage of labour
Change in the length of third stage of labour
Effect on the method of delivery (C/S vs. spontaneous) • Need for C/S prophylactic antibiotic
Perinatal outcomes
Size of the baby at birth: • Weight of baby at birth Low Birth Weight (LBW): Defined as a birth weight of an infant of <2500 grams, irrespective of gestational age, • Length of baby at birth • Head circumference of the baby at birth

• Small for gestational age (SGA): Neonates whose birth weight is below the 10th percentile for that particular gestational age
Gestational age at birth • Preterm birth: being born before 37 weeks' gestation • Need for antenatal corticosteroids for lung maturation
Intrauterine growth restriction (IUGR) • Mild: fetal abdominal circumference 5-10th centile • Moderate: fetal abdominal circumference 1-4th centile • Severe: fetal abdominal circumference < 1st centile
Congenital anomaly (y/n)
Length of stay of the baby in the hospital
Stillbirth • The complete expulsion or extraction from its mother after at least 20 weeks' pregnancy or after attaining a weight of at least 500 grams, of a product of conception in which, after the expulsion or extraction, there is no breathing, heartbeat, pulsation of the umbilical cord, or unmistakable movement of voluntary muscle.

Table 2 Maternal, labour and delivery, and perinatal outcomes. Maternal, labour and delivery, and perinatal outcomes. For further information on how these outcomes and complications affect singleton versus multiple non-IVF and IVF pregnancies, see Table 3 (Chhabra & Kakani, 2007; Kintiraki et al., 2015; Santana et al., 2018).

Non-IVF twin gestation complications and outcomes

Non-IVF multiple pregnancies increase the risk of preterm birth, low birthweight (LBW), small for gestational age (SGA), and perinatal morbidity and mortality, compared with non-IVF singleton pregnancies (Allen et al., 2006; Barda et al., 2017; Bricker et al., 2016; Ingilizova et al., 2021; Okun et al., 2014; Santana et al., 2018). Additional factors include gestational hypertension, preeclampsia (Sibai et al. (2000), antepartum hemorrhage, postpartum hemorrhage (Bricker et al., 2016; Ingilizova et al., 2021), preterm labour, IUGR, and premature rupture of membranes (PROM) (Norwitz et al., 2005), which are also associated more with non-IVF twin births than with non-IVF singleton births (Santana et al., 2018).

IVF singleton and twin gestation complications and outcomes

As mentioned earlier, IVF is a way for couples to conceive in cases of infertility, and can lead to singleton, twin, or other multiple gestations, which depends on an amalgamation of factors, such as maternal demographics, fertility medications used in IVF, and each phase of the IVF procedure (Bricker et al., 2016; Inhorn et al., 2015; Secretariat, 2016; Zollner & Dietl, 2013). There has been speculation that the outcomes of IVF pregnancies (singleton and twin) is significantly different than spontaneous pregnancies, possibly related to a higher degree of perinatal and obstetric risks and complications (Barda et al., 2017; Kallen et al., 2010; Magnusson et al., 2018; Tabs et al., 2004; Zhu, 2016). We now discuss the associated maternal, labour- and delivery-related, and perinatal complications and outcomes, among IVF singleton or twin pregnancies compared with spontaneously conceived pregnancies.

a. Maternal complications of IVF pregnancies

Contrasted to spontaneous pregnancies, IVF pregnancies are more likely to go through obstetric complications and maternal disorders. Noted commonalities among research findings demonstrate that hypertension and preeclampsia were more common in IVF pregnancies than in spontaneous pregnancies (Barda et al., 2017; Jackson et al., 2004; Okby et al., 2018; Sibai et al., 2000; Thomopoulos et al., 2012; Yang et al., 2019; Zhu et al., 2016). Recent studies have linked IVF to an increased incidence of hypertension during pregnancy, gestational diabetes, postpartum hemorrhage, preterm delivery, and low birth weight (Woo et al., 2017). In a retrospective study,

Okby et al. (2018) found that 13.2% of IVF twin pregnancies had preeclampsia compared with 7.6% of non-IVF twins. There was a strong dual association of preeclampsia with obesity, polycystic ovarian syndrome (PCOS), and cesarean section, and Okby et al. (2018) concluded that preeclampsia presents differently in IVF twins compared with non-IVF twins. Antepartum hemorrhage (APH), and placental abruption (PA) are also increased with the births conceived after IVF/ICSI (Healy et al, 2010; Daniel et al., 2000; Perri et al., 2001; Smithers et al., 2003; Romundstad et al., 2006).

Gestational diabetes mellitus (GDM) is associated with hypertension, operative delivery, cesarean delivery, and fetal macrosomia (Practice Bulletin No., 2013). According to Shrafi et al., the incidence of GDM in women who underwent IVF treatment is 43%, but it is 10% in women with spontaneous pregnancies (Shrafi et al., 2014). In comparison with spontaneous pregnancies, Zhu et al. (2016) have demonstrated an increase in GDM in IVF singleton pregnancies. Other studies have also shown a higher risk of maternal complications including gestational diabetes mellitus (GDM) (Practice Bulletin No., 2013; Norwitz et al., 2005; Shrafi et al., 2014; Yang et al., 2019; Zhu et al., 2016), preterm deliveries, low birthweight (Barda et al., 2017; Zhu et al., 2016), preterm premature rupture of membranes (pPROM), PROM, hypertensive disorders in pregnancy (HDP), and antepartum hemorrhage (APH) (Chowdhury & Hussain, 2011) in IVF pregnancies compared to spontaneous pregnancies. Additionally, Healy et al. (2010) found an increase in the prevalence of APH among IVF/ICSI patients compared with the control group. However, they have suggested that further research is required to understand the confounding factors that may lead to hemorrhage, such as infertility status (Healy et al., 2010).

b. Labour and delivery outcomes of IVF pregnancies

Studies of labour- and delivery-related complications among perinatal and maternal complications attempt to understand the association between such complications and IVF pregnancies. Premature rupture of membrane, uterine bleeding, breech and cephalopelvic disproportion at delivery, along with other placental complications are higher in IVF deliveries compared with non-IVF deliveries (Chowdhury, 2011; Luke et al., 2017; Norwitz et al., 2005; Szymusik et al., 2012; Thomopoulos et al., 2016; Yang et al., 2019). However, it is unclear whether these complications were because of the IVF procedure itself, or were a result of infertility.

Luke et al. (2017) collected IVF data from the Society for Assisted Reproductive Technology Clinic Online Data Reporting System between 2004–2010. They showed that there was an increase in uterine bleeding (a type of placental complication) in the IVF singleton group; 0.6% of deliveries in the non-IVF group were complicated with uterine bleeding, compared with the 2.6% of IVF conceived delivery cases. Moreover, they found that IVF singleton deliveries resulted in a two-fold increase in breech or other malpresentations. Furthermore, 2.5% of the non-IVF deliveries had cephalopelvic disproportion at delivery, compared with 3.2% of IVF deliveries. Another finding was that 5.4% of IVF singleton deliveries had placental complications, which was significantly more than the 1.7% of placental complications in non-IVF singleton deliveries (Luke et al., 2017). Although Luke et al. (2017) observed an almost two-fold increase in cesarean section deliveries among IVF vs non-IVF groups, this topic still requires further study.

c. Perinatal outcomes of IVF

Perinatal complications of IVF include an increased rate of preterm births, low birth weight, and congenital abnormalities (Barda et al., 2017; Helmerhorst et al., 2004; Ingilizova et al., 2021; Jackson et al., 2004; Ombelet et al., 2005; Sibai et al., 2000; Zollner & Dietl, 2013). Helmerhorst et al. (2004) conducted a meta-analysis to assess perinatal outcomes between non-IVF birth singletons and twins versus IVF singletons and twins. They found an increased risk for preterm births, longer stay periods in the NICU, and elevated risk for perinatal mortality in the IVF singletons group compared to the non-IVF singletons group, which is relatively higher compared to IVF and non-IVF twin groups (Helmerhorst et al., 2004). Furthermore, in the late 1980s IVF perinatal registries from Australia, France, Denmark, and Sweden, Cohen et al. (2005) also found lower birth weights and preterm births among singletons, compared with their national perinatal statistics.

Kozinszky et al. estimated that 25% of cases involving assisted reproduction result in multiple pregnancies, which may lead to an increased rate of preterm birth, intrauterine growth restriction, and low birthweight, all contributing to the significantly higher morbidity and mortality rates for children born through IVF (Zollner & Dietl, 2013). Meta-analysis of observational studies by Sullivan-Pyke et al. (2017) has confirmed that infants conceived through IVF have an increased

risk of low birth weight compared with those conceived without IVF intervention. Furthermore, the risks of birth defects and perinatal morbidity and mortality associated with IVF monozygotic twin pregnancies (MZT) are higher than those associated with IVF singleton or IVF dizygotic pregnancies (Bricker et al., 2016; Santana et al., 2018; Vaughan et al., 2016). Potential increases in birth defects following IVF are a concern (Hansen M. et al., 2002) but the issue remains controversial to some extent, as there is a lack of robust evidence comparing natural multiple gestations versus multiple gestations following IVF treatment. Some studies have shown that IVF singletons and twins may have an excess of congenital or birth defects, compared with non-IVF singletons and twins (Hansen et al., 2002; Luke et al., 2017; Liberman et al., 2017; Szymusik et al., 2012). Some studies have reported IVF treatment as a risk factor for neurodevelopment anomalies and long-term metabolic outcomes including hypertension, obesity, and type 2 diabetes (Sullivan-Pyke et al., 2017).

Exposure to ART increases the risk of birth defects and aortic arch defects, including Tetralogy Fallot among singletons (Liberman et al., 2017). Szymusik et al. (2012) reported the potential of congenital malformations in IVF twin births with the use of ART and presented a case with atrial septal defect among IVF twins. Davies et al. (2012) found that twins conceived through IVF have double the risk of having significant birth defects compared with non-IVF twins. Hansen et al. (2002) compared the risk of birth defects among IVF singletons and non-IVF conceived singletons and found significant cardiovascular and urogenital birth defects among IVF singletons. In a study conducted by Anthony et al. (2002), IVF techniques contributed to higher rates of cardiovascular malformations. However, the study had significant limitations as it did not distinguish between singleton and twin pregnancies and rates of CNS malformations and neural-tube defects were not significantly different between IVF versus non-IVF births (Anthony et al., 2002). Luke et al. (2017) concluded that there was a significant increase in birth defects among IVF singletons, but again such findings require more research to confirm the association with IVF techniques and birth defects, due to the small study sizes. A few studies have shown that the association between ART and birth defects is confounded by maternal age (Liberman et al., 2017). The higher rate of congenital anomalies among IVF pregnancies could also be due to the parental risk profile (Zollner & Dietl, 2013).

In previous studies, singleton pregnancies conceived through IVF have been observed to deliver earlier, to have lower birth weights and more SGA live births compared with spontaneously conceived singletons (Jackson et al., 2004; Olivennes et al., 1993; Koudstaal, J et al., 2000). A recent study by Szymusik et al. (2019) collected information on perinatal outcomes among IVF singleton pregnancies, and reported an increase in preterm delivery (Szymusik et al., 2019). Qin et al. (2016) completed a meta-analysis on common adverse perinatal outcomes in ART pregnancies in comparison with spontaneously conceived singletons and showed an increase in preterm birth, low birthweight (LBW), perinatal mortality, and also an increased risk of preterm birth and LBW when comparing the IVF and/or ICSI groups versus spontaneously conceived singleton births. Saccone et al. reported that IVF-conceived twins had a significantly higher risk of spontaneous preterm birth, particularly labour before 34 weeks' gestation, and a higher rate of spontaneous onset of labour in comparison with spontaneously conceived twins (Saccone et al., 2017). It has been hypothesized that these complications are caused by the invasiveness, increased placental abnormalities, and abnormal vascular structures seen in IVF pregnancies (Ochsenkhun et al., 2003). However, these events may not be related to extracorporeal fertilization, they may be associated with the risk factors of the patient's age, and other relevant characteristics. Most patients seeking IVF treatment are older, and often report irregular menstrual cycles, uterine anomalies, and obesity (Saccone et al., 2019).

Those born via IVF pregnancies may also have an increased risk of imprinting disorders, which occur when certain genes are expressed only from the maternal or from the paternal gene copy, but not from both parents (Sullivan-Pyke et al., 2017). Imprinting disorders could be due to a stressful uterine environment or embryo culture (Sullivan-Pyke et al., 2017). In some mouse studies, embryo culture has been shown to induc epigenetic changes on certain genes (Sullivan-Pyke et al., 2017). Transfer of fetuses to an intrauterine environment may also be disruptive enough to induce epigenetic changes that result in imprinting (Sullivan-Pyke et al., 2017). Thus, IVF processes may induce epigenetic changes in the embryo that alter the phenotype, resulting in altered health and adverse outcomes (Sullivan-Pyke et al., 2017). Researchers have reported epigenetic changes caused by hormonal and environmental manipulation, handling of embryo transfers, and genetic predisposition, all risk factors for obstetric and perinatal complications. Hormonal stimulation and embryo culture may also play a role in IVF-related adverse outcomes

(De). A recent study by Neubourg et al. (2006) and Sullivan-Pyke et al. (2017) supports this claim. Single embryo transfer will not, however, prevent adverse outcomes since other factors, such as the diagnosis of infertility and the method of treatment itself, could lead to long-term and perinatal health problems. Future studies should consider adverse factors including patient age and pre-existing conditions such as diabetes, hypertension, insulin resistance, thyroid dysfunction, etc. (Kalra et al., 2011). IVF is, therefore, should not be viewed as a single risk factor; several risk factors contribute to the development of long-term outcomes in children (Roseboom et al., 2000; Donjacour et al., 2014).

Current research has indicated that conception via ART including IVF (Allen et al., 2006; Jackson et al., 2004) may increase the risk of stillbirths or perinatal deaths (Henningsen et al., 2014). Wisborg found that a woman's risk of having a stillbirth post IVF/ICSI was 16.2 per 1000 total births, compared with only 2.3 in the non-IVF ART group (Wisborg et al, 2010). Stillbirths and perinatal deaths were more likely after ART compared with spontaneous conception for singleton births (Henningsen et al., 2014) or IVF (Allen et al., 2006; Jackson et al., 2004).

Some adverse perinatal outcomes, including low birthweight and preterm delivery, are more common in patients who have had infertility treatments, such as IVF (Palomba et al., 2016). It has been suggested that every different step or procedure performed may have an impact on the individual and increase the risks of adverse perinatal outcomes (Palomba et al., 2016). Pre-treatment education is recommended for those preparing to undergo infertility treatments so that they are prepared for the potential adverse outcomes (Palomba et al., 2016). Counseling may help to analyze the individual's lifestyle habits and make recommendations to lower risks, such as ceasing smoking, reducing BMI in obese patients, and decreasing alcohol intake (Palomba et al., 2016). Last, those expecting infertility treatments can decrease the risk of adverse perinatal outcomes by optimizing infertility treatments (Palomba et al., 2016). Methods such as providing a milder stimulation may decrease the risk of pregnancy complications in individuals undergoing fertility treatments (Palomba et al., 2016).

As a result of the unclear relationship between complications resulting from IVF techniques or from infertility, we have conducted a study to understand better whether IVF twin pregnancies

have an increased risk of maternal complications, abnormal labour and delivery characteristics, and perinatal outcomes, compared with non-IVF twin pregnancies.

Maternal complications and outcomes					
	IVF Singletons (vs. non-IVF singletons)	Non-IVF Twins (vs. non-IVF singletons)	IVF Twins (vs. non-IVF singletons)	IVF singletons (vs. non-IVF singletons)	IVF Twins (vs. non-IVF Twins)
Preeclampsia (Barda et al., 2017; Sibai et al., 2000; Jackson et al., 2004)	↑	↑	↑		↑
Gestational hypertension (Adele et al., 2018; Barda et al., 2017; Maman et al., 1998; Nassar et al., 2003; Thomopoulos et al., 2012; Sibai et al., 2000; Tabs et al., 2004; Yang et al., 2019) ART results: (Zhu et al., 2016)	↑	↑	↑	↑	↔
Gestational diabetes mellitus (Barda et al., 2017; Nassar et al.,	↔	↔	↔		

2003; Ochsenkuhn et al., 2003)					
Gestational diabetes mellitus (Adele et al., 2018)		↔	↑		
Gestational diabetes mellitus (Yang et al., 2019)	↑				
Antepartum hemorrhage (Healy et al., 2010)		↑			
Proteinuria		↑			
Need for antihypertensive drugs		↑			
Number of antenatal visits		↑			
Hyperestrogenemia (Tabs et al., 2004)	↑	↔	↑		
Maternal hospital stay (Nassar et al., 2003)		↔	↑		
Labour and delivery outcomes					
Cesarean delivery** (Allen et al., 2006;	↑	↔	↑		

Barda et al., 2017; Jackson et al., 2004; Kozinsky et al., 2003; Nassar et al., 2003; Tan et al., 1992)					
Induced labour (Jackson et al., 2004)	↑	↔	↑		
Preterm labour (Ochsenkhun et al., 2003)	↔	↔	↔		
Perinatal outcomes					
Placental abruption*** (Allen et al., 2006 ART results: (Zhu et al., 2016)	↑	↔	↑	↑	↔
Placenta previa (Allen et al., 2006; Lei et al., 2019; Jackson et al., 2004; Tan et al., 1992; (Yang et al., 2019) ART results: (Zhu et al., 2016)	↑	↔	↑	↑	↔
Polyhydramnios (Lei et al., 2019; Zhu et al., 2016)				↑	↔

Oligohydramnios (Lei et al., 2019; Zhu et al., 2016)				↔	↔
Intrauterine growth restriction (IUGR) (Norwitz et al., 2005)		↑			
Small-for-gestational age (SGA) (Bricker et al., 2016; Sibai et al., 2000; Tan et al., 1992)	↑	↑			
Low birthweight (LBW) (Ombelet et al., 2005; Tan et al., 1992) ART results: (Zhu et al., 2016)	↑			↑	↔
Preterm birth (Helmerhorst et al., 2004; Jackson et al., 2004; McGovern et al., 2004; Nassar et al., 2003; Tan et al., 1992) ART results: (Zhu et al., 2016)	↑	↔	↑	↑	↑
Stillbirth (Allen et al., 2006; Jackson et al.,	↑	↔	↑		

2004)					
NICU Admission (Allen et al., 2006; Nassar et al., 2003)	↑	↔	↑		

Table 3 Comparison of maternal, labour and delivery, and perinatal outcomes reported by various studies.

↑ Increased associated risk

↔ Insignificant associated risk

**Koudstaal et al., 2000, and Ochsenkuhn et al., 2003, noted no significant difference in cesarean delivery rates between IVF and non-IVF singleton births, and this may be attributed to fear or anxiety surrounding IVF birth as opposed to the need for it.

***Lei et al., 2019, and Nassar et al., 2003, noted no significant difference in placental abruption between IVF and non-IVF twin births, and this may have been due to a small study size.

Objective

Complications of pregnancy have been widely researched but there is still debate about whether there is a significant difference among complications of IVF twin pregnancies compared with spontaneously conceived twin births.

Observational studies from IVF registry data have demonstrated higher rates of maternal and perinatal complications such as preterm birth in IVF singleton pregnancies compared to non-IVF singletons. Also, there are few studies (e.g., Chen et al. 2019) which have shown IVF twins' complications might not be statistically significantly different from spontaneous twins. Therefore, our objective is to compare the outcomes (maternal and perinatal) of twin pregnancies conceived by IVF, with twin natural pregnancies in the general population of BC, Canada.

Hypothesis

If IVF twins are overall similar to non-IVF twins in pregnancy outcomes, then this suggests that multiple pregnancy is the factor in previous studies showing more complications in IVF pregnancies. However, if IVF twins have higher rates of selected pregnancy outcomes compared to non-IVF twins, then these may be related to the IVF procedure itself or to potential confounders in the IVF population (e.g. history of infertility).

Materials and Methods

An IVF twin pregnancy group was obtained from the former IVF Program of the University of British Columbia Centre for Reproductive Health (UBCCRH), between April 1, 1998, and March 31, 2010 (Table 4). There were a total of 632 mothers (830 babies) in this IVF cohort. After exclusion of those with singletons (453 mothers and 453 babies) and triplet and higher-order multiple pregnancies (18 mothers and 55 babies), our study cohort consisted of 161 mothers with IVF twin pregnancies (322 babies).

Outcome data for the IVF twin pregnancy group were obtained by linking to the British Columbia Perinatal Data Registry (BCPDR). The BCPDR contains data abstracted from obstetrical and neonatal medical records on nearly 100% of births in the province of British Columbia from over 60 hospitals as well as births occurring at home attended by BC registered midwives. Capturing approximately 45,000 births per year, the BCPDR also collects data on maternal postpartum readmissions up to 42 days post-delivery and baby transfers and readmissions up to 28 days after birth. ("Perinatal Data Registry", 2022)

For a comparison non-IVF twin pregnancy group, we identified 5824 mothers with twin pregnancies from the BCPDR, after removing the IVF twin pregnancy group, between April 1, 1998, and March 31, 2010. After excluding patients where there was no baby data linked to mothers' files, we were left with 5525 mothers (11,050 babies). This sample served as a non-IVF twin pregnancy comparison group.

	IVF pregnancies
Singleton Pregnancies	453 mothers (453 babies)
Twin Pregnancies	161 mothers (322 babies)
Multiple (≥3) Pregnancies	18 mothers (55 babies)
Total	632 mothers (830 babies)

Table 4 The distribution of mothers and their babies based on the database of University of British Columbia Centre for Reproductive Health.

For the IVF twin pregnancy group and the non-IVF twin pregnancy comparison group, identification information included detailed pregnancy-related, antenatal, birth, and neonatal outcomes on all enrolled mothers and their babies. This data was then organized into two separate files:

- File 1: Pregnancy and obstetrical outcomes (mothers' database file), which included extensive data on the mothers' demographics, delivery episode of care, past obstetric history, current pregnancy updates, labour and delivery data, and diagnostic information provided by physicians.

- File 2: Perinatal and birth outcomes (babies' database file), which included the newborn demographics, episodes of care, and any congenital anomalies.

These two files were then merged, allowing both the mothers and their babies to be linked and analyzed accordingly. This merging was achieved by using the mother's study ID that was shared between the two files as a link to create a combined file containing the data of the mother and their babies who were delivered from the current pregnancy.

Outcomes

Outcomes were divided into three types: maternal complications, labour and delivery characteristics, and perinatal outcomes. A complete list of variables is listed in Tables 5 to 7.

Maternal complications
Hypertension > (140/90)
Pregnancy-induced hypertension
Antihypertensive drugs in pregnancy
Proteinuria
Gestational diabetes
Antepartum hemorrhage

Table 5 Maternal complications.

Labour and delivery characteristics
Antihypertensive drugs during labour
Steroids for lung maturation
Tocolytics
Length of time between ruptured membranes and delivery (hours)
Length of the first stage of labour (hours)
Length of the second stage (minutes)
Length of the third stage (minutes)
Method of delivery
Cesarean Section prophylactic antibiotics

Table 6 Labour and delivery characteristics.

Perinatal outcomes
Weight of the baby at birth (gram)
Length of the baby at birth (in cm)
Head circumference of the baby at birth (in cm)
IUGR
Stillbirth
Gestational age at birth
Congenital anomaly
Length of stay (hours)

Table 7 Perinatal outcomes. Congenital anomalies described in Appendix 1.

Statistical analysis

Normality of data distribution was assessed by Kolmogorov–Smirnov test (Appendix 2, Table 19) and geometric quantile-quantile plot (Q-Q plot) (Appendix 3). The student's t-test was used for continuous variables with normal distribution and the Mann-Whitney U test was performed for the continuous variables that were not normally distributed. Categorical variables were presented as frequencies and accompanying percentages. They were analyzed using the Chi-square test or Fisher's exact test (for categories with N<5). Each newborn was counted separately for the descriptive analysis and statistics of the neonates, and no average (i.e., mean) of twins' information for each pregnancy was used for the analysis.

To assess the effects of IVF, pregnancy outcomes and labour and delivery events were assessed using multivariate logistic and linear regression models for binary and continuous variables, respectively after adjustment for mothers' age, BMI, and parity. To assess babies' outcomes and address the correlation between fetuses in a paired set, we used generalized estimating equations (GEE) for logistic regression to examine the risk of IUGR, stillbirth, and congenital anomalies in babies after adjustment for mothers' age, BMI, and parity. The GEEs of ordinary linear regression models were used to estimate adjusted mean difference and 95% confidence interval (CI) for continuous babies' outcome variables after the same adjustment.

Gestational age and birth weight were further divided into different subcategories for both groups and compared after adjustment for mothers' age, BMI, and parity. All analyses were conducted using SPSS version 26.0 for Windows (SPSS Inc., Chicago, IL, USA), and a p-value of less than 0.05 was considered statistically significant.

Results

Baseline pre-pregnancy maternal information, i.e., demographic variables, are summarized in Table 8. Mothers in the IVF group had higher age (36.2 ± 3.9 years vs. 31.1 ± 5.47 years, p<0.001), lower BMI ((23.24 ± 4.02 kg/m^2 vs. 24.2 ± 5.07 kg/m^2), p-value=0.045), and a higher rate of nulliparity (75.2% vs. 45.9%, p-value<0.001).

Variables	**IVF Pregnancy**	**N**	**Non-IVF Pregnancy**	**N**	**P-value**
Mother's age at delivery (year) Mean (SD) Median (range)	 36.2 (3.9) 36.2 (26.7-43.7)	161	 31.1 (5.47) 31.3 (14.5-53.7)	5525	<0.001 (7)
Mother's weight before pregnancy (kg) Mean (SD) Median (range)	 62.5 (10.53) 60.9 (45.0-93.4)	127	 66.4 (14.84) 63.6 (35.9-161.0)	4219	0.004 (7)
Mother's height (cm) Mean (SD) Median (range)	 164.2 (6.32) 164.5 (150-183)	136	 165.3 (7.07) 168.0 (132-187)	4579	0.055 (7)
BMI (Body mass index) Mean (SD) Median (range)	 23.24 (4.02) 22.39 (17.95- 39.06)	116	 24.2 (5.07) 23.89 (13.36-57.04)	3845	0.045 (7)
School years Mean (SD) Median (range)	 14.8 (1.98) 16.0 (12-19)	17	 14.4 (2.74) 14.0 (1-22)	608	0.568 (7)
Nulliparous (1)	121 (75.2%)	161	2530 (45.9%)	5525	<0.001 (8)
Previous term pregnancy (>1) (2)	35 (21.7%)	161	2873 (51.9%)	5525	<0.001 (8)
Previous preterm pregnancy (>1) (3)	7(4.3%)	161	248 (4.5%)	5525	0.932 (8)
Previous spontaneous abortions (>1) (4)	41 (27.0%)	160	1326 (24.2%)	5493	0.666 (8)
Prior neonatal death (>1) (5)	1 (0.7%)	133	33 (0.6%)	5525	0.624 (9)
Prior child with major congenital anomalies (>1) (6)	2 (1.2%)	161	63 (1.1%)	5525	0.707 (9)

Table 8 Pre-pregnancy maternal information in IVF and non-IVF twin pregnancy groups.

(1) Parity: Number of previous pregnancies delivered at equal to or greater than 20 completed weeks (140 days) gestation or 500 grams birth weight regardless of the outcome

(2) Previous term pregnancy: Total number of previous pregnancies delivered at greater than or equal to 37 completed weeks gestation, if the mother is not nulliparous.

(3) Previous premature pregnancy: Total number of previous pregnancies delivered before 37 completed weeks of gestation.

(4) Previous spontaneous abortions: Total number of previous pregnancies spontaneously ending prior to 20 completed weeks gestation.

(5) Prior neonatal death: Mother had at least one prior live-born infant, who dies within the first 28 days of life.

(6) Prior child with major congenital anomalies as described in appendix 1: Mother had at least one previous pregnancy in which the baby displayed a major congenital anomaly; The list of congenital anomalies considered for inclusion is provided in appendix 1.

(7) P-Value was calculated using Mann-Whitney U test

(8) P-Value was calculated using Chi-square test

(9) P-Value was calculated using Fisher's exact test

Maternal complications

Maternal complications compared between twins in the IVF and non-IVF groups are summarized in Table 9. Rates of these complications adjusted for maternal age, BMI, and parity, as summarized in Table 10.

After adjustment for age, BMI, and parity, the IVF twin pregnancy group had higher rates of gestational hypertension (aOR = 1.57, 95% CI (1.010, 2.457), p = 0.045), antepartum hemorrhage (APH) (adjusted Odds Ratio (aOR) = 2.32, 95% CI (1.16, 4.68), p = 0.018), and gestational diabetes (aOR = 1.77, 95% CI (1.09, 2.87), p =0.020).

Variables	IVF pregnancy	N	Non-IVF pregnancy	N	P-value
Hypertension > (140/90)	15 (9.3%)	161	320 (5.8%)	5525	0.060 (1)
Pregnancy-induced hypertension	37 (22.9%)	161	733 (13.2%)	5525	0.015 (1)
Antihypertensive drugs in pregnancy	6 (3.7%)	161	155 (2.8%)	5525	0.485 (1)
Proteinuria (Mother had proteinuria > 1g/liter)	10 (6.2%)	161	221 (4.0%)	5525	0.160 (1)
Antepartum hemorrhage	10 (6.2%)	161	173 (3.1%)	5525	0.029 (1)
Gestational diabetes (either insulin or non-insulin-dependent)	35 (21.7%)	161	499 (9.0%)	5525	<0.001 (1)
Number of antenatal visits Mean (SD) and mode Median (range)	 9.3 (3.41) and 9.0 9.0 (2-21)	133	 9.1 (3.72) and 9.0 9.0 (0-30)	4876	0.380 (2)
Gestational age of first ultrasound (weeks) Mean (SD) and mode Median (range)	 8.4 (3.90) and 6.0 7.0 (5-19)	122	 12.5 (4.57) and 18 12.0 (4-20)	3876	<0.001 (2)

Table 9 Pregnancy information in IVF twin pregnancy group compared with non-IVF comparison cohort.

(1) P-Value was calculated using chi-square test

(2) P-Value was calculated using Mann-Whitney test

Outcomes	aOR *	95% CI *	P-value *
Hypertension > (140/90) (y/n)	1.10	(0.56,2.17)	0.78
Gestational hypertension (y/n)	1.57	(1.010,2.457)	0.045
Antihypertensive drugs in pregnancy (y/n)	1.01	(0.39,2.58)	0.99
Proteinuria (Mother had proteinuria > 1g/liter) (y/n)	1.03	(0.46,2.30)	0.96
Antepartum hemorrhage (y/n)	2.32	(1.16,4.68)	0.018
Gestational diabetes (either insulin or non-insulin-dependent) (y/n)	1.77	(1.09,2.87)	0.02

Table 10 Results of logistic regression analyses comparing rates of maternal complications among IVF and non-IVF twins.

* Adjusted for mother's age, BMI, and parity

Labour and delivery events

Labour and delivery events are summarized in Tables 11, with the comparison adjusted for mother's age, BMI, and parity shown in Table 12 and Table 13.

Among labour and delivery characteristics, and after adjustment, there were higher risk of C-section as the method of delivery (aOR = 1.75, CI (1.11, 2.78), p = 0.016) and steroid use for lung maturation (aOR = 1.88, CI (1.18, 3.00), p = 0.008) in IVF mothers compared with non-IVF comparison group.

Variables	IVF Pregnancy	N	Non-IVF Pregnancy	N	P-value
Antihypertensive drugs during labour	15 (9.3%)	161	302 (5.5%)	5525	0.035 (3)
Steroids for lung maturation	33 (20.5%)	161	657 (12.0%)	5525	0.001 (3)
Tocolytics	10 (6.2%)	161	289 (5.3%)	5525	0.583 (3)
Length of time between ruptured membranes and delivery (hours) Mean (SD) Median (range)	 8.13 (12.57) 4.7 (0-97.7)	93	 8.70 (15.70) 4.72 (0-166.3)	3422	0.659 (2)
Length of the first stage of delivery (hours) Mean (SD) Median (range)	 7.7 (4.87) 7.0 (2.11-20.5)	43	 6.1 (5.09) 4.7 (0.00-65.5)	2221	0.006 (2)
Length of the second stage (minutes) Mean (SD) Median (range)	 100 (118.42) 42 (1-490)	43	 62 (73.81) 33 (0-613)	2292	0.017 (2)
Length of the third stage (minutes) Mean (SD) Median (range)	 11 (23.83) 4 (0-150)	141	 18 (84.34) 6 (0-4324)	4398	<0.001 (2)
Method of delivery	C-Section (129, 80.1%)	161	C-Section (3409, 61.7%)	5525	<0.001 (3)
Caesarean section prophylactic antibiotics	Yes (11, 6.8%)	161	Yes (198, 3.6%)	5525	0.110 (3)

Table 11 Labour and delivery information in IVF and non-IVF twin pregnancy groups.

(1) Gestational age from the maternal record: baby's gestational age (in completed weeks) documented by the clinician before delivery, determined by maternal last menstrual period and/or ultrasound.

(2) P-Value was calculated using Mann-Whitney U test

(3) P-Value was calculated using chi-square test

Variables (Twins, Mother)	Mean difference *	95% CI *	P *
Length of time between ROM and delivery – in hours	-2.92	(-10.74,4.89)	0.464
Length of first stage - in hours	0.83	(-0.782,2.442)	0.313
Length of second stage - in minutes	14.417	(-7.68,36.51)	0.201
Length of third stage - in minutes	-5.073	(-23.48,13.33)	0.589

Table 12 Results of logistic regression analyses comparing rates of labour and delivery events among IVF and non-IVF twins.

* Adjusted for mother's age, BMI, and parity

Variables (Twins, Mother)	aOR *	95% CI *	P *
Method of delivery (C/S) (y/n)	1.75	(1.11,2.78)	0.016
Antihypertensive drugs (y/n)	1.24	(0.66,2.34)	0.50
Steroids for lung maturation (y/n)	1.88	(1.18,3.00)	0.008
Tocolytics (y/n)	1.29	(0.58,2.86)	0.54
C/S prophylactic antibiotics (y/n)	1.45	(0.66,3.17)	0.36

Table 13 Results of logistic regression analyses comparing rates of labour and delivery events among IVF and non-IVF twins (continued).

* Adjusted for mother's age, BMI, and parity

Perinatal outcomes

Perinatal outcomes are summarized in Table 14, Table 15, and Table 16 (after adjusting for mother's age, BMI, and parity).

For perinatal outcomes, there were higher risk of IUGR (aOR = 1.41, CI (1.03, 1.96), p = 0.034) and congenital anomaly (aOR = 1.55, CI (1.02, 2.35), p = 0.042) in IVF babies. Furthermore, the IVF twin pregnancy group had a lower weight of baby at birth (in grams) (mean difference = -200.4, 95% CI (-118.6, -36.8), p =0.004), length of baby at birth (in centimeters) (mean difference = -0.63, 95% CI (-1.23, -0.02), p = 0.04), head circumference of baby at birth (in centimeters) (mean difference = -0.35, 95% CI (-0.66, -0.04), p = 0.027), and gestational age at birth (in weeks) (mean difference = -1.014, 95% CI (-1.53, -0.49), p <0.001).

Variables	IVF Twins	N	Non-IVF Twins	N	P-value
Weight of baby at birth (gram)		322		11045	<0.001 (1)
Mean (SD)	2279 (629.328)		2414 (669.56)		
Median (range)	2355 (472-3705)		2520 (280-4350)		
Length of the baby at birth (in cm)		306		10441	0.001 (1)
Mean (SD)	46.23 (4.428)		46.92 (4.40)		
Median (range)	47 (30-57)		48 (20-62)		
Head circumference of the baby at birth (cm)		306		10456	0.001 (1)
Mean (SD)	32.31 (2.518)		32.64 ± 2.73		
Median (range)	33 (21-37)		33 (13-50)		
IUGR	75 (23.2%)	322	1358 (12.3%)	11050	<0.001 (2)
Stillbirth	3 (0.9%)	322	236 (2.2%)	11050	0.167 (3)
Gestational age at birth		236		8674	<0.001 (1)
Mean (SD)	34.52 (3.45)		35.42 (3.24)		
Median (range)	36 (22-40)		36 (17-41)		
Congenital anomaly	34 (10.6%)	322	677 (6.1%)	11050	0.001 (2)
Length of stay (hours)		319		10812	<0.001 (2)
Mean (SD)	267.7 (419.62)		216.1 (344.40)		
Median (range)	124.1 (0.1-922.3)		110.6 (0-922.3)		

Table 14 Perinatal outcomes in IVF and non-IVF twin pregnancy groups.

(1) P-Value was calculated using Mann-Whitney U test

(2) P-Value was calculated using chi-square test

(3) P-Value was calculated using Fisher's exact test

Outcomes	AOR*	95% CI *	P *
IUGR (y/n)	1.41	(1.03,1.96)	0.034
Stillbirth (y/n)	0.32	(0.04,2.28)	0.26
Congenital anomaly (y/n)	1.55	(1.02,2.35)	0.042

Table 15 Results of logistic regression analyses comparing rates of perinatal outcomes among IVF and non-IVF twins.

* Adjusted for mother's age, BMI, and parity

Outcomes	Mean difference *	95% CI *	P *
Weight of baby at birth (grams)	-200.4	(-118.6, -36.8)	0.004
Length of the baby at birth (centimeters)	-0.63	(-1.23, -0.02)	0.04
Head circumference of the baby at birth (centimeters)	-0.35	(-0.66, -0.04)	0.027
Length of stay (hours)	39.9	(-12.66, 92.47)	0.14
Gestational age at birth (weeks)	-1.014	(-1.53, -0.49)	<0.001

Table 16 Results of multiple linear regression analyses comparing rates of perinatal outcomes among IVF and non-IVF twins.

* Adjusted for mother's age, BMI, and parity

Distribution of gestational age of delivery in the IVF twin pregnancy group and non-IVF twin pregnancy comparison group based on term and preterm categories and preterm subcategories, including moderate, very, and extremely preterm, is summarized in Table 17. Its graph is shown in Figure 4.

Among the preterm subcategories, there was a statistically significant increase in the risk of moderate preterm birth in the IVF vs non-IVF twins (62% vs. 39%, p<0.001). Despite the statistically significant increase in the overall rate of preterm birth in the IVF group compared with the non-IVF group (72% vs. 53%, p<0.001), there was no difference in rates of very preterm or extremely preterm birth among IVF vs no-IVF twins.

Maternal records	IVF twins	N	Non-IVF twins	N	P value
Term ≥37 weeks	88 (28%)	322	4946 (47%)	11050	<0.001
Preterm <37 weeks	234 (72%)	322	5659 (53%)	11050	<0.001
Moderate >32 - < 37 weeks	202 (62%)	322	4171 (39%)	11050	<0.001
Very >28 - ≤ 32 weeks	20 (6%)	322	887 (8%)	11050	0.20
Extremely ≤ 28 weeks	12 (3%)	322	601 (6%)	11050	0.17

Table 17 Distribution of gestational age at delivery in IVF and non-IVF twins pregnancy groups.

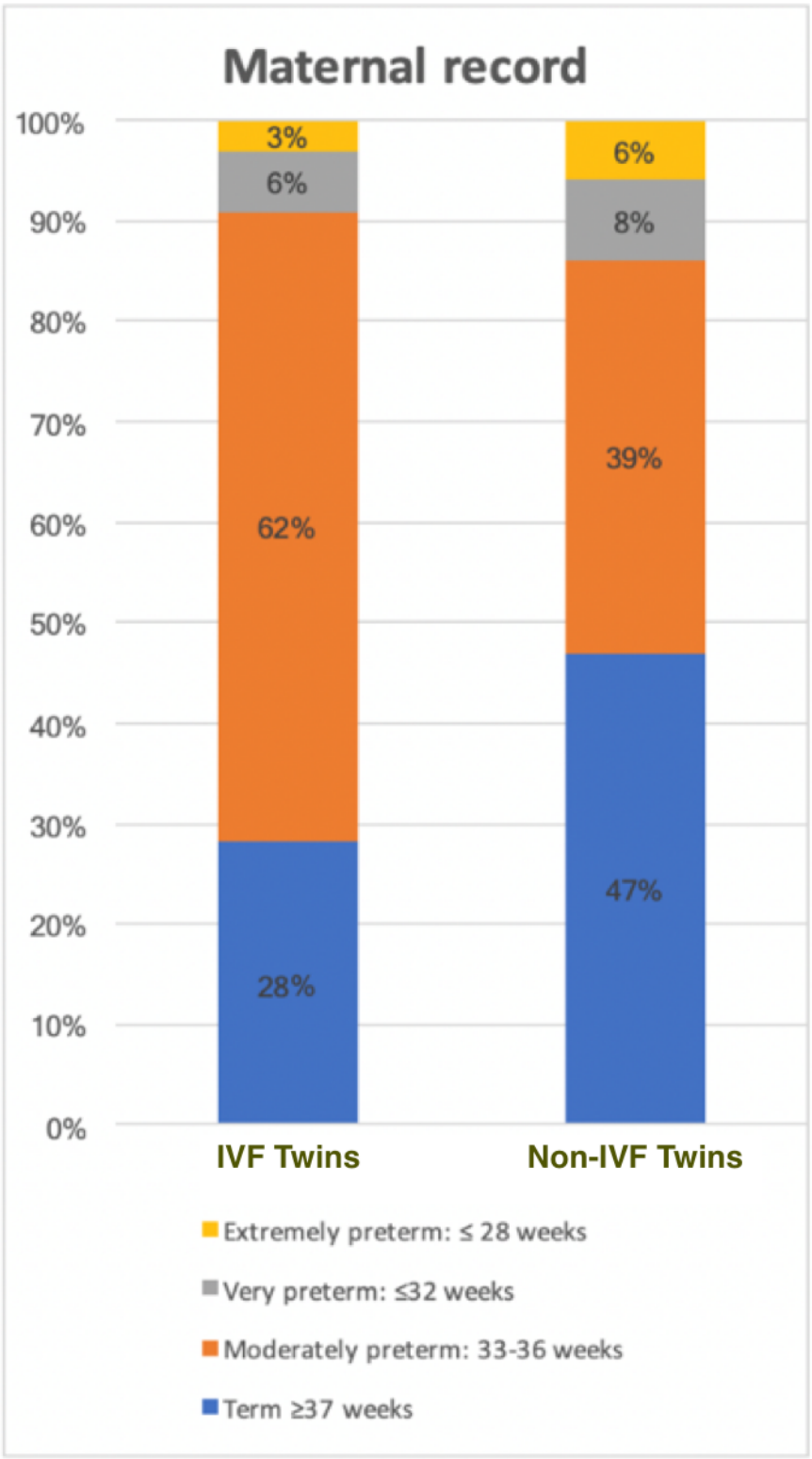

Figure 4 Distribution of gestational age at delivery in IVF and non-IVF twins.

Table 18 and Figure 5 show the distribution of birth weight at delivery in IVF and non-IVF twins based on low birthweight (LBW) categories, including moderately, very, and extremely LBW, and macrosomia. Overall, LBW babies and its moderately LBW subcategory showed a statistically significant increase in the IVF and non-IVF twins (59% vs. 48%, p value = 0.0002 and 47% vs. 39%, p value = 0.003 respectively). Nonetheless, no significant difference was observed in the very (11% vs. 9%, p=0.4714) and extremely (4% vs. 5%, p=0.5968) LBW subcategories. Furthermore, there was no difference in macrosomia (p=0.57).

Birth weight	**IVF Twins**	**N**	**Non-IVF Twins**	**N**	**P value**
Low birth weight (LBW) <2500 g	190 (59%)	322	5344 (48%)	11050	0.0002
Moderately LBW 1500-2499 g	152 (47%)	322	4294 (39%)	11050	0.0030
Very LBW <1500 g	38 (11%)	322	1051 (9%)	11050	0.47
Extremely LBW <1000 g	14 (4%)	322	571 (5%)	11050	0.60
Macrosomia >4000 g	0 (0%)	322	11 (0%)	11050	0.57

Table 18 Distribution of birth weight at delivery in IVF and non-IVF twins.

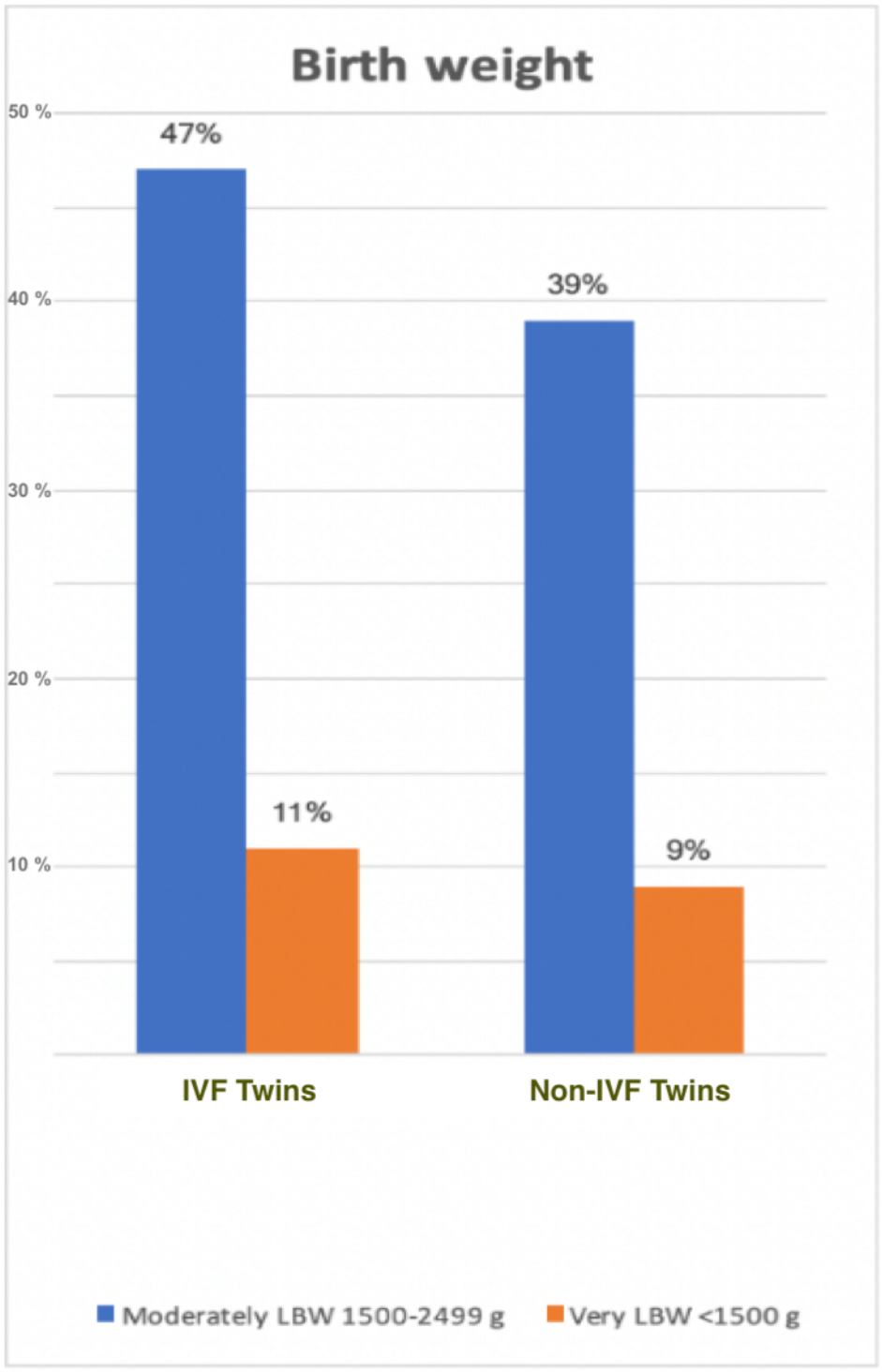

Figure 5 Distribution of birth weight at delivery in IVF and non-IVF twins.

Discussion

Infertility is a continuing issue among couples trying to conceive. This review of the literature on IVF and non-IVF twin pregnancies suggests a correlation between IVF intervention use and an increase in maternal, labour and delivery and perinatal complications. My question was to consider whether twin pregnancies conceived by IVF were associated with a greater risk of maternal complications, abnormal labour and delivery characteristics, and perinatal outcomes when compared with non-IVF twin pregnancies. The study used data from British Columbia and compared IVF and non-IVF twin pregnancies after adjustment for maternal age, BMI, and parity. Outcomes of interest were divided into maternal complications, abnormal labour and delivery characteristics, and perinatal outcomes.

Study results showed that while pregnancy outcomes were similar between IVF and non-IVF groups for a number of variables, there were differences for selected variables. The rates of some maternal complications were higher in IVF mothers compared with non-IVF mothers for gestational hypertension, antepartum hemorrhage, and gestational diabetes. In terms of labour and delivery complications, there was a higher risk of C-section as the method of delivery and steroid use for fetal lung maturation among IVF twin pregnancies. Moreover, there were higher risk of IUGR and congenital anomalies for perinatal outcomes, as well as lower weight and length of babies at birth and lower head circumference of the baby at birth. Furthermore, IVF babies had a lower gestational age at birth compared to non-IVF group. Most of these data could be predicted as women who cannot get pregnant spontaneously are likely to have their first pregnancy and birth at an older age, so pregnancy-related conditions are expected to be higher, although we adjusted for age in the regression analyses/. It should also be emphasized that while there were statistical differences between the IVF and non-IVF groups, the clinical magnitude of the differences was typically small.

Reasons for differences between IVF twins and non-IVF twins

There are a number of potential explanations for the differences between the IVF twin pregnancy group and the non-IVF twin pregnancy comparison group. These potential differences are shown

in Figure 6. Below, I separate the potential differences into maternal characteristics and IVF procedure risks.

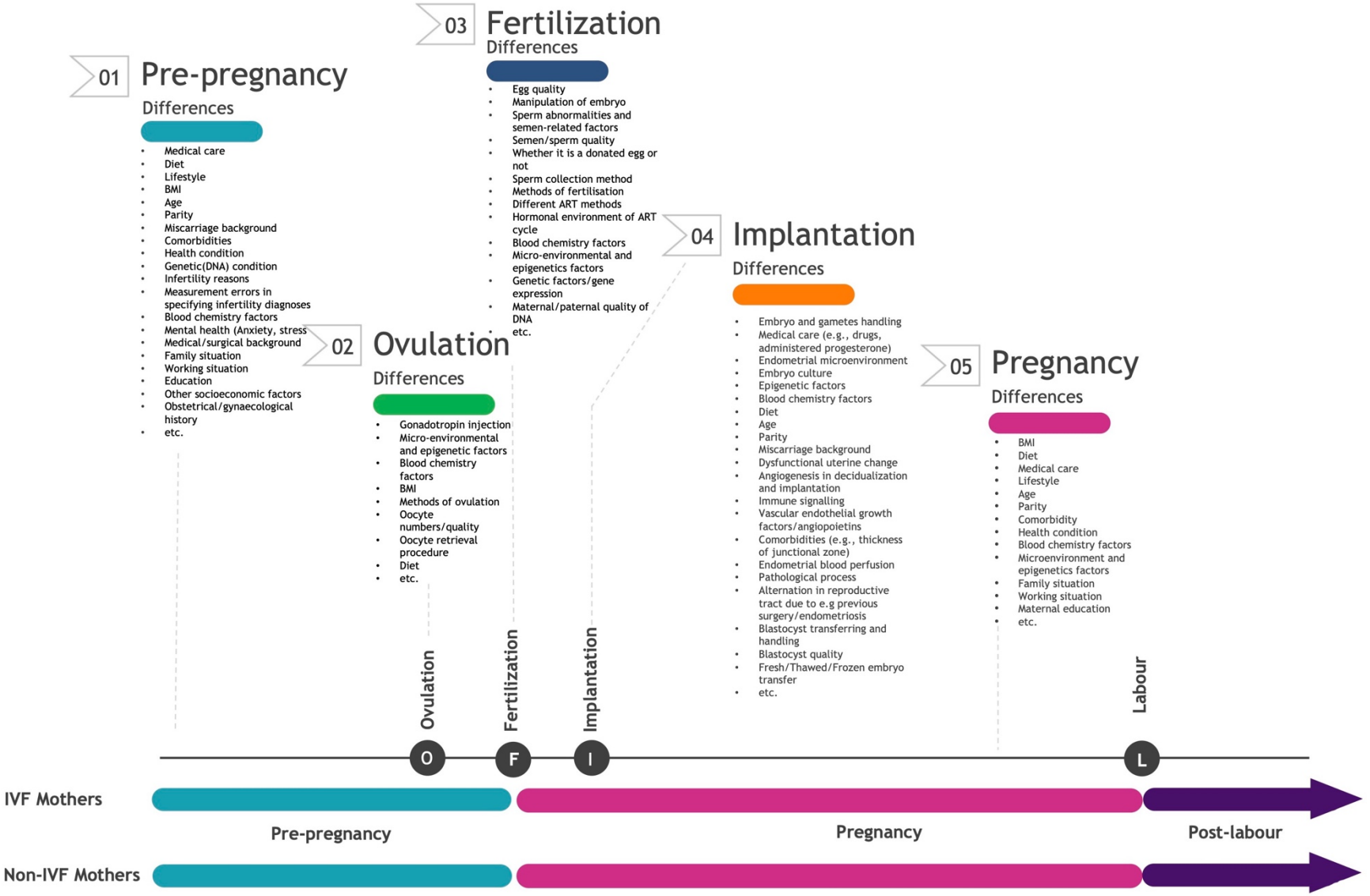
01
Pre-pregnancy
Differences
Medical care
Diet
Lifestyle
BMI
Age
Parity
Miscarriage background
Comorbidities
Health condition
Genetic(DNA) condition
Infertility reasons
Measurement errors in specifying infertility diagnoses
Blood chemistry factors
Mental health (Anxiety, stress
Medical/surgical background
Family situation
Working situation
Education
Other socioeconomic factors
Obstetrical/gynaecological history
etc.
02
Ovulation
Differences
Gonadotropin injection
Micro-environmental and epigenetic factors
Blood chemistry factors
BMI
Methods of ovulation
Oocyte numbers/quality
Oocyte retrieval procedure
Diet
etc.
03
Fertilization
Differences
Egg quality
Manipulation of embryo
Sperm abnormalities and semen-related factors
Semen/sperm quality
Whether it is a donated egg or not
Sperm collection method
Methods of fertilisation
Different ART methods
Hormonal environment of ART cycle
Blood chemistry factors
Micro-environmental and epigenetics factors
Genetic factors/gene expression
Maternal/paternal quality of DNA
etc.
04
Implantation
Differences
Embryo and gametes handling
Medical care (e.g., drugs, administered progesterone)
Endometrial microenvironment
Embryo culture
Epigenetic factors
Blood chemistry factors
Diet
Age
Parity
Miscarriage background
Dysfunctional uterine change
Angiogenesis in decidualization and implantation
Immune signalling
Vascular endothelial growth factors/angiopoietins
Comorbidities (e.g., thickness of junctional zone)
Endometrial blood perfusion
Pathological process
Alternation in reproductive tract due to e.g previous surgery/endometriosis
Blastocyst transferring and handling
Blastocyst quality
Fresh/Thawed/Frozen embryo transfer
etc.
05
Pregnancy
Differences
BMI
Diet
Medical care
Lifestyle
Age
Parity
Comorbidity
Health condition
Blood chemistry factors
Microenvironment and epigenetics factors
Family situation
Working situation
Maternal education
etc.
Ovulation
Fertilization
Implantation
Labour
O
F
I
L
IVF Mothers
Non-IVF Mothers
Pre-pregnancy
Pregnancy
Post-labour

Figure 6 Differences between IVF and non-IVF pregnancies. IVF and non-IVF pregnancies differ in many ways. These differences may involve events and stages, including ovulation, pre-pregnancy, fertilization, implantation, and pregnancy. For example, the infertility indications that lead to IVF, maternal characteristics and comorbidities of women who require IVF, IVF procedures themselves, e.g., manipulation of the embryo, and the differential care given to IVF patients compared with non-IVF mothers may play crucial roles in the outcomes of pregnancy and babies.

Maternal characteristics of women who require IVF

Pre-pregnancy risk factors may affect pregnancy regardless of whether the patient uses ART (Thomopoulos et al., 2013). According to the survey analysis, a research study that included Luke et al. (2017) indicated that women in the subfertile and IVF groups were older than their fertile counterparts and had a 50% higher risk of pregnancy complications. However, there may not be many differences in the maternal characteristics of the people who undergo IVF in the province of BC, Canada, asmaternal pre-existing characteristics probably vary in different countries or regions. Advancing maternal age – precisely age 35 and older – increases the risk of chronic high blood pressure and pregnancy complications. Therefore, preexisting maternal risk, especially cardiovascular risk factors, contribute to long-term implications and risks associated with IVF, and they require close monitoring, particularly during delivery. Counselling may help analyze the individual's lifestyle habits and make recommendations to lower risks, such as decreasing smoking, reducing BMI in obese patients, and decreasing alcohol intake (Palomba et al., 2016).

Once pregnant after IVF, there may also be differential care to patients with IVF twin pregnancies. There may be closer monitoring with ultrasound or other prenatal screening and testing. Elective induction of labour may be considered, based on patient preferences. Cesarean delivery may also be favoured, to avoid the risks of vaginal twin birth.

IVF-procedure risk

Manipulating the hormonal environment and handling female and male gametes during oocyte retrieval and embryo culture may be responsible for obstetric and perinatal complications. One of the early steps in the IVF procedure is to stimulate ovaries by injecting gonadotropins to secret

estradiol. The increased estradiol production from ovaries causes the oocytes and embryos to become exposed to supraphysiological levels of estradiol in the most vulnerable time in embryogenesis (Sullivan-Pyke et al., 2017). The alternation in hormonal level might lead to poor placentation, thereby predisposing to impaired fetal growth and hypertensive disorders of pregnancy. Therefore, providing a milder hormonal stimulation may decrease the risk of pregnancy complications in individuals undergoing fertility treatments (Palomba et al., 2016).

Exposing gametes and embryos to an altered environment may be a driver for epigenetic alterations leading to some IVF offspring adverse outcomes (Sullivan-Pyke et al., 2017). In animal studies, even in the absence of infertility, techniques utilized during IVF/ICSI have resulted in epigenetic changes leading to long-term changes in offspring (de Waal E et al., 2015). In a study by Song et al., ART procedures are associated with DNA methylation differences between in vitro- and in vivo-conceived children (Song et al., 2015) Further study is needed to elucidate the mechanism behind epigenetic alterations.

Maternal complications

Gestational hypertension

The hypertensive disorders of pregnancy are among the leading causes of maternal and fetal morbidity and mortality (Wilkerson et al., 2019) and occur in 6–10% of spontaneous pregnancies (WHO, 2011; Kintiraki et al., 2015), 12% of women 35–40 years and 14% of women 40+ years of age (Keegan et al., 2007). The four categories of hypertensive disorders of pregnancy are chronic hypertension, gestational hypertension, preeclampsia-eclampsia, and chronic hypertension with superimposed preeclampsia (Braunthal et al., 2019). Gestational hypertension (GH) is the development of de novo hypertension after 20 weeks of gestation in the absence of diagnostic criteria for preeclampsia (Wilkerson et al., 2019).

The prevalence of hypertensive disorders of pregnancy is increased in women who undergo IVF procedures, in both IVF singleton and twin pregnancies, compared with spontaneous pregnancies (IVF twin: Adele et al., 2018; Barda et al., 2017; Okby et al., 2018; Sibai et al., 2000; Zhu et al., 2016) (IVF multiple: Jackson et al., 2004) (IVF singleton: Thomopoulos et al., 2016; Yang et al.,

2019; Zhu et al., 2016). While we did not study IVF singletons, a literature review by Thomopoulos et al. (2016) demonstrates the increased rate of gestational hypertension among IVF pregnancies and is applicable to our study and future research. Thomopoulos et al. (2016) looked at the risk of hypertensive disorders in pregnancy following the use of ART. Hypertensive disorders in the study include gestational hypertension, preeclampsia, and eclampsia (Thomopoulos et al., 2016).

According to a study performed by Meister et al., developing hypertensive disorders may be associated with the conditions under which IVF embryos are developed. The in vitro period can involve various non-physiological conditions - temperature, mechanical insults caused by embryo handling, suboptimal culture media, and others which might explain the increase in risk (Meister et al., 2018). Gestational hypertension and preeclampsia saw the highest increase compared with the combination (Wang et al., 2021). The significant increase in hypertensive disorders was found to be independent of gestation order (Thomopoulos et al., 2016).

Antepartum hemorrhage (APH)

Our results show a higher rate of APH in the IVF twin pregnancies compared with the non-IVF comparing cohort. A study by Bhandari et al.'s (2016) study showed that APH rates in IVF pregnancies are higher than the in natural conceptions. Bhandari et al. also found that women who conceive following IVF are significantly more likely to be nulliparous and have significantly greater APH than women who naturally conceive. These results confirm the findings of previous publications which show a strong correlation between IVF pregnancies and APH (Smithers et al., 2003; Healy et al., 2010; Sabban et al., 2017) with a possible association between the development of an APH event in the IVF group and the time of the implantation. For example, Healy et al. examined singleton births conceived via IVF or intracytoplasmic sperm injection and compared these with non-IVF conceptions as well as with conceptions without any ART (Healy et al., 2010). The study found that singleton pregnancies from IVF/ICSI had increased APH complications compared with the control group (Healy et al., 2010). Antepartum hemorrhages are less common in individuals conceiving naturally, which suggests that the increase in prevalence in the assisted-conception groups may be due to the reproductive technologies used (Healy et al., 2010). However, it is unknown if the increase in hemorrhage prevalence is solely due to ART or if the infertile status of the participants acted as a confounding factor (Healy et al., 2010).

Gestational diabetes

As part of our examination of maternal complications, we found those who opted for IVF intervention also had higher rates of gestational diabetes (aOR = 1.77, 95% CI 1.09, 2.87, p =0.02). These results were similar to those found in the literature (Palomba et al., 2016). According to a study by Adele et al. (2018), IVF twin pregnancies are associated with a higher risk of gestational diabetes than spontaneous twin pregnancies. Studies also suggest an increased risk of gestational diabetes in singleton IVF pregnancies but linking the method of conception to GD requires further investigation according to some experts (Ochsenkühn et al., 2003).

Gestational age at first ultrasound

Our studies showed that IVF-induced twin pregnancies had lower gestational age at their first ultrasound than non-IVF pregnancies (Table 9). This could be explained by increased risk factors in this group of mothers, for example, older age in mothers undergoing IVF compared with natural pregnancy (Herman et al. 2021), which is also supported by the demographic profile of mothers in our research (Table 8). IVF-conceived pregnancies may have a higher risk of complications due to increased maternal age. Notably, this increased risk of pregnancy complications may lead to the first ultrasound being earlier than usual. An alternative explanation is that IVF pregnancies are considered high-risk and tend to be more medicalized, leading to earlier investigations and more procedures carried out in the interests of safety.4

Labour and delivery events

Regarding labour and delivery complications, our study concentrated on the risk of cesarean delivery and use of prophylactic antibiotics in C-sections, steroid use for lung maturation, antihypertensive drugs, and use tocolytics for delaying delivery. Additionally, length of time between the rupture of membranes and delivery, and length of the first, second and third stages of delivery were studied.

Cesarean section

It is debatable whether high rates of cesarean sections are necessary and justified in IVF twin births (Koudstaal et al., 2000; Ochsenkuhn et al., 2003; Nassar et al., 2003). We observed a higher rate of C-sections in the IVF twin group compared with the non-IVF group. Our results were comparable to those of Smithers et al. (2003) on the need for emergency cesarean-section delivery. Several previous studies (Hayashi et al., 2012; Koudstaal et al., 2000; Kozinszky et al., 2003; Luke et al., 2017; Ochsenkuhn et al., 2003; Nassar et al., 2003) also document an increase in the rate of cesarean-section deliveries in IVF compared with non-IVF pregnancies. However, this increase has been found among both IVF singleton and IVF twin pregnancies, compared to non-IVF controls. On the other hand, Yang et al. (2019) conducted an analysis of data from the Peking University Third Hospital on singleton pregnancies, in which all patients were IVF patients were matched with two non-IVF patients. They found that there was no difference in the cesarean-section rate of preterm births among IVF singleton pregnancies when compared with non-IVF pregnancies.

According to the literature, cesarean sections may not be linked to IVF twin births but rather to the fear and anxiety associated with IVF births (Nassar et al., 2003; Zollner & Dietl, 2013). In addition to cesarean section, other studies suggest IVF-induced pregnancies are associated with significantly higher rates of induced labour (Chen et al., 2019; Slabinskaya & Sudarikova, 2010). On the other hand, previous studies have described a possible correlation between GDM and cesarean deliveries in IVF singleton pregnancies (Practice Bulletin No., 2013, Shrafi et al., 2014; Yang et al., 2019; Zhu et al., 2016).

Steroid use for lung maturation

A consequence of preterm birth could be underdevelopment of the lungs and insufficient levels of dipalmitoylphosphatidylcholine from Type II pneumocytes in lung surfactant to sustain the lung alveoli. Antenatal corticosteroids are generally provided to the mother to increase the rate of development of the fetal lungs if there is a risk of preterm delivery. In our study, there was a significantly higher rate of steroid use for lung maturation among IVF twin pregnancies compared with the non-IVF group (aOR = 1.88, CI 1.18, 3.00, p = 0.008; Table 13). IVF twin-birth neonates may present with less developed lungs, leading to complications such as respiratory distress

syndrome or pneumothorax (Nassar et al., 2003). As a result of these complications, some previous studies show that mechanical ventilation may be required in as many as 25% of IVF twin births (Nassar et al., 2003).

Perinatal outcomes

Congenital anomalies

We identified a higher rate of congenital anomalies among the IVF group (aOR = 1.55, CI 1.02, 2.35, p = 0.04; Table 16). Since the study examined overall rates of congenital anomalies, further research should be conducted to determine the specific type of anomlies that occurred more frequently in the IVF group. Increasing the number of patients included would make the study more powerful and enable it to determine if there are significant differences among types of congenital anomalies. However, several factors should be taken into consideration when testing for adverse events, like the age of the patient and the presence of pre-existing health problems, including diabetes, hypertension, insulin resistance, and thyroid dysfunction. Overall, these results should be interpreted with caution and require confirmation in future larger studies.

Earlier gestational age at birth

In our study, the IVF twin pregnancy group had a gestational age at birth at least one week lower than the non-IVF twin pregnancy group. An earlier study by Saccone et al. (2017) compared spontaneously conceived twins with IVF-conceived twins and showed that the latter had an earlier gestational age at delivery by about one week. IVF-conceived twins also had a shorter transvaginal ultrasound cervical length than spontaneously conceived twins. Similarly, Helmerhorst et al. (2004) found a 3.27-fold and 2.04-fold higher risk of preterm births among IVF singletons, with preterm birth defined as <32 weeks and <37 weeks' gestation, respectively.

Multiple factors may contribute to the increased risk of spontaneous preterm birth (Saccone et al., 2017). It is possible that the inflammatory response to clinical procedures, such as embryocyte transfer or oocyte retrieval, may trigger spontaneous preterm birth (Saccone et al., 2017). Individuals with endometriosis are more likely to experience spontaneous preterm birth, and many women with endometriosis undergo IVF treatments due to infertility (Saccone et al., 2017). Lastly, IVF pregnancies generally have higher plasma relaxin, which may increase the risk of spontaneous

preterm birth (Saccone et al., 2017). These reasons suggest why spontaneous preterm birth may be more prevalent in IVF-conceived twins in comparison with spontaneously conceived twins (Saccone et al., 2017).

Among preterm births, only moderate preterm birth (>32 and <37 weeks) showed a statistically significant difference between IVF twins and non-IVF twins. However, no statistically significant difference was noticed in either very preterm (>28 and <=32 weeks) or extremely preterm (<=28 weeks) birth rates. This indicates that while preterm birth was statistically higher in IVF twins, the magnitude of the clinical difference is small as there was no increased risk of the more clinically morbid very or extremely pre-term births.

Lower birth weight, length and head circumference at birth, and Intrauterine growth restriction (IUGR)

Our study showed IVF babies had lower birth weights (in grams) (mean difference = -200.4, 95% CI -118.6, -36.8, p =0.004), length (in centimeters) (mean difference = -0.63, 95% CI (-1.23, -0.02), p = 0.04), head circumference (in centimeters) (mean difference = -0.35, 95% CI (-0.66, -0.04), p = 0.027), and gestational age at birth (in weeks) (mean difference = -1.014, 95% CI (-1.53, -0.49), p <0.001). Previous research also indicates earlier delivery and lower birthweight may be seen in IVF pregnancies (Jackson et al., 2004). The lower gestational age at delivery may account for the lower birth weight, length, and head circumference. However, we also observed a higher frequency of IUGR in the IVF group, suggesting there may be other factors for the higher risks in IVF twin pregnancies. IUGR may reflect abnormal placentation, leading to oxidative stress due to inadequate invasion of the spiral arteries and persistent hypoxia (Rana et al., 2019), which perhaps is more frequent in IVF pregnancies.

Similar to pre-term birth, the clinical magnitude of the difference in birth weight between the IVF and non-IVF groups was small. In particular, while there was a difference in moderate LBW, there were similar rates of the more morbid very and extremely LBW infants.

Strengths

The present study includes a sample of women with twin pregnancies who undergoing IVF treatment, with or without intracytoplasmic sperm injection (ICSI), with documented obstetric and perinatal outcomes. The comparison group included a population-based sample of non-IVF twin pregnancies from a perinatal data registry. There are few published studies in Canada about IVF-twin pregnancy and maternal complications after IVF. Our study comprehensively investigates both common and rare complications of IVF pregnancy, using information from a large database.

The use of a large population-based comparison group is an important strength of this study. There are few publications comparing IVF to non-IVF twin pregnancies; most studies cover complications in singleton pregnancies after ART treatment. Another strength of this study is the adjustment for the age, BMI and parity. Unlike many other studies, which have been performed on selected specific populations, our study population consisted of all twin pregnant mothers in the province of British Columbia in Canada. Working on such a study population minimizes selection bias. Accurate ascertainment of IVF treatment can be counted as another strength of this study. Some studies use maternal recall of IVF, which is subject to recall bias.

Limitations

Limitations for our study include its retrospective nature. In addition, there was a relatively small number of patients with IVF-conceived twin pregnancies, as the IVF sample derived from a single centre (although at the time, it was the primary IVF centre for the province). Small sample size also affected the reliability of studies' results because it led to a higher variability and wide confidence intervals for some outcomes (e.g. extremely preterm birth). Also, in regression analysis, we adjusted for BMI and there were some missing data for BMI, such that the sample size was smaller for the regression and the adjusted analysis.

The IVF sample used for this study, was taken from UBCCRH. However, during the there were two other fertility centers, Genesis and in later years the Pacific Centre for Reproductive Medicine (PCRM), that started during the study period, and also there might be people who did IVF outside

BC. Therefore, there may be some IVF twins in the comparison group that would actually bias towards non-difference; Despite this bias, we still were able to observe a significant difference in the IVF vs. non-IVF group in selected pregnancy outcomes.

There are also potential factors that could have been adjusted for in the analysis. Notably, we were not able to obtain information from the study subjects, such as details of the IVF treatment, the couple's main reasons for infertility, their educational level and income, comorbidities (e.g., chronic hypertension and pre-pregnancy diabetes), potential barriers to obstetric services (e.g., rural residence), smoking, alcohol/drug use, number of previous miscarriages, all of which may have affected maternal, labour and perinatal outcomes. In addition, we did not have data on chorionicity, which may differ between the IVF and non-IVF groups. It is possible that with additional adjustment of such potential confounders, there may be fewer statistically significant differences between IVF and non-IVF twin pregnancies. Finally, there were variables with missing values, and also BMI was self-reported and thus subject to inaccuracy.

The study is now over ten years old, and there have been a lot of changes in the IVF practice since then. There are fewer IVF twins now because of the single embryo transfer. There are many other differences in the IVF technique, such as culturing up to the blastocyst stage or using more frozen embryos versus fresh embryo transfers. Also, pre-implantation genetic diagnosis (PGD) is now common. Therefore, the differences between the IVF and non-IVF groups may not be applicable now because of these changes in IVF technique.

During the period of this study, because of transferring more than one embryo, the twins would be primarily dizygotic. However, it is worth noting that following concerns on multiple pregnancies, IVF-induced twin pregnancies have declined significantly in the past ten years, and improvements in elective single embryo transfer have made it a standard practice currently.

Future studies

Over the past decade, increased awareness of the risks involved in multi-fetus pregnancies has created an incentive to reduce such pregnancies from occurring via IVF, with guidelines developed

by professional organizations specifying the advisable number of transferred embryos (SOGC guideline 2014; Min et al., 2010; Okun et al., 2014). Despite existing literature that points to the greater risk of multiple-fetus versus single-fetus pregnancies, there remains a small but growing number of studies into how such pregnancies compare in the context of IVF. However, further research is needed to elucidate whether the increased risk in IVF twin pregnancies is partly due to a diagnosis of infertility or the IVF procedure itself.

In future research studies, more adjustments can be considered. For example, IVF people may have more medical comorbidities, such as chronic hypertension and chronic maternal diseases, which can be adjusted for in the regression analyses. Missing data (e.g. for BMI) could also be imputed in future analyses.

To eliminate the impact of infertility, future research could include studying normal fertility subjects who undergo IVF: e.g. those with previous tubal ligation or lesbian couples. The control group would be mothers with spontaneous pregnancy. If the IVF procedure itself leads to pregnancy complications, then normal fertility subjects undergoing IVF would be expected to have a higher chance of experiencing maternal, delivery, and perinatal complications, compared with non-IVF subjects. Two studies could be one, one in singletons and one in twin pregnancies.

Another example would involve studying women in infertility who undergo fertility treatment and achieve pregnancy. These individuals could be divided into two groups; group A, who got pregnant after IVF +/- ICSI (a high interventional ART procedure), and group B, who got pregnant with less interventional procedures such as ovarian stimulation (OS) +/- intrauterine insemination (IUI). If the IVF procedure itself leads to pregnancy complications, then group A (IVF +/- ICSI) would show a higher risk of pregnancy, labor, and baby complications, compared with group B.

Conclusion

In conclusion, we compared outcome data from IVF twin pregnancies in the former IVF Program of the UBCCRH to that of non-IVF twin pregnancies in the period between April 1, 1998, and March 31, 2010, the data for which were sourced from the BC Perinatal Database. For certain pregnancy outcomes the IVF and non-IVF groups were similar, while there were differences for selected maternal, labour and delivery, and perinatal outcomes. However, it should be emphasized that the clinical magnitude of the differences were small. In addition, the outcomes where there was a difference were themselves interrelated (e.g. a higher rate of hypertension may result in more pre-term birth, need for steroid use for lung maturation, and cesarean sections). Additional prospective studies, preferably multi-centre and inter-provincial, will allow for larger study size and adjustment for other confounders, in order to validate these potential associations between IVF twin pregnancies and pregnancy outcomes.

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Appendices

Appendix 1

List of congenital anomalies considered for this study. Any baby with one or more of the following conditions has been counted as a baby with congenital anomaly:

- Spina bifida no hydrocephalus
- Microcephalus
- Congenital hydrocephalus
- Other specified anomalies brain
- Anomaly of brain/spinal/nervous sys (not otherwise specified)
- Anophthalmos
- Microphthalmos
- Coloboma/other anomalies of anterior segments
- Congenital anomalies of eyelid/lacrim/orbit
- Other specified anomalies eye
- Unspecified anomalies of eye
- Accessory auricle
- Other specified anomalies ear
- Other specified anomalies of face/neck
- Unspecified anomalies of face/neck
- Transposition of great vessels
- Common ventricle
- Ventricular septal defect
- Ostium secundum atrial septum defect
- Endocardial cushion defects
- Other anomalies of cardiac septal closure
- Anomalies of pulmonary valve
- Tricuspid atresia/stenosis congenital
- Hypoplastic left heart syndrome
- Other specified anomalies heart
- Unspecified anomalies of heart
- Patent ductus arteriosus
- Coarctation of aorta
- Anomalies of pulmonary artery

- Anomalies of great veins
- Absence/hypoplasia umbilical artery
- Other anomalies of peripheral vascular system
- Other specific anomalies of circulatory system
- Unspecified anomalies of circulatory system
- Other anomalies of nose
- Other anomalies of larynx/trachea/bronchi
- Congenital cystic lung
- Agenesis/hypoplasia/dysplasia lung
- Other anomalies of lung
- Cleft palate
- Cleft lip
- Cleft palate with cleft lip
- Tongue tie
- Other anomalies of tongue
- Trachea/esophageal fist/atresia/stenosis
- Other specified anomalies of stomach
- Meckel's diverticulum
- Atresia/stenosis of small intestine
- Atresia/stenosis of large intestine/rectum
- Other anomalies of intestine
- Anomalies of gallbladder/bile ducts/liver
- Anomalies of cervix/vagina/external female genital organs
- Undescended testicle
- Hypospadias and epispadias
- Indeterminate sex/pseudohermaphroditism
- Other specified anomalies of genital organs
- Renal agenesis and dysgenesis
- Cystic kidney disease
- Obstruction of renal pelvis/ureter
- Other specified anomalies of kidney
- Other specified anomalies of ureter
- Other specified anomalies of bladder/urethra
- Congenital deformities of skull/face/jaw
- Congenital deformities of sternocleidomastoid
- Congenital dislocation of hip

- Varus deformities of feet
- Valgus deformities of feet
- Other deformities of feet
- Other specified congenital deformities
- Polydactyly
- Syndactyly
- Other anomalies of upper limb/shoulder
- Other anomalies of lower limb/pelvis
- Other specified anomalies unspecific limb
- Anomalies of skull/face bones
- Anomalies of spine
- Other anomalies ribs/sternum
- Osteodystrophies
- Anomalies of diaphragm
- Anomalies of abdominal wall
- Other specified anomalies of muscles/tendons
- Unspecified anomalies of musculoskeletal system
- Hereditary edema of legs
- Ichthyosis congenital
- Other specified anomalies of skin
- Specified anomalies of nails
- Down's syndrome
- Patau's syndrome
- Autosomal deletion syndromes
- Gonadal dysgenesis
- Other sex chromosome anomaly
- Anomalies of spleen
- Situs inversus
- Conjoined twins
- Other specified anomalies
- Congenital anomaly unspecified
- Persistent fetal circulation
- Microcephaly
- Atresia foramina Magendie & Luschka
- Congenital hydrocephalus unspecified
- Congenital malformations corpus callosum

- Other reduction deformities of brain
- Congenital cerebral cysts
- Congenital malformation of brain (not otherwise specified (NOS))
- Spina bifida unspecified
- Arnold-Chiari syndrome
- Congenital ptosis
- Other congenital malformations of eyelid
- Congenital stenosis stricture lacrimal duct
- Other congenital malformation of anterior segment of eye
- Congenital malformation of optic disc
- Accessory auricle
- Microtia
- Other misshapen ear
- Misplaced ear
- Other specified congenital malformations of ear
- Sinus fistula & cyst of branchial cleft
- Preauricular sinus and cyst
- Microstomia
- Other specified congenital malformation of face & neck
- Congenital malformation face & neck NOS
- Double outlet right ventricle
- Complete transposition of great vessels
- Other transposition of great vessels
- Other congenital malformation of cardiac chambers
- Ventricular septal defect
- Atrial septal defect
- Atrioventricular septal defect
- Tetralogy of Fallot
- Congenital pulmonary valve stenosis
- Other congenital malformations of pulmonary valve
- Other congenital malformations of tricuspid valve
- Congenital stenosis of aortic valve
- Congenital insufficiency of aortic valve
- Congenital mitral insufficiency
- Hypoplastic left heart syndrome
- Laevocardia

- Other specified congenital malformations of heart
- Congenital malformation of heart NOS
- Patent ductus arteriosus
- Coarctation of aorta
- Stenosis of aorta
- Other congenital malformations of aorta
- Stenosis of pulmonary artery
- Other congenital malformations of great veins
- Congenital absence / hypoplasia umbilical artery
- Congenital subglottic stenosis
- Congenital laryngomalacia
- Congenital malformation of larynx NOS
- Congenital tracheomalacia
- Sequestration of lung
- Hypoplasia and dysplasia of lung
- Cleft soft palate
- Cleft hard palate with cleft soft palate
- Cleft palate unspecified
- Cleft lip
- Cleft palate with cleft lip
- Congenital malformations of lips NEC
- Ankyloglossia
- Other congenital malformations of tongue
- Congenital malformations of palate NEC
- Other congenital malformations of mouth
- Congenital hypertrophic pyloric stenosis
- Congenital absence / atresia / stenosis of duodenum
- Congenital absence / atresia / stenosis of ileum
- Congenital absence / atresia / stenosis of anus with fistula
- Congenital absence / atresia / stenosis of anus without fistula
- Meckel's diverticulum
- Congenital malformations of intestinal fixation
- Other specified congenital malformations intestine
- Congenital malformation intestine NOS
- Atresia of bile ducts
- Other congenital malformations of liver

- Congenital malformation of digestive system NOS
- Developmental ovarian cyst
- Doubling uterus with doubling cervix vagina
- Imperforate hymen
- Congenital malformation of clitoris
- Undescended testicle unilateral
- Undescended testicle bilateral
- Undescended testicle unspecified
- Hypospadias balanic
- Hypospadias penile
- Hypospadias penoscrotal
- Hypospadias perineal
- Congenital chordee
- Other hypospadias
- Hypospadias unspecified
- Other congenital malformation of testis & scrotum
- Hypoplasia of penis
- Other congenital malformations of penis
- Renal agenesis unilateral
- Renal agenesis unspecified
- Renal hypoplasia bilateral
- Polycystic kidney autosomal recessive
- Polycystic kidney unspecified
- Renal dysplasia
- Other cystic kidney diseases
- Cystic kidney disease unspecified
- Congenital hydronephrosis
- Atresia & stenosis ureteropelvic junction
- Other obstructive defct of renal pelvis / ureter
- Congenital vesico-uretero-renal reflux
- Lobulated fused and horseshoe kidney
- Hyperplastic and giant kidney
- Other specified congenital malformations kidney
- Congenital malformation of kidney NOS
- Congenital posterior urethral valves
- Congenital dislocation hip unilateral

- Congenital dislocation of hip bilateral
- Congenital dislocation of hip NOS
- Congenital subluxation hip unilateral
- Congenital subluxation of hip bilateral
- Congenital subluxation of hip NOS
- Unstable hip
- Other congenital deformities of hip
- Congenital deformity of hip unspecified
- Talipes equinovarus
- Metatarsus varus
- Other congenital varus deformities of feet
- Talipes calcaneovalgus
- Other congenital valgus deformities feet
- Other congenital deformities of feet
- Congenital deformity of feet NOS
- Facial asymmetry
- Compression facies
- Dolichocephaly
- Plagiocephaly
- Other congenital deformities skull face & jaw
- Congenital deformity of spine
- Congenital deformity of sternocleidomastoid muscle
- Other specified congenital musculoskeletal deformity
- Accessory finger(s)
- Accessory thumb(s)
- Accessory toe(s)
- Polydactyly unspecified
- Fused fingers
- Fused toes
- Webbed toes
- Polysyndactyly
- Congenital absence of hand and finger(s)
- Longitudinal reduction defect of radius
- Other reduction defects of upper limb(s)
- Congenital absence of foot and toe(s)
- Longitudinal reduction defect of tibia

- Longitudinal reduction defect of fibula
- Other congenital malformation of upper limb includin shoulder
- Other malformation of lower limb including pelvic girdle
- Craniosynostosis
- Hypertelorism
- Macrocephaly
- Other specified congenital malformation of skull / face bones
- Congenital malformation of skull & face bones NOS
- Other malformation of spine not associated with scoliosis
- Other specified osteochondrodysplasias
- Congenital diaphragmatic hernia
- Exomphalos
- Gastroschisis
- Other congenital malformations of abdominal wall
- Other congenital malformations of musculoskeletal system
- X-linked ichthyosis
- Other congenital ichthyosis
- Congenital non-neoplastic naevus
- Other specified congenital malformations of skin
- Accessory nipple
- Tuberous sclerosis
- Other phakomatoses NEC
- Fetal alcohol syndrome (dysmorphic)
- Congenital syndrome predominantly with short stature
- Congenital malformation syndrome predominantly involving limbs
- Other specified congenital malformation syndromes
- Congenital malformations of spleen
- Situs inversus
- Multiple congenital malformations NEC
- Other specified congenital malformations
- Congenital malformation unspecified
- Trisomy 21 translocation
- Down's syndrome unspecified
- Edwards' syndrome unspecified
- Triploidy and polyploidy
- Trisomy & partial trisomy autosomes NOS

- Karyotype 45,X
- Turner's syndrome unspecified
- Karyotype 47XXX
- Klinefelter's syndrome karyotype 47XXY
- Klinefelter's syndrome male w >2 X chromosome
- Klinefelter's syndrome unspecified
- Other specified sex chromosome abnormality male phenotype
- Chromosomal abnormality unspecified

Appendix 2

Distribution of the following continuous variables were assessed by Kolmogorov–Smirnov test, which showed they are not normally distributed (Table 19). Moreover, density plot, Q-Q (quantile-quantile) plot, and box plot of continuous variables, by splitting IVF cases and non-IVF comparing group, were visualized by ggplot2 package for the statistical programming language R, which are depicted in Appendix 3 (Figure 6 to Figure 21).

Variables	Statistic	Df	P-value
Pre-pregnancy maternal information			
Mother's age at delivery (year)	0.020	11372	<0.001
Mother's weight before pregnancy (kg)	0.080	8692	<0.001
Mother's height (cm	0.122	9430	<0.001
BMI (Body mass index)	0.140	7922	<0.001
School years	0.080	1250	<0.001
Pregnancy information			
Number of antenatal visits	0.080	10018	<0.001
Gestational age of first ultrasound (weeks)	0.143	3998	<0.001
Labour and delivery information			
Length of time between ruptured membranes and delivery (hours)	0.408	7094	<0.001
Length of the first stage of delivery (hours)	0.134	4528	<0.001
Length of the second stage (minutes)	0.202	4670	<0.001
Length of the third stage (minutes)	0.416	9078	<0.001
Babies' outcomes			
Weight of baby at birth (gram)	0.087	11367	<0.001
Length of the baby at birth (in cm)	0.162	10747	<0.001
Head circumference of the baby at birth (cm)	0.195	10762	<0.001
Gestational age at birth	0.218	8910	<0.001
Length of stay (hours)	0.265	11131	<0.001

Table 19 Normality test on continuous variables (Kolmogorov–Smirnov test).

Appendix 3

Density plot, quantile-quantile (Q-Q) plot, and box plot of continuous variables, by splitting IVF cases and non-IVF comparison group, drawn by ggplot2 package for the statistical programming language R, are depicted in the following figures. QQ-plots are not on a straight line for each variable. Moreover, density curves drawn in density plots do not mimic a symmetric histogram, which are confirmed by the box plots' histograms. In addition to the inferential analyses performed by Kolmogorov–Smirnov test, these figures indicate that the continuous variables in this study are not normally distributed.

Pre-pregnancy maternal information

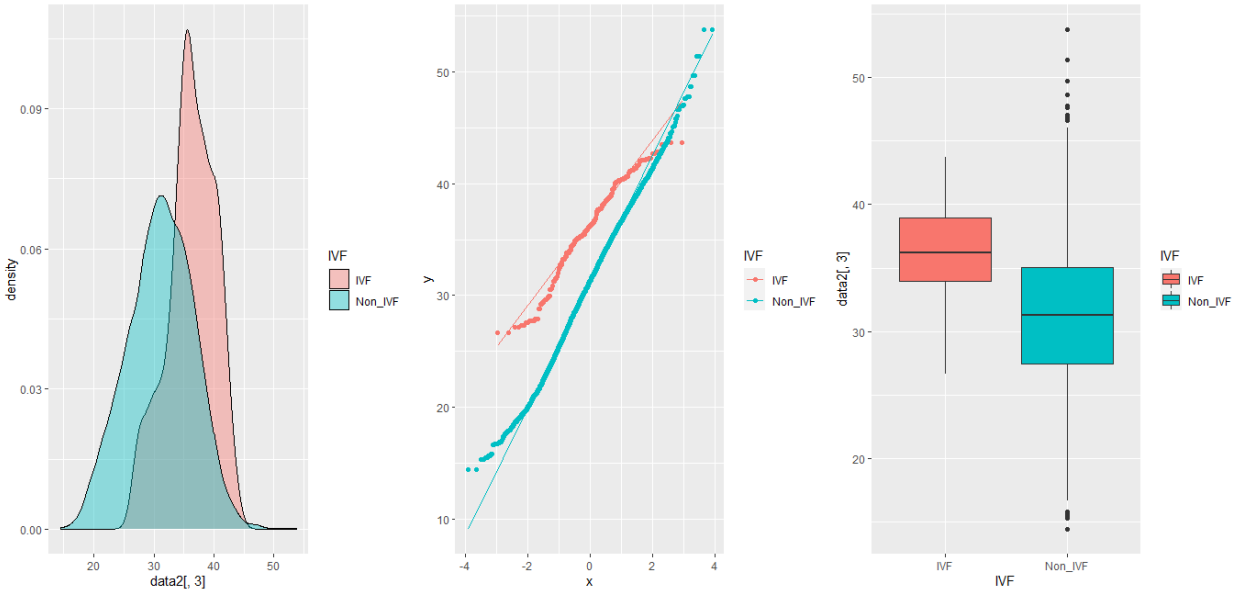

Figure 7 Density plot (left), Q-Q plot (center), and box plot (right) of mothers' age.

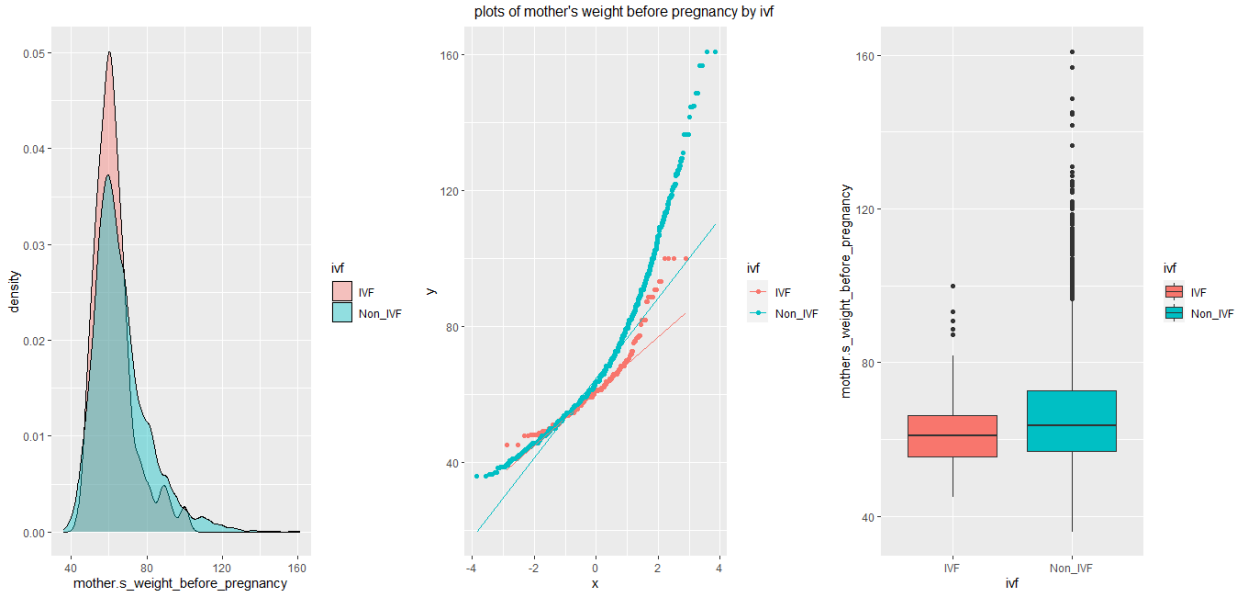

Figure 8 Density plot (left), Q-Q plot (center), and box plot (right) of mothers' weight.

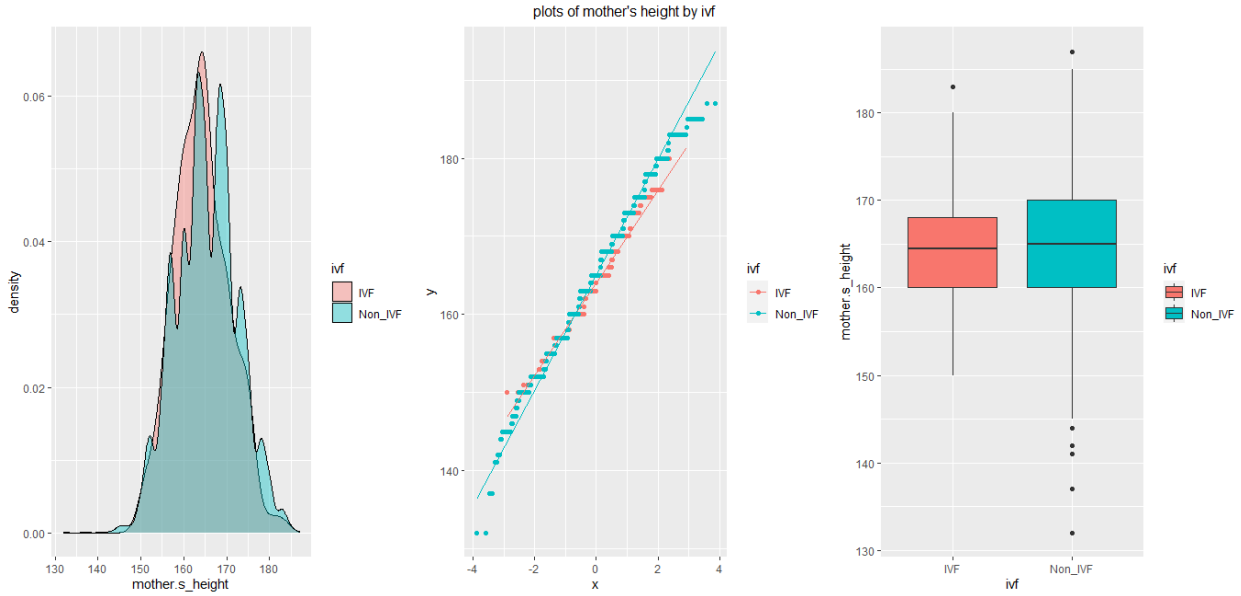

Figure 9 Density plot (left), Q-Q plot (center), and box plot (right) of mothers' height.

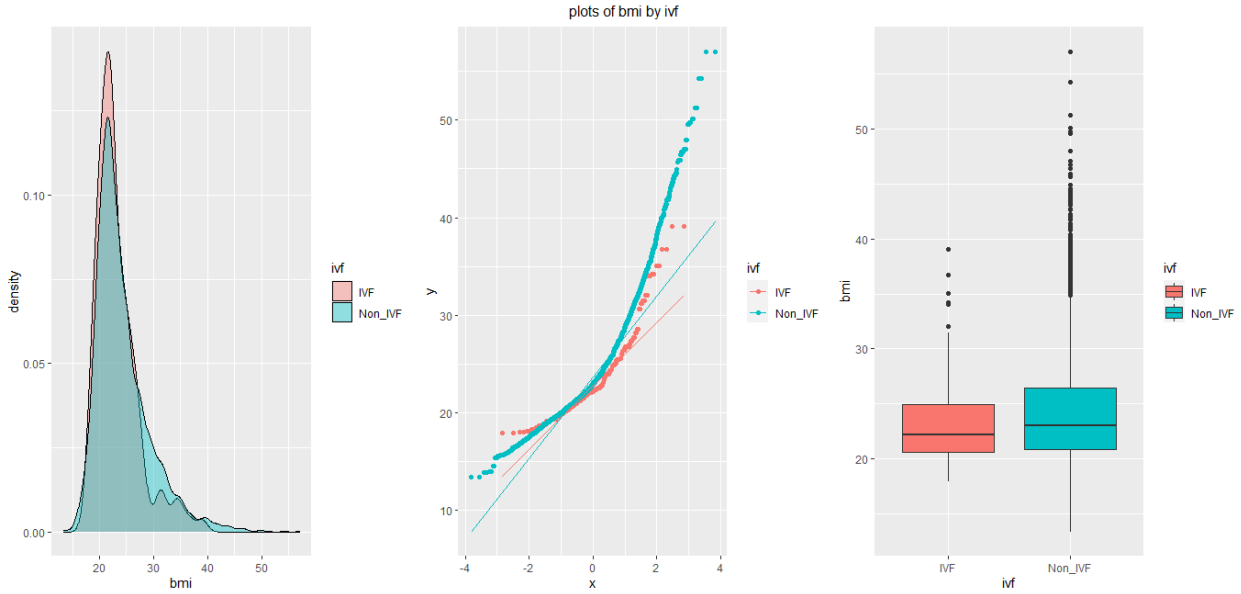

Figure 10 Density plot (left), Q-Q plot (center), and box plot (right) of mothers' BMI

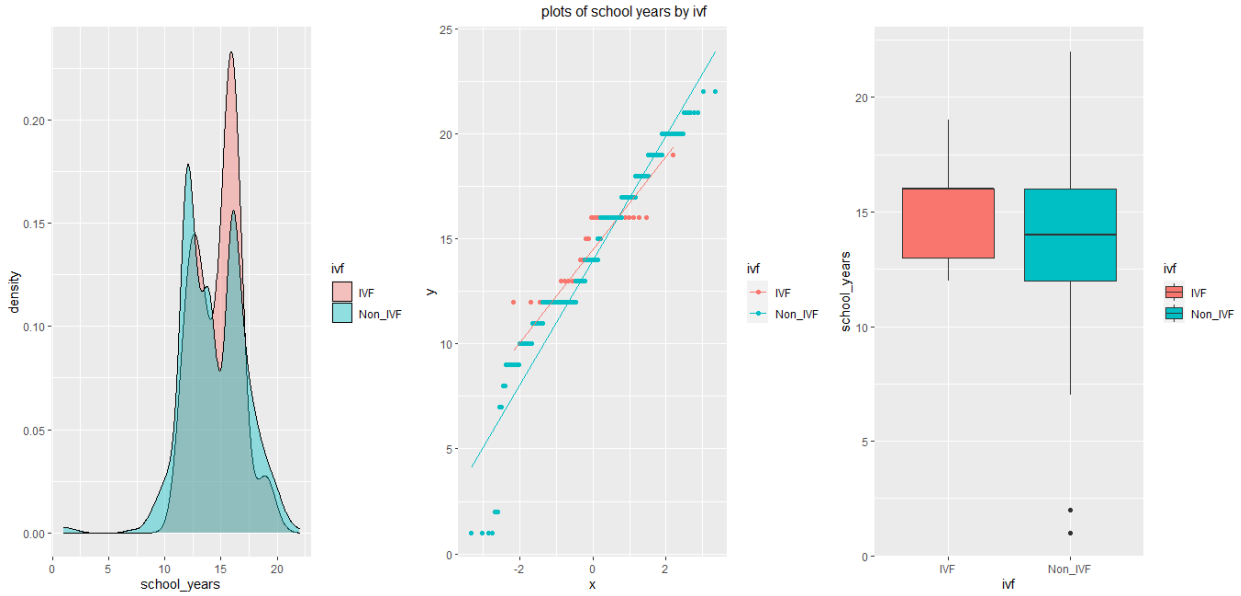

Figure 11 Density plot (left), Q-Q plot (center), and box plot (right) of mothers' school years.

Pregnancy information

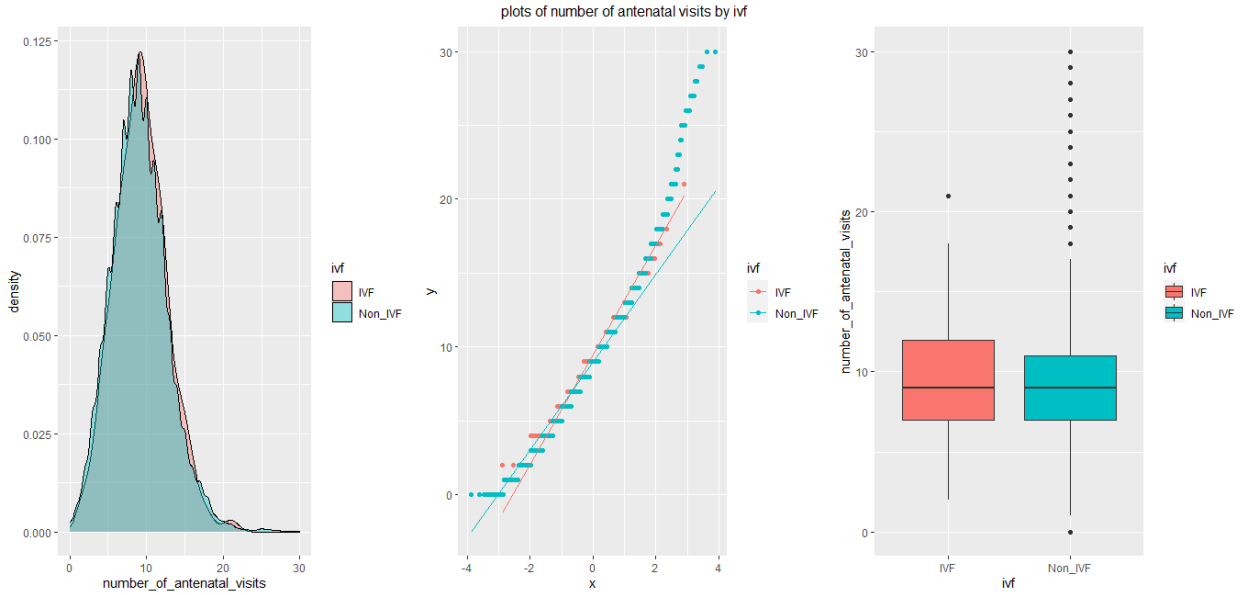

Figure 12 Density plot (left), Q-Q plot (center), and box plot (right) of the number of antenatal visits.

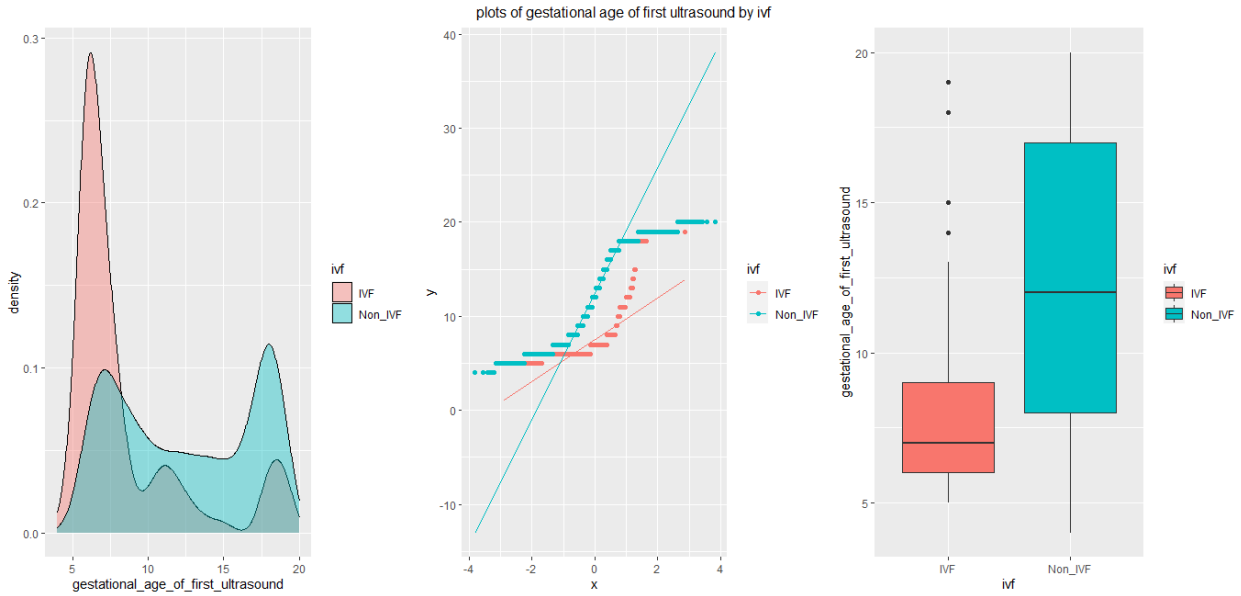

Figure 13 Density plot (left), Q-Q plot (center), and box plot (right) of the gestational age for the first ultrasound.

Labour and delivery information

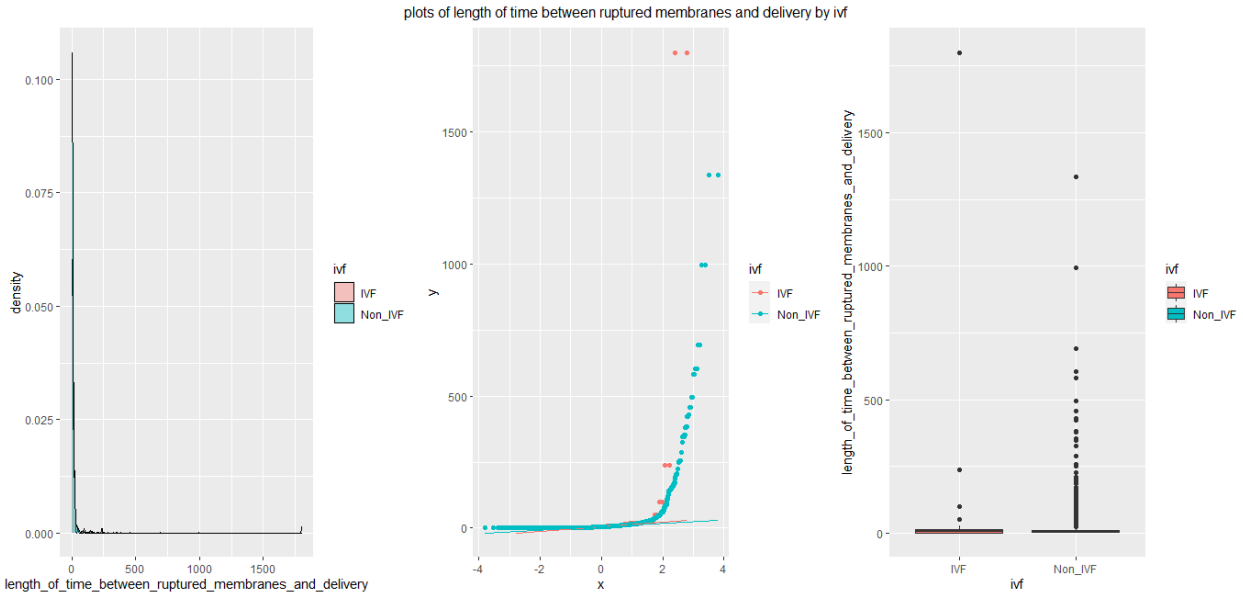

Figure 14 Density plot (left), Q-Q plot (center), and box plot (right) of the length of time between ROM and delivery.

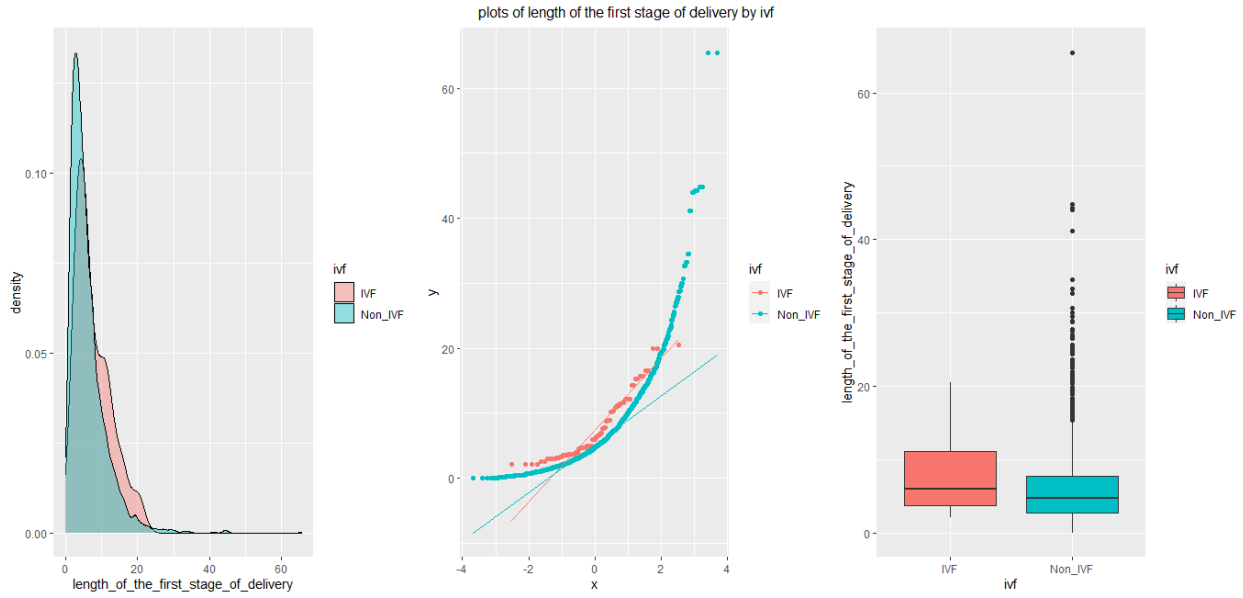

Figure 15 Density plot (left), Q-Q plot (center), and box plot (right) of the length of the first stage of delivery.

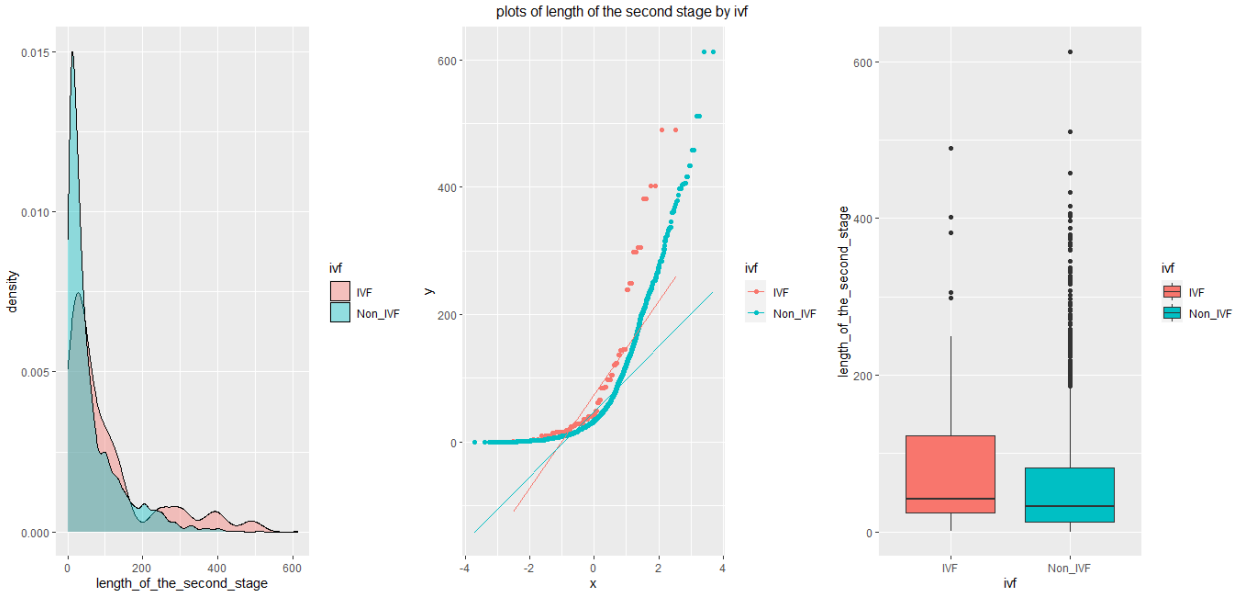

Figure 16 Density plot (left), Q-Q plot (center), and box plot (right) of the length of the second stage of delivery.

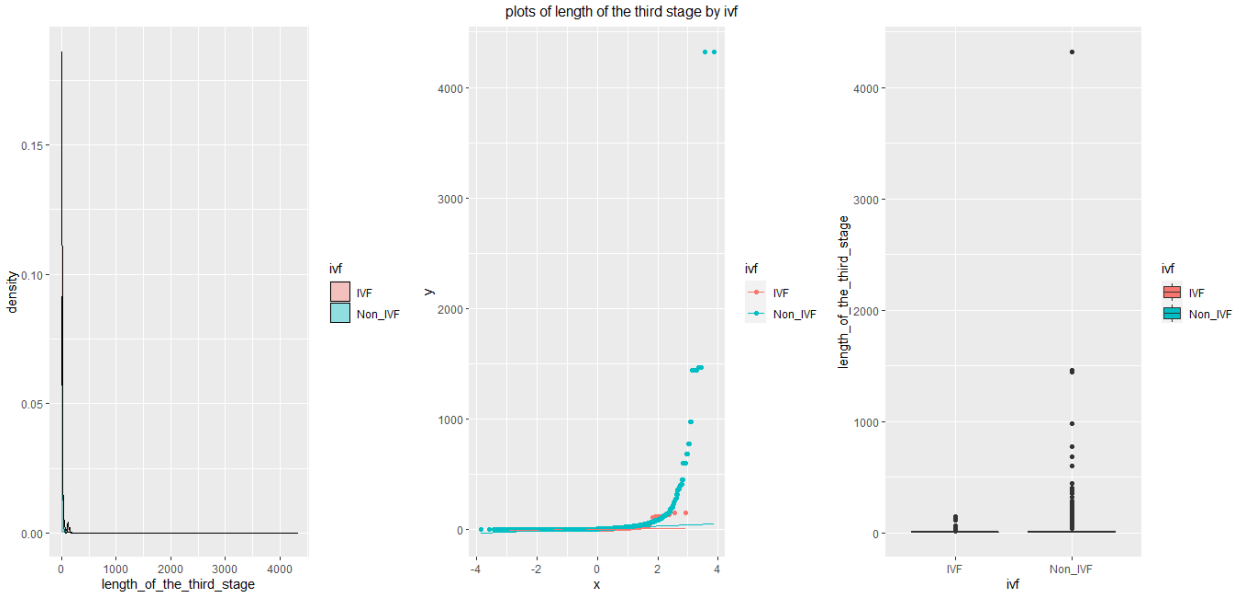

Figure 17 Density plot (left), Q-Q plot (center), and box plot (right) of the length of the third stage of delivery.

Babies' outcomes

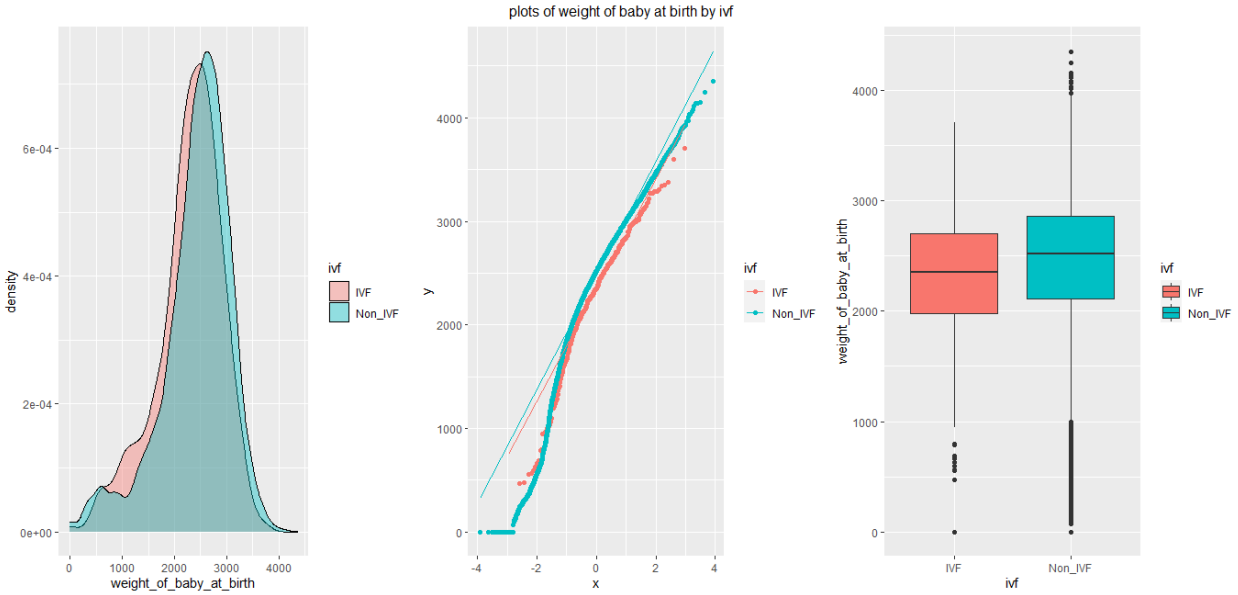

Figure 18 Density plot (left), Q-Q plot (center), and box plot (right) of the weight of baby at birth.

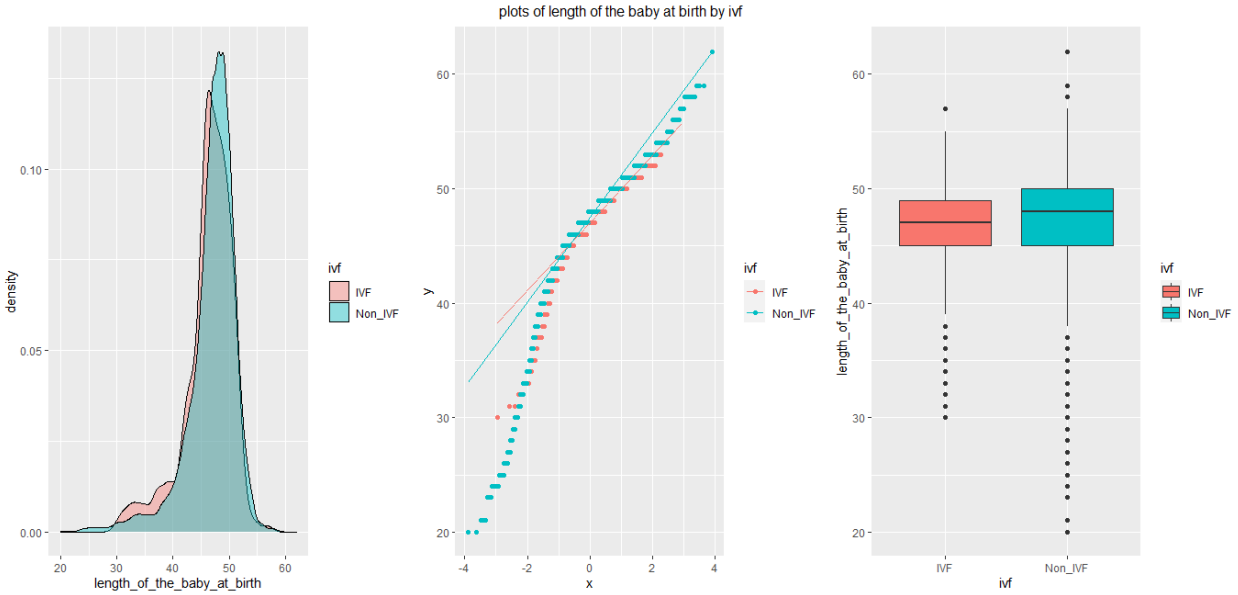

Figure 19 Density plot (left), Q-Q plot (center), and box plot (right) of the length of baby at birth.

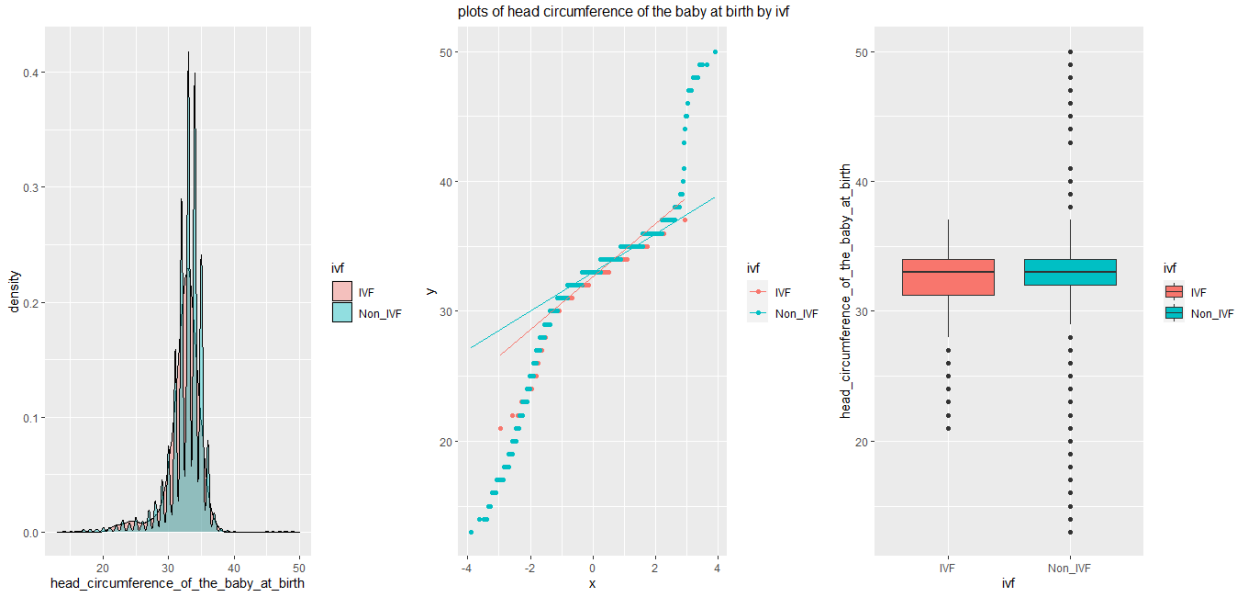

Figure 20 Density plot (left), Q-Q plot (center), and box plot (right) of the head circumference of baby at birth.

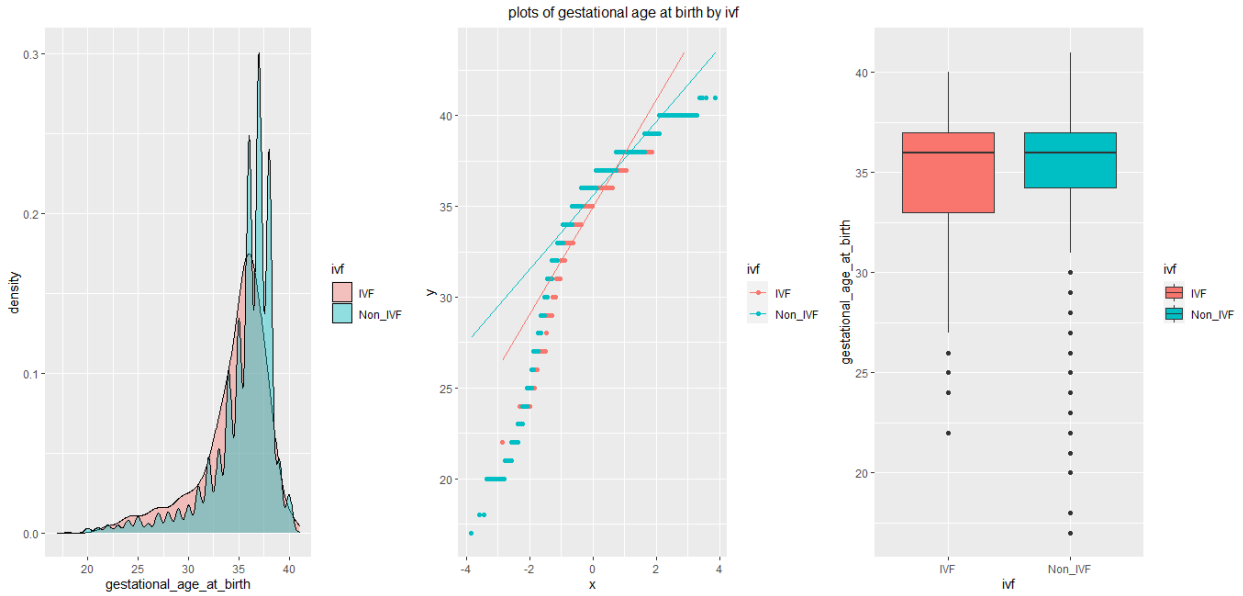

Figure 21 Density plot (left), Q-Q plot (center), and box plot (right) of the gestational age of baby at birth.

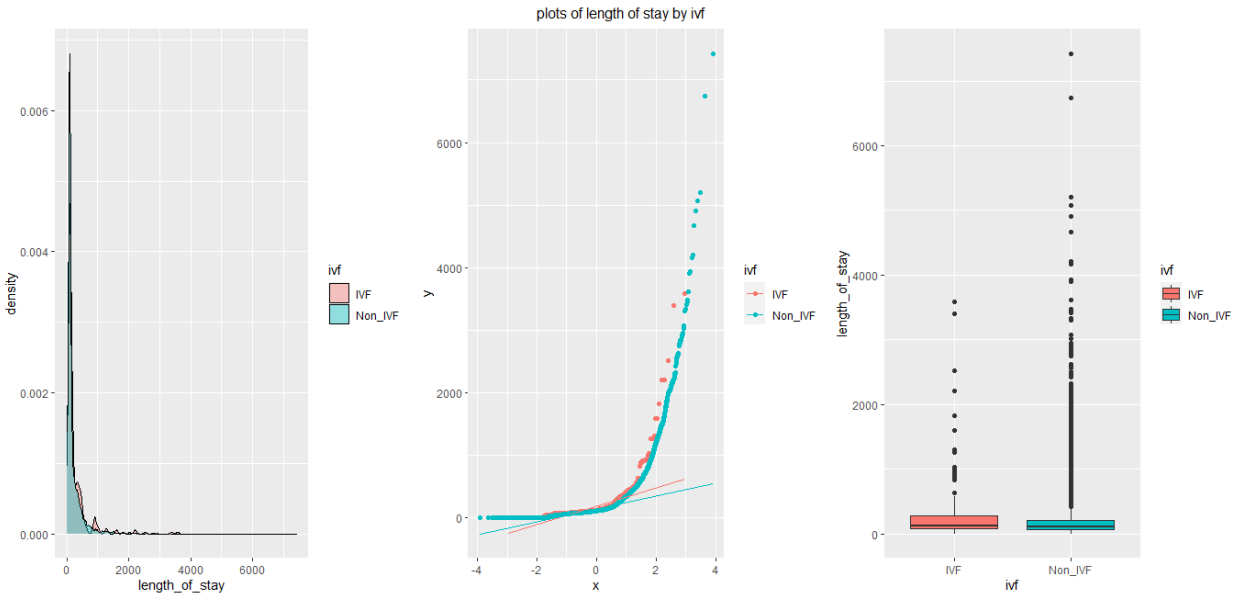

Figure 22 Density plot (left), Q-Q plot (center), and box plot (right) of the length of babies' stay at hospital after birth.