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Sindre Hoff Petersen

Complications in pregnancies after assisted reproduction

Doctoral thesis

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of Philosophiae Doctor
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Department of Public Health and Nursing

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Komplikasjoner i svangerskap etter assistert befruktning

In vitro fertilisering (IVF), også kjent som prøverørsbefruktning eller assistert befruktning, er metoder for å oppnå svangerskap hos par og kvinner som ikke lykkes med å bli gravide på egenhånd. Det første IVF-barnet ble født i 1978, og i dag utgjør IVF-barn nesten 5 % av fødselskullene i de nordiske landene. En vedvarende bekymring knyttet til IVF-behandling er den høyere forekomsten av komplikasjoner, blant annet lav fødselsvekt og for tidlig fødsel, så vel som en høyere risiko for komplikasjoner knyttet til morkaken, inkludert svangerskapsforgiftning, morkakeløsning og forliggende morkake. I dette doktorgradsprosjektet ønsket vi å studere forekomst av svangerskapskomplikasjoner i IVF-svangerskap og om behandlingen bidrar til å øke risikoen. Vi brukte data fra de nordiske landenes nasjonale helseregistre (Danmark, Finland, Norge og Sverige) fra 1988 til 2015, med rundt 6,8 millioner naturlig unnfangede svangerskap og 150 000 IVF-svangerskap.

I studie I fant vi at forekomsten av både svangerskapsforgiftning, morkakeløsning og forliggende morkake var høyere i IVF-svangerskapene enn i de naturlig unnfangede svangerskapene gjennom hele studieperioden. I studie II brukte vi søskenanalyser, der vi dro fordel av at noen kvinner i løpet av studieperioden fikk barn etter både naturlig befruktning og ved hjelp av IVF. I søskenanalyser sammenlikner vi mor med seg selv, og analysen blir dermed automatisk justert for alle faktorer som søsken har felles, for eksempel genetikk og foreldrenes underliggende helse. Her fant vi at risikoen for høyt blodtrykk og svangerskapsforgiftning var nær doblet i IVF-svangerskap etter innsetting av frosset og tint embryo sammenliknet med naturlig unnfangede svangerskap, selv i søskenanalysene. I studie III brukte vi medieringsanalyser for å undersøke om den økte forekomsten av høyt blodtrykk og svangerskapsforgiftning kunne forklare den økte risikoen for for tidlig fødsel i IVF-svangerskapene. Her fant vi at slike tilstander kunne forklare noe (omtrent 20 %) av den økte risikoen for for tidlig fødsel i IVF-svangerskap etter innsetting av frosset og tint embryo sammenliknet med naturlig befruktning, men nærmest ingenting av den økte risikoen for for tidlig fødsel i IVF-svangerskap etter innsetting av ferske embryo.

Samlet sett kan funnene i dette doktorgradsprosjektet bidra til bedre rådgivning og oppfølging til kvinner som gjennomgår IVF-behandling, og de understreker behovet for mer kunnskap om hvordan IVF-behandling påvirker risiko for komplikasjoner i svangerskapet.

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Biveiledere: Ahmed Elhakeem, Bjørn Olav Åsvold og Liv Bente Romundstad

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Complications in pregnancies after assisted reproduction

In vitro fertilization (IVF), also known as the broader term assisted reproductive technology (ART), are methods designed to achieve pregnancy in couples and women who have been unsuccessful in conceiving by their own. The first ART child was born in 1978, and today, ART children account for almost 5% of the birth cohorts in the Nordic countries. A persistent concern associated with ART treatment is the higher incidence of complications, including low birth weight and preterm birth, as well as a higher risk of complications related to the placenta, including preeclampsia, placental abruption, and placenta previa. In this PhD project, we aimed to study the prevalence of pregnancy complications in ART pregnancies, and whether the treatment contributes to increase the risk. We used data from the national health registries of the Nordic countries (Denmark, Finland, Norway, and Sweden) from 1988 to 2015, with approximately 6.8 million naturally conceived pregnancies and 150,000 ART pregnancies.

In study I, we found that the occurrence of both preeclampsia, placental abruption, and placenta previa was higher in ART pregnancies than in naturally conceived pregnancies throughout the study period. In study II, we used sibling analyses, taking advantage of the fact that some women had children conceived naturally and conceived with ART during the study period. In sibling analyses, we compare each mother to herself, and the analysis is thus automatically adjusted for all factors that siblings have in common, such as genetics and underlying parental health. Here, we found that the risk of high blood pressure and preeclampsia was nearly doubled in ART pregnancies after transfer of frozen and thawed embryos compared to naturally conceived pregnancies, even in sibling analyses. In study III, we used mediation analyses to investigate whether the increased occurrence of high blood pressure and preeclampsia could explain the increased risk of preterm birth in ART pregnancies. Here, we found that such conditions could explain some (approximately 20%) of the increased risk of preterm birth in ART pregnancies after the transfer of frozen and thawed embryos compared to natural conception, but almost nothing of the increased risk of preterm birth in ART pregnancies after the transfer of fresh embryos.

Overall, the findings in this PhD project can contribute to better counseling and follow-up of women undergoing ART treatment, and they emphasize the need for more knowledge on how ART treatment influences the risk of pregnancy complications.

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To my highly valued friends and family: Thank you so much for supporting me. To my mom, my dad, my great parents, and my sister, thank you for inspiring me to work hard and pursuing my goals, I am forever grateful to you for providing me with what I need to succeed with my endeavors.

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List of papers

Three studies are included in this thesis and will be referred to as study I, II and III.

Study I:
Time trends in placenta-mediated pregnancy complications after assisted reproductive technology in the Nordic countries
Petersen SH, Bergh C, Gissler M, Åsvold BO, Romundstad LB, Tiitinen A, Spangmose AL, Pinborg A, Wennerholm UB, Henningsen AKA, Opdahl S.

Published in American Journal of Obstetrics and Gynecology: August 2020.
Doi: https://doi.org/10.1016/j.ajog.2020.02.030

Study II:
Risk of hypertensive disorders in pregnancy after fresh and frozen embryo transfer in assisted reproduction: A population-based cohort study with within-sibship analysis
Petersen SH, Westvik-Johari K, Spangmose AL, Pinborg A, Romundstad LB, Bergh C, Åsvold BO, Gissler M, Tiitinen A, Wennerholm UB, Opdahl S.

Published in Hypertension: September 2022.
Doi: https://doi.org/10.1161/HYPERTENSIONAHA.122.19689

Study III:
Preterm birth in assisted reproduction: the mediating role of hypertensive disorders in pregnancy
Petersen SH, Elhakeem A, Lawlor DA, Spangmose AL, Pinborg A, Romundstad LB, Bergh C, Åsvold BO, Gissler M, Tiitinen A, Wennerholm UB, Opdahl S.
Ready for submission to Human Reproduction.

List of abbreviations

aOR	Adjusted odds ratio
AMH	Anti-Müllerian hormone
ART	Assisted reproductive technology
BMI	Body mass index
CI	Confidence interval
CoNARTaS	The Committee of Nordic ART and Safety
DAG	Directed Acyclic Graph
DST	Denmark Statistics
ESHRE	European Society of Human Reproduction and Embryology
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
hCG	Human chorionic gonadotropin
HELLP	Hemolysis, elevated liver enzymes, low platelet count
HMG	Human menopausal gonadotropin
HRT	Hormonal replacement therapy
ICD	International Classifications of Diseases and Related Health Problems
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilization
MAR	Missing at random
MCAR	Missing completely at random
MNAR	Missing not at random
NICE	National Institute for Health and Care Excellence
OHSS	Ovarian hyperstimulation syndrome
OR	Odds ratio
PCOS	Polycystic ovary syndrome
PPROM	Preterm prelabor rupture of membranes
RCT	Randomized controlled trial
TSH	Thyroid-stimulating hormone
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

Table of contents

1 Introduction

In 1978, a great medical achievement was a fact; Louise Brown was the first baby born after in vitro fertilization (IVF), also known as assisted reproductive technology (ART) [1]. ART has since helped millions of infertile couples achieve the family they wish for, and the technology and treatment options are still advancing. Research on the treatment and the technology should always be at the core to optimize effectiveness and to ensure patient safety [2]. Is there a higher risk of complications in pregnancies conceived after assisted reproduction compared to naturally conceived pregnancies? If so, to what degree can the higher risk be attributed to the technology and the treatment itself, and what can be attributed to underlying characteristics in the couples? Such questions are fundamentally questions of causality: What would be the outcome in the counterfactual scenario where the clinician put forward a given clinical intervention instead of what actually took place [3]? Although the randomized controlled trial (RCT) is considered the gold-standard for investigating such research questions, carefully designed observational studies can provide crucial insight as well. Knowledge from causal inference, epidemiology and medical statistics can contribute with methodology and frameworks that can aid the investigator towards valid, well-grounded, and sound conclusions.

2 Background

2.1 Infertility

2.1.1 Definition and epidemiology

Infertility is defined as the failure to achieve a clinical pregnancy after regular intercourse without contraception for 12 months [4]. Correspondingly, fertility can be defined as the ability to become pregnant. Related terms include fecundity, which can be defined as the ability to reproduce, i.e., giving birth to a live offspring, and fecundability, which can be defined as the probability that conception will occur during a specified time interval [5]. A synonymous term to infertility is subfertility, but infertility will be used throughout this thesis. When couples who have not been able to conceive within 12 months seek medical help, it is appropriate to initiate infertility evaluation [5]. Infertility is explained by male factors in 20–30% of the couples, female factors in 20–35% of the couples, a combination of male and female factors in 25–40% of the couples, and unexplained in 10–20% of the couples [6]. Worldwide, one out of six couples will experience infertility in one form or another during their reproductive years [6].

2.1.2 Female infertility

The female infertility evaluation should include a detailed medical history, physical examination, and lab workup. Important components of the lab workup include measurement of levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, androgens, Anti-Müllerian hormone (AMH), thyroid-stimulating hormone (TSH) and prolactin. In addition, to assess potential structural causes of infertility, diagnostic imaging and diagnostic hysteroscopy or laparoscopy can be performed [7]. Female infertility can be divided into four main categories [5]:

- Ovarian factor, also known as ovarian disorders. The cardinal symptom of this group is amenorrhea, i.e., no ovulation or menstruation, or oligomenorrhea where ovulation and menstruation is irregular. Both are related to either insufficient estrogen stimulation of the endometrium or a lacking response from the endometrium. If FSH or LH levels are low, hypogonadotropic hypogonadism is present. If FSH and LH are high, hypergonadotropic hypogonadism is present, which includes reduced ovarian

capacity and few available oocytes associated with either premature ovarian failure or menopause. Levels of AMH is a marker of the ovarian reserves, gradually declines with aging, and is undetectable at menopause [8]. Nonetheless, the far most common condition in this category is normogonadotropic hypogonadism in the form of the polycystic ovary syndrome (PCOS). In PCOS, hyperandrogenism is present along with insulin resistance and polycystic ovarian morphology on ultrasound.

- Blocked Fallopian tubes, also known as tubal factor. After pelvic infections (such as sexually transmitted diseases) as well as post-surgery, the Fallopian tubes can become scarred and blocked. Consequently, the oocyte cannot pass from the ovary into the uterus.
- Endometriosis. Through several mechanisms, including inflammation, adhesions, luteal defects, dysfunctional motility patterns in the tubes and uterus, endometriotic lesions negatively impact the chances of pregnancy.
- Uterine factor. These include structural causes such as congenital malformations, septum formations, myomas, polyps, adhesions and adenomyosis.

Female age is the one single factor that most accurately predicts the chances of having a child [5].

2.1.3 Male infertility

When assessing male infertility, the semen sample is the key. Both quantity (volume, sperm concentration) and quality (motility) are assessed. The World Health Organization (WHO) has defined reference values for human semen characteristics [9], which includes the parameters ejaculate volume, sperm concentration, total sperm number, percentage of motile spermatozoa, percentage of progressively motile spermatozoa, and percentage of morphologically normal spermatozoa. Of note, WHO recommends assessment of at least two sperm samples, ideally at least three months apart. Male infertility causes can be divided into four broad categories:

- Sexual disorders. These include lack of libido, erectile dysfunction and anorgasmia.
- Testicular defects in sperm production. These include structural causes like varicoceles, medical treatments affecting the sperm-producing cells like

chemotherapy, genetic and congenital disorders such as Klinefelter's syndrome, testicular retention, testicular cancer, and infections such as mumps orchitis. This category also includes the most common cause of male infertility, idiopathic primary testicular dysfunction.

- Endocrinopathies that affect spermatogenesis. The most common causes in this category are hypothalamopituitary disorders where spermatogenesis is impaired through lack of stimulation from the hypothalamus and/or the pituitary gland, for example because of tumors in the hypothalamus, obesity, or use of anabolic steroids. Less common causes are hyperprolactinemia, thyroid dysfunction, and Cushing syndrome.
- Defects in sperm transportation. These include absence of the ductus deferens, or obstruction due to structural or functional causes such as diabetes, cystic fibrosis, recurrent urogenital infections, and vasectomy. Causes of ejaculatory dysfunction, for example anejaculation and retrograde ejaculation belong here as well.

The above categorization is often simplified into abnormal spermatogenesis (e.g., hypogonadism, anabolic steroids, primary testicular failure, tobacco smoking) and problems related to sperm delivery (e.g., due to urogenital infections, surgery, retrograde ejaculation) as the two main categories [5]. Irrespective of categorization, in at least 50% of the patients with abnormal semen samples, no cause is found, and these cases are therefore considered idiopathic [5].

2.1.4 Temporal trends in infertility

The total fertility rate, defined as the average number of live-born children per woman in reproductive age, has been steadily decreasing in Norway and other Western countries [10]. Statistics Norway reported that the total fertility rate declined from 2.50 in 1970, to 1.48 in 2020 [11]. Furthermore, the mean age of parenthood has increased considerably for both women and men in many high-income countries [10], likely as a result of sociocultural changes and trends with delayed childbearing [12]. Importantly, already by the age of 32, the ovarian reserves are in significant decline, in parallel with increasing prevalence of gynecological conditions that affect fertility [13]. Declining sperm quality regardless of age has been the subject of concern and research [14, 15], and questions remain about the underlying causes

(which are likely multifactorial), and whether there are environmental causes that could be targets for prevention [16, 17]. For both Norwegian men and women, the increasing prevalence of obesity could also contribute to the decline in fertility [18].

2.2 Assisted reproductive technology

2.2.1 The fundamentals of ART

ART encompasses methods designed to help infertile couples achieve a pregnancy and involves handling of human oocytes and spermatocytes in vitro [19]. The following sections will review the key components of an ART treatment cycle, which includes controlled ovarian stimulation, oocyte retrieval and fertilization, and embryo transfer.

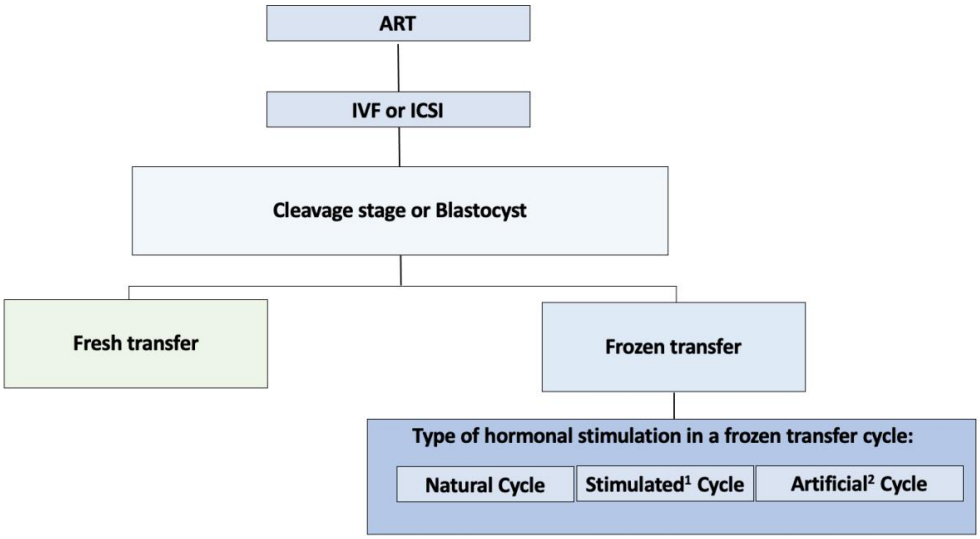

Figure 1. The process of assisted reproductive technology, overview of key components.

[1]Also known as modified .[2]Also known as programmed, HRT (hormonal replacement therapy) and substituted.

2.2.1.1 Controlled ovarian stimulation

In a physiological menstrual cycle, typically only one follicle reaches ovulation. In ART cycles, controlled ovarian stimulation is initiated by the health care provider to increase the number of growing follicles, and for this purpose, either FSH or human menopausal gonadotropin (HMG) is administrated. To avoid spontaneous ovulation, downregulation of endogenous hormone secretion, particularly the LH-surge, is beneficial to ensure full clinician control over the cycle, and for this purpose, gonadotropin-releasing hormone (GnRH) can be administrated

either as an agonist or an antagonist [5]. Following the controlled ovarian stimulation, the development of the growing follicles is then monitored by ultrasound and serum estradiol measurements until a certain number of follicles reach a certain size [20]. The overall aim is that the ovaries should reach an optimal level of stimulation, while minimizing risk of overstimulation.

2.2.1.2 Oocyte and sperm retrieval. Fertilization

Once the optimal level of ovarian stimulation is reached, ovulation is triggered by administration of human chorionic gonadotropin (hCG) or LH [20]. The retrieval of oocytes is typically performed by transvaginal ultrasound-guided follicle aspiration [5]. These retrieved oocyte cells will then rest in an incubator with constant temperature, humidity and gas mixture [5]. Sperm cells are now collected through natural ejaculation or through surgical retrieval. The fertilization itself is achieved using either in vitro fertilization (IVF), where approximately 100,000 spermatozoa are mixed with an oocyte and left to fertilize, or intracytoplasmic sperm injection (ICSI), where a single sperm is injected directly into the oocyte, illustrated in the following figure:

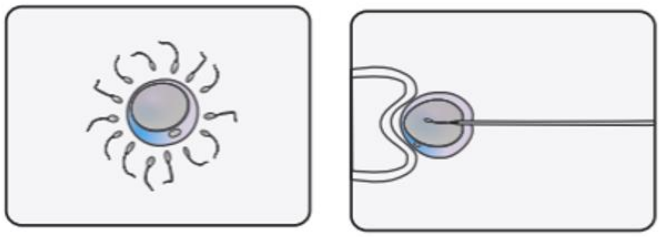

Figure 2. In vitro fertilization (IVF) on the left, intracytoplasmic sperm injection (ICSI) on the right.

Successful fertilization of the oocyte can be confirmed around 18–20 hours later by the identification of two pronuclei and two polar bodies [5]. Around two days after oocyte retrieval, the fertilized oocyte will have cleaved two times, constituting the *cleavage stage* of development. Around five days after oocyte retrieval, the embryo will have reached the *blastocyst stage*, and now consists of at least 100 cells.

2.2.1.3 Embryo transfer

The embryo can be transferred to the uterus at the cleavage stage (2 or 3 days of culturing) or blastocyst stage (5 or 6 days of culturing). Embryo transfer in the same cycle as the retrieval of oocytes is referred to as fresh embryo transfer. If there are surplus embryos from the cycle, these can be frozen (i.e., cryopreserved) for transfer in a later cycle, called frozen embryo transfer. In cycles with fresh embryo transfer, because of the controlled ovarian stimulation, estradiol levels can be many times higher than in a natural cycle [21]. In frozen embryo transfer, a choice is made between transferring the embryo in a natural cycle, in an artificial cycle or in a stimulated cycle [22]. A frozen natural cycle is hormonally similar to a natural cycle, meaning that no medications are used, but this type of cycle is only feasible for women with regular ovulatory cycles. In a frozen natural cycle, careful monitoring is needed through ultrasound scans to verify follicular development and to time the commencement of urine testing for detection of the LH surge [22]. In frozen artificial cycles (also known as frozen programmed cycles, frozen substituted cycles, or HRT (hormonal replacement therapy) cycles) the principle is to suppress natural ovulation, and instead establish an endometrium that is receptive to implantation using exogenous estrogen and progesterone [22]. A key advantage of artificial cycles is a high level of control and flexibility for the clinician and the patient [22]. Lastly, in a frozen stimulated cycle, ovulation is induced by drugs, and is therefore also called ovulation induction frozen cycle [22].

When the cycle regimen has been chosen and embryos are ready for transfer, the clinician transfers the embryo into the uterus using a plastic catheter through the cervix under ultrasound-guidance. An important clinical decision is how many embryos to transfer, either single, double, or multiple embryo transfer. After the embryo transfer, hormonal support with progesterone is administered for 2–10 weeks to optimize the endometrial lining during the luteal phase to promote implantation [5, 19]. In the Nordic countries, after the confirmation of a viable pregnancy by ultrasound in gestational weeks 6–8, the woman follows the same publicly financed antenatal program as other pregnant women [23].

2.2.2 Major developments in ART since 1978, and ART today

Several important changes in practice have taken place since the first child conceived by ART was born in 1978. The following section will summarize the most important developments

and highlight the most important clinical implications, and finally summarize how ART treatment is practiced today.

2.2.2.1 IVF and ICSI

In 1976, the first pregnancy after ART conception was reported, and in 1978, the first baby conceived by ART was born [24, 25], specifically by IVF. The IVF technique has proven a very effective treatment for female infertility. The breakthrough for treatment of male infertility in the form of the ICSI technique was introduced and developed in the late 1980s/early 1990s, and the first pregnancies after ICSI fertilization were reported in 1992 [26]. Although more intervening because the embryologist must choose a sperm and then injecting it into the egg (thereby bypassing some natural selection processes), the ICSI technique would turn out to be very effective, and overall, data on long-term health outcomes including cognitive and motor development are reassuring [27, 28]. Thus, from 1992 onwards, health care providers had effective techniques to treat both female and male infertility.

2.2.2.2 Frozen embryo transfer and single embryo transfer

A major limitation in the early days of ART treatment was that there were no means to store embryos for transfer in cycles after the controlled ovarian stimulation cycle. Consequently, the health care provider typically transferred multiple embryos into the uterus, to increase the chance of at least one successful implantation. This led to a high rate of multifetal pregnancies (twins, triplets, or even higher multiples), which are high-risk pregnancies irrespective of ART conception [29, 30]. Freezing embryos for storing has been known for decades, and in 1983, the first successful pregnancy after transfer of a cryopreserved embryo was reported [31]. It would take many years before frozen embryo transfer gained any popularity, mostly due to the low embryo survival rate from the slow-freezing technique (due to the ice crystal formation on the surface of the embryo) of the early days of cryopreservation [32]. The breakthrough for cryopreservation would be introduced as late as in 2008 in the form of the vitrification technique, which over the years reached a survival rate close to 100% of the thawed embryos [32, 33]. Finally, clinicians had a reliable option of storing embryos for transfer in later cycles, and this in turn facilitated the highly favorable approach of single embryo transfer [33, 34]. Figure 3 shows the consequent developments in multiple pregnancies (twins, triplets, and quadruplets) after ART as a percentage of all ART

pregnancies, and the proportion of ART conceived children born after frozen embryo transfer in the Nordic countries:

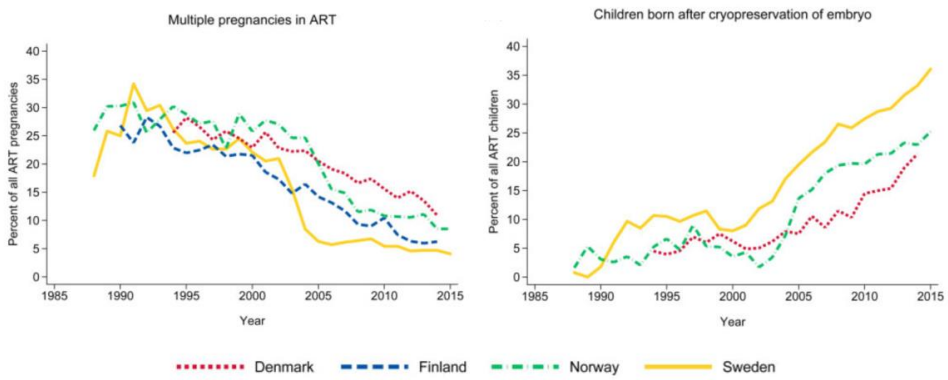

Figure 3. Proportion of multiples (multifetal pregnancies) in ART conceived pregnancies, and the proportion ART children born after frozen embryo transfer.

Reproduced from "Data Resource Profile: Committee of Nordic Assisted Reproductive Technology and Safety (CoNARTaS) cohort" by Opdahl et al. 2020 [35], with permission from Oxford University Press, license number 5462581025966.

Another great advantage of frozen embryo transfer was that clinicians could reduce the risk of one of the most severe complications of ART cycles, the ovarian hyperstimulation syndrome (OHSS) [36]. OHSS occurs due to the hormonal protocols administered during the ART cycles, resulting in enlarged ovaries (>7 cm) and secretions of vascular endothelial growth factor (VEGF) [5]. The hCG used to trigger ovulation can instead trigger the hyperstimulation syndrome, where VEGF results in angiogenesis, with leakage of fluid from the new vascular tissue into the third space, leading to abdominal bloating in the form of ascites. [37]. OHSS is reported to occur in about 1–3 % of fertility treatments in the Nordic countries [5], and is associated with severe morbidity (e.g., renal failure and thromboembolic events) and mortality [6, 38].

Despite the many advantages of frozen embryo transfer, research suggests that risk of hypertensive disorders in pregnancy is higher after this type of transfer compared to both natural conception and fresh embryo transfer [39, 40], whereas risk of preterm birth (birth <37 weeks of gestation) and low birth weight (birth weight <2500 grams) is lower compared to fresh embryo transfer [41].

2.2.2.3 *Blastocyst transfer and cleavage stage transfer*

Another important development in ART treatment was the transition from cleavage stage transfer to blastocyst transfer. Figure 4 shows the difference in morphology of the two stages.

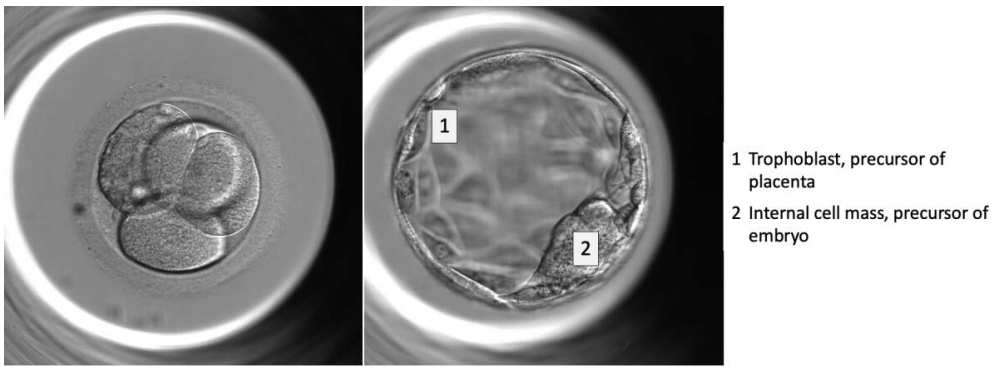

Figure 4. Cleavage stage on the left, versus blastocyst transfer on the right. Credit: Peter M Kragh, Spiren Fertility Clinic, Trondheim.

Although the first report of a pregnancy after blastocyst transfer was published as early as in 1995, cleavage stage was the norm for a long time [42]. This was partly because the uterus was considered the optimal environment for the developing embryo, although in the non-ART setting, implantation typically occurs at the blastocyst stage, not at the cleavage stage. Another important reason was that the in vitro environment (including culture media used) would later turn out to have been more suited to cleavage stage embryos, and thus, there was a low proportion of embryos growing past this stage [42]. Following improvements in culture media and advancements in the in vitro handling of embryos, the option of culturing to the blastocyst stage was facilitated. There are several important advantages with blastocyst transfer [42]. Firstly, it is now considered physiologically premature for the 2- or 3 day-embryo to be exposed to the uterine environment, in particular if the endometrium is hyper-stimulated [42]. The second central argument for blastocyst transfer is that by keeping the embryos in vitro for a longer time, we can take advantage of the self-selection of embryos, where only the most viable embryos will develop past the cleavage stage [42]. Lastly, by allowing for in vitro handling for 4–5 days, the embryologist has more opportunity for observation, and hence more possibilities of scoring and choosing the best embryos for subsequent transfer [42]. Indeed, a policy of blastocyst transfer instead of cleavage stage transfer has been associated with higher pregnancy rates [43]. However, blastocyst culture is also associated with potentially negative outcomes such as a higher rate of monozygotic twins

and a higher risk of preterm birth, perhaps because of longer exposure to the culture media and in vitro handling [44, 45].

2.2.2.4 ART today in the Nordic countries and in the world

So far, over 9 million ART conceived children have been born worldwide [6]. The use of ART treatment is increasing, due to higher availability but also for sociocultural reasons as couples now increasingly tend to postpone having children to ages with lower fertility [46]. According to the European Society of Human Reproduction and Embryology (ESHRE), around one million treatment cycles are reported each year in Europe, resulting in over 200,000 ART conceived live births per year [47]. Worldwide, over 2.5 million ART cycles are performed every year, resulting in over 500,000 deliveries annually [48].

In the Nordic countries, the ART quality registries and ART databases provide the data on ART treatment. Today, approximately 5% of the Nordic birth cohorts are conceived after ART treatment [35]. Importantly, ART treatment is highly subsidized in the public health care systems in the Nordic countries [35]. The number of Nordic children born after some form of ART conception is higher than the official statistics, due to "reproductive tourism", where Nordic couples or single women go abroad to seek ART treatment. If the ART treatment takes place abroad, but the delivery takes place in the mother's country of residence, this will be registered as a naturally conceived delivery (except in Finland and Norway, where the mother can inform the midwife at delivery) [35]. An important motivation for going abroad is that some of aspects of ART treatment has had a conservative legislation in the Nordic countries compared to other European countries [5, 49]. This includes legislation regarding egg donation, which has been allowed in Denmark, Finland, and Sweden for many years, but which was only recently allowed in Norway [5]. Similarly, the legislation of ART treatment to also include single women and same sex female couples were established at different times in the Nordic countries [5].

In Europe, ICSI fertilization accounted for 35% of ART cycles in 1997, 50% in 2002, and around 70% in 2018 [47, 50]. In the Nordic countries, where the technique is mainly used for male infertility, ICSI fertilization accounted for around 50–55% of ART cycles in 2014/2015 [35]. Frozen embryo transfers are increasingly common, and in 2018 in Europe, the proportion of

frozen cycles of all ART cycles was 36% compared to around 15% in 2008 and around 10% in 1997 [47]. Indeed, following reports of the advantages of frozen embryo transfer, some clinicians and researchers have advocated for and implemented the *freeze all embryos approach*, where all embryos are cryopreserved electively, without an initial fresh transfer [51, 52]. Single embryo transfer is now the norm in the Nordic countries, but in Europe and in the rest of the world, multiple embryo transfer (mainly double) is still widely used [35, 47, 53]. Nonetheless, 2018 was the first year in Europe when most transfers involved the transfer of one embryo [47]. Blastocyst transfers are still on the rise, and in 2018 in Europe, 50% of fresh transfers were blastocysts (compared to 44% in 2017), and in 74% of frozen cycles, blastocysts were transferred (compared to 64% in 2017) [47].

2.3 Placenta-mediated pregnancy complications

The placenta is a unique organ, critical for the fetal development, but also important for maternal health. As the interface between the mother and the developing fetus, the placenta serves several crucial functions, most notably transfer of oxygen and nutrients from the mother to the fetus, and transfer of carbon dioxide and waste products from the fetus to the mother. Also, the placenta is an active endocrine agent by its secretion of hormones that modulates both the maternal and fetal organ systems. The following sections will discuss the aspects and phases of *placentation*, referring to the formation and growth of the placenta, that are believed to be tightly linked to the "Great Obstetrical Syndromes", including placenta-mediated pregnancy complications and preterm birth [54]. Placenta-mediated pregnancy complications include hypertensive disorders in pregnancy, placental abruption, and placenta previa. These clinical conditions will be reviewed, with particular emphasis on what is known about pathophysiology and pathogenesis, temporal trends in the general population, and current medical management.

2.3.1 Placentation

Placentation starts with the implantation of the developing embryo at 6–7 days after fertilization [55]. To ensure nutrition and a favorable environment for the embryo, part of the endometrium then differentiates into secretory cells called decidual cells, the process known as the *decidual reaction*. At this point, the embryo has normally reached the blastocyst stage,

consisting of the inner cell mass, the *embryoblast*, and an outer layer, the *trophoblast*. For the future placenta, the trophoblast gives rise to the placental epithelium, while the embryoblast is the precursor for the placental mesenchyme as well as the fetal vascular system [55]. Figure 5 illustrates the further development:

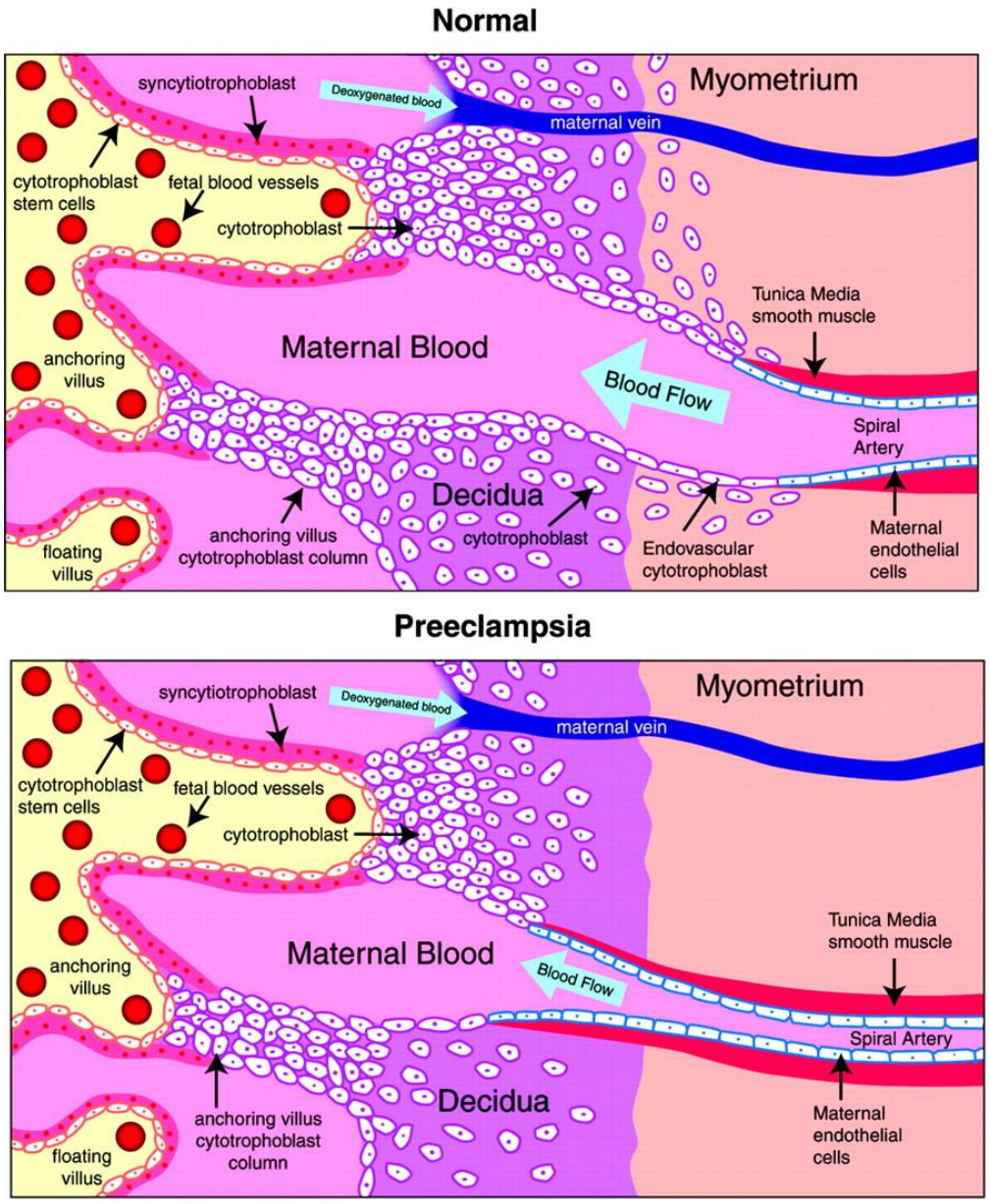

Figure 5. Abnormal placentation in preeclamptic pregnancies.

Reproduced from "Circulating Angiogenic Factors in the Pathogenesis and Prediction of Preeclampsia", by Lam et al. 2005 [56], with permission from Wolters Kluwer Health, Inc. License number 5462560454653.

The trophoblast provides the stem cells of the placenta, the *progenitor cytotrophoblast cells*, which can further develop along two principal paths; the *villous* cytotrophoblast path or the *extravillous* cytotrophoblast path [57]. The villous cytotrophoblast eventually develops into the syncytiotrophoblast (also known as the outer cellular layer), which is a specialized epithelial layer that will facilitate transportation of gases and nutrients and produce hormones that will regulate both the maternal and fetal organ systems. The latter, the extravillous cytotrophoblast (also known as the inner cellular layer), will provide the solution for the fundamental problem that must be overcome; the high pressure in the maternal blood vessels and the vasomotor control they are under. Around four to five weeks of gestation, a *proliferative component* of the extravillous cytotrophoblast will be at the base, and an *invasive component* at the distal part of the column [57]. The invasive component of the extravillous trophoblast will then invade the decidua and the spiral arteries of the mother, displacing the vascular smooth muscles cells and the myometrium of the spiral arteries, transforming them into wide and dilated uteroplacental arteries. Finally, the endometrial veins together with the now reshaped spiral arteries constitute the maternal sinusoids, which will give rise to blood flow into a low resistance vascular network, establishing the uteroplacental circulation.

2.3.2 Hypertensive disorders in pregnancy

Hypertensive disorders in pregnancy include gestational hypertension, preeclampsia, chronic hypertension with superimposed preeclampsia and eclampsia. Gestational hypertension is diagnosed when there is new-onset hypertension presenting after 20 weeks of gestation and occurs in 4–6% of pregnancies in the Nordic countries [58]. Preeclampsia is diagnosed when new-onset hypertension is accompanied by new-onset proteinuria or other signs of end-organ damage (e.g., thrombocytopenia, renal insufficiency, impaired liver function), and occurs in around 3-4% of pregnancies in the Nordic countries [58]. An important clinical distinction is made between early-onset preeclampsia, and late-onset preeclampsia, diagnosed before and after 28 weeks of gestation, respectively. The most serious hypertensive disorders are the HELLP-syndrome and eclampsia. HELLP-syndrome is characterized by hemolysis, elevated liver enzymes and low platelet counts. Eclampsia is a rare and dangerous disorder where the hypertension during pregnancy co-exists with generalized seizures (alternatively at delivery or

during the first week after delivery), where these seizures are not explained by a neurological cause, affecting 5 in 10,000 pregnancies in Scandinavia [58].

Studies on temporal trends of hypertensive disorders in pregnancy indicate that the occurrence might be declining in the Western world, including in the Nordic countries [59, 60].

The exact pathogenesis of hypertensive disorders in pregnancy is still incompletely understood despite extensive research efforts. The so-called two-stage placental model of preeclampsia can illustrate the current understanding of the disease, including how risk factors in the mother and pregnancy-specific characteristics interact and how defective placentation and the maternal clinical syndrome are connected:

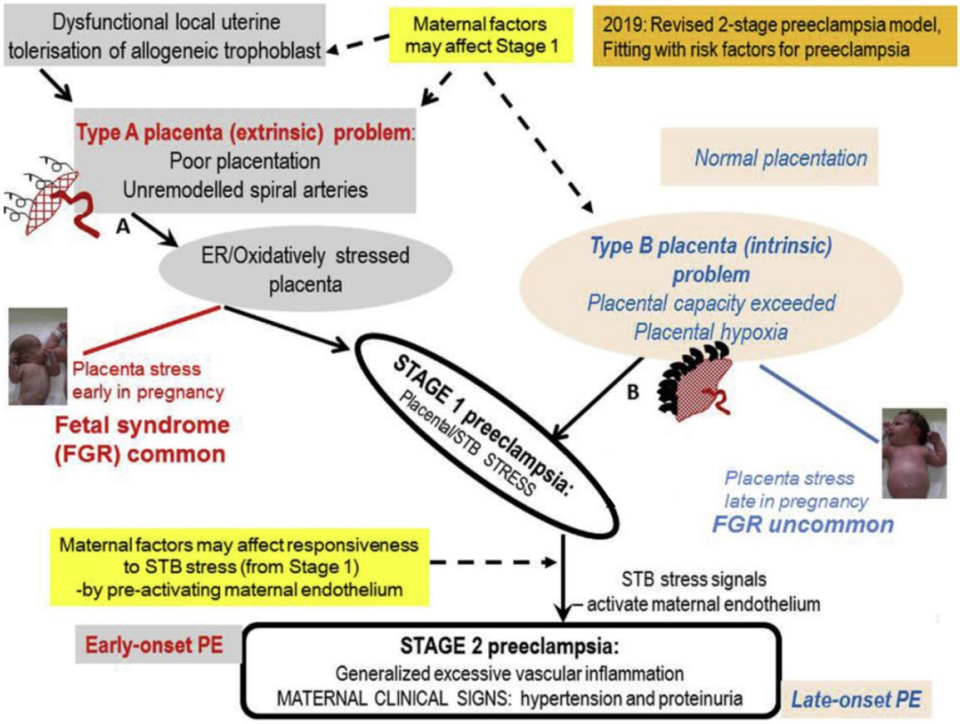

Figure 6. The 2-stage preeclampsia model, integrating maternal risk factors and two main placental pathways to clinical preeclampsia.

Abbreviations: ER, endoplasmic reticulum; FGR, fetal growth restriction; PE, preeclampsia; STB, syncytiotrophoblast.

Simplified and adapted from "The two-stage placental model of preeclampsia: An update" by Staff 2019 [61], with permission from Elsevier under the terms of the Creative Commons Attribution-NonCommercial-No Derivatives License (CC BY NC ND).

In the two-stage model, Stage 1 represents the placental dysfunction stage (with syncytiotrophoblast stress), and Stage 2 represents the maternal clinical phenotype [61]. During Stage 1, the stressed placenta secretes several pro-inflammatory factors into the maternal circulation, resulting in generalized vascular inflammation with endothelial dysfunction and vasoconstriction. In turn, this leads to hypertension, leakage of proteins through the renal glomeruli, as well as other signs of end-organ damage, together constituting Stage 2 [61]. In the two-stage model, two main pathways (A and B) can lead to the placental dysfunction of Stage 1. In Pathway A, called the *extrinsic placental pathway*, there is typically evidence of poor placentation, with defects in the extravillous trophoblast invasion, where the spiral arteries are not invaded at all or only superficially invaded, leading to reduced blood flow into the intervillous space, which is believed to stress the placenta [61]. Pathway A is associated with the fetal growth restriction syndrome, where the fetuses show evidence of poor growth, and is also the pathway which has the strongest association to early-onset preeclampsia [61]. In Pathway B, called the *intrinsic placental pathway*, there is little evidence of poor placentation, and this pathway is rather believed to be due to some intra-placental cause of malperfusion, for instance due to postmature and large placentas which have reached their size limit late in the pregnancy [61]. Pathway B is associated with late-onset preeclampsia, and the growth of the fetus is typically not affected. Important risk factors for developing hypertensive disorders in pregnancy include pregestational (maternal) factors like primiparity, advanced maternal age, chronic hypertension, renal disease, a history of hypertensive disorders in pregnancy, overweight, PCOS and autoimmune diseases (e.g., systemic lupus erythematosus), and pregnancy-specific factors like large placentas, fetal growth restriction, and ART conception [58]. The two-stage model incorporates these different risk factors in the following way: Maternal risk factors can plausibly affect placentation (e.g., through dysfunctional local uterine tolerance of the allogenic trophoblast) and the size of the placenta (e.g., if obese women have larger placentas), which leads to Stage 1 of preeclampsia [61]. Once this stage is reached, maternal risk factors might also play a key role in how susceptible the maternal vascular system is to the systemic inflammation induced by the stressed placenta, leading to Stage 2 of preeclampsia [61].

Currently, the only definitive treatment of preeclampsia is to induce the birth to deliver the placenta [58]. Careful consideration of costs and benefits for both the mother and the fetus is needed before inducing delivery. Delaying delivery may be beneficial for the fetus by a decrease in risk of complications related to being born preterm, but may at the same time increase the risk of further complications of preeclampsia like eclampsia, cerebral hemorrhage, kidney failure, liver damage, fetal growth restriction, placental abruption and stillbirth [58]. According to the National Institute for Health and Care Excellence (NICE) guidelines, women at high risk of developing preeclampsia should be prescribed low-dose aspirin as prophylaxis starting from week 12 [62], as it has been shown to lower risk of preeclampsia in multiple randomized trials [63].

Studies of long-term health for women diagnosed with a hypertensive disorder during pregnancy show higher risk of hypertension, renal disease, and cardiovascular disease later in life [64, 65, 66]. Whether having a hypertensive disorder actually increases the risk of such outcomes, that is, whether the association is causal, remains unanswered [58]. Furthermore, children born after preeclampsia have higher blood pressure and higher BMI compared to non-preeclamptic pregnancies [67], a higher risk of ischemic heart disease and stroke [68], and a higher risk of future chronic diseases [58].

2.3.3 Placental abruption

Placental abruption is a life-threatening condition, affecting around 3–10 per 1000 pregnancies [69]. The condition is characterized by premature detachment of the placenta from the uterus, leading to hemorrhage with potential lethal consequences for both mother and fetus. The most important symptoms and clinical findings include abrupt onset of vaginal bleeding, abdominal pain, uterine stiffness, and uterine contractions, as well as changes in fetal heart rate.

Placental abruption shares many of the same risk factors as preeclampsia [70], including a history of placental abruption, hypertensive disorders in pregnancy, uterine anomalies, and multifetal pregnancy [71, 72, 73]. The pathogenesis is unknown, but some possible pathways include abdominal trauma and long-standing chronic inflammation leading to the physical detachment [74], possibly in combination with suboptimal and/or superficial trophoblast

invasion and placentation [75]. Although the exact etiology and pathogenesis is unknown, the physical detachment of the placenta is the immediate result of rupture of maternal blood vessels (arterial or venous) in the decidua basalis.

Placental abruption can be categorized according to severity, mild abruption (around 1/3 of cases) versus severe abruption (around 2/3 of cases) [76]. Features suggesting severe placental abruption include maternal complications such as disseminated intravascular coagulation, shock and renal failure, and in-hospital death, and fetal/neonatal complications such as fetal growth restriction, death, or preterm birth. Placental abruption can also be categorized according to gestational age, where around 40-60% of neonates born after placental abruption are born preterm [77].

Earlier research on temporal trends of placental abruption indicates that the occurrence might be declining in the Western world, including in the Nordic countries [69].

2.3.4 Placenta previa

Placenta previa is diagnosed when the placenta covers the internal opening of the cervical canal. Worldwide, occurrence of placenta previa approximates 4 to 6 per 1000 pregnancies [78]. The most important symptom is painless antenatal bleeding, but most cases are asymptomatic and are found during routine ultrasound during early pregnancy [79]. Most cases of placenta previa that are detected by ultrasound in weeks 18–20 resolve spontaneously as the uterus grows during the remaining duration of pregnancy [79]. If the placenta still covers the cervical canal later in the pregnancy, delivery by cesarean section is considered mandatory to avoid massive hemorrhage. Major risk factors include a history of placenta previa, a history of cesarean section, and multifetal pregnancy, while moderate risk factors include advanced maternal age, increasing parity and smoking [79]. The pathogenesis of placenta previa is unknown, but hypotheses include defective implantation of the trophoblast because of uterine areas with sequalae from surgery, as well as a surface area hypothesis, in which larger and multiple placentas (e.g., in multifetal pregnancy) will be more likely to cover the cervical os [80].

Earlier research on temporal trends of placenta previa shows that the occurrence might be increasing in the Western world [81].

2.4 Knowledge gap and rationale

The following chapter will review the current understanding of obstetric health and perinatal outcomes after ART conception and present the rationale for each of the studies in this thesis.

2.4.1 Confounding in studies of ART conceived pregnancies vs naturally conceived pregnancies

Studies have shown a higher risk of adverse perinatal outcomes in ART conceived pregnancies compared to naturally conceived pregnancies. Studies comparing infants conceived by ART to naturally conceived infants have reported a higher risk of low birth weight and preterm birth [82, 83, 84]. Furthermore, a higher risk of obstetric complications has also been reported in the literature, including hypertensive disorders in pregnancy [85], placental abruption, placenta previa and gestational diabetes compared to naturally conceived pregnancies [84, 86]. The higher risk of both perinatal and obstetric adverse outcomes is partly attributable to the higher occurrence of multifetal pregnancy (twins, triplets) after ART treatment, but the higher occurrence of adverse outcomes persists when analysing singleton and twin pregnancies separately [84, 86]. Some have argued that the higher risk of adverse outcomes in ART conceived singleton pregnancies compared to naturally conceived singleton pregnancies might be due to the *vanishing twin syndrome*, where the pregnancy starts out as multifetal, but reduces to a singleton pregnancy after spontaneous loss of one or more embryos [87, 88]. Nonetheless, it seems possible that the ART treatment could impact the process of placentation and hence the risk of placenta-mediated pregnancy complications through several mechanisms (regardless of multifetal pregnancy). The hormonal protocols could be of importance, as studies have shown that the estradiol concentrations in ART cycles may be up to 40 times higher than in natural cycles and remain high throughout the pregnancy compared to naturally conceived pregnancies [21]. Furthermore, earlier research has revealed differences in the perinatal and obstetric outcomes according to which ART method is used. There is a higher risk of hypertensive disorders after frozen embryo transfer in artificial cycles compared to frozen embryo transfer in natural cycles [89, 90], and a higher risk of placental

abruption and placenta previa after blastocyst transfer compared to cleavage stage transfer [91].

A major challenge when trying to explain the higher risk of adverse outcomes in ART conceived pregnancies is to disentangle the effect of the ART treatment from confounding, i.e., common causes of the exposure, in our case ART treatment, and the outcome of interest, in our case complications in pregnancy. Important confounding factors in this context are that women who need ART treatment are older and are more often primiparous. The investigator can adjust for these factors in statistical models to the extent that the information is available and accurate. Another important confounder is the cause of infertility [92]. Indeed, important causes of infertility, for example endometriosis, PCOS and uterine anomalies are themselves associated with adverse pregnancy outcomes, irrespective of ART treatment [93, 94, 95, 96, 97]. A systematic review from 2013 found a higher risk of adverse pregnancy outcomes in subfertile women who conceived without ART treatment [98]. Importantly, although randomized trials are considered the gold-standard in clinical research for controlling confounding, sample size would have to be considerable to detect differences when the outcome of interest is rare [99]. Furthermore, randomized trials of ART treatment cannot randomize and compare to natural conception, which is needed to understand the contribution from infertility.

2.4.2 Rationale for time trend analysis

As described in earlier sections, several important temporal changes in ART treatment have taken place in the Nordic countries, including the increasing use of blastocyst transfer, frozen embryo transfer, and single embryo transfer [35]. Such changes could influence the risk of adverse outcomes and complications. In parallel, it seems likely that the population seeking ART treatment is different today compared to thirty years ago, amidst demographical developments described in section 2.1.4 [10, 50]. In 2015, a Nordic study reported that risk of adverse perinatal outcomes in ART conceived pregnancies had declined from 1988 to 2007 in the Nordic countries [100]. Whether this development also applied to obstetric outcomes was unknown prior to this PhD project. Notably though, time trends could also be influenced by temporal changes in diagnostics and detection in the antenatal screening programs, and by temporal changes in registration of the diagnoses in the national health registries [23, 101].

Therefore, before going into more advanced analyses like sibling comparisons and mediation analysis, we aimed to first investigate the time trends in occurrence of placenta-mediated pregnancy complications.

2.4.3 Hypertensive disorders in pregnancy, the sibling comparison design as a tool for causal inference

Prior to this PhD project, observational studies had reported a higher risk of hypertensive disorders in pregnancy after ART conception, in particular after frozen embryo transfer, but these studies might have been influenced by residual confounding [41, 102]. A study design that has been particularly helpful in disentangling parental factors (e.g., causes of infertility) from ART treatment factors is the within sibship analysis, where the investigation benefits from the fact that some couples conceive using different conception methods [103]: Couples who first conceive naturally may need ART treatment later to have more children, and many couples who achieve a pregnancy using ART treatment, will conceive naturally later [104, 105]. In within sibship analyses, each mother serves as her own control, which may strengthen causal inference [106]. This is because the approach controls for both observed and unobserved confounding factors that are shared by the siblings, such as genetics, preconception lifestyle and health, and socioeconomic status on the assumption that these confounders are at the family level, not at the individual level [103]. If the positive associations between ART conception and adverse outcomes found at the population level persist within sibships, this may indicate that treatment factors as opposed to parental preconception factors are responsible. As an example, a Norwegian study with deliveries from 1984 to 2006 comparing perinatal outcomes in ART conceived children to unrelated naturally conceived children (population level analysis) and then to their naturally conceived sibling(s), separately [107]. The authors found an elevated risk of preterm birth and lower birth weight in the population level analysis, but these associations were strongly attenuated within the sibships, suggesting that much of the adverse outcomes among ART conceived children could be attributed to parental factors leading to infertility and not the ART treatment itself. Another sibling design study compared fresh embryo transfer and frozen embryo transfer to natural conceptions, showing that fresh embryo transfer singletons are smaller for gestational age, frozen embryo transfer are larger for gestational age, and both ART methods have a higher risk of preterm birth [108]. Prior to this PhD project, only 2 small studies, both including births

up to 2007, had investigated risk of hypertensive disorders in pregnancy following ART conception using sibling comparison designs. A registry-based study from the Nordic countries showed that risk of hypertensive disorders in pregnancy was higher following frozen embryo transfer compared to fresh embryo transfer in 100 double discordant pairs of singleton siblings born between 1988 and 2007 [40]. In contrast, a Dutch cohort of singletons born between 1999 and 2007 comparing any ART treatment versus natural conception found no clear association between ART treatment and hypertensive disorders in pregnancy [109]. Crucially, while sibling comparisons can strengthen causal inference by accounting for unknown and unmeasured confounders that are shared by the siblings, the investigator should be careful because the analyses may introduce new problems, for example in the form of *carryover effects* (also called *contagion effects*), where the exposure or the outcome in the first sibling influences subsequent siblings [103, 110]. Also, sibling comparisons can be more severely biased by confounding by factors that differ between the siblings, (i.e., non-shared confounding) and misclassification (i.e., information bias) than the conventional population level analysis [111].

2.4.4 Preterm birth – What is the contribution from pregnancy complications?

The higher risk of preterm birth is arguably one of the most severe adverse outcomes associated with ART treatment, given the short- and long-term implications of being born preterm [112, 113, 114]. Preterm birth is still to this day strikingly difficult to prevent and predict [115]. In general, there are three main categories of preterm birth [116]:

(1) 30–35% are medically indicated preterm births, meaning delivery is medically induced or the fetus is delivered with prelabor cesarean section for maternal or fetal indications, generally due to complications in the pregnancy where the health of the mother or fetus is at risk if birth is not accelerated.

(2) 40–45% are after spontaneous preterm labor with intact membranes, defined as regular contractions accompanied by cervical change at less than 37 weeks of gestation. Unfortunately, the pathogenesis of spontaneous preterm labor is incompletely understood, but can be viewed as early activation of the physiological labor, or a consequence of pathological triggers [116].

(3) 25–30% follow preterm prelabor rupture of membranes (PPROM), defined as the spontaneous rupture of membranes at less than 37 weeks of gestation at least one

hour before the onset of contractions [116]. The exact cause of the rupture in a given pregnancy is often unknown, but asymptomatic intrauterine infection is often involved. If labor does not start, a common complication of PPROM is intrauterine infection since the barrier function of the membrane is lost.

Together, the latter two categories are labelled spontaneous preterm births.

A potential explanation for the higher risk of preterm birth after ART treatment could be the higher occurrence of obstetric complications like hypertensive disorders, placental abruption, and placenta previa. These complications are central causes of preterm birth, partly because they can lead to medically indicated preterm birth, and because these complications in themselves are associated with spontaneous preterm birth [117, 118, 119]. However, the causal relationship between ART treatment, obstetric complications and preterm birth is not so straightforward. Obstetric complications in this context are not confounders, but rather mediators, i.e., they are intermediate variables on the causal pathway between ART treatment and preterm birth:

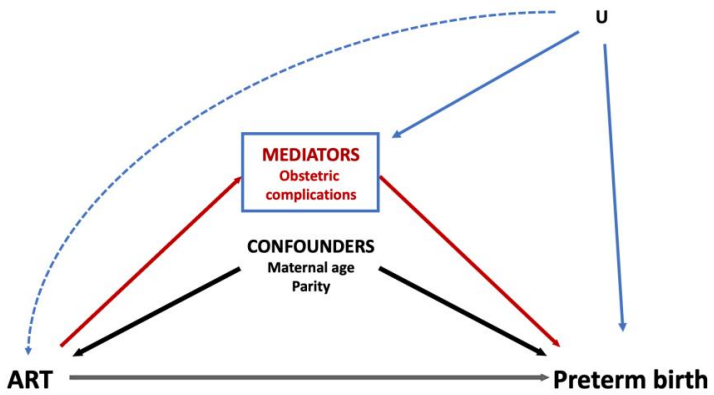

Figure 7. Simplified directed acyclic graph (DAG) showing that obstetric complications (e.g., preeclampsia) are mediators rather than confounders. The U indicate unknown common causes of the mediator and the outcome, and the dotted line indicates that collider bias might arise if the investigator conditions on obstetric complications, because ART and U collide into the mediator.

Hence, if the goal of the investigator is to assess the direct effect of ART on preterm birth not through obstetric complications, simply adjusting for obstetric complications could induce

spurious associations because of so-called collider bias [3]. Briefly, when adjusting for a mediator, the investigator risks opening backdoor paths because of mediator-outcome-confounders, i.e., common causes of obstetric complications and preterm birth [120]. Thus, a different analytical approach is needed when assessing research questions that involve adjustment for potential intermediate variables, also in reproductive and obstetrical epidemiology [121]. Recently, the epidemiological research community has developed improved methods for assessing the relative contribution from an intermediate variable on the causal pathway between some exposure and the outcome of interest [120, 122, 123]. These new methods are built on a counterfactual framework and are called mediation analyses.

3 Aims of the thesis

The overall aim of this thesis was to investigate the occurrence of pregnancy complications after ART treatment.

3.1 Aims of study I

The objective of study I was to investigate temporal changes in risk of placenta-mediated pregnancy complications in ART conceived pregnancies compared to the background population of naturally conceived pregnancies across three decades of ART treatment in the Nordic countries.

3.2 Aims of study II

In study II, a within sibship analysis, we aimed to investigate if there is a higher risk of hypertensive disorders in pregnancy following transfer of fresh and frozen embryos compared to naturally conceived pregnancies.

3.3 Aims of study III

In study III, a mediation analysis, we aimed to investigate to what degree the higher risk of hypertensive disorders in pregnancy could explain the higher risk of preterm birth.

4 Methods and materials

4.1 Data sources

The Committee of Nordic ART and Safety (CoNARTaS) cohort comprises all deliveries in Denmark (1994–2014), Finland (1990–2014), Norway (1984–2015) and Sweden (1985–2015) [35]. Data were obtained from the nationwide Medical Birth Registries in all countries, the national patient registries in Denmark and Finland, and the ART registries or -databases in each country and pooled into a Nordic cohort, in which linkage between the registries was achieved using the mother's national identity number [101]. The Medical Birth Registries record data on all deliveries, sent to them from the delivery units, with maternal information such as age, parity, smoking (yes/no), offspring information like birthweight, gestational age, vital status, conception method (ART/natural conception) and plurality (e.g., singleton pregnancy, twin pregnancy, triplet pregnancy) as well as obstetric information such as occurrence of various complications and medical management. The original purpose of the Medical Birth Registries was to monitor and surveil the occurrence of birth defects, and today these registries are invaluable resources of medical research data [124, 125]. The national patient registries are primarily administrative, to which hospitals submit information on inpatient and outpatient diagnoses and managements. The purposes of the national patient registries include monitoring of hospital and health service utilization, providing tools for health care planning [101]. The ART quality registries have been established to improve the medical aspects of the ART treatment, and to enable research.

4.2 Ethics and data confidentiality

The studies in this PhD project only utilize observational data from registries, and hence no interventions or communication with the study participants. In Denmark and Finland, no ethical approval from ethical bords is required when conducting observational studies from registry data. In Norway, the project has been approved by the Regional Committee for Medical and Health Research Ethics (REK Nord, REK 2010/1909). In Sweden, the project has been approved by the Ethical committee in Gothenburg (Dnr 023-09, T431-09, Dnr 214-12, T-422-12, T-516-15 and T-233-16). To ensure confidentiality, the registries encrypt the all the unique identification numbers before sending data to different research projects. The

CoNARTaS data are stored in a server administered by Denmark Statistics (DST). The researchers gain access to data and analysis software with a remote connection to the DST server, using a personal password as well as a personal, one-time code token. Only result files (e.g., tables, figures) containing no individual-level identifiable data (microdata) are allowed to be exported from the server.

4.3 Study variables

Exposures, outcomes, covariates, and sensitivity analyses for each study are summarized in Table 1.

	Study I	Study II	Study III
Exposure	Time period, and ART-conception compared to natural conception	Fresh and frozen embryo transfer compared to natural conception	Fresh and frozen embryo transfer compared to natural conception
Outcomes	Hypertensive disorders in pregnancy, placental abruption, placenta previa	Hypertensive disorders in pregnancy	Preterm birth (<37 weeks) Spontaneous preterm birth Medically indicated preterm birth Very preterm birth (<32 weeks) Extremely preterm birth (<28 weeks)
Main covariates	Maternal age, parity, country	Maternal age, parity, birth year, country	Maternal age, parity, birth year, country. Hypertensive disorders in pregnancy as a mediator
Sensitivity analyses	BMI & smoking Primiparous Adjustment for history of cesarean section Adjustment for culture duration Adjustment for cryopreservation	BMI & smoking Full siblings < 3 years birth interval IVF (÷ICSI) Single embryo transfers Blastocyst transfers	BMI & smoking Primiparous IVF (÷ICSI) Single embryo transfers Blastocyst transfers Different mediator variables

Table 1. Summary of exposures, outcomes, and covariates in study I, II and III

4.3.1 Exposure variables

In study I, ART conception overall was the exposure, while in study II and III, exposures were defined as ART conception with either fresh embryo transfer or frozen embryo transfer. In all three studies, pregnancies after ovulation induction and insemination were coded as natural conceptions and pregnancies with no registration of ART conception were considered as naturally conceived, and thus non-exposed. In Denmark, ART conception was confirmed from the national ART quality registry (established 1994). This registry also provides information on specific ART method (IVF fertilization vs ICSI fertilization, culture duration, fresh embryo transfer vs frozen embryo transfer). In Finland, there is no national ART quality registry, but in

their Medical Birth Registry, a dichotomous variable could be crossed off for ART conception from 1990 and onwards. In Norway, ART conception is noted on the Medical Birth Registry notification form based on what the mother tells the healthcare provider. Also, Norwegian ART clinics (both public and private) provide information (IVF fertilization or ICSI fertilization, cleavage stage or blastocyst transfer, fresh embryo transfer or frozen embryo transfer, and more) to the Medical Birth Registry on ART cycles resulting in ultrasound verified pregnancies (week 6–7) since 1984. In Sweden, ART conception and ART treatment characteristics IVF fertilization or ICSI fertilization, cleavage stage or blastocyst transfer, fresh embryo transfer or frozen embryo transfer, and more) were notified to National Board of Health and Welfare between 1982 and 2006, and from 2007 all ART cycles are registered in their national ART quality registry with detailed information on ART treatment characteristics.

We used the encrypted maternal and paternal identity numbers to define siblings. For the within sibship analyses, siblings were defined as children with the same maternal identity code in the main analyses, and full siblings as children with the same maternal and paternal identity codes in the sensitivity analyses.

4.3.2 Outcome variables

The outcome variables of interest were defined based on registrations of diagnostic codes according to national adaptations of the International Classifications of Diseases and related Health Problems (ICD). The national adaptations of the different ICD-versions were used in different time periods in each country. Table 2 illustrates the relevant diagnostic codes for our outcomes:

	International Statistical Classification of Diseases and related Health Problems (ICD) classification version			Country			
	ICD-8	ICD-9	ICD-10	Denmark	Finland	Norway	Sweden
Registration practices							
Hypertensive disorders in pregnancy	637	642.3–7	O11, O13–16	ICD-10 (1994–2014)	ICD-9 (1989–1995) ICD-10 (1996–2014)	ICD-8 (1988–1998) Checkbox (1999–2015)	ICD-9 (1988–1996), ICD-10 (1997–2015)
Preeclampsia	637	642.4–7	O11, O14–16	ICD-10 (1994–2014)	ICD-9 (1989–1995) ICD-10 (1996–2014)	ICD-8 (1988–1998) Checkbox (1999–2015)	ICD-9 (1988–1996), ICD-10 (1997–2015)
Placenta previa	632.0	641.0–1	O44	ICD-10 (1994–2014)	ICD-9 (1989–1995) ICD-10 (1996–2014)	ICD-8 (1988–1998) Checkbox (1999–2015)	ICD-9 (1988–1996), ICD-10 (1997–2015)
Placental abruption	632.1	641.2	O45	ICD-10 (1994–2014)	ICD-9 (1989–1995) ICD-10 (1996–2014)	ICD-8 (1988–1998) Checkbox (1999–2015)	ICD-9 (1988–1996), ICD-10 (1997–2015)
Spontaneous preterm birth	–	644.0–1, 658.1	O42.0, O42.1, O42.9, O47, O60.1	ICD-10 (1994–2014)	–	Checkbox (1988–2015)	ICD-9 (1988–1996), ICD-10 (1997–2015) Checkbox (1991–2015)

Table 2. The relevant ICD-codes for the outcomes.

Hypertensive disorders in pregnancy were defined as a combined outcome including preeclampsia, eclampsia, gestational hypertension, and chronic hypertension with superimposed preeclampsia. Preeclampsia was defined as combined outcome including the same hypertensive disorders except for gestational hypertension. For the data from the Medical Birth Registries, any reporting of a relevant ICD-code (or checkbox) was considered an event. For the data from the national patient registries on the other hand, we included only cases of hypertensive disorders if diagnosed after 20 weeks of gestation, and included only cases of placenta previa if diagnosed in the third trimester or within one month of delivery.

Preterm birth was defined as birth before 37 completed weeks of gestation, very preterm birth as birth before 32 completed weeks of gestation, and extremely preterm birth as birth before 28 completed weeks of gestation. In naturally conceived pregnancies, we used gestational age estimated from ultrasound examination, which is routinely performed in weeks 18–20 in Norway and Sweden, and in the late first trimester in Denmark. If the ultrasound estimate was unavailable, we used the date of the last menstrual period. In ART conceived pregnancies, we estimated gestational age from ultrasound examination in the first trimester or second trimester (Denmark and Norway, respectively), or from the date of embryo transfer (Sweden). If the ultrasound estimate was unavailable in the Norwegian or Danish data, we used the estimate based on date of embryo transfer. In Finland, gestational

age is estimated according to the "best clinical estimate", which include ultrasound examination and date of last menstrual period, and for ART conceived pregnancies also date of embryo transfer. Spontaneous preterm birth was defined according to ICD-codes in Denmark, checkboxes in Norway, and ICD-codes in Sweden.

4.3.3 Other study variables

We categorized parity, how many previous deliveries the mother had, as 0, 1, 2 or 3+ in study I. For study II we categorized parity as 0, 1, 2 or 3, and for study III as 0, 1 or 2+.

Smoking during pregnancy was registered throughout the study period in Denmark, Finland, and Sweden, and since 1999 in Norway. Smoking status was self-reported at antenatal visits and could only be harmonized as no versus any smoking during pregnancy across the four registries. Additionally, in Norway, the mother has the option of not consenting to registration of this information, resulting in two categories of missing (not reported versus no consent).

We calculated maternal body mass index (BMI) as weight in kilograms divided by the height in meters squared, and categorized BMI as underweight <18.5 kg/m^2, normal 18.5–24.99 kg/m^2, overweight 25–29.99 kg/m^2, and obese ≥30.0 kg/m^2. Weight was based on either pre-gestational or first trimester weight. In Denmark and Norway, registration of maternal height and weight was implemented from 2004 and 2007, respectively, with substantial missing data during the first years of registration. In Finland, registration took place throughout the whole study period. In Sweden, maternal height and weight were registered in 1988–1989 and 1992–2015, with substantial missing data in the early years of registration.

Cesarean section and induction of labor was recorded in the Medical Birth Registries.

We defined cleavage stage as embryos cultured to day 2 or 3, and blastocysts as embryos cultured to day 5 or 6. We defined single embryo transfers as transfer of one embryo, meaning both pregnancies after *elective single embryo transfer* (i.e., electively transferring only one embryo), and pregnancies where only one embryo was available for transfer were included in this group. In other words, we were not able to distinguish between elective single embryo transfers and other single embryo transfers.

4.4 Study populations

Figure 8 summarizes the key criteria for eligibility, inclusion, and exclusion in the three studies. We defined the study period from 1988 (as there were very few ART conceived deliveries in the years 1984–1988) to 2014 (Denmark and Finland) and 2015 (Norway and Sweden). Study I included data from all four countries, whereas for study II and study III we had to exclude Finnish data, as no details on ART characteristics besides yes/no were available from the Finnish registries.

Eligibility was defined by maternal age between 20 years and 45 years, and in study II and study III we additionally required that (1) only mothers who had their first delivery in the study period were included (to facilitate valid comparison of maternal ages and parities between ART conceiving mothers and naturally conceiving mothers), and (2) we restricted the study population to the first four deliveries for each mother (because there were very few ART conceived deliveries among women with ≥4 deliveries), and (3) only singleton deliveries were included (to facilitate the sibling comparisons). We included only deliveries with known parity and known maternal age, and in study II and study III we additionally required that whether the ART conceived pregnancy was after fresh embryo transfer or frozen embryo transfer had to be known. Furthermore, we only included observations with known birthweight and known gestational age. We also required that birthweight values had to be plausible: >300g, and <6000g (study I) or <6500g (study II and study III), and <6 standard deviations above expected [126].

The complete CoNARTaS cohort from Denmark, Finland, Norway and Sweden						
	All deliveries	**Mothers**	**ART**	**Fresh-ET**	**Frozen-ET**	**Study period**
Denmark (DK)	1,361,470	752,543	46,091	41,305	4786	1994–2014
Finland (FI)	1,473,130	767 ,599	29,859	-	-	1990–2014
Norway (NO)	1,611,901	844,639	34,413	25,936	4478	1984–2015
Sweden (SE)	2,825,472	1,514,853	62,253	48,758	13,495	1985–2015
Total	**7,271,973**	**3,879,594**	**172,616**	**115,999**	**22,759**	1984–2015

	Study I	**Study II**	**Study III**
Study period	1988–2015	1988–2015	1988–2015
Countries	All	DK, NO, SE	DK, NO, SE
1st birth during study period	No	Yes	Yes
Maternal age	22–44 years	20–45 years	20–45 years
Parity (previous deliveries)	All	0–3	0–3
Plurality	All	Singletons only	Singletons only

Inclusion criteria			
	Study I	**Study II**	**Study III**
Exposure	ART and time period	Fresh or frozen transfer (excluded if unknown)	Fresh or frozen transfer (excluded if unknown)
Gestational weeks	22–44	22–44	22-–44
Birthweight, grams	≥300 and ≤6000	≥300 and ≤6500	≥300 and ≤6500
Birthweight, SD	≤+6	≤+6	≤+6

Included in main analyses samples			
	Study I	**Study II**	**Study III**
Mothers	4,160,402	2,379,130	2,379,130
Singletons total	6,720,893	4,533,028	4,533,028
Multiples total	109,685	Not relevant	Not relevant
Natural conception	4,414,703	4,426,691	4,426,691
ART conception	146,998	96,337	96,337
Fresh transfer	Not relevant	78,300	78,300
Frozen transfer	Not relevant	18,037	18,037
Exposure discordant sibships	Not relevant	33,209	Not relevant

Figure 8. Flowchart of observations into study I, II and III.

Abbreviations: Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer.

4.5 Statistical analyses

We used logistic regression models for all our main analyses, where we estimated odds ratios (OR) and adjusted odds ratios (aOR) of the given outcome for ART conceived pregnancies (any ART, fresh or frozen embryo transfer) compared to naturally conceived pregnancies (the reference group). We estimated precision by 95% confidence intervals (CI). To facilitate interpretation, we used post-estimation commands to calculate absolute risks and risk differences with 95% confidence intervals, in addition to the odds ratios.

For the time trend analyses, we used logistic regression within the ART conceiving group and the naturally conceiving group. We analyzed all the pluralities pooled, and then singleton and twin pregnancies separately. We analyzed time trends both in 5-year categories of offspring birth year and as a linear trend throughout the study period (change in absolute risk per 5 years, continuous variable). To investigate whether the risk in the ART conceiving group and the naturally conceiving group converged over time, we also used logistic regression *within* the offspring birth year periods. We accounted for dependency between pregnancies by the same mother with robust standard errors using the maternal ID-variable.

For the sibling comparisons, we compared odds of hypertensive disorders across conception methods in multilevel logistic models, with deliveries as one level and mothers as another, using Stata's xtlogit command with maternal identity codes defining the clusters [103, 127]. We used random effects models to obtain conventional population level estimates and fixed effects models for within sibship estimates (i.e., comparison within mothers). To facilitate comparison with previous studies which typically report frozen embryo transfer versus fresh embryo transfer (not versus natural conception) [128, 129], we also carried out this analysis.

For the mediation analyses, we used logistic regression with to split the *total effect* of ART treatment (fresh embryo transfer or frozen embryo transfer) on preterm birth into the *direct effect* and the *indirect effect* (*mediated effect*) [130], with hypertensive disorders in pregnancy as the mediating variable. The *direct effect* of fresh embryo transfer is an estimate of the influence of fresh embryo transfer or frozen embryo transfer on preterm birth that is independent of hypertensive disorders, whereas the *mediated effect* represents the effect of fresh embryo transfer or frozen embryo transfer that can be attributed to its effect on risk of

hypertensive disorders. The total effect is then the product of the odds ratios for the direct and mediated effects. For a more straightforward interpretation, we calculated the proportion mediated on the risk difference scale [123], where 0% corresponds to no mediation (direct effect only) and 100% corresponds to the entire total effect being mediated (no direct effect at all).

We defined confounders as any factor that could influence the need for ART treatment and the risk of developing the outcome of interest. We adjusted for maternal age, country, and parity as categorical variables. In study II and III we also adjusted for offspring birth year as a categorical variable. In subsamples where we had information on smoking during pregnancy, as well as maternal BMI, we adjusted for these factors as well as categorical variables. In the sibling comparisons, we adjusted for the same factors except for country, which was stable within mothers. In addition to these measured confounders, we considered parental socioeconomic position and cause of infertility as key confounders, but for which we did not have data.

For study II and III, to explore potential systematic differences in preconception characteristics between pregnancies after fresh and frozen embryo transfer, propensity score methodology using logistic regression was performed as an alternative technique of removing confounding [131]. Here, we fitted a logistic regression model with frozen embryo transfer as the dependent variable, with the following variables as the predictors: maternal age, offspring birth year, parity, and country. We used this estimated propensity score for each observation (i.e., the propensity or probability of frozen embryo transfer) in a logistic regression comparing odds of hypertensive disorders in pregnancy for frozen embryo transfer versus fresh embryo transfer. Finally, we designed a figure showing the distribution of these estimated propensity scores to explore the overlap in preconception characteristics.

All analyses were performed in Stata/MP for Windows, versions 15.0, 16.0 and 17.0.

4.5.1 Sensitivity analyses in study I

We analyzed data from each country separately to explore if the time trends differed between them.

We carried out several sensitivity analyses attempting to explain the time trends observed. The following sensitivity analyses were carried out:

(1) In a subsample with available information, we also adjusted for the potentially confounding factors BMI and smoking during pregnancy.
(2) We performed the same analyses restricted to primiparous women only to limit the impact of prior pregnancy experiences [132].
(3) In a subsample where we had detailed information on ART treatment factors, from Denmark (2011–2014), Norway (2011–2015) and Sweden (2006–2015), we adjusted for culture duration, to account for increasing use of blastocyst transfers over time [133].
(4) In a subsample where we had detailed information on the ART treatment, from Denmark (1994–2014), Norway (1988–2015) and Sweden (1988–2015), we adjusted for the temporal effect of cryopreservation (frozen embryo transfer becoming more common over time) [35].
(5) For placenta previa in specific, we explored if associations were similar when restricting the outcome definition to pregnancies with registration of placenta previa and delivery by cesarean section, which is required in cases of complete obstruction [132].
(6) For placenta previa in specific, we identified a subsample of women for which we had information on all previous pregnancies, and adjusted for history of cesarean section, which is an important risk factor for this condition.

4.5.2 Sensitivity analyses in study II

Firstly, to investigate if associations were affected by order and combinations of conception methods, we repeated the random effects models in mothers with singletons in their first two consecutive deliveries and added statistical interaction terms between parity and conception method (bidirectional analysis) [110]. One of the rationales behind this approach was to identify carryover effects in the sibling comparisons, i.e., if the exposure or outcome in the 1st sibling affected the exposure or outcome in the 2nd sibling. This analysis included 1,579,190 sibships belonging to one of nine possible sibship combinations: natural conception/natural conception (n=1,540,571), natural conception/fresh embryo transfer (n=7,873), natural

conception/frozen embryo transfer (n=1,510), fresh embryo transfer/natural conception (n=13,764), frozen embryo transfer/natural conception (n=2,159), fresh embryo transfer/fresh embryo transfer (n=7,507), fresh embryo transfer/frozen embryo transfer (n=3,946), frozen embryo transfer/fresh embryo transfer (n=937), frozen embryo transfer/frozen embryo transfer (n=923).

Secondly, to investigate whether experiencing a hypertensive disorder in pregnancy influenced the selection into the population of double discordant sibships [103, 110], we categorized the first delivery by conception method and occurrence of hypertensive disorders in pregnancy, resulting in six subgroups: Natural conception and no hypertensive disorder (n=1,713,104), natural conception with a hypertensive disorder (n=104,184), fresh embryo transfer and no hypertensive disorder (n=37,667), fresh embryo transfer with a hypertensive disorder (n=2623), frozen embryo transfer and no hypertensive disorder (n=4854), frozen embryo transfer with a hypertensive disorder (n=466). For these subgroups, we calculated the probability of having a second singleton with either conception method within 5 years following the first singleton and estimated odds ratio of hypertensive disorders in pregnancy in the second pregnancy for each subgroup.

Thirdly, to explore whether the higher risk of preterm birth after ART conception reduced the probability of hypertensive disorders in pregnancy [108], we repeated analyses using Cox regression with gestational duration as the time scale. The rationale behind this analysis was that pregnancies ending preterm have less time to develop the outcome [132]. We estimated hazard ratios, adjusting for the same covariates as the main analyses. For population level estimates, we used robust standard errors to account for dependency of observations within mothers, and for within sibship estimates, we used stratified models with maternal identity in separate strata. No clear violations of the proportional hazard assumption were found when inspecting log-log plots.

We also carried out several sensitivity analyses in subgroups to explore the robustness of our results:

(1) In a subsample with available information, we also adjusted for the potentially confounding factors BMI and smoking during pregnancy.

(2) We repeated analyses for full siblings (same mother and father) to ensure constant paternal factors.
(3) We repeated analyses for siblings born within a 3-year interval as their parents' health should be more similar than for siblings born further apart in time.
(4) We repeated our main models for each country separately to see if associations were similar enough to justify pooling.
(5) We restricted the ART conceived population to those conceived after IVF fertilization, in other words excluding ICSI fertilized pregnancies (ICSI fertilization is mainly used for male infertility in the Nordic countries) to explore the potential impact of male infertility [134].
(6) We restricted the ART conceived population to those conceived after single embryo transfers to limit the potential impact of vanishing twins [87, 135, 136].
(7) We restricted the ART conceived population to blastocyst transfers, to take into account the prolonged exposure to culture media and in vitro handling [137].

4.5.3 Sensitivity analyses in study III

We carried out several sensitivity analyses to explore the robustness of our estimates of the mediation analysis. The following sensitivity analyses were carried out:

(1) In a subsample with available information, we also adjusted for the potentially confounding factors BMI and smoking during pregnancy.
(2) We performed the same analyses restricted to primiparous women only to limit the impact of prior pregnancy experiences [132].
(3) We restricted the ART conceived population to those conceived after IVF fertilization, in other words excluding ICSI fertilized pregnancies (ICSI fertilization is mainly used for male infertility in the Nordic countries) to explore the potential impact of male infertility [134].
(4) We restricted the ART conceived population to those conceived after single embryo transfers to limit the potential impact of vanishing twins [87, 135, 136].
(5) We restricted the ART conceived population to blastocyst transfers, to take into account the prolonged exposure to culture media and in vitro handling [137].
(6) We performed analyses where we explored the effect of using other, related mediator variables: preeclampsia, placental abruption, and the combination of placental

abruption and hypertensive disorders in pregnancy. We also carried out an analysis with any diabetes (pregestational or gestational) included as a mediator-outcome confounder, as gestational diabetes increases the risk of hypertensive disorders in pregnancy [138]. In the analysis with placental abruption as the mediator, we included hypertensive disorders in pregnancy as a potential mediator-outcome-confounder, because these are risk factors for placental abruption [77].

5 Results

The following sections will present summaries of the main results in the three studies. For closer details, please see the results sections in the respective studies.

5.1 Baseline characteristics of the study populations

Although the study populations in the studies differed somewhat with regards to inclusion and exclusion criteria, they are comparable in their essence and are therefore presented collectively with regards to baseline characteristics.

Children born after ART conception constituted increasing fractions of the total birth cohorts over time. During the study period overall, ART conceived deliveries constituted 2.2% of all deliveries in the Nordic countries. ART conceiving mothers were older and more commonly primiparous than mothers who conceived naturally. Furthermore, the ART conceiving mothers tended to smoke less during pregnancy, whereas mean BMI was similar to naturally conceiving mothers. Smoking rates declined in both ART conceived pregnancies and naturally conceived pregnancies throughout the study period.

ART conceived pregnancies were more frequently delivered with induction and cesarean section. In study I, the frequency of twin and higher order pregnancy was consistently higher in the ART conceiving group, but this difference declined throughout the study period.

Frozen embryo transfers comprised around 20% of the ART conceived deliveries in study II and III. Among frozen embryo transfer pregnancies, 36.7% were fertilized by ICSI, 64.3% were single embryo transfers, and 20.8% were blastocyst transfers. Fresh embryo transfer pregnancies had similar proportions of ICSI fertilization and single embryo transfers, but only 5.7% were blastocyst transfers.

5.2 Main results in study I

5.2.1 Time trends in risk of placental abruption

Risk of placental abruption remained higher in ART conceived pregnancies compared to naturally conceived pregnancies throughout the study period. Overall, risk of placental abruption declined in both conception groups, and in all pluralities. The risk in naturally conceived pregnancies decreased by 0.06 percentage points per 5 years, while the risk in ART conceived pregnancies decreased by 0.16 percentage points per 5 years. In subgroup analyses, adjusting for BMI and smoking did not impact the time trend estimates substantially, neither did adjusting for ART characteristics (ICSI fertilization versus IVF fertilization, fresh embryo transfer versus frozen embryo transfer, cleavage stage versus blastocyst transfer).

5.2.2 Time trends in risk of placenta previa

ART conceived pregnancies remained at a higher risk of developing placenta previa compared to naturally conceived pregnancies throughout the study period. The risk of placenta previa increased weakly over time in naturally conceived pregnancies, whereas risk seemed to rise more for ART conceived pregnancies. ART conceived twin pregnancies seemed to have the strongest risk increase, increasing by 0.30 percentage points per 5 years, while the risk in ART conceived singleton pregnancies increased by 0.21 percentage points per 5 years. These findings were robust in our sensitivity analyses, except an attenuated time trend in all ART conceived pregnancies when adjusting for the increasing use of blastocyst transfer.

5.2.3 Time trends in risk of hypertensive disorders in pregnancy

Risk of hypertensive disorders was higher after ART conception when comparing singleton and multiples combined. However, when restricting to singleton and twin pregnancies separately, this association was weakened and reversed, respectively. Risk of hypertensive disorders seemed to increase over time in twin pregnancies, regardless of conception method. The risk increase during the study period was slightly stronger in ART conceived twin pregnancies, 1.73 percentage points per 5 years, than in naturally conceived twin pregnancies, 0.75 percentage points per 5 years. Risk of hypertensive disorders increased over time for the singleton pregnancies as well, but not to the same degree, 0.13 percentage points per 5 years for ART conceived singletons and 0.16 percentage points per 5 years for naturally conceived

singletons. In sensitivity analysis, adjusting for BMI and smoking reversed the time trend for naturally conceived singleton pregnancies and substantially attenuated the time trend in naturally conceived twin pregnancies, but did not substantially impact the time trend estimates for ART conceived pregnancies. In contrast, adjusting for the increasing use of blastocyst transfer moderately attenuated the point estimate in ART conceived singletons, and reversed the point estimate in ART conceived twin pregnancies, but precision was very limited. When adjusting for the increasing use of embryo cryopreservation over time, all estimates attenuated.

5.3 Main results in study II

The unadjusted risk of hypertensive disorders in pregnancy after frozen embryo transfer was 7.4%, compared to a risk of 5.8% after fresh embryo transfer and 4.3% after natural conception. Odds of hypertensive disorders in pregnancy was markedly higher (roughly doubled) after frozen embryo transfer compared to natural conception, both at the population level and within sibships. For fresh embryo transfer, risk was similar to natural conception, both at the population level and within sibships. Frozen embryo transfer pregnancies had higher odds of hypertensive disorders in pregnancy compared to fresh embryo transfer pregnancies, both at population level and within sibships.

Sensitivity analyses with adjustment for BMI and smoking, restriction to full siblings and siblings born within a three-year interval, were consistent with our main findings. Results were not driven by order of conception method, or by other treatment procedures (ICSI fertilization, culture duration, or number of embryos transferred). Results were also very similar when using Cox regression with gestational age as the time variable.

When accounting for the potential selection imposed by experiencing a hypertensive disorder in the first pregnancy, results remain consistent, with frozen embryo transfer showing higher odds of hypertensive disorders in pregnancy compared to natural conception in most subgroups, whereas fresh embryo transfer showed no or weak positive associations. However, precision was limited for some of these subgroups.

5.4 Main results in study III

Compared to naturally conceived pregnancies, the total effect of frozen embryo transfer on any preterm birth (adjusted for offspring birth year, parity, maternal age, and country) was aOR 1.29 (95% CI 1.21 to 1.37), with 19.5% of this total effect mediated by hypertensive disorders in pregnancy. Frozen embryo transfer pregnancies also had higher adjusted odds of spontaneous and medically indicated preterm birth, but mediation by hypertensive disorders in pregnancy was restricted to medically indicated preterm birth.

The total effect of fresh embryo transfer on any preterm birth compared to natural conception was aOR 1.49 (95% CI 1.45 to 1.63), of which ≈0% could be explained by hypertensive disorders in pregnancy, meaning that the association was entirely independent of hypertensive disorders. Fresh embryo transfer pregnancies had higher adjusted odds of both spontaneous and medically indicated preterm birth, but neither of these associations could be explained by hypertensive disorders in pregnancy.

Results for both frozen embryo transfer and fresh embryo transfer were similar when adjusting for BMI and smoking, when restricting to only primiparous women, and when restricting the ART conceived population to IVF fertilization (i.e., excluding ICSI fertilization), single embryo transfer and blastocyst transfer. In the analysis with placental abruption as the sole mediator, 14.2% of the effect of fresh embryo transfer was mediated.

5.5 Additional analysis not included in the studies

Figure 9 shows the distribution of propensity scores (probability of being exposed, i.e., frozen embryo transfer) when using offspring birth year, maternal age, parity, and country as the independent predictors, and illustrates a high degree of overlap between the two conception groups:

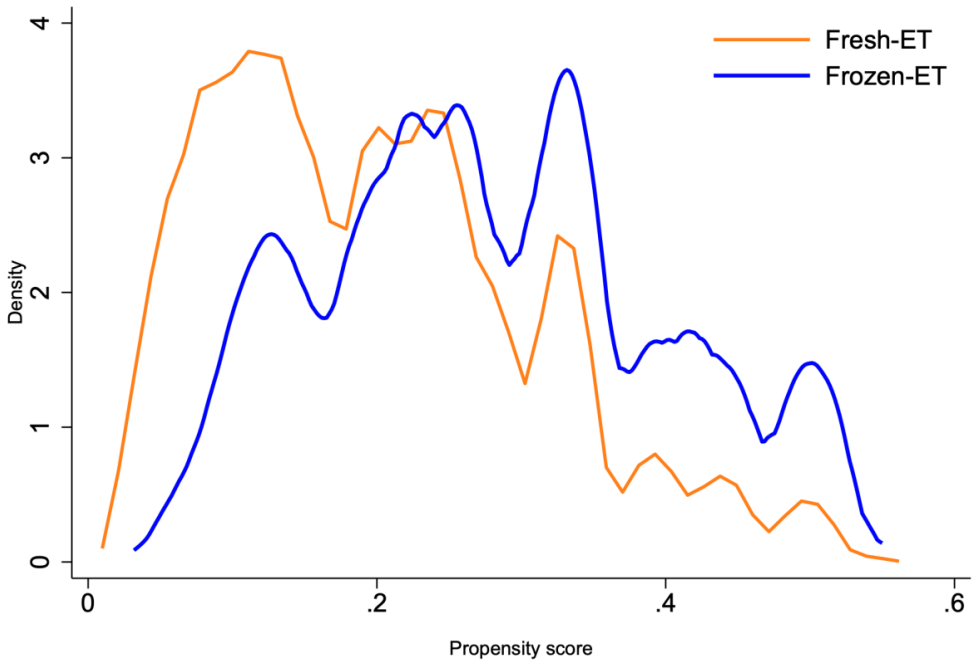

Figure 9. Propensity score distributions for fresh embryo transfer and frozen embryo transfer.

The results in study II and study III remained very similar when using the estimated propensity score to adjust for potential preconception differences between pregnancies after fresh embryo transfer and frozen embryo transfer.

6 Discussion

6.1 Summary of findings

The overall aim of this thesis was to explore the association between ART conception and risk of pregnancy complications.

The main findings from our studies were:

Study I:

- The risk of hypertensive disorders in pregnancy, placental abruption, and placenta previa was consistently higher in ART conceived pregnancies compared to naturally conceived pregnancies throughout the study period. The only notable exception was risk of hypertensive disorders in pregnancy in twin pregnancies, which was similar between the two conception groups.
- When considering all ART conceived pregnancies combined, risk of all complications declined considerably and approached that in the background population during the study period.
- Risk of placental abruption declined over time in all groups. Hence, the ART conceived group followed the time trend patterns of the naturally conceived group.
- Risk of placenta previa rose over time in ART conceived singleton pregnancies and increased strongly in the ART conceived twin pregnancies, while risk in the naturally conceived pregnancies remained stable over time.
- Risk of hypertensive disorders in pregnancy increased over time in twin pregnancies in both conception groups, but more strongly for the ART conceived group. No consistent time patterns were found for singleton pregnancies in risk of hypertensive disorders in pregnancy.

Study II:

- In pregnancies following frozen embryo transfer, risk of hypertensive disorders in pregnancy was higher than in naturally conceived pregnancies, even after accounting for parental factors through offspring sibling comparison. In contrast, in pregnancies following fresh embryo transfer, risk was similar to that after natural conception.

Study III:

- In pregnancies following frozen embryo transfer, hypertensive disorders in pregnancy could explain around 20% of the higher risk of preterm birth compared to naturally conceived pregnancies. In contrast, very little of the association between fresh embryo transfer and preterm birth was mediated by hypertensive disorders in pregnancy.

6.2 Methodological considerations

The findings from our studies must be interpreted with caution. In general, errors in estimation (i.e., the difference between the estimated value and the "true" value) can be categorized as either random or systematic, see Figure 10. The following sections will explore these errors further.

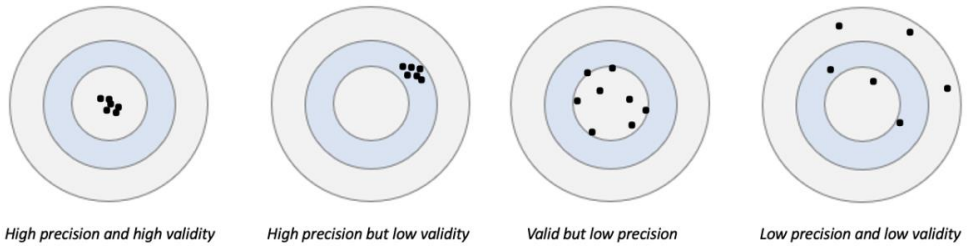

Figure 10. Illustration of high precision (little random error) and high validity (little systematic error).

6.2.1 Random error and precision

Random error can be defined as the variability in estimation that is due to chance alone [92]. Random error is in some sense predictable in that it will get smaller as one increases the sample size, which is equivalent to increasing statistical precision. One can quantify and communicate the degree of statistical precision using confidence intervals. A simple way to view confidence intervals is that they display a range of results that could be considered reasonable given the data. More precisely, 95% confidence intervals should be interpreted in the following way: given that the statistical model is correctly fitted, and the study is free of bias (i.e., no systematic errors), we would expect the confidence intervals from numerous correctly performed studies to include the "true value" in 95% of the studies. 95% here is an a priori chosen confidence level, which by convention is set to 95%, but other confidence

levels (for example 1% or 10%) can also be used. Notably, a relative risk of 1.50 with 95% confidence intervals from 1.40 to 1.60 does not mean that we can be 95% confident that the true value of association is between 1.40 and 1.60, nor can we say there is only 5% possibility that the real relative risk is <1.40 or >1.60. Interpretation of confidence intervals should include the totality of evidence for or against the hypothesis.

The CoNARTaS cohort is one of largest resources for research on health following ART conception in the world. We consider the large sample size as of one of the main strengths of our studies, enabling us to estimate the associations with high precision, as indicated by reasonably narrow confidence intervals. This was particularly true for study I, in which we had ≈150,000 ART conceived pregnancies and ≈6.5 million naturally conceived pregnancies in our main analyses. However, in the earliest part of the study period, and in some of the sensitivity analyses according to specific ART characteristics, sample sizes were limited, and hence statistical precision was also limited. For study II and III, statistical precision for our main results was reasonable as we had data on 78,300 pregnancies after fresh embryo transfer, 18,700 pregnancies after frozen embryo transfer, and ≈4.4 million naturally conceived pregnancies available for analysis. Nonetheless, in several of the sensitivity analyses in study II and study III, sample sizes were limited, mainly because of few pregnancies after frozen embryo transfer.

6.2.2 Systematic error and internal validity

Systematic errors are errors that arise not due to chance, but rather due to some systematic process within the data [139]. Systematic errors in estimation are commonly referred to as biases. The opposite of bias is validity, meaning the more the investigator controls systematic errors, the higher the internal validity of the study. Internal validity implies validity of inference for the source population of the study [139]. The following sections will explore the main types of systematic errors that can threaten internal validity: confounding, information bias and selection bias.

6.2.2.1 Confounding

A confounder or a confounding variable can be defined as a common cause of the exposure and the outcome, but which is not a consequence of either [139]. When conducting

observational studies with a causal inference aim, the researcher looks for associations between the exposure and the outcome, but there is always a possibility that the associations found are not causation, because the exposed and the unexposed are not *exchangeable* [3]. Exchangeability means that the risk of the outcome in the unexposed would have been the same as the risk of the outcome in the exposed if *they* had been exposed [140]. Lack of exchangeability occurs when risk factors differ between the exposed and the unexposed, which can arise through confounding, but also through selection bias [140]. In our studies, we defined confounders as any factor that could influence the need for ART and cause the outcome of interest. Importantly, identification and selection of confounders should be guided by substantive knowledge in the field [139], to correctly classify potential covariates as either causes, mediators or colliders on the proposed causal pathways. A way of visualizing and communicating these choices is directed acyclic graphs (DAGs), where assumptions about the direction of causality are made explicit:

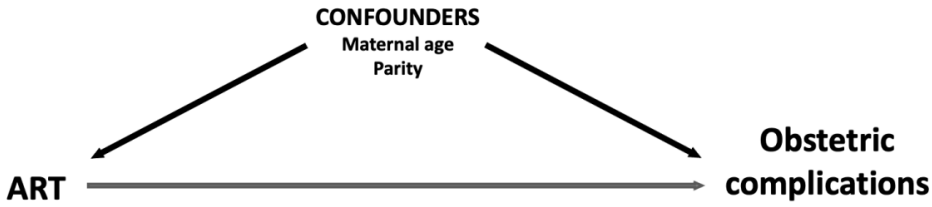

Figure 11. Simplified directed acyclic graph (DAG) showing selected, major confounders in our studies.

We were able to adjust for potentially important confounding factors like maternal age, parity, offspring birth year and country. In subsamples with available information, we were also able to adjust for BMI and smoking during pregnancy. However, as in all observational studies, there is a possibility of *residual confounding*. In study I and III, we were not able to adjust for causes of infertility. Some important causes for infertility like endometriosis and PCOS are themselves (independent of ART treatment) positively associated with risk of complications during pregnancy [93]. This would imply that the positive association between ART treatment and risk of complications would likely attenuate if we were able to control for cause of infertility. Another key unmeasured confounder was socioeconomic status, but here we suspect that if we had data on this variable and included it in the regression models, the

positive association between ART conception and adverse outcomes would strengthen. This is because ART conceiving mothers on average have a higher socioeconomic status, which likely has a *protective* effect on adverse outcomes [89, 141]. Cause of infertility and socioeconomic status are examples of *known, but unmeasured* confounders. Another key issue in observational studies in general is that they can be affected by *unknown and unmeasured* confounding as well.

In study II, we aimed to limit the residual confounding from both unmeasured and unknown confounding by using each mother as her own control. Sibling comparisons control for observed and unobserved confounders that are shared by the siblings [103]. Thus, we were able to account for unmeasured confounders such as genetics, preconception lifestyle and health, as well as socioeconomic status, but also unknown confounders. We also carried out several sensitivity analyses where we took into account the potential impact of birth order, male infertility, full siblings versus half siblings, and interpregnancy interval [110, 134, 142], as well as different ART treatment characteristics like increasing use of frozen embryo transfers, single embryo transfers, and blastocyst transfers [35, 133]. Still, there are some limitations to study II in terms of confounder control.

Even though we could control for smoking during pregnancy and BMI, factors that need not be constant between siblings, confounder control was limited by a large degree of missingness and potential measurement error (6.2.2.2). Secondly, we had no data on other lifestyle factors. Although sibling comparisons provide optimal control for factors that are shared between siblings, non-shared residual confounding can result in stronger bias within sibships compared to population estimates between unrelated individuals [111].

Causes of infertility were largely unknown. Nonetheless, to the degree that major causes of infertility like endometriosis and PCOS have an important genetic component [143, 144], the sibling comparison should be able to deal with parts of this confounding. For instance, if a woman first conceived naturally, and then via ART treatment due to onset of clinical infertility, it seems possible that some part of the underlying condition leading to infertility was present all along, but may have been subclinical (latent) before the first pregnancy. As described by Olsen & Basso, risk of recurrence is relatively high for most reproductive outcomes, including preeclampsia, suggesting that at least some of the important causes are *time-stable* as opposed to *time-varying* [132]. Thus, we do believe that

the sibling comparison design, together with our sensitivity analyses (especially the analysis accounting for birth order) will control for the confounding from cause of infertility.

Still, residual confounding from both the cause and severity of infertility might still be present in study II, because couples conceiving after fresh embryo transfer might not be exchangeable with those who conceive after frozen embryo transfer. Although couples who conceive after fresh and frozen cycles may be more similar than couples who conceive naturally and after ART, causes and severity of infertility are nonetheless likely to influence the couple's probability of having embryos for freezing in the first place. In other words: more severe infertility might reduce the chance of having any surplus embryos to cryopreserve. Unfortunately, we did not have data on number of embryos obtained from the stimulation cycle, and we could therefore not determine whether couples who conceived after fresh embryo transfer had surplus embryos eligible for freezing. Nor could we determine if the frozen embryo transfer pregnancies were after an initial, unsuccessful fresh embryo transfer or from an elective freezing approach. During our study period, elective freezing was still relatively uncommon and most frozen embryo transfers would have been preceded by an unsuccessful fresh transfer. Furthermore, results from randomized controlled trials show that the chances of a successful pregnancy are similar or slightly higher after elective freezing compared to fresh transfer [145, 146, 147, 148], which could mean that for couples with surplus embryos eligible for freezing in our cohort, the chances of pregnancy after either transfer type might be comparable. This was supported by the approach using propensity scoring methods, which showed that there was a high degree of overlap of propensity scores for fresh embryo transfer pregnancies and frozen embryo transfer pregnancies.

In study III we used mediation analysis to estimate how much of the total effect of fresh and frozen embryo transfer on the risk of preterm birth was mediated by hypertensive disorders in pregnancy. In addition to the assumption in study I and II of no exposure-outcome-confounding, including no confounding from underlying infertility, this approach relies on additional key assumptions about confounding. Specifically, for our estimates of the direct and mediated effects (and hence the proportion mediated) to be valid, we must also control exposure-mediator-confounding and mediator-outcome-confounding. We were able to adjust for the important confounding of offspring birth year, parity, maternal age, and country, which can all plausibly affect both the conception method, risk of hypertensive

disorders and risk of preterm birth. For mediator-outcome-confounding, any factor that (1) differs between pregnancies affected by hypertensive disorders and pregnancies not affected by hypertensive disorders, (2) is associated with risk of preterm birth, (3) is *unrelated* to need of ART could potentially be such a confounder. Importantly, although the confounding factors that we could adjust for were considered to be likely to be related to both the exposure, the mediator, and the outcome, we cannot exclude that such mediator-outcome-confounders are present, and hence we cannot exclude residual confounding.

6.2.2.2 Information bias

Information bias arises when the measurement, reporting or classification of the study variables is inaccurate, and includes bias from measurement error, misclassification, and missing data [139]. In general, we can divide this bias in two main categories: differential and non-differential. In *differential misclassification*, the misclassification depends on other study factors, while *non-differential misclassification* is independent of other study variables. Notably, misclassification of confounding variables can potentially induce residual confounding [139].

We suspect there could be a degree of non-differential misclassification because of typing errors from the reporting health care personnel. The non-differential nature of this would drive the association between ART conception and the outcomes in our studies toward the null if there was misclassification of the exposure or the outcome, in other words an underestimation. Furthermore, when the ART treatment had taken place outside of the Nordic countries, these pregnancies will be misclassified as naturally conceived pregnancies. Depending on the risk of pregnancy complications in these abroad-conceiving women, this misclassification could drive the overall association between ART conception and obstetric complications either way. However, the absolute number of these pregnancies should be low compared to the large number of correctly classified naturally conceived pregnancies.

Another issue is the potential misclassification of smoking status in pregnancy. Smoking was self-reported, and it seems possible that women with ART conceived pregnancies could have provided false information to the clinician when asked (e.g., because of guilt or fear of being denied treatment). Noting that smoking has an apparently protective effect on the risk of

preeclampsia [149]), the association between ART conception and hypertensive disorders in pregnancy would be biased towards the null if actual smoking was more prevalent than the information provided.

Another possibility for error comes from detection bias, where detection of pregnancy complications could happen disproportionately more often in ART conceived pregnancies, constituting differential misclassification. For example, clinicians might consider these pregnancies as high-risk pregnancies, and women conceiving through ART might have a lower threshold for seeking medical attention. However, practically all pregnant women attend the publicly financed antenatal care program in the Nordic countries, which should ensure that the initial screening for pregnancy complications is equal in ART conceived and naturally conceived pregnancies. For the findings in study II and III, it also seems unlikely that an increased detection would differ between fresh embryo transfer and frozen embryo transfer.

Gestational age can be a difficult study variable to handle correctly. Gestational age was obviously a very important variable in study III, where preterm birth was the outcome of interest. We chose to use the gestational age estimates that were used to guide the clinical decision-making in the respective countries during the study period. This was in line with the general recommendation in the reproductive epidemiology literature, which is to use the same method for assessing gestational age in the exposed and unexposed groups [132]. The only exception from this rule was for the Swedish data, where clinical practice is to use transfer date for ART conceived pregnancies.

Next, the validity of the complication diagnoses we studied should be considered. Earlier validation studies have found the diagnoses provided by the registries to be of acceptable validity [150, 151, 152, 153, 154]. It is nonetheless important to recognize that our data are very much dependent on the way the Medical Birth Registries, national patient registries and ART registries are organized and how they receive their data, which could have influence on the time trends we found. In the countries where we had information from more than one source (both Medical Birth Registry and national patient registries), namely Denmark and Finland, the overall occurrence of complications was higher than in Norway and Sweden, from which we had Medical Birth Registry information only. Furthermore, from 1995, outpatient

visits in public hospitals have been included in the national patient registry in Denmark. In Finland, outpatient visits from both public hospitals and private clinics have been reported to the national patient registry since 1998. These inclusions could have led to increased registration of the outcomes of interest. Another potentially important source of measurement error stems from the revision of the registration form in Norway in 1999, when reporting of pregnancy complications changed from writing diagnoses in a free text field, to checkboxes for the most relevant diagnoses. Still, we do not suspect that these temporal changes in registration practice would influence ART conceived and naturally conceived pregnancies differently.

Missing data is a common problem in all observational studies, in particular for routinely collected data. Missing data can be categorized as: (1) *missing completely at random* (MCAR) where the missing data is independent of the observed and unobserved data; (2) *missing at random* (MAR), where the missingness is systematically related to observed but not unobserved data; and (3) *missing not at random* (MNAR), where the missingness is systematically related to the unobserved data [155]. It is an information bias in the sense that it involves information, but when we condition on it, then selection bias might arise [92, 156].

The sensitivity analyses where we excluded pregnancies with missing information on BMI and smoking could potentially be somewhat biased. The main reason for missing data on these variables was that they were not part of the registration procedure in all countries throughout the study period. However, even during the study period when these factors should have been recorded, the missing proportion was still as high as 18%. In general, a potentially viable solution to the problem of missing data is *multiple imputation* [157]. This method relies on using other variables that are reasonably good predictors of the missing values. We did not go forward with multiple imputation for two main reasons. Firstly, multiple imputation requires that the data contain variables that can predict BMI, which is likely not the case in the current CoNARTaS cohort. Secondly, running a computationally intensive procedure like multiple imputation on already complex multilevel logistic regression models is likely to be unfeasible in the current dataset solution in Stata on Denmark Statistics' server.

6.2.2.3 *Selection bias. Collider bias, mediation.*

Selection bias can be defined as bias that occurs when the parameter of interest in a population differs from the parameter in the subset of individuals from the population that is available to the investigator [156]. The consequence of selection bias is that the estimated associations are not the same associations that would be estimated with the entire source population, which similar to confounding, results in lack of exchangeability between the exposed and the unexposed groups [140, 158]. It is important to recognize that selection bias and confounding are distinct from one another [159]. Whereas confounding is *inherent* to the source population (the population that gave rise to the cases), and could not be avoided even if everyone in the source population took part in the study, selection bias arises exactly because not everyone ended up participating *and* completing the study. The structure of selection bias can be illustrated as follows:

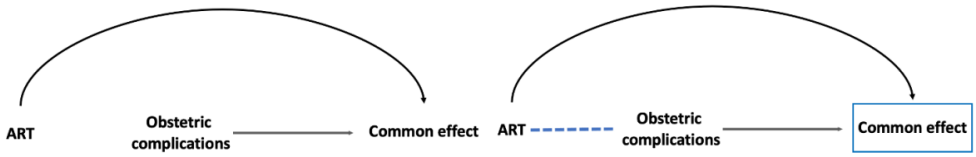

Figure 12. Selection bias. The right part of the figure shows that an association will arise when conditioning on a common effect.

There are many forms of selection bias, including non-random sampling and recruitment, self-selection (volunteer bias), missing data, and loss to follow-up [156, 159].

Since the CoNARTaS cohort is a population-based cohort, we expect selection bias related to recruitment to be small because this will be based on residency as opposed to health factors. In the Nordic countries, the public health system strongly subsidizes ART treatment, thereby ensuring that the couple's financial situation should not be a major determinant of access to ART treatment. This contrasts with other parts of the world, where couples with lower socioeconomic status may not be able to access this treatment.

Furthermore, we used inclusion and exclusion criteria that aimed to minimize selection bias. For study II, we compared the excluded and included observations in the eligible study

population, which revealed that these were largely similar on characteristics that were not part of the exclusion criteria.

Although our sibling comparisons were intended to increase internal validity (specifically by reducing confounding) by accounting for all shared factors between the siblings, simultaneously we risk introducing new problems, especially with regards to selection. This is because in the fixed effects models, the fixed intercept (unique to each mother) "absorbs" all covariates that are shared by the siblings, i.e., not only confounders, but also mediators and colliders [103, 110]. These selection phenomena have special names in this context. *Sibling carryover* or *contagion effects* arise when the exposure or outcome of the first sibling affects the exposure or outcome in the second sibling [110]. For example, if the exposure was cesarean section, there would be exposure-to-exposure carryover present in sibling comparisons because a prior cesarean section is a major risk factor for a new cesarean section [110]. Next, *selective fertility* occurs when the outcome of the first pregnancy directly affects the probability of new pregnancies [160]. In a classical example of selective fertility, women who experience a stillbirth will be likely to replace the fetal loss, potentially leading to very counterintuitive research results if not taken into account [160]. In our case, women experiencing serious complications in the form of a hypertensive disorder in their first pregnancy may avoid going forward with more pregnancies. Indeed, we found some indications of selection into the sibling comparison population (the double discordant sibships). There was a lower probability of continued reproduction among women with a hypertensive disorder in their first pregnancy and differential probability of a second, naturally conceived singleton for women with a hypertensive disorder and fresh embryo transfer or frozen embryo transfer in their first pregnancy. Although this may have biased the sibling comparisons somewhat, overall conclusions appeared robust in our attempts to control for this selection.

The lack of positive association between fresh embryo transfer and hypertensive disorders was unexpected given the earlier literature [40, 128]. This lack of association was the rationale for repeating the main analyses using Cox regression instead of logistic regression. As described by Olsen & Basso, a factor causing preterm birth may appear to protect from preeclampsia simply because women with a shortened pregnancy have had less opportunity

to develop preeclampsia [132]. Pregnancies after fresh embryo transfer indeed have shorter pregnancies [108], and by using Cox regression with gestational age as the time-scale, the differences in time at risk might be overcome [132]. These analyses gave results very similar to the logistic regression, for both fresh embryo transfer and frozen embryo transfer.

In all analyses where we restricted to singleton pregnancies (or analyzed singleton and twin pregnancies separately), we condition on a mediator on the causal pathway between ART conception and pregnancy complications, since ART treatment increases occurrence of multifetal pregnancy. A strength of the mediation analysis framework is that it can avoid the collider bias that might arise if adjustment for the mediator was performed [120, 123]. In other words, multifetal pregnancy is a mediator and not a confounder in this causal pathway. Strictly speaking, a more valid approach would thus be to utilize mediation analysis, like Öberg and colleagues did in a Swedish study investigating the association between ART, multiple pregnancies, and adverse outcomes [161]. However, since elective single embryo transfer is becoming more and more common, the analyses with restriction to singleton pregnancies will to a larger extent reflect contemporary clinical practice. Along similar themes, the higher risk of complications even among ART conceived singleton pregnancies could in part be explained by the spontaneous reduction of multifetal pregnancy to a singleton pregnancy, also known as the vanishing twin phenomenon [87, 136, 162, 163]. To illustrate, in a US ART database, 8% of all ART conceived singleton pregnancies originated from an initially multifetal pregnancy [164]. Although it seems most valid to consider vanishing twins as a mediator, and not a confounder, we took the vanishing twin phenomenon into account by restricting the ART conceived pregnancies to those after single embryo transfers, and results were very similar.

6.2.3 Other methodological considerations

6.2.3.1 Statistical interaction (effect measure modification)

Statistical interaction, also known as *effect measure modification* in the epidemiology literature, is the situation where the effect of a covariate (typically the exposure) changes according to the value of another covariate [92]. In fact, when adjusting for covariates in regression models, one of the underlying assumptions is that there is no statistical interaction between the covariates (unless the investigator has added interaction terms in the models), also known as *homogeneity of effects across strata* [165].

In study III, the question of statistical interaction is central because one of the advantages of mediation analysis is that the investigator can allow for statistical interaction between the exposure and the mediator, i.e., exposure-mediator-interaction, unlike the traditional methods [120]. In our case, this would mean that experiencing a hypertensive disorder in an ART conceived pregnancy is somehow different with respect to risk of preterm birth than in a natural conception pregnancy. In other words, hypertensive disorders would modify the association between ART conception and preterm birth. A priori, we did not expect statistical interaction for this association.

6.2.3.2 Reflections on the non-collapsibility of the odds ratio

Collapsibility is a feature of certain effect measures in epidemiology, where the estimate of the exposure's effect on the population level (aggregate level) can be expressed as a weighted average of the stratum-specific measures [166]. In other words, the stratum-specific estimates "collapse" into the population estimate. Stratum-specific in this context typically refers to the covariates the investigator chooses to adjust for. An example of collapsibility can run as follows: the risk ratio of death in women is 2.0, risk ratio in men 3.0, and on the population level (both sexes) 2.5, i.e., 2.0 and 3.0 collapse into 2.5. The population effect is also known as the *marginal, unconditional, crude, or unadjusted effect*, and the stratum-specific effect can be viewed as the *conditional or adjusted effect*. Correspondingly, *non-collapsibility* refers to the phenomenon that some effect measures, in particular the odds ratio, does not have the feature of collapsibility (unlike risk ratios). At first glance, if epidemiologists find a difference between the unadjusted and adjusted estimates in a logistic regression model, they will generally conclude that confounding was present. However, this is not necessarily the case. The crucial point is that even in a randomized controlled trial (i.e., no confounding), the marginal and the conditional odds ratios can still differ from each other, and generally in a predictable direction. Generally, adding more and more covariates to a logistic regression model will move the adjusted odds ratio away further and further away from 1 (the null effect): downward when the original estimate is below 1, and upward when the original estimate is above 1 [167, 168]. This is not confounding or bias, it is simply a non-causal arithmetical consequence of how odds ratios are calculated [167, 168], and is also closely related to *sparse data bias* (sometimes called *small sample bias*) [169], and *Simpson's paradox*

[170]. We say that odds ratios are not a collapsible effect measure because the stratum-specific odds ratios do not readily collapse into the marginal odds ratio. This non-collapsibility has important implications for interpretation of studies that use logistic regression and odds ratios. In our case, when running fixed effects models (the sibling comparisons), even the *unadjusted* estimate of the odds ratio between the conception methods should be regarded as a sort of conditional odds ratio, and the *adjusted* estimate of the odds ratio even more so. This is because in the fixed effects models, the fixed intercept (unique for each mother) absorbs all covariates that are shared by the siblings [103]. From the causal inference literature on non-collapsibility and confounding, particularly an influential article by Greenland, Robins and Pearl from 1999 [166], it is thus not surprising that the odds ratio derived from the fixed effects model (the sibling comparisons) will tend to be further away from 1 (the null value, i.e., no association) than the odds ratio from the random effects model (the population level analysis) [127]. Thus, although the difference between the estimates from sibling comparisons and the conventional population level analysis can be used to disentangle parental factors from treatment factors, this should be done with caution. Not only because of systematic errors (i.e., bias) associated with sibling comparisons, but also for technical reasons in how the odds ratio is calculated and in turn its feature of non-collapsibility. Study I and study III are also subject to this non-collapsibility, as the odds ratio is the effect measure in these studies as well. All this being said, the effect of non-collapsibility is fortunately limited if the outcome of interest is rare [171], which should apply to all three studies.

6.2.4 External validity/generalizability

External validity can be defined as the degree to which the findings of a given study can provide a valid basis for generalizations to other populations or circumstances [139]. Importantly, internal validity is a prerequisite for external validity.

There are some important differences in how ART treatment is practiced in the Nordic countries compared to the rest of the world. These differences should be considered before generalizing our results to other parts of the world, particularly the time trend results from study I. An important difference is that in the Nordic countries, ART treatment is subsidized by the public health system. In other parts of the world, access to ART treatment can be

determined by the couple's socioeconomic status and finances. It therefore seems likely that the population seeking ART treatment can be different in the Nordic countries compared to parts of the world where ART treatment is costly and unavailable to some couples. Furthermore, the publicly financed antenatal care program in the Nordic countries is the same for ART conceived pregnancies as for naturally conceived pregnancies. This may be different from other parts of the world, where couples who conceive through ART treatment may be more resourceful and receive different antenatal care compared to those who conceive naturally. Another important factor is systematic differences in the ART techniques that are in use. The Nordic countries today mainly use single embryo transfers, illustrated in the latest ESHRE-report with 2018 data where 80.6%, 94.3% and of ART cycles used single embryo transfer in Denmark, Finland, and Sweden, respectively [47]. In other parts of the world, multiple embryo transfer (mostly double) is still standard procedure [47, 53]. Furthermore, in the Nordic countries, ICSI fertilization is mainly used in cases of male infertility, while in the rest of the world, ICSI fertilization is used for all infertility causes and in up to three-fourths of all ART cycles [47, 53, 172]. For these reasons, the time trends we found might not be fully applicable to other parts of the world. In addition, differences in the distribution of pregnancy-specific factors like parity and pre-existing maternal characteristics like BMI, smoking, diabetes, and ethnicity might be of importance, as well as differences in cesarean section policies between countries [69, 78, 173, 174].

An important question regarding study II is whether the results from the sibling comparisons where we found higher risk of hypertensive disorders in pregnancy after frozen embryo transfer can be generalized to couples who only have one ART conceived child. The 33,209 double discordant sibling groups are after all a comparatively small fraction of the overall ART conceived group. Firstly, in the sensitivity analysis with interaction terms between parity and conception method (bidirectional analysis), the higher risk of hypertensive disorders in pregnancy after frozen embryo transfer seemed to apply to all the subgroups. This indicates that frozen embryo transfer was associated with higher risk of hypertensive disorders in pregnancy regardless of birth order and which conception method occurred first or second. Secondly, even in the sensitivity analysis in subgroups according to conception method and outcome in the 1st pregnancy (diagnosed with a hypertensive disorder during pregnancy or not), frozen embryo transfer was associated with higher risk of hypertensive disorders in

pregnancy in most subgroups. In sum, it thus seems likely that these results are indeed generalizable.

For study III, it is important to note that the occurrence of preterm birth in the Nordic countries around 6% is among the lowest in Europe and in the world [175]. Worldwide, the overall occurrence of preterm birth is estimated to be around 10%, but with large geographical differences [176]. Of note, preterm birth is prevalent also in some developed countries, for instance in the US with an overall occurrence around 12%, but with large inequalities within the population. These occurrence differences likely reflect the complex etiology that is behind preterm birth, as well as differences in antenatal care between countries [175]. It thus seems reasonable to be careful before using the findings from study III to generalize to other countries where the distribution of risk factors is different.

6.3 Comparison to other studies and interpretation of the main findings

To our knowledge, few published studies have investigated time trends in risk of placenta-mediated pregnancy complications after ART conception. However, some studies have investigated the time trends in the general population. Several studies on the general association between ART conception and adverse outcomes have been published, but few have used sibling designs and mediation analysis. The following sections will put study I–III into the broader current literature as well as proposing possible mechanisms and explanations behind our findings.

6.3.1 Time trends in risk of placenta-mediated pregnancy complications in ART conceived pregnancies

A crucial point before attempting to interpret the time trends findings from study I is to appraise the role of multiple gestations. A well-designed cohort study from Öberg and colleagues analyzing data from the Swedish Medical Birth Registry (hence, an overlapping study population to our studies), aimed to disentangle the role of multiple gestation in the association between ART conception and pregnancy complications using mediation analysis, outcomes being (among others) placental abruption, placenta previa and preeclampsia [161]. They found a higher risk of all three complications in twin pregnancies compared to singleton

pregnancies, which is in accordance with our findings (except we found a lower risk of hypertensive disorders in pregnancy in ART conceived twin pregnancies compared to naturally conceived twin pregnancies). Furthermore, they found that the higher risk of placental abruption and placenta previa after ART conception was largely independent of multiple gestations. The higher risk of preeclampsia after ART conception on the other hand seemed to be mediated to a large extent by the multiple gestations. Hence, they argue that interventions aimed at lowering the occurrence of multifetal pregnancy (e.g., the single embryo transfer approach) would presumably attenuate the positive association between ART conception and preeclampsia, but not for placental abruption and placenta previa. Their main findings support the results from study I, since we found that the higher risk of complications after ART conception became substantially weaker when we analyzed singletons and twin pregnancies separately. Since multifetal pregnancy is an important risk factor for many adverse outcomes, it thus would seem likely that the occurrence of these adverse outcomes would decrease as the Nordic countries implemented elective single embryo transfer. Indeed, in study I, when considering all ART conceived pregnancies combined, the risk of hypertensive disorders in pregnancy and placental abruption declined markedly throughout the study period and approached that in the background population. The overall interpretation is thus that the implementation of single embryo transfer in the Nordic countries has been successful in decreasing the multifetal pregnancy rate, and in turn risk of adverse outcomes.

6.3.2 Time trends in occurrence of placental abruption and placenta previa

A study from 2015 with an overlapping study population to ours, including pregnancies from Denmark, Finland, Norway, and Sweden from 1978 to 2010, reported a decline in incidence of placental abruption in many European countries [69]. This is in line with our study, where we found declining rates in all conception and plurality groups. Ananth et al. suggested several possible explanations, including temporal changes in the distribution of risk factors, particularly the declining smoking rates among pregnant women [69]. This potential explanation was however not supported by our sensitivity analysis, where adjustment for smoking did not substantially change the time trend estimate in neither naturally conceived pregnancies nor ART conceived pregnancies. When also considering that our sensitivity analysis for culture duration and cryopreservation did not impact time trend estimates for the

ART conceived pregnancies, it seems possible that the underlying reasons for the declining abruption rates may apply to both naturally conceived pregnancies and ART conceived pregnancies. When it comes to the overall higher risk of abruption in ART conceived pregnancies, we do not know the respective contributions from parental factors and ART treatment factors.

A Swiss population-based cohort study from 2017 reported an increase in the incidence of placenta previa from 0.3% in 1993 to 0.5% in 2014 [177]. An Australian cohort study analyzing Australian births from 2001 to 2009 found an increase in risk of placenta previa from 0.69% to 0.87% [81]. Some researchers have pointed to the temporal increase in cesarean section as a possible driver of increasing risk of placenta previa in the general population [174]. In our study, risk of placenta previa increased strongly in ART conceived pregnancies over the last decades. This finding seemed robust in our sensitivity analyses (adjusting for BMI and smoking, restricting analysis to primiparous women (who have no previous caesarean section), using a stricter definition of placenta previa and adjusting for a history of cesarean section.). This would suggest that temporal changes in the distribution of these important risk factors cannot explain the temporal increase in risk among ART conceived pregnancies. However, when adjusting for the temporal changes in culture duration (increasing use of blastocyst transfer), the increasing risk of placenta previa over time became weaker in both ART conceived singleton pregnancies and ART conceived twin pregnancies. Hence, it seems possible that the increasing use of blastocyst transfer can explain some of the increasing placenta previa risk in ART conceived pregnancies. This is in line with a Swedish study from 2016, reporting a higher risk of placenta previa after blastocyst transfer compared to cleavage stage transfer [91]. When it comes to the higher risk of placenta previa in ART conceived pregnancies compared to naturally conceived pregnancies in general, a Norwegian study from 2006 showed that the higher risk of placenta previa persisted in sibling comparisons, indicating that factors related to the ART treatment may contribute to the higher risk [178].

6.3.3 Time trends in occurrence of hypertensive disorders in pregnancy.

A 2021 global study of 204 countries and territories reported an increase in incidence of hypertensive disorders in pregnancy in Western countries from 1990 to 2019 regardless of conception method [179]. A study from 2011 on other hand reported a decline in risk of

gestational hypertension and preeclampsia in Northern Europe from 1997 to 2007 [60]. A recent US study reported a doubling of incidence of hypertensive disorders in pregnancy from 6.0% in 2000 to 12.0% in 2018 [180], whereas a very recent Norwegian study reported a decrease in overall preeclampsia occurrence from 4.3% in 1999–2002 to 2.7% in 2015-2018 [59]. In our study, risk of hypertensive disorders increased somewhat in singleton pregnancies and strongly over time in twin pregnancies regardless of conception method. The driving factors behind this remain unknown. Our sensitivity analysis adjusting for BMI and smoking during pregnancy reversed the time trend in the naturally conceived singleton pregnancies and substantially attenuated the time trend in the naturally conceived twin pregnancies, suggesting that temporal changes in BMI and smoking status could explain some of the time trends we observed for naturally conceived pregnancies. Furthermore, sensitivity analysis in ART conceived pregnancies revealed a moderately attenuated time trend when adjusting for increasing use of frozen embryo transfer. Unlike the Norwegian study of births from 1999 to 2018 [59], we did not find any clear decrease in occurrence of hypertensive disorders in pregnancy in singleton pregnancies, but it seems likely that this difference reflects our data from three other Nordic countries, and that we did not have data on the years 2016–2018, wherein low-dose aspirin was starting to make its way as prophylactic treatment.

6.3.4 Risk of hypertensive disorders after fresh and frozen embryo transfer

On the population level, several studies have reported a higher risk of hypertensive disorders in pregnancy after frozen embryo transfer compared to fresh embryo transfer and compared to natural conception [39, 40, 128].

A CoNARTaS sibling analysis study with pregnancies from 1988 to 2007 found a higher risk of hypertensive disorders in pregnancy after frozen embryo transfer in both twin and singleton pregnancies compared to fresh embryo transfer [40]. Our results from study II are in line with this study. In contrast, a Dutch sibling study comparing any ART conception versus natural conception for deliveries between 1999 and 2007, found higher crude risk of hypertensive disorders in pregnancy within sibships but no clear association after adjustments [109]. However, these results may be biased by adjustment for level of care, which could be a common consequence of ART conception and hypertensive disorders in pregnancy, thereby inducing collider bias [121].

The biological mechanism behind a potentially higher risk after frozen embryo transfer remains unanswered. A study from 2019 with authors from our research group used Swedish registry data from 2005 to 2015 to analyze the risk of neonatal and maternal outcomes after frozen embryo transfer, where they also found a higher risk of hypertensive disorders, in particular following frozen transfer in artificial cycles as compared to frozen transfer in natural cycles [89]. Our Danish colleagues have done a similar study with Danish data from 2006 to 2014 where they reported similar findings [90]. A 2022 multicenter cohort study with data from 2016 to 2019 on ovulatory women also found a higher risk of hypertensive disorders in frozen artificial cycles compared to frozen natural cycles [181]. Von Versen-Höynck and colleagues (and other researchers) have pointed to the absence of a corpus luteum as a possible explanation for these patterns [39, 182]. In ovulatory cycles (like in natural conception, fresh cycles, and frozen natural and frozen stimulated cycles), the corpus luteum with its secretion of relaxin (and possibly other vasoactive hormones) likely facilitates regulation and adaptation of the maternal circulatory system during early pregnancy [182]. Another hormone that may be involved is prorenin, which might influence the maternal renin-angiotensin-aldosterone-system and hence the renal adaptation to pregnancy [183, 184]. In non-ovulatory cycles (notably frozen artificial cycles), the formation of corpus luteum is bypassed altogether by the clinician, possibly attenuating the physiological adaptation. Indeed, several perinatal and obstetric outcomes seem to be negatively affected when comparing frozen artificial cycles to frozen natural cycles and frozen stimulated cycles [89, 90].

However, a potentially higher risk of hypertensive disorders after frozen embryo transfer in our study seems unlikely to be due to the missing corpus luteum alone. Unfortunately, we did not have data on type of frozen cycle, so we could not explore this hypothesis directly. However, from the Danish and Swedish studies where they had data on type of frozen cycle, we can infer that in the CoNARTaS cohort, around 17% of the frozen embryo transfer pregnancies were from frozen artificial cycles [89, 90] (since we did not have direct data on this, we here assumed that the proportion of Norwegian ART pregnancies was somewhere between the Danish and Swedish ART pregnancies with regards to frozen artificial cycle proportions). If the remaining 83% of frozen embryo transfer pregnancies (which were in either natural or stimulated cycles) had a similar risk profile to fresh embryo transfer and to

natural conception, the artificial cycles would have to have a relative risk (compared to natural conception) of around 5–6 to drive the association to the doubled risk. 5–6 is many times higher than what was found in the Danish and Swedish studies. Hence, it seems likely that other treatment characteristics of the frozen embryo transfer process contribute as well. Essentially, if there is some treatment characteristic behind, this would have to differ from the fresh cycles. Indeed, our study's lack of clear association between fresh embryo transfer and hypertensive disorders in pregnancy was somewhat surprising given the earlier literature [128]. Common features between fresh and frozen embryo transfer include the fertilization method, to a large degree the ovarian hormone stimulation (except for artificial frozen cycles) and to some degree culture duration and media. It thus seems valuable to put the findings from study II into the perspective of the 2-stage placental model of preeclampsia to find potential explanations [61]. It seems plausible that maternal preconception characteristics like advanced maternal age, primiparity and underlying diseases predisposing for infertility could affect both Stage 1 preeclampsia (i.e., inducing placental stress), but also affect responsiveness to the resulting inflammation leading to Stage 2 preeclampsia. Some have also pointed to immunological factors, for instance through poor local uterine tolerance of the trophoblast, notably very relevant when the ART conceived pregnancy is after using donor oocytes or donor sperms [185]. Although the 2-stage model can shed light over these possible mechanisms, it nonetheless seems implausible that many of these explanations could plausibly differ between pregnancies after fresh embryo transfer and pregnancies after frozen embryo transfer. Some important features that are unique to frozen embryo transfers include embryo selection [186], wherein the better-quality embryos are chosen for the initial fresh transfer, and also, only the better-quality embryos will endure the blastocyst stage and cryopreservation. Furthermore, epigenetic or other changes inherent to the freezing and thawing [187, 188, 189] can possibly influence the trophoblast invasion, in turn leading to abnormal placentation [190]. Finally, the higher risk of large for gestational age after frozen embryo transfer [108], could be compatible with the Type B placenta (intrinsic) problem, where the placental capacity is exceeded because of larger fetuses, and hence larger placentas [61].

6.3.5 Preterm birth after fresh embryo transfer and frozen embryo transfer and hypertensive disorders

Although many published studies have investigated the association between ART conception and preterm birth, few have investigated the relationship between fresh embryo transfer/frozen embryo transfer, hypertensive disorders in pregnancy, and preterm birth using mediation analysis. Stern et al. used meditation analysis to investigate the influence of placental abnormalities and pregnancy-induced hypertension in pregnancy on prematurity, and reported that compared to a fertile reference group, fresh embryo transfer pregnancies had 39% (95% CI 1.36 to 1.60) higher odds of late preterm birth, of which 4.1% (proportion mediated) could be explained by pregnancy-induced hypertension, while frozen embryo transfer pregnancies had 42% (95% CI 1.21 to 1.62) higher odds of late preterm birth, of which 25.9% (proportion mediated) could be explained by pregnancy-induced hypertension [191]. Our main findings in study III are in line with this study. Unlike the study by Stern et al., we were able to conduct analyses where a distinction could be made between spontaneous preterm birth and medically indicated preterm birth. These analyses illustrated that both frozen embryo transfer and fresh embryo transfer was associated with higher risks of both types of preterm birth. Importantly though, the influence of hypertensive disorders on these associations was to a large degree restricted to medically indicated preterm birth after frozen embryo transfer. We were unfortunately not able to explore further what mechanisms might be behind these findings. Furthermore, as described in earlier sections, the pathogenesis of preterm birth itself (outside the ART conception setting) is poorly understood, and preterm birth is difficult to both prevent and predict [115].

6.4 Implications

In study I, when considering all ART conceived pregnancies combined, risk of all the complications declined considerably and approached that in the background population during the study period, mainly due to declining occurrence of multifetal pregnancies, a major risk factor for adverse outcomes. Thus, the implementation of single embryo transfer policies in the Nordic countries has been highly successful in reducing risk of adverse outcomes. Our results therefore further emphasize the importance of single embryo transfer. Furthermore, it seems pertinent to inform clinicians and couples seeking ART treatment that ART conceived pregnancies still are at higher risk of placenta-mediated pregnancy complications despite

increasing success rates and improving neonatal outcomes in ART conceived pregnancies [47, 100]. Future studies should aim to further disentangle the role of the ART treatment versus the role of the parental factors in the higher risk of placenta-mediated pregnancy complications. Such research will be important, as identifying potentially modifiable risk factors or treatment components can provide opportunities for monitoring or prevention of pregnancy complications in ART conceived pregnancies, in turn contributing to overall better fetal and maternal health. The increasing risk of placenta previa in ART conceived pregnancies is a matter of concern and could only partly be explained by the concurrent increase in blastocyst transfers. Whether other treatment-related and thus potentially modifiable factors are involved, or whether changes in characteristics of the ART conceiving population contribute to this trend, is not yet known.

Although cryopreservation has facilitated elective single embryo transfer, thereby reducing risk of hypertensive disorders in pregnancy after ART treatment through reduction of multifetal pregnancy [34, 161], careful consideration of all benefits and harms is needed before freezing all embryos as routine (*the freeze all embryos approach*), rather than for couples with clinical indications, such as high risk of ovarian hyperstimulation syndrome [192]. Hypertensive disorders in pregnancy are relatively common, and with the estimated adjusted absolute risk difference from Table S3 in study II compared to fresh embryo transfer (and compared to natural conception), we can calculate a number needed to harm: $Number\ needed\ to\ harm = \frac{1}{Risk\ difference} = \frac{1}{0.0218} = 46$

46 in this case can be interpreted as follows: for every 46th pregnancy where the health care provider chooses a frozen embryo transfer instead of a fresh embryo transfer, that would entail 1 extra case of a hypertensive disorder in pregnancy. Hypertensive disorders in pregnancy can have severe maternal and fetal consequences [67, 193, 194]. Children born after preeclamptic pregnancies have higher blood pressures and BMIs [67], and mothers experiencing hypertensive disorders in pregnancy have a higher risk of cardiovascular diseases later in life [193]. As well as highlighting the seriousness of each single case, this also suggests that identifying subgroups at higher risk could provide opportunities for more targeted monitoring and interventions. The need for preventive measures is further emphasized by the

fact that the associations in our study were not driven by isolated gestational hypertension but were even somewhat strengthened when only considering the more serious hypertensive disorders. Furthermore, the previously reported increase in birthweight and risk of being born large for gestational age [108], should also be included in the balance sheet if a choice between fresh and frozen transfer is possible. Finally, although clinical guidelines have already identified ART conception as a major risk factor for hypertensive disorders in pregnancy [58], our findings can possibly nuance the picture by identifying frozen embryo transfer pregnancies as the driving factor, but future research should nonetheless investigate which treatment factors associated with frozen embryo transfer that might be involved in the development of hypertensive disorders in pregnancy.

Preventive strategies for preterm birth are very important given the high risk of short- and long-term adverse outcomes for the children [112, 113, 175]. Since our results indicate that most of the excess risk of preterm birth in ART conceived pregnancies was independent of hypertensive disorders in pregnancy, future research should explore which aspects of the ART treatment that might be responsible, and how this knowledge may be translated into preventive measures. For pregnancies after frozen embryo transfer, where our results indicate that a proportion of excess risk of preterm birth is mediated by hypertensive disorders, preventive strategies targeted towards hypertensive disorders in pregnancy such as prophylactic aspirin might contribute to prevention of preterm birth, and studies investigating this are warranted. For pregnancies after fresh embryo transfer, the higher risk of medically indicated preterm birth despite no higher risk of hypertensive disorders in pregnancy (and hence, no mediation) warrants further attention.

7 Conclusions

The risk of placenta-mediated pregnancy complications in the form of hypertensive disorders in pregnancy, placental abruption, and placenta previa has remained higher following ART conception compared to naturally conceived pregnancies during the last three decades of ART treatment in the Nordic countries. The risk remains higher despite declining rates of multifetal pregnancies and improvements in perinatal health and is a cause of concern which should be communicated from the clinicians to their patients. The underlying causes behind these findings are largely unknown. Nonetheless, our findings strongly favor the policy of elective single embryo transfer. When considering hypertensive disorders in pregnancy and placental abruption, pregnancies conceived through assisted reproduction followed the same time trends as the background population. Occurrence of placenta previa on the other hand seems to be temporally increasing strongly in ART conceived pregnancies and warrants further attention.

Pregnancies following frozen embryo transfer are at a higher risk of hypertensive disorders compared to naturally conceived pregnancies, even after accounting for shared parental factors. The mechanism behind this higher risk is unknown and warrants further attention. In contrast, pregnancies following fresh embryo transfer are not at a higher risk of hypertensive disorders than naturally conceived pregnancies. The higher risk of hypertensive disorders in pregnancies after frozen embryo transfer can explain some of the higher risk of preterm birth compared to naturally conceived pregnancies. In contrast, hypertensive disorders in pregnancy cannot explain the higher risk of preterm birth after fresh embryo transfer compared to natural conception. Further investigations into mechanisms and identification of potential preventive strategies of these findings are warranted.

Future studies should aim to further disentangle the role of the ART treatment versus the role of the parental factors in the higher risk of pregnancy complications. Such research will be important, as identifying potentially modifiable risk factors or treatment components can provide opportunities for prevention of pregnancy complications in ART conceived pregnancies, in turn contributing to overall better maternal and fetal health.

8 References

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Paper I

GYNECOLOGY

Time trends in placenta-mediated pregnancy complications after assisted reproductive technology in the Nordic countries

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BACKGROUND: The use of assisted reproductive technology is increasing worldwide and conception after assisted reproduction currently comprises 3%—6% of birth cohorts in the Nordic countries. The risk of placenta-mediated pregnancy complications is greater after assisted reproductive technology compared with spontaneously conceived pregnancies. Whether the excess risk of placenta-mediated pregnancy complications in pregnancies following assisted reproduction has changed over time, is unknown.

OBJECTIVES: To investigate whether time trends in risk of pregnancy complications (hypertensive disorders in pregnancy, placental abruption and placenta previa) differ for pregnancies after assisted reproductive technology compared with spontaneously conceived pregnancies during 3 decades of assisted reproduction treatment in the Nordic countries.

STUDY DESIGN: In a population-based cohort study, with data from national health registries in Denmark (1994—2014), Finland (1990—2014), Norway (1988—2015) and Sweden (1988—2015), we included 6,830,578 pregnancies resulting in delivery. Among these, 146,998 (2.2%) were pregnancies after assisted reproduction (125,708 singleton pregnancies, 20,668 twin pregnancies and 622 of higher order plurality) and 6,683,132 (97.8%) pregnancies were conceived spontaneously (6,595,185 singleton pregnancies, 87,106 twin pregnancies and 1,289 of higher order plurality). We used logistic regression with post-estimation to estimate absolute risks and risk differences for each complication. We repeated analyses for singleton and twin pregnancies, separately. In subsamples with available information, we also adjusted for maternal body mass index, smoking during pregnancy, previous cesarean delivery, culture duration, and cryopreservation.

RESULTS: The risk of each placental complication was consistently greater in pregnancies following assisted reproductive technology compared with spontaneously conceived pregnancies across the study period, except for hypertensive disorders in twin pregnancies, where risks were similar. Risk of hypertensive disorders increased over time in twin pregnancies for both conception methods, but more strongly for pregnancies following assisted reproductive technology (risk difference, 1.73 percentage points per 5 years; 95% confidence interval, 1.35—2.11) than for spontaneously conceived twins (risk difference, 0.75 percentage points; 95% confidence interval, 0.61—0.89). No clear time trends were found for hypertensive disorders in singleton pregnancies. Risk of placental abruption decreased over time in all groups. Risk differences were —0.16 percentage points (95% confidence interval, —0.19 to —0.12) and —0.06 percentage points (95% confidence interval, —0.06 to —0.05) for pregnancies after assisted reproduction and spontaneously conceived pregnancies, respectively, for singletons and multiple pregnancies combined. Over time, the risk of placenta previa increased in pregnancies after assisted reproduction among both singletons (risk difference, 0.21 percentage points; 95% confidence interval, 0.14—0.27) and twins (risk difference, 0.30 percentage points; 95% confidence interval, 0.16—0.43), but remained stable in spontaneously conceived pregnancies. When adjusting for culture duration, the temporal increase in placenta previa became weaker in all groups of assisted reproductive technology pregnancies, whereas adjustment for cryopreservation moderately attenuated trends in assisted reproductive technology twin pregnancies.

CONCLUSIONS: The risk of placenta-mediated pregnancy complications following assisted reproductive technology remains higher compared to spontaneously conceived pregnancies, despite declining rates of multiple pregnancies. For hypertensive disorders in pregnancy and placental abruption, pregnancies after assisted reproduction follow the same time trends as the background population, whereas for placenta previa, risk has increased over time in pregnancies after assisted reproductive technology.

Key words: assisted reproduction, gestational hypertension, hypertensive disorders in pregnancy, in vitro fertilization, placenta previa, placental abruption, preeclampsia, reproductive medicine, temporal changes, twins

Assisted reproductive technology (ART) comprises conception methods in which fertilization takes place outside the female body. Risk of placenta-mediated pregnancy complications, including preeclampsia, placental abruption, and placenta previa, is greater in pregnancies after ART treatment compared with spontaneously conceived (SC) pregnancies.[1,2] Risk of adverse perinatal outcomes such as preterm birth, low birthweight, and perinatal death is also greater.[3,4] This has been attributed partly to the high occurrence of multiple pregnancies after ART treatment. Still, singleton ART pregnancies also carry a greater risk of adverse outcomes compared with SC singletons.[1,2] The underlying causes of infertility, as well as the ART treatment itself, may both contribute to the greater risk.[5—8] It has been hypothesized that the super-physiological hormone levels seen in ART cycles may alter early placentation and thereby contribute to adverse outcomes.[9]

Worldwide, ART treatment has increased steadily over the past decades,

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AJOG at a Glance

Why was this study conducted?

Use of assisted reproductive technology increases worldwide with improving perinatal outcomes. We aimed to investigate changes in occurrence of placenta-mediated complications in ART pregnancies compared to the background population over three decades.

Key findings

Assisted reproductive technology pregnancies continue to be at greater risk, despite declining rates of multiple pregnancies. Risk of hypertensive disorders in twin pregnancies is increasing regardless of conception method, while risk of placenta previa has increased more strongly in assisted reproductive technology pregnancies. Risk of placental abruption risk has decreased in both populations.

What does this add to what is known?

Recent improvements in perinatal outcomes after assisted reproductive technology have not been accompanied by a corresponding improvement in maternal pregnancy health in this population. Increasing risk of placenta previa requires further attention.

due to increasing availability and success rates in combination with sociodemographic changes with postponement of childbearing.[10] Simultaneously, perinatal outcomes after ART conception have improved and are approaching the levels of the background population, mainly due to reduction of multiple births, but also due to the improved health in ART singletons.[11]

It seems likely that the increasing use and success rates of ART would be accompanied by changes in the population of women seeking medical attention for infertility. Women treated with ART today comprise a larger proportion of the total population and may therefore be more comparable with the background population than women treated some decades ago. Conversely, advances in ART[12] over time may also have enabled more severely infertile women to become pregnant. Previous studies indicate that risk of some placenta-mediated pregnancy complications, namely preeclampsia and placental abruption, are declining in the general population.[13,14] Whether this development also concerns ART pregnancies is unknown.

The objective of this study was to investigate whether time trends in occurrence of placenta-mediated pregnancy complications, hypertensive disorders in pregnancy (HDP), placental abruption, and placenta previa, differ for ART pregnancies compared with SC pregnancies during 3 decades of ART treatment in the Nordic countries.

Materials and Methods

Study population and data sources

The Committee of Nordic ART and Safety (CoNARTaS) study population comprises all deliveries in Denmark (1994−2014), Finland (1990−2014), Norway (1984−2015), and Sweden (1985−2015). Data were obtained from the nationwide Medical Birth Registries (MBRs) in each country, where detailed information on maternal, fetal, and neonatal health for all deliveries is recorded. Individual-level data from MBRs can be linked to other data sources through the unique national identity number assigned to all residents in the Nordic countries.[15] ART conception was determined through direct reporting to MBRs (Finland 1990−2014, Norway 1984−2015, and Sweden 1985−2006), in separate notifications of all ART pregnancies at gestational week 6-7 (Norway 1984−2015) or through linkage with cycle-based ART registries (Denmark 1994−2014 and Sweden 2007−2015).

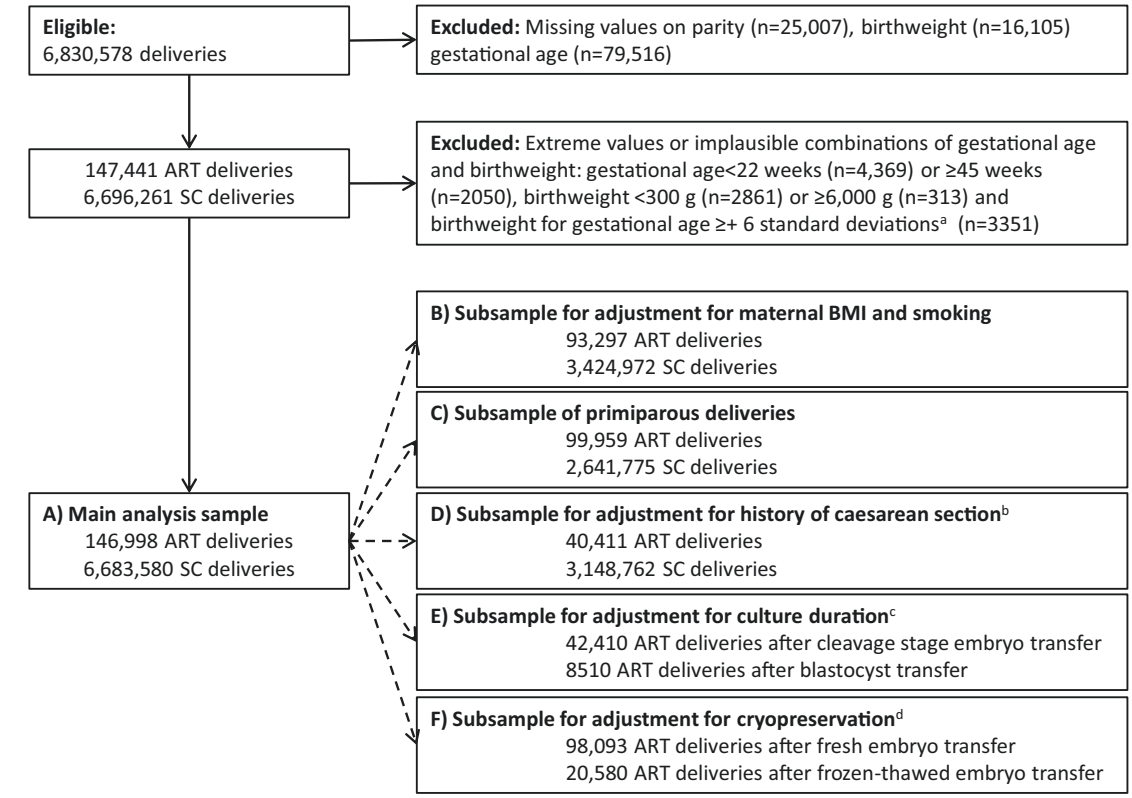
From the MBRs we obtained information on birth year, plurality, birthweight, gestational age, offspring sex, parity, maternal age, smoking status in pregnancy and body mass index (BMI, measured prepregnancy or in first trimester). For SC pregnancies, gestational age was estimated based on ultrasound examination or on last menstrual period if information from ultrasound examination was unavailable. For ART pregnancies, gestational age was estimated based on ultrasound examination or on date of embryo transfer and culture duration, according to clinical practice in each country.

Information on pregnancy complications was obtained directly from MBRs in Finland (2004−2014), Norway (1984−2015), and Sweden (1985−2015) and from data linkage with national patient registries (NPRs) in Denmark (1994−2014) and Finland (1989−2014). In the MBRs, complications are reported at delivery with limited information on gestational age at diagnosis. In Norway, the MBR revised the notification form in 1998, changing the reporting of pregnancy complications from free text to checkboxes. For NPR data, diagnoses from each prenatal visit, delivery and postpartum controls were linked to each pregnancy using maternal identity and date of delivery. The Danish NPR comprised data from hospital admissions and outpatient visits in public specialist health care during the entire study period, and from private specialist health care since 2003. The Finnish NPR expanded its data collection in 1998 from hospital admissions only to include also hospital outpatient visits.

Because there were very few ART deliveries during the first years of registration, and among women of young or high reproductive ages, we restricted the study to 1988−2015 and deliveries with maternal age 22−44 years. Thus, a total of 6,830,578 deliveries among 4,160,402 women were eligible.

We excluded 120,628 deliveries with missing information on one or more study variables and 12,944 deliveries with gestational age <22 or ≥ 45 weeks, birthweight <300 g or ≥ 6000 g and birthweight for gestational age $\geq +6$ standard deviations.[16] Multiple pregnancies were excluded when at least one

FIGURE 1
Selection of the study population and subsamples for sensitivity analyses



Solid-line arrows pointing to the right indicate exclusions, and *dashed-line arrows* pointing to the right indicate subsample selection. [a]Using z-scores from Marsal et al[16] where the authors developed growth curves based on ultrasonography from Swedish centers and made exclusive curves according to offspring sex. [b]Deliveries among parous women whose first delivery was included in the main analysis sample and thus had information on delivery mode in all previous deliveries. [c]Data from Denmark (2011–2014), Norway (2011–2015), and Sweden (2006–2015). [d]Data from Denmark (1994–2014), Norway (1988–2015), and Sweden (1988–2015).

ART, assisted reproductive technology, *BMI*, body mass index; *SC*, spontaneous conception.

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child met the exclusion criteria. Our main analysis sample included 146,998 deliveries after ART and 6,683,580 deliveries of SC pregnancies. Selection of the study population and subsamples for sensitivity analyses are described in Figure 1.

Outcome variables

Pregnancy complications were registered according to national adaptations of the *International Classification of Diseases and related Health Problems* classification as outlined in Supplemental Table 1. We considered HDP as a combined outcome including preeclampsia, eclampsia, gestational hypertension, and chronic hypertension with superimposed preeclampsia. We did not consider chronic hypertension as a hypertensive disorder in pregnancy because prepregnancy conditions cannot be a consequence of ART. For MBR data, any reporting of relevant *International Classification of Diseases and related Health Problems* codes was considered as events, whereas the following diagnoses were included from NPRs: Diagnoses of HDP registered after 20 weeks gestation, any diagnosis of placental abruption, and any diagnosis of placenta previa in the third trimester or within one month before delivery.

Statistical analyses

We used logistic regression to estimate time trends in occurrence of pregnancy complications within the ART and SC populations. To facilitate interpretation, we used post-estimation commands to calculate absolute risks and risk differences (RDs) with 95% confidence intervals (CIs). We estimated trends over birth year categories (1988–1992, 1993–1997, 1998–2002, 2003–2007,

TABLE 1
Characteristics of the study population according to conception method and year of birth

	Total study period		ART pregnancies			Spontaneously conceived pregnancies		
	ART	SC	1988–1997	1998–2007	2008–2015	1988–1997	1998–2007	2008–2015
N = 6,830,578	146,998 (2.2)	6,683,132 (97.8)	17,878 (0.8)	59,215 (2.3)	69,905 (3.4)	2,206,123 (99.2)	2,481,325 (97.7)	1,996,132 (96.6)
Country								
Denmark	37,230 (3.0)	1,200,360 (97.0)	3161 (1.3)	17,796 (3.0)	16,273 (4.2)	244,601 (98.7)	583,124 (97.0)	372,635 (95.8)
Finland	25,207 (1.8)	1,350,409 (98.2)	4345 (0.9)	11,287 (2.2)	9575 (2.5)	465,306 (99.1)	510,810 (97.8)	374,293 (97.5)
Norway	28,839 (2.0)	1,446,218 (98.0)	3412 (0.7)	10,628 (2.0)	14,799 (3.3)	488,567 (99.3)	519,083 (98.0)	438,568 (96.7)
Sweden	55,722 (2.0)	2,686,593 (98.0)	6960 (0.7)	19,504 (2.2)	29,258 (3.5)	1,007,649 (99.3)	868,308 (97.8)	810,636 (96.5)
Mean maternal age, y (SD)	33.8 (4.2)	30.3 (4.7)	33.6 (3.8)	33.6 (4.1)	34.1 (4.3)	29.5 (4.6)	30.6 (4.6)	30.9 (4.8)
Parity								
Nullipara	99,974 (68.0)	2,641,775 (39.5)	13,542 (75.8)	41,224 (69.6)	45,208 (64.7)	841,256 (38.1)	986,562 (39.8)	813,957 (39.5)
Para 1	38,820 (26.4)	2,485,615 (37.2)	3624 (20.3)	14,651 (24.7)	20,545 (29.4)	818,657 (37.1)	919,106 (37.0)	747,852 (37.2)
Para 2	6305 (4.3)	1,065,203 (15.9)	554 (3.1)	2521 (4.3)	3230 (4.6)	378,509 (17.2)	390,943 (15.8)	295,751 (14.8)
Para 3+	1899 (1.3)	490,987 (7.4)	158 (0.9)	819 (1.4)	922 (1.3)	167,701 (7.6)	184,714 (7.4)	138,572 (6.9)
Mean maternal BMI, kg/m^2 (SD)	24.2 (4.1)	24.3 (4.6)	24.0 (3.6)	24.4 (4.2)	24.2 (4.1)	23.4 (3.8)	24.4 (4.5)	24.6 (4.8)
Missing[a]	51,967 (35.4)	3,180,859 (47.6)	12,646 (70.7)	30,739 (51.9)	8582 (12.3)	1,590,714 (72.1)	1,317,466 (53.1)	272,679 (13.7)
Smoking in pregnancy	8395 (5.7)	789,825 (11.8)	1784 (10.0)	4065 (7.8)	2006 (2.9)	313,034 (14.2)	311,304 (12.6)	165,487 (8.3)
Missing[b]	9980 (6.8)	818,702 (12.3)	5030 (28.1)	3364 (5.7)	1586 (2.3)	717,524 (28.0)	156,580 (6.3)	44,598 (2.2)
Cesarean delivery	45,400 (30.9)	1,027,247 (15.4)	6224 (34.8)	18,722 (31.6)	20,454 (29.3)	273,740 (12.4)	404,380 (16.3)	349,127 (17.5)
Induction of labor	30,482 (20.7)	893,632 (13.4)	2977 (16.7)	10,975 (18.5)	16,530 (23.7)	220.96 (10.0)	321,128 (12.9)	352,408 (17.7)
Plurality								
Singleton	125,708 (85.5)	6,595,185 (98.7)	13,337 (74.6)	48,527 (82.0)	63,844 (91.3)	2,178,651 (98.8)	2,447,637 (98.6)	1,968,897 (98.7)
Twins	20,668 (14.1)	87,106 (1.3)	4168 (23.3)	10,508 (17.8)	5992 (8.6)	26,998 (1.2)	33,240 (1.3)	26,868 (1.4)
Higher order	622 (0.4)	1289 (0.02)	373 (2.1)	180 (0.3)	69 (0.1)	474 (0.02)	448 (0.02)	367 (0.02)
Preterm birth								
Singletons	10,038 (8.0)	319,385 (4.8)	1286 (9.6)	3983 (8.2)	4769 (7.5)	106,913 (4.9)	121,037 (4.6)	91,435 (4.6)
Twins	9535 (46.1)	37,309 (42.8)	1891 (45.4)	4974 (47.3)	2670 (44.6)	11,049 (40.9)	14,511 (43.7)	11,749 (43.7)

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(continued)

TABLE 1
Characteristics of the study population according to conception method and year of birth *(continued)*

	Total study period		ART pregnancies				Spontaneously conceived pregnancies			
	ART	SC	1988–1997	1998–2007	2008–2015		1988–1997	1998–2007	2008–2015	
Mean gestation in days (SD)										
Singletons	275.6 (15.6)	278.8 (12.9)	275.7 (17.1)	276.5 (15.8)	276.8 (15.1)		279.0 (13.1)	278.7 (12.9)	278.7 (12.6)	
Twins	253.8 (20.9)	255.4 (20.5)	254.8 (21.4)	253.8 (20.8)	253.2 (20.8)		256.9 (20.7)	255.1 (20.4)	254.3 (20.1)	

Unless otherwise specified, numbers of observations and percent are presented.

ART, assisted reproductive technology; *BMI*, body mass index; *SC*, spontaneous conception; *SD*, standard deviation.

[a] No data available from Denmark before 2003, Finland before 2003, and Norway before 2006; [b] No data available from Norway before December 1998.

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2008–2012, 2013–2015) and as linear trends across the study period (change per 5 years, continuous variable). We also compared risk of each complication in ART versus SC pregnancies within each period as a measure of whether risks in the 2 populations converged over time. Analyses were performed on the all pregnancies, and for singletons and twins, separately. We adjusted for parity, maternal age and country. To investigate whether time trends differed between countries, we repeated analyses for each country separately.

We performed several sensitivity analyses to investigate potential explanations for the observed trends: We repeated analyses for primiparous women. In subsamples with available information, we adjusted for maternal BMI and smoking. Within the ART population, we also adjusted for embryo cryopreservation (restricted to Denmark, Norway and Sweden) and culture duration (cleavage stage 2–3 days vs blastocyst stage 5–6 days, restricted to Denmark and Sweden). Next, we restricted diagnosis of placenta previa to pregnancies with delivery by cesarean section, which is required in cases of complete obstruction. Furthermore, to investigate the potential impact of a previous cesarean delivery, a known risk factor for placenta previa subjected to marked time trends, we adjusted for this in a subsample of deliveries among parous women whose first delivery was included in the study. Statistical analyses were performed using Stata/MP for Windows, Version 15.0 (StataCorp LLC, College Station, TX).

Ethical considerations and approvals

Approvals for data retrieval and linkage were obtained in each country. In Denmark and Finland, ethical approval is not required for research solely based on registry data. In Norway, ethical approval was given by the Regional Committee for Medical and Health Research Ethics (REC North, 2010/1909). In Sweden approval was obtained from the Ethical committee in Gothenburg, Dnr 214-12, T422-12, T516-15, T233-16, T300-17, T1144-17, T121-18.

Results

For the total period, deliveries after ART constituted 3.0% of birth cohorts in Denmark, 1.8% in Finland, 2.0% in Norway, and 2.0% in Sweden (Table 1). There was a clear increase in ART deliveries over time from 0.8% of all deliveries in 1988−1997 to 3.4% in 2008−2015, accompanied by a reduction of multiple pregnancies in ART from 26% in 1988−1997 to 8.7% in 2008−2015. The proportion of SC multiple pregnancies remained stable around 1.3%.

Overall, parity was lower (68.0% vs 39.5% primiparous) and mean maternal age higher (33.8 vs 30.3 years) in ART compared with SC pregnancies, whereas BMI was similar between the 2 groups. ART mothers smoked less (5.7%) than spontaneously conceiving mothers (11.8%). Cesarean deliveries (30.9% vs 15.4%) and labor inductions (20.7% vs 13.4%) were more common in ART compared with SC pregnancies.

Hypertensive disorders in pregnancy

Risk of HDP in SC pregnancies was 4.4% (Table 2). For all pregnancies (ie, singletons and multiples combined), risk of HDP was greater in ART compared with SC pregnancies throughout the study period (odds ratio [OR], 1.25, 95% CI, 1.23−1.28, corresponding to a RD of 1.06 percentage points [*pp*]). In SC pregnancies, risk increased with 0.17 *pp* per 5 years (95% CI, 0.16−0.18). The increase was stronger in twin compared with singleton pregnancies (RD 0.75 and 0.16 *pp* per 5 years, respectively). When adjusting for maternal smoking and BMI in a subsample, time trends were reversed in SC singletons and substantially attenuated in SC twins (Supplemental Table 2). For all ART pregnancies combined, there was no clear time trend. However, in separate analyses of singleton and twin pregnancies, development followed that in SC pregnancies (Figure 2A−B), with strongly increasing risk in twin pregnancies (RD 1.73 *pp* per 5 years, 95% CI, 1.35−2.11) in all countries. Adjustment for maternal smoking and BMI had little influence on trends in ART pregnancies, but adjustment for cryopreservation moderately attenuated trends in ART twin pregnancies (Supplemental Table 2).

Placental abruption

Risk of placental abruption in SC pregnancies was 0.43% (Table 3). Throughout the study period, risk of placental abruption was consistently greater in ART compared with SC pregnancies, both overall (OR, 1.95 across the study period; 95% CI, 1.83−2.07, corresponding to a RD of 0.40 *pp*) and when separating singleton and twin pregnancies. Risk of placental abruption decreased weakly over time in SC pregnancies (RD −0.06 *pp* per 5 years, 95% CI, −0.06 to −0.05), with similar trends for singleton and twin pregnancies. In ART pregnancies, the risk decrease was somewhat stronger than in SC pregnancies (RD −0.16 *pp* per 5 years, 95% CI, −0.19 to −0.12) and of similar magnitude in singletons and twins. Country specific analyses were compatible with results from pooled analyses (Figure 2C−D). In all groups, time trends remained broadly similar after additional adjustment for BMI and smoking (Supplemental Table 3).

Placenta previa

Risk of placenta previa in SC pregnancies was 0.34% (Table 4). Placenta previa was considerably more common in ART compared with SC pregnancies across the study period for all pregnancies combined (OR, 3.87; 95% CI, 3.70−4.04 corresponding to a RD of 0.95 *pp*), and for singleton and twin pregnancies separately. In SC pregnancies, risks did not substantially differ between singletons and twins, whereas for ART pregnancies, risk was somewhat greater for singletons than for twins. In SC pregnancies, risk increased weakly over time for singleton pregnancies (RD 0.03 *pp* per 5 years) but remained stable for twins (Figure 2E). In contrast, risk increased strongly with time in ART pregnancies (RD 0.24 *pp* per 5 years for all pluralities combined, 95% CI, 0.18−0.30). Trends in ART pregnancies were similar for singletons and twins and were most pronounced in Denmark and Finland (Figure 2F).

In sensitivity analyses, results remained similar when adjusting for smoking and BMI (Supplemental Table 4), when restricting analyses to primiparous women, when restricting diagnoses of placenta previa to those accompanied by cesarean delivery, and when adjusting for previous cesarean delivery. When we adjusted for culture duration, the temporal increase in placenta previa became weaker in all groups of ART pregnancies.

Comment

Main findings

In this registry-based cohort with nationwide data from 4 countries across almost 3 decades, we found a greater risk of placenta-mediated pregnancy complications in ART pregnancies compared with the background population of SC children throughout the study period. For placenta previa, risk increased substantially over time in ART pregnancies, in contrast to a weakly increasing risk in the background population. For HDP, ART pregnancies followed the trends of the background population, with weakly increasing occurrence in singletons and strongly increasing occurrence in twins. Risk of placental abruption decreased over time in all groups.

Results

Recent meta-analyses of observational studies show positive associations between ART conception and gestational hypertension, preeclampsia, placental abruption, and placenta previa.[1,17] Our results are largely consistent with these studies, apart from lower risk of HDP in ART twin pregnancies compared with SC twin pregnancies.

We are not aware of previous studies investigating time trends in pregnancy complications following ART conception. However, some studies of time trends in the general population exist for these complications. In contrast to the weakly increasing rates of HDP in the general population that we observed from 1988 to 2015, Roberts et al[14] reported declining rates of gestational hypertension and preeclampsia in

TABLE 2
Risk of hypertensive disorders in pregnancy, according to conception method and birth year

	Within the ART population				Within the SC population				Within birth year period: ART versus SC[a]		
	Cases	Risk, %	RD,[b] *pp*	(95% CI)	Cases	Risk, %	RD,[b] *pp*	(95% CI)	RD,[b] *pp*	OR[b]	(95% CI)
All pregnancies											
88–92	259	8.33	0.07	(−0.09 to 1.1)	35,857	3.73	−0.80	(−0.86 to −0.74)	1.45	1.42	(1.24 to 1.61)
93–97	1108	7.50	−0.50	(−0.98 to −0.02)	47,672	3.83	−0.76	(−0.81 to −0.70)	1.15	1.32	(1.24 to 1.40)
98–02	2192	8.58	0.85	(0.42 to 1.27)	57,112	4.73	0.06	(−0.01 to −0.12)	1.47	1.34	(1.28 to 1.40)
03–07	2774	8.24	0.75	(0.37 to 1.14)	61,722	4.85	0.08	(0.03 to 0.13)	1.43	1.32	(1.26 to 1.37)
08–12	3297	7.38	0	Ref	63,525	4.82	0	Ref	0.75	1.16	(1.12 to 1.21)
13–15	1842	7.29	−0.01	(−0.49 to 0.33)	30,688	4.53	−0.21	(−0.27 to −0.15)	0.86	1.20	(1.14 to 1.26)
Per 5 years			−0.06	(−0.16 to −0.05)			0.17	(0.16 to 0.18)			
Total	11,472	7.80			296,587	4.44			1.06	1.25	(1.23 to 1.28)
Singleton pregnancies											
88–92	161	7.18	−0.07	(−1.10 to 0.96)	34,972	3.66	−0.77	(−0.82 to −0.71)	0.75	1.21	(1.03 to 1.43)
93–97	687	6.19	−0.94	(−1.43 to −0.45)	46,088	3.76	−0.73	(−0.78 to −0.68)	0.29	1.08	(1.00 to 1.17)
98–02	1395	7.05	0.16	(−0.27 to 0.59)	55,232	4.63	0.07	(0.02 to 0.13)	0.38	1.09	(1.03 to 1.15)
03–07	2010	7.00	0.35	(−0.03 to 0.74)	59,537	4.74	0.08	(0.03 to 0.13)	0.55	1.12	(1.07 to 1.18)
08–12	2650	6.56	0	Ref	61,203	4.70	0	Ref	0.23	1.05	(1.01 to 1.10)
13–15	1545	6.59	0.06	(−0.34 to 0.47)	29,494	4.42	−0.21	(−0.27 to −0.15)	0.37	1.11	(1.06 to 1.11)
Per 5 years			0.13	(0.01 to 0.24)			0.16	(0.15 to 0.18)			
Total	8448	6.72			286,346	4.32			0.38	1.09	(1.07 to 1.12)
Twin pregnancies											
88–92	78	11.02	−6.77	(−9.09 to −4.44)	1,041	9.50	−3.23	(−3.96 to −2.50)	−1.37	0.84	(0.65 to 1.09)
93–97	400	11.56	−5.40	(−6.93 to −3.87)	1,559	9.72	−2.71	(−3.38 to −2.04)	−1.44	0.84	(0.74 to 0.96)
98–02	787	13.96	−2.43	(−3.89 to −0.99)	1,858	11.79	−0.69	(−1.39 to 0.00)	−1.05	0.90	(0.82 to 1.00)
03–07	747	15.33	−0.35	(−1.88 to 1.12)	2,175	12.44	−0.22	(−0.90 to 0.46)	0.18	1.02	(0.92 to 1.12)
08–12	641	15.19	0	Ref	2,296	12.85	0	Ref	−0.24	0.98	(0.88 to 1.08)
13–15	294	16.59	0.80	(−1.30 to 2.90)	1,176	13.06	−0.03	(−0.85 to 0.79)	0.51	1.05	(0.91 to 1.22)
Per 5 years			1.73	(1.35 to 2.11)			0.75	(0.61 to 0.89)			
Total	2947	14.26			10,105	11.60			−0.55	0.95	(0.90 to 0.99)

ART, assisted reproductive technology; *CI*, confidence interval, *OR*, odds ratio; *RD*, risk difference; *Ref*, reference group; *SC*, spontaneous conception.

[a] SC pregnancies are the reference group within each birth year category; [b] Adjusted for parity (0, 1, 2, 3+), age at birth (22–25, 25–28, 28–31, 31–34, 34–37, 37–40, 40–45), and country.

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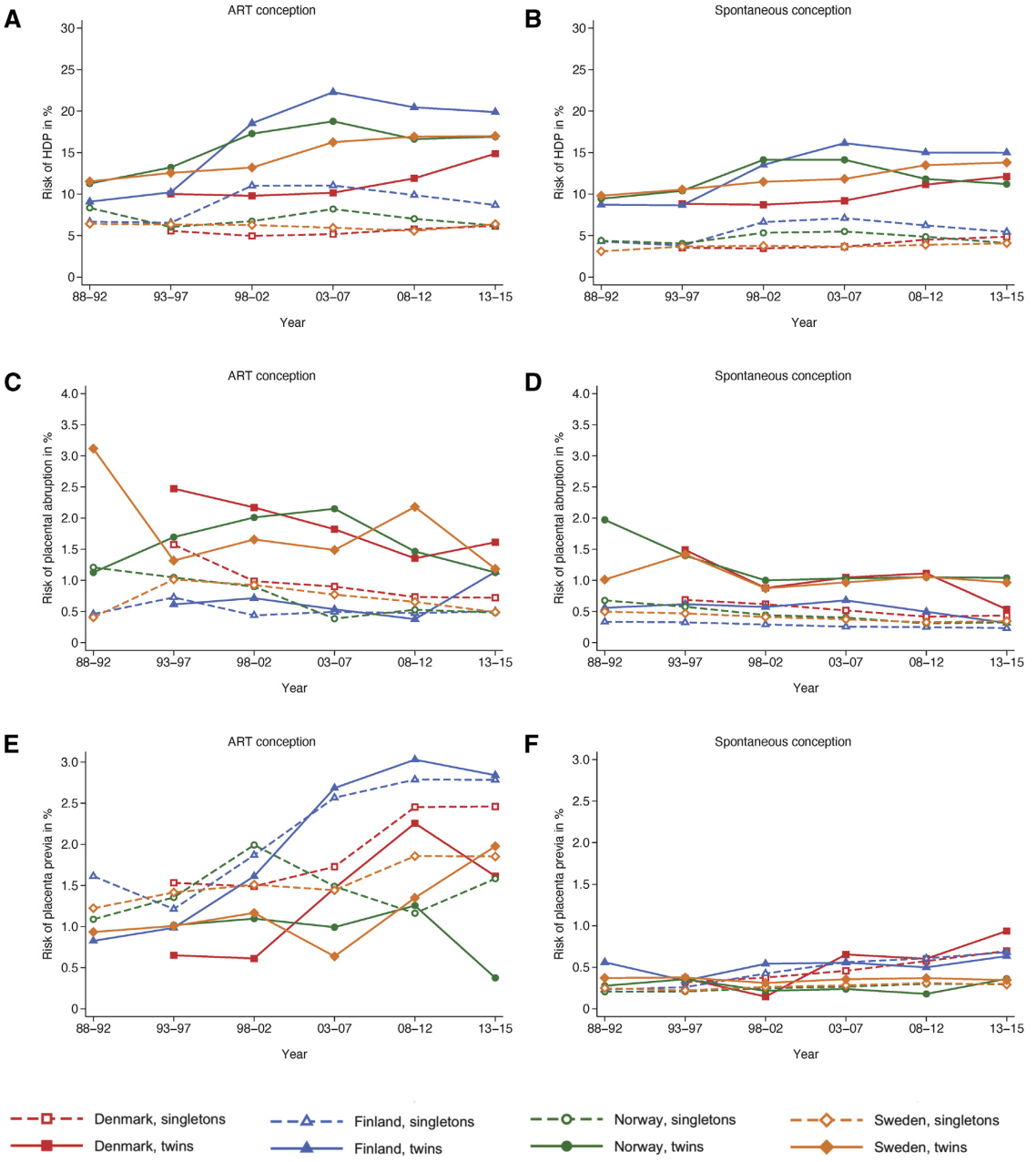
FIGURE 2
Time trends in risk of placenta-mediated pregnancy complications



Shown are time trends in risk of HDP, placental abruption, and placenta previa according to conception method, plurality, and country. (**A**) HDP in ART pregnancies. (**B**) HDP in SC pregnancies. (**C**) Placental abruption in ART pregnancies. (**D**) Placental abruption in SC pregnancies. (**E**) Placenta previa in ART pregnancies. (**F**) Placenta previa in SC pregnancies. Estimates are adjusted for parity and maternal age.

ART, assisted reproductive technology, *HDP*, hypertensive disorders in pregnancy; *SC*, spontaneous conception.

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TABLE 3
Risk of placental abruption, according to conception method and birth year

	Within the ART population				Within the SC population				Within birth year period: ART vs SC[a]		
	Cases	Risk, %	RD,[b] *pp*	(95% CI)	Cases	Risk, %	RD,[b] *pp*	(95% CI)	RD,[b] *pp*	OR[b]	(95% CI)
All pregnancies											
88–92	31	1.00	0.42	(0.02 to 0.82)	5030	0.52	0.24	(0.22 to 0.26)	0.36	1.70	(1.19 to 2.43)
93–97	172	1.16	0.54	(0.34 to 0.74)	6371	0.51	0.19	(0.17 to 0.21)	0.51	2.19	(1.88 to 2.43)
98–02	262	1.03	0.34	(0.20 to 0.49)	5442	0.45	0.11	(0.10 to 0.13)	0.51	2.14	(1.88 to 2.43)
03–07	279	0.83	0.12	(0.00 to 0.24)	5064	0.40	0.06	(0.04 to 0.07)	0.35	1.88	(1.66 to 2.13)
08–12	314	0.70	0	Ref	4433	0.34	0	Ref	0.33	1.98	(1.76 to 2.23)
13–15	148	0.59	−0.11	(−0.23 to 0.01)	2341	0.35	0.01	(−0.01 to 0.03)	0.20	1.57	(1.32 to 1.87)
Per 5 years			−0.16	(−0.19 to −0.12)			−0.06	(−0.06 to −0.05)			
Total	1206	0.82			28,681	0.43			0.40	1.95	(1.83 to 2.07)
Singleton pregnancies											
88–92	16	0.71	0.18	(−0.22 to 0.58)	4895	0.51	0.24	(0.22 to 0.26)	0.11	1.22	(0.75 to 2.01)
93–97	118	1.06	0.48	(0.27 to 0.70)	6167	0.50	0.19	(0.17 to 0.21)	0.51	2.02	(1.67 to 2.43)
98–02	164	0.83	0.21	(0.06 to 0.36)	5308	0.45	0.11	(0.10 to 0.13)	0.32	1.73	(1.47 to 2.02)
03–07	199	0.69	0.07	(−0.05 to 0.19)	4896	0.39	0.06	(0.04 to 0.07)	0.23	1.60	(1.39 to 1.85)
08–12	252	0.62	0	Ref	4261	0.33	0	Ref	0.27	1.82	(1.59 to 2.07)
13–15	125	0.53	−0.09	(−0.21 to 0.34)	2264	0.34	0.01	(−0.03 to 0.03)	0.15	1.46	(1.21 to 1.75)
Per 5 years			−0.11	(−0.15 to −0.07)			−0.06	(−0.06 to −0.05)			
Total	874	0.70			27,791	0.42			0.29	1.70	(1.58 to 1.82)
Twin pregnancies											
88–92	13	1.84	0.62	(−0.52 to 1.76)	129	1.18	0.20	(−0.05 to 0.46)	0.96	1.85	(0.99 to 3.46)
93–97	51	1.47	0.25	(−0.33 to 0.83)	201	1.25	0.30	(0.07 to 0.52)	0.59	1.50	(1.06 to 2.13)
98–02	96	1.70	0.39	(−0.10 to 0.88)	133	0.84	−0.12	(−0.33 to 0.08)	1.04	2.30	(1.70 to 3.10)
03–07	80	1.64	0.24	(−0.25 to 0.72)	166	0.95	−0.02	(−0.22 to 0.19)	0.67	1.72	(1.28 to 2.31)
08–12	61	1.45	0	Ref	172	0.96	0	Ref	0.50	1.53	(1.11 to 2.11)
13–15	23	1.30	−0.15	(−0.76 to 0.45)	74	0.82	−0.16	(−0.39 to 0.07)	0.55	1.69	(1.01 to 2.83)
Per 5 years			−0.15	(−0.29 to 0.00)			−0.08	(−0.12 to −0.03)			
Total	324	1.57			875	1.00			0.72	1.75	(1.51 to 2.02)

ART, assisted reproductive technology; *CI*, confidence interval, *OR*, odds ratio; *RD*, risk difference; *Ref*, reference group; *SC*, spontaneous conception.

[a] SC pregnancies are the reference group within each birth year category; [b] Adjusted for parity (0, 1, 2, 3+), age at birth (22–25, 25–28, 28–31, 31–34, 34–37, 37–40, 40–45), and country.

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TABLE 4
Risk of placenta previa, according to conception method and birth year

	Within the ART population				Within the SC population				Within birth year period: ART versus SC[a]		
	Cases	Risk, %	RD,[b] *pp*	(95% CI)	Cases	Risk, %	RD,[b] *pp*	(95% CI)	RD[b], *pp*	OR[b]	(95% CI)
All pregnancies											
88–92	34	1.09	−0.81	(−1.23 to −0.40)	2,277	0.24	-0.11	(-0.12 to -0.09)	0.64	3.72	(2.62 to 5.26)
93–97	185	1.25	−0.76	(−0.98 to −0.54)	3,089	0.25	-0.14	(-0.16 to -0.13)	0.71	3.89	(3.33 to 4.56)
98–02	390	1.53	−0.50	(−0.69 to −0.30)	3,867	0.32	-0.09	(-0.10 to -0.08)	0.89	3.82	(3.42 to 4.27)
03–07	562	1.67	−0.32	(−0.51 to −0.14)	4,973	0.38	-0.04	(-0.06 to -0.03)	1.04	3.79	(3.46 to 4.16)
08–12	891	2.00	0	Ref	5,578	0.42	0	Ref	1.22	3.94	(3.65 to 4.25)
13–15	498	1.97	0.04	(0.18 to 0.26)	2,806	0.41	0.02	(-0.00 to 0.03)	1.20	3.93	(3.54 to 4.35)
Per 5 years			0.24	(0.18 to 0.30)			0.03	(0.03 to 0.04)			
Total	2,560	1.74			22,410	0.34			0.95	3.87	(3.70 to 4.04)
Singleton pregnancies											
88–92	28	1.25	−0.65	(−1.17 to −0.14)	2,235	0.23	−0.11	(−0.13 to −0.09)	0.74	4.20	(2.87 to 6.14)
93–97	153	1.38	−0.65	(−0.90 to −0.39)	3,030	0.25	−0.14	(−0.16 to −0.13)	0.79	4.22	(3.56 to 5.01)
98–02	329	1.66	−0.39	(−0.61 to −0.16)	3,821	0.32	−0.09	(−0.10 to −0.07)	0.98	4.08	(3.62 to 4.60)
03–07	494	1.72	−0.29	(−0.49 to −0.09)	4,715	0.38	−0.04	(−0.06 to −0.03)	1.08	3.93	(3.56 to 4.33)
08–12	810	2.01	0	Ref	5,504	0.42	0	Ref	1.25	3.99	(3.69 to 4.32)
13–15	471	2.01	0.06	(−0.16 to 0.29)	2,762	0.41	0.01	(0.00 to 0.03)	1.24	4.04	(3.64 to 4.49)
Per 5 years			0.21	(0.14 to 0.27)			0.03	(0.03 to 0.04)			
Total	2285	1.82			22,067	0.33			0.99	4.00	(3.82 to 4.19)
Twin pregnancies											
88–92	4	0.56	−1.36	(−2.07 to −0.65)	42	0.38	0.06	(−0.10 to 0.23)	0.21	1.57	(0.51 to 4.80)
93–97	32	0.92	−1.05	(−1.58 to −0.52)	58	0.36	−0.02	(−0.15 to 0.11)	0.63	2.79	(1.67 to 4.65)
98–02	60	1.06	−0.87	(−1.37 to −0.37)	46	0.29	−0.10	(−0.22 to 0.02)	0.61	3.01	(1.95 to 4.65)
03–07	67	1.38	−0.52	(−1.05 to 0.02)	78	0.45	0.04	(−0.09 to 0.17)	0.70	2.43	(1.70 to 3.48)
08–12	81	1.92	0	Ref	73	0.41	0	Ref	1.15	3.73	(2.62 to 5.30)
13–15	26	1.47	−0.40	(−1.12 to 0.31)	44	0.49	0.10	(−0.07 to 0.27)	0.71	2.41	(1.41 to 4.11)
Per 5 years			0.30	(0.16 to 0.43)			0.01	(−0.02 to 0.04)			
Total	270	1.31			341	0.39			0.76	2.92	(2.43 to 3.50)

ART, assisted reproductive technology; *CI*, confidence interval, *OR*, odds ratio; *RD*, risk difference; *Ref*, reference group; *SC*, spontaneous conception.

[a] SC pregnancies are the reference group within each birth year category; [b] Adjusted for parity (0, 1, 2, 3+), age at birth (22–25, 25–28, 28–31, 31–34, 34–37, 37–40, 40–45), and country.

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several Western populations, including Denmark, Norway, and Sweden, during the shorter time span from 1997 to 2007. Causes of the increasing incidence of HDP in twin pregnancies are unknown but are not likely due to increasing gestational age, since mean gestational age did not increase in twin pregnancies across time in our study, in line with the previously reported stable occurrence of preterm birth for twin pregnancies.[11]

Ananth et al[13] reported declining rates of placental abruption in singleton pregnancies in several Western populations, including the Nordic countries, from 1978 to 2008. They hypothesized that this might be due to changes in smoking. Our results are consistent with their study but additionally show that risk of placental abruption has declined regardless of conception methods and multiplicity, suggesting that the development might be driven by reduction in risk factors common to all subgroups. However, smoking seemed not to explain this development, since adjustment for smoking had very little influence on trends, both for ART and SC pregnancies.

An Australian cohort study showed that the risk of placenta previa in the general population increased from 0.69% to 0.87% in the years 2001–2009,[18] whereas a Swiss population-based cohort study showed an increase in the yearly incidence of placenta previa from 0.3% to 0.5% between 1993 and 2014.[19] Although our results support an overall increasing trend, the increase of placenta previa in the background population was much weaker. The increase in risk in ART pregnancies was considerably stronger, a finding not previously reported. Consistent with expectations from a Swedish study showing greater risk of placenta previa after blastocyst transfer[20] in a subsample of our Swedish study population, the increasing risk of placenta previa over time attenuated moderately after adjustment for culture duration.

Clinical implications

When considering all ART pregnancies combined, risk of all complications declined considerably and approached that in the background population during the study period, mainly due to declining occurrence of multiple pregnancies, a major risk factor for adverse outcomes, after ART conception. Elective single embryo transfer policies in the Nordic countries have led to substantial reductions in multiple pregnancies after ART, thereby also reducing risk of adverse outcomes in ART pregnancies. Thus, our results further emphasize the importance of single embryo transfer.

The increasing risk of placenta previa in ART pregnancies is a matter of concern and could only partly be explained by the concurrent increase in blastocyst culture. Whether other treatment-related and thus potentially modifiable factors are involved, or whether changes in characteristics of the ART population contribute to this trend, is not yet known.

Furthermore, informing clinicians and infertile couples that ART pregnancies are still at greater risk of placenta-mediated pregnancy complications despite increasing success rates and improving neonatal outcomes in ART,[11,21] is important.

Research implications

Future studies should investigate underlying causes for the increasing occurrence of HDP in twin pregnancies. In addition, reasons behind the increasing incidence of placenta previa in ART pregnancies warrant further investigation.

Strengths and limitations

A key strength of this study is the large study sample with data on all deliveries in 4 Nordic countries over 3 decades, which enabled precisely estimated time trends in most analyses. Nonetheless, there were few events in the ART population in the earliest study period and that power was limited also in some sensitivity analyses.

Another strength is that we could adjust for potential confounders such as parity, maternal age, and country, as well as BMI, smoking, and cesarean delivery in subsamples. Still, we cannot exclude residual confounding by unmeasured factors such as causes for infertility or from misclassification of self-reported information such as smoking status.

In the Nordic countries, ART treatment is strongly subsidized, ensuring that the couple's financial situation is not a major determinant of ART conception. In combination with nationwide data sources with a very low proportion of missing data, this suggests that selection bias should be minimal. Furthermore, practically all pregnant women attend the publicly financed antenatal care program. In consequence, opportunities to detect pregnancy complications should not differ between the 2 conception methods, and the overall validity of such diagnoses is acceptable in all countries.[22–26] However, it is possible that women who conceive through ART have a lower threshold for seeking medical attention during pregnancy, and detection bias thus cannot be excluded.

Occurrence of pregnancy complications was generally greater when extracted from patient registries (Denmark, Finland) than from MBRs only (Norway, Sweden). Changes in registration and coding practice over time may also have influenced the observed trends but should not affect ART and SC pregnancies differently.

Conclusion

Risk of placenta-mediated pregnancy complications following ART conception is greater than for SC pregnancies in the Nordic countries. For HDP and placental abruption, ART pregnancies follow the same trends as the background population. Risk of HDP is increasing in both ART and SC twin pregnancies. Placenta previa risk has increased strongly over time in ART pregnancies. ■

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SUPPLEMENTAL TABLE 1

Overview of coding systems in use in the Nordic countries during the study period and selection of codes from each system

	International Classification of Diseases and related Health Problems (ICD) classification version		
	ICD-8	ICD-9	ICD-10
Year in use			
Denmark	—	—	1994—2014
Finland	—	1989—1995	1996—2014
Norway	1988—1998	—	1999—2015
Sweden	—	1988—1996	1997—2015
Diagnostic codes			
Hypertensive disorders in pregnancy	637	642.3—7	O11, O13—16
Placental abruption	632.1	641.2	O45
Placenta previa	632.0	641.0, 641.1	O44

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SUPPLEMENTAL TABLE 2

Time trends in hypertensive disorders in pregnancies conceived after ART and spontaneous conception

Analysis sample		Cases/deliveries	RD[a] (95% CI)	Cases/deliveries	RD[a] (95% CI)
All pregnancies	**Model**	**ART**		**Spontaneous conception**	
B) BMI & smoking	Basic model[b]	6928/93,295	−0.26 (−0.44 to −0.08)	149,946/3,424,972	0.06 (0.04 to 0.08)
B) BMI & smoking	Basic model[b] + smoking[c]		−0.27 (−0.45 to −0.09)		0.03 (0.01 to 0.05)
B) BMI & smoking	Basic model[b] + BMI[d]		−0.29 (−0.46 to −0.11)		−0.10 (−0.12 to −0.08)
B) BMI & smoking	Basic model[b] + smoking[c] + BMI[d]		−0.31 (−0.49 to −0.13)		−0.13 (−0.15 to −0.11)
C) Primiparous	Basic model[e]	8979/99,974	−0.02 (−0.15 to 0.12)	172,609/2,641,775	0.29 (0.27 to 0.31)
E) Culture duration	Basic model[b]	3658/53,230	0.04 (−0.05 to 0.13)		
E) Culture duration	Basic model[b] + culture duration[f]		0.00 (−0.09 to 0.10)		
F) Cryopreservation	Basic model[b]	8737/121,987	−0.03 (−0.05 to −0.03)		
F) Cryopreservation	Basic model[b] + cryopreservation[g]		−0.05 (−0.08 to −0.03)		
Singletons		**ART**		**Spontaneous conception**	
B) BMI & smoking	Basic model[b]	5401/82,867	0.07 (-0.18 to 0.25)	144,510/3,380,732	0.05 (0.03 to 0.07)
B) BMI & smoking	Basic model[b] + smoking[c]		0.07 (-0.11 to 0.26)		0.03 (0.01 to 0.04)
B) BMI & smoking	Basic model[b] + BMI[d]		0.05 (-0.14 to 0.23)		−0.10 (−0.12 to −0.09)
B) BMI & smoking	Basic model[b] + smoking[c] + BMI[d]		0.03 (-0.15 to 0.22)		−0.14 (−0.16 to −0.12)
C) Primiparous	Basic model[e]	6578/85,404	0.23 (0.09 to 0.37)	166,549/2,607,276	0.28 (0.26 to 0.30)
E) Culture duration	Basic model[b]	3019/49,285	0.07 (-0.02 to 0.16)		
E) Culture duration	Basic model[b] + culture duration[f]		0.05 (-0.04 to 0.14)		
F) Cryopreservation	Basic model[b]	6349/104,085	0.02 (0.00 to 0.04)		
F) Cryopreservation	Basic model[b] + cryopreservation[g]		0.00 (-0.03 to 0.02)		
Twins		**ART**		**Spontaneous conception**	
B) BMI & smoking	Basic model[b]	1496/10,240	1.52 (0.83 to 2.22)	5375/43,690	0.50 (0.24 to 0.75)
B) BMI & smoking	Basic model[b] + smoking[c]		1.47 (0.76 to 2.17)		0.41 (0.15 to 0.67)
B) BMI & smoking	Basic model[b] + BMI[d]		1.46 (0.76 to 2.16)		0.29 (0.03 to 0.55)
B) BMI & smoking	Basic model[b] + smoking[c] + BMI[d]		1.39 (0.68 to 2.10)		0.19 (-0.07 to 0.45)
C) Primiparous	Basic model[e]	2340/14,093	1.95 (1.46 to 2.43)	5963/33,856	1.19 (0.92 to 1.46)

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SUPPLEMENTAL TABLE 2

Time trends in hypertensive disorders in pregnancies conceived after ART and spontaneous conception *(continued)*

Analysis sample		Cases/deliveries	RD[a] (95% CI)	Cases/deliveries	RD[a] (95% CI)
All pregnancies	Model	ART		Spontaneous conception	
E) Culture duration	Basic model[b]	632/3896	0.03 (−0.51 to 0.58)		
E) Culture duration	Basic model[b] + culture duration[f]		−0.14 (−0.69 to 0.41)		
F) Cryopreservation	Basic model[b]	2334/17,164	0.30 (0.21 to 0.38)		
F) Cryopreservation	Basic model[b] + cryopreservation[g]		0.23 (0.14 to 0.31)		

Estimates of time trends are risk differences in percentage points.

ART, assisted reproductive technology; *BMI*, body mass index; *CI*, confidence interval; *RD*, risk difference.

[a] Per 5 years in sample B-D and per year in sample E-F; [b] Basic model includes adjustment for parity, maternal age and country; [c] Smoking (yes/no); [d] BMI: <20, 20−24, 25−29, ≥30 kg/m^2; [e] Adjusted for maternal age and country; [f] Cleavage stage vs blastocyst transfer; [g] Fresh vs frozen embryo transfer.

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SUPPLEMENTAL TABLE 3

Time trends in placental abruption in pregnancies conceived after ART and spontaneous conception

Analysis sample		Cases/deliveries	RD[a] (95% CI)	Cases/deliveries	RD[a] (95% CI)
All pregnancies	**Model**	**ART**		**Spontaneous conception**	
B) BMI & smoking	Basic model[b]	683/93,295	−0.11 (−0.16 to −0.05)	12,454/3,424,972	−0.02 (−0.03 to −0.02)
B) BMI & smoking	Basic model[b] + smoking[c]		−0.10 (−0.15 to −0.04)		−0.01 (−0.02 to −0.00)
B) BMI & smoking	Basic model[b] + BMI[d]		−0.11 (−0.16 to −0.05)		−0.02 (−0.03 to −0.02)
B) BMI & smoking	Basic model[b] + smoking[c] + BMI[d]		−0.10 (−0.15 to −0.05)		−0.01 (−0.02 to −0.00)
C) Primiparous	Basic model[e]	816/99,974	−0.14 (−0.18 to −0.10)	11,079/2,641,775	−0.06 (−0.06 to −0.05)
E) Culture duration	Basic model[b]	384/53,230	−0.05 (−0.08 to −0.02)		
E) Culture duration	Basic model[b] + culture duration[f]		−0.05 (−0.09 to −0.02)		
F) Cryopreservation	Basic model[b]	1073/121,987	−0.04 (−0.04 to −0.03)		
F) Cryopreservation	Basic model[b] + cryopreservation[g]		−0.03 (−0.04 to −0.02)		
Singletons		**ART**		**Spontaneous conception**	
B) BMI & smoking	Basic model[b]	532/82,867	−0.07 (−0.12 to −0.02)	12,046/3,380,732	−0.02 (−0.03 to −0.02)
B) BMI & smoking	Basic model[b] + smoking[c]		−0.06 (−0.11 to −0.00)		−0.01 (−0.02 to −0.01)
B) BMI & smoking	Basic model[b] + BMI[d]		−0.07 (−0.12 to −0.02)		−0.02 (−0.03 to −0.02)
B) BMI & smoking	Basic model[b] + smoking[c] + BMI[d]		−0.06 (−0.11 to −0.00)		−0.01 (−0.02 to −0.01)
C) Primiparous	Basic model[e]	592/85,404	−0.11 (−0.15 to −0.06)	10,779/2,607,276	−0.06 (−0.06 to −0.05)
E) Culture duration	Basic model[b]	317/49,282	−0.04 (−0.07 to −0.01)		
E) Culture duration	Basic model[b] + culture duration[f]		−0.05 (−0.08 to −0.02)		
F) Cryopreservation	Basic model[b]	763/104,085	−0.02 (−0.03 to −0.02)		
F) Cryopreservation	Basic model[b] + cryopreservation[g]		−0.02 (−0.03 to −0.02)		
Twins		**ART**		**Spontaneous conception**	
B) BMI & smoking	Basic model[b]	149/10,240	−0.07 (−0.31 to 0.17)	405/43,690	−0.05 (−0.12 to 0.02)
B) BMI & smoking	Basic model[b] + smoking[c]		−0.07 (−0.31 to 0.17)		−0.04 (−0.11 to 0.03)
B) BMI & smoking	Basic model[b] + BMI[d]		−0.07 (−0.31 to 0.17)		−0.06 (−0.13 to 0.01)
B) BMI & smoking	Basic model[b] + smoking[c] + BMI[d]		−0.07 (−0.32 to 0.17)		−0.05 (−0.12 to 0.02)
C) Primiparous	Basic model[e]	217/14,093	−0.07 (−0.24 to 0.10)	293/33,856	−0.03 (−0.10 to 0.04)

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(continued)

SUPPLEMENTAL TABLE 3

Time trends in placental abruption in pregnancies conceived after ART and spontaneous conception *(continued)*

Analysis sample		Cases/deliveries	RD[a] (95% CI)	Cases/deliveries	RD[a] (95% CI)
All pregnancies	Model	ART		Spontaneous conception	
E) Culture duration	Basic model[b]	66/3896	−0.15 (−0.35 to 0.05)		
E) Culture duration	Basic model[b] + culture duration[f]		−0.13 (−0.33 to 0.01)		
F) Cryopreservation	Basic model[b]	303/17,164	−0.03 (−0.07 to −0.00)		
F) Cryopreservation	Basic model[b] + cryopreservation[g]		−0.03 (−0.06 to −0.00)		

Estimates of time trends are risk differences in percentage points.

ART, assisted reproductive technology; *BMI*, body mass index; *CI*, confidence interval; *RD*, risk difference.

[a] Per 5 years in sample B-D and per year in sample E-F; [b] Basic model includes adjustment for parity, maternal age, and country; [c] Smoking (yes/no); [d] BMI: <20, 20–24, 25–29, ≥30 kg/m^2; [e] Adjusted for maternal age and country; [f] Cleavage stage vs blastocyst transfer; [g] Fresh vs frozen embryo transfer.

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SUPPLEMENTAL TABLE 4

Time trends in placenta previa in pregnancies conceived after ART and spontaneous conception

Analysis sample		Cases/deliveries	RD[a] (95% CI)	Cases/Deliveries	RD[a] (95% CI)
All pregnancies	**Model**	**ART**		**Spontaneous conception**	
B) BMI & smoking	Basic model[b]	1759/93,295	0.23 (0.13 to 0.34)	12,492/3,424,972	0.03 (0.02 to 0.03)
B) BMI & smoking	Basic model[b] + smoking[c]		0.22 (0.11 to 0.32)		0.03 (0.02 to 0.04)
B) BMI & smoking	Basic model[b] + BMI[d]		0.24 (0.14 to 0.34)		0.03 (0.02 to 0.03)
B) BMI & smoking	Basic model[b] + smoking[c] + BMI[d]		0.22 (0.12 to 0.32)		0.03 (0.03 to 0.04)
C) Primiparous	Basic model[e]	1584/99,974	0.25 (0.18 to 0.31)	6993/2,641,775	0.03 (0.02 to 0.03)
D) History of cesarean section	Basic model[b]	847/40,421	0.25 (0.10 to 0.38)	11,668/3,148,760	0.04 (0.03 to 0.05)
D) History of cesarean section	Basic model[b] + history of cesarean section		0.24 (0.10 to 0.40)		0.04 (0.03 to 0.04)
E) Culture duration	Basic model[b]	986/53,230	0.06 (0.00 to 0.10)		
E) Culture duration	Basic model[b] + culture duration[f]		0.03 (−0.02 to 0.08)		
F) Cryopreservation	Basic model[b]	1993/121,987	0.04 (0.03 to 0.05)		
F) Cryopreservation	Basic model[b] + cryopreservation[f]		0.05 (0.04 to 0.06)		
Singletons		**ART**		**Spontaneous conception**	
B) BMI & smoking	Basic model[b]	1588/82,867	0.24 (0.13 to 0.35)	12,302/3,380,732	0.03 (0.02 to 0.03)
B) BMI & smoking	Basic model[b] + smoking[c]		0.22 (0.11 to 0.34)		0.03 (0.02 to 0.04)
B) BMI & smoking	Basic model[b] + BMI[d]		0.24 (0.13 to 0.35)		0.03 (0.02 to 0.03)
B) BMI & smoking	Basic model[b] + smoking[c] + BMI[d]		0.23 (0.11 to 0.34)		0.03 (0.03 to 0.04)
C) Primiparous	Basic model[e]	1402/85,404	0.22 (0.15 to 0.30)	6874/2,607,276	0.03 (0.02 to 0.03)
D) History of cesarean section	Basic model[b]	771/34,972	0.18 (0.03 to 0.33)	11,503/3,107,064	0.04 (0.04 to 0.05)
D) History of cesarean section	Basic model[b] + history of cesarean section		0.17 (0.02 to 0.33)		0.04 (0.03 to 0.04)
E) Culture duration	Basic model[b]	930/49,285	0.06 (0.00 to 0.11)		
E) Culture duration	Basic model[b] + culture duration[f]		0.03 (−0.02 to 0.08)		
F) Cryopreservation	Basic model[b]	1787/104,085	0.03 (0.02 to 0.05)		
F) Cryopreservation	Basic model[b] + cryopreservation[f]		0.04 (0.03 to 0.06)		

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SUPPLEMENTAL TABLE 4

Time trends in placenta previa in pregnancies conceived after ART and spontaneous conception *(continued)*

Analysis sample		Cases/deliveries	RD[a] (95% CI)	Cases/Deliveries	RD[a] (95% CI)
All pregnancies	Model	ART		Spontaneous conception	
Twins		**ART**		**Spontaneous conception**	
B) BMI & smoking	Basic model[b]	170/10,240	0.08 (−0.22 to 0.37)	189/43,690	−0.00 (−0.06 to 0.05)
B) BMI & smoking	Basic model[b] + smoking[c]		0.05 (−0.25 to 0.35)		0.01 (−0.04 to 0.06)
B) BMI & smoking	Basic model[b] + BMI[d]		0.08 (−0.22 to 0.37)		0.00 (−0.05 to 0.06)
B) BMI & smoking	Basic model[b] + smoking[c] + BMI[d]		0.05 (−0.25 to 0.35)		0.01 (−0.04 to 0.07)
C) Primiparous	Basic model[e]	178/14,093	0.29 (0.14 to 0.44)	118/33,856	0.03 (−0.01 to 0.08)
D) History of cesarean section	Basic model[b]	75/5360	0.34 (0.04 to 0.65)	164/41,196	0.00 (−0.04 to 0.05)
D) History of cesarean section	Basic model[b] + history of cesarean section		0.34 (0.04 to 0.65)		0.00 (−0.05 to 0.05)
E) Culture duration	Basic model[b]	56/3896	0.01 (−0.17 to 0.20)		
E) Culture duration	Basic model[b] + culture duration[f]		0.00 (−0.20 to 0.20)		
F) Cryopreservation	Basic model[b]	202/17,164	0.05 (0.02 to 0.07)		
F) Cryopreservation	Basic model[b] + cryopreservation[g]		0.05 (0.02 to 0.08)		

Estimates of time trends are risk differences in percentage points.

ART, assisted reproductive technology; *BMI*, body mass index; *CI*, confidence interval; *RD*, risk difference.

[a] Per 5 years in sample B-D and per year in sample E-F; [b] Basic model includes adjustment for parity, maternal age, and country; [c] Smoking (yes/no); [d] BMI: <20, 20–24, 25–29, ≥ 30 kg/m^2; [e] Adjusted for maternal age and country; [f] Cleavage stage vs blastocyst transfer; [g] Fresh vs frozen embryo transfer.

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Paper II

Hypertension

ORIGINAL ARTICLE

Risk of Hypertensive Disorders in Pregnancy After Fresh and Frozen Embryo Transfer in Assisted Reproduction: A Population-Based Cohort Study With Within-Sibship Analysis

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BACKGROUND: Frozen embryo transfer (frozen-ET) is increasingly common because of improved cryopreservation methods and elective freezing of all embryos. Frozen-ET is associated with higher risk of hypertensive disorders in pregnancy than both natural conception and fresh embryo transfer (fresh-ET), but whether this is attributable to parental factors or treatment is unknown.

METHODS: Using the Medical Birth Registries of Denmark (1994–2014), Norway, and Sweden (1988–2015), linked to data from national quality registries and databases on assisted reproduction, we designed a population-based cohort study with within-sibship comparison. We included 4 426 691 naturally conceived, 78 300 fresh embryo transfer, and 18 037 frozen-ET singleton pregnancies, of which 33 209 sibships were conceived using different conception methods. Adjusted odds ratios (aOR) of hypertensive disorders in pregnancy for fresh embryo transfer and frozen-ET versus natural conception with 95% CI were estimated using multilevel logistic regression, where random effects provided conventional population-level estimates and fixed effects gave within-sibship estimates. Main models included adjustment for birth year, maternal age, parity, and country.

RESULTS: Risk of hypertensive disorders in pregnancy was higher after frozen-ET compared to natural conception, both at population level (7.4% versus 4.3%, aOR, 1.74 [95% CI, 1.61–1.89]) and within sibships (aOR, 2.02 [95% CI, 1.72–2.39]). For fresh embryo transfer, risk was similar to natural conception, both at population level (aOR, 1.02 [95% CI, 0.98–1.07]) and within sibships (aOR, 0.99 [95% CI, 0.89–1.09]).

CONCLUSIONS: Frozen-ET was associated with substantially higher risk of hypertensive disorders in pregnancy, even after accounting for shared parental factors within sibships. **(*Hypertension*. 2022;79:00–00. DOI: 10.1161/HYPERTENSIONAHA.122.19689.)** ● **Supplemental Material**

Key Words: cryopreservation ■ embryo transfer ■ fertilization in vitro ■ hypertension ■ pre-eclampsia ■ pregnancy ■ siblings

Worldwide, use of assisted reproductive technology (ART) has increased, and today, children born after ART constitute up to 7% of birth cohorts in several Western countries.[1] The conventional approach in ART involves either in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), followed by fresh embryo transfer (fresh-ET). If there are surplus embryos, these are frozen and can be thawed and transferred in subsequent cycles. In the last decade, the number of pregnancies after frozen embryo transfer (frozen-ET) has increased substantially,[1] due to improved cryopreservation methods that facilitate single embryo transfer.[2,3] Moreover, initial reports showed better perinatal and obstetric outcomes after frozen-ET than fresh-ET.[4] Elective freezing, where

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Hypertension is available at www.ahajournals.org/journal/hyp

NOVELTY AND RELEVANCE

What Is New

This study is the largest sibship design study to date to explore the risk of hypertensive disorders in pregnancies after assisted reproduction.

What Is Relevant?

In this Nordic population-based cohort study, frozen embryo transfer was associated with a substantially higher risk of hypertensive disorders compared to natural conception, even after accounting for shared parental factors within sibships.

Clinical/Pathophysiological Implications?

The high risk of hypertensive disorders following frozen embryo transfer raises concerns about the increasingly popular elective freezing of all embryos.

Nonstandard Abbreviations and Acronyms

ART	assisted reproductive technology
CoNARTaS	The Committee of Nordic ART and Safety
Fresh-ET	fresh embryo transfer
Frozen-ET	frozen embryo transfer
HDP	hypertensive disorders in pregnancy
ICSI	intracytoplasmic sperm injection

ovarian stimulation is not followed by fresh transfer, but instead freezing of all embryos for transfer in later cycles, has been successful in preventing ovarian hyperstimulation while achieving similar or higher live birth rate compared to fresh cycles.[5] Consequently, elective freezing of all embryos is increasingly used, and in many high-income countries, most embryo transfers are now from frozen cycles.[1,6,7] Despite the advantages of frozen-ET, observational studies raise concern about treatment safety due to higher risk of hypertensive disorders in pregnancy (HDP) after frozen-ET compared with both natural conception and fresh-ET.[8,9] Importantly, conventional observational studies are prone to residual confounding, particularly from factors associated with infertility.[10-12] An increased risk of HDP after frozen compared with fresh transfer is supported by a recent meta-analysis of 3 randomized trials comparing 1193 pregnancies after elective freezing to 1205 after fresh transfer.[13] However, comparison with natural conception, which is needed to understand the potential contribution from infertility, cannot be investigated through randomization.

Within sibship analyses may strengthen causal inference by controlling for unmeasured or unknown confounders at the parental level.[14] Using a sibship design, we have recently shown that singletons conceived by fresh-ET are smaller, and singletons conceived by frozen-ET are larger for gestational age than their naturally conceived siblings, whereas risk of preterm birth is higher after both ART treatments.[15] So far, only 2 small studies, both including births up to 2007, have investigated risk of HDP after ART using a sibship design. In a Nordic registry-based cohort, risk of HDP was higher after frozen-ET compared to fresh-ET in 100 double discordant pairs of singleton siblings.[16] In a Dutch cohort comparing risk of HDP after any ART versus natural conception within 1813 sibling pairs, no clear association was found.[17] HDP is associated with severe morbidity in mother[18,19] and child[20,21] and identification of ART treatments that influence risk contributes to informed decision-making but could also reveal valuable opportunities for prevention.

The objective of this study was to investigate whether the risk of HDP following fresh-ET and frozen-ET is higher compared to naturally conceived pregnancies. We used within-sibship comparison to control for confounding from unmeasured and unknown parental factors, such as genetics, preconception lifestyle and health, as well as socioeconomic status.

METHODS

Data availability

The data cannot be shared publicly due to national data protection regulations but may be accessed from a server at Statistics Denmark, after approval by the relevant Ethics Committees and registry-keeping authorities in each country.

Materials

The CoNARTaS (Committee of Nordic ART and Safety) cohort includes all deliveries registered in the Medical Birth Registries in four Nordic countries, described in detail elsewhere.[22] We included data from Denmark (1994–2014), Norway (1984–2015), and Sweden (1985–2015), but not from Finland, where details on ART treatment were not registered. Using the national identity number assigned to each resident in the Nordic countries,[23] we linked data from the Medical Birth Registries and national ART registries and databases, as well as the National Patient Registry in Denmark.

In Denmark, all ART cycles in public and private clinics have been recorded in the national ART quality registry since 1994. In Norway, public and private ART clinics have provided information to the Medical Birth Registry since 1984 on all ART cycles resulting in an ultrasound verified pregnancy (week 6–7). In Sweden, conception method was reported to the National

Board of Health and Welfare between 1982 and 2006, and from 2007 all ART cycles have been registered in the national ART quality registry.

Exposures, Outcome, and Other Factors

The exposures were frozen-ET or fresh-ET versus natural conception (reference group). Natural conception comprised all pregnancies with no registration of ART conception (including ovulation induction and insemination).

Diagnoses during pregnancy, delivery, and the puerperal period were registered in the Danish National Patient Registry and the Norwegian and Swedish Medical Birth Registries according to national adaptations of the *International Statistical Classifications of Diseases and Related Health Problems*, as outlined in Table S1. We defined HDP as a combined outcome, including gestational hypertension, preeclampsia, eclampsia, and chronic hypertension with superimposed preeclampsia. We also repeated the analyses after restricting the outcome to preeclampsia, superimposed preeclampsia, and eclampsia, that is, pregnancies with isolated gestational hypertension were considered as not having the outcome.

In Denmark and Norway, gestational age was estimated from ultrasound scans in the first or second trimester. If unavailable, we used transfer date for ART pregnancies and last menstrual period for naturally conceived pregnancies. For Sweden, gestational age was based on transfer date for ART pregnancies, and second-trimester ultrasound scans for natural conception, and if these were unavailable, the date of the last menstrual period was used.

Smoking status was self-reported and registered throughout the study period in Denmark and Sweden and since 1999 in Norway and harmonized across the countries as no versus any smoking during pregnancy. Maternal height and weight were registered from 1988 to 1989 and 1992 to 2015 in Sweden and since 2004 and 2007 in Denmark and Norway, respectively. In all countries, the proportion of observations with missing data on these variables was considerable during the first years of registration.

Study Population

We defined the study period from 1988 (the first year with a registered delivery after frozen-ET) to 2014 (Denmark) or 2015 (Norway and Sweden). The eligible cohort was defined as all singleton deliveries with mothers who were ≥20 years and had their first delivery within the study period, comprising 4 637 605 pregnancies in 2 392 505 women (Figure 1). We excluded observations with missing parity, maternal age, birthweight, or gestational age. Next, we excluded pregnancies with parity ≥4 (as there were very few ART conceptions among mothers with ≥5 deliveries) and/or maternal age ≥45 years, as well as observations with extreme values on birthweight (<300 or >6500 g, >6 SDs above expected)[24] or gestational age (<22 or >44 weeks). Available data on the 114 577 excluded pregnancies are presented in Table S2. Our main sample comprised 4 523 028 deliveries from 2 379 130 mothers, including 78 300 after fresh-ET and 18 037 after frozen-ET. In total, 33 209 singleton sibships with the same mother and conceived from at least 2 of the 3 conception methods were defined from maternal identity codes.

Statistical Analyses

We compared odds of HDP across conception methods in multilevel logistic models, with deliveries as one level and mothers as another (using Stata's xtlogit command with maternal identity codes defining the clusters).[25,26] We used random-effects models to obtain conventional population-level estimates and fixed effects models for within-sibship estimates (ie, comparison within mothers). Precision was estimated by 95% CIs. To increase interpretability of odds ratios, we used postestimation commands to obtain risk differences. To facilitate comparison with previous studies,[27,28] we also compared frozen-ET to fresh-ET.

We defined potential confounders as factors that could influence the need for ART and risk of developing HDP. In random effect models, we adjusted for birth year, maternal age, parity, and country as categorical variables. Fixed effects models were adjusted for the same covariates except for country (which is stable within mothers). Within-sibship analyses control for unknown and unmeasured confounding under the assumption that most of these confounders are at the family level, not at the individual level.[25]

To investigate if associations were affected by which conception method occurred first, we restricted the random effect models to mothers with singletons in their first 2 consecutive deliveries and added interaction terms between parity and conception method (bidirectional analysis).[25] This analysis included 1 579 190 sibships belonging to 1 of 9 possible sibship combinations. Furthermore, to investigate whether experiencing HDP influenced the selection into the population of double discordant sibships,[29] we categorized the first delivery by conception method and HDP occurrence (resulting in 6 subgroups). For these subgroups, we calculated the probability of having a second singleton with either conception method within 5 years following the first singleton and estimated odds ratio of HDP in the second pregnancy for each subgroup.

We conducted several sensitivity analyses to evaluate the robustness of our findings (Figure 1). First, we adjusted for maternal smoking and body mass index in the subsample with available information. Second, we repeated our main models for full siblings (same mother and father) to account for constant paternal factors, for siblings born within a 3-year interval as their parents' health might be more constant than for singletons born further apart in time, and for each country separately. Finally, we restricted deliveries after ART to explore the impact of other treatment factors: fertilization by IVF (ie, excluding fertilization by ICSI, which is used mainly for male infertility in the Nordic countries),[30] single embryo transfers (to limit the potential impact of vanishing twins),[31,32] and blastocyst transfers (to account for prolonged culture media and in vitro exposure).[33] During the study period, most frozen blastocysts were vitrified whereas most cleavage stage embryos were slow-frozen.[33]

To explore whether the higher risk of preterm birth after ART reduced the probability of being diagnosed with HDP,[15] we repeated analyses using Cox regression with gestational duration as the time scale. We estimated hazard ratios, adjusting for the same covariates as the main analyses. For population-level estimates, we used robust standard errors to account for dependency of observations within mothers, and for within-sibship estimates, we used stratified models with maternal identity in separate strata. The proportional hazards assumption was examined by inspection of log-log plots.

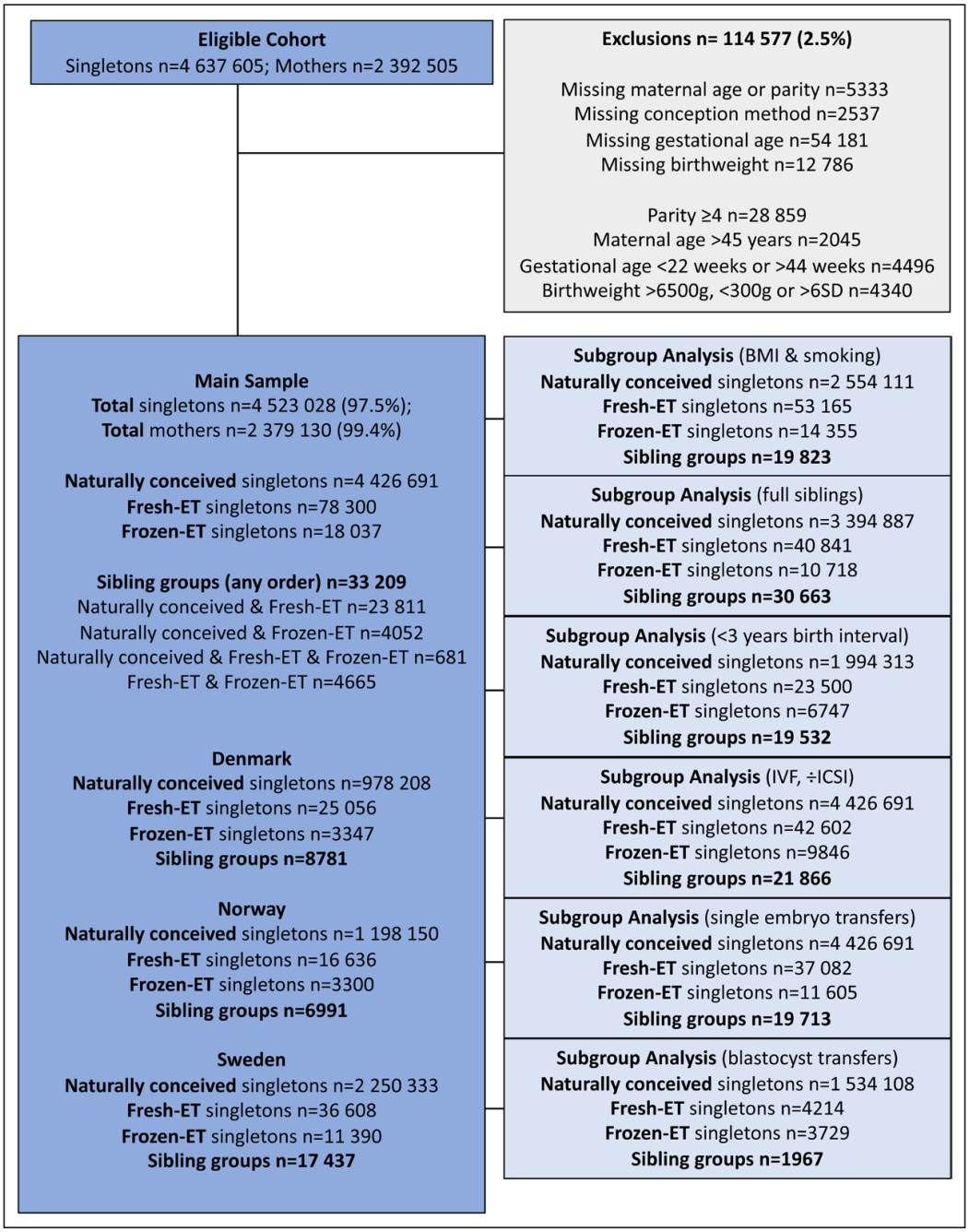

Figure 1. Flowchart of the study population.
The subgroup with only blastocyst transfers was restricted to birth years 2005–2015. BMI indicates body mass index; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; ICSI, intracytoplasmic sperm injection; and IVF, in vitro fertilization.

Statistical analyses were performed using Stata/MP for Windows, Version 17.0 (StataCorp LLC, College Station, TX).

This study is reported according to The Reporting of Studies Conducted Using Observational Routinely Collected Health Data Statement guideline (Supporting document: Reporting of

Studies Conducted Using Observational Routinely Collected Health Data checklist).

RESULTS

Table 1 shows that women who gave birth after frozen-ET (mean age 34.3 years) or fresh-ET (33.8 years) were older than women with natural conception (29.6 years). Parity was lower after fresh-ET (75.3% primiparous) than frozen-ET (58.0%) and natural conception (51.2%). A lower proportion of ART conceiving mothers smoked during pregnancy, whereas mean body mass index was similar between all conception groups. Prevalence of chronic hypertension was low for all conception methods (0.6% to 0.9%).

Pregnancies after frozen-ET and fresh-ET were more frequently preterm (6.6% and 8.1%, respectively) compared to naturally conceived pregnancies (5.0%) and more frequently induced and/or delivered by cesarean section. Among frozen-ET pregnancies, 36.7% were fertilized by ICSI, 64.3% were single embryo transfers, and 20.8% were blastocyst transfers. Fresh-ET pregnancies had similar proportions of ICSI and single embryo transfers, but only 5.7% were blastocyst transfers.

Main Analyses

The unadjusted risk of HDP was 7.4% after frozen-ET, 5.9% after fresh-ET, and 4.3% after natural conception (Table 2). In population-level analysis, frozen-ET was associated with higher odds of HDP compared with naturally conceived pregnancies, adjusted odds ratio of 1.74 (95% CI, 1.61–1.89), with a corresponding adjusted risk difference of 1.95 percentage points (95% CI, 1.61–2.28). There was little difference in adjusted odds of HDP between fresh-ET and naturally conceived pregnancies. Within sibships, the odds of HDP after frozen-ET were twice as high as for their naturally conceived siblings. Pregnancies after fresh-ET had similar odds of HDP as their naturally conceived siblings. When the more restricted outcome definition was used (preeclampsia, superimposed preeclampsia, and eclampsia), associations remained similar or were slightly strengthened. Frozen-ET pregnancies had higher odds of HDP compared to fresh-ET pregnancies, both at population-level and within sibships (Table S3).

Among women with 2 consecutive singletons, risk of HDP declined from the first to the second pregnancy for all combinations of conception methods (Figure 2, and Table S4). The highest risk in first pregnancy and the largest decline was seen for frozen-ET/natural conception, whereas the highest risk in second pregnancy and the smallest decline was seen for natural conception/frozen-ET, indicating that a single subgroup did not drive the within-sibship estimates.

For all conception methods, women with HDP in their first pregnancy were less likely to have a second pregnancy than women without HDP (Figure S1). Among women with frozen-ET in their first pregnancy, continuation with natural conception was more common if they had experienced HDP, whereas those with fresh-ET in their first pregnancy were less likely to have natural conception in their second pregnancy, than women without HDP. When accounting for this selection by estimating risk in the second pregnancy for women with similar experience in their first pregnancy, frozen-ET showed higher odds of HDP compared to natural conception in most subgroups, whereas fresh-ET showed no or weak positive associations. However, power was limited in some of these subgroups.

Sensitivity Analyses

Subgroup analyses with adjustment for body mass index and smoking, restriction to full siblings and siblings born within a 3-year interval, yielded results consistent with our main findings (Figure 3, Table S5, and Table S6). Results were also similar when restricting the ART pregnancies to IVF fertilization (ie, excluding ICSI), single embryo transfer, and blastocyst transfers. Both population-level and within-sibship estimates consistently indicated higher odds of HDP among frozen-ET compared to natural conception. For fresh-ET, population-level estimates overall indicated similar odds of HDP as in naturally conceived pregnancies, whereas most within-sibship estimates tended towards lower odds of HDP compared to naturally conceived siblings. Results were also similar between countries (Table S7).

Cox regression gave broadly similar results as our main analyses (Table S8), with a higher risk of HDP after frozen-ET in both population-level and sibship analyses and no strong associations for fresh-ET. No clear violations of the proportional hazard assumption were found, indicating that associations were similar throughout pregnancy.

DISCUSSION

In this population-based cohort study with nationwide data from 3 countries over almost 3 decades, the risk of HDP following frozen-ET was substantially higher than after natural conception, even when controlling for constant parental characteristics within sibships. In contrast, pregnancies following fresh-ET were at a similar or lower risk than naturally conceived. Considering the robustness of these findings across subgroups and with different regression models, our study provides strong indications that treatment factors may contribute to the higher risk of HDP observed in frozen-ET pregnancies.

Table 1. Baseline Characteristics of the Study Population (Main Sample) by Conception Method*

	Fresh-ET (n=78 300)	Frozen-ET (n=18 037)	Natural conception (n=4 426 691)
Country, N (%)			
Denmark	25 056 (32.0)	3347 (18.6)	978 208 (22.1)
Norway	16 636 (21.3)	3300 (13.8)	1 198 150 (27.1)
Sweden	36 608 (46.8)	11 390 (63.2)	2 250 33 (50.8)
Birth year, N (%)			
1988–1996	5802 (7.4)	498 (2.8)	1 024 162 (23.1)
1997–2001	11 208 (14.3)	1101 (6.1)	808 644 (18.3)
2002–2006	17 766 (22.7)	2545 (14.1)	911 965 (20.6)
2007–2011	24 405 (31.2)	6517 (36.1)	967 295 (21.9)
2012–2015	19 119 (24.4)	7376 (40.9)	714 625 (16.1)
Parity, N (%)			
0	58 919 (75.3)	10 454 (58.0)	2 265 209 (51.2)
1	16 999 (21.7)	6545 (36.3)	1 589 045 (35.9)
2	2041 (2.6)	920 (5.1)	478 062 (10.8)
3	341 (0.4)	1180.4)	94 375 (2.1)
Maternal age, mean (SD), y	33.8 (4.2)	34.3 (4.1)	29.6 (4.8)
Chronic hypertension, N (%)	707 (0.9)	159 (0.9)	26 936 (0.6)
Maternal BMI, mean (SD), kg/m^2	24.2 (4.1)	24.2 (4.0)	24.2 (4.5)
Missing outside registration period, N (%)	15 183 (19.4)	1196 (6.6)	1 314 826 (29.7)
Missing during registration period, N (%)	8950 (11.4)	2290 (12.7)	502 475 (11.4)
Maternal smoking in pregnancy, N (%)	4055 (5.2)	540 (3.0)	449 538 (10.2)
Missing outside registration period, N (%)	2006 (2.6)	115 (0.6)	378 584 (8.6)
Missing during registration period, N (%)	5357 (6.8)	996 (5.5)	305 443 (6.9)
Cesarean section, N (%)	19 910 (25.4)	5133 (28.5)	670 949 (15.2)
Induction of labor, N (%)	14 720 (18.8)	4500 (25.0)	567 072 (12.8)
Sex, N (%)			
Male	40 019 (51.1)	9215 (51.1)	2 275 150 (51.4)
Female	38 257 (48.9)	8822 (48.9)	2 151 084 (48.6)
Birthweight, mean (SD), g	3406.4 (620.7)	3578.0 (614.6)	3537.2 (565.2)
Gestational age, mean (SD), d	276.5 (15.8)	278.0 (14.9)	279.0 (13.0)
Preterm birth,† N (%)	6351 (8.1)	1198 (6.6)	219 461 (5.0)
ART fertilization method, N (%)			
IVF	44 602 (57.0)	9846 (54.6)	…
ICSI	32 239 (41.2)	6616 (36.7)	…
Unknown	1459 (1.9)	1575 (8.7)	…
Embryos transferred, N (%)			
1	37 082 (47.4)	11 605 (64.3)	…
2	29 987 (38.3)	4209 (23.3)	…
3	1891 (2.4)	131 (0.7)	…
Unknown	9340 (11.9)	2092 (11.6)	…
Embryo culture, N (%)			
2–3 d (cleavage)	61 772 (78.6)	11 707 (64.9)	…
5–6 d (blastocyst)	4450 (5.7)	3756 (20.8)	…
Unknown	12 178 (15.6)	2574 (14.3)	…

BMI: calculated as weight in kilograms divided by height in meters squared. ART indicates assisted reproductive technology; BMI, body mass index; ET, embryo transfer; ICSI, intracytoplasmic sperm injection; and IVF, in vitro fertilization.

*Percentages may not total 100% on account of rounding.

†Preterm birth was defined as birth before 37 wk of gestation.

Table 2. Risk of Hypertensive Disorders in Pregnancy by Conception Method: Population-Level Estimates and Within-Sibship Comparison

	Population-level estimates (random effects)					Within-sibship estimates (fixed effects)		
	Cases/deliveries (%)	RD,* pp	RD, pp (95% CI)†	OR*	Adjusted OR (95% CI)†	Cases/deliveries‡ (%)	OR*	Adjusted OR (95% CI)†
HDP								
Natural conception	191 287/4 426 691 (4.32)	0	0 (Ref.)	1	1 (Ref.)	1364/34 151 (3.99)	1	1 (Ref.)
Fresh-ET	4600/78 300 (5.87)	1.55	0.06 (−0.07 to 0.19)	1.54	1.02 (0.98 to 1.07)	1539/30 333 (5.11)	1.46	0.99 (0.89 to 1.09)
Frozen-ET	1326/18 037 (7.35)	3.09	1.95 (1.61 to 2.28)	2.18	1.74 (1.61 to 1.89)	590/9651 (6.11)	1.91	2.02 (1.72 to 2.39)
Preeclampsia§								
Natural conception	142 195/4 426 691 (3.21)	0	0 (Ref.)	1	1 (Ref.)	933/34 151 (2.73)	1	1 (Ref.)
Fresh-ET	3371/78 300 (4.31)	1.11	0.18 (0.01 to 0.30)	1.48	1.08 (1.03 to 1.13)	1133/30 333 (3.74)	1.56	1.06 (0.94 to 1.19)
Frozen-ET	991/18 037 (5.49)	2.38	1.91 (1.59 to 2.22)	2.11	1.93 (1.77 to 2.12)	440/9651 (4.56)	2.11	2.45 (2.02 to 2.96)

Fresh-ET indicates fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP, hypertensive disorders in pregnancy; OR, odds ratio; pp, percentage points; RD, risk difference; and Ref, reference group.

*Unadjusted.

†Adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44) and parity (0, 1, 2 or 3). Random effects are additionally adjusted for countries (Denmark, Norway, or Sweden).

‡Refers to deliveries that are part of a sibling group with at least 2 different conceptions methods within the group.

§Preeclampsia, chronic hypertension with superimposed preeclampsia, and eclampsia.

Comparison With Other Studies and Interpretation of Findings

Our study is in agreement with earlier population-level studies showing a higher risk of HDP after frozen-ET transfer.[9,16,28] On the population-level, it has been demonstrated that in our study cohort, the association between frozen-ET and HDP is similar for blastocyst transfers.[33,34] However, our study's lack of a clear association between fresh-ET and HDP differs from other studies.[16,28]

We are not aware of other studies that could separate fresh and frozen transfers and compare risk of HDP to naturally conceived siblings. However, the higher risk of HDP after frozen-ET is in agreement with an earlier CoNARTaS study comparing siblings born after fresh-ET and frozen-ET between 1988 and 2007.[16] In contrast, a Dutch study comparing siblings conceived after any ART versus natural conception between 1999 and 2007, found higher crude risk of HDP within sibships and no clear association after adjustments.[17] However, the results from that study may have been unintentionally biased by adjustment for level of care, which could be a common consequence of ART and HDP.[35]

Several recent cohort studies found that odds of HDP or preeclampsia in frozen cycles were from 43% to 173% higher for transfer in programmed cycles (substituted with estrogen and progesterone, but no ovulation) than transfer in natural, ovulatory cycles.[8,36-38] It has been suggested that these observations could be attributed to the absence of a corpus luteum in programmed cycles.[27,38] Unfortunately, we did not have information on type of

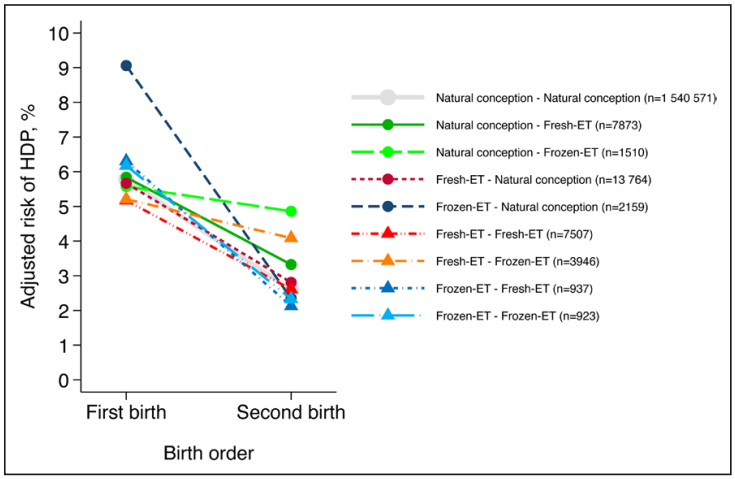

Figure 2. Risk of hypertensive disorders in pregnancy in consecutive sibling pairs according to birth order and conception method.
Absolute risks are obtained using random-effects logistic models with postestimation commands. All estimates are adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012, or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39, or 40–44), and country (Denmark, Norway, or Sweden). HDP indicates hypertensive disorders in pregnancy; ICSI, intracytoplasmic sperm injection; and IVF, in vitro fertilization.

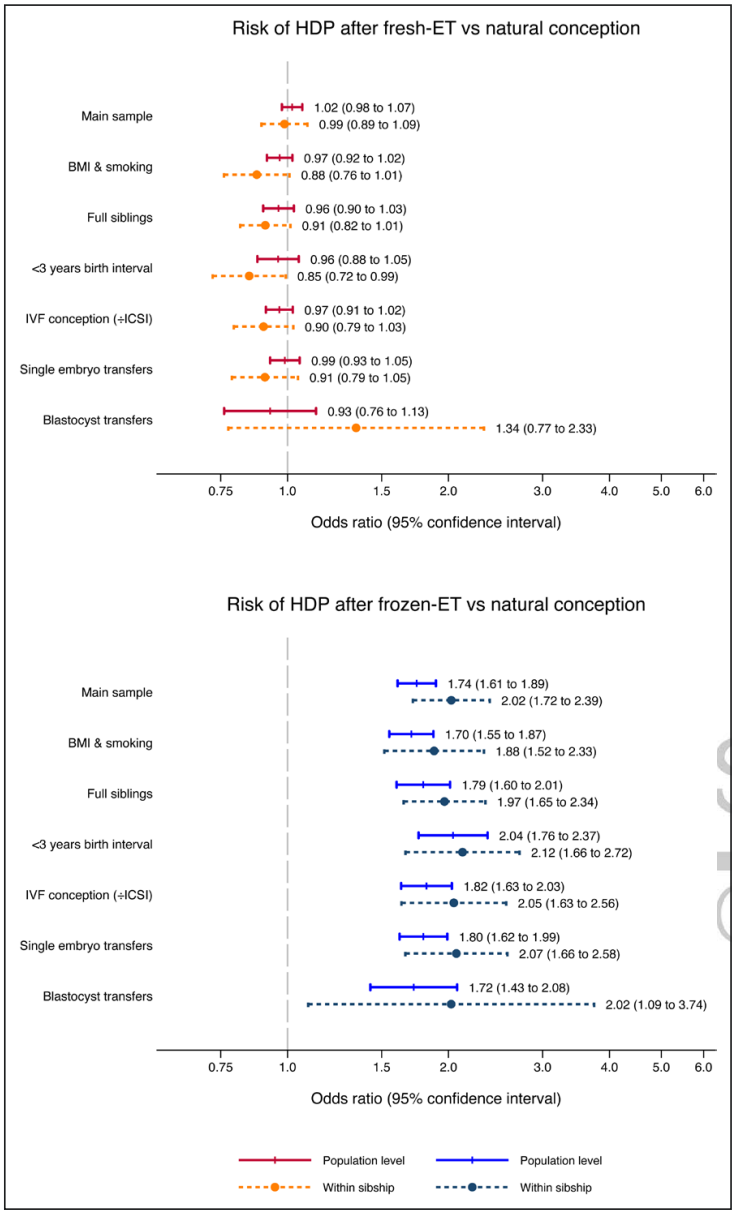

Figure 3. Risk of hypertensive disorders in pregnancy (HDP) by conception method: population-level estimates and within-sibship comparisons in subgroups. Adjusted odds ratios with 95% CIs for fresh embryo transfer (fresh-ET) vs natural conception and frozen embryo transfer (frozen-ET) vs natural conception in our subgroups. All estimates are adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012, or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39, or 40–44), and parity (0, 1, 2, or 3). Random effects are additionally adjusted for countries (Denmark, Norway, or Sweden). Estimates for the body mass index (BMI) and smoking subgroup are additionally adjusted for BMI as a categorical variable (underweight <18.5 kg/m^2, normal 18.5–24.99 kg/m^2, overweight 25–29.99 kg/m^2, or obese ≥30 kg/m^2) and smoking status as a dichotomous variable (yes/no). Blastocyst analyses were restricted to 2005–2015. ICSI, intracytoplasmic sperm injection; and IVF, in vitro fertilization.

cycle in our data, but previous studies from the Nordic countries indicate that only 15% to 30% of frozen-ET were programmed cycles during our study period.[8,36] This suggests that the strong association between frozen-ET and HDP in our study was not driven by cycle programming alone. Additional explanations that have been proposed include embryo selection,[39] and epigenetic or other changes associated with freezing and thawing,[40-42] possibly affecting trophoblast invasion, in turn leading to abnormal placentation.[43] Lastly, differential obstetric management seems unlikely to play a role, as we found similar results when accounting for preterm birth using survival analysis.

Strengths and Limitations

A strength of our study is the sibship design which allowed control for confounding shared by siblings (observed and nonobserved), such as genetics, preconception parental health, and socioeconomic status.[25] The use of nationwide, prospectively collected registry data of high quality from 3 countries,[44-46]

ensured a large and unselected study population with opportunities for a range of sensitivity analyses that supported the main findings.

Despite the extra control for shared confounders provided by the within-sibship analyses, we cannot exclude residual confounding from nonshared confounders, such as smoking and body mass index where confounder control was limited by a large proportion of missingness, and causes of infertility, which were largely unknown. Although couples who conceive after fresh and frozen cycles may be expected to be more similar than couples who conceive naturally and after ART, causes and severity of infertility are likely to influence the couple's probability of having embryos for freezing. Unfortunately, we did not have data on number of embryos obtained from the stimulation cycle, and we could, therefore, not determine whether couples with fresh-ET conception had surplus embryos eligible for freezing. Nor could we determine if the frozen-ET pregnancies were after an initial, unsuccessful fresh-ET or from an elective freezing approach. However, during our study period, elective freezing was still relatively uncommon and most frozen-ET conceptions would have been preceded by a fresh transfer. Results from randomized controlled trials show that the chances of a successful pregnancy are similar or slightly higher after elective freezing compared to fresh transfer.[47-50] This suggests that for couples with surplus embryos eligible for freezing in our cohort, the chances of pregnancy after either transfer type might be comparable.

Another limitation is that most pregnancies conceived after ART treatment abroad would be misclassified as naturally conceived in our data, but these would be greatly outnumbered by the correctly classified naturally conceived pregnancies. Furthermore, it is possible that increased parental awareness and a lower threshold for seeking medical attention could increase detection of HDP after ART conception. However, in the Nordic countries, ART-conceived pregnancies are followed in the same antenatal program as the background population. It also seems unlikely that a potentially increased detection of HDP after ART would differ for fresh-ET versus frozen-ET.

We found some evidence of selection into the within-sibship population (double discordant sibships), due to lower probability of continued reproduction among women with HDP in first pregnancy and differential probability of a second, naturally conceived singleton for women with HDP and fresh-ET or frozen-ET in their first pregnancy. Although this may have biased the within-sibship estimates, overall conclusions appeared robust in our attempts to control for this selection.

Although we consider pooling of data from 3 Nordic countries justifiable because all are high-income countries with publicly financed, accessible health care systems and similar ART policies and antenatal care programs,[22] these characteristics of our societies may also limit generalizability to other populations.

PERSPECTIVES

Although cryopreservation has facilitated elective single embryo transfer, thereby reducing risk of HDP after ART through reduction of multiple pregnancies,[2,51,52] careful consideration of all benefits and harms is needed before freezing all embryos as routine, rather than for couples with clinical indications, such as high risk of ovarian hyperstimulation syndrome.[53] HDP is relatively common and can have severe maternal and fetal consequences,[18-21] suggesting that identifying subgroups at higher risk could provide opportunities for more targeted monitoring and interventions. The need for preventive measures is further emphasized by the fact that associations in our study were not driven by isolated gestational hypertension. Furthermore, the previously reported increase in birthweight and risk of being born large for gestational age,[15] should also be included in the balance sheet. Future research should investigate which treatment factors associated with frozen-ET might be involved in the development of HDP.

ARTICLE INFORMATION

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Disclosures

None.

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The RECORD statement – checklist of items, extended from the STROBE statement, that should be reported in observational studies using routinely collected health data.

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	(a): Title. (b): Abstract: Methods and results.	RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	1.1: Abstract: Methods. 1.2: Abstract: Methods. 1.3: Abstract: Methods.
Introduction					
Background rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction, first and second paragraph.		
Objectives	3	State specific objectives, including any prespecified hypotheses	Introduction, third paragraph.		
Methods					
Study Design	4	Present key elements of study design early in the paper	Introduction, second and third paragraph.		
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods, second section: Materials.		
Participants	6	*(a) Cohort study* - Give the eligibility criteria, and the sources and methods	(a): Methods, fourth section: Study population.	RECORD 6.1: The methods of study population selection (such as codes or algorithms used to identify subjects) should	6.1: Methods, second section: Materials. Supplementary table 1.

		of selection of participants. Describe methods of follow-up *Case-control study* - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants *(b) Cohort study* - For matched studies, give matching criteria and number of exposed and unexposed *Case-control study* - For matched studies, give matching criteria and the number of controls per case	(b): Not applicable.	be listed in detail. If this is not possible, an explanation should be provided. RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided. RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	6.2: Discussion, third section: Strengths and limitations. 6.3: Methods, second section: Materials. We refer to a Data resource profile paper describing the study cohort and linkages.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	Methods, third section: Exposures, outcome, and other factors. Methods, fifth section: Statistical analyses. Supplementary table 1.	RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	7.1: Methods, third section: Exposures, outcome, and other factors. Supplementary table 1.
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods, second section: Materials. Methods, third section: Exposures, outcome, and other factors.		
Bias	9	Describe any efforts to address potential sources of bias	Methods, fifth section: Statistical analyses.		

Study size	10	Explain how the study size was arrived at	Methods, fourth section: Study population. Figure 1.		
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	Methods, third section: Exposures, outcome, and other factors. Methods, fifth section: Statistical analyses. Table 2.		
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses	(a): Methods, fifth section: Statistical analyses. (b): Methods, fifth section: Statistical analyses, third paragraph, fourth and fifth paragraph. (c): Methods, fourth section: Study population. Figure 1. (d): Not applicable. (e): Methods, fifth section: Statistical analyses.		
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population. RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	12.1: Methods, fourth section: Study population. Figure 1. 12.2: Methods, fourth section: Study population. Figure 1.

Linkage		..		RECORD 12.3: State whether the study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	12.3: Methods, second section: Materials.
Results					
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram	(a): Methods, fourth section: Study population. Figure 1. (b): Methods, fourth section: Study population. Figure 1. (c) Figure 1.	RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.,* study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	13.1: Methods, fourth section: Study population. Figure 1.
Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)	(a): Results: first section. Table 1. (b): Methods, fourth section: Study population. Figure 1. (c): Not applicable.		
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or summary measures	Table 2.		

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	(a): Results, second section: Main analyses. (b): Results, third section: Sensitivity analyses. Table 2. (c): Table 2.		
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	Results, third section: Sensitivity analyses. Figure 2 and Figure 3. Supplementary table 3-8. Supplementary figure 1.		
Discussion					
Key results	18	Summarise key results with reference to study objectives	Discussion, first section.		
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Discussion, third section: Strengths and limitations.	RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported.	19.1: Discussion, third section: Strengths and limitations.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Discussion, second section: Comparison with other studies and interpretation of findings.		

Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion, third section: Strengths and limitations.		
Other Information					
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Statements: Sources of funding.		
Accessibility of protocol, raw data, and programming code		..		RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	22.1: Methods, first section.

*Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM, the RECORD Working Committee. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Medicine* 2015; in press.

Table S1. Overview of coding systems in use in the Nordic countries during the study period and selection of relevant codes for hypertensive disorders in pregnancy from each system.

	International Statistical Classification of Diseases and related Health Problems (ICD) version		
	ICD-8	**ICD-9**	**ICD-10**
Year in use			
Denmark	–	–	1994–2014
Norway	1988–1998	–	1999–2015
Sweden	–	1988–1996	1997–2015
Diagnostic codes for HDP	637	642.3–7	O11, O13–16
Diagnostic codes for preeclampsia	637	642.4–7	O11, O14–16

Abbreviations: HDP, hypertensive disorders in pregnancy.

Table S2. Characteristics of the eligible study population according to inclusion vs exclusion into main sample.*

	Fresh-ET		Frozen-ET		Natural conception		Unknown conception method
	Included (n=78 300)	Excluded (n=774)	Included (n=18 037)	Excluded (n=168)	Included (n=4 426 691)	Excluded (n=111 098)	Excluded (n=2537)
Country, No. (%)							
Denmark	25 056 (32.0)	456 (58.9)	3347 (18.6)	73 (43.5)	978 208 (22.1)	30 105 (27.1)	0
Norway	16 636 (21.3)	158 (20.4)	3300 (13.8)	42 (25.0)	1 198 150 (27.1)	57 110 (51.1)	2537 (100.0)
Sweden	36 608 (46.8)	160 (20.7)	11 390 (63.2)	53 (31.6)	2 250 333 (50.8)	23 883 (21.5)	0
Birth year, No. (%)							
1988–1996	5802 (7.4)	52 (6.7)	498 (2.8)	5 (3.0)	1 024 162 (23.1)	36 855 (33.2)	243 (9.6)
1997–2001	11 208 (14.3)	180 (23.3)	1101 (6.1)	22 (13.1)	808 644 (18.3)	25 422 (22.9)	654 (25.8)
2002–2006	17 766 (22.7)	159 (20.5)	2545 (14.1)	27 (16.1)	911 965 (20.6)	16 473 (14.8)	376 (14.8)
2007–2011	24 405 (31.2)	222 (28.7)	6517 (36.1)	59 (35.1)	967 295 (21.9)	19 418 (17.5)	692 (27.3)
2012–2015	19 119 (24.4)	161 (20.8)	7376 (40.9)	55 (32.7)	714 625 (16.1)	12 930 (11.6)	572 (22.6)
Parity, No. (%)							
0	58 919 (75.3)	513 (70.6)	10 454 (58.0)	78 (50.7)	2 265 209 (51.2)	42 906 (40.5)	1827 (72.0)
1	16 999 (21.7)	119 (16.4)	6545 (36.3)	31 (20.1)	1 589 045 (35.9)	24 085 (22.8)	591 (23.3)
2	2041 (2.6)	23 (3.2)	920 (5.1)	7 (4.6)	478 062 (10.8)	7749 (7.3)	86 (3.4)
3 or higher	341 (0.4)	72 (9.9)	118 0.4)	38 (24.7)	94 375 (2.1)	31 088 (29.4)	33 (1.3)
Maternal age, mean (SD), years	34.3 (4.1)	34.6 (5.0)	33.8 (4.2)	35.9 (5.2)	29.6 (4.8)	30.7 (6.0)	33.8 (4.6)
Chronic hypertension, No. (%)	707 (0.9)	11 (1.4)	159 (0.9)	–‡	26 936 (0.6)	780 (0.70)	21 (0.8)
Maternal BMI, mean (SD), kg/m²	24.2 (4.0)	24.8 (4.5)	24.2 (4.1)	25.5 (4.5)	24.2 (4.5)	26.0 (5.4)	24.8 (4.7)
Missing outside registration period, No. (%)	15 183 (19.4)	239 (30.9)	1196 (6.6)	30 (17.9)	1 314 826 (29.7)	62 812 (56.4)	1273 (50.2)
Missing during registration period, No. (%)	8950 (11.4)	160 (20.7)	2290 (12.7)	42 (25.0)	502 475 (11.4)	16 061 (14.5)	433 (17.1)
Maternal smoking in pregnancy, No. (%)	4055 (5.2)	47 (6.1)	540 (3.0)	6 (3.6)	449 538 (10.2)	7139 (6.4)	197 (7.8)
Missing outside registration period, No. (%)	2006 (2.6)	202 (26.1)	115 (0.6)	37 (22.0)	378 584 (8.6)	21 309 (19.2)	221 (8.7)
Missing during registration period, No. (%)	5357 (6.8)	21 (2.7)	996 (5.5)	–‡	305 443 (6.9)	41 220 (37.1)	350 (15.8)
Caesarean section, No. (%)	19 910 (25.4)	173 (22.4)	5133 (28.5)	50 (30.0)	670 949 (15.2)	16 184 (14.6)	665 (26.2)
Induction of labor, No. (%)	14 720 (18.8)	190 (24.6)	4500 (25.0)	45 (26.8)	567 072 (12.8)	19 137 (17.2)	494 (19.5)
Sex, No. (%)							
Male	40 019 (51.1)	395 (54.8)	9215 (51.1)	81 (50.0)	2 275 150 (51.4)	56 493 (51.5)	1268 (50.1)

Female	38 257 (48.9)	326 (45.2)	8822 (48.9)	81 (50.0)	2 151 084 (48.6)	53 146 (48.5)	1265 (49.9)
Birthweight, mean (SD), grams	3406.4 (620.7)	2125.3 (1836.1)	3578.0 (614.6)	2762.1 (1707.4)	3537.2 (565.2)	3338.1 (1051.9)	3421.4 (696.8)
Gestational age, mean (SD), days	276.5 (15.8)	242.2 (79.5)	278.0 (14.9)	246.7 (66.4)	279.0 (13.0)	263.0 (43.0)	282.0 (284.5)
Preterm birth[†], No. (%)	6351 (8.1)	280 (44.0)	1198 (6.6)	48 (32.7)	219 461 (5.0)	10 989 (20.7)	211 (8.7)
ART fertilization method, No. (%)							
IVF	44 602 (57.0)	461 (59.6)	9846 (54.6)	79 (47.0)	–	–	–
ICSI	32 239 (41.2)	286 (37.0)	6616 (36.7)	55 (32.7)	–	–	–
Unknown	1459 (1.9)	27 (3.5)	1575 (8.7)	34 (20.4)	–	–	2537 (100.0)
Embryos transferred, No. (%)							
Single embryo transfer	37 082 (47.4)	246 (31.8)	11 605 (64.3)	75 (44.6)	–	–	–
Multiple embryo transfer	31 878 (40.7)	451 (58.3)	4340 (24.1)	60 (35.7)	–	–	–
Unknown	9340 (11.9)	77 (10.0)	2092 (11.6)	33 (19.6)	–	–	2537 (100.0)
Embryo culture, No. (%)							
2–3 days (Cleavage)	61 772 (78.6)	–	11 707 (64.9)	–	–	–	–
5–6 days (Blastocyst)	4450 (5.7)	–	3756 (20.8)	–	–	–	–
Unknown	12 178 (15.6)	–	2574 (14.3)	–	–	–	2537 (100.0)
HDP diagnosis, No. (%)	4600 (5.9)	45 (5.8)	1326 (7.4)	16 (9.5)	191 287 (4.3)	4734 (4.3)	182 (7.2)

[*]Percentages may not total to 100% on account of rounding.
[†]Preterm birth was defined as birth before 37 weeks of gestation.
[‡]Omitted due to small numbers.
Abbreviations: ART, assisted reproductive technology; BMI, body mass index; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP, hypertensive disorders in pregnancy; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; SD, standard deviation.

Table S3. Risk of hypertensive disorders in pregnancy for frozen-ET versus fresh-ET: population level estimates and within sibship comparison.

	Population level estimates (Random effects)					Within sibship estimates (Fixed effects)		
	Cases/Deliveries (%)	RD*, *pp*	RD, *pp* (95% CI)†	OR*	Adjusted OR (95% CI)†	Cases/Deliveries‡ (%)	OR*	Adjusted OR (95% CI)†
HDP								
Fresh-ET	4600/78 300 (5.87)	0	0 (Ref.)	1	1 (Ref.)	256/5544 (4.62)	1	1 (Ref.)
Frozen-ET	1326/18 037 (7.35)	1.51	2.18 (1.73 to 2.63)	1.40	1.62 (1.47 to 1.79)	279/5466 (5.10)	1.14	2.00 (1.57 to 2.54)
Preeclampsia§								
Fresh-ET	3371/78 300 (4.31)	0	0 (Ref.)	1	1 (Ref.)	181/5544 (3.26)	1	1 (Ref.)
Frozen-ET	991/18 037 (5.49)	1.22	1.93 (1.52 to 2.33)	1.40	1.68 (1.51 to 1.87)	200/5466 (3.66)	1.14	2.21 (1.67 to 2.92)

*Unadjusted.
†Adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44) and parity (0, 1, 2 or 3). Random effects are additionally adjusted for country (Denmark, Norway, or Sweden).
‡Refers to deliveries that are part of a sibling group with the two different conceptions methods within the group.
§Preeclampsia, chronic hypertension with superimposed preeclampsia, and eclampsia.
Abbreviations: CI, confidence interval; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP, hypertensive disorders in pregnancy; OR, odds ratio; pp, percentage points; RD, risk difference; Ref, reference group.

Table S4. Risk of hypertensive disorders in pregnancy according to birth order and combination of conception methods.

			First birth		Second birth	
First birth	**Second birth**	**Sibling groups (n)**	**Adjusted risk**	**(95% CI)**	**Adjusted risk**	**(95% CI)**
Natural conception	Natural conception	1 540 571	5.78	(5.74 to 5.82)	2.58	(2.56 to 2.61)
Natural conception	Fresh-ET	7873	5.84	(5.32 to 6.36)	3.32	(2.95 to 3.69)
Natural conception	Frozen-ET	1510	5.57	(4.42 to 6.73)	4.86	(3.85 to 5.87)
Fresh-ET	Natural conception	13 764	5.67	(5.29 to 6.05)	2.81	(2.55 to 3.07)
Frozen-ET	Natural conception	2159	9.06	(7.87 to 10.25)	2.35	(1.75 to 2.95)
Fresh-ET	Fresh-ET	7507	5.17	(4.62 to 5.67)	2.61	(2.28 to 2.95)
Fresh-ET	Frozen-ET	3946	5.21	(4.53 to 5.89)	4.09	(3.51 to 4.67)
Frozen-ET	Fresh-ET	937	6.31	(4.78 to 7.83)	2.12	(1.27 to 2.98)
Frozen-ET	Frozen-ET	923	6.18	(4.67 to 7.69)	2.33	(1.43 to 3.22)

Absolute risks are obtained using random effects logistic models with post-estimation commands.
All estimates are adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44), and country (Denmark, Norway, or Sweden).

Abbreviations: CI, confidence interval; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer.

Table S5. Risk of HDP by conception method: population level estimates and within sibship comparison in subgroups.

	Population level estimates (Random effects)			Within sibship estimates (Fixed effects)		
	Cases/Deliveries (%)	OR*	aOR (95% CI)†	Cases/Deliveries‡ (%)	OR*	aOR (95% CI)†
Smoking & BMI						
Natural conception	104 439/2 554 111 (4.09)	1	1 (Ref.)	666/18 983 (3.51)	1	1 (Ref.)
Fresh-ET	3022/53 165 (5.68)	1.61	0.97§ (0.92 to 1.02)	840/17 550 (4.79)	1.56	0.88§ (0.76 to 1.01)
Frozen-ET	1025/14 355 (7.14)	2.30	1.70§ (1.55 to 1.87)	369/6547 (5.64)	2.00	1.88§ (1.52 to 2.33)
Full siblings						
Natural conception	132 146/3 394 887 (3.89)	1	1 (Ref.)	1213/30 774 (3.95)	1	1 (Ref.)
Fresh-ET	1957/40 841 (4.79)	1.35	0.96 (0.90 to 1.03)	1382/27 385 (5.05)	1.45	0.91 (0.82 to 1.01)
Frozen-ET	637/10 718 (5.94)	1.86	1.80 (1.60 to 2.01)	548/9031 (6.07)	1.94	1.97 (1.65 to 2.34)
<3 years birth interval						
Natural conception	74 568/1 994 313 (3.74)	1	1 (Ref.)	534/16 828 (3.17)	1	1 (Ref.)
Fresh-ET	1113/23 500 (4.82)	1.53	0.96 (0.88 to 1.05)	857/16 742 (5.12)	2.42	0.85 (0.72 to 0.99)
Frozen-ET	410/6747 (6.08)	2.14	2.04 (1.76 to 2.37)	358/5627 (6.36)	3.16	2.12 (1.66 to 2.72)
IVF (÷ICSI)						
Natural conception	191 287/4 426 691 (4.32)	1	1 (Ref.)	1014/25 450 (3.98)	1	1 (Ref.)
Fresh-ET	2587/44 602 (5.80)	1.49	0.97 (0.91 to 1.02)	893/17 997 (4.96)	1.35	0.90 (0.79 to 1.03)
Frozen-ET	765/9846 (7.77)	2.37	1.82 (1.63 to 2.03)	331/4947 (6.69)	2.09	2.05 (1.63 to 2.56)
Single embryo transfers						
Natural conception	191 287/4 426 691 (4.32)	1	1 (Ref.)	880/22 763 (3.87)	1	1 (Ref.)
Fresh-ET	2100/37 082 (5.66)	1.45	0.99 (0.93 to 1.05)	716/14 892 (4.81)	1.34	0.91 (0.79 to 1.05)
Frozen-ET	860/11 605 (7.41)	2.18	1.80 (1.62 to 1.99)	346/5545 (6.24)	1.87	2.07 (1.66 to 2.58)
Blastocyst transfers‖						
Natural conception	66 577/1 534 108 (4.34)	1	1 (Ref.)	45/1461 (3.08)	1	1 (Ref.)
Fresh-ET	218/4214 (5.17)	1.32	0.93 (0.76 to 1.13)	59/1310 (4.50)	1.98	1.34 (0.77 to 2.33)
Frozen-ET	280/3729 (7.51)	2.27	1.72 (1.43 to 2.08)	55/973 (5.65)	1.88	2.02 (1.09 to 3.74)

[*]Unadjusted.
[†]Adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44), and parity (0, 1, 2 or 3). Random effects are additionally adjusted for country (Denmark, Norway, or Sweden).
[‡]Refers to deliveries that are part of a sibling group with at least two different conceptions methods within the group.
[§]Additionally adjusted for BMI as a categorical variable (underweight <18.5 kg/m^2, normal 18.5–24.99 kg/m^2, overweight 25–29.99 kg/m^2, or obese ≥30 kg/m^2) and smoking status as a dichotomous variable (yes/no).
[||]Blastocyst analyses were restricted to 2005-2015.

Abbreviations: aOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP; hypertensive disorders in pregnancy; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; OR, odds ratio; Ref, reference group.

Table S6. Risk of preeclampsia by conception method: population level estimates and within sibship comparison in subgroups.

	Population level estimates (Random effects)			Within sibship estimates (Fixed effects)		
	Cases/Deliveries (%)	OR*	aOR (95% CI)†	Cases/Deliveries‡ (%)	OR*	aOR (95% CI)†
Smoking & BMI						
Natural conception	78 091/2 554 111 (3.06)	1	1 (Ref.)	458/18 983 (2.41)	1	1 (Ref.)
Fresh-ET	2223/53 165 (4.18)	1.54	1.02§ (0.96 to 1.09)	634/17 550 (3.61)	1.75	0.97§ (0.82 to 1.14)
Frozen-ET	764/14 355 (5.32)	2.20	1.88§ (1.69 to 2.08)	279/6547 (4.26)	2.43	2.58§ (2.01 to 3.32)
Full siblings						
Natural conception	97 748/3 394 887 (2.88)	1	1 (Ref.)	820/30 774 (2.67)	1	1 (Ref.)
Fresh-ET	1408/40 841 (3.45)	1.30	1.02 (0.95 to 1.10)	1005/27 385 (3.67)	1.59	0.94 (0.83 to 1.07)
Frozen-ET	468/10 718 (4.37)	1.80	2.01 (1.77 to 2.28)	401/9031 (4.44)	2.16	2.28 (1.86 to 2.80)
<3 years birth interval						
Natural conception	55 605/1 994 313 (2.79)	1	1 (Ref.)	351/16 828 (2.09)	1	1 (Ref.)
Fresh-ET	833/23 500 (3.54)	1.48	1.05 (0.95 to 1.16)	639/16 742 (3.82)	2.90	0.94 (0.78 to 1.14)
Frozen-ET	305/6747 (4.52)	2.05	2.30 (1.95 to 2.71)	268/5627 (4.76)	3.82	2.51 (1.88 to 3.34)
IVF (÷ICSI)						
Natural conception	142 195/4 426 691 (3.21)	1	1 (Ref.)	686/25 450 (2.70)	1	1 (Ref.)
Fresh-ET	1922/44 602 (4.31)	1.47	1.03 (0.97 to 1.10)	658/17 997 (3.66)	1.48	0.99 (0.86 to 1.16)
Frozen-ET	575/9846 (5.84)	2.30	2.03 (1.80 to 2.29)	252/4947 (5.09)	2.34	2.50 (1.93 to 3.24)
Single embryo transfers						
Natural conception	142 195/4 426 691 (3.21)	1	1 (Ref.)	595/22 763 (2.61)	1	1 (Ref.)
Fresh-ET	1503/37 082 (4.05)	1.35	1.05 (0.98 to 1.13)	507/14 892 (3.40)	1.40	0.97 (0.82 to 1.14)
Frozen-ET	634/11 605 (5.46)	2.07	1.99 (1.78 to 2.23)	253/5545 (4.56)	1.97	2.37 (1.85 to 3.05)
Blastocyst transfers‖						
Natural conception	44 929/1 534 108 (2.93)	1	1 (Ref.)	30/1461 (2.05)	1	1 (Ref.)
Fresh-ET	149/4214 (3.54)	1.31	0.96 (0.76 to 1.21)	35/1310 (2.67)	1.79	1.03 (0.52 to 2.07)
Frozen-ET	196/3729 (5.26)	2.31	1.89 (1.52 to 2.35)	42/973 (4.32)	2.36	2.69 (1.25 to 5.77)

*Unadjusted.
†Adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44), and parity (0, 1, 2 or 3). Random effects are additionally adjusted for country (Denmark, Norway, or Sweden).
‡Refers to deliveries that are part of a sibling group with at least two different conceptions methods within the group.
§Additionally adjusted for BMI as a categorical variable (underweight <18.5 kg/m^2, normal 18.5–24.99 kg/m^2, overweight 25–29.99 kg/m^2, or obese ≥30 kg/m^2) and smoking status as a dichotomous variable (yes/no).
||Blastocyst analyses were restricted to 2005-2015.

Caption: Subgroup analyses after using the more restricted outcome definition (i.e., excluding isolated gestational hypertension).

Abbreviations: aOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; ICSI,

intracytoplasmic sperm injection; IVF, in vitro fertilization; OR, odds ratio; Ref, reference group.

Table S7. Risk of hypertensive disorders in pregnancy by conception method: population level estimates and within sibship comparison in each country separately.

	Population level estimates (Random effects)			Within sibship estimates (Fixed effects)		
	Cases/Deliveries (%)	OR*	Adjusted OR (95% CI)†	Cases/Deliveries‡ (%)	OR*	Adjusted OR (95% CI)†
Denmark						
Natural conception	40 816/978 208 (4.17)	1	1 (Ref.)	331/9173 (3.61)	1	1 (Ref.)
Fresh-ET	1376/25 056 (5.49)	1.49	0.97 (0.89 to 1.06)	398/8510 (4.68)	1.49	0.95 (0.78 to 1.16)
Frozen-ET	195/3347 (5.83)	1.64	1.24 (1.01 to 1.53)	78/1846 (4.23)	1.39	1.58 (1.05 to 2.38)
Norway						
Natural conception	61 375/1 198 150 (5.12)	1	1 (Ref.)	422/7421 (5.69)	1	1 (Ref.)
Fresh-ET	1099/16 636 (6.61)	1.41	1.00 (0.91 to 1.09)	420/6516 (6.45)	1.26	0.96 (0.80 to 1.15)
Frozen-ET	325/3300 (9.85)	2.57	2.35 (1.99 to 2.79)	170/1851 (9.18)	1.96	2.32 (1.69 to 3.18)
Sweden						
Natural conception	89 096/2 250 333 (3.96)	1	1 (Ref.)	611/17 557 (3.48)	1	1 (Ref.)
Fresh-ET	2125/36 608 (5.80)	1.71	1.06 (0.99 to 1.13)	731/15 307 (4.78)	1.57	1.01 (0.87 to 1.7)
Frozen-ET	806/11 390 (7.08)	2.35	1.73 (1.55 to 1.92)	342/5954 (5.74)	2.07	2.06 (1.64 to 2.58)

*Unadjusted.
†Adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44), and parity (0, 1, 2 or 3).
‡Refers to deliveries that are part of a sibling group with at least two different conceptions methods within the group.
Abbreviations: aOR, adjusted odds ratio; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; OR, odds ratio; Ref, reference group.

Table S8. Risk of hypertensive disorders in pregnancy by conception method: population level estimates and within sibship comparison using Cox regression.

	Cases/Deliveries (%)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)*
Population level estimates			
Natural conception	191 287/4 426 691 (4.32)	1 (Ref.)	1 (Ref.)
Fresh-ET	4600/78 300 (5.87)	1.50 (1.46 to 1.54)	1.12 (1.08 to 1.15)
Frozen-ET	1326/18 037 (7.35)	1.73 (1.64 to 1.82)	1.40 (1.32 to 1.48)
Within sibship estimates			
Natural conception	1364/34 151† (3.99)	1 (Ref.)	1 (Ref.)
Fresh-ET	1539/30 333† (5.11)	1.40 (1.27 to 1.65)	1.06 (0.95 to 1.17)
Frozen-ET	590/9651† (6.11)	1.68 (1.41 to 1.99)	1.81 (1.51 to 2.17)

*Adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44), and parity (0, 1, 2 or 3). Population level analyses are also adjusted for country (Denmark, Norway, or Sweden).
†Refers to deliveries that are part of a sibling group with at least two different conceptions methods within the group.

Caption: Cox regression with gestational duration as the time scale (i.e., pregnancies without HDP were censored at delivery) on Main sample.

Abbreviations: CI, confidence interval; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP, hypertensive disorders in pregnancy; HR, hazard ratio; Ref, reference group.

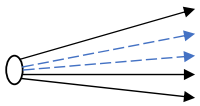
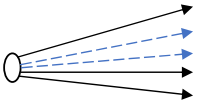
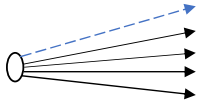
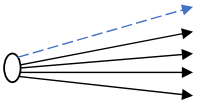
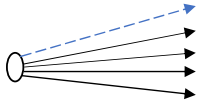
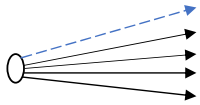
Conception method and outcome in 1st delivery	Continuation rate within 5 years in %, by conception method in 2nd delivery	HDP in 2nd delivery, aOR (95% CI)
Natural conception No HDP **n = 1 713 104**	 Natural conception **77.42%** Fresh-ET **0.41%** Frozen-ET **0.08%** No siblings **20.97%** Multiples **1.12%**	Natural conception: 1 (Ref.) Fresh-ET: 1.36 (1.18 to 1.57) Frozen-ET: 2.24 (1.74 to 2.89)
Natural conception HDP **n = 104 184**	 Natural conception **74.40%** Fresh-ET **0.42%** Frozen-ET **0.07**% No siblings **23.97%** Multiples **1.14%**	Natural conception: 1 (Ref.) Fresh-ET: 1.12 (0.90 to 1.39) Frozen-ET: 1.36 (0.85 to 2.20)
Fresh-ET No HDP **n = 37 667**	 Natural conception **28.23%** Fresh-ET **16.16%** Frozen-ET **7.43%** No siblings **43.09%** Multiples **5.09%**	Natural conception: 1 (Ref.) Fresh-ET: 0.92 (0.75 to 1.14) Frozen-ET: 1.69 (1.36 to 2.09)
Fresh-ET HDP **n = 2623**	 Natural conception **25.77%** Fresh-ET **13.02%** Frozen-ET **6.56%** No siblings **50.40%** Multiples **4.19%**	Natural conception: 1 (Ref.) Fresh-ET: 1.04 (0.78 to 1.38) Frozen-ET: 1.08 (0.75 to 1.54)
Frozen-ET No HDP **n = 4854**	 Natural conception **28.72%** Fresh-ET **14.28%** Frozen-ET **12.09%** No siblings **40.75%** Multiples **4.16%**	Natural conception: 1 (Ref.) Fresh-ET: 1.05 (0.56 to 1.98) Frozen-ET: 0.99 (0.52 to 1.91)
Frozen-ET HDP **n = 466**	 Natural conception **31.55%** Fresh-ET **9.44%** Frozen-ET **9.23%** No siblings **45.49%** Multiples **4.29%**	Natural conception: 1 (Ref.) Fresh-ET: 1.01 (0.43 to 2.36) Frozen-ET: 1.52 (0.72 to 3.25)

Figure titles and captions. Supporting information.

"Figure 1: Flowchart of the study population"

The subgroup with only blastocyst transfers was restricted to birth years 2005–2015.

Abbreviations: Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.

"Figure 2: Risk of hypertensive disorders in pregnancy in consecutive sibling pairs according to birth order and conception method"

Absolute risks are obtained using random effects logistic models with post-estimation commands. All estimates are adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44), and country (Denmark, Norway, or Sweden).

Table S4 presents the full results from Figure 2.

Abbreviations: Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP, hypertensive disorders in pregnancy.

"Figure 3: Risk of HDP by conception method: Population level estimates and within sibship comparisons in subgroups"

Adjusted odds ratios with 95% confidence intervals for fresh embryo transfer (fresh-ET) versus natural conception and frozen embryo transfer (frozen-ET) versus natural conception in our subgroups. All estimates are adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44) and parity (0, 1, 2 or 3). Random effects are additionally adjusted for country (Denmark, Norway, or Sweden). Estimates for the body mass index (BMI) and smoking subgroup are additionally adjusted for BMI as a categorical variable (underweight <18.5 kg/m^2, normal 18.5–24.99 kg/m^2, overweight 25–29.99 kg/m^2, or obese

≥30 kg/m^2) and smoking status as a dichotomous variable (yes/no). Blastocyst analyses were restricted to 2005–2015.

Table S5 presents the full results from Figure 3.

Abbreviations: BMI, body mass index; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP, hypertensive disorders in pregnancy; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.

"Figure S1: Risk of hypertensive disorders in pregnancy by conception method when accounting for selection"

Adjusted odds ratios with 95% confidence intervals for fresh embryo transfer (fresh-ET) and frozen embryo transfer (frozen-ET) versus natural conception (NC). All estimates are adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44) and country (Denmark, Norway, or Sweden).

Dashed blue lines indicate subgroups that contribute to within sibship estimates (fixed effects).

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP, hypertensive disorders in pregnancy; Ref, reference group.

Paper III

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