Safety and quality aspects of IVF - neonatal and maternal outcomes following advanced techniques

Erica Ginström Ernstad

Department of Obstetrics and Gynecology
Institute of Clinical Science
Sahlgrenska Academy
University of Gothenburg
Gothenburg, Sweden

UNIVERSITY OF GOTHENBURG

Gothenburg 2020

Cover illustration: Tubies by Stina Rosén

Safety and quality aspects of IVF –
neonatal and maternal outcomes following advanced techniques

erica.ernstad@vgregion.se

ISBN 978-91-7833-782-8 (PRINT)
ISBN 978-91-7833-783-5 (PDF)
http://hdl.handle.net/2077/63272

Printed in Borås, Sweden 2020
Printed by Stema Specialtryck AB

"If we knew what it was we were doing,

it would not be called research, would it?"

Albert Einstein

Safety and quality aspects of IVF - neonatal and maternal outcomes following advanced techniques

Erica Ginström Ernstad

Department of Obstetrics and Gynecology, Institute of Clinical Science
Sahlgrenska Academy, University of Gothenburg
Gothenburg, Sweden

ABSTRACT

Background: Singletons born following assisted reproductive technology (ART) have adverse neonatal outcome compared to singletons born following spontaneous conception (SC). Moreover, the women undergoing ART are at an increased risk of hypertensive disorders in pregnancy (HDP) and placental complications.
Aim: To study the neonatal and maternal outcomes following the introduction of advanced techniques in ART.
Material and methods: All papers were population-based register studies in Sweden with cross linkage of the national ART registers and national health data registers. In *paper III* also Danish register data were included. *Paper I* Singletons born after blastocyst transfer (n=4819), singletons born after cleavage stage transfer (n=25,747) and singletons born after SC (n=1,196,394) were included. The main outcome was birth defects. Moreover, other neonatal and maternal outcomes were assessed. *Paper II* Neonatal and maternal outcomes in different cycle regimens in frozen embryo transfer (FET) (n=6297 in natural cycles, n=1983 in stimulated cycles, n=1446 in programmed cycles) were studied. FET was also compared to fresh embryo transfer and to SC. The primary outcomes were preterm birth (PTB, <37 weeks), low birth weight (LBW, <2500 grams), HDP and postpartum hemorrhage (PPH, >1000 mL). *Paper III* Singleton pregnancies following transfer of vitrified blastocysts (n=3650) were compared to singleton pregnancies following slow-frozen cleavage stage transfer (n=8123) and fresh blastocyst transfer (n=4469). Main outcomes were PTB, LBW, macrosomia, HDP and PPH. *Paper IV* Singletons born following preimplantation genetic testing (PGT) (n=267) were compared to singletons born following in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) (n=55,355) and to SC (n=26,535). Main outcomes were PTB and LBW. Moreover, maternal outcomes and early childhood outcome were assessed.
Results: *Paper I* No difference in the rate of birth defects were observed between the groups. However, there was an increased risk of placenta previa and placental abruption following blastocyst transfer compared to transfer of cleavage stage embryos and SC. *Paper II* Programmed cycles were associated with a higher risk of HDP (adjusted odds ratio [AOR] 1.6-1.8), PPH (AOR 2.6-2.9), post term birth (AOR 1.6-2.0) and macrosomia (≥4500 grams) (AOR 1.4-1.6) compared to other cycle regimens. The rates of PTB and LBW were similar independently of cycle regimen. *Paper III* Transfer of vitrified blastocysts was associated with a higher risk of PTB (AOR 1.3). No other differences were found. *Paper IV* For PGT singletons no differences in PTB and LBW were observed in comparison to other IVF/ICSI singletons yet higher rates compared to SC. The early childhood outcomes were reassuring but should be interpreted cautiously due to few cases and short follow-up time.
Conclusion: Blastocyst transfer is associated with a higher risk of placenta previa and placental abruption compared to cleavage stage transfer. Programmed cycles were associated with higher risks of HDP and PPH compared to other cycle regimens. The freezing technique or the embryo biopsy used for PGT do not seem to alter the neonatal and maternal outcomes.

Keywords: blastocyst transfer, frozen embryo transfer, vitrification, preimplantation genetic testing, neonatal outcome, maternal outcome

ISBN 978-91-7833-782-8 (PRINT)
ISBN 978-91-7833-783-5 (PDF) http://hdl.handle.net/2077/63272

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Ginström Ernstad E, Bergh C, Khatibi A, Källén K, Westlander G, Nilsson S, Wennerholm UB. Neonatal and maternal outcome after blastocyst transfer: a population-based registry study. *Am J Obstet Gynecol. 2016 Mar;214(3):378.e1-378.e10*

II. Ginström Ernstad E, Wennerholm UB, Khatibi A, Petzold M, Bergh C. Neonatal and maternal outcome after frozen embryo transfer: Increased risks in programmed cycles. *Am J Obstet Gynecol. 2019 Aug;221(2):126.e1-126.e18*

III. Ginström Ernstad E, Spangmose AL, Opdahl S, Henningsen AK, Romundstad LB, Tiitinen A, Gissler M, Wennerholm UB, Pinborg A, Bergh C, Malchau SS. Perinatal and maternal outcome after vitrification of blastocysts: a Nordic study in singletons from the CoNARTaS group. *Hum Reprod. 2019 Nov 1;34(11):2282-2289*

IV. Ginström Ernstad E, Hanson C, Wånggren K, Thurin Kjellberg A, Hulthe C, Syk Lundberg E, Petzold M, Wennerholm UB, Bergh C. Perinatal, maternal and early childhood outcome following preimplantation genetic testing: a national register-based study. *In manuscript.*

CONTENT

SAMMANFATTNING PÅ SVENSKA

Infertilitet drabbar 10-15% av alla par och idag föds i Sverige ca 5000 barn årligen efter assisterad befruktning (ART). ART innebär hantering av könsceller utanför kroppen och innefattar bl.a. provrörsbefruktning (in vitro fertilisering, IVF) och mikroinjektion ("intracytoplasmic sperm injection", ICSI). Det är sedan tidigare känt att barn födda efter IVF har en ökad risk för förtidsbörd och låg födelsevikt jämfört med barn födda efter spontan befruktning. Denna riskökning kan till stor del förklaras av den ökade risken för flerbörd efter IVF men ökade risker har även visats för barnen födda i enkelbörd. Sannolikt bidrar såväl behandlingstekniken som den bakomliggande infertiliteten till detta.

Under senare år har flera nya tekniker införts. Vid blastocyståterföring har embryot odlats utanför kroppen i 5-6 dagar istället för i 2-3 dagar som tidigare varit rutin. Vid en färsk behandlingscykel återförs ett embryo i livmodern i samma cykel som ägget utplockas. I vissa fall väljer man dock att inte göra ett färskt återförande utan att frysa samtliga embryon. Väljer man att göra ett färskt återförande, fryses övriga embryon ned för att kunna tinas upp och återföras vid ett senare tillfälle. Vid en fryscykel kan embryot återföras i olika typer av cykler. Vid en naturlig cykel återförs embryot i kvinnans naturliga menscykel. Vid en stimulerad cykel stimuleras kvinnans äggstockar till en ägglossning varpå embryot återförs. Vid en programmerad cykel ges kvinnan läkemedel som förbereder livmoderns slemhinna att ta emot ett embryo utan att stimulera till ägglossning. Därtill har en ny frysteknik, vitrifiering med ultrasnabb nedfrysning av embryot, införts på bred front. Tidigare studier visar att graviditeter efter blastocyståterföring är behäftade med en ökad risk för förtidsbörd och, enligt en tidigare svensk studie, även med missbildningar i jämförelse med återförande av dag 2-3 embryon. Barn födda efter frysåterförande har visats ha en ökad risk för att födas "stora för tiden" i jämförelse med barn födda efter färska cykler och spontan befruktning. Kvinnor som blivit gravida efter IVF drabbas i högre utsträckning av hypertoni/havandeskapsförgiftning (preeklampsi) med störst risk för hypertoni/preeklampsi vid behandling med frysta/tinade embryon. Därtill har kvinnorna en ökad risk för placentakomplikationer så som föreliggande/lågt sittande moderkaka och moderkaksavlossning.

Syftet med denna avhandling var att studera utfallet för barnen och deras mödrar efter introduktionen av avancerade tekniker inom ART. Endast barn födda i enkelbörd inkluderades. Samtliga delarbeten utfördes som populationsbaserade registerstudier i

Sverige med korskörning av de nationella registren för assisterad befruktning mot nationella hälsoregister. I den tredje studien inkluderades även danska registerdata.

I *delarbete I* jämfördes barn födda efter blastocyståterföring med barn födda efter återförande av dag 2-3 embryon och spontan befruktning. Studien visade ingen skillnad i missbildningsfrekvens men däremot en ökad risk för föreliggande/lågt sittande moderkaka och avlossning av moderkakan efter blastocyståterförande. *Delarbete II* visade att utfallet varierar med vilken typ av fryscykel som används och att riskerna för mor och barn var störst efter programmerad cykel med ökad risk för så väl hypertoni/preeklampsi och stor blödning i samband med förlossning samt även för överburenhet och stora barn. I *delarbete III* jämfördes de två frystekniker som används idag, vitrifiering och den äldre tekniken, "slow-freezing", och resultaten visade att frystekniken inte påverkar utfallet för varken mor eller barn. I *delarbete IV* jämfördes barn födda efter preimplantatorisk genetisk testing (PGT) mot barn födda efter IVF/ICSI och spontan befruktning. PGT erbjuds par som är drabbade av eller bärare till en genetisk sjukdom och möjliggör återförande av ett embryo som inte bär på denna sjukdom. För att utföra PGT krävs dels IVF eller ICSI men även embryobiopsi där en eller två, ibland upptill tio celler, avlägsnas från embryot och analyseras för den specifika genetiska sjukdomen. Resultaten i delarbete IV talar för att embryobiopsin som används vid PGT inte påverkar utfallet för mor och barn. Även långtidsutfallen, inkluderande bl.a. astma, psykiatrisk sjuklighet och cerebral pares, för PGT barnen var betryggande. Dock skall resultaten tolkas med försiktighet p.g.a. få fall men även p.g.a. kort uppföljningstid.

ABBREVIATIONS AND DEFINITIONS

ADHD	Attention deficit hyperactivity disorder
AID	Artificial insemination of donated sperm
AOR	Adjusted odds ratio
Array-CGH	Array-comparative genome hybridization
ART	Assisted reproductive technology; includes treatment where human gametes are handled outside the body to achieve a pregnancy
ASD	Autism spectrum disorders
ATC codes	Anatomic Therapeutic Chemical codes; a drug classification system that classifies the drugs according to the organ or system on which they act
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CL	Corpus luteum; a mass of cells that forms in an ovary following ovulation and is responsible for the production of mainly progesterone during early pregnancy.
CoNARTaS	Committee of Nordic ART and Safety
Controlled ovarian hyperstimulation	Use of fertility medication to induce ovulation of several ovarian follicles
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
EIM	European IVF Monitoring Consortium
ESHRE	European Society of Human Reproduction and Embryology
EUROCAT	European network of population-based registers for epidemiological surveillance of birth defects
FET	Frozen embryo transfer
FISH	Fluorescence in situ hybridization
FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone; a hormone released from the hypothalamus and responsible for the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary
hCG	Human chorionic gonadotropin
hMG	Human menopause gonadotropin

HDP	Hypertensive disorders in pregnancy; includes pregnancy induced hypertension and preeclampsia/eclampsia
HR	Hazard ratio
ICD 9, ICD 10	International Statistical Classification of Diseases and Related Health Problems-ninth and tenth revision
ICMART	The International Committee Monitoring Assisted Reproductive Technologies
ICSI	Intracytoplasmic sperm injection; a technique to fertilize an oocyte by injecting one single sperm directly in the oocyte
Infant mortality	Death of child within the first year of life
IQR	Interquartile range
IVF	*In vitro* fertilization
LBW	Low birth weight, <2500 grams, VLBW very low birth weight, <1500 grams
LGA	Large for gestational age (more than two standard deviations above Swedish growth standard)
MBR	Medical Birth Register
Monozygotic twinning	When one fertilized oocyte divides into two (or even three or four)
Neonatal mortality	Death of a live-born child within the first 28 days of life. *Early neonatal mortality*, death of live-born child within the first 7 days of life. *Late neonatal mortality*, death of live-born child covering the time after the first seven days of life until 28 days
NGS	Next generation sequencing
NPR	National Patient Register
OHSS	Ovarian hyperstimulation syndrome
OR	Odds ratio
PCOS	Polycystic ovarian failure syndrome; a hormonal disorder causing infrequent, irregular or prolonged menstrual periods, usually accompanied with high levels of androgens (male hormones). Numerous small follicles are developed but often fail to ovulate regularly
PCR	Polymerase chain reaction
Perinatal mortality	Stillbirths and deaths in the first week of life
PGD	Preimplantation genetic diagnosis
PGS	Preimplantation genetic screening
PGT	Preimplantation genetic testing; preimplantation genetic testing for monogenic disorders (PGT-M), preimplantation genetic

	testing for structural rearrangements (PGT-SR), preimplantation genetic testing for aneuploidy (PGT-A)
PIN	Personal identification number
Placenta previa	Low-lying placenta or a placenta that partially or completely covers the inner part of cervix
Placental abruption	When placenta partially or completely separates from the uterine wall
PPH	Postpartum hemorrhage, >1000mL in Sweden
Preeclampsia	High blood pressure (≥ 140/90) with significant amounts of protein in the urine (≥0,3g in a 24-hour urine specimen or a protein/creatinin ratio ≥0,3mg/dL) in a pregnant woman after 20 weeks of gestation. *ICD 10 code O14-O15.* According to new definitions, proteinuria is no longer considered necessary for PE (ACOG, 2017, SFOG, 2019)
Pregnancy induced hypertension	High blood pressure (≥140/90) without significant amounts of protein in the urine (≥0,3g in a 24-hour urine specimen or a protein/creatinin ratio ≥0,3mg/dL) in a pregnant woman after 20 weeks of gestation. *ICD 10 code O13*
Q-IVF	The National Registry of Assisted Reproduction
RCT	Randomized controlled trial
RNA	Ribonucleic acid
RR	Risk ratio (or relative risk)
SART	Society for Assisted Reproductive Technology
SC	Spontaneous conception
SCB	Statistics Sweden
SD	Standard deviation
SET	Single embryo transfer
SGA	Small for gestational age (more than two standard deviations below Swedish growth standard)
SNQ	Swedish Neonatal Quality Register
Stillbirth	Intrauterine fetal death ≥ 22 weeks of gestation from July 1, 2008 (≥28 weeks of gestation before July 1, 2008)
WHO	World Health Organization

1 INTRODUCTION

1.1 Infertility - an overview

The use of assisted reproductive technology (ART) has increased rapidly since the birth of the first child following *in vitro* fertilization (IVF) in 1978 (Steptoe and Edwards, 1978) and today approximately 8 million children have been born following ART (Adamson *et al.*, 2019). ART involves all treatment where human gametes are handled outside the body to achieve a pregnancy. IVF, intracytoplasmic sperm injection (ICSI) as well as oocyte donation are all such treatments whereas artificial insemination with husbands or donated sperm is not (Zegers-Hochschild *et al.*, 2017). Medically assisted reproduction (MAR) is a wider term including ART but also ovulation induction, ovulation triggering, insemination and uterine transplantation. In total, 10-15% of heterosexual couples suffer from infertility defined by the World Health Organization (WHO) as "failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse without any other reason, such as breastfeeding or postpartum amenorrhea". According to WHO, infertility is a public health problem and the United Nations has included infertility and its treatment as part of Sexual and Reproductive Human Rights. Regarding the increasing demand on ART treatment it is of utmost importance to offer women/couples suffering from infertility safe and effective treatment.

There are several reasons behind infertility. Around 1/3 are attributable to female factors, 1/3 to male factors and 1/3 a combination of both female and male factors or unknown factors. Infertility rates have increased in the last century, mainly due to problems associated with increasing maternal age and postponing childbearing (Schmidt *et al.*, 2012). The women´s age is the most important predictive factor for the chance of a live birth, depending on decreased ovarian reserve and also an increase in chromosomal abnormalities, resulting in failed implantation and/or increased miscarriage rate. Accordingly, the chance of a live birth following ART decreases with increased maternal age with a 26% chance of live birth per initiated fresh cycle for women aged 30-35 but only 8% for women aged ≥42 years. Per embryo transfer, the live birth rate in fresh cycles is substantially higher, 35% for women aged 30-35 years and 11% for women aged ≥42 years (www.qivf.se). Access to and financing of infertility treatment varies globally, as does the percentages of ART children in the national birth cohorts. In Sweden, infertility treatment is offered both in public and private care, in 6 and 13 centers, respectively. The first ART child

in Scandinavia was born 1982 in Gothenburg. Following 18,639 treatment cycles with autologous gametes, 5137 children were born in 2017, comprising 4.3% of the total national birth cohort in Sweden. Additionally, 350 children were born following 1233 donor cycles (www.qivf.se).

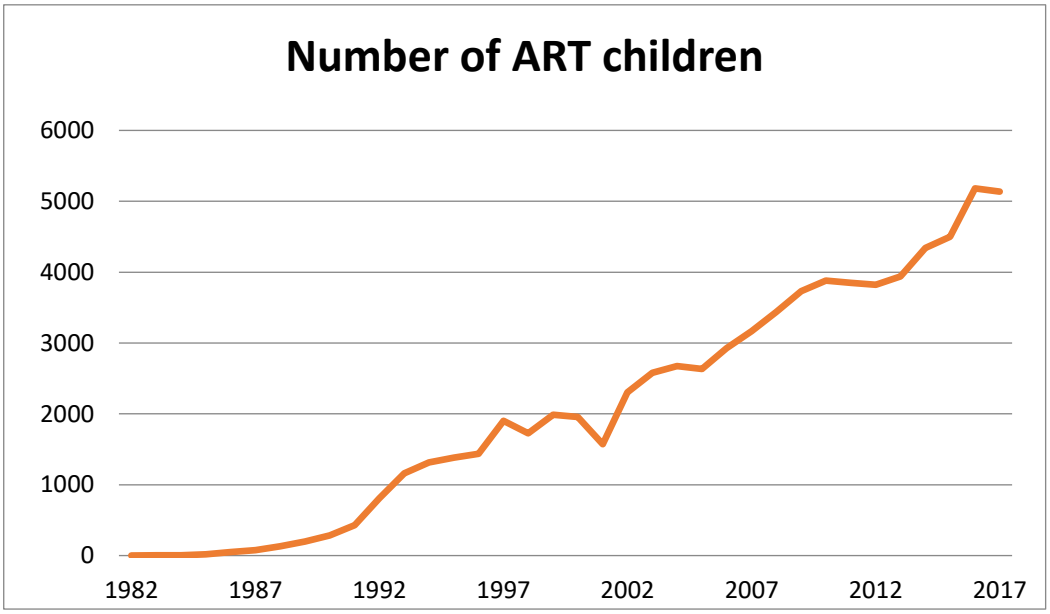

Figure 1. Number of ART children per year in Sweden 1982-2017 (www.qivf.se)

1.2 Legal aspects on ART in Sweden

In Sweden, insemination with donated sperm is regulated since 1985 (1984:1140) and ART since 1988 (1988:711). Today, ART is regulated by The Genetic Integrity Act (2006:351). Other acts and regulations such as the Bio banks in Medical Care Act, however, have an impact on the regulations. In addition, as a member of the European Union, Sweden is obliged to comply with the European Tissues and Cells Directive (2004/23/EC). This directive applies to the donation, testing, preservation, storage and distribution of human tissues and cells intended for human use and includes gametes used in ART.

Embryos can be frozen up to ten years following new regulations in Sweden in 2019. There are no regulations on freezing time for frozen oocytes or frozen sperm.

Infertility treatment with donor oocytes/sperm has previously only been performed at University Hospitals but following January 1, 2019 private clinics can get permission for donation treatment. Both known and anonymous donors are allowed in Sweden. Information regarding the anonymous donors is stored for 70 years, however, the donor is anonymous to the parents. The child has the right to know its genetic origin and parents undergoing infertility treatment with donated oocytes/sperm are thus highly recommended to tell the child its origin. The child conceived by donated oocytes or sperm has the right to access this data when the child has reached sufficient maturity. No obligations for the donor against the child exist.

Table 1. Regulations for different ART treatments in Sweden

PGT-M PGT-SR	Allowed since 1994 for monogenic diseases, chromosomal aberrations or sex-linked disorders.
PGT-HLA	Allowed in specific cases following permission from the National Board of Health and Welfare.
PGT-A	Only allowed in research following ethical approval.
Oocyte donation	Allowed since 2003
IVF/ICSI with donated sperm	Allowed since 2003
AID and IVF/ICSI in female same-sex couples	Allowed since 2005
IVF/ICSI in single women	Allowed since 2016
Use of donated oocytes + donated sperm in the same treatment cycle, embryo donation	Allowed since 2019. The National Board of Health and Welfare is working on guidelines and the combination of sperm and oocyte donation is not practiced yet, neither is embryo donation.
Surrogacy	Not allowed

PGT-M preimplantation genetic testing for monogenic disorders, ***PGT-SR*** preimplantation genetic testing for structural rearrangements, ***HLA*** human leukocyte antigen, ***PGT-A*** preimplantation genetic testing for aneuploidy, ***IVF*** in vitro fertilization, ***ICSI*** intracytoplasmic sperm injection, ***AID*** artificial insemination using donated sperm

1.3 The IVF and ICSI procedure

IVF is a series of procedures enabling fertilization of an oocyte with sperm outside the body (*in vitro)*. In controlled ovarian hyperstimulation, follicle stimulating hormone (FSH) or human menopause gonadotropin (hMG) is given to enable development of multiple follicles. In parallel, a gonadotropin releasing hormone (GnRH) agonist or antagonist is given to inhibit premature luteinization. The development of the follicles is monitored through vaginal ultrasound and/or serum estradiol levels. To enable the final oocyte maturation, human chorionic gonadotropin (hCG) is given as a single dose. In the next step, approximately 36 hours after the administration of hCG, ovum pick up is performed transvaginally with guidance of transvaginal ultrasound in local anesthesia combined with analgesics and sometimes light sedation. Following the ovum pick up the oocytes are fertilized with sperm on specific dishes and then cultured between 2 and 5-6 days. In Sweden, in around 50% of cycles, mainly due to e.g. low sperm count or low sperm motility, a more invasive technique called ICSI, where a single sperm is injected into the oocyte, is used. In the last step of IVF, the embryo is transferred to the uterine cavity either at culture day 2 to 3 or 5 and surplus good quality embryos are cryopreserved.

During the 90s and in the beginning of this century, many countries reported on multiple birth rates of 20-30% following IVF treatment. In a large Swedish population-based study, published in Lancet 1999, the multiple birth rate was 27% in the IVF group compared to 1% in the background population (Bergh *et al.*, 1999). The same study, including 5856 children after ART showed a 5-folded increase in preterm birth (PTB, <37 weeks) and low birth weight (LBW, <2500 grams) in the ART cohort, mainly explained by the higher rate of multiples. When comparing only singletons, a 2-3 folded increase in adverse outcome was noted. Later, several meta-analyses have confirmed adverse outcomes in regards of PTB and LBW also in singletons born following ART as summarized in Table 2 (Helmerhorst *et al.*, 2004, Jackson *et al.*, 2004, McDonald *et al.*, 2009, Pandey *et al.*, 2012, Qin *et al.*, 2017).

Single embryo transfer (SET) is the most effective way of reducing multiple births and the associated adverse outcomes. A Finnish study was the first study to demonstrate SET as a successful option in IVF (Vilska *et al.*, 1999). In 2004 a large randomized control trial (RCT) including 661 women <36 years compared live birth rate in women with at least two good-quality embryos. The women were randomized either to transfer of a single fresh embryo and a subsequent transfer of a single frozen embryo if there was no live birth, or to undergo a single transfer of two fresh

embryos. The results showed a dramatic reduction in the rate of multiple births following SET (33% in double embryo transfer, 1% in SET) without substantially reducing the live birth rate (Thurin *et al.*, 2004). In Sweden, SET is highly recommended by the National Board of Health and Welfare and the numbers of cycles with SET are amongst the highest in Europe (Figure 2). Worldwide, the use of SET has also gained in popularity, though, the latest numbers from Europe still show a multiple birth rate of 17% in fresh cycles and 12% in frozen cycles in 2015 (De Geyter *et al.*, 2020) whereas the latest report from the United States reveals a multiple birth rate of 16% in 2018 (www.cdc.gov/art).

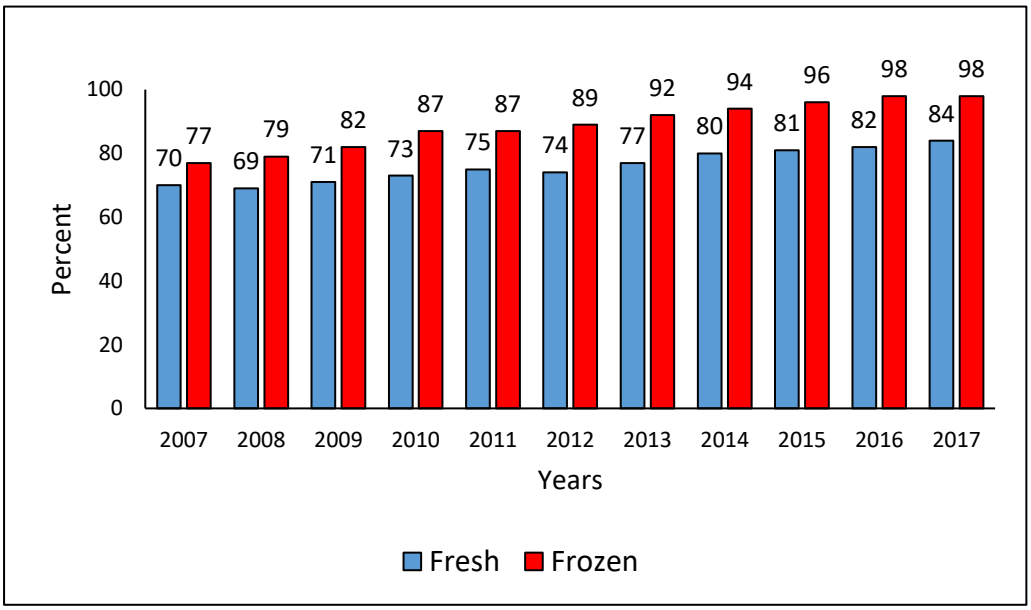

Figure 2. Single embryo transfer in fresh and frozen cycles in Sweden 2007-2017 (www.qivf.se)

The use of ICSI varies in different countries. In Sweden around 50% of all ART cycles are ICSI cycles while European data shows a predominance of ICSI, 71.3 % ICSI versus 28.7% IVF (De Geyter *et al.*, 2020). In the beginning, following the birth of the first ICSI baby in 1992 (Palermo *et al.*, 1992), ICSI was used only for severe male infertility but today ICSI is also used for mild male infertility and in cases of mixed or unexplained infertility.

1.4 Advanced techniques

Blastocyst transfer

An embryo is considered a blastocyst when it reaches the 64-cell stage which occurs at day 5-6 post fertilization. Blastocyst transfer, i.e. transferring the embryo into the uterine cavity on day 5 or 6 is, compared to transferring the embryo at the cleavage stage (i.e. day 2-3 at the 4 - or 8-cell stage), considered more physiological since embryos at an earlier developmental stage are usually located in the fallopian tube. In spontaneous conception (SC), the embryo reaches the uterine cavity around day 4-5 post fertilization (morula or blastocyst stage). The embryo can be morphologically graded at day 2 at the earliest, hence allowing selection and transfer of good quality embryos from that day.

In the early days of IVF, transfer of cleavage stage embryos was the standard since studies during the 80s showed that few embryos survived culturing *in vitro* until the blastocyst stage (Bolton *et al.*, 1989). In the late 90s stage-specific culture medias were developed and the embryos developing *in vitro* were transferred from one media to another at day 2 or 3, increasing both the development from a cleavage stage embryo to a blastocyst and the implantation/pregnancy rate (Gardner *et al.*, 1998a, Gardner *et al.*, 1998b). The advantage with blastocyst transfer is the possibility to choose the most viable embryos for transfer whereas the major disadvantage is having no embryos developing to this stage and thus no embryos available for transfer.

The first pregnancies and live births following transfer of human blastocysts were reported in 1991 (Bolton *et al.*, 1991). Following the introduction of a new freezing technique, vitrification, described more closely on pages 24-25, survival and implantation rates for frozen blastocysts improved significantly (Stehlik *et al.*, 2005) and hence, blastocyst transfer is today clinical routine in many countries. In 2017, more than 30% of fresh cycles and almost 90% of frozen cycles were performed as blastocyst transfer in Sweden (Figure 3) (www.q-ivf.se). A Cochrane review reported that blastocyst transfer, in comparison to cleavage stage transfer, is associated with increased delivery rates in fresh cycles (adjusted odds ratio [AOR] 1.48, 95% confidence interval [CI] 1.20-1.82). The review also suggested that if 29% of women deliver a live birth following a cleavage stage transfer in a fresh cycle, then around 40% would do so following a fresh blastocyst transfer. Yet, even though there is a difference for fresh transfers, the cumulative live birth rate when including

both fresh and frozen cycles from the same oocyte pick up, was not significantly different (Glujovsky *et al.*, 2016).

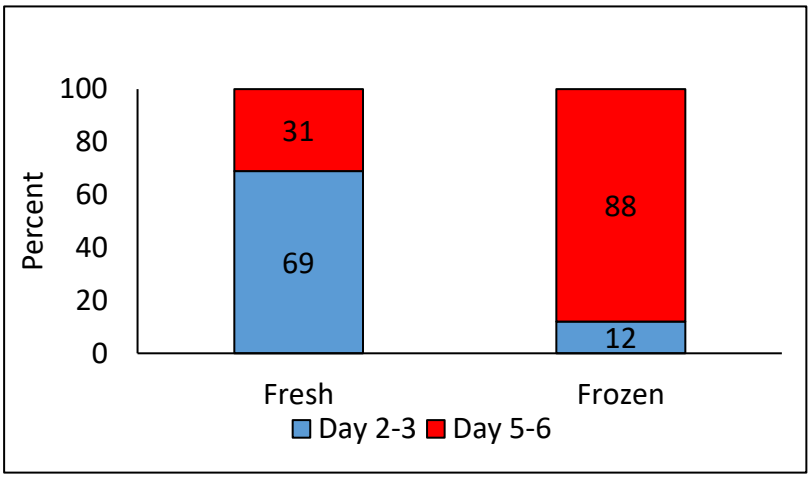

Figure 3. Distribution of day 2-3 and day 5-6 embryos in Sweden in 2017 for fresh and frozen transfers (www.q-ivf.se)

Frozen embryo transfer

Since the early 80s, when the first baby was born following frozen embryo transfer (FET) (Trounson and Mohr, 1983, Zeilmaker *et al.*, 1984) the technique has improved and success rates increased, and FET is today more widely used. A major contribution to this development is SET, leaving a number of supernumerary embryos available for freezing. Recently, the freeze-all technique has been introduced which reduces the risk of ovarian hyperstimulation syndrome (OHSS), a potentially life-threatening complication for the woman.

In Europe, the rate of FET has steadily been rising and in 2015, cryopreservation constituted 25.7% of all cycles with the highest rate in Switzerland being 45% (De Geyter *et al.*, 2020). In the United States the rate of FET have been doubled since 2015, being almost 70% of the non-donor ART in 2017 (www.cdc.gov/art). The latest Swedish report from 2017, reported FET in 6718 out of a total of 15,294 embryo transfers with autologous gametes, i.e. in 44% of all cycles (www.q-ivf.se).

Even though fresh embryo transfer is still the norm in many countries, FET including the freeze-all strategy has emerged as an alternative and previous studies have shown better perinatal outcome in terms of lower rates of PTB, LBW and small for gestational age (SGA) following FET in comparison to fresh transfer (Wennerholm

et al., 2013, Maheshwari *et al.*, 2018, Zhang *et al.*, 2018). It has been suggested that the supra-physiological environment in the uterus and the endometrium, caused by controlled ovarian hyperstimulation, is a possible reason for the poorer perinatal outcome in singletons born following fresh embryo transfer in comparison to FET and elevated estrogen levels have been shown to be associated with a higher risk of PTB, LBW and preeclampsia (Imudia *et al.*, 2012, Pereira *et al.*, 2017).

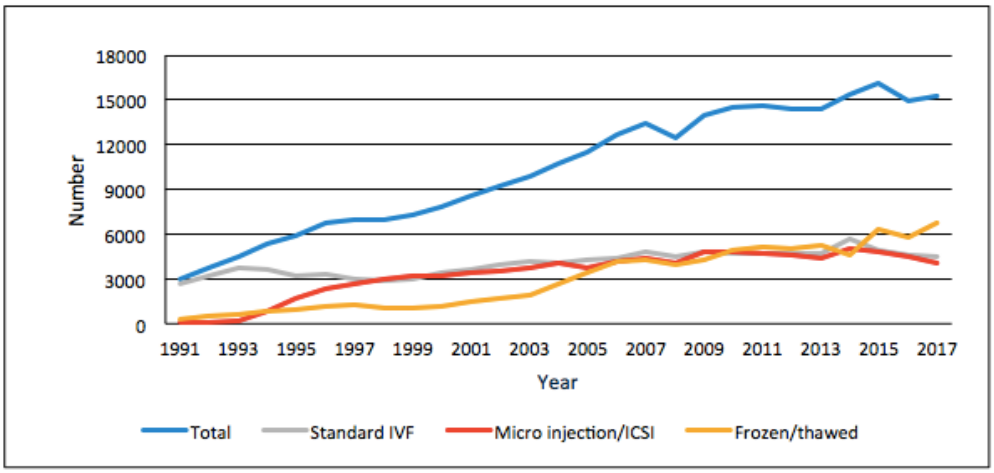

Figure 4. Distribution of embryo transfers following different techniques in ART in Sweden 1991-2017 (from Q-IVF, www.qivf.se)

Four large RCTs have investigated the differences in live birth rate following fresh and FET in freeze-all cycles. The first study investigated live birth rate in 1508 women with polycystic ovary syndrome (PCOS) randomized to either a fresh transfer or cryopreservation of all embryos with a subsequent frozen transfer. The results showed a higher live birth rate following frozen transfer with a relative risk (RR) of 1.17 (95% CI 1.05-1.31) (Chen and Legro, 2016). Later studies by Shi *et al.* and Vuong *et al.* investigated live birth rate in 2157 and 782 ovulatory women, respectively, randomized to a fresh or frozen transfer. These studies showed no differences with a RR of 0.97 (95% CI 0.89-1.06) and 1.07 (95% CI 0.88-1.31), respectively (Shi *et al.*, 2018, Vuong *et al.*, 2018). In a systematic review and meta-analysis, published last year, it was concluded that there is currently no overall support for a higher live birth rate following FET in comparison to fresh transfer in the overall IVF/ICSI population. However, in a subgroup analysis, a higher live birth rate was seen in hyper-responders, but not in normo-responders (Roque *et al.*, 2019).

The latest RCT on live birth rate, published in Lancet 2019, showed a significantly higher live birth rate following frozen transfer in comparison to fresh transfer (RR 1.26, 95% CI 1.14-1.41) in 825 ovulatory women (Wei *et al.*, 2019). Moreover, in an RCT, there was no difference in ongoing pregnancy rate in 460 normo-ovulatory women following the first single blastocyst transfer in a freeze-all cycle compared to a fresh embryo transfer (Stormlund *et al.*, 2019). The reasons for the differences in live birth rate between the studies are not completely known. However, the included women differ in regards of ovulatory function. Further, the study by Wei *et al.* included only blastocysts while the others included only cleavage stage embryos. Additionally, a maximum of two embryos were transferred in the first three studies, while in the study by Wei *et al.* only SET was conducted.

Different cycle regimens used in FET

FET can be performed in either a natural, a stimulated or a programmed cycle. In a natural cycle the woman's endogenous menstrual cycle is used without exposure to exogenous hormones leading to a natural folliculogenesis and ovulation. The stimulated cycle in FET differs from the one in fresh cycles where the stimulation is aimed at producing large number of oocytes. In FET, the stimulation is rather aimed at ovulation induction and subsequently preparing the lining of the uterine cavity for embryo implantation. The primary approach in Sweden for stimulated FET is aromatase inhibitors while gonadotropins are only given in exceptional cases. An alternative, the programmed cycle, means preparing the endometrium for implantation by giving estrogen and progesterone and sometimes adding a GnRH agonist/antagonist to suppress natural ovulation. A Cochrane review, including 18 RCTs, comparing different cycle regimens in FET in a total of 3815 women did not find evidence for supporting one treatment modality in preference for another when investigating live birth rate, however, with low quality of evidence (Ghobara *et al.*, 2017). In the review, natural cycles including modified natural cycles were compared to programmed cycles with and without GnRH suppression but also subtypes of ovulation induction with hMG alone and clomiphene+hMG were compared.

Recently, an interest has raised concerning the role of corpus luteum (CL) in frozen cycles. Four studies have evaluated the risks for altered vascular adaptation associated with pregnancies following FET according to the presence or absence of CL. CL is known to produce estrogen and progesterone, but also relaxin, a hormone that can regulate the maternal cardiovascular and renal systems and hence mediates the hemodynamic changes occurring during pregnancy. A study on 184 women revealed undetectable serum relaxin concentrations in women where no CL was

present (von Versen-Hoynck *et al.*, 2019). Furthermore, two smaller studies showed that the drop in mean arterial pressure was lacking in women with 0 or >3 CL and that the perturbations in blood pressure remained in the third trimester (von Versen-Hoynck *et al.*, 2019, von Versen-Hoynck *et al.*, 2020). Finally, in a prospective cohort study on almost 700 women, programmed cycles in FET with no CL were associated with an almost 3-folded risk of preeclampsia compared to modified natural cycles with one CL (von Versen-Hoynck *et al.*, 2019).

In programmed cycles the ovaries are not stimulated, no CL is present and hence luteal phase support is given in early pregnancy. In addition to these cycles, modified natural cycles can be performed by using hCG as ovulation trigger for controlling the timing of ovulation in an otherwise natural cycle. In natural cycles including the modified natural cycle, the cycle is associated with physiological estradiol levels in the presence of one CL and in stimulated FET at least one CL is present. In a programmed cycle the pituitary-ovarian hormonal axis is suppressed by exogenous estradiol leading to the absence of a CL.

Different freezing techniques

Various protocols for freezing have been introduced, differing particularly in type and concentration of cryoprotectant, cooling rates and type of device used. Today, two freezing techniques are available in the IVF laboratory. In the older one, used for more than 20 years and called slow-freezing, the embryo is frozen slowly at a decrease of approximately 1° Celsius (C) per minute and then stored in liquid nitrogen at a temperature of -196°C. The idea of the technique is to permit cellular dehydration while minimizing intracellular ice formation (Lassalle *et al.*, 1985, Testart *et al.*, 1986). Over the last decade, there has been a shift from conventional slow-freezing towards vitrification of human embryos, a cryopreservation method which turns the embryo into a glass-like state without formation of ice (Mukaida *et al.*, 1998, Kuwayama *et al.*, 2005). Vitrification is considered as an ultra-rapid freezing method, 600 times faster than slow-freezing, and minimizing the time the embryo is exposed to the most harmful temperatures between +15°C and -5°C.

Compared to slow-freezing, vitrification has resulted in improved embryo survival rates and improved clinical pregnancy/live birth rates (Balaban *et al.*, 2008, Fasano *et al.*, 2014, Levron *et al.*, 2014, Li *et al.*, 2014, Debrock *et al.*, 2015, Rienzi *et al.*, 2017). Today, the majority of embryos transferred in frozen cycles are vitrified blastocysts since the combination of blastocysts and vitrification have turned out to be more successful than blastocysts and slow-freezing (Stehlik *et al.*, 2005). If

freezing is performed on cleavage stage embryos, slow-freezing is usually performed.

Preimplantation genetic testing

More than 10,000 children have been born following preimplantation genetic testing (PGT) for monogenic disorders (PGT-M), structural rearrangements (PGT-SR) and aneuploidy (PGT-A) (De Rycke *et al.*, 2017) since the birth of the first PGT child in 1990 (Handyside *et al.*, 1990). The technique has recently been re-named. Previously the terms preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) were used. Nowadays, PGD has been re-named into PGT-M and PGT-SR and PGS re-named into PGT-A, the name now also containing the indication for the procedure. The terms PGD and PGS have been used for over 25 years and are also the terms that are referred to in the Swedish law.

Couples carrying or suffering from monogenic diseases, inherited chromosomal aberrations or X-linked disorders can be offered PGT-M/PGT-SR to limit the risk of the child inheriting the disorder. Regarding X-linked disorders the defect gene is located on the X-chromosome and the X-linked disorders are thus categorized under PGT-M. Couples undergoing PGT- M and PGT-SR are usually not suffering from sub fertility. PGT-A, the parallel technique, is used to screen for aneuploid embryos in presumed genetically and chromosomally normal couples. In women of advanced age, the higher rate of implantation failure and recurrent pregnancy loss is thought to be due to a higher proportion of aneuploid embryos. The technique aims at excluding embryos with aneuploidy, hence increasing the chance of a live birth. Today, there is, however, conflicting results whether PGT-A improves pregnancy and live birth rates or not. The first large RCT on 408 women randomized to IVF with or without PGT-A showed a reduction in live birth rate following IVF with PGT-A in women 35-41 years of age (Mastenbroek *et al.*, 2007). In a meta-analysis, including nine RCTs on IVF with PGT-A and IVF without PGT-A in mainly cleavage stage embryos, no difference in live birth rate could be found (Mastenbroek *et al.*, 2011). In a later RCT on 396 women, PGT-A did not affect live birth rate in comparison to ICSI without PGT-A (RR 1.07, 95% CI 0.75-1.51) (Verpoest *et al.*, 2018). The latest, and largest, RCT on almost 700 women did not observe any differences in ongoing pregnancy rate at 20 weeks of gestation in women randomized to a PGT-A cycle versus a non-PGT-A cycle in blastocysts (p=0.32) (Munné *et al.*, 2019). However, several observational studies, summarized in a systematic review (Lee *et al.*, 2015)

show improved implantation and pregnancy rates. Owing to the lack of evidence, PGT-A is not allowed in Sweden in clinical practice.

To enable PGT, fertilization is performed either with IVF or ICSI. Usually ICSI is used for fertilization, avoiding contamination of the oocyte's zona pellucida with extraneous sperms leading to possibly false results. In the latest European report on PGT, ICSI was used in 91% of cycles (De Rycke *et al.*, 2017). Following fertilization, one or two, or up to ten, cells are removed from the embryo at day 3 or day 5-6 and analyzed for the specific disorder using fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR)-based techniques, array-comparative genome hybridization (CGH) or next generation sequencing (NGS). PCR is most commonly used for monogenic disorders while FISH, array-CGH and NGS are used in the majority of cases with chromosomal aberrations and sex-linked disorders.

Every human, animal, plant, bacteria or virus contains genetic material such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequences that are unique to their species and to the individual member of that species. PCR is a method used to amplify, i.e. make many identical copies, of these unique sequences, sequences that can then be analyzed and compared to sequences in a person not carrying the specific disease, enabling transfer of an unaffected embryo.

FISH is applied to detect genetic abnormalities including the presence of an abnormal number of chromosomes in a cell or loss of a chromosomal region or a whole chromosome. A fluorescent copy (probe) of the DNA sequence of interest is produced. Following denaturation of the DNA in both the probe and the target, by either high temperature or chemicals, the probe and target sequences are then mixed together. The probe specifically hybridizes to its complementary sequence on the chromosome (the abnormal part) and it will be possible to detect the site of hybridization directly and hence sort out the affected embryo/embryos.

In array-CGH even small deletions or duplications in the genome can be detected. However, CGH is only able to detect unbalanced chromosomal abnormalities. Balanced chromosomal abnormalities, such as reciprocal translocations and inversions, do not affect copy number which is what is detected by CGH. NGS is a relatively novel method for DNA sequencing and all NGS platforms are able to sequence millions of small fragments of DNA in parallel. NGS can be used to sequence entire genomes or a small number of individual genes. NGS is, in parallel to CGH, used for detection of deletions and duplications. In accordance to array-

CGH, NGS is not able to separate normal chromosomes from balanced abnormalities.

Following the genetic analysis, an unaffected embryo, or in some cases two, is transferred to the uterine cavity. The rate of transferable embryos varies with indication for PGT with the lowest rate, 19%, in reciprocal translocations (De Rycke *et al.*, 2017). PGT-M and PGT-SR are today considered as a good alternative to invasive prenatal tests, including chorionic villus sampling and amniocentesis, performed during first and early second trimester and more psychologically acceptable for many couples than termination of pregnancy. PGT-M and PGT-SR offers the couples an alternative to donation treatment or adoption. However, the disadvantage of PGT is the need of IVF/ICSI, a treatment with associated risks but also costs. In addition, a delivery/live birth rate per aspiration around 20% is rather low taken into account that these couples are not suffering from sub fertility. In general, the risk for a misdiagnosis, i.e. transferring an affected embryo, is limited. According to the annual report from the European Society of Human Reproduction and Embryology (ESHRE) PGT Consortium, no misdiagnoses for the latest dataset on 2066 live born were reported and a decline in the rate of misdiagnosis has been seen over the years (De Rycke *et al.*, 2017). Whether the decline is a true decline following better laboratory quality or an effect of unwillingness to report misdiagnosis is unknown. In the early days of PGT in Sweden, a confirmative amniocentesis was recommended. Considering the accuracy of the new techniques used for genetic analysis (Harton *et al.*, 2011), no such confirmation is recommended any longer, except for in exceptional cases. To date, no misdiagnosis have been reported in Sweden (personal communication, Charles Hanson and Elisabeth Syk Lundberg, PGT offering clinics in Sweden).

The first child following PGT in Sweden was born in 1997 in Gothenburg and today more than 300 children have been born in Sweden following the technique. PGT-M and PGT-SR are currently performed at two centers, at university hospitals in Gothenburg and Stockholm. For the first couple of years following 1994, PGT was only allowed in cases with "severe genetic diseases leading to death in early childhood". Today PGT is regulated in the Genetic Integrity Act (2006:351) and allowed for monogenic diseases, inherited chromosomal aberrations and X-linked and gender-dependent disorders.

Delivery rates per oocyte aspiration are comparable for PGT and IVF/ICSI pregnancies, being around 20% (De Rycke *et al.*, 2017, De Geyter *et al.*, 2020).

1.5 Surveillance

Several national and international surveillance systems regarding ART have been established.

Following the birth of the first IVF child in Sweden, data on ART treatments has been collected. During 1982-2006 aggregated data were collected by the National Board of Health and Welfare annually. In addition, at four occasions during these years, data for all ART cycles resulting in a delivery, were collected for research purpose. These data include full maternal identity and are stored at the Medical Birth Registry (MBR), thus named MBR-IVF in this thesis. In 2007, the Swedish National Quality Register of Assisted Reproduction (Q-IVF) was founded (www.q-ivf.se). The Q-IVF collects information from all IVF clinics with full parental identification and enables follow-up on treatment efficacy and safety for the children as well as the mothers. The register is primarily a base for developmental and quality work but can also be used for research. The coverage rate is close to 100%. Q-IVF is presented more thoroughly in the Methods section.

ESHRE was founded in 1985 by Sir Robert Edwards, the Nobel Prize winner in 2010 for the development of IVF. ESHRE has the main aim to promote interest in, and understanding of human reproduction and embryology (ESHRE). ESHRE also promotes research in the field and enhance safety and quality in ART. The European IVF Monitoring (EIM) Consortium, introduced in 1999, collects data from the national IVF registries (EIM). The IVF Consortium was established to collect national data for Europe including complications such as OHSS and to a smaller extent also follow-up children's well-being and moreover, report on the availability and the structure of services in the different countries. Data are published in annual reports and covers approximately 84% of European countries active in ART. The latest report published in 2020 contains data for 2015 (De Geyter *et al.*, 2020). Since 1999, the PGT Consortium of ESHRE collects data on the accuracy, reliability, effectiveness and safety of PGT and the 14th report was published in 2017 (De Rycke *et al.*, 2017). In addition, the Consortium establishes guidelines.

The International Committee Monitoring Assisted Reproductive Technologies (ICMART) is an independent, international organization covering 63-70% of the world’s ART activity with data on almost 2 million ART cycles and 350,000 deliveries in 2015. However, many countries are still missing in the numbers, especially in the Middle East and Asia. ICMART provides data on availability,

effectiveness and safety regarding ART. The first ICMART World Report was published in 1989 and the latest annual report in 2018 cover data for 2011 (Adamson *et al.*, 2018).

Centers for Disease Control and Prevention (CDC) is a public health institute in the United States responsible for control and prevention of diseases, including infertility, with annual reports published on efficacy and safety in ART (www.cdc.gov/art). The Society for Assisted Reproductive Technology (SART), founded in 1985, includes >90% of all ART clinics in the United States and reports on birth outcome data from all member clinics in annual reports and also sets national guidelines for best practice in ART. The data collected by SART is also included in the annual reports from the CDC.

In summary, the main aim for the national and international registries is to follow treatment success as well as medical risks for the children conceived through ART and the mothers undergoing ART. However, individual linkage is usually not possible. In the Nordic countries all citizens are given a unique personal identification number (PIN) either at birth or at immigration. The PIN is used by authorities, health care, schools and universities (both public and private). PIN is the unique identifier and the key variable when linking health- and quality registers and enables large scale register-based studies involving several registers.

1.6 Neonatal outcome after ART

Besides efficacy data, i.e. pregnancy- and live birth rates, it is of utmost importance to study the safety of the children and their mothers in connection to the different procedures introduced in ART.

IVF/ICSI versus SC

Neonatal outcomes following IVF/ICSI have been extensively analyzed. The reason for the increased risk of adverse neonatal outcome in ART has mainly been associated with the higher rates of multiple births in ART as discussed in chapter 1.3. However, several meta-analyses and large cohort studies consistently show compromised outcomes also for singleton pregnancies following ART in comparison to SC, even after adjustment for relevant confounders. The latest meta-analysis, including 52 cohort studies with a total of 180,000 IVF/ICSI singletons and 4.6

million SC singletons born worldwide, showed increased risks for particularly very PTB (VPTB) and very LBW (VLBW) (Qin *et al.*, 2017). Several previous meta-analysis (Helmerhorst *et al.*, 2004, Jackson *et al.*, 2004, McDonald *et al.*, 2009, Pandey *et al.*, 2012) are consistent with the study by Qin *et al*. The AORs or RRs for these meta-analyses are summarized in Table 2.

Regarding birth defects, several studies including systematic reviews and meta-analyses indicate a 30-40% higher risk for birth defects following ART as opposed to SC, in the study by Pandey *et al*. as much as a 70% increase (Pandey *et al.*, 2012, Hansen *et al.*, 2013, Qin *et al.*, 2017, Zhao *et al.*, 2020). A summary on these meta-analyses is presented in Table 3.

A large Nordic cohort study including 61,281 ART singletons and 350,811 spontaneous singletons reported major birth defects in 3.4% of ART singletons vs. 2.9% in SC singletons with an AOR 1.14 (1.08-1.20). For specific organ systems, significantly increased risks were found in the nervous system, the eye, ear, face and neck, the heart, the gastrointestinal and urinary systems as well as in the musculo-skeletal system (Henningsen *et al.*, 2018). For cardiac defects, an increased risk following IVF/ICSI has also been summarized in a systematic review and meta-analysis (Giorgione *et al.*, 2018). In an earlier Swedish study (n=15,570), the risks of certain specific birth defects, such as neural tube and cardiovascular defects and limb reduction, were also significantly increased in adjusted analyses (Kallen *et al.*, 2010).

Table 2. A summary on adverse perinatal outcome in singletons. Systematic reviews and meta-analyses

Author **Year of publication**	**Helmerhorst** **2004**	**Jackson** **2004**	**McDonald** **2009**	**Pandey** **2012**	**Qin** **2017**
ART vs SC Adjusted for at least maternal age	RR, 95% CI	OR, 95% CI	RR, 95% CI	RR, 95% CI	ART vs SC, %
No of studies No of ART singletons	12 5361	14 12,114	27 14,748	22 27,819	52 181,741
PTB <37 weeks	2.0 (1.8-2.3)	2.0 (1.7-2.2)	1.8 (1.5-2.2)	1.5 (1.5-1.6)	10.9 vs 6.4
VPTB <32 weeks	3.3 (2.0-5.3)	3.1 (2.0-4.8)	2.3 (1.7-3.0)	1.7 (1.5-1.9)	2.4 vs 1.2
LBW <2500 grams	1.7 (1.5-1.9)	1.8 (1.4-2.2)	1.6 (1.3-2.0)	1.6 (1.6-1.8)	8.7 vs 5.8
VLBW <1500 grams	3.0 (2.1-4.4)	2.7 (2.3-3.1)	2.6 (1.8-3.8)	1.9 (1.7-2.2)	2.0 vs 1.0
SGA	1.4 (1.2-2.7)	1.6 (1.3-2.0)	1.4 (1.0-2.0)	1.4 (1.3-1.5)	7.1 vs 5.7
Perinatal mortality	1.7 (1.1-2.6)	2.2 (1.6-3.0)	-	1.9 (1.5-2.4)	1.1 vs 0.6

ART assisted reproductive technology, ***SC*** spontaneous conception, ***RR*** relative risk, ***CI*** confidence interval, ***OR*** odds ratio, ***PTB*** preterm birth <37 weeks, ***VPTB*** very preterm birth <32 weeks, ***LBW*** low birth weight <2500 grams, ***VLBW*** very low birth weight <1500 grams, ***SGA*** small for gestational age

Considering the increased use of ICSI worldwide it is of interest to study whether this technique poses higher perinatal risks compared to IVF. In ICSI, the natural selection of sperm is by-passed. Moreover, the sperm injection could potentially harm the oocyte and contamination of the oocyte´s cytoplasm with culture media might occur following the sperm insertion. These mechanisms have led to a concern regarding the perinatal outcomes. Reassuringly, studies have shown comparable or even slightly better, perinatal outcomes following ICSI compared to IVF. In a systematic review and meta-analysis including five studies for PTB, a significantly lower risk for PTB following ICSI in comparison to IVF in singleton pregnancies was found (AOR 0.80, 95% CI 0.69-0.93) (Pinborg *et al.*, 2013). Regarding birth defects, most studies found no differences between IVF and ICSI children (Wen *et al.*, 2012, Zhu *et al.*, 2019). However, in one meta-analysis, a higher risk of genitourinary malformations following ICSI was found. Yet, when including only high qualitative studies the difference disappeared (Massaro *et al.*, 2015).

Table 3. A summary on birth defects in singletons.
Systematic reviews and meta-analyses.

Author **Year of publication**	**Pandey** **2012**	**Hansen** **2013**	**Qin** **2017**	**Zhao** **2020**
ART vs SC	RR, 95% CI	RR, 95% CI	ART vs SC, %	RR, 95% CI
No of studies No of ART singletons	7 4382	23 48,944	29 77,630	46 112,913
Birth defects	1.7 (1.3-2.1)	**Any** 1.4 (1.3-1.4) **Major** 1.4 (1.3-1.5)	5.7 vs 3.9	1.4 (1.3-1.5)

ART assisted reproductive technology, ***SC*** spontaneous conception,
RR relative risk, ***CI*** confidence interval

Frozen embryo transfer versus fresh embryo transfer

Singletons born after FET have better perinatal outcomes than singletons born following fresh embryo transfer regarding PTB, LBW and SGA (Wennerholm *et al.*, 2013, Maheshwari *et al.*, 2018, Zhang *et al.*, 2018). However, several studies have reported on a higher risk of being born as large for gestational age (LGA) and macrosomic (≥4500 grams) following FET both compared to fresh transfer and to SC (Wennerholm *et al.*, 2013, Pinborg *et al.*, 2014). In two meta-analyses (Berntsen and Pinborg, 2018, Maheshwari *et al.*, 2018) a 1.5-folded risk for LGA and an almost two-folded risk for macrosomia was seen when comparing FET to fresh transfer. In a large "freeze-all" RCT on PCOS women, an almost 1,5-folded risk was seen for both LGA and macrosomia following FET in comparison to fresh transfer (Zhang *et al.*, 2018). Moreover, FET seems to be associated with higher rates of post term birth (Wennerholm *et al.*, 2013). No differences in the incidence of birth defects have been demonstrated when comparing fresh and frozen transfer (Pelkonen *et al.*, 2014, Maheshwari *et al.*, 2018).

The current evidence is not indicating any major differences in perinatal outcome using different freezing techniques. A Finnish study comparing 276 children, both singletons and multiples, born from vitrified and slow-frozen day 2-3 embryos did not show any significant differences in rate of PTB (Kaartinen *et al.*, 2016). Neither did a study on 4721 singletons from vitrified blastocysts and 1965 singletons from slow-frozen blastocysts reveal any differences in perinatal outcome (Li *et al.*, 2014). The latest study, comparing 297 pairs of newborns from vitrified and slow-frozen

day 3 embryos, showed similar results (Gu *et al.*, 2019).

Blastocyst transfer versus cleavage stage transfer

Extended culturing to the blastocyst stage has been found to be associated with a slightly increased risk for PTB compared to cleavage stage transfer. Three meta-analyses have shown an increased risk for PTB and VPTB (<32 weeks) (Table 4) (Dar *et al.*, 2014, Martins *et al.*, 2016, Alviggi *et al.*, 2018). Studies also indicate that extended culture is associated with increased birth weight (Makinen *et al.*, 2013, Zhu *et al.*, 2014). A Swedish register-based study was the first larger study (n=1311) reporting on neonatal outcome following blastocyst transfer and showed an increased risk of birth defects compared to cleavage stage transfer (Kallen *et al.*, 2010). In that study, also including multiples, any birth defect was defined as all diagnoses with an International Classification of Diseases (ICD) code starting with Q. Any birth defect was present in 6.9% of infants born following blastocyst transfer and 5.1% of infants born following cleavage stage transfer (AOR 1.43, 95% CI 1.14-1.81). In a sub-group analysis, including only relatively severe birth defects with exclusion of some minor and common birth defects with little clinical relevance, the corresponding risks were 4.6% and 4.1%, respectively, and the significant difference persisted (AOR 1.33, 95% CI 1.01-1.75). A Canadian study on 3206 singletons from blastocyst transfer did not find any difference in the rates of birth defects between day 2 to 3 and day 5 to 6 transfers (Dar *et al.*, 2013). The drawbacks on that study are the collection of data, mainly via telephone calls or mail to the parents. Finally, no difference for birth defects was seen in two systematic reviews and meta-analyses including the same four studies with 8737 singletons from blastocyst transfer and 36,097 singletons from cleavage stage transfer (RR 0.97, 95% CI 0.85-1.12) (Martins *et al.*, 2016, Alviggi *et al.*, 2018).

Following extended culture, several studies have reported an altered male-female ratio in favor for male (Luna *et al.*, 2007, Chang *et al.*, 2009, Dean *et al.*, 2010, Maalouf *et al.*, 2014, Ding *et al.*, 2018, Hattori *et al.*, 2019) as well as a doubled risk for monozygotic twinning, from around 1% to 2% per pregnancy (Chang *et al.*, 2009, Kawachiya *et al.*, 2011, Ding *et al.*, 2018, Hviid *et al.*, 2018, Hattori *et al.*, 2019, Spangmose *et al.*, 2019).

Table 4. Preterm birth <37 weeks and very preterm birth <32 weeks following blastocyst transfer compared to cleavage stage transfer

Author **Year of publication**	**Dar** **2014**	**Martins** **2016**	**Alviggi** **2018**
Blastocyst vs cleavage stage transfer	AOR 95% CI	RR 95% CI	RR 95% CI
No of studies No of children	6 studies 75,516	12 studies 195,325	14 studies 193,827
PTB <37 weeks	1.3 (1.2-1.5)	1.1 (1.02-1.2)	1.2* (1.1-1.3)
VPTB <32 weeks	1.2 (0.9-1.5)	1.1 (1.04-1.2)	1.2* (1.02-1.3)

AOR adjusted odds ratio, ***CI*** confidence interval, ***RR*** relative risk,
PTB preterm birth <37 weeks, ***VPTB*** very preterm birth <32 weeks

*only fresh cycles

Preimplantation genetic testing versus IVF/ICSI

The number of studies that have investigated the neonatal and maternal outcomes following PGT in comparison to IVF/ICSI and SC is limited and most studies include small cohorts of children.

When comparing singletons born following PGT-M, PGT-SR and PGT-A to singletons born following IVF/ICSI, studies have shown similar or even slightly lower rates for PTB and LBW in PGT children (Table 5, including only studies on PGT-M and PGT-SR) (Liebaers *et al.*, 2010, Eldar-Geva *et al.*, 2014, Sunkara *et al.*, 2017, He *et al.*, 2019, Zhang *et al.*, 2019). Likewise, a meta-analysis on four studies with 375 singletons following PGT-M/PGT-SR and 24,844 singletons following ICSI did not find any significant differences of PTB (AOR 0.85, 95% CI 0.59-1.22) (Hasson *et al.*, 2017). As opposed to SC, a Danish study on 149 PGT-M/PGT-SR children, reported on a higher risk of PTB (AOR of 1.6, 95% CI 1.0-2.7), following adjustment for multiplicity (Bay *et al.*, 2016). Regarding birth defects, the results have also been reassuring, with similar risks in comparison to IVF/ICSI (Liebaers *et al.*, 2010, Desmyttere *et al.*, 2012, Bay *et al.*, 2016, Hasson *et al.*, 2017, He *et al.*, 2019, Zhang *et al.*, 2019). However, in the Danish study, inexplicably high rates of birth defects were seen (13.5% among PGT children and 8.4% among IVF/ICSI children), even though the study included multiples and the follow-up was up to one year of age (Bay *et al.*, 2016).

Table 5. Preterm birth <37 weeks and low birth weight <2500 g in singletons born following preimplantation genetic testing for monogenic disorders and structural rearrangements versus IVF/ICSI

Author **Year of publication**	**Eldar-Geva** **2014**	**Bay** **2016**	**Sunkara** **2017**	**Hasson** **2017**
PGT-M / PGT-SR vs IVF/ICSI	RR, 95% CI	RR, 95% CI	OR, 95% CI	RR, 95% CI
No of PGT children	242	149	439	51
PTB <37 weeks	0.6 (0.3-1.1)	1.01 (0.5-2.0)	0.7 (0.5-0.98)	1.3 (0.5-3.2)
LBW <2500 grams	0.4 (0.2-0.8)	1.00 (0.5-2.2)	0.6 (0.4-0.9)	1.02 (0.4-2.9)

PGT-M preimplantation genetic testing for monogenic disorders,
PGT-SR preimplantation genetic testing for structural rearrangements,
IVF in vitro fertilization, ***ICSI*** intracytoplasmic sperm injection,
RR relative risk, ***CI*** confidence interval, ***OR*** odds ratio,
PTB preterm birth <37 weeks, ***LBW*** low birth weight <2500 grams

Lumping together PGT-M, PGT-SR and PGT-A in studies might, however, cause a bias. In general, patients undergoing PGT-M and PGT-SR are not sub fertile while PGT-A aims at increasing pregnancy- and live birth rates in elderly women and women suffering from recurrent implantation failure and miscarriage by excluding aneuploid embryos. In a study, carried out by an Israeli research group, there was a lower risk of LBW in the PGT children when comparing 158 PGT-M and PGT-SR singletons to 158 IVF/ICSI singletons (Eldar-Geva *et al.*, 2014), a finding that was also shown in a later and larger study (Sunkara *et al.*, 2017). These finding might be due to the underlying involuntary childlessness among IVF/ICSI patients, while patients undergoing PGT-M and PGT-SR are not sub fertile. Subfertility *per se* also affect pregnancy outcome (Pinborg *et al.*, 2013, Luke *et al.*, 2016, Luke, 2017). At date, there are no indications that the embryo biopsy *per se* would have a negative impact on the neonatal outcome following PGT.

1.7 Maternal outcome after ART

Several studies have reported on maternal outcomes following ART. In general, placenta-mediated pregnancy complications, including preeclampsia, placental abruption and placenta previa seems to be more common in ART pregnancies than in spontaneous pregnancies.

Regarding hypertensive disorders in pregnancy (HDP), defined as either pregnancy induced hypertension or preeclampsia or a combination of both, a higher risk of hypertensive disorders in singleton ART pregnancies have been shown when compared to singleton pregnancies following SC with an AOR of 1.2-1.5 (Pandey *et al.*, 2012, Thomopoulos *et al.*, 2013, Opdahl *et al.*, 2015, Gui *et al.*, 2020). No differences for HDP have been seen when comparing IVF and ICSI pregnancies separately (Kallen *et al.*, 2005, Buckett *et al.*, 2007, Farhi *et al.*, 2013), neither has blastocyst transfer been shown to influence the rate of HDP (Fernando *et al.*, 2012, Ishihara *et al.*, 2014, Oron *et al.*, 2015). Unlike ICSI and blastocyst transfer, FET seems to be associated with an increased risk of HDP both in comparison to fresh transfer and SC (Sazonova *et al.*, 2012, Ishihara *et al.*, 2014, Opdahl *et al.*, 2015, Maheshwari *et al.*, 2018). However, prior history of hypertensive disease, an important confounder, have not been adjusted for in any of these studies except for the study by Farhi *et al.* (2013). The reasons for the higher incidence of HDP following FET has not been clear but recent studies from the United States noted higher rates of preeclampsia in pregnancies lacking a CL (von Versen-Hoynck *et al.*, 2019, von Versen-Hoynck *et al.*, 2020). Likewise, a large Japanese study on almost 8000 children following a natural cycle and 16,000 children following FET in programmed cycles, found a 40% increase in HDP following programmed cycles lacking a CL (Saito *et al.*, 2019). In addition to HDP, FET further seems to be associated with increased risk for postpartum hemorrhage (PPH) compared to fresh transfer and SC and possibly associated with a decreased risk of placenta previa in comparison to fresh transfer (Sazonova *et al.*, 2012, Sha *et al.*, 2018).

More than a 3-folded risk for placenta previa and an almost doubled risk for placental abruption could be found in two large meta-analyses when analyzing ART versus spontaneous pregnancies (Qin *et al.*, 2016, Vermey *et al.*, 2019). In the first of these meta-analysis also a 1,3-folded risk for PPH could be seen (Qin *et al.*, 2016). Moreover, when comparing ART pregnancies to non-ART pregnancies in sub fertile patients a 2,5-folded risk for placenta previa and an almost twofold risk of placental abruption was observed in ART pregnancies, suggesting that the ART procedure *per se* might have an effect on placentation (Vermey *et al.*, 2019).

A recently published Nordic register-based study found a doubled risk of placenta previa following fresh blastocyst transfer as compared to fresh cleavage stage transfer in singletons pregnancies (Spangmose *et al.*, 2019). Other studies on singleton pregnancies (Fernando *et al.*, 2012, Ishihara *et al.*, 2014, Oron *et al.*, 2015) have not shown any differences in the rate of placenta previa or placental abruption

when comparing blastocyst to cleavage stage transfer in fresh and/or frozen cycles, results that are in accordance with a systematic review and meta-analysis (Maheshwari *et al.*, 2013). Neither was any differences observed in the study by Fernando *et al.* (2012), including both fresh and frozen cycles, in regards of PPH. In all studies adjustment was either made for relevant confounders known to influence the obstetric outcome or the study was designed as a matched cohort study.

Studies on maternal outcome in PGT pregnancies are scarce. A retrospective cohort study (Hasson *et al.*, 2017) on 51 PGT-M/PGT-SR and 83 ICSI singleton pregnancies matched for maternal age and body mass index (BMI) prior to pregnancy, showed no differences regarding HDP or in a composite outcome of placenta related complications (Hasson *et al.*, 2017). An American study on 177 singleton pregnancies following PGT including PGT-A, found a 3-folded risk of preeclampsia and a 4.5-folded risk of placenta previa when comparing PGT to IVF/ICSI pregnancies, whereas the rate of pregnancy induced hypertension and PPH was similar following adjustment for several relevant confounders including history of hypertensive disease, parity and fresh, frozen natural or frozen programmed cycle (Zhang *et al.*, 2019). Additionally, when comparing 149 PGT-M/PGT-SR and SC pregnancies, a 9-folded increase in risk of placenta previa was found in comparison to SC whereas no difference was found in a sub analysis on PGT versus IVF/ICSI pregnancies. In that study, the absolute risk for placenta previa was 3.6% in PGT pregnancies, 1.6% in IVF/ICSI pregnancies and 0.3% in spontaneous pregnancies and adjustment was made for important confounders including multiplicity (Bay *et al.*, 2016).

1.8 Sibling studies

Both sub fertility and the ART technique including ovarian stimulation and embryo culture have been suggested to contribute to the adverse perinatal and maternal outcome. In the majority of studies a healthy background population with spontaneous pregnancies has been used as a reference group, making it impossible to distinguish between the "chicken or the egg", the sub fertility or the ART treatment (Berntsen *et al.*, 2019). A time to pregnancy exceeding one year as well as ovulation induction without IVF has been shown to increase the risk of PTB as opposed to SC (Pinborg *et al.*, 2013, Luke *et al.*, 2017). Some researchers have tried to overcome this problem by a study design named sibling studies comparing children born following IVF and SC in the same mother. The first sibling study was published in

Lancet in 2008. This Norwegian study on 2546 women with at least one ART singleton and one spontaneous singleton did not find any differences in the rate of PTB, SGA and perinatal mortality following adjustment for relevant confounders including time from previous birth, suggesting that the adverse outcome found in ART children could rather be attributable to the underlying infertility than to the ART technique (Romundstad *et al.*, 2008). On the contrary, a larger Danish study on almost 14,000 sibling pairs examined the perinatal outcomes showing a higher risk of PTB and LBW in siblings born following ART compared to SC. Moreover, the same study showed that children born following FET were heavier than their siblings born from fresh cycles thus suggesting that both patient characteristics as well as the ART technique contribute to the outcome (Henningsen *et al.*, 2011). Adjustments were made for known confounders such as maternal age, parity, year of birth and child's sex. In another study, the rate of placenta previa was assessed in consecutive pregnancies in 1349 women with one pregnancy following SC and another following assisted reproduction. Following adjustment for maternal age, parity, prior Cesarean section and time interval between pregnancies the AOR was 2.9 (95% CI 1.4-6.1) following ART in comparison to SC (Romundstad *et al.*, 2006). In the latest sibling study, the authors concluded that a decline in fertility status increases the risks for adverse perinatal outcome, irrespective of which ART treatment is used (Luke *et al.*, 2016). In conclusion, sibling studies indicate that the adverse outcome in ART pregnancies is related to both maternal characteristics and the IVF technique *per se.*

1.9 Trends over time

When comparing perinatal outcomes over time a significant improvement has been noticed. The trend toward SET leading to fewer multiple births has contributed to the improvement but also for singleton births the rate of PTB, LBW and SGA has declined considerably over the last decades as shown in a large Nordic population-based matched cohort study on 92,000 ART and half a million spontaneously conceived children. In the Nordic study, the rate of PTB and SGA declined by half in ART singletons during 1988-2007, a trend that was not seen in SC (Henningsen *et al.*, 2018). A single center study from the United Kingdom, showed a significant increase in birth weight in IVF singletons over a time period of 25 years, a trend that has not been seen in spontaneous pregnancies (Castillo *et al.*, 2019). To determine trends in the rate of birth defects, the Committee of Nordic ART and Safety (CoNARTaS) group investigated the risks of major birth defects between 1988 and 2007. The number of children diagnosed with a major birth defect increased over the

years in both ART and SC pregnancies, considered to be a consequence of better data quality, yet the relative risk of being born with a major birth defect following ART in comparison to SC did not change over the time period (Henningsen *et al.*, 2018).

When investigating maternal outcomes the rate of placenta previa has increased among ART pregnancies during the last 30 years, a trend that has not been observed for spontaneous pregnancies and results that remained significant when restricting analyses to nulliparous women. For HDP, no clear trend has been found whereas the rate of placental abruption has decreased in both IVF and SC pregnancies (Petersen *et al.*, 2020). This study from the CoNARTaS group included almost 7 million pregnancies of which almost 150,000 were conceived through ART.

1.10 Long-term health of children born following ART

While the neonatal outcome of ART is well documented, large studies on long-term follow-up are scarce. Since the intrauterine milieu has been suggested to influence long-term health, as described by the Barker hypothesis, follow-up studies on ART children are warranted (Barker *et al.*, 1993). Even though the number of ART children is growing rapidly, the vast majority are still young and drawing conclusions on life-time risks is thus hard at date. However, there are some studies published on health for children and adolescents following ART.

Cardiovascular risks

Several risk factors for the future development of cardiovascular diseases (CVD) have been described. Some of the risk factors for developing metabolic syndrome and CVD, e.g. PTB, LBW and SGA, are also associated with ART (Barker *et al.*, 1993, Helmerhorst *et al.*, 2004, Jackson *et al.*, 2004, McDonald *et al.*, 2009, Pandey *et al.*, 2012, Pinborg *et al.*, 2013, Qin *et al.*, 2017). Further, the risk of HDP is elevated following ART (Sazonova *et al.*, 2012, Thomopoulos *et al.*, 2013, Ishihara *et al.*, 2014, Opdahl *et al.*, 2015, Chen *et al.*, 2016, Maheshwari *et al.*, 2018), not least in frozen cycles, thus ART might also be a potential risk factor for the development of CVD in the offspring. A meta-analysis including 36 studies on 53,000 individuals showed that in utero exposure to preeclampsia was associated with a 5.17 mm Hg higher mean systolic and 4.06 mm Hg higher mean diastolic blood pressure as well as a slightly higher (0.36 kg/m^2) mean BMI in children and young adults (Andraweera and Lassi, 2019). Both elevated blood pressure and changes in body composition have been found after ART in a few studies (Ceelen *et*

al., 2009, Scherrer *et al.*, 2015, Meister *et al.*, 2018) and summarized in systematic reviews and meta-analyses (Hart and Norman, 2013, Guo *et al.*, 2017). In the study by Meister, vascular function was reassessed in 54 ART children and 43 age- and sex-matched controls born following SC five years following the initial assessment. The mean age of the adolescents examined were 17 years in both groups. The reassessment revealed persisting premature vascular aging in the ART children and increased blood pressure (Meister *et al.*, 2018).

Type 1 diabetes

ART has been shown to alter the glucose metabolism in mice and in children (Ceelen *et al.*, 2009, Chen *et al.*, 2014, Vrooman and Bartolomei, 2017). Two Danish studies comparing children born to sub fertile parents or parents undergoing infertility treatment found no association between fertility status and/or fertility treatment and type 1 diabetes (Hargreave *et al.*, 2016, Kettner *et al.*, 2016). A newly published large Swedish cohort study on 48,000 ART singletons and 3 million spontaneously conceived singletons investigated the development of type 1 diabetes. In the unadjusted analysis there was an increased risk for type 1 diabetes following ART, yet when adjusting for confounders including parental diabetes, no significant difference was found in the main analysis. In sub-analyses on singletons after frozen versus fresh transfer and singletons after frozen transfer versus SC, significantly higher risks of type 1 diabetes were observed following FET (hazard ratio [HR] 1.52, 95% CI 1.08-2.14 and HR 1.41, 95% CI 1.05-1.89, respectively) whereas ICSI versus IVF in fresh cycles did not seem to affect the risk of developing type 1 diabetes (Norrman *et al.*, 2020). The reason for the higher risk for developing diabetes following FET might be due to larger babies, also shown to be a risk factor for diabetes later in life (Haynes *et al.*, 2007, Ievins *et al.*, 2007).

Cancer

In a large cohort study including just above 100,000 children born in Britain after ART and with a mean follow-up time of seven years, no increase in the overall risk of cancer was found (Williams *et al.*, 2013). Accordingly, in an almost equally large Nordic study with a mean follow-up of ten years, similar risks for overall cancer was observed (Sundh *et al.*, 2014). A systematic review and meta-analysis including 16 cohort and 13 case-control studies, showed an increased risk for overall cancer (RR 1.16, 95% CI 1.01-1.32), hematological malignancies (RR 1.39, 95% CI 1.21-1.60) and other solid tumors (RR 1.57, 95% CI 1.14-2.16) when comparing ART offspring to non-ART offspring (Wang *et al.*, 2019). In a later large retrospective study from the United States, including almost 300,000 ART children and above 2 million non-

ART controls, there was no difference for overall cancer risk in the first decade of life, yet an association between ART and embryonal tumors, in particular hepatic tumors (HR 2.46, 95% CI 1.29-4.70) (Spector *et al.*, 2019). A recent Danish cohort study, including 37,000 ART children born during 1996 to 2012, showed no association between any type of childhood cancer and any ART treatment or use of any type of fertility drugs in comparison to children born from fertile women. However, based on 14 cases, an increased risk of childhood cancer following FET was reported (incidence rate difference 26.9 [95% CI 1.28-51.0] per 100,000), mainly due to the elevated risk for leukemia and sympathetic nervous tumors (Hargreave *et al.*, 2019).

Altogether, studies show some divergent results. Differences have mainly been found in subgroups of patients and for specific types of malignancies. Regarding FET and cancer risk, some studies have shown an association between high birth weight, more common following FET, and an increased risk of especially leukemia but possibly also Hodgkin's lymphoma (Roman *et al.*, 2013, Crump *et al.*, 2015, Petridou *et al.*, 2015, Triebwasser *et al.*, 2016, Groves *et al.*, 2018). However, further studies are warranted to elucidate this association.

Cerebral palsy

For cerebral palsy, a Swedish study on 5680 singletons, not adjusting for gestational age, found a 2.8-folded risk following IVF (Stromberg *et al.*, 2002) whereas a larger Danish study on 33,139 IVF singletons and multiples reported similar risks following adjustment for multiplicity and gestational age (Hvidtjorn *et al.*, 2011). Both studies presented an incidence of approximately 2 per 1000 for singletons. In a smaller study, a doubled risk for cerebral palsy was seen following fresh transfer in comparison to non-ART singletons, yet no adjustment was made for gestational age (Pinborg *et al.*, 2010). The fact that children born following a fresh transfer are more prone to PTB as well as the results in the study by Hvidtjorn *et al.* could suggest that the increased risk for cerebral palsy is driven by the higher incidence of PTB rather than the treatment or the infertility *per se* (Hvidtjorn *et al.*, 2011).

Neurodevelopment, cognitive health and school performances

Considering the elevated risks for PTB and LBW following ART also neurodevelopment and cognitive health is of great interest.

A Swedish cohort study found a 4-folded increase for developmental delay following IVF in comparison to SC but when restricting the analyses to singletons, the

significance disappeared (Stromberg *et al.*, 2002). Other studies have also shown comparable results for neurodevelopment in ART and SC children. A Danish study, adjusting for multiplicity, showed no differences in mental retardation (Bay *et al.*, 2013) and neither was any differences seen in a Swedish study when restricting the analysis to singletons (Sandin *et al.*, 2013). In a European collaboration study, including a substantial number of singletons from five European countries, no differences regarding cognitive and motor development were found when comparing 424 IVF, 511 ICSI and 488 SC children (Ponjaert-Kristoffersen *et al.*, 2005). Finally, a systematic review and meta-analysis summarized studies on cognitive development following different ART techniques concluding that, at date, there is some evidence to suggest that ICSI might influence cognitive health yet, most studies on the subject suffer from methodological limitations (Rumbold *et al.*, 2017). Among high-quality studies, there was no difference in cognitive outcome among children born following conventional IVF and those conceived spontaneously. Findings among high-quality studies of children conceived with ICSI were, however, inconsistent in comparison to IVF and ranged from no differences observed in intelligence quotient (IQ) scores to a 50% higher risk of severe intellectual disability.

Further, four large Nordic population-based studies on school performances in children at age 15-16 years have been published in the last couple of years. Giving the fact that all children, with a few exceptions, attend school in the Nordic countries, school performances is a good way of assessing cognitive development. In a Swedish study, on 8323 ART children and almost 1.5 million spontaneously conceived children, higher total mean scores were seen in ART singletons compared to SC singletons in the crude analysis. When adjusting for relevant confounders, the total mean score was, however, significantly lower in the ART singletons but not considered to be clinically relevant (Norrman *et al.*, 2018). A Danish study showed similar results (Spangmose *et al.*, 2017). The third study, performed by the same Danish study group, showing no significant differences in performance, compared children born following FET (n=423) and fresh transfer (n=6072) (Spangmose *et al.*, 2019). The latest Swedish study compared ICSI singletons (n=6953) to IVF singletons (n=11,713) and spontaneously conceived singletons (n=2,022,995). Similar school results were seen for IVF and ICSI children. Compared to children born after SC, small differences were observed in the 3rd grade in favor of SC children whereas no differences between ICSI and SC children were observed in the 9th grade (Norrman *et al.*, 2020). Overall the differences are small and probably not of clinical relevance.

Autism spectrum disorders and attention deficit hyperactivity disorder

Concerns have been raised whether there is an association between ICSI and autism spectrum disorders (ASD). The findings are, however, still inconclusive. A study on ART children in California in 1997-2006 with a five year observational time found an association between ICSI singletons in fresh cycles and ASD (Kissin *et al.*, 2015). In this study it was also shown that among the children with ASD, a greater percentage was of male sex, born in a multiple birth and more often also born preterm or with LBW. In contrast to the Californian study, a large Danish cohort study did not find any association between ICSI and ASD (Bay *et al.*, 2013). A meta-analysis published three years ago including around 8 million children in 11 studies, revealed an increased risk of ASD following any ART treatment (RR 1.35, 95% CI 1.09-1.68) but in a subgroup analyses on singletons, no difference was seen (Liu *et al.*, 2017). Regarding attention deficit hyperactivity disorder (ADHD), a Swedish study suggested a slight increase following ART. Though, when adjusting for length of infertility or restricting the analysis to singletons only, the statistical significance was lost (Kallen *et al.*, 2011). Additionally, a greater risk for ADHD was reported in children born to women with fertility problems in comparison to children born to fertile women but without adjustment for multiplicity (HR 1.36, 95% CI 1.29-1.45) (Svahn *et al.*, 2015).

In summary, both regarding ASD and ADHD, diseases involving both genetic and environmental factors, there might be a slight increase following ART treatment. Noteworthy, in the majority of studies the risk differences disappeared when restricting the analyses to singletons.

2 AIMS OF THE THESIS

General aim

The aim of this thesis is to evaluate the risks for the children born through ART and the mothers undergoing ART with advanced techniques.

Specific aims

- To analyze the neonatal and maternal outcome after blastocyst transfer (day 5-6) compared to cleavage stage transfer (day 2-3) and SC.
- To compare the obstetric outcome after FET depending on protocol used. Additionally, we compare FET to fresh transfer and SC.
- To investigate neonatal and maternal outcome following transfer of vitrified blastocysts in comparison to slow-frozen cleavage stage embryos and fresh blastocysts.
- To study neonatal, maternal and early childhood outcomes following PGT in Sweden compared to children born after IVF/ICSI and SC.

3 PATIENTS AND METHODS

All four papers included in this thesis were carried out as retrospective population-based register studies. The majority of Swedish health care is publicly financed and for a lot of diseases and conditions the quality of the care is followed through nationwide health- and quality registers. In Sweden, two types of national registers including information on health outcome are used. The National Board of Health and Welfare manage the health registers whereas National Quality Registers are managed by the Swedish Association of Local Authorities and Regions (SKR). For the health registers run by the National Board of Health and Welfare, regulated by the Health Data Law, reporting is compulsory for all care providers and the patients have no possibility to ask for their data to be withdrawn. Regarding the National Quality Registers, regulated by the Patient Data Law, participation is voluntary. Registers run by the National Board of Health and Welfare used in this thesis are: the MBR-IVF, the MBR, the National Patient Register (NPR), the Register of Birth Defects, the Prescribed Drug Register and the Cause of Death Register. Of the nearly 100 Quality Registers in Sweden, two were used in this thesis: the Q-IVF and the Swedish Neonatal Quality Register (SNQ). Due to the PIN given to every legal citizen in Sweden, but also other Nordic countries, it is possible to cross-link these registers thus enabling epidemiological research (Ludvigsson *et al.*, 2009). For all Nordic countries, the PIN is given either at birth or at immigration.

An overview of the study cohorts and registers used in the different papers is presented in Table 6.

3.1 Settings and study design

Table 6. Study settings and study populations

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Setting	Population-based register study			
Study design	Retrospective register study			
Countries	Sweden	Sweden	Sweden and Denmark	Sweden
Study period	2002-2013	2005-2015	2002-2015 for Sweden 2009-2014 for Denmark	1996-2017
Study group	All singletons born following blastocyst transfer (n=4813)	All singletons born following natural (n=6297), stimulated (n=1983) and programmed (n=1446) cycles in FET	All singletons born following transfer of vitrified blastocysts (n=3650)	Singletons born following PGT (n=267)
Control groups	All singletons born following cleavage stage transfer (n=25,747) All singletons born following SC (n=1,196,394)	All singletons born following fresh embryo transfer (n=24,365) All singletons born following SC (n=1,127,566)	All singletons born following transfer of slow-frozen cleavage stage transfer (n=8123) All singletons born following transfer of fresh blastocysts (n=4469)	All singletons born following IVF/ICSI (n=55,355) Singletons born following SC, (n=26,535). One hundred matched controls per PGT child. Matched for year of birth of child, maternal age (+/- 5 years), parity and delivery hospital. In five cases we did not find 100 controls per PGT child, these PGT children had 41, 64, 66, 68 and 96 controls each.
Registers	Q-IVF, MBR-IVF, MBR, NPR, Register of Birth Defects	Q-IVF, MBR-IVF, MBR, NPR, Register of Birth Defects, SNQ, Prescribed Drug Register	CoNARTaS cohort based on the Swedish and Danish ART-registers, MBRs and NPRs	Q-IVF, MBR-IVF, MBR, NPR, Register of Birth Defects, Cause of Death Register
Primary outcome	Major birth defects	PTB, LBW, HDP, PPH	PTB, LBW, macrosomia, HDP, placenta previa	PTB, LBW
Secondary outcomes	Male gender, PTB, VPTB, EPTB, LBW, VLBW, macrosomia, SGA, LGA, perinatal mortality, placenta previa, placental abruption, preeclampsia, PPH	VPTB, EPTB, post term birth (≥42 weeks), VLBW, macrosomia, SGA, LGA, perinatal/neonatal mortality, major birth defects, placenta previa, placental abruption	VPTB, post term birth (≥42 weeks), VLBW, SGA, LGA, perinatal/neonatal mortality, any birth defects, placenta previa, placental abruption	VPTB, post term birth (≥42 weeks), VLBW, macrosomia, SGA, LGA, perinatal/neonatal/infant mortality, any and major birth defects at birth, HDP, placenta previa, placental abruption, PPH. Early childhood outcomes: asthma, allergic disorders, sepsis, hypothyroidism, ADHD, ASD, affective disorders, schizophrenia, mental retardation, cerebral palsy, epilepsy, mortality

FET frozen embryo transfer, ***PGT*** preimplantation genetic testing, ***IVF*** in vitro fertilization, ***ICSI*** intracytoplasmic sperm injection, ***SC*** spontaneous conception, ***Q-IVF*** National Quality Register for Assisted Reproduction, ***MBR*** Medical Birth Register, ***NPR*** National Patient Register, ***SNQ*** Swedish Neonatal Quality Register, ***CoNARTaS*** Committee of Nordic ART and Safety, ***ART*** Assisted Reproductive Technology, ***PTB*** preterm birth <37 weeks, ***VPTB*** very preterm birth <32 weeks, ***EPTB*** extreme preterm birth <28 weeks, ***LBW*** low birth weight <2500 grams, ***VLBW*** very low birth weight <1500 grams, ***HDP*** hypertensive disorders in pregnancy, ***SGA/LGA*** small/large for gestational age, ***PPH*** postpartum hemorrhage (>1000mL), ***ADHD*** attention deficit hyperactivity disorder, ***ASD*** autism spectrum disorders

3.2 Data sources

For register-based studies in Sweden, ethical permission from the Ethical Review Authority is required. Following ethical approval, an application is sent to the National Board of Health and Welfare providing a support service for all registers and researchers. This application shall contain information on which health - and quality registers should be included in the data set but also all variables the researchers request shall be listed. Following a disclosure, all requested registers are cross-linked by the National Board of Health and Welfare using the PIN and subsequently, the researcher get a pseudoanonymized data set with serial numbers instead of PIN. A key containing the PINs is stored at the National Health and Welfare for three years enabling updates in the data file in case of longitudinal studies.

In Sweden ICD 9 codes were used through 1996 and ICD 10 codes from the beginning of 1997.

The MBR-IVF and Q-IVF

Following the birth of the first ART child in Sweden in 1982 and until 2006, all ART cycles were annually reported to the National Board of Health and Welfare as aggregated data. Further, for research purpose, identified data for all cycles resulting in deliveries were collected by the National Board of Health and Welfare at four occasions during 1982 and 2006 and stored in a separate file named MBR-IVF in this thesis.

In 2007, the Q-IVF was established to collect nationwide, identified data on all ART treatments. All public (n=6) and private (n=13) IVF clinics in Sweden report individual data with full identification to the register and the results are public and published at the website (www.qivf.se). The ART registers include full identification of the mother, information on donated or autologous gametes, number of oocytes retrieved, fertilization method (IVF or ICSI), culture duration, treatment (fresh or frozen-thawed transfer), freezing method (vitrification or slow-freeze), date of embryo transfer, number of embryos transferred, number of gestational sacs at first ultrasound and date of delivery as well as number of children born. Since 2014 also complications to ART treatment are registered. In addition to medical results, the Q-IVF also collect data on patient satisfaction via questionnaires. Five variables are

assessed and the results published on the web page at clinic level. The patients are informed about the Q-IVF and may choose to opt-out, although this is very rare. Subsequently, the coverage rate is very close to 100%.

The Swedish MBR

MBR started in 1973 as an act of Swedish parliament and its basic structure has remained the same since the start even though major modifications has taken place, especially in the way data are collected (MBR). Initially, a Medical Report was filled in by the secretaries at each unit, these reports were mainly used for communication between the antenatal, the delivery and the pediatric/neonatal units. However, one copy of the report, containing only information that was considered relevant for the register, was sent to the National Board of Health and Welfare for registration. Today, the process is computerized and the information is based on information recorded at maternity care units, delivery wards and pediatric examinations of the newborn by filling in standardized medical records. It is mandatory for all health care providers to report to the MBR. Approximately 115,000-120,000 children are born in Sweden annually, MBR covering the vast majority of these. A summary publication of the content and quality of the Swedish MBR published in 2003 by the Centre of Epidemiology (EpC), reported that 1-3% of infants were completely missing, for some others there might be some missing data owing to incomplete records from the antenatal units and pediatric wards (Research Report from Centre for Epidemiology (EpC), 2003). Every year, infants reported to the MBR are compared with infants reported to Statistics Sweden (SCB) and in case of missing data in the MBR, a request on obtaining copies of the medical records are sent to the health care provider, however, not always successfully. Reasons for complete missing data in the MBR might be emigration or incomplete reporting from the health care providers.

The quality of the register has been investigated at three times, in 1976, 1988 and in 2001 with the first two evaluations summarized in a publication from 1990 by Cnattingius *et al.* (Cnattingius *et al.*, 1990, Research Report from Centre for Epidemiology (EpC), 2003). In these quality controls the original medical records were compared to the data in the register but also to information available at the SCB, and the MBR was shown to have high validity.

The MBR contains data on maternal characteristics i.e. age, parity, BMI, smoking habits, years of involuntary childlessness, all recorded at first antenatal visit at the maternity unit. Moreover, data on delivery and neonatal outcomes including child´s sex, birth weight, gestational age, Apgar score and stillbirth/live birth is included. In addition, maternal complications such as HDP, PPH, lacerations as well as mode of delivery is recorded. Both click-boxes and ICD codes can be used for the neonates and the mothers.

According to the Summary Report in Swedish in 2002 (Forskningsrapport från Epidemiologiskt Centrum (EpC), 2002), published in English in 2003 (Research Report from Centre for Epidemiology (EpC), 2003), the registration on PIN for both mothers and infants, gestational age, birth weight and incidence of birth defects is fairly reliable. For maternal smoking, information on smoking at first antenatal visit is missing in 4-9%. Maternal pre-pregnancy weight and length are recorded at the first visit to the antenatal unit during the first trimester, with missing data in 20-30% of cases. Hence, BMI is possible to calculate in 65-85%. No involuntary childlessness was stated in almost 94% of pregnancies. Parity seems to be incorrect in 9% of cases, yet well documented in women who have not immigrated. In the majority of the incorrect cases, the first birth in the MBR is by mistake listed as the first child even though the woman has one or several deliveries abroad. Regarding mode of delivery, Cesarean section is not recorded in around 2% of cases. When comparing these figures to the information available for our studies with missing information on smoking in 4-6% and on BMI in 6-10% we might assume that the quality of the Swedish MBR has not declined in the last decade. For BMI, there is of course a risk that the heaviest women, not wanting their weight to be recorded, cause a bias especially in the spontaneous group. Additionally, women with BMI >35 are not offered publicly funded ART treatment even though national guidelines on BMI limits do not exist and different policies are adopted in different county councils but also on a clinic level.

The Swedish NPR

In the 1960's the National Board of Health and Welfare started to collect information regarding inpatient care at public hospitals, registered in the NPR (NPR). Since 1984 the participation is mandatory for all county councils. From 1987 NPR includes all inpatient care in Sweden. Following 2001 the register also covers outpatient visits including day surgery and psychiatric care from both private and public caregivers.

The NPR is divided into the In Patient Register, also called the Hospital Discharge Register and the Out Patient Register. The coverage rate of the In Patient Register is almost 100% whereas it is considerably lower for the outpatient part (80%). Primary care is not covered in the NPR. The register is based on ICD codes and also includes information on hospital, age, gender, admission and discharge dates. The NPR has been shown to have high validity (Ludvigsson *et al.*, 2011).

The Register of Birth Defects

The Register of Birth Defects and chromosomal abnormalities (formerly Register of Congenital Malformation) (www.socialtyrelsen.se/en), established in 1964 in conjunction with the Thalidomide scandal, contains information on anatomical defects and chromosomal abnormalities according to ICD codes. Birth defects are supposed to be reported to the register at birth, at latest within a month from date of birth. Since 1999 the register also include data on induced abortions following pre-natal screening and the detection of malformations, however, without the women's PIN due to legal regulations in Sweden regarding identification and abortion. According to the latest report the coverage rate on birth defects in born children is good. However, missing data on induced abortions due to malformations is rather high, yet considered random (Annual report Register of Birth Defects, 2018).

The Prescribed Drug Register

The Prescribed Drug Register (www.socialtyrelsen.se/en) was established in 2005 and, based on anatomic therapeutic chemical (ATC) codes, includes information on all purchased prescription drugs. Besides ATC codes, the register contains information on the patient's PIN, gender, age, prescription amount as well as date for prescription and date when the drugs are purchased. Data are collected electronically with little missing data. Medications used in inpatient care are not recorded.

The SNQ

The SNQ was founded in 2001 and collects data for infants admitted to neonatal units during the first four postnatal weeks (SNQ). All pediatric hospitals in Sweden with a neonatal unit (n=37) are affiliated to SNQ. The register contains data on 34 interventions and information on 25 outcomes ranging from infant mortality and

neonatal morbidity to health at follow-up. The registration of health at follow-up started in 2016 and covers children with complicated neonatal periods such as extremely preterm birth (<28 weeks), children with hypoxic-ischemic encephalopathy and meningitis. Moreover, parental reported experience measures are included. The register has been shown to have excellent completeness and high validity (Norman *et al.*, 2019).

The Cause of Death Register

From 1961 the Cause of Death register (www.socialtyrelsen.se/en) is updated annually based on the medical death certificate. The medical death certificate is filled in by the physician and sent to the National Board of Health and Welfare within three weeks of death. The underlying cause/causes of death are given according to the ICD system. For the years 1952-1960 there is a historical register collected retrospectively from medical records. Until 2011 only Swedish citizens, including death abroad, were registered but following 2012 also foreign residents dying in Sweden are registered. The number of deaths registered is considered complete. However, a small proportion of deaths are lacking the information on cause of death. In summary, the register is considered of high quality (Brooke *et al.*, 2017).

Statistics Sweden (SCB)

SCB is a national administrative authority and responsible for official statistics including, for example, information on income, socio-economic conditions, educational level and foreign background. In addition, population statistics show both population size and changes such as number of children born and number of deaths but also information on both emigration and immigration (SCB).

The CoNARTaS cohort

CoNARTaS was established in 2008 and is a Nordic collaborative research group (www.conartas.com). Information on the Danish, Finnish, Norwegian and Swedish ART patients in ART registers or ART databases has been linked to MBRs to determine ART deliveries. Furthermore, linkage has been made to other national health registers, disease-specific quality registers and other nationwide databases. Data are linked at an individual level owing to the PIN given to every legal citizen in the Nordic countries. Data linkage has been performed twice, the first covering

years 1982-2007 with four spontaneous controls from the MBR for each ART child within their own country and the second 1985-2015 including all children in the MBRs. However, due to differences in availability of data on ART conception in each country some differences for the coverage period exist between the countries (Opdahl *et al.*, 2019). In paper III the data from the second data linkage were used. At the moment the cohort consist of 170,000 ART children, live and still born, and 7.7 million spontaneously conceived children as well as 130,000 mothers with at least one delivery after ART and 4 million mothers with deliveries only following SC during 1984 through 2015 (Opdahl *et al.*, 2019). The Nordic countries are comparable in terms of demography, health care and social security systems including reimbursement of ART treatment, which enables analyses on pooled data. Initially, the database was set up to study perinatal health in ART offspring. At date, the aim is also to study pregnancy complications for the mothers as well as long-term health for the children in terms of diabetes, CVD, cancer, mental health, puberty problems and imprinting disorders. In addition, long-term morbidity for the mothers, such as cancer and cardiovascular disease, can be assessed.

3.3 Definitions of birth defects

Several definitions for birth defects can be used. According to the ICD system, ICD 9 codes 740-759 A-X and ICD 10 codes beginning with Q involves birth defects, deformations and chromosomal abnormalities. Major birth defects are those defects that in general cause functional impairment and/or require surgical repair whereas minor defects are considered of little clinical relevance, yet sometimes also require surgical correction (e.g. tongue tie). EUROCAT, founded in 1979, is a European network of population-based registers for epidemiological surveillance of birth defects. The main objective of the network is surveillance on congenital anomalies, mainly in Europe. Several countries report anonymous data to EUROCAT for surveillance and research purpose (Tucker *et al.*, 2018). Besides, the EUROCAT classification system differentiates between major and minor malformations and is updated with regularity. All minor birth defects with limited clinical impact that should be excluded are listed according to ICD codes (www.eurocat-network.eu).

In Sweden, birth defects are recorded at birth in both the MBR and in the Register of Birth Defects following the pediatric examination of the newborn at the delivery unit. Birth defects that are detected during childhood are recorded in the NPR. The following definitions and registers have been used for birth defects in this thesis:

Paper I: Major birth defects; all ICD 10 codes beginning with Q but with exclusion of minor defects with little clinical relevance. The same classification system has been used in previous Swedish studies and the exclusion is based on "conditions that are relatively common, variable in registration, and sometimes associated with preterm birth and low birth weight. The following such conditions that are excluded: preauricular appendix, patent ductus arteriosus at preterm birth (<37 weeks), single umbilical artery, undescended testicle, congenital hip (sub) luxation, and minor skin malformations (mainly nevus). The remaining malformations are classified as "weeded" and will contain mainly major malformations but also some less severe conditions" (Kallen *et al.*, 2005). Birth defects were recorded up to one year of age using the Swedish MBR, the Register of Birth Defects and the Swedish NPR.

Paper II: Classification of major birth defects according to EUROCAT. Birth defects were recorded up to one year of age using the Swedish MBR, the Register of Birth Defects and the Swedish NPR.

Paper III: Any birth defects with an ICD 10 code beginning with Q were included, i.e. both major and minor birth defects. Only birth defects at birth were recorded using the CoNARTaS cohort.

Paper IV: Any birth defect with an ICD 9 code 740-759 or ICD 10 code beginning with Q at birth were included, i.e. both major and minor birth defects. Moreover, we report major birth defects at birth according to EUROCAT separately.

3.4 Statistical analyses

Statistical analyses for Paper I were done in collaboration with Karin Källen, PhD statistics, Professor, Department of Reproduction Epidemiology, Tornblad Institute, Institution of Clinical Science, Lund University, Lund, Sweden. The analyses were conducted by using software Gauss (Gauss TM, Aptech Systems Inc, Maple Valley, WA, USA, http://www.aptech.com). For Paper II-IV, the statistical analyses were done in collaboration with Max Petzold, PhD biostatistics, Professor, Swedish National Data Service & Health Metrics Unit, University of Gothenburg, Gothenburg, Sweden. Software STATA version 15 and SPSS version 24 and version 26 were used for the analyses.

Descriptive statistics are given by number (n) and percentages for categorical variables and by mean and standard deviation (SD) or median and interquartile range (IQR) for categorical variables.

For all four papers, logistic regression analyses were used to examine the association between exposure and outcome. The results from the regression analyses are presented as crude as well as adjusted ORs, i.e. uni- and multivariable logistic analyses. Two-sided tests were used and the significance level was set to 0.05. Corresponding 95% CI are presented. In paper IV, to assess the risks of adverse early childhood outcome, Cox-regression was used to calculate the HRs. Adjustment was made for known and possible covariates as listed in Table 7.

A summary on statistical methods used in the different papers is presented in Table 7.

Logistic regression could be used to analyze how one or several predictors (independent variables) are associated with an outcome (dependent variable). The predictors can be either continuous or categorical while the outcome variable is binary/dichotomous (yes/no, sick/healthy etc.). The result of a regression analysis is presented as OR and 95% CI. Odds is the probability of a certain event to occur divided by the probability of a certain event not to occur and the OR is the ratio between odds. A logistic regression analysis, both in its simple and its multivariable form, does not require enormous data sets. A "rule of a thumb" has suggested ten events per added independent variable, however, this rule is by some considered too conservative suggesting 5-9 events per independent variable (Vittinghoff and McCulloch, 2007). In a regression analysis, the independent variables should not be correlated causing multicollinearity and can be checked by calculating the Pearson´s or Spearman´s correlation coefficient.

Cox-regression, or the Cox proportional-hazards model, is used in time-to-event analysis, also called survival analysis. A time scale is added in the regression analysis with a starting point when the observations enter the study and an ending either when a certain event occur, at emigration, death or end of follow-up, whichever occur first. The hazard rate is the number of cases of a specified outcome per population at risk per unit of time. The HR is the ratio between hazards and can be explained as the probability of an event in one group divided by the probability of an event in another group occurring at a given interval of time. A HR >1 means that the event is more likely to happen whereas a HR <1 means it is less likely to occur. A HR of one

indicates that both groups experience an equal risk for the event at any point of time, i.e. the exposure has no effect on the outcome over time.

Table 7. Overview on statistical methods

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Descriptive categorical variables	Numbers, percentages	Numbers, percentages	Numbers, percentages	Numbers, percentages
Descriptive continuous variables	Year of birth, maternal age, parity, maternal smoking, maternal BMI, years of involuntary childless-ness, child´s sex.	Mean, SD	Mean, SD, median, IQR	Mean, SD
Analytical statistics	Uni- and multivariable logistic regression analysis, OR and AOR with 95% CI			Uni- and multivariable logistic regression analysis, OR and AOR with 95% CI Uni- and multivariable Cox-regression, HR and adjusted HR with 95% CI
Covariates	When comparing blastocyst and cleavage stage transfer adjustment was also made for number of oocytes retrieved, number of embryos transferred and for fresh or frozen transfer. In sub analysis on birth defects during 2008-2013 adjustment was moreover made for IVF or ICSI.	Year of birth, maternal age, parity, maternal smoking, maternal BMI, years of involuntary childless-ness, child´s sex, level of maternal education, chronic hypertension. For comparison between ART pregnancies adjustment was also made for cause of infertility, IVF/ ICSI, culture duration and number of gestational sacs. For programmed and stimulated vs. natural cycles adjustment was moreover made for freezing method (vitrification or slow-freeze).	Child´s country and year of birth, maternal age, parity, maternal smoking, maternal BMI, parental educational level, child´s sex, fertilization method (IVF/ICSI), SET, number of gestational sacs.	Year of birth, maternal age, parity. When comparing PGT to IVF/ICSI, adjustment was also made for fresh or frozen transfer. For early childhood outcome adjustment was made for year of birth, maternal age, parity and smoking
Missing data	Replaced by overall mean for smoking and BMI. Set as 0 for years of involuntary childlessness	Missing data was not imputed.	Missing data was not imputed.	No missing data for confounders used for regression analysis.

SD standard deviation, ***IQR*** interquartile range, ***OR*** odds ratio, ***AOR*** adjusted odds ratio, ***CI*** confidence interval, ***HR*** hazard ratio, ***BMI*** body mass index, ***IVF*** in vitro fertilization, ***ICSI*** intracytoplasmic sperm injection, ***ART*** assisted reproductive technology, ***SET*** single embryo transfer, ***PGT*** preimplantation genetic testing

3.5 Ethics

All studies were approved by the Regional Ethical Review Board in Gothenburg.
Ethical approval for paper I: Dnr 304/06, T876-14.
Ethical approval for paper II: Dnr 304/06, T109-08, T087-12 and Dnr 214-12, T422-12, T516-15, T233-16, T300-17, T1144-17, T121-18.
Ethical approval paper III: Dnr 304/06, T T109-08, T087-12 and Dnr 214-12, T422-12, T516-15, T233-16, T300-17, T1144-17, T121-18.
Regarding Danish data, ethical approval is not required for register-based research.
Ethical approval paper IV: Dnr 086/18.

4 RESULTS AND COMMENTS

4.1 Paper I

Neonatal and maternal outcome after blastocyst transfer: a population-based registry study.

During 2002-2013, 4819 singletons were born following blastocyst transfer, 25,747 singletons following cleavage stage transfer and 1,196,394 singletons following SC in Sweden.

Neonatal outcome

Birth defects and other neonatal outcomes are summarized in Table 8, 9 and 10. Birth defects were defined according to ICD 10 codes beginning with Q. Minor birth defects such as preauricular appendix, tongue tie, patent ductus arteriosus in preterm infants, single umbilical artery, undescended testicle, hip luxation/ subluxation, clicking hip and nevus were excluded.

Table 8. Birth defects in singleton pregnancies after blastocyst transfer, cleavage stage transfer and spontaneous conception in Sweden 2002-2013.

Outcome	**Blastocyst transfer** n=4819	**Cleavage stage transfer** n=25,747	**SC** n=1,196,394
Any major birth defect, n (%)	153 (3.2)	874 (3.4)	32117 (2.7)
Neural tube defects*, n (%)	<5** (<0.1)	10 (0.04)	287 (0.02)
Orofacial clefts*, n (%)	8 (0.2)	55 (0.2)	2026 (0.2)
Cardiac malformations*, n (%)	60 (1.2)	325 (1.3)	12201 (1.0)
Gastrointestinal malformations*, n (%)	4 (0.1)	46 (0.2)	1080 (0.1)
Limb reduction defects*, n (%)	<5** (<0.1)	17 (0.1)	526 (0.04)
Hypospadias*, n (%)	13 (0.3)	87 (0.3)	3363 (0.3)

SC spontaneous conception
*One child could have more than one birth defect
**The exact numbers are not reported to ensure anonymity of the participants

(Ginström Ernstad *et al.*, AJOG, 2016)

Birth defects occurred in 3.2% of singletons born after blastocyst transfer, in 3.4% of singletons born after cleavage stage transfer and in 2.7% of singletons in the general population. Following adjustment for relevant confounders listed in Table 7, the ORs for birth defects were not significant (AOR 0.94, 95% CI 0.79-1.13 versus cleavage stage and AOR 1.09, 95% CI 0.92-1.28 versus SC). In the crude analyses, when specific groups of birth defects were analyzed, the rate of neural tube defects was significantly higher after blastocyst transfer compared to SC (0.08% versus 0.02%, OR 3.46; 95% CI 1.29-9.29). However, no adjustment could be done due to few events.

Table 9. Birth defects (crude and adjusted ORs) for singleton pregnancies after blastocyst transfer (n=4819) versus cleavage stage transfer (n=25,747) and versus spontaneous conception (n=1,196,394).

Outcome	**Blastocyst vs cleavage stage** Crude OR (95% CI)	**Blastocyst vc cleavage stage** Adjusted OR* (95% CI)	**Blastocyst vs SC** Crude OR (95% CI)	**Blastocyst vs SC** Adjusted OR* (95% CI)
Any birth defect	0.93 (0.78-1.11)	0.94 (0.79-1.13)	1.19 (1.01-1.40)	1.09 (0.92-1.28)
Neural tube defects	2.14 (0.67-6.82)	NA	**3.46 (1.29-9.29)**	NA
Orofacial clefts	0.78 (0.37-1.63)	NA	0.98 (0.49-1.96)	NA
Cardiac malformations	0.99 (0.75-1.30)	0.99 (0.74-1.31)	1.22 (0.95-1.58)	1.16 (0.90-1.51)
Gastrointestinal malformations	0.46 (0.17-1.29)	NA	0.92 (0.34-2.46)	NA
Limb reduction defects	0.31 (0.04-2.36)	NA	0.47 (0.07-3.36)	NA
Hypospadias	0.80 (0.45-1.43)	NA	0.96 (0.56-1.66)	NA

OR odds ratio, ***CI*** confidence interval, ***SC*** spontaneous conception, ***NA*** no adjustment made because of few events reported

*Adjusted ORs were obtained after stratification for year of birth of child, maternal age, parity, smoking, BMI, years of involuntary childlessness, child's sex and, for comparison to cleavage stage, also for number of oocytes retrieved, number of embryos transferred and frozen/fresh embryo transfer

(Ginström Ernstad *et al.*, AJOG, 2016)

Regarding other neonatal outcomes the rate of male gender was significantly higher and the rate of SGA significantly lower in the blastocyst group compared to both cleavage stage transfer and SC. Moreover, the singletons born following blastocyst transfer were more often born preterm (<37 weeks) and macrosomic compared to SC and the risk for perinatal mortality was higher compared to cleavage stage transfer.

Maternal outcome

Maternal outcomes are summarized in Table 10. The rate of placenta previa and placental abruption was significantly higher in singleton pregnancies after blastocyst transfer when compared both to singleton pregnancies after cleavage stage transfer and SC. The rate of PPH was higher in the blastocyst group compared to the SC group whereas there were no differences in the risk of preeclampsia between the groups.

Comments

Birth defects is an extremely important outcome after ART and following a previous Swedish study showing an increased risk of birth defects in children born after blastocyst transfer compared to cleavage stage transfer concerns were raised both among patients and IVF clinics (Kallen *et al.*, 2010).

The present study, including a much larger cohort of singletons born following blastocyst transfer, yet partly overlapping with the study by Källen *et al*., did not show any differences in the rate of birth defects when comparing blastocyst transfer to cleavage stage transfer. Neither were there any significant differences in subgroups of birth defects. Our results are in accordance with two meta-analysis including the same four observational studies with a total of 8737 singletons born following blastocyst transfer and 36,097 singletons following cleavage stage transfer and reporting on similar risks between the groups (Martins *et al.*, 2017, Alviggi *et al.*, 2018).

The major strength in the present study is the inclusion of a complete national birth cohort of singletons born following blastocyst transfer and cleavage stage transfer during 2002-2013. Data were collected in the same way for all children and nationwide registers with high coverage rates were used. In addition, to minimize the risk of missing out on any diagnoses, birth defects recorded up to one year of age were included for analyses. However, data on birth defects leading to termination of pregnancy were not available, the main limitation of this study.

Table 10. Neonatal and maternal outcomes (adjusted ORs) for singleton pregnancies after blastocyst transfer (n=4819) versus cleavage stage transfer (n=25,747) and versus spontaneous conception (n=1,196,394).

Outcome	Blastocyst vs cleavage stage Adjusted OR* (95% CI)	Blastocyst vs SC Adjusted OR* (95% CI)
Male gender	**1.10 (1.03-1.17)**	**1.06 (1.10-1.12)**
PTB <37 weeks	1.12 (0.99-1.27)	**1.17 (1.05-1.31)**
LBW <2500 grams	**0.83 (0.71-0.97)**	0.89 (0.77-1.04)
SGA < -2 SD	**0.71 (0.56-0.88)**	**0.70 (0.57-0.87)**
Macrosomia ≥4500 grams	1.16 (0.98-1.37)	**1.24 (1.07-1.44)**
LGA > +2 SD	1.06 (0.91-1.23)	1.14 (0.997-1.31)
Perinatal mortality	**1.61 (1.14-2.29)**	0.76 (0.57-1.02)
Placenta previa	**2.18 (1.79-2.65)**	**6.38 (5.31-7.66)**
Placental abruption	**1.68 (1.20-2.35)**	**2.31 (1.70-3.13)**
Preeclampsia	1.00 (0.85-1.17)	1.02 (0.88-1.18)
PPH	0.95 (0.84-1.08)	**1.19 (1.07-1.33)**
Cesarean section	**1.21 (1.13-1.31)**	**1.24 (1.16-1.33)**

OR odds ratio, ***CI*** confidence interval, ***SC*** spontaneous conception, ***PTB*** preterm birth <37 weeks, ***LBW*** low birth weight <2500 grams, ***SGA/LGA*** small/large for gestational age, ***SD*** standard deviation, ***PPH*** postpartum hemorrhage

Maternal outcomes included placenta previa (ICD 10 code O44), placental abruption (ICD 10 code O45), preeclampsia (ICD 10 code O14-O15), postpartum hemorrhage after vaginal delivery (ICD 10 code O72; >1000mL)

*Adjusted ORs were obtained after stratification for year of birth of child, maternal age, parity, smoking, BMI, years of involuntary childlessness, child's sex and for comparison to cleavage stage, also for number of oocytes retrieved, number of embryos transferred, and frozen/fresh embryo transfer

4.2 Paper II

Neonatal and maternal outcome after frozen embryo transfer: Increased risks in programmed cycles.

In total, 9726 singletons were born following FET, 24,365 following fresh transfer and 1,127,566 following SC in Sweden during 2005 to 2015. Of the singletons born following FET, 6297 were born following a natural cycle, 1983 following a stimulated cycle and 1446 following a programmed cycle.

Outcome following different cycle regimens in FET

Maternal and neonatal outcomes following different cycle regimens in FET are summarized in Table 11 and 12.

Table 11. Maternal and neonatal outcome in singleton pregnancies after frozen embryo transfer (natural, stimulated and programmed cycles) in Sweden 2005-2015

Outcome	All FET n=9726	FET, natural n=6297	FET, stimulated n=1983	FET, programmed n=1446
Placenta previa, n (%)	107 (1.1)	73 (1.2)	21 (1.1)	13 (0.9)
Placental abruption, n (%)	44 (0.5)	29 (0.5)	8 (0.4)	7 (0.5)
HDP, n (%)	663 (6.8)	381 (6.1)	130 (6.6)	152 (10.5)
PPH, n (%)	943 (9.7)	497 (7.9)	165 (8.3)	281 (19.4)
Cesarean section, n (%)	2719 (28.0)	1665 (26.4)	573 (28.9)	481 (33.3)
Post term ≥42 weeks, n (%)	584 (6.0)	363 (5.8)	93 (4.7)	128 (8.9)
PTB <37 weeks, n (%)	592 (6.1)	377 (6.0)	117 (5.9)	98 (6.8)
LBW <2500 grams, n (%)	346 (3.6)	222 (3.5)	76 (3.8)	48 (3.3)
SGA < -2 SD, n (%)	271 (2.8)	179 (2.8)	54 (2,7)	39 (2.7)
Macrosomia ≥4500 grams, n (%)	503 (5.2)	292 (4.6)	104 (5.2)	107 (7.4)
LGA > +2 SD, n (%)	637 (6.5)	386 (6.1)	139 (7.0)	112 (7.8)
Major birth defects, n (%)	370 (3.8)	242 (3.8)	92 (4.6)	67 (4.6)

FET frozen embryo transfer, ***HDP*** hypertensive disorders in pregnancy, ***PPH*** postpartum hemorrhage, ***PTB*** preterm birth <37 weeks, ***LBW*** low birth weight <2500 grams, ***SGA/LGA*** small/large for gestational age, ***SD*** standard deviation

Maternal outcomes included placenta previa (ICD 10 code O44), placental abruption (ICD 10 code O45), hypertensive disorders in pregnancy (ICD 10 code O13-O15) and postpartum hemorrhage after vaginal delivery (ICD 10 code O72; >1000mL)

Major birth defects according to EUROCAT (www.eurocat-network.eu)
(Ginström Ernstad *et al.*, AJOG, 2019)

For maternal outcomes, the rate of HDP and PPH differed in the groups. HDP occurred in 6.1% of women following a natural cycle, in 6.6% following a stimulated cycle and in 10.5% following a programmed cycle. When comparing programmed cycles to natural and stimulated cycles, the risk was significantly higher in the programmed cycles both compared to natural cycles and stimulated cycles following adjustment for several confounders including PCOS (listed in Table 7) (AOR 1.78, 95% CI 1.43-2.21 and AOR 1.61, 95% CI 1.22-2.10, respectively).

Table 12. Maternal and neonatal outcomes (adjusted ORs) for singleton pregnancies after stimulated (n=1983) versus natural cycles (n=6297 natural cycles) in frozen embryo transfer, programmed (n=1446) versus natural cycles (n=6297) in frozen embryo transfer and programmed (n=1446) versus stimulated cycles (n=1983) in frozen embryo transfer.

Outcome	**Stimulated vs natural cycles** Adjusted* OR (95% CI)	**Programmed vs natural cycles** Adjusted* OR (95% CI)	**Programmed vs stimulated cycles** Adjusted* OR (95% CI)
Placenta previa	0.82 (0.50-1.36)	0.71 (0.36-1.37)	1.00 (0.46-2.16)
Placental abruption	0.75 (0.32-1.76)	0.93 (0.37-2.35)	0.56 (0.16-1.99)
HDP	1.05 (0.84-1.31)	**1.78 (1.43-2.21)**	**1.61 (1.22-2.10)**
PPH	1.05 (0.86-1.28)	**2.63 (2.20-3.13)**	**2.87 (2.29-3.60)**
Cesarean section	**1.15 (1.01-1.30)**	**1.39 (1.21-1.60)**	**1.27 (1.08-1.50)**
Post term >42 weeks	0.87 (0.68-1.11)	**1.59 (1.27-2.01)**	**1.98 (1.47-2.68)**
PTB < 37 weeks	0.94 (0.74-1.19)	1.09 (0.85-1.40)	1.15 (0.84-1.57)
LBW < 2500 grams	1.04 (0.77-1.40)	0.88 (0.62-1.26)	0.83 (0.55-1.27)
SGA < -2 SD	1.06 (0.76-1.47)	0.91 (0.62-1.35)	0.89 (0.56-1.43)
Macrosomia ≥ 4500 grams	1.14 (0.89-1.46)	**1.62 (1.26-2.09)**	**1.40 (1.03-1.90)**
LGA > +2 SD	1.11 (0.89-1.38)	1.27 (0.99-1.61)	1.10 (0.82-1.47)
Major birth defects	1.03 (0.79-1.33)	0.999 (0.74-1.34)	0.94 (0.66-1.34)

OR odds ratio, ***CI*** confidence interval, ***HDP*** hypertensive disorders in pregnancy, ***PPH*** postpartum hemorrhage, ***PTB*** preterm birth <37 weeks, ***LBW*** low birth weight <2500 grams, ***SGA/LGA*** small/large for gestational age, ***SD*** standard deviation

Maternal outcomes included placenta previa (ICD 10 code O44), placental abruption (ICD 10 code O45), hypertensive disorders in pregnancy (ICD 10 codes O13-O15) and postpartum hemorrhage after vaginal delivery (ICD 10 code O72; >1000mL)

*Adjusted ORs were obtained after stratification for maternal age, BMI, parity, year of birth of child, smoking, chronic hypertension, child´s sex, level of maternal education, years of involuntary childlessness, cause of infertility, IVF/ICSI, freezing method (vitrification or slow-freeze), culture duration and number of gestational sacs

Major birth defects according to EUROCAT (www.eurocat-network.eu)
(Ginström Ernstad *et al.*, AJOG, 2019)

Regarding PPH, an increase in risk was also seen in programmed cycles in comparison to natural cycles (AOR 2.63, 95% CI 2.20-3.13) as well as in comparison to stimulated cycles (AOR of 2.87, 95% CI 2.29-3.60) with a risk of almost 20% among programmed cycles. For placenta previa and placental abruption, we report on no significant differences in regards of cycle regimen used with absolute risks of around 1% for placenta previa and around 0.5% for placental abruption in all three groups.

For neonatal outcomes, the rate of post term birth was significantly higher following programmed cycles in comparison to natural cycles (AOR 1.59, 95% CI 1.27-2.01) as well as in comparison to stimulated cycles (AOR 1.98, 95% CI 1.47-2.68). Moreover, programmed cycles had a higher risk of macrosomia in comparison to both natural and stimulated cycles (AOR 1.62, 95% CI 1.26-2.09 and AOR 1.40, 95% CI 1.03-1.90, respectively). No significant differences were seen for PTB, LBW, perinatal mortality or major birth defects.

Outcome following FET in comparison to fresh transfer and SC

In comparison to fresh transfer, FET was associated with increased risks of HDP and PPH (1.5-1.6) but decreased risks of placenta previa and placental abruption (AOR 0.4-0.6) in comparison to fresh IVF. In comparison to SC, FET was associated with increased risks of placenta previa, HDP and PPH (AOR 1.2-1.8) whereas the risk of placental abruption did not significantly differ between the groups.

For neonatal outcomes, FET was associated with a 1.6-1.9-folded increase for post term birth, macrosomia and LGA whereas the risk estimates for PTB, LBW and SGA were lower (AOR 0.6-0.8) in the adjusted analyses. When comparing FET to SC, the rate of macrosomia and LGA was increased 1.6-folded whereas the rate of LBW and SGA was decreased (AOR 0.7-0.8) following adjustment.

Comments

We conclude that pregnancies following programmed cycles are more prone to HDP, PPH, post term birth and macrosomia as opposed to natural and stimulated cycles. Preeclampsia is a multisystem disorder with poorly understood etiology and pathophysiology. Since delivery is the only cure for the disorder, the placenta is considered to play a crucial role. Interestingly, four recently published studies reveal an association between the absence of CL in programmed cycles and HDP (von Versen-Hoynck *et al.*, 2019, von Versen-Hoynck *et al.*, 2019, von Versen-Hoynck *et al.*, 2019, von Versen-Hoynck *et al.*, 2020). The CL, lacking in programmed cycles due to suppression of the pituitary-ovarian hormonal axis, is the sole source of relaxin in early pregnancy. Subsequently, the lack of relaxin but also other, yet unidentified vasodilators from the CL, could have physiologic importance altering the vascular adaptation in early pregnancy and potentially inhibit the drop in mean arterial

pressure thereby causing HDP during the second half of the pregnancy. Our study support these findings.

In addition, in our study we saw a 3-folded risk of PPH following programmed cycles in comparison to natural and stimulated cycles. Several risk factors for PPH exist including large babies, more common in programmed cycles. Moreover, the increased risk of post term birth and HDP in programmed cycles might increase the rate of labor induction, also a risk factor for PPH. In conclusion, the increased risk of PPH in programmed cycles could at least partly be explained by other risks associated with the cycle regimen.

Since information on medication used for IVF treatment is lacking in the Q-IVF, information on medication used for stimulation was collected through the Prescribed Drug Register. All medication purchased 70 days prior to embryo transfer was assumed to belong to the analyzed treatment cycle which is considered the main limitation in this study. Programmed cycles have gained in popularity mainly due to decreased demand on extensive monitoring and the possibility to schedule transfers to weekdays. However, owing to the increased risks in programmed cycles, this cycle regimen should only be performed when ovulation fails. In our study we were able to adjust for several relevant confounders including chronic hypertension and cause of infertility.

4.3 Paper III

Perinatal and maternal outcome after vitrification of blastocysts: a Nordic study in singletons from the CoNARTaS group.

In this register-based study on Swedish and Danish data covering the years 2002 to 2015 we had 3650 singletons born following transfer of vitrified blastocysts, 8123 singletons born following transfer of slow-frozen cleavage stage transfers and 4469 singletons born following transfer of fresh blastocysts. The majority of children, 13,991 (86.1%), were born in Sweden.

Neonatal and maternal outcomes are summarized in Table 13 and 14. For PPH only Swedish data were analyzed due to the differences in definition. In Sweden, PPH is defined as a bleeding exceeding 1000mL, whereas the definition is >500mL in Denmark.

Outcome following different freezing techniques

Following transfer of a vitrified blastocyst, 7.4% of singletons were born preterm. For the slow-frozen cleavage stage group the estimate was 6.3% and for the fresh blastocyst group 8.9%. After adjustment for relevant confounders, the rate of PTB was significantly higher following transfer of vitrified blastocysts in comparison to slow-frozen cleavage stage transfer (AOR 1.33, 95% CI 1.09-1.62). No other significant differences for neonatal and maternal outcome could be found when comparing the two groups.

Table 13. Perinatal and maternal outcome in singleton pregnancies after vitrified blastocyst transfer (n=3650) compared with slow-frozen cleavage stage transfer (n=8123) and fresh blastocyst transfer (n=4469), 2002-2015.

Outcome	**Vitrified blastocysts** n=3650	**Slow-frozen cleavage stage transfer** n=8123	**Fresh blastocysts** n=4469
Post term ≥42 weeks, n (%)	281 (7.7)	620 (7.6)	190 (4.3)
PTB <37 weeks, n (%)	271 (7.4)	513 (6.3)	398 (8.9)
LBW <2500 grams, n (%)	127 (3.5)	304 (3.8)	256 (5.7)
Macrosomia ≥4500g, n (%)	183 (5.0)	410 (5.1)	117 (2.6)
SGA < -2 SD, n (%)	92 (2.5)	233 (2.9)	198 (4.4)
LGA > +2 SD, n (%)	247 (6.8)	513 (6.3)	181 (4.1)
Birth defects, any, n (%)	152 (4.2)	338 (4.2)	217 (4.9)
Placenta previa, n (%)	58 (1.6)	82 (1.0)	182 (4.1)
Placental abruption, n (%)	20 (0.5)	42 (0.5)	49 (1.1)
HDP, n (%)	258 (7.1)	534 (6.6)	231 (5.2)
PPH*, n (%)	362 (10.1)	645 (9.4)	231 (6.6)
Cesarean section, n (%)	1078 (29.6)	2205 (27.1)	1205 (27.0)

PTB preterm birth <37 weeks, ***LBW*** low birth weight <2500 grams, ***SGA/LGA*** small/large for gestational age, ***SD*** standard deviation, ***HDP*** hypertensive disorders in pregnancy, ***PPH*** postpartum hemorrhage

Birth defects were defined according to ICD 10 codes beginning with Q with no exclusion for minor defects

Maternal outcomes included placenta previa (ICD 10 code O44), placental abruption (ICD 10 code O45), hypertensive disorders in pregnancy (ICD 10 codes O13-O15) and postpartum hemorrhage after vaginal delivery (ICD 10 code O72; >1000mL).
*Only Swedish data
(Ginström Ernstad *et al.*, Hum Reprod, 2019)

Outcome following transfer of frozen vs fresh blastocysts

Transfer of frozen blastocysts was associated with an increased risk of larger babies with a 1.5 and 1.8-folded risk for LGA and macrosomia, respectively, and a 40% decrease in risk for both LBW and SGA in comparison to fresh blastocyst transfer, results that are in accordance with paper II for comparisons between fresh and frozen transfers. Regarding maternal outcomes, the rate of HDP (AOR 1.47, 95% CI 1.19-1.81) and PPH (AOR 1.68, 95% CI 1.39-2.03) were significantly higher and the rate of placenta previa significantly lower (AOR 0.35, 95% CI 0.25-0.48) following frozen blastocyst transfer, results also in line with paper II.

Comments

In this study we aimed at investigating whether the introduction of vitrification poses any risks for the children and their mothers when compared to slow-freezing. Using the CoNARTaS cohort, we included all singletons born following transfer of vitrified blastocysts, slow-frozen cleavage stage embryos and fresh blastocysts during 2002-2015 in Sweden and Denmark yielding a reasonable large sample size. Since the new freezing technique, vitrification, has improved success rates in terms of implantation and pregnancy rates particularly for blastocysts (Stehlik *et al.*, 2005), a comparison of vitrified and slow-frozen blastocysts was not feasible.

The risk of PTB was slightly higher following transfer of vitrified blastocysts compared to slow-frozen cleavage stage transfer, a finding that might be attributable to the extended culture more than to the freezing technique as meta-analyses has shown an increased risk of PTB in blastocyst compared to cleavage stage transfers (Dar *et al.*, 2014, Martins *et al.*, 2017, Alviggi *et al.*, 2018). No other differences in outcomes were seen, indicating that the freezing technique *per se* does not seem to influence the neonatal or maternal outcome. At date, neither have other studies observed a difference in neonatal or maternal outcomes following the introduction of vitrification when compared to slow-freezing of blastocysts (Li *et al.*, 2014) or cleavage stage embryos (Kaartinen *et al.*, 2016, Gu *et al.*, 2019).

The major drawback in this study is the comparison of vitrified blastocysts and slow-frozen cleavage stage embryos. However, since vitrification was introduced simultaneously with blastocyst transfer in Sweden and Denmark, it was not possible to explore the effect of vitrification *per se* in this study. Even though there are some children born after transfer of vitrified cleavage stage embryos, the number was too

small for statistical comparison.

Table 14. Adjusted odds ratios of perinatal and maternal outcome in singleton deliveries after vitrified blastocyst transfer (n=3650) compared with slow-frozen cleavage stage transfer (n=8123) and fresh blastocyst transfer (n=4469), 2002-2015

Outcome	**Vitrified blastocyst vs slow-frozen cleavage stage transfer** Adjusted OR* (95% CI)	**Vitrified vs fresh blastocyst transfer** Adjusted OR* (95% CI)
Post term birth ≥42 weeks	1.01 (0.85-1.20)	**1.61 (1.31-1.98)**
PTB <37 weeks	**1.33 (1.09-1.62)**	0.86 (0.71-1.04)
LBW <2500 grams	0.91 (0.70-1.19)	**0.57 (0.44-0.74)**
SGA < -2 SD	0.85 (0.63-1.13)	**0.58 (0.44-0.78)**
Macrosomia ≥4500 grams	0.93 (0.76-1.15)	**1.77 (1.35-2.31)**
LGA > +2 SD	1.10 (0.91-1.32)	**1.48 (1.18-1.84)**
Birth defects, any	1.16 (0.91-1.47)	0.99 (0.77-1.27)
Placenta previa	1.48 (0.98-2.24)	**0.35 (0.25-0.48)**
Placental abruption	1.78 (0.93-3.40)	0.65 (0.37-1.16)
HDP	0.97 (0.81-1.17)	**1.47 (1.19-1.81)**
PPH**	1.03 (0.88-1.25)	**1.68 (1.39-2.03)**
Cesarean section	**1.17 (1.05-1.30)**	**1.15 (1.03-1.29)**

OR odds ratio, ***CI*** confidence interval, ***PTB*** preterm birth <37 weeks, ***LBW*** low birth weight <2500 grams, ***SGA/LGA*** small/large for gestational age, ***SD*** standard deviation, ***HDP*** hypertensive disorders in pregnancy, ***PPH*** postpartum hemorrhage

Birth defects were defined according to ICD 10 codes beginning with Q with no exclusion for minor defects.

Maternal outcomes included placenta previa (ICD 10 code O44), placental abruption (ICD 10 code O45), hypertensive disorders in pregnancy (ICD 10 codes O13-O15) and postpartum hemorrhage after vaginal delivery (ICD 10 code O72; >1000mL)

*Adjusted for country of birth of child, year of birth of child, maternal age, BMI, parity, smoking, parental educational level, fertilization method (IVF/ICSI), single embryo transfer, number of gestational sacs and child´s sex
**Only Swedish data
(Ginström Ernstad *et al*., Hum Reprod, 2019)

4.4 Paper IV

Perinatal, maternal and early childhood outcome following preimplantation genetic testing: a national register-based study

This study included singletons born following PGT in Sweden (n=267) since the birth of the first child and as far as information was available, i.e. 1996-2017. The PGT children were compared to two control groups:

I. All IVF/ICSI singletons (n=55,355) born in Sweden during 1996-2017.

II. Matched control group of singletons born following SC (n=26,535) during 1996-2017. The SC controls were matched for year of birth of child, maternal age +/- 5 years, parity and for delivery hospital or nearest delivery hospital. We aimed for 100 controls per PGT child. However, in five cases, we did not find 100 matched controls, these cases had 41, 64, 66, 68 and 96 controls each.

The majority of PGT treatments were performed for monogenic disorders (65.2%). More details are presented in the Results section in paper IV.

Neonatal and maternal outcome

When comparing PGT pregnancies to IVF/ICSI pregnancies there were no significant differences for PTB and LBW. Regarding maternal outcomes, the rates of HDP, placenta previa and PPH were comparable between the groups. The AORs are presented in paper IV.

When comparing PGT to spontaneously conceived singletons, significantly higher risks for PTB and LBW were found. For maternal outcomes, the rate of placenta previa was significantly higher following PGT. Regarding HDP and PPH, there were no significant differences. The AORs are presented in paper IV.

Early childhood outcome

The mean follow-up time was 3.3 years for PGT offspring and the matched spontaneous controls. For IVF/ICSI offspring, the follow-up time was twice as long, 7.7 years. Data on early childhood outcome were collected from the Swedish NPR covering in- and outpatient care, yet not primary care.

Complete data on early childhood outcome is presented in paper IV. Shortly, in the crude analysis the risk of sepsis was significantly higher in PGT singletons compared to both IVF/ICSI and spontaneous singletons. However, the analysis is based on few cases in the PGT group, no adjustment was made and the results should thus be taken with precaution. No other differences in early childhood outcome were observed.

Comments

Our study shows that neonatal and maternal outcomes following PGT are comparable to pregnancies following IVF/ICSI yet, there are higher risks in comparison to SC, in line with other IVF/ICSI pregnancies. Data on early childhood health in PGT singletons are sparse, yet reassuring compared to singletons conceived through IVF/ICSI and spontaneously conceived singletons. However, a drawback is the short follow-up time.

Two control groups were set up for this study. The first group consisted of all IVF/ICSI singleton pregnancies. The comparison between PGT and IVF/ICSI pregnancies gave us the possibility to study the effect of the embryo biopsy *per se* on the outcome. The second control group consisted of matched spontaneous controls from the Swedish MBR. The main reason for using matched controls instead of the entire spontaneous population in the MBR, was that we did not plan to do additional analysis or sub analyses that would have been affected by the matching. One hundred matched controls for each case can be considered sufficient in terms of generalizability. The matching variables chosen, year of birth of child, maternal age, parity and delivery hospital, were chosen to balance the groups for important predictors of outcome.

Since the proportion of children born following PGT increased throughout the study period, the majority of children were born later in the study period. The short follow-up time for all children but particularly for the PGT children and their matched spontaneous controls is the major drawback in the study considering early childhood outcome. For example, children with a mild case of cerebral palsy might not get the diagnosis until the age of four years. However, data on early childhood outcomes were collected using national health registers and the data was collected in the same way for all three groups. There are no previous studies published investigating the early childhood outcome for PGT children concerning somatic and psychiatric diseases. The few earlier studies on growth, neurological development, body

composition and blood pressure (Desmyttere *et al.*, 2009, Belva *et al.*, 2018, Kuiper *et al.*, 2018) all suffer from small sample sizes and methodological problems such as unclear selection of controls, data reported by questionnaires to the parents and low participation rates.

5 DISCUSSION

The goal of this thesis was to evaluate the outcome for the children born through ART and the mothers undergoing ART following advanced techniques. The increased demand on ART, with up to 7.1% of national birth cohorts in Europe being ART children (de Geyter *et al.*, 2020), necessitates follow-up of both the children and their mothers. The results are in general reassuring even though some differences exist both between different techniques but also as opposed to SC.

5.1 Neonatal outcome following advanced techniques in ART

Singletons born following ART treatment are more often born preterm, with LBW and they also have a slightly increased risk of birth defects in comparison to spontaneously conceived singletons (Helmerhorst *et al.*, 2004, Jackson *et al.*, 2004, McDonald *et al.*, 2009, Pandey *et al.*, 2012, Hansen *et al.*, 2013, Qin *et al.*, 2017, Zhao *et al.*, 2020). However, the risks associated with ART has decreased markedly during the last decades as discussed in chapter 1.9. Due to the increased use of extended culture and FET including vitrification as well as PGT we ought to study the neonatal outcome following these advanced techniques.

Blastocyst transfer and birth defects

Following the introduction of blastocyst transfer, concerns were raised whether this technique increase the rate of birth defects among the newborns. In 2010, a Swedish register-based study on 1311 children born after blastocyst transfer revealed a significantly higher risk in blastocyst offspring compared to offspring born following cleavage stage transfer (Kallen *et al.*, 2010). A Canadian retrospective study (n=3206) did, however, not reveal any differences (Dar *et al.*, 2013) and these two studies have then been summarized in a meta-analysis showing an increased risk following blastocyst transfer, mainly driven by the results in the Swedish study (Dar *et al.*, 2014). Finally, two more recent meta-analyses including the same four studies, one of them being our study, reported on similar risks of birth defects following transfer with blastocysts and cleavage stage embryos (Martins *et al.*, 2016, Alviggi *et al.*, 2018). In our study, we found similar risks for birth defects following blastocyst transfer as opposed to cleavage stage transfer, divergent to the results in the previous Swedish study, yet in accordance with the study by Dar *et al.* (2013)

and the meta-analyses. There are some possible explanations behind these divergent results. In the Swedish study by Källen *et al.*, partly covering the same cohort as our study, both singletons and multiples (236 multiples out of 1311 children, 18%) were included, potentially causing a bias in consideration of the almost doubled risk of birth defects in multiples as opposed to singletons (Li *et al.*, 2003, Glinianaia *et al.*, 2008, Zhang *et al.*, 2011). Moreover, our study was almost five times larger in regards of singletons. In the study by Källen *et al.*, the absolute risk for a relatively severe birth defect was 4.7% in the blastocyst group and 4.1% in the cleavage stage group whereas the corresponding numbers in our study were 3.2% and 3.4%, respectively. In the Canadian study on singletons, the main limitation was the way of data collection. The information on major and minor birth defects were collected directly via the parents, either by telephone or by mail, not through medical records, a possible explanation to the somewhat lower absolute risks (2.4% following transfer of a day 5-6 embryo and 2.3% following transfer of a day 2-3 embryo) in comparison to our study. In our study, the follow-up time for birth defects was up to one year of age whereas the follow-up time in the study by Källen *et al.* and Dar *et al.* is not stated. When comparing blastocyst transfer to SC, neither was any significant differences for birth defects found in our study (3.2% in the blastocyst group and 2.7% in the SC group), possibly due to a relatively small sample size.

Yet, for specific birth defects, previous studies have shown that there might be an association between ART and some specific birth defects such as neural tube defects, gastrointestinal and cardiac malformations (Kallen *et al.*, 2005, Chen *et al.*, 2018, Giorgione *et al.*, 2018). In embryonal development, blastogenesis occurs during the first four weeks, followed by the organogenesis. Some defects, sometimes called the blastogenesis defects, occur during the first four weeks and involves neural tube defects, abdominal wall defects as well as esophageal and anal atresia. ART treatment, manipulating with the embryo at an early developmental stage, could thus be hypothesized to be associated with the blastogenesis defects as shown in an Australian study (Halliday *et al.*, 2010).

Blastocyst transfer and neonatal outcome

Regarding being born preterm, we found similar rates for PTB when comparing blastocyst to cleavage stage transfer, yet higher risks when compared to SC. However, the lower CI for PTB, when comparing blastocyst and cleavage stage transfer, was close to one (95% CI 0.99-1.27). Recent meta-analyses consistently show a higher risk

of PTB following extended culture compared to day 2-3 transfers (Dar *et al.*, 2014, Martins *et al.*, 2016, Alviggi *et al.*, 2018). In the meta-analysis by Alviggi *et al*, the results were, however, only seen in fresh and fresh plus frozen cycles but not in frozen cycles alone. In the other meta-analyses these cycles were lumped together. In our study on blastocyst transfer, including both fresh and frozen cycles, the absolute risk was 7.8% in fresh and 6.5% in frozen cycles, yet no statistical comparisons were made for frozen blastocyst versus frozen day 2-3 transfer or fresh blastocyst versus fresh day 2-3 transfer. The results in our study, pointing in the same direction as the meta-analyses, however, not reaching a significant level, could be attributable to the study size/power. Additionally, the PTB rate varies in different ethnicities with relatively low rates in white populations, e.g. the Nordic countries.

Extended culture has been shown to influence birth weight, with possibly larger babies as compared to cleavage stage transfer. In our study, when analyzing fresh and frozen cycles together, we found a higher risk of LGA in the crude analysis between blastocyst and cleavage stage transfer, however, following adjustment for confounders including fresh or frozen transfer, this difference disappeared. Even though the rate of LGA in the adjusted analysis was similar there were other indications that extended culture might influence birth weight. The rate of macrosomia was higher in comparison to SC, the risk of LBW was lower in comparison to day 2-3 transfer and the risk of SGA lower both in comparison to day 2-3 transfer and SC. In another study, there were neither any differences regarding LGA found in 4000 singletons born following blastocyst transfer adjusting for relevant confounders including fresh or frozen cycle (Fernando *et al.*, 2012) while two studies in fresh cycles observed an increase in LGA and a higher mean birth weight following blastocyst transfer (Makinen *et al.*, 2013, Zhu *et al.*, 2014). In accordance with our study, a lower risk of SGA has also been observed in other studies when comparing day 5-6 transfer and day 2-3 transfer (Maheshwari *et al.*, 2013, Ishihara *et al.*, 2014). Animal studies have shown increased risk for intrauterine overgrowth, called the large offspring syndrome, in cattle and sheep cultured *in vitro* to the blastocyst stage (Young *et al.*, 1998). However, the large offspring syndrome in animals is associated with organ anomalies, findings that are not in line with our study showing similar risks for birth defects following extended culture in comparison to day 2-3 transfers.

According to a recent study, based on WHO data, there are 106 males born for each 100 females (Grech and Mamo, 2019), very close to the male-female ratio seen in the spontaneously conceived children in our studies. In IVF, extended culture has been

shown to skew the ratio in favor for male (Luna *et al.*, 2007, Chang *et al.*, 2009, Dean *et al.*, 2010, Maalouf *et al.*, 2014, Ding *et al.*, 2018, Hattori *et al.*, 2019). Likewise, in our study on blastocyst transfer, we found an altered male/female ratio both in comparison to cleavage stage transfer and SC with almost 53% male offspring following blastocyst transfer corresponding to 112 males for each 100 females. A possible explanation is faster cleavage in embryos of male origin causing a selection of male embryos for transfer at the blastocyst stage (Tarín *et al.*, 1995, Ménézo *et al.*, 1999, Alfarawati *et al.*, 2011). Another explanation might be a higher post-implantation fetal demise in female embryos following blastocyst transfer (Tarín *et al.*, 2014).

Frozen embryo transfer and neonatal outcome

In recent years, there has been emerging evidence that FET is associated with larger babies. In our studies on frozen transfers, paper II and III, the risk of LGA and macrosomia was increased 1.5-1.9-folded compared to fresh transfer but also compared to SC. Our findings of larger babies following FET are supported by several previous studies (Wennerholm *et al.*, 2013, Pinborg *et al.*, 2014, Maheshwari *et al.*, 2018, Berntsen *et al.*, 2019) as well as a sibling study (Henningsen *et al.*, 2011). In such a study, the outcome is compared in siblings born to the same mother with one sibling born following SC and another following ART or one sibling born following one ART technique and another following another ART technique. The Danish sibling study, published in 2011, showed significantly higher birth weights in siblings born following FET than for those born following fresh IVF, indicating that the cryo technique may induce changes in embryo development and intrauterine growth (Henningsen *et al.*, 2011).

FET seems to be associated with an elevated risk of post term rather than preterm birth (Wikland *et al.*, 2010, Wennerholm *et al.*, 2013). In our studies on frozen transfers (paper II and III), we report on a 1.6-folded increase for post term birth in comparison to fresh transfer. The definition of post term birth is a pregnancy lasting for more than ≥294 days, i.e. ≥42 weeks and has been shown to be associated with an increase in perinatal mortality (Ingemarsson and Källén, 1997, Smith, 2001, Nakling and Backe, 2006, Wennerholm *et al.*, 2019). At date, in Sweden, induction is recommended at 42+0 weeks at the latest whereas in Denmark, induction is recommended at 41+2-41+6 weeks. Gestational age for SC pregnancies is determined from the 1st or 2nd trimester ultrasound or from the first day of the last

menstrual period if information on the ultrasound is unavailable. In IVF pregnancies gestational age is rather calculated from the day of embryo transfer and culture duration. Whether the accuracy of these two calculations of gestation age differ is not fully known but with today's knowledge, calculating gestational age in ART pregnancies should be based on fertilization date, i.e. day of embryo transfer and culture duration (Butt *et al.*, 2014).

Altogether, for both blastocyst transfer and FET, the findings of larger babies could be due to selection of good quality embryos surviving extended culture and freezing/thawing. On the other hand, poor embryo quality has not been shown to be associated with adverse neonatal and maternal outcome in two smaller studies (Oron *et al.*, 2014, Akamine *et al.*, 2018). Moreover, possible epigenetic changes and differences in culture conditions including different culture medias and variations in oxygen concentrations might matter (Dumoulin *et al.*, 2010, Gardner, 2016, Mani and Mainigi, 2018). In addition, parental characteristics might influence the outcome and thus need to be adjusted for. Regarding larger babies and FET, one hypothesis is that *in vitro* culture is prone to intrauterine overgrowth (Young *et al.*, 1998, Grace and Sinclair, 2009) but that changes in steroid profile following the stimulation used in fresh cycles out rules these overgrowing tendencies. It is also important to keep in mind that the divergent results seen in studies could be explained by differences in obstetric management including surveillance and interventions during pregnancy and labor.

Frozen embryo transfer: Freezing techniques and neonatal outcome

When investigating the effect of freezing techniques on gestational age, we think that the increased risk of PTB following transfer of vitrified blastocysts shown in paper III (AOR 1.33, 95% CI 1.09–1.62) might be attributable to the extended culture rather than to the vitrification owing to the fact that blastocyst transfer seems to be associated with PTB as discussed before. Few studies have investigated the effect of freezing technique on outcome. In a Finnish study, no differences in the rate of PTB were seen in 276 children, both singletons and multiples, born following transfer of cleavage stage embryos that were vitrified or slow-frozen (Kaartinen *et al.*, 2016). Neither were any differences in neonatal outcome seen in a larger study on 4721 vitrified blastocyst and 1965 slow-frozen blastocysts in singleton pregnancies (Li *et al.*, 2014) nor in a later study on 297 pairs of newborns born following vitrified or slow-frozen day 3 embryos (Gu *et al.*, 2019). Regarding our study, a comparison

between vitrification and slow-freeze in blastocysts would have been more appropriate than the comparison we did. Since vitrification of blastocysts have been shown to be much more successful than slow-frozen blastocysts, increasing both survival-, implantation- and pregnancy rates (Stehlik *et al.*, 2005), such a study is, however, hardly feasible, at least not in the Nordic countries.

Frozen embryo transfer and birth defects

There were no differences in the rate of birth defects between different cycles regimens (paper II), neither did the freezing technique seem to influence the rate of birth defects (paper III). Likewise, when comparing frozen and fresh embryo transfer, similar risks were detected (paper II and III). These results are in line with several other studies showing similar rates for birth defects in fresh and frozen cycles (Pelkonen *et al.*, 2014, Maheshwari *et al.*, 2018).

Preimplantation genetic testing and neonatal outcome

In comparison to singletons born following IVF/ICSI, PGT singletons had comparable outcomes in terms of PTB, LBW and birth defects, results that are in line with previous studies (Liebaers *et al.*, 2010, Desmyttere *et al.*, 2012, Hasson *et al.*, 2017, Sunkara *et al.*, 2017, He *et al.*, 2019, Zhang *et al.*, 2019). At date, there are no indications that the invasiveness of the technique used in PGT have detrimental effects on the neonatal outcome.

In summary, extended culture might be associated with a higher risk of PTB whereas FET seems to reduce the risk, yet increase the risk of post term birth, especially in programmed cycles. Singletons born following FET are more prone to macrosomia and LGA both in comparison to fresh transfer and SC and blastocyst transfer might be associated with larger babies. The freezing technique or the embryo biopsy used in PGT does not seem to alter the neonatal outcome.

5.2 Maternal outcome following advanced techniques in ART

In general, placental complications including HDP seems to be more common in ART pregnancies compared to spontaneous pregnancies (Pandey *et al.*, 2012, Opdahl *et al.*,

2015, Qin *et al.*, 2016, Vermey *et al.*, 2019) and for placenta previa the risk has increased over time in ART pregnancies (Petersen *et al.*, 2020). Also, in a Norwegian sibling study there was a 3-folded increased rate of placenta previa in ART pregnancies as opposed to SC pregnancies within the same mother, indicating that the ART technique *per se* may play a role (Romundstad *et al.*, 2006).

Blastocyst transfer: placenta previa and placental abruption

Following blastocyst transfer, we found a two-folded risk of placenta previa in comparison to cleavage stage transfer and a 6-folded risk in comparison to SC. When comparing transfer of blastocysts to day 2-3 embryos, one recent study revealed a higher risk of placenta previa following blastocyst transfer (Spangmose *et al.*, 2019) while other studies showed no differences (Fernando *et al.*, 2012, Ishihara *et al.*, 2014). The study by Spangmose *et al*., covering the years of 2002-2015 for Swedish data, partially overlaps with the study cohort used in our study (paper I), however, only including fresh cycles. In the Australian study (Fernando *et al.*, 2012), 70% of transfers were performed in a fresh cycle and adjustment made for cycle type. In the Japanese study (Ishihara *et al.*, 2014) the vast majority of blastocyst transfers were performed in frozen cycles and revealed no association between extended culture or FET and placenta previa. In our study, 37% of blastocyst transfers were performed in frozen cycles and adjustment was made for fresh or frozen transfer. Interestingly, two studies, including both day 2-3 and day 5-6 embryos, have shown a potentially protective effect of FET on placenta previa (Sazonova *et al.*, 2012, Sha *et al.*, 2018). A previous Australian study suggested an association between endometrial thickness >9 mm and placenta previa (Rombauts *et al.*, 2014). One could hypothesize that extended culture and the stimulation in fresh cycles causes a thicker lining in the uterine cavity, thus predisposing to placenta previa. Besides different distributions of fresh and frozen transfers, the reasons for the divergent results between the studies mentioned are probably many. First, the diagnostic criteria may vary. Today there are different criteria for diagnosis (complete/partial/marginal placenta previa and low-lying placenta) and the diagnosis is also dependent on frequency of surveillance during pregnancy, e.g. a possibly closer surveillance of ART pregnancies could contribute to the differences. Moreover, there might also be differences in registration practices, e.g. at what time during pregnancy the diagnosis of placenta previa is recorded. Second, the reason for infertility might matter. Endometriosis, independently of ART treatment, has been shown to be associated with placenta previa (Rombauts *et al.*, 2014). In addition, advanced maternal age, multiparity,

smoking, male fetuses, multiple pregnancies, prior cesarean delivery as well as spontaneous and induced abortions might contribute to the incidence of placenta previa (Faiz and Ananth, 2003, Karami *et al.*, 2018). Noteworthy, according to a recently published CoNARTaS study, the risk for placenta previa has increased substantially in ART pregnancies for the last three decades whereas this trend has not been seen in SC pregnancies. The reason for this trend is not known and could only partly be explained by the increasing use of blastocyst transfer as discussed by the authors (Petersen *et al.*, 2020). In regards of placental abruption we found a 1.6-folded risk following blastocyst transfer in comparison to cleavage stage transfer, contradictory to other studies (Fernando *et al.*, 2012, Ishihara *et al.*, 2014, Oron *et al.*, 2015). Placental abruption, a clinical diagnosis, is even harder to diagnose than placenta previa. Several risk factors including advanced maternal age, multiparity, smoking, HDP and previous Cesarean section exist (Baumann *et al.*, 2000, Tikkanen *et al.*, 2006a, Tikkanen *et al.*, 2006b). Regarding both placenta previa and abruption, we were only able to partly adjust for the confounders mentioned which is also the case in other studies listed above.

Blastocyst transfer: HDP and PPH

In paper I, we found no association between extended culture and the risk of preeclampsia neither in comparison to cleavage stage pregnancies nor SC. Similar findings have also been observed in other studies on blastocyst transfer compared to cleavage stage transfer (Fernando *et al.*, 2012, Oron *et al.*, 2015). Neither has any differences been seen for pregnancy induced hypertension (Ishihara *et al.*, 2014). PPH, defined as a postpartum bleeding of >500mL or >1000mL depending on country, is the leading cause of maternal mortality, not least in developing countries. For Sweden, the definition used is > 1000mL. Several risk factors for PPH exist, among them ART and a meta-analysis have reported on a 1.3-folded risk for PPH following an ART pregnancy in comparison to a non-ART pregnancy (Qin *et al.*, 2016). In our study, we found no association between blastocyst transfer and PPH, findings in line with a previous Australian study (Fernando *et al.*, 2012).

Frozen embryo transfer: HDP

For autologous oocytes, a higher risk of HDP have been shown in singleton ART pregnancies when compared to singleton pregnancies following SC with the highest risks following FET (Pandey *et al.*, 2012, Opdahl *et al.*, 2015). Considering the trend

towards FET, including the freeze-all strategy, this is a reason for concern. The reasons behind these findings are yet to be understood.

The etiology behind preeclampsia is not fully elucidated, though thought to be multifactorial. Nonetheless, an inadequate placentation is crucial for the development. In ART pregnancies, an asynchrony between the endometrium and the embryo could potentially result in inadequate placentation leading to a higher incidence of HDP in ART pregnancies. Also placental oxidative stress and subsequently, a generalized inflammatory response (Myatt and Cui, 2004, Redman and Sargent, 2010, Pereira and Martel, 2014) have been discussed as contributors.

Four recent studies, investigating the association between physiological changes during pregnancy and pregnancy outcome to the presence/absence of CL revealed increased risks for abnormal vascular adaptation and preeclampsia in pregnancies lacking a CL (von Versen-Hoynck *et al.*, 2019, von Versen-Hoynck *et al.*, 2019, von Versen-Hoynck *et al.*, 2019, von Versen-Hoynck *et al.*, 2020). The CL produces not only estrogen and progesterone but is also the sole source of relaxin in early pregnancy. Since relaxin plays an important part in vasodilation, consequently the absence of a CL might yield inappropriate vascular adaptations in early pregnancy. The results from the studies by von Versen-Höynck *et al*. are supported by our findings revealing an increased risk of HDP in programmed cycles both compared to natural (AOR 1.78, 95% CI 1.43-2.21) and stimulated cycles (AOR 1.61, 95% CI 1.22-2.10) (paper II). Similar results have then also been confirmed in a large Japanese register-based study comparing outcomes in natural and hormone replacement cycles. In a sensitivity analysis, restricting the analysis to singletons, the results remained significant (Saito *et al.*, 2019).

In this thesis, oocyte donation pregnancies were excluded since they differ from ART pregnancies with autologous oocytes and poses different risks. However, the findings of adverse outcomes in programmed cycles, reminding of oocyte donation pregnancies with lacking CL, are interesting. Oocyte donation pregnancies have been thought to be more prone to adverse outcome due to immunological reasons, with a pregnancy immunologically completely foreign to the mother in case of an unrelated donor. Since oocyte donation pregnancies are primarily offered to women with premature ovarian failure and thus lacking a CL, the adverse outcome, similar to that of programmed cycles, might at least partly be explained by the absence of CL rather than by immunological responses.

The novel findings by von Versen-Höynck and her research team, supported by results in our study as well as in the Japanese study by Saito *et al.* (2019), should enhance further studies on the mechanisms underlying the association between obstetric outcome and endometrial preparation. In regards of these results, at date, programmed cycles ought only to be used in women when ovulation fails.

Frozen embryo transfer: *Placenta previa and PPH*

Frozen transfer has been shown to have a potential protective effect on placenta previa. In paper II and III we reported on significantly lower risks of placenta previa in frozen cycles compared to fresh cycles (AOR 0.38 and AOR 0.35, respectively), findings that are in line with a previous Swedish study (Sazonova *et al.*, 2012) as well as a meta-analysis (Sha *et al.*, 2018). The cohorts in our study and the study by Sazonova *et al.* are partially overlapping. However, another systematic review and meta-analysis did not show any differences in the incidence of placenta previa depending on cycle type (Roque *et al.*, 2019). In the meta-analysis by Sha *et al*. showing a lower risk of placenta previa in frozen cycles, six studies were included with a total of 72,000 pregnancies whereas the meta-analysis by Roque *et al*. included four studies and a total of 69,000 pregnancies revealing similar rates in the two treatment modalities. Three of the four studies in the meta-analysis by Roque *et al*. were also included in the study by Sha *et al*. Following different cycle regimens in FET, we did not see any differences in the rate of placenta previa, findings that are supported by the large Japanese study (Saito *et al.*, 2019). Interestingly, in the Japanese study by Saito *et al*. the risk of placenta accreta was 7-folded following a programmed cycle in FET when compared to a natural cycle and in the study by Ishihara *et al*., FET was associated with a 3-folded increase in risk for placenta accreta in comparison to fresh transfer (Ishihara *et al.*, 2014, Saito *et al.*, 2019). However, placenta accreta is a rare outcome, the results have limited precision and should thus be taken with caution.

On the other hand, for PPH, FET seems to be associated with an increased risk (Sazonova *et al.*, 2012, Sha *et al.*, 2018). In our study comparing different endometrial preparation methods used in FET, PPH was shown to be more common in programmed cycles in comparison to natural and stimulated cycles with almost 20% of pregnancies in programmed cycles yielding a bleeding >1000mL. When comparing all frozen cycles to fresh cycles and SC, the rate of PPH was moreover higher following a frozen transfer. These findings could be due to the increased risk of post

term birth in especially programmed cycles leading to more induction of labor. Moreover, the increased risk of PPH could be attributable to larger babies.

Frozen embryo transfer: Freezing techniques and maternal outcome

Today, there is no evidence that the freezing technique would affect the rate of HDP as shown in paper III, a result that is supported by a small Swedish study (Wikland *et al.*, 2010). For other maternal outcomes, no significant differences were seen in the risk of placenta previa, placental abruption and PPH comparing the vitrified blastocyst versus the slow frozen cleavage stage group. However, due to the sample size, precision is limited meaning that there might be a clinically relevant difference which this study could not identify.

Preimplantation genetic testing and maternal outcome

In our study, we found no differences in maternal outcome following PGT as opposed to IVF/ICSI. Few studies have investigated maternal outcome following PGT. In a small study from the United States on singletons there were no differences in placenta related complications and HDP (Hasson *et al.*, 2017). However, in another study from the United States a 3-folded risk of preeclampsia was found following PGT in comparison to IVF/ICSI (Zhang *et al.*, 2019). Yet, 70% of transfers in the PGT and IVF/ICSI group were performed in frozen cycles, a confounder not adjusted for. In a sub-analysis, only including frozen transfers, there was no difference in the rate of preeclampsia between women who have undergone PGT with IVF and women who have only undergone IVF. In our study, showing similar risks for HDP between the groups, only 30% of transfers were performed in frozen cycles and adjustment was made for fresh/frozen cycle. Altogether, at date, there is no evidence that the embryo biopsy *per se* would have any effect on maternal outcome.

In summary, not only ART but also the advanced technique chosen might influence placentation. Blastocyst transfer seem to be associated with abnormal placentation in terms of increased risks of placenta previa and eventually also placental abruption whereas FET seems to be protective against placenta previa, yet increase the risk of HDP especially in programmed cycles. The freezing technique or the embryo biopsy used in PGT have not been shown to be associated with altered maternal outcome.

5.3 General discussion

In general, the majority of children born through ART are healthy and even though the maternal risks are increased in ART pregnancies the absolute risks are still low. The adverse effect on neonatal and maternal outcome following ART is, however, well known. Nonetheless, the reasons behind these findings are not fully elucidated. The infertility *per se* as well as the technical aspects have been suggested as contributors for the adverse outcome. The infertile population comprise a unique study population and hence picking out the optimal control population is a challenge. In the majority of studies performed, including the studies in this thesis, a healthy background population with a spontaneously conceived pregnancy have been used as the comparison group making it hard to distinguish whether the adverse outcomes observed in the children are a result of the infertility treatment, or rather the underlying infertility that indicated treatment. One way to overcome the problem concerning controls might be sibling studies comparing children born following SC and ART in the same mother. Another possible way is to establish a sub fertile control group with a spontaneous pregnancy but with a time to pregnancy for example >1 year. Such studies indicate that both maternal characteristics and the technique *per se* contribute to the adverse neonatal and maternal outcome (Romundstad *et al.*, 2006, Romundstad *et al.*, 2008, Henningsen *et al.*, 2011, Luke *et al.*, 2016, Luke *et al.*, 2017, Zhang *et al.*, 2019). One might though hypothesize that the patients in sibling studies comparing ART and SC pregnancies as well as in a sub fertile control group suffer from less severe infertility and are thus not representative for the general ART population. In paper I and II we have adjusted for years of involuntary childlessness, however, this variable is missing in a very large proportion of the SC group but also for 20-25% of women having ART treatment. The missing values for the spontaneously conceived women could be interpreted as no involuntary childlessness but could also be due to the question not being asked at the maternity unit. In several published studies, no adjustment for years of involuntary childlessness has been made.

In addition, other parental characteristics differ between the groups, IVF parents usually being older, having lower parity and higher socioeconomic status, factors that also need to be accounted and adjusted for. Moreover, paternal characteristics could influence the outcome (Oldereid et al., 2018), confounders that are very rarely adjusted for in studies on ART outcome. There is also evidence that the diagnosis behind infertility on its own can alter the outcome. Endometriosis has been shown to alter perinatal and maternal outcome irrespective of ART or not (Stephansson *et al.*,

2009, Lalani *et al.*, 2018). PCOS, another common reason for infertility treatment, also alters the outcome irrespective of age, BMI or ART (Roos *et al.*, 2011). In Sweden, but presumably also abroad, infertility treatment is sometimes offered following minor infertility investigation and hence the cause of the underlying infertility is not available and thus impossible to take into consideration.

Epigenetic changes are changes in the gene expression without changes in the DNA, i.e. a change in the phenotype without a change in the genotype. Epigenetic programming is a natural occurrence in both gametogenesis and during early embryonal development. Epigenetic modification can be influenced by age, lifestyle factors and diseases but also due to altered hormonal environment and manipulation of the oocytes, sperm and early embryos as shown in animal studies (Doherty *et al.*, 2000, Grace and Sinclair, 2009, Market-Velker *et al.*, 2010, Mainigi *et al.*, 2016). In recent years epigenetic differences in gene expression have been reported in frozen/thawed embryos. A previous study on mice have shown loss of methylation in genes responsible for growth following transfer of fresh and vitrified embryos with a greater loss in the vitrified embryos leading to normalization of gene expression (Wang *et al.*, 2010). Other studies support these findings by showing that frozen embryos in human present epigenetic patterns more similar to natural conception (Estill and Krawetz, 2016, Ghosh *et al.*, 2017). Furthermore, a recent meta-analysis showed an association between birth weight and DNA methylation (Küpers *et al.*, 2019). Whether epigenetic modifications related to methylation of genes in the human fetus and the maternal tissue during ART alters fetal growth and placentation and to what extent, however, needs further investigation.

For singleton IVF pregnancies in general, the rate of birth defects seems to be 30-40%, or even up to 70%, higher as compared to SC, according to a number of systematic reviews and meta-analyses (Pandey *et al.*, 2012, Hansen *et al.*, 2013, Qin *et al.*, 2017, Zhao *et al.*, 2020). Interestingly, one paper suggests a larger difference in singleton pregnancies if excluding minor anomalies (Hansen *et al.*, 2013). In our studies, paper I, II and IV, we found no differences when comparing singletons born following advanced techniques in ART to SC. Reasons for these results might be differences in study size and patient characteristics. In addition, the prevalence of birth defects in different regions of the world seem to differ due to unknown reasons, also a possible contributor to differences in results depending on study population (Qin *et al.*, 2017). Unfortunately, there is no universal definition of major birth defects and in the majority of studies major and minor defects have been lumped together. The EUROCAT classification system, described in the Material and Method section, is updated with

regularity and differentiates between major and minor birth defects, excluding all minor defects (www.eurocat-network.eu). To enable adequate comparisons and meta-analyses it is desirable that major birth defects would be reported according to EUROCAT in future studies. There are several reasons for only reporting on major birth defects in studies comparing ART and non-ART offspring. Children born following ART might be more closely examined due to parental anxiety, increasing the detection of minor anomalies. Moreover, it is assumed that major birth defects are more consistently reported whereas reports on minor are often more incomplete. Furthermore, major birth defects have more clinical relevance and the inclusion of minor birth defects could ultimately hide potential associations of ART and major birth defects. In addition, it is important that information on birth defects is collected in the same way for all groups, preferably via medical records and registers, not through parents by e-mail or phone calls. In paper II and IV we classified birth defects according to EUROCAT whereas in paper I, we used the same classification system as Källen *et al*. including all ICD 10 codes beginning with a Q but with exclusion of minor defects with little clinical relevance as described in the Material and Method section and also listed in the paper. The reason for this was to be able to compare our results to the results in Källen's study. In paper III all birth defects beginning with a Q according to ICD 10 codes were included, i.e. both major and minor birth defects. The reason for not categorizing major and minor birth defects in paper III was that we had no suspicions that the freezing technique *per se* would affect the rates.

In Sweden, following 1999, termination of pregnancy due to birth defects should be reported to the Register of Birth Defects, however, according to a summary report from the register 35-50% of such terminations are not reported (the Swedish Pregnancy Register, Annual report Register of Birth Defects, 2018). Due to legal regulations on abortions in Sweden, these reports have only included the mothers date of birth, not the PIN, and can thus not be cross-linked to health and quality registers. One might assume that the diagnostics of birth defects as well as chromosomal abnormalities during pregnancy is more precise today than 10-20 years ago and in a recently published Nordic paper it was shown that the rate of birth defects has risen throughout the years, both following ART and SC, however the relative risk of major birth defects between ART and SC children remained unchanged. As suggested by the authors', the increase in birth defects is most likely explained by better data quality and better prenatal diagnostics (Henningsen *et al.*, 2018). The terminations due to chromosomal abnormalities, especially trisomy 21, have more than doubled in Sweden between 1999 and 2016, yet no such trend has been seen for birth defects (the Swedish Pregnancy Register, Annual report Register of Birth Defects, 2018). In

Sweden, legal abortion on own request is allowed prior to 18 weeks of pregnancy (≤17+6). During 18+0 and 21+6 legal abortion is allowed following written permission from the National Board of Health and Welfare due to special reasons including birth defects. Following infertility and an ART pregnancy, the inclination to terminate a pregnancy could be assumed to be lower if detecting a birth defect, which could influence the numbers and the analyses when comparing birth defects in ART and spontaneously conceived children. However, this has not been shown in studies from Finland and France, indicating similar rates on termination of pregnancies in ART and SC pregnancies (Pelkonen *et al.*, 2014, Tararbit *et al.*, 2015).

Following blastocyst transfer and PGT a higher risk of PTB with AOR 1.2 and 2.0, respectively, was found in comparison to SC, results that are in line with several studies reporting on an increased risk of PTB (ORs/RRs 1.5-2.0) following ART (Helmerhorst *et al.*, 2004, Jackson *et al.*, 2004, McDonald *et al.*, 2009, Pandey *et al.*, 2012, Qin *et al.*, 2017). Moreover, ART singletons have been shown to be smaller (Helmerhorst *et al.*, 2004, Jackson *et al.*, 2004, McDonald *et al.*, 2009, Pandey *et al.*, 2012, Qin *et al.*, 2017). These findings are, however, particularly seen for fresh cycles whereas children born following FET are more often born post term and large as discussed before. The short- and long-term effects of being born small and preterm are widely recognized and described. These children are at a greater risk of birth asphyxia, CVD and metabolic syndrome (Osmond and Barker, 2000, Parkinson *et al.*, 2013, Bellou *et al.*, 2018, Wu *et al.*, 2018). Whether these long-term effects are also attributable to ART children born small and preterm is less studied and needs large follow-up studies. On a short-term basis large babies are more likely to suffer from intrapartal asphyxia, hypoglycemia as well as labor and shoulder dystocia. The long-term effects of being born macrosomic and LGA are less clear but some studies have shown an elevated risk of obesity and CVD (Ornoy, 2011, Gu *et al.*, 2012, Derraik *et al.*, 2020) as well as a higher risk for leukemia and Hodgkin's lymphoma (Roman *et al.*, 2013, Crump *et al.*, 2015, Petridou *et al.*, 2015, Triebwasser *et al.*, 2016, Groves *et al.*, 2018). Given the fact that an increasing amount of ART cycles are performed as FET, large scale studies on the long-term effects are warranted. An unanswered question is also whether being born LGA but preterm, risks that might both be associated with extended culture (Martins *et al.*, 2016, Alviggi *et al.*, 2018) is better or worse than being born normal/small for gestational age, yet preterm.

In comparison to spontaneously conceived pregnancies, ART pregnancies in general, independently of technique used, were associated with an increased risk of placenta previa (AOR 1.8-6.6, paper I, II and IV). For placental abruption, the difference was

only significant when comparing blastocyst transfer and SC (paper I). Pregnancies following assisted reproduction are prone to more placental complications (Romundstad *et al.*, 2006, Kallen, 2008, Healy *et al.*, 2010, Vermey *et al.*, 2019), a reason for concern considering the potentially detrimental effects on the health of both the newborn and the mother following both placenta previa and placental abruption. Sub fertility can be part of the explanation but the technique probably also matters as shown in a meta-analysis (Vermey *et al.*, 2019), but also in a sibling study from Norway (Romundstad *et al.*, 2006).

In Sweden, the rates of Cesarean section are rather low compared to other parts of the world with a total rate of 17% in 2017 according to the MBR (MBR). For IVF pregnancies the rates are generally higher (25-33%) as shown in our papers. The reasons for this can probably be explained by several factors. The increased risk for PTB, small and large babies as well as placental complications might contribute. Moreover, patient´s and doctor´s anxiety, possibly higher in an ART pregnancy, might matter. Since infertility and/or ART treatment increases the risk of placenta previa as does Cesarean section, it is of great importance to avoid the first Cesarean section, not least in ART pregnancies.

Choosing the optimal treatment is a challenge. A treatment leading to larger babies born at term, or possibly post term, and in parallel leading to more HDP but less placenta related complications? A treatment leading to possibly larger babies born preterm with a doubled risk for placenta previa? The answer is probably depending on person asked with different points of view from health care workers working in the reproductive field, obstetrics and neonatology. Nevertheless, it is important to keep in mind that all treatment options are not available for all patients and especially PCOS patients poses a challenge with a balance between excessive response with a high risk of OHSS and unexpected poor response with no embryos available for transfer. When choosing the best option for each woman/couple, consideration of risk factors such as medical history should be discussed thoroughly between the patient and the physician to enable individualized treatment.

Even though pregnancies following ART are more prone to adverse perinatal and maternal outcome, most children are healthy and there has been a decline in the risk following ART as discussed in chapter 1.9, mainly depending on the decrease in multiple birth rate but probably also due to development and refinement of the technical procedures. As today, knowledge is though mainly based on observational studies and few RCTs have included neonatal and maternal outcome following ART.

Yet, to study neonatal and maternal as primary outcomes following ART in RCTs, is hardly feasible. In conclusion, several possible factors, including parental characteristics, infertility treatment *per se* and sub fertility, contribute to the perinatal and maternal outcome following ART and potentially affects the long-term health of the ART children. In the future further epidemiological studies are warranted to study the long-term outcomes for the children and their mothers undergoing ART.

5.4 Strengths and limitations

The major strength in these nationwide population-based studies, is including large national birth cohorts of singletons conceived following different ART techniques and SC during the same time period. Information on outcomes were collected in similar ways for ART and spontaneously conceived children. In addition, data to the health and quality registers are primarily collected for quality reasons, not for the actual study, minimizing the risk of selection bias and re-call bias. Further, the number of children included in paper 1-III is large, yielding rather precise estimates for many outcomes and a possibility to discover even modest differences between groups. For paper IV, a national birth cohort of PGT children were included. Thus, low risk of selection bias and large sample sizes are the two most important strengths in the present studies.

Limitations

Yet, register-based studies also carry some limitations. Even though we were able to adjust for several confounders there is a risk of residual confounding of unknown and unmeasured confounders. Since the data is not collected for research purpose only and not by the researches themselves, important information might be lacking. One example of this is missing data on BMI. One might hypothesize that overweight (BMI >30) or obese (BMI >35) women do not want their BMI to be recorded. Since ART is generally not offered to obese women the missing data could cause a bias in favor for SC. In paper I, missing values on BMI were replaced by the overall mean whereas in paper II and III these women were excluded in the analyses. In paper IV BMI was not adjusted for. Another example is the lack of information on cause of infertility in the Swedish IVF register, thus this information was collected from the NPR. IVF is sometimes, however, performed following minor infertility investigation and therefore the diagnosis might be missing in the NPR. Moreover,

data on medication used during ART treatment is not included in the Q-IVF and collected from the Prescribed Drug Register. In addition, in the included studies we have used a healthy background population as a control group while a sub fertile population with spontaneous pregnancies would have been more adequate since subfertility *per se* has been shown to have an impact on the outcome (Pinborg *et al.*, 2013, Luke *et al.*, 2016, Luke *et al.*, 2017). Such a group is hard to define, though. Finally, even though associations may be found in observational studies, causality can never be addressed.

Strengths and limitations paper I

The main strength in paper I is, apart from the population-based study design, the way we collected data on birth defects. This information was retrieved from three different sources, the MBR, the NPR and the Register of Birth Defects, and the follow-up time for the children was set to one year of age, minimizing the risk to miss any major birth defects. However, information on pregnancies leading to termination due to major fetal anomalies were not available, which is considered the main limitation in paper I.

Strengths and limitations paper II

In paper II, we were able to include all singletons born following frozen and fresh embryo transfer and SC and moreover divide the pregnancies in FET according to protocol used. However, since data on medication during ART treatment is not yet covered by Q-IVF, data on medication used was retrieved from the Prescribed Drug Register. We assumed that medication redeemed 70 days prior to ET belonged to the analyzed cycle, which is the major limitation of this study.

Strengths and limitations paper III

In this study, the main strength is combining two national birth cohorts in the Nordic countries, the Swedish and the Danish. The main limitation is investigating both the effect of freezing technique and culture duration at the same time, which makes it impossible to separate their effects on the outcome. Due to the rapid implementation of vitrification of blastocysts following the higher success rates (Stehlik *et al.*, 2005), studies comparing perinatal and maternal outcomes between vitrified and slow-frozen blastocysts are rare and hardly feasible.

Strengths and limitations paper IV

For the study on outcome following PGT, we included singletons born following PGT in Sweden since the birth of the first child and for as long as data were available, i.e. 1996-2017. The main limitation is the rather small sample size, which makes it impossible to adjust for all relevant confounders including fertilization method, culture duration and length of infertility. Moreover, the follow-up time is short for the majority of children especially in the PGT group lowering the absolute risks for diagnosis in early childhood.

In conclusion, the large populations and minimizing selection bias by using national health registries are the main strengths of this thesis. Different techniques used in ART poses different neonatal and maternal risks.

5.5 Ethical aspects of register-based research

January 1, 2019 the Swedish Ethical Review Authority replaced the Regional Ethical Review Boards. The Ethical Review Authority is responsible for conducting ethical reviews of research on humans and consist of both researchers and nonprofessionals.

The health registers run by the National Board of Health and Welfare include all citizens in Sweden and according to Swedish regulations individual patients cannot withhold or withdraw their medical data from the health registers, neither can they ask the data to be anonymized. The individual patient is identified using the unique PIN. Participation in the Swedish Quality Registers (www.kvalitetsregister.se) is not mandatory. However, by default, patients are presumed by the legislation to consent to registration but have the right to opt-out.

For conducting register-based research in Sweden, ethical permission is required. Written consent from the patients is generally not required since it is assumed that the study participants do not object to research if it is accepted by the Ethical Review Authority. Several arguments for this manner exist. First, individual approvals would have a negative effect on the study size and statistical power. Second, in high-risk populations, one might hypothesize that consent would be difficult to obtain, thus causing a selection bias and a less generalizable result. A third reason is that some individuals would be dead by that time and consent impossible to obtain while a

fourth reason is the increased costs for obtaining written consent for millions of individuals. Nevertheless, publication of data must guarantee the anonymity of the individuals and data should be presented at group level. This is particularly important for rare outcomes. Regarding rare outcomes, register-based studies with large study populations are needed to reveal any possible associations. However, it is important to weigh the statistically significant results against clinical relevance.

In 2017, a Swedish report, based on surveys and interviews, found that the majority of the Swedish population is positive to the use of digital data in healthcare and research (Report ”For safety's sake”, 2017). In Finland, also a country with a tradition of health registers and offering a health care system very similar to the Swedish, similar results were found. In general, the participants were supportive of register-based research. However, the opinion for informed consent was divergent and many would at least like to get information of the research use of their data (Eloranta and Auvinen, 2015).

All studies in this thesis were approved by the Regional Ethical Committee at the University of Gothenburg. Ethical approvals are listed in section 3.4.

6 CONCLUSION

Advanced techniques introduced in ART have increased success rates and the majority of children born following these techniques are healthy. In addition, the majority of women undergoing ART with advanced techniques do not suffer from pregnancy complications. Yet, some differences between techniques exist.

- No increased risk of birth defects was found in singletons born after blastocyst transfer compared to cleavage stage transfer. The risk of placenta previa and placental abruption was higher in the blastocyst group as compared to the cleavage stage group.
- In frozen/thawed cycles, an increased rate of HDP and PPH were detected in programmed cycles. Moreover, programmed cycles were associated with a higher risk of post term birth and macrosomia. Stimulated cycles had outcomes similar with natural cycles. The results suggest a link between the absence of corpus luteum in programmed cycles and adverse obstetric outcomes. These findings are important in view of the increasing use of frozen cycles including the freeze-all policy.
- Transfer of vitrified blastocysts is associated with a slightly higher risk of PTB when compared with slow-frozen cleavage stage embryos. For other neonatal and maternal outcomes, no significant differences were found and at date, there is no indication that the freezing technique *per se* has major influence on the perinatal and maternal outcomes. Transfer of vitrified blastocysts compared to transfer of fresh blastocysts entails a higher risk of large babies, HDP and PPH, yet a lower risk of placenta previa.
- There is no indication that the embryo biopsy used in PGT alters the perinatal, maternal or early childhood outcome. Singleton pregnancies following PGT had similar outcomes as IVF/ICSI pregnancies, yet a higher risk of PTB, LBW and placenta previa in comparison to SC. The limited data on early childhood outcomes is so far reassuring. However, there were only 267 PGT children included in the study and the mean follow-up time for these children was only 3.3 years.

7 FUTURE PERSPECTIVES

The reasons for the adverse outcome following ART are still not fully understood. Technical aspects, the underlying sub fertility, differences in parental characteristics as well as epigenetic changes following ART have been suggested as potential contributors.

In the future, studies should focus on investigating the outcome according to the exposure since grouping all ART treatments together will create a very heterogeneous group, thus different techniques used in ART should be examined separately. Previously programmed and stimulated cycles in FET have been lumped together. The novel findings by von Versen-Höynck *et al.*, suggesting a link between the presence/absence of CL and altered maternal outcome, shows the importance of not lumping all ART treatments in one group. These findings should enhance further studies on potential circulating compounds, such as relaxin or other yet unknown vasodilators, from the CL. Ultimately, adding relaxin in the luteal support regimen might be considered.

Since the sub fertility *per se* has been shown to influence the outcome, sub fertility should also be accounted for, either by adjustment or by creating adequate comparison groups such as in sibling studies or creating a control group of sub fertile couples with for example time to pregnancy > 1 year. Other confounders that might be worth taking into consideration, yet seldom included/adjusted for in studies, are paternal characteristics influencing the outcome (Oldereid *et al.*, 2018).

According to the Barker-hypothesis, the intrauterine milieu has been shown to affect long-term health (Barker *et al.*, 1993). Studies on the long-term consequences of ART treatment are still scarce and a large knowledge gap needs to be filled. The underlying reasons for the differences in long-term health might be attributable to the increased risks for adverse neonatal outcomes. Although some differences in long-term health have been described, the majority of ART children are healthy.

In conclusion, quality and health registers offers a "goldmine" for further research on both short - and long- term consequences of ART. Since the technical development is fast, new advanced techniques introduced and the number of ART children born worldwide is steadily increasing, it is of great importance to continue the surveillance with large scale studies. The majority of the children born following ART are still rather young and follow-up on long-term consequences is thus warranted.

ACKNOWLEDGEMENT

There are many persons that, in different ways, have helped me with the work of this thesis. I am grateful to all of you! In particular I would like to thank:

All of the fertility clinics in Sweden for reporting data on ART procedures throughout the years.

Professor **Christina Bergh**, my main supervisor, for introducing me to research with your vast knowledge. For your honest and encouraging feedback and for your patience with my shortcomings throughout the years.

Associate professor **Ulla-Britt Wennerholm**, my co-supervisor, for your endless enthusiasm, your positive attitude to problem solving and your knowledge. For being a role model as a clinical obstetrician.

Professor **Max Petzold**, my co-supervisor and statistician, for your never-ending patience regarding my statistical failings. Your short stories on meeting other Swedish speaking Finns have lighten up any statistical session.

Nils Crona, Head of Clinical Department of Obstetrics at Sahlgrenska University Hospital, Gothenburg, for your support and allowing me time off clinical work as well as time off being Head of Resident Physicians at Department of Obstetrics and Gynecology during the last year of my PhD-studies.

The **CoNARTaS-group**, for sharing the CoNARTaS database and for many interesting meetings and discussions accompanied with lovely food at Hotel Ocean.

All co-authors, this thesis would not be as it is without you, thank you!

Karin Sundfeldt, Katja Stenström Bohlin and Klaus Groth, for constructive discussions during my half-time seminar.

Anja Andersson and Annette Nattland, for always being helpful and friendly and sorting things out. A special thanks to Annette for help with the layout of the thesis book.

Nona Sargisian, for stepping in for me as Head of Resident Physicians at Department of Obstetrics and Gynecology during my last year of PhD-studies.

Ylva Carlsson, for giving me the basics in research and for your support during my residency as well as my first years as a consultant.

All colleagues at the Department of Obstetrics, Sahlgrenska University Hospital, Gothenburg, Sweden, for covering for me during my PhD-studies and for always bringing a smile on my face while drinking coffee in our “fikarum”. A special thanks to **Maria Lycke** for the daily chats during the last months, I will always think of you when I look back at this spring.

Linnea and Jenny, my partners in crime. For being a shoulder to cry on, for never ending laughter and for serving me great wine during late hours. I would not be where I am without you! #Obtrion
All my wonderful friends, especially **Helena, Heidi, Mia, Pia, Hannah, Emma** and **Maria**, for being there for me and for endless laughter. Emma, I still think I am faster than you.
Mami och pappa, Sinikka and Tage, for always supporting, encouraging and believing in me. For helping with your grandchildren while I have been busy working in clinic and writing this thesis.
Henrica, my sister, for teaching me how to debate and for never letting me get away easily during our intense discussions. Goes for you as well, **Stefan**. **Edward**, you are the cutest nephew.
Andreas, my rock and love of my life. For always supporting me in (almost) everything I do and for being a great father to our children. For reminding me of what really matters. And last but not least, for your great skills in hacking computer systems. **Amanda and Edvin**, our wonderful children, full of energy, full of life. Thank you for all the happiness, laughter and gray hairs you bring to my life.

The Sahlgrenska University Hospital LUA/ALF and the Hjalmar Svensson Research Foundation for financial support of the work with this thesis.

REFERENCES

Adamson GD, de Mouzon J, Chambers GM, Zegers-Hochschild F, Mansour R, Ishihara O, Banker M, Dyer S. International Committee for Monitoring Assisted Reproductive Technology: world report on assisted reproductive technology, 2011. *Fertil Steril* 2018;110: 1067-1080.

Adamson GD, de Mouzon J, Zegers-Hochschild F, Chambers GM, Ishihara O, Banker M, Mansour R, Dyer S. International Committee for Monitoring Assisted Reproductive Technologies (ICMART) Preliminary World Report on ART, 2015. Abstract (O-144) presented at: Annual meeting of European Society of Human Reproduction and Embryology (ESHRE), 2019 June 23-26, Vienna, Austria. 2019.

Akamine K, Mekaru K, Gibo K, Nagata C, Oishi S, Miyagi M, Heshiki C, Kinjo T, Masamoto H, Aoki Y. Comparative study of obstetric and neonatal outcomes of live births between poor- and good-quality embryo transfers. *Reprod Med Biol* 2018;17: 188-194.

Alfarawati S, Fragouli E, Colls P, Stevens J, Gutiérrez-Mateo C, Schoolcraft WB, Katz-Jaffe MG, Wells D. The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. *Fertil Steril* 2011;95: 520-524.

Alviggi C, Conforti A, Carbone IF, Borrelli R, de Placido G, Guerriero S. Influence of cryopreservation on perinatal outcome after blastocyst- vs cleavage-stage embryo transfer: systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2018;51: 54-63.

Andraweera PH, Lassi ZS. Cardiovascular Risk Factors in Offspring of Preeclamptic Pregnancies-Systematic Review and Meta-Analysis. *The Journal of pediatrics* 2019;208: 104-113.e106.

Annual report Register of Birth Defects. Available at: https://www.socialstyrelsen.se/globalassets/sharepoint-dokument/artikelkatalog/statistik/2018-3-13.pdf. 2018.

Balaban B, Urman B, Ata B, Isiklar A, Larman MG, Hamilton R, Gardner DK. A randomized controlled study of human Day 3 embryo cryopreservation by slow freezing or vitrification: vitrification is associated with higher survival, metabolism and blastocyst formation. *Hum Reprod* 2008;23: 1976-1982.

Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 1993;36: 62-67.

Baumann P, Blackwell SC, Schild C, Berry SM, Friedrich HJ. Mathematic modeling to predict abruptio placentae. *Am J Obstet Gynecol* 2000;183: 815-822.

Bay B, Ingerslev HJ, Lemmen JG, Degn B, Rasmussen IA, Kesmodel US. Preimplantation genetic diagnosis: a national multicenter obstetric and neonatal follow-up study. *Fertil Steril* 2016;106: 1363-1369.e1361.

Bay B, Mortensen EL, Hvidtjørn D, Kesmodel US. Fertility treatment and risk of childhood and adolescent mental disorders: register based cohort study. *BMJ (Clinical research ed)* 2013;347: f3978-f3978.

Bellou V, Belbasis L, Tzoulaki I, Evangelou E. Risk factors for type 2 diabetes mellitus: An exposure-wide umbrella review of meta-analyses. *PLoS One* 2018;13: e0194127-e0194127.

Belva F, Roelants M, Kluijfhout S, Winter C, De Schrijver F, Desmyttere S, De Rycke M, Tournaye H, Liebaers I, Bonduelle M. Body composition and blood pressure in 6-year-old singletons born after pre-implantation genetic testing for monogenic and structural chromosomal aberrations: a matched cohort study. *Hum Reprod Open* 2018;2018: hoy013-hoy013.

Bergh T, Ericson A, Hillensjo T, Nygren KG, Wennerholm UB. Deliveries and children born after in-vitro fertilisation in Sweden 1982-95: a retrospective cohort study. *Lancet* 1999;354: 1579-1585.

Berntsen S, Pinborg A. Large for gestational age and macrosomia in singletons born after frozen/thawed embryo transfer (FET) in assisted reproductive technology (ART). *Birth Defects Res* 2018;110: 630-643.

Berntsen S, Söderström-Anttila V, Wennerholm U-B, Laivuori H, Loft A, Oldereid NB, Romundstad LB, Bergh C, Pinborg A. The health of children conceived by ART: 'the chicken or the egg?'. *Hum Reprod Update* 2019;25: 137-158.

Bolton VN, Hawes SM, Taylor CT, Parsons JH. Development of spare human preimplantation embryos in vitro: an analysis of the correlations among gross morphology, cleavage rates, and development to the blastocyst. *J In Vitro Fert Embryo Transf* 1989;6: 30-35.

Bolton VN, Wren ME, Parsons JH. Pregnancies after in vitro fertilization and transfer of human blastocysts. *Fertil Steril* 1991;55: 830-832.

Brooke HL, Talback M, Hornblad J, Johansson LA, Ludvigsson JF, Druid H, Feychting M, Ljung R. The Swedish cause of death register. *Eur J Epidemiol* 2017;32: 765-773.

Buckett WM, Chian R-C, Holzer H, Dean N, Usher R, Tan SL. Obstetric outcomes and congenital abnormalities after in vitro maturation, in vitro fertilization, and intracytoplasmic sperm injection. *Obstet Gynecol* 2007;110: 885-891.

Butt K, Lim K, Diagnostic Imaging C. Determination of gestational age by ultrasound. *Journal of obstetrics and gynaecology Canada: JOGC=Journal d'obstetrique et gynecologie du Canada: JOGC* 2014;36: 171-181.

Castillo CM, Horne G, Fitzgerald CT, Johnstone ED, Brison DR, Roberts SA. The impact of IVF on birthweight from 1991 to 2015: a cross-sectional study. *Hum Reprod* 2019;34: 920-931.

Ceelen M, van Weissenbruch MM, Prein J, Smit JJ, Vermeiden JPW, Spreeuwenberg M, van Leeuwen FE, Delemarre-van de Waal HA. Growth during infancy and early childhood in relation to blood pressure and body fat measures at age 8-18 years of IVF children and spontaneously conceived controls born to subfertile parents. *Human reproduction (Oxford, England)* 2009;24: 2788-2795.

Chang HJ, Lee JR, Jee BC, Suh CS, Kim SH. Impact of blastocyst transfer on offspring sex ratio and the monozygotic twinning rate: a systematic review and meta-analysis. *Fertil Steril* 2009;91: 2381-2390.

Chen L, Yang T, Zheng Z, Yu H, Wang H, Qin J. Birth prevalence of congenital malformations in singleton pregnancies resulting from in vitro fertilization/intracytoplasmic sperm injection worldwide: a systematic review and meta-analysis. *Arch Gynecol Obstet* 2018;297: 1115-1130.

Chen M, Wu L, Zhao J, Wu F, Davies MJ, Wittert GA, Norman RJ, Robker RL, Heilbronn LK. Altered glucose metabolism in mouse and humans conceived by IVF. *Diabetes* 2014;63: 3189-3198.

Chen Z-J, Shi Y, Sun Y, Zhang B, Liang X, Cao Y, Yang J, Liu J, Wei D, Weng N *et al.* Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome. *The New England journal of medicine* 2016;375: 523-533.

Chen ZJ, Legro RS. Fresh versus Frozen Embryos in Polycystic Ovary Syndrome. *N Engl J Med* 2016;375: e42.

Cnattingius S, Ericson A, Gunnarskog J, Kallen B. A quality study of a medical birth registry. *Scand J Soc Med* 1990;18: 143-148.

Crump C, Sundquist J, Sieh W, Winkleby MA, Sundquist K. Perinatal and familial risk factors for acute lymphoblastic leukemia in a Swedish national cohort. *Cancer* 2015;121: 1040-1047.

Dar S, Lazer T, Shah PS, Librach CL. Neonatal outcomes among singleton births after blastocyst versus cleavage stage embryo transfer: a systematic review and meta-analysis. *Hum Reprod Update* 2014;20: 439-448.

Dar S, Librach CL, Gunby J, Bissonnette F, Cowan L. Increased risk of preterm birth in singleton pregnancies after blastocyst versus Day 3 embryo transfer: Canadian ART Register (CARTR) analysis. *Hum Reprod* 2013;28: 924-928.

De Geyter C, Calhaz-Jorge C, Kupka MS, Wyns C, Mocanu E, Motrenko T, Scaravelli G, Smeenk J, Vidakovic S, Goossens V *et al.* ART in Europe, 2015: results generated from European registries by ESHRE. *Hum Reprod Open* 2020;2020: hoz038-hoz038.

De Rycke M, Goossens V, Kokkali G, Meijer-Hoogeveen M, Coonen E, Moutou C. ESHRE PGD Consortium data collection XIV-XV: cycles from January 2011 to December 2012 with pregnancy follow-up to October 2013. *Hum Reprod* 2017;32: 1974-1994.

Dean JH, Chapman MG, Sullivan EA. The effect on human sex ratio at birth by assisted reproductive technology (ART) procedures--an assessment of babies born following single embryo transfers, Australia and New Zealand, 2002-2006. *BJOG* 2010;117: 1628-1634.

Debrock S, Peeraer K, Fernandez Gallardo E, De Neubourg D, Spiessens C, D'Hooghe TM. Vitrification of cleavage stage day 3 embryos results in higher live birth rates than conventional slow freezing: a RCT. *Hum Reprod* 2015;30: 1820-1830.

Derraik JGB, Maessen SE, Gibbins JD, Cutfield WS, Lundgren M, Ahlsson F. Large-for-gestational-age phenotypes and obesity risk in adulthood: a study of 195,936 women. *Sci Rep* 2020;10: 2157-2157.

Desmyttere S, Bonduelle M, Nekkebroeck J, Roelants M, Liebaers I, De Schepper J. Growth and health outcome of 102 2-year-old children conceived after preimplantation genetic diagnosis or screening. *Early Hum Dev* 2009;85: 755-759.

Desmyttere S, De Rycke M, Staessen C, Liebaers I, De Schrijver F, Verpoest W. Neonatal follow-up of 995 consecutively born children after embryo biopsy for PGD. *Hum Reprod* 2012;27.

Ding J, Yin T, Zhang Y, Zhou D, Yang J. The effect of blastocyst transfer on newborn sex ratio and monozygotic twinning rate: an updated systematic review and meta-analysis. *Reprod Biomed Online* 2018;37: 292-303.

Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biol Reprod* 2000;62: 1526-1535.

Dumoulin JC, Land JA, Van Montfoort AP, Nelissen EC, Coonen E, Derhaag JG, Schreurs IL, Dunselman GA, Kester AD, Geraedts JP *et al.* Effect of in vitro culture of human embryos on birthweight of newborns. *Human reproduction (Oxford, England)* 2010;25: 605-612.

EIM. Available at: https://www.eshre.eu/eim.

Eldar-Geva T, Srebnik N, Altarescu G, Varshaver I, Brooks B, Levy-Lahad E. Neonatal outcome after preimplantation genetic diagnosis. *Fertil Steril* 2014;102.

Eloranta K, Auvinen A. Population attitudes towards research use of health care registries: a population-based survey in Finland. *BMC Med Ethics* 2015;16: 48.

ESHRE. Available at: https://www.eshre.eu.

Estill MS, Krawetz SA. The Epigenetic Consequences of Paternal Exposure to Environmental Contaminants and Reproductive Toxicants. *Curr Environ Health Rep* 2016;3: 202-213.

Faiz AS, Ananth CV. Etiology and risk factors for placenta previa: an overview and meta-analysis of observational studies. *J Matern Fetal Neona* 2003;13: 175-190.

Farhi A, Reichman B, Boyko V, Hourvitz A, Ron-El R, Lerner-Geva L. Maternal and neonatal health outcomes following assisted reproduction. *Reprod Biomed Online* 2013;26: 454-461.

Fasano G, Fontenelle N, Vannin AS, Biramane J, Devreker F, Englert Y, Delbaere A. A randomized controlled trial comparing two vitrification methods versus slow-freezing for cryopreservation of human cleavage stage embryos. *J Assist Reprod Genet* 2014;31: 241-247.

Fernando D, Halliday JL, Breheny S, Healy DL. Outcomes of singleton births after blastocyst versus nonblastocyst transfer in assisted reproductive technology. *Fertil Steril* 2012;97: 579-584.

Forskningsrapport från Epidemiologiskt Centrum (EpC). Utvärdering av det svenska Medicinska födelseregistret. Stockholm. 2002.

Gardner DK. The impact of physiological oxygen during culture, and vitrification for cryopreservation, on the outcome of extended culture in human IVF. *Reprod Biomed Online* 2016;32: 137-141.

Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. *Hum Reprod* 1998b;13: 3434-3440.

Gardner DK, Vella P, Lane M, Wagley L, Schlenker T, Schoolcraft WB. Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. *Fertil Steril* 1998a;69: 84-88.

Ghobara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. *Cochrane Database Syst Rev* 2017;7: Cd003414.

Ghosh J, Coutifaris C, Sapienza C, Mainigi M. Global DNA methylation levels are altered by modifiable clinical manipulations in assisted reproductive technologies. *Clin Epigenetics* 2017;9: 14-14.

Giorgione V, Parazzini F, Fesslova V, Cipriani S, Candiani M, Inversetti A, Sigismondi C, Tiberio F, Cavoretto P. Congenital heart defects in IVF/ICSI pregnancy: systematic review and meta-analysis. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology* 2018;51: 33-42.

Glinianaia SV, Rankin J, Wright C. Congenital anomalies in twins: a register-based study. *Hum Reprod* 2008;23: 1306-1311.

Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *The Cochrane database of systematic reviews* 2016: CD002118-CD002118.

Grace KS, Sinclair KD. Assisted reproductive technology, epigenetics, and long-term health: a developmental time bomb still ticking. *Semin Reprod Med* 2009;27.

Grech V, Mamo J. What is the sex ratio at birth? *Early Hum Dev* 2019: 104858-104858.

Groves FD, Watkins BT, Roberts DJ, Tucker TC, Shen T, Flood TJ. Birth Weight and Risk of Childhood Acute Lymphoblastic Leukemia in Arizona, Illinois, and Kentucky. *South Med J* 2018;111: 579-584.

Gu F, Li SZ, Zheng LY, Gu J, Li TT, Du HZ, Gao CF, Ding CH, Quan S, Zhou CQ *et al.* Perinatal outcomes of singletons following vitrification versus slow-freezing of embryos: a multicenter cohort study using propensity score analysis. *Hum Reprod* 2019;34: 1788-1798.

Gu S, An X, Fang L, Zhang X, Zhang C, Wang J, Liu Q, Zhang Y, Wei Y, Hu Z *et al.* Risk factors and long-term health consequences of macrosomia: a prospective study in Jiangsu Province, China. *J Biomed Res* 2012;26: 235-240.

Gui J, Ling Z, Hou X, Fan Y, Xie K, Shen R. In vitro fertilization is associated with the onset and progression of preeclampsia. *Placenta* 2020;89: 50-57.

Guo XY, Liu XM, Jin L, Wang TT, Ullah K, Sheng JZ, Huang HF. Cardiovascular and metabolic profiles of offspring conceived by assisted reproductive technologies: a systematic review and meta-analysis. *Fertil Steril* 2017;107: 622-631.e625.

Halliday JL, Ukoumunne OC, Baker HW, Breheny S, Jaques AM, Garrett C, Healy D, Amor D. Increased risk of blastogenesis birth defects, arising in the first 4 weeks of pregnancy, after assisted reproductive technologies. *Hum Reprod* 2010;25: 59-65.

Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature* 1990;344: 768-770.

Hansen M, Kurinczuk JJ, Milne E, de Klerk N, Bower C. Assisted reproductive technology and birth defects: a systematic review and meta-analysis. *Hum Reprod Update* 2013;19: 330-353.

Hargreave M, Jensen A, Hansen MK, Dehlendorff C, Winther JF, Schmiegelow K, Kjær SK. Association Between Fertility Treatment and Cancer Risk in Children. *JAMA* 2019;322: 2203-2210.

Hargreave M, Kjaer SK, Jørgensen ME, Jensen A. Type 1 diabetes risk in children born to women with fertility problems: a cohort study in 1.5 million Danish children. *Acta Obstet Gynecol Scand* 2016;95: 1441-1446.

Hart R, Norman RJ. The longer-term health outcomes for children born as a result of IVF treatment: Part I--General health outcomes. *Hum Reprod Update* 2013;19: 232-243.

Harton GL, De Rycke M, Fiorentino F, Moutou C, SenGupta S, Traeger-Synodinos J, Harper JC, European Society for Human R, Embryology PGDC. ESHRE PGD consortium best practice guidelines for amplification-based PGD. *Human reproduction (Oxford, England)* 2011;26: 33-40.

Hasson J, Limoni D, Malcov M, Frumkin T, Amir H, Shavit T, Bay B, Many A, Almog B. Obstetric and neonatal outcomes of pregnancies conceived after preimplantation genetic diagnosis: cohort study and meta-analysis. *Reprod Biomed Online* 2017;35: 208-218.

Hattori H, Kitamura A, Takahashi F, Kobayashi N, Sato A, Miyauchi N, Nishigori H, Mizuno S, Sakurai K, Ishikuro M *et al.* The risk of secondary sex ratio imbalance and increased monozygotic twinning after blastocyst transfer: data from the Japan Environment and Children's Study. *Reprod Biol Endocrinol* 2019;17: 27.

Haynes A, Bower C, Bulsara MK, Finn J, Jones TW, Davis EA. Perinatal risk factors for childhood Type 1 diabetes in Western Australia--a population-based study (1980-2002). *Diabetic medicine : a journal of the British Diabetic Association* 2007;24: 564-570.

He H, Jing S, Lu CF, Tan YQ, Luo KL, Zhang SP, Gong F, Lu GX, Lin G. Neonatal outcomes of live births after blastocyst biopsy in preimplantation genetic testing cycles: a follow-up of 1,721 children. *Fertil Steril* 2019;112: 82-88.

Healy DL, Breheny S, Halliday J, Jaques A, Rushford D, Garrett C, Talbot JM, Baker HWG. Prevalence and risk factors for obstetric haemorrhage in 6730 singleton births after assisted reproductive technology in Victoria Australia. *Hum Reprod* 2010;25: 265-274.

Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ* 2004;328.

Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ* 2004;328: 261.

Henningsen A-KA, Bergh C, Skjaerven R, Tiitinen A, Wennerholm U-B, Romundstad LB, Gissler M, Opdahl S, Nyboe Andersen A, Lidegaard Ø *et al.* Trends over time in congenital malformations in live-born children conceived after assisted reproductive technology. *Acta Obstet Gynecol Scand* 2018;97: 816-823.

Henningsen A-KA, Pinborg A, Lidegaard Ø, Vestergaard C, Forman JL, Andersen AN. Perinatal outcome of singleton siblings born after assisted reproductive technology and spontaneous conception: Danish national sibling-cohort study. *Fertil Steril* 2011;95: 959-963.

Hvidtjorn D, Grove J, Schendel D, Schieve LA, Svaerke C, Ernst E, Thorsen P. Risk of autism spectrum disorders in children born after assisted conception: a population-based follow-up study. *J Epidemiol Community Health* 2011;65: 497-502.

Hviid KVR, Malchau SS, Pinborg A, Nielsen HS. Determinants of monozygotic twinning in ART: a systematic review and a meta-analysis. *Hum Reprod Update* 2018;24: 468-483.

ICMART. Availble at: https://www.icmart.com.

Ievins R, Roberts SE, Goldacre MJ. Perinatal factors associated with subsequent diabetes mellitus in the child: record linkage study. *Diabetic medicine : a journal of the British Diabetic Association* 2007;24: 664-670.

Imudia AN, Awonuga AO, Doyle JO, Kaimal AJ, Wright DL, Toth TL, Styer AK. Peak serum estradiol level during controlled ovarian hyperstimulation is associated with increased risk of small for gestational age and preeclampsia in singleton pregnancies after in vitro fertilization. *Fertil Steril* 2012;97: 1374-1379.

Ingemarsson I, Källén K. Stillbirths and rate of neonatal deaths in 76,761 postterm pregnancies in Sweden, 1982-1991: a register study. *Acta Obstet Gynecol Scand* 1997;76: 658-662.

Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. *Fertil Steril* 2014;101.

Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. *Fertil Steril* 2014;101: 128-133.

Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol* 2004;103: 551-563.

Kaartinen N, Kananen K, Huhtala H, Keranen S, Tinkanen H. The freezing method of cleavage stage embryos has no impact on the weight of the newborns. *J Assist Reprod Genet* 2016;33: 393-399.

Kallen AJ, Finnstrom OO, Lindam AP, Nilsson EM, Nygren KG, Otterblad Olausson PM. Is there an increased risk for drug treated attention deficit/hyperactivity disorder in children born after in vitro fertilization? *Eur J Paediatr Neurol* 2011;15: 247-253.

Kallen B. Maternal morbidity and mortality in in-vitro fertilization. *Best Pract Res Clin Obstet Gynaecol* 2008;22: 549-558.

Kallen B, Finnstrom O, Lindam A, Nilsson E, Nygren KG, Olausson PO. Blastocyst versus cleavage stage transfer in in vitro fertilization: differences in neonatal outcome? *Fertil Steril* 2010;94: 1680-1683.

Kallen B, Finnstrom O, Lindam A, Nilsson E, Nygren KG, Otterblad PO. Congenital malformations in infants born after in vitro fertilization in Sweden. *Birth Defects Res A Clin Mol Teratol* 2010;88: 137-143.

Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: risk for congenital malformations after different IVF methods. *Birth Defects Res A Clin Mol Teratol* 2005;73: 162-169.

Kallen B, Finnstrom O, Nygren KG, Otterblad Olausson P, Wennerholm UB. In vitro fertilisation in Sweden: obstetric characteristics, maternal morbidity and mortality. *BJOG* 2005;112: 1529-1535.

Karami M, Jenabi E, Fereidooni B. The association of placenta previa and assisted reproductive techniques: a meta-analysis. *J Matern Fetal Neona* 2018;31: 1940-1947.

Kawachiya S, Bodri D, Shimada N, Kato K, Takehara Y, Kato O. Blastocyst culture is associated with an elevated incidence of monozygotic twinning after single embryo transfer. *Fertil Steril* 2011;95: 2140-2142.

Kettner LO, Matthiesen NB, Ramlau-Hansen CH, Kesmodel US, Bay B, Henriksen TB. Fertility treatment and childhood type 1 diabetes mellitus: a nationwide cohort study of 565,116 live births. *Fertil Steril* 2016;106: 1751-1756.

Kissin DM, Zhang Y, Boulet SL, Fountain C, Bearman P, Schieve L, Yeargin-Allsopp M, Jamieson DJ. Association of assisted reproductive technology (ART) treatment and parental infertility diagnosis with autism in ART-conceived children. *Human reproduction (Oxford, England)* 2015;30: 454-465.

Kuiper D, Bennema A, la Bastide-van Gemert S, Seggers J, Schendelaar P, Mastenbroek S, Hoek A, Heineman MJ, Roseboom TJ, Kok JH *et al.* Developmental outcome of 9-year-old children born after PGS: follow-up of a randomized trial. *Hum Reprod* 2018;33: 147-155.

Kuwayama M, Vajta G, Ieda S, Kato O. Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. *Reprod Biomed Online* 2005;11: 608-614.

Küpers LK, Monnereau C, Sharp GC, Yousefi P, Salas LA, Ghantous A, Page CM, Reese SE, Wilcox AJ, Czamara D *et al.* Meta-analysis of epigenome-wide association studies in neonates reveals widespread differential DNA methylation associated with birthweight. *Nat Commun* 2019;10: 1893-1893.

Lalani S, Choudhry AJ, Firth B, Bacal V, Walker M, Wen SW, Singh S, Amath A, Hodge M, Chen I. Endometriosis and adverse maternal, fetal and neonatal outcomes, a systematic review and meta-analysis. *Human reproduction (Oxford, England)* 2018;33: 1854-1865.

Lassalle B, Testart J, Renard JP. Human embryo features that influence the success of cryopreservation with the use of 1,2 propanediol. *Fertil Steril* 1985;44: 645-651.

Lee E, Illingworth P, Wilton L, Chambers GM. The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review. *Human reproduction (Oxford, England)* 2015;30: 473-483.

Levron J, Leibovitz O, Brengauz M, Gitman H, Yerushalmi GM, Katorza E, Gat I, Elizur SE. Cryopreservation of day 2-3 embryos by vitrification yields better outcome than slow freezing. *Gynecol Endocrinol* 2014;30: 202-204.

Li SJ, Ford N, Meister K, Bodurtha J. Increased risk of birth defects among children from multiple births. *Birth Defects Res A Clin Mol Teratol* 2003;67: 879-885.

Li Z, Wang YA, Ledger W, Edgar DH, Sullivan EA. Clinical outcomes following cryopreservation of blastocysts by vitrification or slow freezing: a population-based cohort study. *Hum Reprod* 2014;29: 2794-2801.

Liebaers I, Desmyttere S, Verpoest W, De Rycke M, Staessen C, Sermon K, Devroey P, Haentjens P, Bonduelle M. Report on a consecutive series of 581 children born after blastomere biopsy for preimplantation genetic diagnosis. *Hum Reprod* 2010;25: 275-282.

Liu L, Gao J, He X, Cai Y, Wang L, Fan X. Association between assisted reproductive technology and the risk of autism spectrum disorders in the offspring: a meta-analysis. *Sci Rep* 2017;7: 46207.

Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, Heurgren M, Olausson PO. External review and validation of the Swedish national inpatient register. *BMC Public Health* 2011;11: 450.

Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. *Eur J Epidemiol* 2009;24: 659-667.

Luke B. Pregnancy and birth outcomes in couples with infertility with and without assisted reproductive technology: with an emphasis on US population-based studies. *Am J Obstet Gynecol* 2017;217: 270-281.

Luke B, Gopal D, Cabral H, Diop H, Stern JE. Perinatal outcomes of singleton siblings: the effects of changing maternal fertility status. *J Assist Reprod Genet* 2016;33: 1203-1213.

Luke B, Gopal D, Cabral H, Stern JE, Diop H. Pregnancy, birth, and infant outcomes by maternal fertility status: the Massachusetts Outcomes Study of Assisted Reproductive Technology. *Am J Obstet Gynecol* 2017;217: 327.e321-327.e314.

Luna M, Duke M, Copperman A, Grunfeld L, Sandler B, Barritt J. Blastocyst embryo transfer is associated with a sex-ratio imbalance in favor of male offspring. *Fertil Steril* 2007;87: 519-523.

Maalouf WE, Mincheva MN, Campbell BK, Hardy ICW. Effects of assisted reproductive technologies on human sex ratio at birth. *Fertil Steril* 2014;101: 1321-1325.

Maheshwari A, Kalampokas T, Davidson J, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of blastocyst-stage versus cleavage-stage embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* 2013;100: 1615-1621.e1610.

Maheshwari A, Pandey S, Amalraj Raja E, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? *Hum Reprod Update* 2018;24: 35-58.

Mainigi M, Rosenzweig JM, Lei J, Mensah V, Thomaier L, Talbot CC, Jr., Olalere D, Ord T, Rozzah R, Johnston MV *et al.* Peri-Implantation Hormonal Milieu: Elucidating Mechanisms of Adverse Neurodevelopmental Outcomes. *Reproductive sciences (Thousand Oaks, Calif)* 2016;23: 785-794.

Makinen S, Soderstrom-Anttila V, Vainio J, Suikkari AM, Tuuri T. Does long in vitro culture promote large for gestational age babies? *Hum Reprod* 2013;28: 828-834.

Mani S, Mainigi M. Embryo Culture Conditions and the Epigenome. *Semin Reprod Med* 2018;36: 211-220.

Market-Velker BA, Fernandes AD, Mann MRW. Side-by-side comparison of five commercial media systems in a mouse model: suboptimal in vitro culture interferes with imprint maintenance. *Biol Reprod* 2010;83: 938-950.

Martins WP, Nastri CO, Rienzi L, van der Poel SZ, Gracia C, Racowsky C. Blastocyst vs cleavage-stage embryo transfer: systematic review and meta-analysis of reproductive outcomes. *Ultrasound Obstet Gynecol* 2017;49: 583-591.

Martins WP, Nastri CO, Rienzi L, van der Poel SZ, Gracia CR, Racowsky C. Obstetrical and perinatal outcomes following blastocyst transfer compared to cleavage transfer: a systematic review and meta-analysis. *Hum Reprod* 2016;31: 2561-2569.

Massaro PA, MacLellan DL, Anderson PA, Romao RLP. Does intracytoplasmic sperm injection pose an increased risk of genitourinary congenital malformations in offspring compared to in vitro fertilization? A systematic review and meta-analysis. *The Journal of urology* 2015;193: 1837-1842.

Mastenbroek S, Twisk M, van der Veen F, Repping S. Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. *Hum Reprod Update* 2011;17: 454-466.

Mastenbroek S, Twisk M, van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, Vogel NEA, Arts EGJM, de Vries JWA, Bossuyt PM *et al.* In vitro fertilization with preimplantation genetic screening. *The New England journal of medicine* 2007;357: 9-17.

MBR. Available at: https://www.socialstyrelsen.se/en/statistics-and-data/registers/register-information/the-swedish-medical-birth-register/.

McDonald SD, Han Z, Mulla S, Murphy KE, Beyene J, Ohlsson A. Preterm birth and low birth weight among in vitro fertilization singletons: a systematic review and meta-analyses. *Eur J Obstet Gynecol Reprod Biol* 2009;146: 138-148.

Meister TA, Rimoldi SF, Soria R, von Arx R, Messerli FH, Sartori C, Scherrer U, Rexhaj E. Association of Assisted Reproductive Technologies With Arterial Hypertension During Adolescence. *J Am Coll Cardiol* 2018;72: 1267-1274.

Ménézo YJ, Chouteau J, Torelló J, Girard A, Veiga A. Birth weight and sex ratio after transfer at the blastocyst stage in humans. *Fertil Steril* 1999;72: 221-224.

Mukaida T, Wada S, Takahashi K, Pedro PB, An TZ, Kasai M. Vitrification of human embryos based on the assessment of suitable conditions for 8-cell mouse embryos. *Hum Reprod* 1998;13: 2874-2879.

Munné S, Kaplan B, Frattarelli JL, Child T, Nakhuda G, Shamma FN, Silverberg K, Kalista T, Handyside AH, Katz-Jaffe M *et al.* Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. *Fertil Steril* 2019;112: 1071-1079.e1077.

Myatt L, Cui X. Oxidative stress in the placenta. *Histochem Cell Biol* 2004;122: 369-382.

Nakling J, Backe B. Pregnancy risk increases from 41 weeks of gestation. *Acta Obstet Gynecol Scand* 2006;85: 663-668.

Norman M, Kallen K, Wahlstrom E, Hakansson S. The Swedish Neonatal Quality Register - contents, completeness and validity. *Acta Paediatr* 2019;108: 1411-1418.
Norrman E, Petzold M, Bergh C, Wennerholm U-B. School performance in singletons born after assisted reproductive technology. *Human reproduction (Oxford, England)* 2018;33: 1948-1959.

Norrman E, Petzold M, Bergh C, Wennerholm U-B. School performance in children born after ICSI. *Human reproduction (Oxford, England)* 2020: dez281.

Norrman E, Petzold M, Clausen TD, Henningsen AK, Opdahl S, Pinborg A, Rosengren A, Bergh C, Wennerholm UB. Type 1 diabetes in children born after assisted reproductive technology: a register-based national cohort study. *Human reproduction (Oxford, England)* 2020;35: 221-231.

NPR. Available at: https://www.socialstyrelsen.se/en/statistics-and-data/registers/register-information/the-national-patient-register/.

Oldereid NB, Wennerholm U-B, Pinborg A, Loft A, Laivuori H, Petzold M, Romundstad LB, Söderström-Anttila V, Bergh C. The effect of paternal factors on perinatal and paediatric outcomes: a systematic review and meta-analysis. *Hum Reprod Update* 2018;24: 320-389.

Opdahl S, Henningsen A-KA, Bergh C, Gissler M, Romundstad LB, Petzold M, Tiitinen A, Wennerholm U-B, Pinborg AB. Data resource profile: the Committee of Nordic Assisted Reproductive Technology and Safety (CoNARTaS) cohort. *Int J Epidemiol* 2019: dyz228.

Opdahl S, Henningsen AA, Tiitinen A, Bergh C, Pinborg A, Romundstad PR, Wennerholm UB, Gissler M, Skjærven R, Romundstad LB. Risk of hypertensive disorders in pregnancies following assisted reproductive technology: a cohort study from the CoNARTaS group. *Human reproduction (Oxford, England)* 2015;30: 1724-1731.

Ornoy A. Prenatal origin of obesity and their complications: Gestational diabetes, maternal overweight and the paradoxical effects of fetal growth restriction and macrosomia. *Reproductive toxicology (Elmsford, NY)* 2011;32: 205-212.

Oron G, Nayot D, Son WY, Holzer H, Buckett W, Tulandi T. Obstetric and perinatal outcome from single cleavage transfer and single blastocyst transfer: a matched case–control study. *Gynecol Endocrinol* 2015;31.

Oron G, Son W-Y, Buckett W, Tulandi T, Holzer H. The association between embryo quality and perinatal outcome of singletons born after single embryo transfers: a pilot study. *Human reproduction (Oxford, England)* 2014;29: 1444-1451.

Osmond C, Barker DJ. Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. *Environ Health Perspect* 2000;108 Suppl 3: 545-553.

Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 1992;340: 17-18.

Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. *Hum Reprod Update* 2012;18: 485-503.

Parkinson JRC, Hyde MJ, Gale C, Santhakumaran S, Modi N. Preterm birth and the metabolic syndrome in adult life: a systematic review and meta-analysis. *Pediatrics* 2013;131: e1240-e1263.

Pelkonen S, Hartikainen AL, Ritvanen A, Koivunen R, Martikainen H, Gissler M, Tiitinen A. Major congenital anomalies in children born after frozen embryo transfer: a cohort study 1995-2006. *Hum Reprod* 2014;29: 1552-1557.

Pereira AC, Martel F. Oxidative stress in pregnancy and fertility pathologies. *Cell Biol Toxicol* 2014;30: 301-312.

Pereira N, Elias RT, Christos PJ, Petrini AC, Hancock K, Lekovich JP, Rosenwaks Z. Supraphysiologic estradiol is an independent predictor of low birth weight in full-term singletons born after fresh embryo transfer. *Hum Reprod* 2017;32: 1410-1417.

Petersen SH, Bergh C, Gissler M, Åsvold BO, Romundstad LB, Tiitinen A, Spangmose AL, Pinborg A, Wennerholm U-B, Henningsen A-KA *et al.* Time trends in placenta-mediated pregnancy complications after assisted reproductive technology in the Nordic countries. *Am J Obstet Gynecol* 2020: S0002-9378(0020)30218-30210.

Petridou ET, Sergentanis TN, Skalkidou A, Antonopoulos CN, Dessypris N, Svensson T, Stephansson O, Kieler H, Smedby KE. Maternal and birth anthropometric characteristics in relation to the risk of childhood lymphomas: a Swedish nationwide cohort study. *European journal of cancer prevention: the official journal of the European Cancer Prevention Organisation (ECP)* 2015;24: 535-541.

Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN. Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique? *Hum Reprod* 2014;29: 618-627.

Pinborg A, Loft A, Aaris Henningsen A-K, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995-2006. *Fertil Steril* 2010;94: 1320-1327.

Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Soderstrom-Anttila V. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. *Hum Reprod Update* 2013;19.

Ponjaert-Kristoffersen I, Bonduelle M, Barnes J, Nekkebroeck J, Loft A, Wennerholm UB, Tarlatzis BC, Peters C, Hagberg BS, Berner A *et al.* International collaborative study of intracytoplasmic sperm injection-conceived, in vitro fertilization-conceived, and naturally conceived 5-year-old child outcomes: cognitive and motor assessments. *Pediatrics* 2005;115: e283-e289.

Qin J, Liu X, Sheng X, Wang H, Gao S. Assisted reproductive technology and the risk of pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies: a meta-analysis of cohort studies. *Fertil Steril* 2016;105.

Qin J-B, Sheng X-Q, Wu D, Gao S-Y, You Y-P, Yang T-B, Wang H. Worldwide prevalence of adverse pregnancy outcomes among singleton pregnancies after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Arch Gynecol Obstet* 2017;295: 285-301.

Redman CWG, Sargent IL. Immunology of pre-eclampsia. *American journal of reproductive immunology (New York, NY : 1989)* 2010;63: 534-543.

Report ”For safety's sake”. The population's attitude to the benefits and risks of digital health data. (För säkerhets skull, Befolkningens inställning till nytta och risker med digitala hälsouppgifter) Stockholm. 2017.

Research Report from Centre for Epidemiology (EpC). The Swedish Medical Birth Register - A summary of content and quality. The National Board of Health and Welfare. 2003.

Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, Vanderpoel S, Racowsky C. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. *Hum Reprod Update* 2017;23: 139-155.

Roman E, Lightfoot T, Smith AG, Forman MR, Linet MS, Robison L, Simpson J, Kaatsch P, Grell K, Frederiksen K *et al.* Childhood acute lymphoblastic leukaemia and birthweight: insights from a pooled analysis of case-control data from Germany, the United Kingdom and the United States. *European journal of cancer (Oxford, England : 1990)* 2013;49: 1437-1447.

Rombauts L, Motteram C, Berkowitz E, Fernando S. Risk of placenta praevia is linked to endometrial thickness in a retrospective cohort study of 4537 singleton assisted reproduction technology births. *Hum Reprod* 2014;29: 2787-2793.

Romundstad LB, Romundstad PR, Sunde A, von During V, Skjaerven R, Vatten LJ. Increased risk of placenta previa in pregnancies following IVF/ICSI; a comparison of ART and non-ART pregnancies in the same mother. *Hum Reprod* 2006;21: 2353-2358.

Romundstad LB, Romundstad PR, Sunde A, von Düring V, Skjaerven R, Gunnell D, Vatten LJ. Effects of technology or maternal factors on perinatal outcome after assisted fertilisation: a population-based cohort study. *Lancet (London, England)* 2008;372: 737-743.

Roos N, Kieler H, Sahlin L, Ekman-Ordeberg G, Falconer H, Stephansson O. Risk of adverse pregnancy outcomes in women with polycystic ovary syndrome: population based cohort study. *BMJ* 2011;343: d6309.

Roque M, Haahr T, Geber S, Esteves SC, Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. *Hum Reprod Update* 2019;25: 2-14.

Rumbold AR, Moore VM, Whitrow MJ, Oswald TK, Moran LJ, Fernandez RC, Barnhart KT, Davies MJ. The impact of specific fertility treatments on cognitive development in childhood and adolescence: a systematic review. *Human reproduction (Oxford, England)* 2017;32: 1489-1507.

Saito K, Kuwahara A, Ishikawa T, Morisaki N, Miyado M, Miyado K, Fukami M, Miyasaka N, Ishihara O, Irahara M *et al.* Endometrial preparation methods for frozen-thawed embryo transfer are associated with altered risks of hypertensive disorders of pregnancy, placenta accreta, and gestational diabetes mellitus. *Hum Reprod* 2019;34: 1567-1575.

Sandin S, Nygren KG, Iliadou A, Hultman CM, Reichenberg A. Autism and mental retardation among offspring born after in vitro fertilization. *JAMA* 2013;310: 75-84.

SART. Available at: https://www.sart.org.

Sazonova A, Kallen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos. *Hum Reprod* 2012;27: 1343-1350.

Sazonova A, Källen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos. *Hum Reprod* 2012;27.

SCB. Available at: scb.se/en.

Scherrer U, Rexhaj E, Allemann Y, Sartori C, Rimoldi SF. Cardiovascular dysfunction in children conceived by assisted reproductive technologies. *Eur Heart J* 2015;36: 1583-1589.

Schmidt L, Sobotka T, Bentzen JG, Nyboe Andersen A. Demographic and medical consequences of the postponement of parenthood. *Hum Reprod Update* 2012;18: 29-43.
Sha T, Yin X, Cheng W, Massey IY. Pregnancy-related complications and perinatal outcomes resulting from transfer of cryopreserved versus fresh embryos in vitro fertilization: a meta-analysis. *Fertil Steril* 2018;109: 330-342.e339.

Shi Y, Sun Y, Hao C, Zhang H, Wei D, Zhang Y, Zhu Y, Deng X, Qi X, Li H *et al.* Transfer of Fresh versus Frozen Embryos in Ovulatory Women. *N Engl J Med* 2018;378: 126-136.

Smith GC. Life-table analysis of the risk of perinatal death at term and post term in singleton pregnancies. *Am J Obstet Gynecol* 2001;184: 489-496.

SNQ. Available at: https://www.medscinet.com/PNQ/.

Spangmose AL, Malchau SS, Henningsen AA, Forman JL, Rasmussen S, Loft A, Schmidt L, Pinborg A. Academic performance in adolescents aged 15-16 years born after frozen embryo transfer compared with fresh embryo transfer: a nationwide registry-based cohort study. *BJOG : an international journal of obstetrics and gynaecology* 2019;126: 261-269.

Spangmose AL, Malchau SS, Schmidt L, Vassard D, Rasmussen S, Loft A, Forman J, Pinborg A. Academic performance in adolescents born after ART-a nationwide registry-based cohort study. *Human reproduction (Oxford, England)* 2017;32: 447-456.

Spangmose AL, Opdahl S, Malchau S, Ginström Ernstad E, Romundstad LB, Wennerholm UB, Bergh C, Henningsen AK, Pinborg A. Obstetric and perinatal risks i 8368 singletons and 1167 twins conceived after fresh and frozen blastocyst transfers in the Nordic countries - a CoNARTaS collaboration. Abstract (O-035) presented at: Annual meeting of European Society of Human Reproduction and Embryology (ESHRE), 2019 June 23-26, Vienna, Austria. 2019.

Spector LG, Brown MB, Wantman E, Letterie GS, Toner JP, Doody K, Ginsburg E, Williams M, Koch L, Schymura MJ *et al.* Association of In Vitro Fertilization With Childhood Cancer in the United States. *JAMA Pediatr* 2019;173: e190392-e190392.

Stehlik E, Stehlik J, Katayama KP, Kuwayama M, Jambor V, Brohammer R, Kato O. Vitrification demonstrates significant improvement versus slow freezing of human blastocysts. *Reprod Biomed Online* 2005;11: 53-57.

Stephansson O, Kieler H, Granath F, Falconer H. Endometriosis, assisted reproduction technology, and risk of adverse pregnancy outcome. *Human reproduction (Oxford, England)* 2009;24: 2341-2347.

Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. *Lancet* 1978;2: 366.

Stormlund S, Sopa N, Zedeler A, Bogstad J, Prætorius L, Nielsen H, Kitlinski M, Skouby S, Mikkelsen A, Polyzos N *et al.* Freeze-all versus fresh embryo transfer in ART: A multicentre randomised controlled trial in normo-ovulatory women. Abstract (O-069) presented at: Annual meeting of European Society of Human Reproduction and Embryology (ESHRE), 2019 June 23-26, Vienna, Austria. 2019.

Stromberg B, Dahlquist G, Ericson A, Finnstrom O, Koster M, Stjernqvist K. Neurological sequelae in children born after in-vitro fertilisation: a population-based study. *Lancet* 2002;359: 461-465.

Sundh KJ, Henningsen AK, Kallen K, Bergh C, Romundstad LB, Gissler M, Pinborg A, Skjaerven R, Tiitinen A, Vassard D *et al.* Cancer in children and young adults born after assisted reproductive technology: a Nordic cohort study from the Committee of Nordic ART and Safety (CoNARTaS). *Hum Reprod* 2014;29: 2050-2057.

Sunkara SK, Antonisamy B, Selliah HY, Kamath MS. Pre-term birth and low birth weight following preimplantation genetic diagnosis: analysis of 88 010 singleton live births following PGD and IVF cycles. *Hum Reprod* 2017;32: 432-438.

Svahn MF, Hargreave M, Nielsen TSS, Plessen KJ, Jensen SM, Kjaer SK, Jensen A. Mental disorders in childhood and young adulthood among children born to women with fertility problems. *Human reproduction (Oxford, England)* 2015;30: 2129-2137.

Tararbit K, Lelong N, Jouannic JM, Goffinet F, Khoshnood B, Group ES. Is the probability of prenatal diagnosis or termination of pregnancy different for fetuses with congenital anomalies conceived following assisted reproductive techniques? A population-based evaluation of fetuses with congenital heart defects. *BJOG : an international journal of obstetrics and gynaecology* 2015;122: 924-931.

Tarín JJ, Bernabeu R, Baviera A, Bonada M, Cano A. Sex selection may be inadvertently performed in in-vitro fertilization-embryo transfer programmes. *Human reproduction (Oxford, England)* 1995;10: 2992-2998.

Tarín JJ, García-Pérez MA, Hermenegildo C, Cano A. Changes in sex ratio from fertilization to birth in assisted-reproductive-treatment cycles. *Reproductive biology and endocrinology : RB&E* 2014;12: 56-56.

Testart J, Lassalle B, Belaisch-Allart J, Hazout A, Forman R, Rainhorn JD, Frydman R. High pregnancy rate after early human embryo freezing. *Fertil Steril* 1986;46: 268-272.

the Swedish Pregnancy Register. Available at: https://www.medscinet.com/gr/engelska.aspx.

Thomopoulos C, Tsioufis C, Michalopoulou H, Makris T, Papademetriou V, Stefanadis C. Assisted reproductive technology and pregnancy-related hypertensive complications: a systematic review. *J Hum Hypertens* 2013;27: 148-157.

Thurin A, Hausken J, Hillensjo T, Jablonowska B, Pinborg A, Strandell A, Bergh C. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. *N Engl J Med* 2004;351: 2392-2402.

Tikkanen M, Nuutila M, Hiilesmaa V, Paavonen J, Ylikorkala O. Prepregnancy risk factors for placental abruption. *Acta Obstet Gynecol Scand* 2006a;85: 40-44.

Tikkanen M, Nuutila M, Hiilesmaa V, Paavonen J, Ylikorkala O. Clinical presentation and risk factors of placental abruption. *Acta Obstet Gynecol Scand* 2006b;85: 700-705.

Triebwasser C, Wang R, DeWan AT, Metayer C, Morimoto L, Wiemels JL, Kadan-Lottick N, Ma X. Birth weight and risk of paediatric Hodgkin lymphoma: Findings from a population-based record linkage study in California. *European journal of cancer (Oxford, England : 1990)* 2016;69: 19-27.

Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. *Nature* 1983;305: 707-709.

Tucker FD, Morris JK, Committee JRCM, Neville A, Garne E, Kinsner-Ovaskainen A, Lanzoni M, Loane MA, Martin S, Nicholl C *et al.* EUROCAT: an update on its functions and activities. *J Community Genet* 2018;9: 407-410.

Vermey BG, Buchanan A, Chambers GM, Kolibianakis EM, Bosdou J, Chapman MG, Venetis CA. Are singleton pregnancies after assisted reproduction technology (ART) associated with a higher risk of placental anomalies compared with non-ART singleton pregnancies? A systematic review and meta-analysis. *BJOG : an international journal of obstetrics and gynaecology* 2019;126: 209-218.

Verpoest W, Staessen C, Bossuyt PM, Goossens V, Altarescu G, Bonduelle M, Devesa M, Eldar-Geva T, Gianaroli L, Griesinger G *et al.* Preimplantation genetic testing for aneuploidy by microarray analysis of polar bodies in advanced maternal age: a randomized clinical trial. *Human reproduction (Oxford, England)* 2018;33: 1767-1776.

Vilska S, Tiitinen A, Hydén-Granskog C, Hovatta O. Elective transfer of one embryo results in an acceptable pregnancy rate and eliminates the risk of multiple birth. *Human reproduction (Oxford, England)* 1999;14: 2392-2395.

Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. *Am J Epidemiol* 2007;165: 710-718.

von Versen-Hoynck F, Häckl S, Selamet Tierney ES, Conrad KP, Baker VL, Winn VD. Maternal Vascular Health in Pregnancy and Postpartum After Assisted Reproduction. *Hypertension (Dallas, Tex : 1979)* 2020;75: 549-560.

von Versen-Hoynck F, Narasimhan P, Selamet Tierney ES, Martinez N, Conrad KP, Baker VL, Winn VD. Absent or Excessive Corpus Luteum Number Is Associated With Altered Maternal Vascular Health in Early Pregnancy. *Hypertension* 2019;73: 680-690.

von Versen-Hoynck F, Schaub AM, Chi YY, Chiu KH, Liu J, Lingis M, Stan Williams R, Rhoton-Vlasak A, Nichols WW, Fleischmann RR *et al.* Increased Preeclampsia Risk and Reduced Aortic Compliance With In Vitro Fertilization Cycles in the Absence of a Corpus Luteum. *Hypertension* 2019;73: 640-649.

von Versen-Hoynck F, Strauch NK, Liu J, Chi YY, Keller-Woods M, Conrad KP, Baker VL. Effect of Mode of Conception on Maternal Serum Relaxin, Creatinine, and Sodium Concentrations in an Infertile Population. *Reprod Sci* 2019;26: 412-419.

Vrooman LA, Bartolomei MS. Can assisted reproductive technologies cause adult-onset disease? Evidence from human and mouse. *Reproductive toxicology (Elmsford, NY)* 2017;68: 72-84.

Vuong LN, Dang VQ, Ho TM, Huynh BG, Ha DT, Pham TD, Nguyen LK, Norman RJ, Mol BW. IVF Transfer of Fresh or Frozen Embryos in Women without Polycystic Ovaries. *N Engl J Med* 2018;378: 137-147.

Wang T, Chen L, Yang T, Wang L, Zhao L, Zhang S, Ye Z, Chen L, Zheng Z, Qin J. Cancer risk among children conceived by fertility treatment. *Int J Cancer* 2019;144: 3001-3013.

Wang Z, Xu L, He F. Embryo vitrification affects the methylation of the H19/Igf2 differentially methylated domain and the expression of H19 and Igf2. *Fertil Steril* 2010;93: 2729-2733.

Wei D, Liu JY, Sun Y, Shi Y, Zhang B, Liu JQ, Tan J, Liang X, Cao Y, Wang Z *et al.* Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial. *Lancet* 2019;393: 1310-1318.

Wen J, Jiang J, Ding C, Dai J, Liu Y, Xia Y, Liu J, Hu Z. Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. *Fertil Steril* 2012;97: 1331-1337.e1374.

Wennerholm U-B, Saltvedt S, Wessberg A, Alkmark M, Bergh C, Wendel SB, Fadl H, Jonsson M, Ladfors L, Sengpiel V *et al.* Induction of labour at 41 weeks versus expectant management and induction of labour at 42 weeks (SWEdish Post-term Induction Study, SWEPIS): multicentre, open label, randomised, superiority trial. *BMJ (Clinical research ed)* 2019;367: l6131-l6131.

Wennerholm UB, Henningsen AK, Romundstad LB, Bergh C, Pinborg A, Skjaerven R, Forman J, Gissler M, Nygren KG, Tiitinen A. Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTaS group. *Hum Reprod* 2013;28: 2545-2553.

Wikland M, Hardarson T, Hillensjo T, Westin C, Westlander G, Wood M, Wennerholm UB. Obstetric outcomes after transfer of vitrified blastocysts. *Hum Reprod* 2010;25: 1699-1707.

Williams CL, Bunch KJ, Stiller CA, Murphy MF, Botting BJ, Wallace WH, Davies M, Sutcliffe AG. Cancer risk among children born after assisted conception. *N Engl J Med* 2013;369: 1819-1827.

Wu P, Gulati M, Kwok CS, Wong CW, Narain A, O'Brien S, Chew-Graham CA, Verma G, Kadam UT, Mamas MA. Preterm Delivery and Future Risk of Maternal Cardiovascular Disease: A Systematic Review and Meta-Analysis. *J Am Heart Assoc* 2018;7: e007809.

www.cdc.gov/art.

www.conartas.com.

www.eurocat-network.eu.

www.kvalitetsregister.se.

www.q-ivf.se.

www.socialtyrelsen.se/en.

Young LE, Sinclair KD, Wilmut I. Large offspring syndrome in cattle and sheep. *Rev Reprod* 1998;3: 155-163.

Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, Rienzi L, Sunde A, Schmidt L, Cooke ID *et al.* The International Glossary on Infertility and Fertility Care, 2017. *Hum Reprod* 2017;32: 1786-1801.

Zeilmaker GH, Alberda AT, van Gent I, Rijkmans CM, Drogendijk AC. Two pregnancies following transfer of intact frozen-thawed embryos. *Fertil Steril* 1984;42: 293-296.

Zhang B, Wei D, Legro RS, Shi Y, Li J, Zhang L, Hong Y, Sun G, Zhang T, Li W *et al.* Obstetric complications after frozen versus fresh embryo transfer in women with polycystic ovary syndrome: results from a randomized trial. *Fertil Steril* 2018;109: 324-329.

Zhang WY, von Versen-Hoynck F, Kapphahn KI, Fleischmann RR, Zhao Q, Baker VL. Maternal and neonatal outcomes associated with trophectoderm biopsy. *Fertil Steril* 2019;112: 283-290.e282.

Zhang X-H, Qiu L-Q, Huang J-P. Risk of birth defects increased in multiple births. *Birth defects research Part A, Clinical and molecular teratology* 2011;91: 34-38.

Zhao J, Yan Y, Huang X, Li Y. Do the children born after assisted reproductive technology have an increased risk of birth defects? A systematic review and meta-analysis. *J Matern Fetal Neona* 2020;33: 322-333.
Zhu J, Lin S, Li M, Chen L, Lian Y, Liu P, Qiao J. Effect of in vitro culture period on birthweight of singleton newborns. *Hum Reprod* 2014;29: 448-454.

Zhu J, Zhu Q, Wang Y, Wang B, Lyu Q, Kuang Y. Comparative study on risk for birth defects among infants after in vitro fertilization and intracytoplasmic sperm injection. *Syst Biol Reprod Med* 2019;65: 54-60.