

# Long-term outcome of children born after Assisted Reproductive Technology

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UNIVERSITY OF GOTHENBURG

Gothenburg 2020

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ISBN 978-91-8009-038-4 (PRINT)

ISBN 978-91-8009-039-1 (PDF)

<http://hdl.handle.net/2077/65149>

Printed in Borås, Sweden 2020

Printed by Stema Specialtryck AB

“All statistical models are wrong but some are useful”

*George Box*



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**Background:** While the short-term outcome after assisted reproductive technology (ART) is broadly examined, studies on the long-term outcome of ART children are limited.

**Aim:** To examine the long-term outcome in children born after ART and study whether different ART techniques affect the outcome in the children differently.

**Material and methods:** All papers were national population-based register studies, performed by cross-linking national ART registers with health data registers. *Paper I-III* included all singletons born in Sweden: after ART (n=8 323) and spontaneous conception (SC) (n=1 499 667) between 1985 and 2001 (*Paper I*), after intracytoplasmic sperm injection (ICSI) (n=6 953), standard *in vitro* fertilization (IVF) (n=11 713) and SC (n=2 022 995) between 1985 and 2006 (*Paper II*) and after ART (n=47 938) and SC (n=3 090 602) between 1985 and 2015 (*Paper III*). In *Paper IV* all singletons born after ART (n=122 429) and SC (n=7 574 685) in Sweden, Norway, Finland and Denmark between 1984 and 2015 were included. The primary outcomes were school performance (*Paper I and II*), type 1 diabetes (*Paper III*), cardiovascular disease (ischemic heart disease, cardiomyopathy, heart failure or cerebrovascular disease), obesity and type 2 diabetes (*Paper IV*).

**Results:** *Paper I:* ART children had significantly better school results than SC children in the crude analyses. After adjustment, a small but significant difference was observed in total scores in favour of SC children (adjusted mean difference [percentiles] -0.72; 95% confidence interval -1.31 to -0.12; p=0.018). *Paper II:* ICSI children had similar school performance as IVF and SC children in the ninth grade. In the third grade, ICSI children had lower chance of passing all the subtests in mathematics and Swedish compared to SC children. *Paper III:* ART children had no increased risk of type 1 diabetes after adjustment for important covariates. In a subgroup analysis, children born after frozen embryo transfer had increased risk of type 1 diabetes compared to children born after fresh embryo transfer and SC. *Paper IV:* No increased risk of cardiovascular disease or type 2 diabetes were found among ART children in the adjusted analyses. A small but significantly increased risk of obesity was found.

**Conclusion:** School performance up to ninth grade is reassuring for ART children. Cardiometabolic outcomes in ART children are also generally reassuring. However, the number of events were limited for several diseases and small negative differences were observed in a few analyses. Previous studies of ART children have repeatedly suggested small differences in cardiometabolic surrogate outcomes, emphasizing a need for further studies.

**Keywords:** assisted reproductive technology, IVF, children, long-term outcome, school performance, neurodevelopmental, diabetes, cardiovascular disease, obesity

ISBN 978-91-8009-038-4 (PRINT)

ISBN 978-91-8009-039-1 (PDF)



# List of papers

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

- I. Norrman E, Petzold M, Bergh C, Wennerholm UB. School performance in singletons born after assisted reproductive technology.  
*Hum Reprod* 2018;**33**:1948-1959.
- II. Norrman E, Petzold M, Bergh C, Wennerholm UB. School performance in children born after ICSI.  
*Hum Reprod* 2020;**35**:340-354.
- III. 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.  
*Hum Reprod* 2020;**35**:221-231.
- IV. Norrman E, Petzold M, Gissler M, Spangmose AL, Opdahl S, Pinborg A, Henningsen AK, Tiitinen A, Rosengren A, Romundstad LB, Wennerholm UB, Bergh C. Cardiovascular disease, obesity and type 2 diabetes in children born after assisted reproductive technology: a population based cohort study.  
*In manuscript.*

# Sammanfattning på svenska

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Infertilitet, eller ofrivillig barnlöshet, definieras av WHO som utebliven graviditet trots regelbundna oskyddade samlag under ett års tid. Detta drabbar omkring ett av sex par och efter utredning kan assisterad befruktning (ART) erbjudas. Det första barnet efter ART i Sverige föddes 1982 och efter det har tekniken utvecklats enormt och ökat drastiskt. Idag föds det mer än 5000 barn efter ART i Sverige årligen. Det är välkänt att barn födda efter ART har ökade risker för bland annat förtidsbörd och låg födelsevikt. Denna riskökning kan till stor del förklaras av en högre andel flerbördsgraviditeter efter ART men även enkelbördsgraviditeter är förbundna med ökade perinatale och obstetriska risker. Medan de perinatale och obstetriska riskerna, associerade med ART, är väl undersökta finns det fortfarande relativt lite studier på hur det går för ART barnen på lång sikt. Syftet med denna avhandling var att undersöka ART barnens långtidsutfall och även att undersöka om olika tekniker som intracytoplasmatisk spermieinjektion (ICSI) och återföring av frysta/tinade embryon kan påverka barnen på lång sikt.

I *delarbete I* jämfördes skolbetyg i årskurs 9 hos alla barn födda efter ART (enkelbörd) med alla barn födda efter spontan befruktning, mellan 1985 till 2001 i Sverige. De ojusterade resultaten visade att ART barnen presterade cirka 10% bättre i skolan jämfört med barn födda efter spontan befruktning. Efter justering för viktiga kovariater, som föräldrarnas utbildningsnivå, fanns små men signifikanta skillnader till fördel för barn födda efter spontan befruktning. Skillnaderna var dock så små att de inte bedömdes vara kliniskt relevanta. I *delarbete II* jämfördes skolbetyg i årskurs 9 hos alla barn födda efter ICSI med alla barn födda efter standard in vitro fertilisering (IVF) och alla barn födda efter spontan befruktning mellan 1985 och 2006 i Sverige. Dessutom jämfördes de nationella proven i matematik och svenska i trean mellan samma grupper. Resultaten visade att barn födda efter ICSI presterade lika bra som barn födda efter standard IVF. När ICSI barnen jämfördes med barn födda efter spontan befruktning var det ingen skillnad på betygen i nionde klass men i de nationella proven i tredje klass hade ICSI barnen något lägre chans att klara alla deltest i matematik och svenska. I *delarbete III* jämfördes risken för att utveckla typ 1 diabetes mellan alla barn födda 1985 till 2015 efter ART och spontan befruktning i Sverige. Även två subanalyser gjordes. Den första subanalysen jämförde barn födda efter återföring av frysta/tinade embryon med barn födda efter återföring av färska embryon och barn födda efter spontan befruktning. Den andra subanalysen jämförde



barn födda efter ICSI med barn födda efter standard IVF och barn födda efter spontan befruktning. Resultaten visade att barn födda efter ART inte hade någon ökad risk för att utveckla typ 1 diabetes i de justerade analyserna. Inte heller barn födda efter ICSI hade någon ökad risk för att utveckla typ 1 diabetes. Barn födda efter återföring av frysta/tinade embryon hade dock en ökad risk för att utveckla typ 1 diabetes jämfört med barn födda efter återföring av färska embryon och spontan befruktning. Resultaten var dock baserade på få individer och ska därför tolkas med försiktighet. I *delarbete IV* jämfördes risken för att utveckla kardiovaskulär sjukdom (ischemisk hjärtsjukdom, kardiomyopati, hjärtsvikt eller cerebrovaskulär sjukdom), fetma och typ 2 diabetes hos ART barn jämfört med barn födda efter spontan befruktning. Resultaten visade att ART barnen inte hade någon ökad risk för att utveckla kardiovaskulär sjukdom eller typ 2 diabetes efter att justering gjorts för viktiga kovariater. Däremot noterades en liten men signifikant ökad risk för fetma hos ART barnen jämfört med barn födda efter spontan befruktning. Alla resultat i delarbete IV ska tolkas med försiktighet då det fanns en del metodologiska begränsningar med studien.

# Abbreviations and definitions

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ADHD	Attention deficit hyperactivity disorder
AHR	Adjusted hazard ratio
ALL	Acute lymphoblastic leukemia
AOR	Adjusted odds ratio
ARR	Adjusted relative risk
ART	Assisted reproductive technology
ASD	Autism spectrum disorders
BMI	Body mass index (kg/m <sup>2</sup> )
BORIS	Swedish National Register for Treatment of Childhood Obesity
CDC	Centers for Disease Control and Prevention
CDR	Cause of Death Register
CI	Confidence interval
CNS	Central nervous system
CoNARTaS	Committee of Nordic ART and Safety
CP	Cerebral palsy
CPUP	Cerebral Palsy Follow Up Register
DET	Double embryo transfer
EIM	European IVF Monitoring Consortium
ESHRE	European Society of Human Reproduction and Embryology
FET	Frozen embryo transfer
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin releasing hormone
hCG	Human chorionic gonadotrophin
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 version
ICMART	The International Committee Monitoring Assisted Reproductive Technologies
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilization
LBW	Low birth weight (<2500 grams)

LGA	Large for gestational age (more than two standard deviations above Swedish growth standard)
LH	Luteinizing hormone
MBR	Medical Birth Register
NDR	National Diabetes Register
NPR	National Patient Register
OD	Oocyte donation
OHSS	Ovarian hyperstimulation syndrome
OR	Odds ratio
PCOS	Polycystic ovarian syndrome
Perinatal mortality	Stillbirths and deaths in the first week of life
PESA	Percutaneous epididymal sperm aspiration
PIN	Personal identification number
PTB	Preterm birth (<37 weeks of gestation)
Q-IVF	The Swedish National Register for Assisted Reproduction
RR	Relative ratio
SC	Spontaneous conception; spontaneously conceived
SCB	Statistics Sweden
SD	Standard deviation
SET	Single embryo transfer
SGA	Small for gestational age (more than two standard deviations below Swedish growth standard)
SIR	Standardized incidence ratio
SPDR	Swedish Prescribed Drug Register
Stillbirth	Definition in Sweden: intrauterine fetal death $\geq 22$ weeks of gestation from July 1, 2008 ( $\geq 28$ weeks of gestation before July, 2008)
SWEDIABKIDS	Swedish National Register for Child and Juvenile Diabetes
TESA	Testicular sperm aspiration
VLBW	Very low birth weight (<1500 grams)
VPTB	Very preterm birth (<32 weeks of gestation)
WHO	World Health Organization



# Contents

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Introduction.....	15
Infertility.....	15
IVF procedure .....	16
ART legislation in Sweden .....	20
ART monitoring .....	21
Results after ART .....	22
Complications after ART .....	22
Perinatal and obstetric outcomes after ART .....	23
Trends over time in ART .....	28
Long-term outcome in children born after ART .....	29
Aims of the thesis .....	41
Methods .....	43
Study design and settings .....	43
Ethical aspects .....	43
Data collection.....	45
Paper I-III .....	45
Paper IV.....	47
Outcomes.....	49
Paper I and II .....	49
Paper III and IV.....	50
Statistical methods .....	50
Paper I and II.....	51
Paper III and IV.....	51
Results.....	53
Paper I .....	53
Paper II .....	56
Paper III.....	61
Paper IV.....	64
Discussion.....	67
The specific outcomes in the papers .....	67
General discussion .....	73
Conclusion .....	77
Future perspectives .....	79
Acknowledgements.....	81
References.....	83



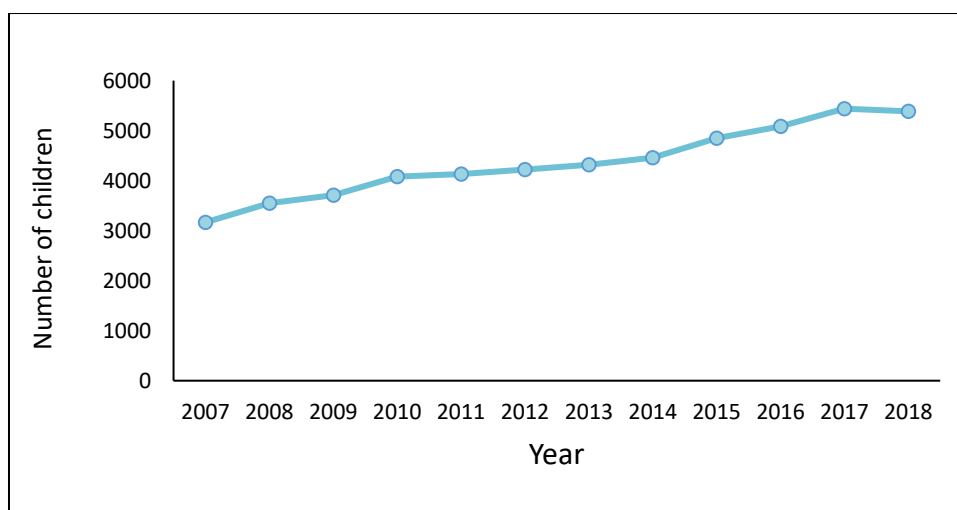
# Introduction

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## Infertility

The World Health Organization (WHO) defines infertility as failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Today, some form of infertility problems affects approximately one in six couples. The causes of infertility may be female factors (20-35%), male factors (20-30%) or both female and male factors (25-40%). In about 10-20% of couples no cause is found, thus named unexplained infertility (ESHRE art fact sheet, 2020). Female factors include ovarian conditions, endometriosis, tubal or uterine conditions. Male factors include poor semen quality or quantity or a combination of both. Lifestyle factors such as smoking and bodyweight are also important aspects, as well as increased age of the female partner, which is essential.

After medical investigation, patients can be offered assisted reproductive technology (ART). ART comprise all interventions that include *in vitro* handling of both human oocytes and sperm, or of embryos for the purpose of reproduction (Zegers-Hochschild *et al.*, 2017). This includes standard *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), freezing and thawing of embryos, as well as sperm and oocyte donation (OD).

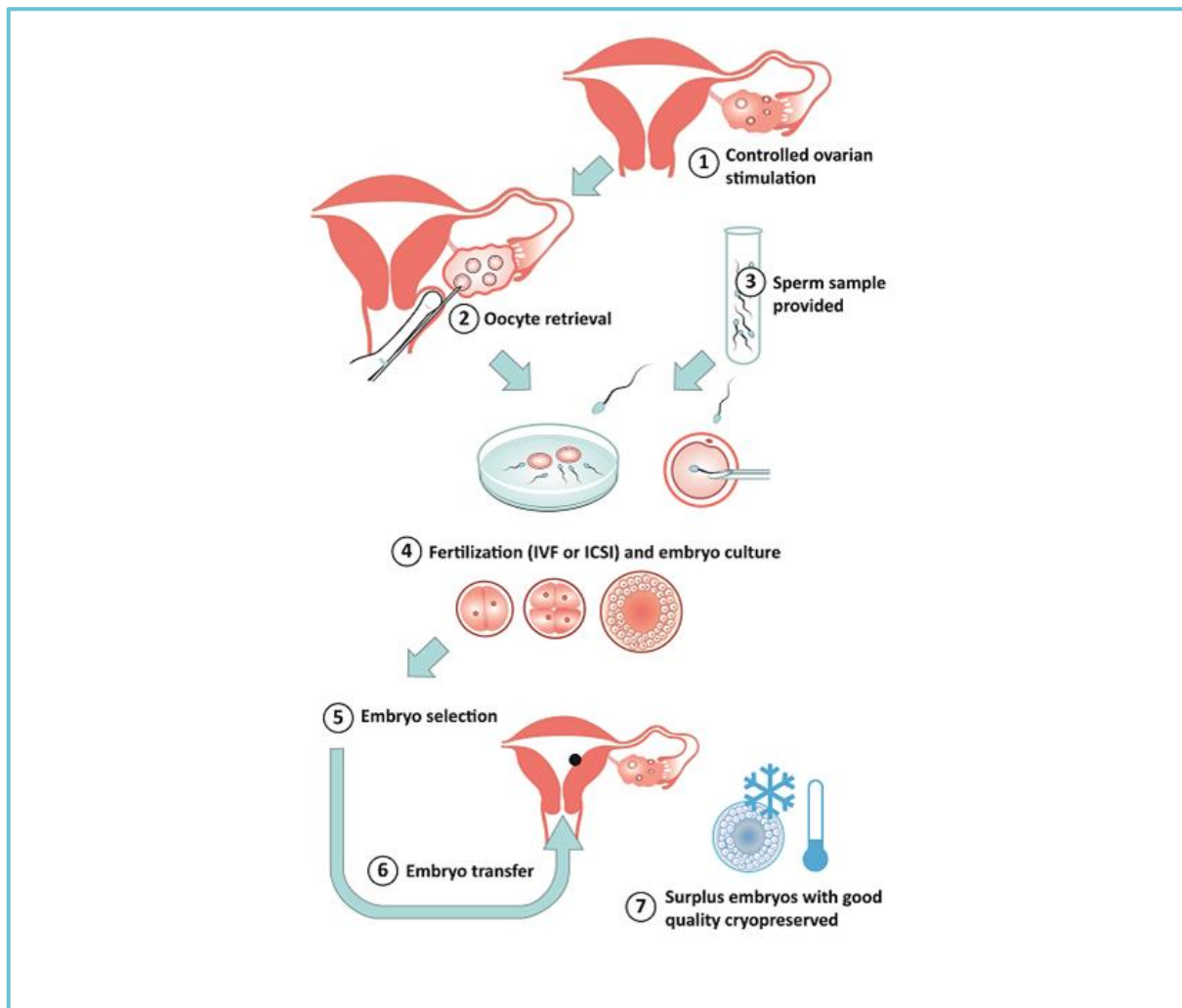


**Figure 1.** Number of live-born ART children per year in Sweden 2007-2018. From Q-IVF (<http://www.medscinet.com/qivf>)

The first IVF baby was born in 1978 (Steptoe and Edwards, 1978), and since then more than 9 million children have been born after ART (ESHRE art fact sheet, 2020). The use of ART has increased significantly during the past decades with more than 390 000 children born worldwide annually (Adamson *et al.*, 2018). In Sweden, the first child after IVF was born in 1982, and more than 5 000 children are born after ART each year. Thereby, ART children constituted 4.6% of all newborn children in Sweden in 2018 (<http://www.medscinet.com/qivf>).

## IVF procedure

The main steps in IVF are controlled ovarian stimulation, oocyte retrieval, fertilization (standard IVF/ICSI), embryo culture, embryo selection and embryo transfer to the uterus.



**Figure 2.** The IVF procedure. Illustration: Jan Funke



### **Controlled ovarian stimulation**

There are two main approaches of ovarian stimulation for IVF: the long gonadotrophin releasing hormone (GnRH) agonist protocol and the short GnRH antagonist protocol. The long GnRH agonist protocol, which traditionally has been used, starts with approximately two weeks of downregulation of ovarian function and synchronizing of the follicles. The short GnRH antagonist protocol, which has gained popularity during the last years, starts in early follicular phase. The GnRH antagonist is usually added on day 5, when follicle growth is initiated, to prevent spontaneous ovulation. Ovarian stimulation is in both protocols performed with daily injections of follicle stimulating hormone (FSH) or human menopausal gonadotropin (hMG), monitored by serum estradiol levels and/or vaginal ultrasound. To induce the final oocyte maturation, human chorionic gonadotropin (hCG) is given as a single injection.

Initially, the short GnRH antagonist protocol was used for polycystic ovarian syndrome (PCOS) patients and high responders due to the lower risk of ovarian hyperstimulation syndrome (OHSS). However, in recent years, studies have shown that also patients with normal ovarian reserve could benefit from the antagonist protocol since the side-effects are less and the risk of OHSS is lower but the pregnancy rate and live birth rate are similar to those with the GnRH agonist protocol (Wang *et al.*, 2017). Another advantage with the antagonist protocol, is the possibility to induce oocyte maturation by a GnRH agonist instead of hCG, resulting in an endogenous luteinizing hormone (LH)-surge. In this way, the risk of OHSS is almost completely prevented since severe events observed in OHSS are mediated by hCG (Kol and Humaidan, 2013). However, all embryos are cryopreserved in such cycles and used for subsequent frozen embryo transfer (FET) since it has been shown that the luteal phase in these fresh cycles is insufficient. This management has been called the freeze-all strategy.

### **Oocyte retrieval**

Approximately 36 hours after the administration of hCG, the oocytes are collected from the ovaries with transvaginal ultrasound-guided follicle aspiration. Several large cohort studies have shown increased cumulative live birth rates, including one fresh cycle and all subsequent FET cycles from that oocyte retrieval, with increased numbers of oocytes retrieved (Stanger and Yovich 2013; Ji *et al.*, 2013, Steward *et al.*, 2014, Magnusson *et al.*, 2018). However, the balance between efficacy and safety during IVF is a delicate issue. Between 15 and 18 oocytes have been found to be

optimal for cumulative live birth rate while keeping the safety during the treatment. If a higher number of oocytes are retrieved, there is increased risk of maternal complications such as OHSS and also thromboembolic events (Magnusson *et al.*, 2018). However, with the possibility to use “freeze all strategy”, the risk of severe OHSS has decreased.

### **Fertilization**

Fertilization can occur with standard IVF or ICSI. In standard IVF, a fixed concentration of sperm (usually 100 000-200 000 sperms/ml) is added to the culture medium containing oocytes, allowing the sperm to penetrate and fertilize the egg. In ICSI, a single sperm is injected directly into the cytoplasm of an egg, allowing for treatment of men with poor semen quality or quantity. ICSI can further be separated into ICSI with ejaculated sperm or ICSI with non-ejaculated sperm (microsurgical sperm retrieval procedures from the epididymis [PESA] or testis [TESA]).

ICSI was introduced in 1992 (Palermo *et al.*, 1992) and is now the most common fertilization technique, not only used in the treatment of male infertility but also in the treatment of unexplained infertility. Today, ICSI accounts for two-thirds of all treatments worldwide (Adamson *et al.*, 2018) and 49% of all treatments in Sweden (<http://www.medscinet.com/qivf>). ICSI is in its nature invasive and can potentially influence a series of biological mechanisms. For example, ICSI bypasses the natural selection of sperm, can potentially damage the egg and may introduce foreign material (culture media) when the sperm is injected to the egg (Retzlöff and Hornstein, 2003). Furthermore, it is unclear what effect poor quality spermatozoa have on the health of ICSI children and their future fertility (Catford *et al.*, 2018). Altogether this has led to a concern about the general health of ICSI children (Lacamara *et al.*, 2017; Catford *et al.*, 2018).

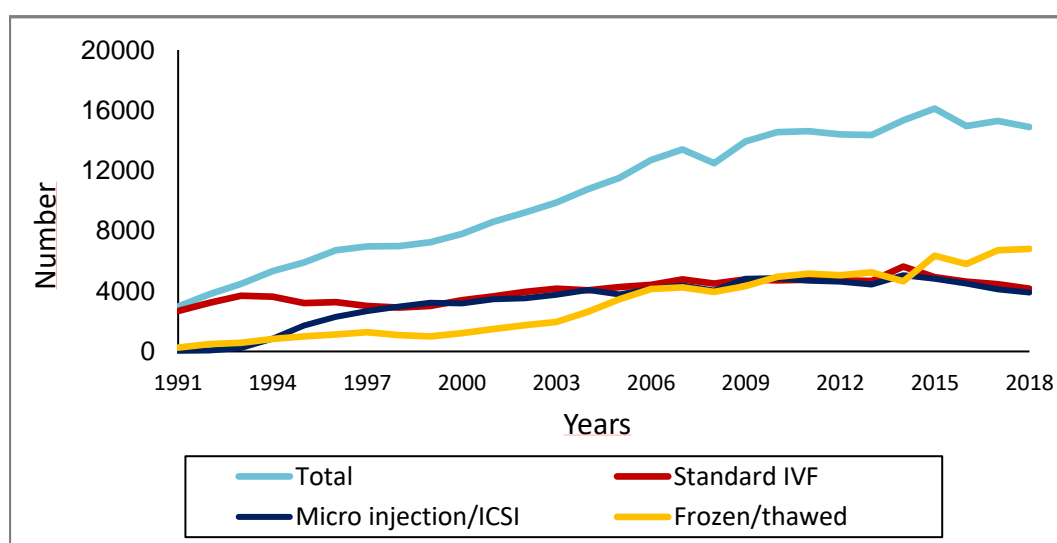
### **Embryo culture, embryo selection and embryo transfer**

Zygotes, the diploid cells resulting from the fusion of a sperm and an oocyte, are stored in a culture medium in a 37° C incubator with low oxygen levels until they reach the cleavage stage (4-8 cells, two to three days after oocyte retrieval) or the blastocyst stage (five to six days after oocyte retrieval). In recent years, advances in cell culture media have led to a shift from cleavage stage embryo transfer to blastocyst stage embryo transfer. A Cochrane analysis was performed in 2016 in which live birth rate between blastocyst stage transfer and cleavage stage embryo

transfer was compared (Glujovsky *et al.*, 2016). This analysis showed that blastocyst transfer increases delivery rates in fresh cycles, although not cumulatively. Yet, the majority of studies included in that Cochrane analysis, used slow freezing as the freezing method for blastocysts. Nowadays, a new freezing method have been introduced, vitrification, with considerably improved results.

### Cryopreservation

Embryos with good quality can be cryopreserved. There are two different methods used for cryopreservation. The traditional technique is called “slow freezing” in which cryopreservation is performed in a stepwise process and embryos are stored in liquid nitrogen at a temperature of minus 196° C (Lassalle *et al.*, 1985, Testart *et al.*, 1986). In recent years, the slow freezing technique has rapidly been replaced with a new technique called vitrification (Mukaida *et al.*, 1998, Kuwayama *et al.*, 2005). This is an ultra-rapid freezing method that is 600 times faster than conventional cryopreservation, with a very short exposure of embryos to the most critical temperature zones and less trauma to the cells. This has resulted in improved survival frequencies for the embryos and higher pregnancy/live birth rates per thawed embryo (Balaban *et al.*, 2008; Fasano *et al.*, 2014; Li *et al.*, 2014; Debrock *et al.*, 2015; Rienzi *et al.*, 2017). The rates of FET have increased steadily and in 2016, FET accounted for 27% of all treatment cycles in Europe (Wyns *et al.*, 2020). A similar trend exists in Sweden where the rate of FET was 40% of all treatment cycles with ET in 2018 (<http://www.medscinet.com/qivf>).



**Figure 3.** Number of embryo transfers per year for different treatment methods in Sweden. From Q-IVF (<http://www.medscinet.com/qivf>)

In recent years, the freeze-all strategy has gained popularity. Several, large randomized controlled studies from Asia and Europe have been performed in which the live-birth rate, the rate of OHSS and other obstetric and perinatal complications have been compared between fresh embryo transfer and the freeze-all strategy. The studies have shown preserved (Vuong *et al.*, 2018; Shi *et al.*, 2018; Stormlund *et al.*, 2020) or even higher live birth rates (Chen *et al.*, 2016; Wei *et al.*, 2019) and decreased rates of OHSS when the freeze-all strategy has been used, results which have a major impact on how ART is conducted today.

## ART legislation in Sweden

Since the first child was born in 1982 in Sweden, the legislation regarding ART has undergone several changes. Today, ART in Sweden is regulated by The Genetic Integrity Act (SFS 2006:351). Some of the most important changes in Swedish legislation in recent years are summarized below:

- 2003: oocyte donation is allowed
- 2005: treatment of lesbian couples is allowed
- 2016: treatment of single women is allowed
- 2019: use of embryo donation is allowed
- 2019: embryos are allowed to be kept in freeze up to 10 years (5 years was allowed before)
- 2019: surrogacy is continuously not allowed

ART legislation differs considerably worldwide. For example, surrogacy is only allowed in some countries in Europe, some US states and a limited number of other countries in the world. The differences in ART legislation has led to the so called “reproductive tourism” in which singles/couples, who cannot get the treatment they wish in their own country, travels to another country where the fertility treatment is available.

## ART monitoring

Several national registers and international monitoring committees have been established to collect data on efficacy and safety of ART.

Since the birth of the first IVF child in Sweden in 1982 and until 2006, aggregated data on all women who delivered after IVF treatment were reported to the National Board of Health and Welfare. However, at three times during this period, data with full identification for deliveries after IVF were collected for research purposes. Since the file is stored at MBR we have decided to name the file MBR-IVF in the studies of this thesis. In 2007, the Swedish National Quality Register for Assisted Reproduction (Q-IVF) was established which includes results of all IVF cycles that has started in Sweden with full patient identification, i.e. Personal Identification Number (PIN-code). The purpose of the register is to continuously monitor results of ART treatments and possible medical risks for ART children and the treated women/couples. The register is also a platform for research (<http://www.medscinet.com/qivf>).

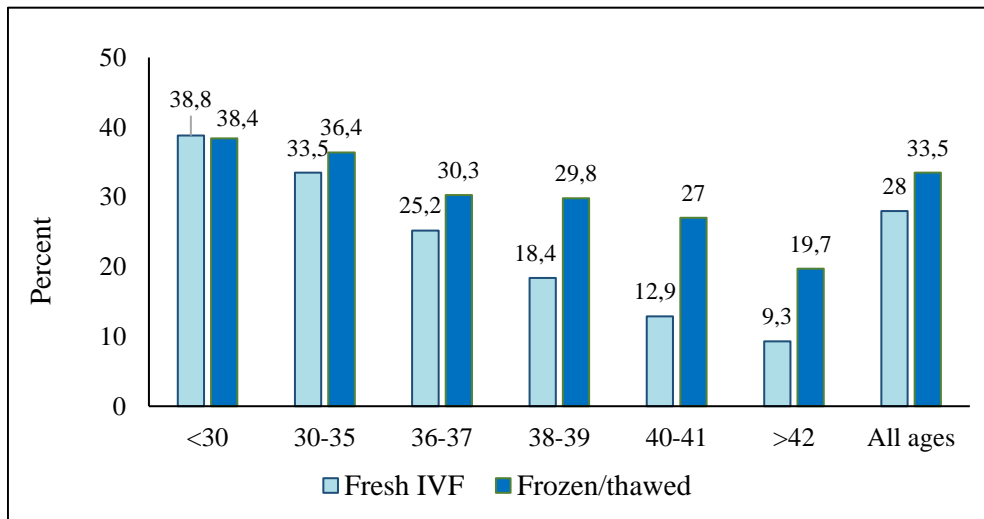
The European Society of Human Reproduction and Embryology (ESHRE) was established in 1985 by Sir Robert Edwards, who was awarded the Nobel Prize in 2010 in Medicine, due to the development of IVF. The main aim of ESHRE is to promote interest in and understanding of reproductive biology and medicine. The activities of ESHRE include teaching and training as well as developing and maintaining data registers (<http://www.eshre.eu>). ESHRE also enhances safety and quality in clinical and laboratory practices. In 1999, The European IVF Monitoring (EIM) Consortium was introduced. The aim of the IVF Consortium is to collect data from national registers on ART in Europe. The data are published in an annual report and the 20th report from 40 participating European countries for 2016 was published recently (Wyns *et al.*, 2020).

The International Committee for Monitoring Assisted Reproductive Technologies (ICMART) is an independent, international organization, which has generated annual world reports concerning ART utilization, effectiveness and safety, since 1989 (<http://www.icmartivf.org>). The last ICMART report (Adamson *et al.*, 2018) included 65 countries and 2 560 ART clinics, which accounted for 72.7% of existing clinics worldwide.

Centers for Disease Control and Prevention (CDC) is a public health institute in the United States that provides health information including information about the safety and efficacy of ART that is published in annually reports (<http://www.cdc.gov/art>).

## Results after ART

In Sweden for year 2018, the delivery rate per embryo transfer was 28.0% for fresh cycles and 33.5% for FET cycles (<http://www.medscinet.com/qivf/>). The higher numbers of delivery rates for FET cycles primarily depends on a higher rate of blastocysts in these cycles. The age of the female partner is the most important factor for the chance of having a live birth, mainly since the number of oocytes and quality of the oocytes rapidly deteriorate with higher age (<http://www.medscinet.com/qivf/>).



**Figure 4.** Delivery rate per embryo transfer in different age groups. Fresh IVF and frozen/thawed cycles. Own gametes. From Q-IVF (<http://www.medscinet.com/qivf/>)

## Complications after ART

Severe maternal complications related to the ART procedure are rare. The most common, and one of the most serious complications, is OHSS. Severe OHSS (associated with severe illness and hospitalization) is in Sweden reported to 0.4% (<http://www.medscinet.com/qivf/>) and worldwide reported to 0.5% (Adamson *et al.*, 2018). Other complications associated with ART are thromboembolic events (Rova *et al.*, 2012; Sennström *et al.*, 2017; Magnusson *et al.*, 2018), which in turn are related to OHSS (Sennström *et al.*, 2017), severe intra-abdominal hemorrhages and ovarian abscesses. Severe intra-abdominal hemorrhages and ovarian abscesses have

been reported in 0.06% and 0.003% respectively (Aragona *et al.*, 2011). The risk of ovarian cancer in women treated with ART has been investigated for almost 30 years and the findings have been inconclusive. A Norwegian population-based cohort study (Reigstad *et al.*, 2015) found an increased risk of ovarian cancer in women treated with ART but the results were not significant after correcting for multiple analyses. Furthermore, a large population-based register study from the United Kingdom (Williams *et al.*, 2018) found an increased risk of invasive and borderline ovarian tumours in women treated with ART (standardized incidence ratio [SIR] 1.39; 95% confidence interval [CI] 1.26 to 1.53). The tumours were limited to women with endometriosis, low parity or both, suggesting that the increased risk could be due to patient characteristics. The results of Williams *et al.*, was supported by a Danish population-based register study in which an increased risk of ovarian cancer was found in ART treated women (HR 1.20; 95% CI 1.10 to 1.31). In subgroup analyses, the increased risk was restricted to women with endometriosis (HR 3.78; 95% CI 2.45 to 5.84) while no increased risk of ovarian cancer was found in ART treated women with other infertility diagnoses (Vassard *et al.*, 2019). Recently, a Swedish population-based register study investigated 38 025 women treated with ART, 49 208 women with an infertility diagnosis but not treated with ART and 1 252 864 women without an infertility diagnosis, between 1982 and 2012 (Lundberg *et al.*, 2019). The results showed an increased risk of ovarian cancer in women treated with ART compared to women without an infertility diagnosis (adjusted hazard ratio [AHR] 2.43; 95% CI 1.73 to 3.42) but also compared to women with an infertility diagnosis (AHR 1.79; 95% CI 1.18 to 2.71), suggesting that the increased risk of ovarian cancer may be due to both the infertility and ART treatment per se. Still, that study suffered from methodological limitations since only cycles leading to live-birth was investigated and thus no possibility to investigate whether a dose-response relationship existed between the number of ART cycles and ovarian cancer risk. Furthermore, the registration of many infertility diagnoses was missing in the beginning of the study period (registration of specialized outpatient care started 2001 in Sweden).

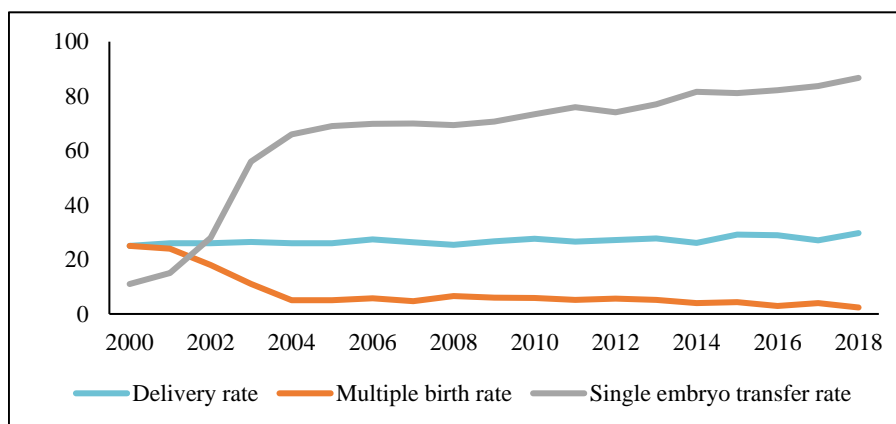
## Perinatal and obstetric outcomes after ART

### **Literature search and selection of studies in the tables of the introduction in this thesis**

Search has been done in PubMed and Cochrane (Sept 2020). The overviews are narrative reviews based on systematic reviews/meta-analyses and large observational studies, when relevant.

## Multiple pregnancies

Most pregnancies after ART are uncomplicated and result in the birth of healthy children (Wennerholm and Bergh, 2020). Yet, it is well documented that ART pregnancies are associated with higher risks of adverse perinatal and obstetric outcomes (Bergh *et al.*, 1999; Qin *et al.*, 2017). Many of the perinatal and obstetric complications are a result from multiple pregnancies and can be reduced by using single embryo transfer (SET) (Thurin *et al.*, 2004; Sazonova *et al.*, 2013). When ART was introduced, multiple embryos were often transferred to achieve “sufficient” pregnancy rates. As the IVF-technique developed, attention shifted into the increased risk multiple pregnancies entail. A Nordic randomized control study was performed (Thurin *et al.*, 2004), in which fresh SET followed by a single FET was compared to double embryo transfer (DET). The results showed no substantial difference in cumulative live births when SET was compared to DET (difference 4.1 percentage points; 95% CI -3.4 to 11.6 percentage points) and the reduction in multiple births was substantial (0.8% vs 33.1%;  $p=0.001$ ). The result had a major impact on future ART treatment and today many countries have adopted the strategy of SET (Bergh *et al.*, 2020). Still, considerable geographical differences exist. For example, the Scandinavian countries and Australia used SET in 90% of embryo transfers in 2017 (Newman *et al.*, 2019; <http://www.medscinet.com/qivf/>) and the United States also report an increase up to 71% of all embryo transfers today (CDC, 2020). However, the East European countries still use SET in a minority of the transfers, thus the overall SET rate for Europe was 41.5% in 2016 (Wyns *et al.*, 2020). Yet, several systematic reviews consistently show that also singleton pregnancies after ART are associated with higher risks of obstetric and perinatal complications, although to a much lesser extent than multiples. (Helmerhorst *et al.*, 2004; McDonald *et al.*, 2009; Pandey *et al.*, 2012, Sazonova *et al.*, 2013; Qin *et al.*, 2017).



**Figure 5.** Delivery rate per embryo transfer, multiple birth rate and single embryo transfer per year in Sweden. Fresh IVF. From Q-IVF (<http://www.medscinet.com/qivf/>)



## Perinatal complications in singleton pregnancies

It is well documented that singletons born after ART have increased risks of preterm birth (PTB), very preterm birth (VPTB), low birth weight (LBW) and very low birth weight (VLBW) when compared to singletons conceived spontaneously (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004, McDonald *et al.*, 2009, Pandey *et al.*, 2012; Qin *et al.*, 2017). The adjusted risks have been estimated to 1.5-2.0 for PTB, 1.7-3.3 for VPTB, 1.6-1.7 for LBW and 1.9-3.0 for VLBW (Helmerhorst *et al.*, 2004; McDonald *et al.*, 2009; Pandey *et al.*, 2012). Most studies also show an increased risk of small for gestational age (SGA) (adjusted risk of 1.4-1.5) (Helmerhorst *et al.*, 2004; McDonald *et al.*, 2009; Pandey *et al.*, 2012). An overview of systematic reviews and meta-analyses investigating perinatal outcomes in singleton ART children is shown in Table 1.

**Table 1.** An overview of systematic reviews and meta-analyses investigating perinatal outcomes in singletons born after ART.

First author and year of publication	Number of studies	Number of ART singletons	PTB <37 weeks	VPTB <32 weeks	LBW <2500 grams	VLBW <1500 grams	SGA	Perinatal mortality
<b>ART vs SC adjusted at least for maternal age</b>								
Helmerhorst, 2004 ARR (95 % CI)	12	5 361	2.0 (1.8 to 2.3)	3.3 (2.0 to 5.3)	1.7 (1.5 to 1.9)	3.0 (2.1 to 4.4)	1.4 (1.2 to 2.7)	1.7 (1.1 to 2.6)
Jackson, 2004 AOR (95% CI)	14	12 114	2.0 (1.7 to 2.2)	3.1 (2.0 to 4.8)	1.8 (1.4 to 2.2)	2.7 (2.3 to 3.1)	1.6 (1.3 to 2.0)	2.2 (1.6 to 3.0)
McDonald, 2009 ARR (95% CI)	27	14 748	1.8 (1.5 to 2.2)	2.3 (1.7 to 3.9)	1.6 (1.3 to 2.0)	2.6 (1.8 to 3.8)	1.4 (1.0 to 2.0)	-
Pandey, 2012 ARR (95% CI)	22	27 819	1.5 (1.5 to 1.6)	1.7 (1.5 to 1.9)	1.6 (1.6 to 1.8)	1.9 (1.7 to 2.2)	1.4 (1.3 to 1.5)	1.9 (1.5 to 2.4)
Qin, 2017 ART vs SC, %	52	181 741	10.9 vs 6.4	2.4 vs 1.2	8.7 vs 5.8	2.0 vs 1.0	7.1 vs 5.7	1.1 vs 0.6

ART, assisted reproductive technology; SC, spontaneous conception; vs, versus; ARR, adjusted relative risk; AOR; adjusted odds ratio; PTB; preterm birth; VPTB, very preterm birth; LBW, low birth weight; VLBW, very low birth weight; SGA, small for gestational age

Several studies, including systematic reviews and meta-analyses, have also found increased risks of birth defects in ART singletons (adjusted odds ratios [AORs] between 1.3 to 1.6) (Hansen *et al.*, 2013; Pandey *et al.*, 2012; Qin *et al.*, 2017; Zhao *et al.*, 2020). A register-based cohort study from the The Committee of Nordic ART and Safety (CoNARTaS) group (Henningsen *et al.*, 2018) in which trends over time in birth defects in live-born children conceived after ART was investigated, found that the absolute risk for a major birth defect in singleton ART and SC children was 3.4% and 2.9% respectively. They also found that the relative risk of being born with

a major birth defect between children born after ART and SC remained similar over the past 20 years ( $p=0.39$ ). However, they noticed significantly increased risks of major birth defects in the nervous system, eye-ear-face-and neck-, heart-, gastrointestinal-, urinary- and musculoskeletal system in ART singletons compared to SC singletons. Correspondingly, a systematic review and meta-analysis of eight cohort studies on congenital heart defects in ART children (Giorgione *et al.*, 2018), observed an increased risk of congenital heart defects in ART children compared to SC children. Three of the eight studies reported AORs with adjustment for maternal age and parity in singleton ART pregnancies, and the meta-analysis of these studies still showed an increased risk of congenital heart defects in ART children compared with controls (pooled odds ratio [OR] 1.29; 95% CI 1.03 to 1.60). An overview of systematic reviews and meta-analyses investigating birth defects is shown in Table 2. Lastly, increased perinatal mortality has been found in several studies in ART singletons (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004; Pandey *et al.*, 2012; Qin *et al.*, 17) (adjusted risks 1.7-1.9).

**Table 2.** An overview of the most recent systematic reviews and meta-analyses investigating birth defects in singletons born after ART.

First author and year of publication	Number of studies	Number of ART singletons	Birth defects RR (95% CI)
ART vs SC			
Pandey, 2012	7	4 382	1.7 (1.3 to 2.1)
Hansen, 2013	23	48 944	Any: 1.4 (1.3 to 1.4) Major: 1.4 (1.3 to 1.5)
Qin, 2017 ART vs SC, %	29	77 630	5.7% vs 3.9%*
Zhao, 2020	46	112 913	1.4 (1.3 to 1.5)

ART, assisted reproductive technology; SC, spontaneous conception; RR, relative risk; vs, versus

\* No relative risks or odds ratios presented

When comparing ICSI with standard IVF, most large studies have found similar or lower risks of PTB, LBW and perinatal mortality in singletons born after ICSI (Romundstad *et al.*, 2008; Nelson *et al.*, 2011; Pinborg *et al.*, 2013). In a meta-analysis of five studies (Pinborg *et al.*, 2013) comparing ICSI with IVF singletons, the pooled estimate showed a lower risk of PTB in ICSI singletons (AOR 0.80; 95% CI 0.69 to 0.93). A possible explanation for these results could be a higher proportion of reproductively healthy women getting ICSI, possibly leading to a better intrauterine environment and thus a better perinatal outcome. Moreover, several large

studies and systematic reviews have found that perinatal outcomes are better in children born after FET compared to fresh embryo transfer with lower risks of PTB and LBW (Wennerholm *et al.*, 2009; Sazonova *et al.*, 2012; Wennerholm *et al.*, 2013; Pinborg *et al.*, 2013; Maheshwari *et al.*, 2012; Zhao *et al.*, 2016). However, the results have also shown that children born after FET have increased risks of being born large for gestational age (LGA) and macrosomic, compared to children born after both fresh embryo transfer and SC (Wennerholm *et al.*, 2013; Berntsen and Pinborg, 2018; Maheshwari *et al.*, 2018). A meta-analysis of ten studies on LGA and six studies on macrosomia (Berntsen and Pinborg, 2018) confirmed that there was an increased risk of LGA in FET compared to fresh embryo transfer (AOR 1.5; 95% CI 1.44 to 1.57) and SC (1.3; 95% CI 1.20 to 1.43) and an increased risk of macrosomia in FET compared to fresh embryo transfer (AOR 1.71; 95% CI 1.59 to 1.83) and SC (AOR 1.42; 95% CI 1.17 to 1.71). Possible explanatory mechanisms to this increase in birth weight include epigenetic modifications and/or an embryo selection mechanism, where better embryos survive the freezing/thawing procedure. A third theory is that the uterine environment in a FET cycle is more natural than in a fresh IVF cycle (Wennerholm and Bergh, 2020).

Several systematic reviews and meta-analyses have also examined the neonatal and maternal outcomes after blastocyst versus cleavage stage transfer (Maheshwari *et al.*, 2013; Dar *et al.*, 2014; Martins *et al.*, 2016; Wang *et al.*, 2017; Alviggi *et al.*, 2018). The meta-analyses have shown increased risks of PTB when blastocysts have been used (relative risk [RR] 1.11; 95% CI 1.01 to 1.22) (Wang *et al.*, 2017). In the latest meta-analysis (Alviggi *et al.*, 2018) the increased risk of PTB was only observed in fresh cycles. Abnormal placentation and implantation are possible explanations to the increased risk of PTB in blastocysts (Wennerholm and Bergh, 2020). Studies also show higher rate of monozygotic twins (Ding *et al.*, 2018; Hviid *et al.*, 2018; Liu *et al.*, 2018) and altered male-female ratio with predominance for male (Chang *et al.*, 2009; Ding *et al.*, 2018; Hattori *et al.*, 2019; Ginström Ernstad *et al.*, 2016) after blastocyst transfer.

### **Obstetric outcomes in singleton pregnancies**

Several systematic reviews and large cohort studies have shown that ART pregnancies have significantly increased risks of placenta related complications such as hypertensive disorders in pregnancy (HDP), placenta previa, antepartum hemorrhage, postpartum hemorrhage and placental abruption (Pandey *et al.*, 2012; Thomopoulos *et al.*, 2013; Opdahl *et al.*, 2015; Qin *et al.*, 2016; Gui *et al.*, 2020;

Petersen *et al.*, 2020). In recent years, it has been shown that pregnancies following FET, in particular, seems to be at highest risk of HDP (Thomopoulos *et al.*, 2013; Ishihara *et al.*, 2014; Opdahl *et al.*, 2015; Maheshwari *et al.*, 2018; Ginström Ernstad *et al.*, 2019; Gui *et al.*, 2020). The reasons for the higher incidence of HDP after FET is not clear but recent studies found a link between the absence of corpus luteum and higher risks of preeclampsia (von Versen-Hoynck *et al.*, 2019; von Versen-Hoynck *et al.*, 2020). Accordingly, a large population-based Swedish register study (Ginström Ernstad *et al.*, 2019), and later a large Japanese cohort study (Saito *et al.*, 2019), found that pregnancies after FET using programmed cycles (no corpus luteum) were associated with higher risks of HDP compared to natural or stimulated cycles (with a corpus luteum). These findings suggest that some substance, secreted from corpus luteum may influence cardiovascular adaptation during pregnancy.

Regarding placenta previa, two large meta-analyses found a more than 3-folded higher risk for placenta previa and an almost doubled risk for placental abruption when comparing pregnancies after ART to pregnancies after SC (Qin *et al.*, 2016; Vermey *et al.*, 2019). In the study of Vermey *et al.* (2019), ART pregnancies were also compared to non-ART pregnancies in sub-fertile patients and also in this comparison they noted a significantly higher risk for placenta previa (OR 2.51; 95% CI 2.12 to 2.98) and placental abruption (OR 1.61; 95% CI 1.33 to 1.95) in ART pregnancies, suggesting that the ART procedure *per se* could have an effect on placentation. When comparing blastocyst transfer with cleavage stage transfer, there have been conflicting results about the risk of placental complications. A Swedish population-based register study (Ginström Ernstad *et al.*, 2016) found that the risk of placenta complications was higher in the blastocyst group as compared to the cleavage stage group, while other studies did not find any differences in placental complications (Fernando *et al.*, 2012; Ishihara *et al.*, 2014).

## Trends over time in ART

The trend of increased use of SET have led to fewer multiple pregnancies and consequently improved perinatal and obstetric outcomes after ART. However, also for singletons born after ART the rate of PTB, LBW and SGA has declined over time. The CoNARTaS group has published three large population-based cohort studies in recent years, evaluating time-trends in ART pregnancies compared to SC pregnancies (Henningsen *et al.*, 2015; Henningsen *et al.*, 2018; Petersen *et al.*, 2020). The first two studies investigated perinatal complications (Henningsen *et al.*, 2015)

and birth defects (Henningsen *et al.*, 2018) and compared approximately 90 000 ART children with almost 500 000 SC controls born between 1988 and 2007. The results showed a substantial decline in the absolute and relative risks of PTB and VPTB as well as a decline in the absolute and relative risks of LBW and VLBW in ART singletons over time. The number of children diagnosed with a major birth defect increased in pregnancies after both ART and SC, probably related to improved diagnostics, but the relative risk of being born with a major birth defect in ART children compared to SC was not changed over time. In the latest study (Petersen *et al.*, 2020), 146 998 pregnancies after ART and 6 683 132 pregnancies after SC between 1988 and 2015 were compared concerning HDP and placental complications. Pregnancies after ART followed the same time trends as the SC pregnancies concerning HDP and placental abruption (stable for HDP, a decline for placental abruption) whereas the risk for placenta previa had increased over time for ART pregnancies in contrast to SC pregnancies.

## Long-term outcome in children born after ART

### **Neurodevelopmental and psychomotor health after ART**

Three systematic reviews examining the neurodevelopmental function of children conceived after ART, have concluded that long-term emotional, social and mental health, including language development, is reassuring (Middelburg *et al.*, 2008; Bay *et al.*, 2013; Hart and Norman, 2013). Yet, individual studies, summarized below and in Table 3a and 3b, have shown diverging results and many suffer from methodological limitations.

#### *Cognitive development after ART*

A systematic review, exploring the impact of specific fertility treatments on cognitive development in childhood and adolescence, reported conflicting results and emphasized methodological limitations with less than a third of studies rated as high quality (Rumbold *et al.*, 2017). The authors identified five high quality studies when comparing ART versus SC. Among the five studies, two reported poorer cognitive ability among children born after ART. The first one, was a Swedish population-based study, that included 5 680 children born after ART (both singletons and multiples). The study showed an increased risk of developmental delay and use of habilitation service compared to children born after SC. However, when only singletons were compared, the risk was not increased (Stromberg *et al.*, 2002). The second study, a Swedish population-based register study of 30 959 children born

after ART, found a small but significantly increased risk of mental retardation among ART children (RR 1.18; 95% CI 1.01 to 1.36). When the analysis was restricted to singletons, the significance disappeared. When a subgroup analysis was performed, an increased risk of mental retardation was found for children born after FET with ICSI. Yet, the analysis was only based on seven children (Sandin *et al.*, 2013). The remaining three studies of high quality found no difference in the risk of mental retardation or mental disorders (Pinborg *et al.*, 2003; Bay *et al.*, 2013b) or in performance on standardized school tests (Wagenaar *et al.*, 2009). Likewise, a European collaboration project between five European countries, in which 5-year old children (511 ICSI, 424 IVF and 488 SC) were followed up regarding cognitive assessments, found no differences in cognitive or motor development (Ponjaert-Kristoffersen *et al.*, 2005). Notable is that only children born after 32 gestational weeks were included in the study.

A limited number of controlled studies have been published about ART children and school performance (Levy-Shiff *et al.*, 1998; Wagenaar *et al.*, 2008; Wagenaar *et al.*, 2009; Mains *et al.*, 2010, Spangmose *et al.*, 2017; Spangmose *et al.*, 2019). Spangmose *et al.* (2017) explored the school performance in a Danish ART cohort (n=2836 singletons and n=1930 twins) with matched singletons and twins born after SC, aged 16-17 years. After adjustment for relevant confounders, they found that ART singletons had slightly lower overall mean test scores than controls. Thereafter, the same research group compared the school performance of adolescents aged 16-17 years born after FET (n=423) with adolescents born after fresh embryo transfer (n=6 072), and found similar academic performance in the groups (Spangmose *et al.*, 2019).

#### *Autism spectrum disorders and attention deficit hyperactivity disorder*

The association between ART and autism spectrum disorders (ASD) has been examined in several studies, but the results are still inconclusive. A population-based cohort study of 42 383 ART infants in California (Kissin *et al.*, 2015) found higher risk of ASD in ICSI singletons compared with standard IVF with fresh embryo transfer (adjusted hazard risk ratio 1.65; 95% CI 1.08 to 2.52). No comparison between ART and SC was performed in that study. Conversely, a Swedish population-based register study of 30 959 children born after ART did not find any increased risk of ASD neither in ICSI singletons compared with standard IVF singletons, nor in ART children compared to SC children (Sandin *et al.*, 2013). A recent meta-analysis of 11 studies showed an increased risk of ASD in ART children

compared to SC children (RR 1.35; 95% CI 1.09 to 1.68). However, there were no increased risk in ART singletons (Liu *et al.*, 2017).

Regarding attention deficit hyperactivity disorder (ADHD), a weak association with IVF was found in a Swedish register-based study which included 28 158 children born after IVF (Källén *et al.*, 2011). After adjustment for years of infertility or restricting the analysis to only singletons, there was no statistical significance between the groups. A Danish study (Svahn *et al.*, 2015), in which 124 269 children born to women with fertility problems were included, reported a higher risk of ADHD in the children born to women with fertility problems. Yet, they did not perform any adjustment for multiplicity.

### *Cerebral palsy*

Several large studies from the Nordic countries have observed an increased risk of cerebral palsy (CP) in children born after ART compared to children born after SC (Strömberg *et al.*, 2002; Klemetti *et al.*, 2006; Hvidtjorn *et al.*, 2006; Hvidtjorn *et al.*, 2010; Källén *et al.*, 2010). Strömberg *et al.* (2002) compared 5680 children born after IVF with controls in a population-based cohort study from Sweden and found an increased risk of CP in the total ART group (singletons and multiples) (AOR 3.7; 95% CI 2.0 to 6.6) and for ART singletons (AOR 2.8; 95% CI 1.3 to 5.8). Klemetti *et al.* (2006) and Hvidtjorn *et al.* (2010) performed retrospective cohort studies in Finland and Denmark, respectively, and found increased risks of CP in the total ART groups (singletons and multiples). Still, none of the studies found any increased risk when the analyses were restricted to singletons. A systematic review and meta-analysis (Hvidtjorn *et al.* 2009) showed that ART children (singletons and multiples) had an increased risk of CP (OR 2.18; 95% CI 1.71 to 2.77 for the total ART group and OR 1.82; 95% CI 1.31 to 2.52 for ART singletons). The increased risk was partly explained by the higher risk of PTB in the ART group. In a recent published Australian register study (Goldsmith *et al.*, 2018), which included 2 914 ART children, the prevalence of CP was more than doubled in ART singletons born very preterm (<32 gestational weeks).

### *Neurodevelopmental and cognitive health after ICSI*

A couple of large population-based register studies, described above, (Sandin *et al.*, 2013; Kissin *et al.*, 2015) have reported modest increases in the risk of mental retardation and ASD in ICSI children. Furthermore, a follow-up study 83 ICSI, 83 IVF and 85 SC singletons (5-8 years old) reported lower mean IQ-scores in ICSI children than in their SC counterparts (adjusted mean difference 5.6; 95% CI 0.9 to

10.3 vs 7.1; 95% CI 1.7 to 12.5 depending on which covariates that were included in the model) (Knoester *et al.*, 2008). A systematic review (Rumbold *et al.*, 2017) concluded that findings among high quality studies on the cognitive ability of ICSI children were inconsistent, leading to a general concern for ICSI children and their neurodevelopmental outcomes (Rumbold *et al.*, 2019). The findings of lower IQ, small increases in mental retardation and ASD in ICSI children, have however not been replicated in other studies. A large Danish cohort study of mental disorders, such as autism and mental retardation, in 33 000 children born after fertility treatment, did not find any association between ICSI and ASD or mental retardation (Bay *et al.*, 2013). Furthermore, two other studies of 4-6-year-old children did not find any significant difference in intelligence quotient (IQ) between ICSI and IVF children (Leslie *et al.*, 2003) or ICSI and SC children (Ponjaert-Kristoffersen *et al.*, 2005). Since ASD and mental retardation are severe neurodevelopmental disorders and the results are diverging, there is a need for more and larger studies on the subject.

**Table 3a.** Summary of the cognitive and neurodevelopmental outcome in ART children. Cohort studies.

First author and year of publication	Country	Number of children	Exposure	Age of child (year)	Study design	Outcome AOR/ARR/AHR/mean difference (95% CI)
<b>Cognitive</b>						
Strömberg, 2002	Sweden	5680 IVF (3 620 singletons 2060 twins), 15 397 SC	IVF/ICSI	1.5-14	Retrospective cohort study (register)	Suspected developmental delay All: AOR 4.0 (1.9 to 8.3) Singletons: AOR 2.0 (0.7 to 5.4)
Leslie, 2003	Australia	97 ICSI, 80 IVF, 110 SC	ICSI	5	Prospective cohort study	No significant difference in IQ-scores
Place and Englert, 2003	Belgium	66 ICSI, 52 IVF, 59 SC	ICSI	5	Prospective cohort study	No significant difference in IQ-scores
Pinborg, 2003	Denmark	1 106 ART (634 singletons 472 twins), 1 132 SC twins	IVF/ICSI	3-4	Retrospective cohort study	No significant difference in mental retardation
Ponjaert-Kristoffersen, 2004	Belgium, Sweden, USA	300 ICSI, 260 SC	ICSI	5	Prospective cohort study	No significant difference in IQ-scores
Ponjaert-Kristoffersen, 2005	Belgium, Denmark, Greece, Sweden, UK	511 ICSI, 424 IVF, 488 SC	ICSI	4-6	Prospective cohort study	No significant difference in IQ-scores
Bonduelle, 2005	Belgium, Denmark, Greece, Sweden, UK	540 ICSI, 437 IVF, 538 SC	ICSI and IVF	5	Several retrospective and prospective cohorts combined	Increased use of speech/language therapy in the IVF-group
Knoester, 2008	Netherlands	86 ICSI, 83 IVF, 85 SC	ICSI	5-8	Retrospective cohort study	Lower mean IQ-scores in ICSI compared to SC Singletons: Adj. mean diff 5,6 (0.9 to 10.3) vs 7.1 (1.7 to 12.5) depending on model of adjustment



Wagenaar, 2008	Netherlands	233 IVF, 233 SC of parents with fertility problems	IVF/ICSI	8-18, mean age 12	Retrospective cohort study	No significant difference in school performance
Wagenaar, 2009	Netherlands	233 IVF, 233 SC	IVF	12	Retrospective cohort study	No significant difference in standardized school tests
Goldbeck, 2009	Germany	35 ICSI, 34 IVF	ICSI	5,10	Retrospective cohort study	ICSI significantly lower IQ-scores compared to IVF Mean IQ 94.1 (SD 13.8) vs 102 (SD 9.1) p=0.005 ICSI group more likely to have delayed cognitive development (23.5% vs 2.9%, p=0.011)
Mains, 2010	USA	423 ART, number of controls unclear	ART	8-17	Retrospective cohort study	No significant difference in standardized school tests
Sandin, 2013	Sweden	11 514 ICSI, 19 445 IVF, 2 510 166 SC	IVF, ICSI	0-26.5. Mean 10.	Retrospective cohort study (register)	Increased risk of mental retardation. All: ARR 1.18 (1.01 to 1.36) Singletons: ARR 1.01 (0.83 to 1.24) Singleton ICSI with FET: ARR 2.36 (1.04 to 5.36)
Bay, 2013b	Denmark	14 991 ART vs 555 828 SC	IVF, ICSI, OI, IUI	8-17	Retrospective cohort study (register)	No significant difference in mental disorders
Punamaki, 2015	Finland	76 ICSI, 164 IVF, 278 SC	ICSI, IVF	7-8	Prospective cohort study	No significant difference in cognitive developmental problems reported by parents
Spangmose, 2017	Denmark	4 766 ART (2 836 singletons, 1 930 twins) SC	ART	15-16	Retrospective cohort study (register)	Test scores, in standardized school tests, significantly lower for ART singletons vs SC singletons. Adj mean diff: -0.15 (-0.29 to -0.02).
Spangmose, 2019	Denmark	423 FET vs 6 072 fresh ET	FET	15-16	Retrospective cohort study (register)	No significant difference in standardized school tests
<b>Autism</b>						
Hvidtjorn, 2011	Denmark	14 991 IVF/ICSI, 18 148 OI, 555 828 SC	ART	4-13	Retrospective cohort study (register)	No increased risk of ASD
Sandin, 2013	Sweden	11 514 ICSI, 19 445 IVF, 2 510 166 SC	ICSI, ART	0-26.5. Mean 10.	Retrospective cohort study	No increased risk of ASD in main analysis or in singletons.
Kissin, 2015	USA	21 728 ICSI vs 19 926 IVF	ICSI	≥5	Retrospective cohort study	Increased risk of ASD in ICSI vs IVF. AHR 1.65 (1.08 to 2.52)
<b>ADHD</b>						
Källén, 2011	Sweden	28 158 IVF, 2 417 886 SC	IVF/ICSI	5-28	Retrospective cohort study (register)	No increased risk after adjustment for years of infertility
<b>Cerebral palsy</b>						
Strömberg, 2002	Sweden	5 680 IVF singletons, 11 360 SC singletons 2060 IVF twins 4 120 SC twins	IVF/ICSI	1.5-14	Register-based retrospective cohort study (register)	Use of habilitation services: All: AOR 1.7 (1.3 to 2.2) Singletons: aOR 1.4 (1.0 to 2.1) CP: All: AOR: 3.7 (2.0 to 6.6) Singletons: AOR 2.8 (1.3 to 5.8)
Klemetti, 2006	Finland	4 559 IVF, two control groups: 190 398 SC (ovulation induction excluded) and a random sample with 26 877 SC	IVF	≤4	Retrospective cohort study (register)	CP: All: AOR 2.92 (1.63 to 5.26) Singletons: AOR 1.15 (0.40 to 3.27)

## Introduction

Hvidtjorn, 2010	Denmark	14 991 ART, 18 148 OI, 555 828 SC	ART	5-13	Retrospective cohort study (register)	CP: All: HRR 2.00 (1.51 to 2.65) Singletons: HRR 1.21 (0.75-1.94)
Källén, 2010	Sweden	31 587 IVF/ICSI	IVF/ICSI	0-25	Retrospective cohort study (register)	CP: All: AOR 1.81 (1.52 to 2.13) Singletons: 1.23 (0.96 to 1.58)
Goldsmith, 2018	Australia	2 835 ART (1927singletons, 908 twins), 208 571 SC (203 352 singletons, 5219 twins)	ART		Retrospective cohort study (register)	CP: All: AOR 2.6 (1.6 to 4.0) Singletons <32w AOR 2.7 (1.0 to 6.9) Singletons ≥37w AOR 1.0 (0.3 to 3.1)

ART, assisted reproductive technology; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; FET, frozen embryo transfer; SC, spontaneous conception; CP, cerebral palsy; IQ, intelligence quotient; ASD, autism spectrum disorder; CI, confidence intervals; ARR, adjusted relative ratio; OR, odds ratio; AOR, adjusted odds ratio; AHR, adjusted hazard ratio; HRR, hazard relative ratio; adj. mean diff., adjusted mean difference

**Table 3b.** Summary of the cognitive and neurodevelopmental outcome in ART children. Systematic reviews and meta-analyses.

First author and year of publication	Subject	Number of studies	Exposure	Study design	Outcome/Conclusion
Middelburgh, 2008	Cognitive and neurodevelopmental outcomes	23	IVF/ICSI	Systematic review	The IVF/ICSI technique per se did not have an effect on the neurodevelopment
Hvidtjorn, 2009	CP. ASD. Mental retardation	41	ART	Systematic review and meta-analysis	CP: Overall: OR 2.18 (95% CII.71 to 2.77) Singletons: OR 1.82 (95% CI 1.31 to 2.52) ASD and mental retardation: inconsistent results. Methodological limitations in many studies
Bay, 2013	Neurodevelopmental outcomes	80	ART	Systematic review	Neurodevelopment comparable between ART and SC
Hart and Norman, 2013	Mental health and development	87	IVF	Systematic review	Increased risk of CP and neurodevelopmental delay related to PTB and LBW. Cognitive development, social functioning and behavior reassuring
Rumbold, 2017	Cognitive development	35	IVF/ICSI	Systematic review	Cognitive development of conventional IVF reassuring. Conflicting results for ICSI. Methodological limitations in the majority of studies
Liu, 2017	ASD	11	ART	Systematic review and meta-analysis	ASD: Overall: RR 1.35 (1.09 to 1.68) Singletons: RR 0.94 (0.77 to 1.14)

ART; assisted reproductive technology; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; SC, spontaneous conception; CP, cerebral palsy; PTB, preterm birth; LBW, low birth weight; ASD, autism spectrum disorder; OR, odds ratio; RR, relative risk

### **Type 1 diabetes after ART**

Type 1 diabetes is one of the most common chronic diseases in childhood (Dabelea, 2009) and the incidence of type 1 diabetes has increased during the past decades (Mayer-Davis *et al.*, 2017; Patterson *et al.*, 2019; Mobasseri *et al.*, 2020) although several centers in high-incidence countries have shown a slower increase in more recent years (Mayer-Davis *et al.*, 2017; Patterson *et al.*, 2019). Type 1 diabetes is a chronic autoimmune disorder that leads to progressive pancreatic  $\beta$ -cell destruction and ends in absolute insulin deficiency and stable hyperglycemia (DiMeglio *et al.*, 2018). The etiology is complex and seems to be a combination between environmental factors and microbiome, genome, metabolism, and the immune system. Experimental and epidemiological studies have suggested that a number of environmental factors could modify gene expression through epigenetic mechanisms, thereby leading to an abnormal immune response and islet autoimmunity (Zullo *et al.*, 2017). Studies have also shown that epigenetic modifications are more common in individuals with autoimmune diseases such as type 1 diabetes (Wang *et al.*, 2017). Gametogenesis, fertilization and early embryo development are stages vulnerable to epigenetic dysregulation, coinciding with ART treatments (Pinborg *et al.*, 2016). Altered glucose metabolism has been found in ART children (Ceelen *et al.*, 2008a, Chen *et al.*, 2014) but few studies have explored whether ART children have an increased risk of developing type 1 diabetes. Two Danish cohort studies found no association between fertility problems or fertility treatment and type 1 diabetes. (Kettner *et al.*, 2016; Hargreave *et al.*, 2016). In one of the studies (Hargreave *et al.*, 2016), there was no information on whether the children were born after ART or not.

### **Cardiovascular and metabolic diseases after ART**

Some studies raise concerns as to whether fertility treatment may influence metabolic and cardiovascular risk factors in ART children (Ceelen *et al.*, 2007; Ceelen *et al.*, 2008a, Ceelen *et al.*, 2009; Sakka *et al.*, 2010; Chen *et al.*, 2014; Scherrer *et al.*, 2012; Zhou *et al.*, 2014; Liu *et al.*, 2015; Meister *et al.*, 2018). A systematic review and meta-analysis of 19 studies (Guo *et al.*, 2017) concluded that the blood pressure levels of ART children were significantly higher than those of SC children (weighted mean differences 1.88 mmHg; 95% CI 0.27 to 3.49 for systolic blood pressure and 1.51 mmHg; 95% CI 0.33 to 2.70 for diastolic blood pressure). In addition, cardiac diastolic function was suboptimal and vessel thickness was higher among ART children.

Apart from these findings, it is also well-known that PTB, LBW and SGA, all associated with ART children, are risk factors for future metabolic syndrome (Barker *et al.*, 1993). Furthermore, emerging evidence implies that children born to mothers with preeclampsia may be at increased risk of cardiovascular disease in adult life. A long-term register-based follow-up study from Finland reported that children born to mothers with preeclampsia were at twice the risk of stroke compared with children to normotensive mothers (Kajantie *et al.*, 2009). Further, one systematic review (Davies *et al.*, 2012) and one meta-analysis (Andraweera and Lassi, 2019) concluded that children born to mothers with preeclampsia demonstrate higher systolic blood pressure and diastolic blood pressure and a small increase in BMI, compared with controls. Since elevated blood pressure during childhood has been shown to predict the development of hypertension later in life (Chen *et al.*, 2008) and hypertension is a risk factor for future cardiovascular disease, the findings are essential.

Altogether, the data described have led to a concern for future cardiovascular disease in ART children. Yet, it should be emphasized that the majority of studies published so far, are based on small cohorts with high risk of selection bias. Additionally, the ART population is still young and most cardiovascular diseases develop later in life.

### **Growth after ART**

Several studies have compared growth in ART children to growth in children born after SC (Ceelen *et al.*, 2008; Knoester *et al.*, 2008; Bonduelle *et al.*, 2005; Basatemur *et al.*, 2010). A recent meta-analysis, including 3 972 children born after ART and 11 012 children born after SC (Bay *et al.*, 2019), did not find any difference in weight or height.

### **Respiratory disorders after ART**

There are limited data about respiratory disorders in ART children. A Swedish register-based study (Källén *et al.*, 2013) found an increased risk of asthma in ART children. However, after adjustment for duration of infertility, the effect was non-significant. Another prospective study from the UK found an increased risk of asthma in ART children but the result was based on few ART children (Carson *et al.*, 2013).

## Cancer after ART

There is a controversy whether the exposure to ART leads to an increased risk of childhood cancer. Higher risks have mainly been observed in subgroups of patients and for specific types of cancers (Bergh and Wennerholm, 2020). Three large population-based cohort studies from the United Kingdom (Williams *et al.*, 2013), the Nordic countries (Sundh *et al.*, 2014) and the Netherlands (Spaan *et al.*, 2019) did not find any increased risk of overall cancer rates among children born after ART whereas a cohort study from the United States (Spector *et al.*, 2019) found a marginally increased risk for overall cancers in ART children (HR 1.17; 95% CI 1.00 to 1.36). Spector *et al.* (2019) also observed an increased risk of embryonal cancers, particularly hepatic tumors (HR 2.46; 95% CI 1.29 to 4.70). A recent population-based cohort study from Denmark (Hargreave *et al.*, 2019) also did not find any overall risk of cancers in children born after ART but an increased risk of cancer was observed among children born after FET (AHR 2.43, 95% CI 1.44 to 4.1). Yet, the observation was based on 14 cases. One meta-analysis of 13 cohort studies (Gilboa *et al.*, 2019) did not reveal any increased risk of childhood cancers (RR 0.99; 95% 0.85 to 1.15). Another, recently published, meta-analysis found no increased risk of childhood cancer in IVF or ICSI children in general, whereas an increased risk was observed in children born after FET compared to children born after SC (five studies with 25 563 children born after FET, RR 1.37; 95% CI 1.04 to 1.81) (Zhang *et al.*, 2020). A summary of the largest published studies and systematic reviews on the risks of cancer in ART children is presented in Table 4.

**Table 4.** Summary of large observational studies, systematic reviews and meta-analyses on the risk of cancer in ART children.

First author and year of publication	Country	Number of ART children	Overall cancer risk ART vs SC (95% CI)	Specific cancers at increased risk
<b>Observational studies</b>				
Williams, 2013	United Kingdom	106 013	SIR 0.98 (0.81 to 1.19)	Hepatoblastoma, rhabdomyosarcoma
Sundh, 2014	Sweden, Denmark, Finland, Norway	91 796	AHR 1.08 (0.91 to 1.27)	CNS, malignant epithelial neoplasms
Reigstad, 2016	Norway	25 782	HR 1.21 (0.90 to 1.63)	
Gilboa, 2019	Israel	64 317	RR 1.09 (0.79 to 1.48)	
Spaan, 2019	Netherland	24 269	SIR 1.11 (0.90 to 1.36) vs SC HR 1.00 (0.72 to 1.38) vs subfertile women	
Spector, 2019	United States	275 686	HR 1.17 (1.0 to 1.36)	Hepatic tumors
Hargreave, 2019	Denmark	19 448 IVF 13 427 ICSI 3 356 FET	ART: AHR 1.20 (0.96 to 1.49) IVF: AHR 0.96 (0.70 to 1.32) ICSI: AHR 1.33 (0.94 to 1.89) FET: AHR 2.43 (1.44 to 4.11)	Leukemia
<b>Systematic reviews and meta-analyses</b>				
Gilboa, 2019		13/750 138	RR 0.99 (0.85 to 1.15)	
Zhang, 2020		27 (16 cohort studies and 11 case-control studies)/591 192	Cohort studies: IVF: RR 1.01 (0.80 to 1.28) ICSI: RR 0.97 (0.80 to 1.17) FET: RR 1.37 (1.04 to 1.81)	Not reported

ART; assisted reproductive technology; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; FET, frozen embryo transfer; SIR, standardized incidence ratio; OR, odds ratio; HR, hazard ratio; AHR, adjusted hazard ratio; RR, relative risk; CNS, central nervous system; ALL, acute lymphoblastic leukemia

### Imprinting diseases after ART

Epigenetics refers to changes in gene expression or phenotype, without changes in the DNA sequence. Different mechanisms of epigenetic control exist and several studies have suggested an association with imprinting disorders in ART children but it is difficult to separate the influence of the ART technique from the effect of the reproductive disease of the parents (Pinborg *et al.*, 2016). Imprinting disorders are also rare diseases which makes them difficult to study. One recently published register-based cohort study from the Nordic countries (Henningesen *et al.*, 2020) showed an association between ART and Beckwith-Widemann syndrome (AOR 2.84; 95% CI 1.34 to 6.01) while there was no association between ART and Prader-Willi syndrome, Silver-Russel syndrome or Angelman syndrome. Several earlier case-control studies have also found an association between ART and Beckwith-Widemann syndrome (Hiura *et al.*, 2012; Mussa *et al.*, 2017; Hattori *et al.*, 2019).

**Reproductive health after ART**

There are limited data on the reproductive health in children born after ART. One study found that pubertal development in terms of menarche, genital development in males and pubic hair development was similar in the ICSI and SC group. However, breast development was less advanced in ICSI females compared to SC females (Belva *et al.*, 2012). Another follow-up project from Belgium with a cohort of 54 men (18-22 years old) born after ICSI and 57 men born after SC, showed that the total sperm count and total motile sperm count were significantly lower in the men born after ICSI (Belva *et al.*, 2016), whereas the reproductive hormones were normal compared to the SC controls (Belva *et al.*, 2017a). In another study from the same study group, 71 young adult women (18-22 years old) conceived by ICSI, because of male infertility in their parents, were explored. No difference in reproductive hormone levels or antral follicle count was found compared to peers born after SC (Belva *et al.*, 2017b).





# Aims of the thesis

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The overall aim of the thesis was to examine the long-term outcome in children born after ART and to study whether different ART techniques affect the outcome in the children differently. The specific aims were:

- To examine whether children born after ART have similar school performance as children born after spontaneous conception (Paper I).
- To examine whether children born after ICSI have similar school performance as children born after standard IVF. A secondary aim was to examine whether children born after ICSI have similar school performance as children born after spontaneous conception (Paper II).
- To explore whether children born after ART, including specific ART treatments, have an increased risk of developing type 1 diabetes (Paper III).
- To explore whether children born after ART, including specific ART treatments, have an increased risk of developing cardiovascular disease, obesity or type 2 diabetes (Paper IV).



## Study design and settings

The thesis comprises four population-based register studies. Paper I-III are national register-based cohort studies and include all singletons born after ART and SC in Sweden from 1985 to 2001 in Paper I, 1985 to 2006 in Paper II and 1985 to 2015 in Paper III. In Paper IV the CoNARTaS cohort was used. The CoNARTaS cohort comprise all children born after ART and SC between 1985 and 2015 in Sweden, 1994 and 2014 in Denmark, 1990 and 2014 in Finland and 1984 and 2015 in Norway. The unique PIN-code assigned to each Nordic citizen enabled individual level data linkage between registers and between children and parents. The study designs, settings and analyses for each study are summarized in Table 5.

## Ethical aspects

All studies obtained ethical permission from the Regional Ethical Committee in Gothenburg (Dnr 214-12, T422-12, T516-15, T233-16, T300-17, T1144-17, T121-18, T1071-18). In Norway, ethical approval was given by the Regional Committee for Medical and Health Research Ethics (REC North, 2010/1909). According to the national laws in Denmark and Finland studies exclusively based on register data do not need ethical approval from the Scientific Ethical Committees.

**Table 5.** Study designs, settings and analyses.

	<b>Paper I</b>	<b>Paper II</b>	<b>Paper III</b>	<b>Paper IV</b>
Setting	National population-based register study in Sweden	National population-based register study in Sweden	National population-based register study in Sweden	National population-based register study in Sweden, Denmark, Finland, Norway
Study design	Retrospective population-based cohort study	Retrospective population-based cohort study	Retrospective population-based cohort study	Retrospective population-based cohort study
Study period	1985-2001	1985-2006	1985-2015	1984-2015
Study group and number of children	All singletons born after ART (n=8 323)	All singletons born after ICSI (n=6 953)	All singletons born after ART (n=47 938)	All singletons born after ART (n=122 429)
Control group and number of children	All singletons born after SC (n=1 499 667)	All singletons born after IVF (n=11 713) and SC (n=2 022 995)	All singletons born after SC (n=3 090 602)	All singletons born after SC (n=7 574 685)
Data sources	MBR-Q-IVF, Q-IVF, NPR, CDR. National School Register, Statistics Sweden	MBR-Q-IVF, Q-IVF, NPR, CDR. National School Register, Statistics Sweden	MBR-Q-IVF, Q-IVF, NPR, CDR, NDR, SWEDIABKIDS, SPDR, Statistics Sweden	CoNARTaS cohort based on ART-registers and MBRs in respective country. NPRs, CDRs, Statistic Bureaus in respective country. In Sweden also: NDR, SWEDIABKIDS, SPDR, BORIS
Primary outcome	School performance based on mean total score of 16 subjects (0-320) at the end of ninth grade	School performance based on mean total score of 16 subjects (0-320) at the end of ninth grade	Type 1 diabetes	Cardiovascular disease (ischemic heart disease, cardiomyopathy, heart failure or cerebrovascular disease), obesity, type 2 diabetes
Secondary outcomes	Mean school grade in specific subjects (mathematics, Swedish, English, physical education). "Qualified to secondary school". "Poor school performance" (total score < 160).	Mean school grade (0-20) in specific subjects (mathematics, Swedish, English, physical education). "Qualified to secondary school". "Poor school performance" (total score < 160). Results of national tests (mathematics, Swedish) in third grade (proportion who passed all subtests).		
Main analysis	ART vs SC	ICSI vs IVF ICSI vs SC	ART vs SC	ART vs SC
Subgroup analyses		Non-Ejaculated ICSI vs Ejaculated-ICSI	FET vs fresh embryo transfer ICSI fresh vs IVF fresh	FET vs fresh embryo transfer

ART, assisted reproductive technology; SC, spontaneous conception; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; FET, frozen embryo transfer; MBR, Medical Birth Register; Q-IVF, National Quality Register for Assisted Reproduction; NPR; National Patient Register; CDR, Cause of Death Register; SWEDIABKIDS, Swedish National Register for Child and Juvenile Diabetes; SPDR, Swedish Prescribed Drug Register; BORIS, Swedish National Register for Treatment of Childhood Obesity

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## Data collection

### Paper I-III

Data for Paper I, II and III were collected from the following national registers: The Swedish Medical Birth Register IVF (MBR/IVF), The Swedish Q-IVF, the Swedish Medical Birth Register (MBR), the Swedish National Patient Register (NPR) and the Swedish Cause of Death Register (CDR). Additionally, the National School Register and the Cerebral Palsy Follow-up Register (CPUP) were used in Paper I and II. Lastly, the National Diabetes Register (NDR), the National Register for Child and Juvenile Diabetes (SWEDIABKIDS) and the Swedish Prescribed Drug Register (SPDR) were used in Paper III. In addition to these registers, The Longitudinal Integration Database for Health Insurance and Labour Market Studies (LISA) and Multi-Generation Register at Statistics Sweden (SCB), provided information on emigration and parental sociodemographic background factors.

**The MBR-IVF** Since the birth of the first IVF child in Sweden in 1982, and until 2006 cycles were only reported to the National Board of Health and Welfare as aggregated data without possibility of cross linking with other registers. All women who delivered after IVF were however collected in a separate file at the National Board of Health and Welfare at three occasions during the period as a research file. For the purpose of these studies and since this file is stored at the MBR, it was decided to name this file MBR/IVF.

**The Q-IVF** was established in 2007 and is funded by the Swedish Association of Local Authorities and Regions (SKR). The register includes results of all IVF cycles that started in Sweden and has full patient identification, i.e. PIN-codes. All public and private IVF-clinics report their data to the register. The patients are informed about the Q-IVF and may choose not to have their data included, although this is very rare. Thus, the register has almost 100% completeness (<https://www.medscinet.com/qivf/>).

**The Swedish MBR** was established in 1973. Reporting to the register is compulsory and the information is collected from medical records from antenatal, delivery and neonatal care. The quality of MBR has been evaluated in 1976, 1988 (Cnattingius *et al.*, 1990) and in 2001. More detailed information about the quality of the register was published in a report in Swedish in 2002 (Forskningsrapport från Epidemiologiskt Centrum (EpC), 2002-112-4) and in English in 2003 (Research report from Centre for Epidemiology, 2003-112-3). The register is considered to be

of high quality and covers 97-99.5% of all births in Sweden. Thus, the external data loss is approximately 0.5-3% yearly. However, the internal data loss, when information is missing for an individual variable, varies considerably more (<https://www.socialstyrelsen.se/statistik-och-data/register/alla-register/medicinska-fodelseregistret/bortfall-och-kvalitet>). For example, information about maternal smoking was missing in 4-9% and maternal BMI at first antenatal visit was missing in 35% according to the Research report from Centre for Epidemiology in 2003 ([https://www.socialstyrelsen.se/globalassets/sharepointdokument/artikelkatalog/ovrigt/2003-112-3\\_20031123.pdf](https://www.socialstyrelsen.se/globalassets/sharepointdokument/artikelkatalog/ovrigt/2003-112-3_20031123.pdf).)

**The NPR** is nationwide and includes diagnoses on all patients admitted to in-hospital care in Sweden since 1987. The NPR is divided into the In-Patient-Register and the Out-Patient-Register. Starting in 2001, the Out-Patient-Register covers specialized outpatient care, including day surgery and psychiatric disorders, treated by both private and public caregivers. Primary care is not yet included. The In-Patient-Register was validated in 2011 and the positive predictive value was 85-95% for most diagnoses (Ludvigsson *et al.*, 2011). In 2019, only 1% of main diagnoses were missing in the In-Patient-Register and the underreporting for inpatient data is low. The underreporting for the Out-Patient-Register is higher which largely depends on missing information from private caregivers. In recent years the proportion of missing information on main diagnoses has decreased significantly, to 3% of reported doctor visits (<https://www.socialstyrelsen.se/statistik-och-data/register/alla-register/patientregistret/bortfall-och-kvalitet/>).

**The CDR** includes all Swedish citizens who have died since 1952, either in the country or abroad, and who were registered in Sweden at the time of death (<http://www.socialstyrelsen.se/statistik-och-data/register/alla-register/dodsorsaksregistret/>).

**The National School Register** is administered cooperatively by the Swedish School Authority (Skolverket) and Statistics Sweden (SCB). It includes information on individual educational achievements (grades by subject as well as summary scores) for all students graduating from ninth grade in public schools since 1988. Non-public schools, which have become more common in recent years and accounted for 15% of all primary schools in 2019 (<http://www.friskola.se>), have been included in the National School Register since 1993. The register also contains information about national tests. Summary statistics are published regularly (<http://www.skolverket.se>).

**The CPUP** has been a national quality register in Sweden since 2005. Since 2011, the register also includes adults with cerebral palsy (<http://www.socialstyrelsen.se>). According to a report from the National Board of Health and Welfare in 2015 the register covered 90% of children with cerebral palsy, born between 2002 and 2009 (<http://www.socialstyrelsen.se>).

**The NDR** was established in 1996. All individuals over 18 years of age with diabetes mellitus, except from gestational diabetes, are included. About two thirds of all patients today are reported directly from computerized medical record systems, reducing the amount of duplication work (Eliasson *et al.*, 2014). In 2006 the register covered 50% of all cases with diabetes in Sweden. Today, 94% of all cases with diabetes are included in the register (<http://www.ndr.nu>).

**The SWEDIABKIDS** is under the umbrella of NDR. It was established in year 2000 and from 2007 the register is complete i.e. all clinics treating children and adolescents with diabetes report data to the register. The SWEDIABKIDS is now covering 98% of children and adolescents that are diagnosed with diabetes mellitus until 18 years of age (<http://www.ndr.nu>).

**The SPDR** contains personal identity number since July 2005. The SPDR registers all redeemed prescriptions for drugs and contains information such as Anatomic Therapeutic Chemical (ATC) -code, prescription amount, date of prescription and date when the product was redeemed (<http://www.socialstyrelsen.se>). The quality of the register is generally very good since all registrations are computerized.

**Statistics Sweden** is the central authority for recording statistics on all Swedish citizens. The LISA contains information such as highest educational level, disposable income, immigration and emigration (<http://www.scb.se>).

## Paper IV

Data for Paper IV were collected from the CoNARTaS cohort.

**The CoNARTaS cohort**, is a Nordic collaborative research group, which was established in 2008, with the purpose to examine the health of children born after ART and with this, the safety of ART. Data in the CoNARTaS cohort are obtained from the national ART registers and MBRs in Sweden, Denmark, Norway and Finland, all with high quality (Opdahl *et al.*, 2019). Linkage has also been made to

other national health registers, quality registers and nationwide data bases. Using the PIN-code, given to each citizen in the Nordic countries, data are linked at an individual level. In Paper 4, the CoNARTaS cohort was linked with data from the NPRs and the CDRs in each country. In Sweden, the data were also cross-linked with information from the national quality registers; the Swedish NDR, the SWEDIABKIDS, the SPDR and the Swedish National Register for Treatment of Childhood Obesity (BORIS).

**The MBRs** covers nearly all births in the Nordic countries since decades. Reporting to the registers is mandatory (Langhoff-Roos *et al.*, 2014).

**ART Registration.** The ART registration for Sweden has been described above. In Denmark, the national ART register was established in 1994. Registration of all ART cycles for both public and private ART clinics have been compulsory since then. Thus, the register has almost 100% coverage (Andersen *et al.*, 1999). In Norway public and private ART clinics report detailed information to the MBR on all ART cycles that result in pregnancies verified by ultrasound in gestational week 6-7 (Opdahl *et al.*, 2019). In Finland, there is no national ART register, but ART conception has been recorded at an individual level at delivery as a dichotomous variable in the MBR from 1990 (Opdahl *et al.*, 2019).

**The NPRs** are nationwide and include diagnoses on all patients admitted to in-hospital care since 2008 in Norway, 1967 in Finland and 1977 in Denmark. Outpatient visits in public hospitals and specialized health care in private clinics have been included since 1998 in Finland and 2008 in Norway. In Denmark, outpatient visits in public hospitals have been registered since 1995 and information about specialized health care in private clinics since 2003 (Opdahl *et al.*, 2019). The registers have high coverage rates and high validity with positive predictive values in the range of 81-94% for Denmark (Schmidt *et al.*, 2015), 75-99% for common diagnoses in Finland (Sund, 2012) and 80-95% in Norway (Govatsmark *et al.*, 2020; Varndal *et al.*, 2016; Hollung *et al.*, 2017; Nesvag *et al.*, 2017). The Swedish NPR has been described above.

**The CDRs** includes all citizens who have died, either in the country or abroad, and who were registered in the country at the time of death. The register started 1951 in Norway (<http://www.fhi.no>), 1936 in Finland (<http://www.stat.fi>) and 1973 in Denmark (<http://www.sundhedsstyrelsen.dk>). The Swedish CDR has been described above.



**The Swedish BORIS** was established in 2005. The register includes children until 18 years of age who are treated for obesity at pediatric clinics or in-hospital care. According to the annual report of BORIS, the coverage ratio increased rapidly from 2008 and from 2013 almost 80% of children treated for obesity are included in the register. (<http://www.e-boris.se>).

The Swedish NDR, the SWEDIABKIDS and the SPDR has been described above. Socio-economic data were retrieved from the Statistic Bureau in each country.

## Outcomes

### Paper I and II

The primary outcome for Paper I and II was school performance at the end of nine years in regular Swedish school, based on a mean total score of 16 subjects. Secondary outcomes were the mean school grade (0-20) of specific subjects (mathematics, Swedish, English and physical education), “qualified to secondary school” (approved in mathematics, Swedish and English) and “poor school performance” (total score <160). In Paper II, we also obtained results from national tests in third grade for the children who were too young to have ended ninth grade.

During the study period, a grading system was used with school grades ranging from 0 to 20. With 16 different subjects, the total score was between 0 and 320. Between 1997 and 2012 the grade system consisted of four different grades (fail=0 points, pass=10 points, pass with distinction=15 points, pass with special distinction=20 points). From 2013 the grade system consisted of six different grades (F=0 points, E=10 points, D=12.5 points, C=15 points, B=17.5 points, A=20 points). F meant fail, E to A were all passes. It was not possible to separate between “fail” or “not participated” as both groups of students received a total score of 0 points. To qualify for secondary school a passing grade in the core subjects of mathematics, Swedish and English is required in Sweden. The percentage of students that passed in these three subjects were therefore measured. Furthermore, “poor school performance” was assessed, which was defined as a total score below 160, i.e., the mean grade of the individual was below “pass” (Lambe *et al.*, 2006).

The national tests in third grade consisted of seven or eight subtests in mathematics and Swedish. To pass the national tests, passing all the subtests was required. Thus,

we calculated the number of children who passed all subtests in mathematics and Swedish.

## Paper III and IV

The primary outcome in Paper III was type 1 diabetes in the children. In Paper IV the primary outcomes were any cardiovascular disease (ischemic heart disease, cardiomyopathy, heart failure or cerebrovascular disease), obesity and type 2 diabetes in the children.

### *Definition of type 1 diabetes (Paper III)*

The criteria were set up as a diagnostic hierarchy with criteria 1 as the most reliable in the hierarchy and 4 as the least reliable. Date of onset was defined as the first date when the diagnosis was set in either SWEDIABKIDS, NDR or NPR.

1. Type 1 diabetes diagnosis in SWEDIABKIDS or NDR
2. Diabetes diagnosis of unknown type, exclusively treated with insulin in SWEDIABKIDS or NDR (i.e. insulin treatment recorded, but no information on oral antidiabetics in SWEDIABKIDS or NDR)
3. Diabetes diagnosis of unknown type in SWEDIABKIDS or NDR, exclusively treated with insulin or insulin analogues as recorded in the prescription database ( $\geq 2$  prescriptions of insulin or insulin analogues for males and  $\geq 3$  prescriptions for females [for exclusion of gestational diabetes] during the whole study period and  $< 2$  prescriptions of oral antidiabetics during the whole study period)
4. Discharge diagnosis from hospital (NPR) with type 1 diabetes/unspecific diabetes and information from the prescription database similar to criteria 3.

### *Definition of cardiovascular disease, obesity and type 2 diabetes (Paper IV)*

The definitions of any cardiovascular disease, obesity and type 2 diabetes are described in detail in Paper IV.

## Statistical methods

All statistical analyses were performed in collaboration with professional statistician Max Petzold, PhD, Professor, School of Public Health and Community Medicine, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden. The

statistical methods used in the different papers are summarized in Table 6. Descriptive statistics were given by mean and standard deviation (SD), median and range for continuous variables, and by number (n) and percent for categorical variables. Significance level was set to 5%. The Analyses were performed in STATA version 15 and 15.1.

## Paper I and II

The statistical methods are described in detail in Paper I and II. School scores were categorized into percentiles (1-100). Simple and multivariable linear regression were used for the analysis of percentiles whereas logistic regression was used for the analysis of binary outcomes. Crude and adjusted mean differences in percentiles with 95% CI were presented for school grades while OR and AOR with 95% CI were presented for qualified to enter secondary school and poor school performance.

## Paper III and IV

The statistical methods are described in detail in Paper III and IV. The risk of type 1 diabetes, any cardiovascular disease, obesity and type 2 diabetes was estimated as HRs using Cox proportional hazards models. Age was used as the time scale and included each child's time at risk computed from the date of birth until whichever event occurred first: diagnosis of type 1 diabetes (in Paper III), any cardiovascular disease, obesity, type 2 diabetes (in Paper IV), emigration, death, or end of the follow-up period. In Paper IV the outcomes any cardiovascular disease, obesity and type 2 diabetes were analyzed separately i.e. if a child developed obesity it continued to be followed up in the study for the outcomes any cardiovascular disease and type 2 diabetes. We estimated crude and adjusted HRs and 95% CIs. A fixed set of covariates was selected based on medical knowledge. Different models of adjustment were used.

**Table 6.** Overview of statistical methods used in the papers.

	Paper I	Paper II	Paper III	Paper IV
Descriptive statistics	Categorical variables: number and percentage  Continuous variables: mean and SD, median and range	Categorical variables: number and percentage  Continuous variables: mean and SD, median and range	Categorical variables: number and percentage  Continuous variables: mean and SD, median and range	Categorical variables: number and percentage  Continuous variables: mean and SD, median and range
Analytical statistics	For analysis of percentiles: simple and multivariable linear regression. Crude and adjusted mean difference with 95% CI  For analysis of binary outcomes: logistic regression. OR and AOR with 95% CI	For analysis of percentiles: simple and multivariable linear regression. Crude and adjusted mean difference with 95% CI  For analysis of binary outcomes: logistic regression. OR and AOR with 95% CI	The risk of type 1 diabetes was estimated using Cox proportional hazards model. HR and AHR with 95% CI	The risk of any cardiovascular disease, obesity and type 2 diabetes was estimated using Cox proportional hazards model. HR and AHR with 95% CI
Covariates and their definitions	Child: sex, year of birth (continuous variable)  Pregnancy characteristics: parental age ( $\leq 24$ , 25-34, 35-44, $\geq 45$ years) at birth of child, parity (1, 2, 3, 4, $\leq 5$ ), maternal smoking at first antenatal visit (yes/no)  Parental characteristics: region of birth (Sweden, Nordic outside Sweden, European outside Nordic Region, African origin, Asian origin, other origin), educational level ( $\leq 9$ , 10-12, higher education $< 3$ years, higher education $\geq 3$ years), socioeconomic class working class, middle class, high class	Child: sex, year of birth (continuous variable)  Pregnancy characteristics: parental age ( $\leq 24$ , 25-34, 35-44, $\geq 45$ years) at birth of child, parity (1, 2, 3, 4, $\leq 5$ ), maternal smoking at first antenatal visit (yes/no)  Parental characteristics: region of birth (Sweden, Nordic outside Sweden, European outside Nordic Region, African origin, Asian origin, other origin), educational level ( $\leq 9$ , 10-12, higher education $< 3$ years, higher education $\geq 3$ years)  In the comparison between the different ART-groups we also adjusted for FET and in an additional analysis we also adjusted for vanishing twin.	Child: sex, year of birth (1985-1990, 1991-1995, 1996-2000, 2001-2005, 2006-2010, 2011-2015)  Pregnancy characteristics: maternal age (continuous variable) at birth of child, parity (primiparous or multiparous), maternal smoking at first antenatal visit (yes/no)  Parental characteristics: region of birth (Sweden, Nordic outside Sweden, European outside Nordic Region, other origin), parental educational level, parental type 1 diabetes.  Different models of adjustments were made. In one of the models we adjusted for macrosomia. In the subgroup analyses of FET we adjusted for fertilization method (IVF/ICSI).	Child sex, year of birth (continuous variable), country of birth  Pregnancy characteristics: maternal age (continuous variable) at birth of child, maternal smoking at first antenatal visit (yes/no), highest educational level (basic $\leq 9$ years, secondary 10-12 years, tertiary $\leq 13$ years)  Maternal characteristics: any cardiovascular disease, obesity, any diabetes before or at birth of child

ART, assisted reproductive technology; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; SD, standard deviation; OR, odds ratio, AOR, adjusted odds ratio; CI, confidence intervals, HR, hazard ratio; AHR, adjusted hazard ratio; FET, frozen embryo transfer

## Paper I

### *School performance in singletons born after assisted reproductive technology*

A total of 8 323 singletons born after ART and 1 499 667 singletons born after SC were included in the study.

### *Maternal and perinatal characteristics*

Mean maternal age at birth was 33.9 years in ART mothers and 28.6 years in SC mothers. Higher education  $\geq 3$  years was seen in 29.3% of ART mothers compared to 24.0% of SC mothers. Nulliparity characterized 76.2% of ART mothers and 41.2% of SC mothers. PTB was seen in 9.3% of ART children and 4.8% of SC children whereas LBW occurred in 7.0% of ART children and 3.0% of SC children.

### *School performance*

School performance in ninth grade for children graduated 2000-2016 is summarized in Table 7.

In the crude analyses, ART children performed significantly better than SC children in total score for all subjects, as well as in the specific subjects mathematics, Swedish, English and physical education. ART children also had a significantly higher proportion who managed to qualify for secondary school and a significantly lower proportion of poor school performance than children born after SC. After adjustment, however, ART children had significantly lower total score for all subjects compared to their SC counterparts (adjusted mean difference [percentiles] -0.72; 95% CI -1.31 to -0.12;  $p=0.018$ ). There were no significant differences between children born after ART and children born after SC in the specific subjects mathematics (adjusted mean difference [percentiles] -0.56; 95% CI, -1.19 to 0.06;  $p=0.08$ ), Swedish (adjusted mean difference [percentiles] -0.51 (95% CI, -1.1 to 0.09;  $p=0.10$ ), English (adjusted mean difference [percentiles] 0.03; 95% CI -0.62 to 0.69;  $p=0.92$ ) or physical education (adjusted mean difference [percentiles] -0.48; 95% CI, -1.2 to 0.23;  $p=0.19$ ). Neither were there any differences between the two groups regarding qualifying for secondary school (AOR 1.05; 95% CI 0.95 to 1.17;  $p=0.35$ ), nor for poor school performance (AOR 0.98; 95% CI 0.89 to 1.09;  $p=0.73$ ). When separating boys and girls, a significantly lower total score for all subjects (adjusted mean

difference [percentiles] -1.22; 95% CI -2.1 to -0.36; p=0.005) and a significantly lower score in the specific subject Swedish (adjusted mean difference [percentiles] -1.2; 95% CI -2.2 to -0.29; p=0.01) was seen in ART girls compared to SC girls.

### Missing data

There were 42 274 children without any registration at the Swedish School Authority. Of children born after ART, 2.7% had no registered education. The corresponding figure for children born after SC was 2.8%. By cross-linking the children without any registration with the NPR, using the ICD 9 and ICD 10 codes for mental disability, we found that 35% and 34% of the ART and SC children respectively, were registered with a code for mental disability. Additionally, the PTB/VPTB and LBW/VLBW rates were high in the group of children with missing data, especially among children born after ART.

**Table 7.** School performance in ninth grade, children graduated 2000-2016 and born in Sweden. Adjusted mean difference in total score and in mathematics, English, Swedish and physical education in percentiles, 1-100. Adjusted odds ratio for qualified to enter secondary school and poor school performance.

	ART vs SC Adjusted mean difference <sup>a</sup> (95% CI) p-value	ART vs SC Adjusted OR <sup>a</sup> (95% CI) p-value
Total score, all subjects	-0.72 (-1.31 to -0.12) p=0.018	
Mathematics	-0.56 (-1.19 to 0.06) p=0.08	
Swedish	-0.51 (-1.10 to 0.09) p=0.10	
English	0.03 (-0.62 to 0.69) p=0.92	
Physical education	-0.48 (-1.20 to 0.23) p=0.19	
Qualified to enter secondary school <sup>b</sup>		1.05 (0.95 to 1.17) p=0.35
Poor school performance <sup>c</sup>		0.98 (0.89 to 1.09) p=0.73

<sup>a</sup> Adjusted for maternal age, paternal age, parental region of birth, parity, maternal smoking during pregnancy, parental level of education, socioeconomic status, year of birth

<sup>b</sup> Qualified to enter secondary school (approved in mathematics, English and Swedish)

<sup>c</sup> Poor school performance (total score <160)

### Comments

Interestingly, and in alignment with a previous study from Denmark (Spangmose *et al.*, 2017), ART children performed better in all crude analyses compared to children born after SC. However, the differences were attenuated considerably after adjustment for important covariates and instead a small negative difference was seen for ART children in the total score for all subjects. The sociodemographic and perinatal characteristics varied between the children born after ART and SC. High educational level of the parents and low number of siblings were covariates associated with good school performance, covariates that parents of ART children to a larger extent were associated with. In contrast, young maternal age, smoking, high number of siblings and low educational level of the parents were covariates associated with lower school performance, characteristics more common in parents to children born after SC. These differences probably explain why the crude and adjusted results differed.

The covariates were selected *a priori* and from existing literature (Svensson *et al.*, 2011; Ahlsson *et al.*, 2015). Some of the covariates were essential to adjust for, such as gender, year of birth and parental educational level while others were more questionable. For example, socioeconomic status is likely correlated with parental educational level and could probably have been ruled out (which it was in the subsequent studies of the thesis). Another question was whether we should use highest educational level in the parents or educational level at birth. We chose highest educational level since this has been shown to be a stable socioeconomic variable over time (Liberatos *et al.*, 1988). Still, we are aware of the disadvantage with parents to children in the younger cohorts, who may not have reached their highest educational level yet. Further, a question was if we were going to adjust for PTB, LBW and Apgar score. Since these covariates are potential causal pathway characteristics, we preferred not to adjust for those. In a supplementary analysis, however, adjustment for gestational age and LBW was performed which changed the estimates and 95% CI only marginally (Paper I).

One can discuss if the crude or the adjusted results should have most credit. From a pure scientific point of view, for sure, it is the adjusted results. However, the parents might care more of what their children actually achieve in comparison to their friends in school and be less interested in possible confounders and adjusted estimates. The adjusted results are crucial since these, to a higher extent, evaluate the ART technique *per se*.

## Paper II

### *School performance in children born after ICSI*

A total of 6 953 ICSI children, 11 713 IVF children and 2 022 995 SC children were included in the study.

#### **The ninth grade**

In the analysis of ninth grade, 2 571 ICSI children (2 440 children born after ICSI with ejaculated sperm and 131 children born after ICSI with non-ejaculated sperm), 5 766 IVF children and 1 500 709 SC children were included.

#### *Maternal and perinatal characteristics*

Mean maternal age at birth was 33.2 years in ICSI mothers and 34.3 years in IVF mothers. Higher education  $\geq 3$  years was seen in 30.3% of ICSI mothers and in 29.0% of IVF mothers. Nulliparity characterized 78.4% of ICSI mothers and 75.3% of IVF mothers. Smoking at first antenatal visit was reported in 7.4% of ICSI mothers whereas the corresponding figure for IVF mothers was 13.4%. PTB occurred in 8.7% of ICSI children, 9.6% of IVF children and 4.8% of SC children whereas LBW occurred in 6.2% of ICSI children, 7.3% of IVF children and 3.0% of SC children.

#### *School performance*

School performance in ninth grade for singletons born after ICSI, IVF and SC is presented in Table 8.

#### *ICSI versus IVF*

The crude total mean scores and the mean scores in specific subjects were significantly higher in ICSI children compared to IVF children and the risk of poor school performance was lower in ICSI children. In the adjusted analyses, however, there was no significant difference between ICSI and IVF children neither for total score or specific subjects nor for approved to secondary school or poor school performance. The adjusted mean difference (percentiles) for total score was 1.03 (95% CI -0.22 to 2.28;  $p=0.11$ ).

#### *ICSI versus SC*

The crude mean total score and mean scores for specific subjects were significantly higher in ICSI children. A significantly higher proportion of ICSI children also managed to qualify for secondary school and a significantly lower proportion of ICSI children was classified with poor school performance compared to children born



after SC. After adjustment, however, there were no significant differences between the groups in any of the analyses.

**Table 8.** School performance in ninth grade for singletons born after ICSI, IVF and spontaneous conception, graduated 2000 -2016 and born in Sweden. Adjusted mean difference in total score and in mathematics, English, Swedish and physical education in percentiles, 1-100.

	All ICSI (n=2 571) vs IVF (n=5 766)	All ICSI (n=2 571) vs SC (n=1 500 709)	Non-Ejaculated ICSI (n=131) vs Ejaculated ICSI (n=2 440)
	Adjusted mean difference <sup>a</sup> (95% CI) p-value	Adjusted mean difference <sup>b</sup> (95% CI) p-value	Adjusted mean difference <sup>a</sup> (95% CI) p-value
Total score, all subjects	1.03 (-0.22 to 2.28) 0.11	0.52 (-0.45 to 1.50) 0.29	-3.01 (-7.25 to 1.22) 0.16
Mathematics	0.98 (-0.36 to 2.31) 0.15	0.60 (-0.42 to 1.62) 0.25	0.96 (-3.58 to 5.51) 0.68
Swedish	0.60 (-0.71 to 1.83) 0.39	0.66 (-0.34 to 1.65) 0.20	-5.07 (-9.41 to -0.74) 0.02
English	0.38 (-0.97 to 1.73) 0.58	0.95 (-0.12 to 2.03) 0.08	-5.53 (-10.10 to -0.96) 0.02
Physical education	0.44 (-1.01 to 1.89) 0.55	0.47 (-0.68 to 1.63) 0.42	1.26 (-3.63 to 6.15) 0.61

ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; SC, spontaneous conception; SD standard deviation

<sup>a</sup>Adjusted for sex, year of birth, parental age, parental region of birth, parity, maternal smoking during pregnancy, parental level of education, frozen embryo transfer

<sup>b</sup>Adjusted for sex, year of birth, parental age, parental region of birth, parity, maternal smoking during pregnancy, parental level of education

### *ICSI with non-ejaculated sperm versus ICSI with ejaculated sperm*

In a subgroup analysis, children born after ICSI with non-ejaculated sperm were compared to children born after ICSI with ejaculated sperm. In the adjusted analyses, children born after ICSI with non-ejaculated sperm had significantly lower results in Swedish (adjusted mean difference [percentiles] -5.07; 95% CI -9.41 to -0.74; p=0.02) and English (adjusted mean difference [percentiles] -5.53; 95% CI -10.10 to -0.96; p=0.02) as well as a significantly higher risk of poor school performance (AOR 1.87; 95% CI 1.01 to 3.45; p=0.045) than children born after ICSI with ejaculated sperm.

*FET versus fresh embryo transfer*

An analysis of the school performance in ninth grade in children born after FET versus children born after fresh embryo transfer was also made for study II, but not included in the published paper. No significant differences were found between children born after FET and children born after fresh embryo transfer in the crude or adjusted analyses. The analysis of children born after FET versus children born after fresh embryo transfer is presented in Table 9.

**Table 9.** School performance in ninth grade for singletons born after frozen and fresh embryo transfer in Sweden, graduated 2000-2016.

	FET N=873	Fresh embryo transfer N=7 464	Crude mean difference <sup>e</sup> (95% CI) p-value	Adjusted mean difference <sup>e,f</sup> (95% CI) p-value	FET vs fresh embryo transfer OR (95% CI) p-value	FET vs fresh embryo transfer Adjusted OR <sup>f</sup> (95% CI) p-value
Total score <sup>a</sup> , all subjects						
Mean score (SD)	231.7 (56.0)	230.2 (57.4)	0.61 (-1.33 to 2.54)	-0.04 (-1.77 to 1.69)		
Mean percentile	60.8	60.2	0.54	0.96		
Mathematics <sup>b</sup>						
Mean score (SD)	13.4 (4.5)	13.3 (4.6)	0.77 (-1.21 to 2.75)	0.16 (-1.69 to 2.02)		
Mean percentile	61.9	61.1	0.45	0.86		
Swedish <sup>b</sup>						
Mean score (SD)	14.4 (4.1)	14.2 (4.4)	0.95 (-0.99 to 2.88)	0.16 (-1.74 to 1.77)		
Mean percentile	63.8	62.9	0.34	0.99		
English <sup>b</sup>						
Mean score (SD)	15.2 (4.1)	15.1 (4.3)	0.36 (-1.61 to 2.33)	-0.23 (-2.10 to 1.64)		
Mean percentile	66.8	66.4	0.72	0.81		
Physical education <sup>b</sup>						
Mean score (SD)	14.9 (4.6)	14.6 (4.7)	2.04 (-0.01 to 4.11)	1.53 (-0.48 to 3.54)		
Mean percentile	64.1	62.0	0.05	0.14		
Qualified to enter secondary school <sup>c</sup>					1.36 (0.99 to 1.86) 0.06	1.30 (0.93 to 1.81) 0.12
Poor school performance <sup>d</sup>					1.01 (0.77 to 1.33) 0.94	1.10 (0.83 to 1.46) 0.50

FET, frozen embryo transfer

<sup>a</sup> Total score 0-320

<sup>b</sup> Score 0-20 (not pass=0 p, pass=10 p, pass with distinction=15 p, pass with special distinction=20 p)

<sup>c</sup> Qualified to enter secondary school (approved in mathematics, English and Swedish)

<sup>d</sup> Poor school performance (total score <160)

<sup>e</sup> In percentiles, 1-100

<sup>f</sup> Adjusted for sex, year of birth, maternal age, paternal age, parental region of birth, parity, smoking during pregnancy, parental level of education

### **The third grade**

In the analysis of third grade, 4 382 ICSI children (4 051 children born after ICSI with ejaculated sperm and 331 children born after ICSI with non-ejaculated sperm), 5 947 IVF children and 552 286 SC children were included.

#### *Maternal and perinatal characteristics*

Mean maternal age at birth was 33.4 years in ICSI mothers and 34.5 years in IVF mothers. Smoking at first antenatal visit was registered in 4.4% and 4.2% of ICSI and IVF mothers respectively whereas higher education  $\geq 3$  years was seen in 37.7% and 43.7% of ICSI and IVF mothers, respectively. PTB occurred in 6.5% of ICSI children, 7.8% of IVF children and 4.2% of SC children whereas LBW occurred in 4.7% of ICSI children, 5.5% of IVF children and 2.9% of SC children.

#### *School performance*

##### *ICSI versus IVF*

ICSI and IVF children had comparable performance regarding passing all national subtests in mathematics (AOR 0.98; 95% CI 0.89 to 1.08;  $p=0.71$ ) and Swedish (AOR 0.96; 95% CI 0.87 to 1.07;  $p=0.49$ ).

##### *ICSI versus SC*

The crude analyses showed that ICSI children to a greater extent passed all the national subtests in mathematics and Swedish. In contrast, the adjusted analyses showed that ICSI children to a lower extent than SC children passed all the subtests in mathematics (AOR 0.89; 95% CI 0.83 to 0.96;  $p=0.002$ ) and Swedish (AOR 0.92; 0.85 to 0.99;  $p=0.02$ ).

#### *Missing data*

A number of 45 951 children did not have any registered education by the Swedish School Authority. The proportion of children born after ICSI, IVF and SC with no registered education was 2.1%, 2.0% and 2.3% respectively. The children without registered education was cross-linked with the NPR, using the ICD 9 and ICD 10 codes for mental disability. The analysis showed that 29.9% of ICSI, 32.6% of IVF and 35% of SC children without registered education were diagnosed with mental disability. Furthermore, the children without registered education was cross-linked with the CPUP, which showed that 2.7% of ICSI, 5.7% of IVF and 1.7% of SC children without registered education were diagnosed with cerebral palsy.

### Comments

The findings showed that children born after ICSI had comparable school performance at the end of ninth grade to those born after IVF and SC. In fact, ICSI children had higher scores than children in both these groups in the crude analyses but the differences disappeared after adjustment for measured confounders. However, when comparing ICSI children with SC children in the third grade, ICSI children had significantly lower chance of passing all the subtests in mathematics and Swedish. A specific group with increased risk of poor school performance was identified among children born after ICSI with non-ejaculated sperm. Clearly, further studies are required to look into this potential association, particularly since the result was based on a subgroup analysis with limited data (131 individuals, 14 events).

To summarize, the findings of Paper I and II were reassuring. Even if some of the analyses suggested minor differences between the groups, the differences were small and probably smaller than the sum of unmeasured confounders. Furthermore, we did not adjust for multiple comparisons (as the outcomes were not independent and therefore formal adjustment for multiple comparison was not possible). If standard methods of multiple testing had been applied, the outcomes would probably not have been significant. A register-based study from Denmark (Spangmose *et al.*, 2017), in which the school performance of 2 836 ART singletons and 1 930 ART twins in the ninth grade were evaluated, found similar results between children born after ART and SC.

The main limitation of Paper I and II, was the missing data of children without a registration at the Swedish School Authority, since this group contains children at high risk of cognitive impairment. Nevertheless, the missing data was comparable between the different groups in the studies and it was low compared to most other published studies on school performance in ART children. Another limitation was the change in grading system during the study period, from four different grades between 1997 and 2012 to six different grades from 2013. The minimum and maximum score was the same throughout the period (0-20 points for specific subjects and 0-320 points for total score), as well as the score for passing in a subject (10 points). Yet, the purpose and requirements of knowledge changed when the grading system was reformed, since the grades were related to specific aims in the first system but related to specific knowledge in the later system. Clearly, the changes were the same for the ART and SC children but since the ART population accounted for a larger proportion in the latest grading system, it is difficult to estimate how and if this has affected the results of Paper I and II.

In the statistical analyses we chose to categorize the school scores into percentiles (1-100) since the distribution of school grades was skewed towards higher scores and this method better allowed for comparison between birth cohorts without interference from changes in the grading system. Recent publications about Swedish school grades have used the same method (Ahlsson *et al.*, 2015; Shen *et al.*, 2016). Another option could have been to estimate the percentage of children in each category and subsequently perform a Chi2-test.

## Paper III

### *Type 1 diabetes in children born after assisted reproductive technology: a register-based national cohort study*

A total of 47 938 singletons born after ART and 3 090 602 children born after SC were included in the study.

#### *Maternal and perinatal characteristics*

Mean maternal age at birth was 34.2 years for ART mothers and 29.7 years for SC mothers. BMI was 24.4 (SD 3.9) in ART mothers and 24.0 (SD 4.3) in SC mothers whereas smoking at first antenatal visit was registered in 3.9% of ART mothers and 13.9% of SC mothers. Type 1 diabetes occurred in 0.6% of mothers and 0.8% of fathers to children born after ART and in 0.4% of mothers and 0.6% of fathers to children born after SC. The majority of parents to ART and SC children were born in Sweden (between 80-85% in both groups). In ART children, PTB occurred in 7.6% (7.9% for fresh embryo transfer, 6.3% for FET) whereas it occurred in 5.0% of SC children. LBW characterized 5.4% of children born after ART (5.9% of fresh embryo transfer, 3.7% of FET) and 3.2% of children born after SC. The proportion of macrosomia (>4000g) was 16.2% in ART children (14.3% in fresh embryo transfer, 22.3% in FET) and 18.7% in SC children. Mean follow-up time differed considerably between the two groups (9.7 [SD 6.4] years for children born after ART and 16.3 [SD 9.2] years for children born after SC).

#### *Main analysis*

##### *ART versus SC*

The association between ART, including different ART techniques, and type 1 diabetes is shown in Table 10.

During the study period, 202 (0.42%) of children born after ART and 17 916 (0.58%) of children born after SC developed type 1 diabetes. The crude analysis showed an increased risk of type 1 diabetes in ART children (HR 1.23; 95% CI 1.07 to 1.42). However, after adjustment for calendar year of birth the association disappeared (AHR 1.13; 95% CI 0.98 to 1.30). After additional adjustment for maternal age, sex, maternal country of birth, maternal educational level and parental type 1 diabetes, the estimate and 95% CI changed only marginally (AHR 1.07; 95% CI 0.93 to 1.23). The most important predictor of type 1 diabetes was paternal type 1 diabetes (AHR 8.82; 95% CI 8.23 to 9.45) and maternal type 1 diabetes (AHR 6.42; 95% CI 5.85 to 7.05). Further, macrosomia was found to be a predictor of type 1 diabetes (AHR 1.10; 95% CI 1.07 to 1.15).

*Subgroup analyses*

*FET versus fresh embryo transfer and SC*

When children born after FET (n=11 211) were compared to children born after fresh embryo transfer (n=36 727) and compared to children born after SC (n=3 090 602), an increased risk of type 1 diabetes was found in FET children, also after adjustment (AHR 1.52; 95% CI 1.08 to 2.14 and AHR 1.41; 95% CI 1.05 to 1.89).

*ICSI versus IVF and SC*

Children born after fresh ICSI had similar risk of developing type 1 diabetes as children born after fresh IVF.

**Table 10.** Association between ART, including frozen embryo transfer, and type 1 diabetes in singletons born in Sweden between 1985 and 2015.

	ART* N= 47 938	SC N= 3 090 602	FET N= 11 211	Fresh ET N= 36 727	ART vs SC HR crude (95% CI)	ART vs SC AHR <sup>a</sup> (95% CI)	FET versus fresh ET HR crude (95% CI)	FET versus fresh ET AHR <sup>b</sup> (95% CI)	FET vs SC HR crude (95% CI)	FET vs SC AHR <sup>a</sup> (95% CI)
Number of events (%)	202 (0.42)	17 916 (0.58)	45 (0.40)	157 (0.43)	1.23 (1.07 to 1.42)	1.07 (0.93 to 1.23)	1.48 (1.06 to 2.07)	1.52 (1.08 to 2.14)	1.65 (1.23 to 2.21)	1.41 (1.05 to 1.89)
Person-years at risk	465 450	50 471 136	83 181	382 269						

N, number; ET, embryo transfer; FET, frozen embryo transfer; SC, spontaneous conception; ART: assisted reproductive technology; HR, hazard ratio; AHR, adjusted hazard ratio

<sup>a</sup> Adjusted for maternal age, sex, year of birth, maternal smoking, maternal region of birth and educational level and type 1 diabetes of the parents

<sup>b</sup> Adjusted for maternal age, sex, year of birth, maternal smoking, maternal region of birth and educational level and type 1 diabetes of the parents, IVF/ICSI

\*ART=Fresh ET+FET

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### Comments

The findings showed no association between ART and type 1 diabetes after adjustment for important covariates. Type 1 diabetes in ART children has been studied to a very small extent earlier (Kettner *et al.*, 2016).

Except for a strong association with parental type 1 diabetes and a relatively weak association with macrosomia, a higher risk of type-1 diabetes in the children was also associated with Swedish region of birth in the parents, male gender and a more recent calendar year of birth in the children. These findings are supported by other epidemiological studies published on type 1 diabetes (Mayer-Davis *et al.*, 2017; Patterson *et al.*, 2019; Mobasseri *et al.*, 2020). Furthermore, a slight protecting effect of smoking was noticed, which might appear unexpected. However, in an earlier study (Robertson and Harrild, 2010) where risk factors for diabetes in children were investigated, a reduced risk of diabetes was found in mothers who smoked compared to non-smoking mothers. The effect might be mediated by low birth weight, which was suggested in a meta-analysis of Cardwell *et al.* (2010) in which a reduced risk of diabetes was seen in children with low birth weight.

An important limitation with our study was the considerable difference in follow-up time between the ART and SC children (9.7 [SD 6.4] years for ART children and 16.3 [SD 9.2] years for SC children). Since the most common age of onset for type 1 diabetes is 5 to 14 years (peak 10-14 years) (Rawshani *et al.*, 2014), the majority of SC children in our study had past the most critical years of developing type 1 diabetes, while the majority of ART children had not. Consequently, there could be an underestimation of the risk of type 1 diabetes in the ART population.

In a subgroup analysis, we found a significant association between FET and type 1 diabetes, however based on few events (n=45). Therefore, these results should be interpreted with caution.

## Paper IV

### *Cardiovascular disease, obesity and type 2 diabetes in children born after assisted reproductive technology: a population-based cohort study*

The number of children included in the study are described in Paper IV.

#### *Maternal and perinatal characteristics*

Mean maternal BMI was 24.2 (SD 4.1) in ART mothers and 24.1 (SD 4.5) in SC mothers. There were differences between ART children and SC children in their proportions of PTB and LBW, analogous with the earlier studies. Mean follow-up time was considerably shorter for ART children compared to children born after SC. The results are presented in Paper IV.

#### *Main analysis*

##### *ART versus SC*

Children born after ART had significantly increased risks of any cardiovascular disease (ischemic heart disease, cardiomyopathy, heart failure or cerebrovascular disease), obesity and type 2 diabetes in the crude analyses. After adjustment, however, the risk of any cardiovascular disease and type 2 diabetes were comparable between the groups whereas the risk of obesity was still increased after ART. A secondary analysis was performed in which data from Norway was excluded, since their NPR did not start until 2008, with comparable results to the main analysis. The crude and adjusted HRs are presented in paper IV.

#### *Subgroup analysis*

No association between FET and any cardiovascular disease or obesity was found in the adjusted main analysis. However, an association between FET and obesity was found in the secondary analysis where data from Norway was excluded. For the outcome type 2 diabetes, there were too few events to be presented and to perform any analysis.

#### *Comments*

After adjustment for important covariates the results showed no association between ART children and any cardiovascular disease or type 2 diabetes whereas an increased risk of obesity was found in ART children. The findings are generally reassuring.

When covariates were selected for adjustment, there was a discussion whether adjustment should be made for maternal cardiovascular disease, type 2 diabetes and



obesity at birth or during the lifetime of the mother. Both alternatives had pros and cons but we chose to assess these diseases at birth of the child since the follow up time for ART and SC groups differed substantially. Another question was whether we should adjust for type 2 diabetes or any diabetes in the mothers. Since a recent study (Rolandsson *et al.*, 2020) had shown that the differences between type 1 and type 2 diabetes may not be as great as previously thought, we chose to adjust for any maternal diabetes, after consulting a specialist in diabetology.

The cardiovascular diagnoses can be considered reliable since they were based on inpatient data from the NPRs or data from the CDRs and all diagnoses had to be principal diagnoses except cardiomyopathy and heart failure. Additionally, the type 2 diabetes diagnosis in Sweden was based on both the NPR and quality registers for diabetes, which is a strength. The analyses on cardiovascular disease and type 2 diabetes were, however, limited by the relatively short follow-up time and consequently limited number of events. Since cardiovascular disease and type 2 diabetes also increase markedly with age ([https://sdb.socialstyrelsen.se/if\\_par\\_val.aspx](https://sdb.socialstyrelsen.se/if_par_val.aspx)) and the follow-up time differed considerably between the ART and SC children, it cannot be ruled out that there is an underestimation of cardiovascular diseases and type 2 diabetes in the ART children.

Another important limitation was the late start of the Outpatient Registers, since obesity primarily is an outpatient diagnosis. Furthermore, the registration of obesity depends on the motivation to seek care for a condition that often is not seen as a disease. Consequently, there is undoubtedly a large number of individuals with obesity, which are not registered in this study. Further, it is possible that ART parents and children to a greater extent seek care for obesity than the general population do. Nevertheless, the finding of a higher risk of obesity in ART children is important and needs to be explored further in the future.



# Discussion

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## The specific outcomes in the papers

The aim of the thesis was to evaluate the long-term outcome in children born after ART and study whether different ART techniques had special impact on the outcome. By using large population-based national registers we evaluated school performance, risk of developing type 1 diabetes, cardiovascular disease, obesity and type 2 diabetes in ART children. Overall, the results were reassuring even though some differences were found between children born after ART and SC and between different ART techniques.

### **School performance**

Several studies have shown a strong correlation between intelligence (IQ) and school performance (Naglieri and Bornstein, 2003; Furnham *et al.*, 2009). Furthermore, earlier studies have shown that school performance and intelligence are directly related to health later in life (Gottfredson and Deary, 2004; Batty *et al.*, 2007a; Batty *et al.*, 2007b; Herd, 2010). A report from Statistics Sweden a few years ago, showed a relationship between educational level and life expectancy, where the most obvious differences were seen between individuals with 9-12 years of education and individuals with more than twelve years of education (Demographic report 2016:2, Statistics Sweden). The report showed a life expectancy in women of 81.5 years for 9 years or less of education, 84.5 years for 10-12 years of education and 86.7 years for more than 12 years of education. The corresponding figures for men were 78.1 years, 81.2 years and 83.8 years, respectively. Studies on intelligence and health suggest that the reason behind these associations might be that intelligence, and good school performance, represent reasoning and problem-solving, skills valuable to prevent chronic disease and accidental injury (Gottfredson and Deary, 2004). This could be relevant in a number of ways such as having access to the most current information regarding health risk factors, being able to understand the pertinence of such factors and recognize when a condition requires an intervention. A cohort study from the United Kingdom (Batty *et al.*, 2007b) showed that higher IQ-score at 10 years of age was associated with a reduced prevalence of smoking, obesity and hypertension as well as an increased likelihood of having given up smoking by the age of 30 years. The grades at the end of primary school are crucial, since they decide

whether a student is eligible for secondary school or not ([www.skolverket.se](http://www.skolverket.se)). Since school education in Sweden is compulsory and due to its important predicting of future health, school performance is a highly important and relevant outcome measure to evaluate.

Previous studies of the cognitive development in ART children have generally been reassuring but the majority of the studies are small and with high risk of selection bias due to the exclusion of children at highest risk of cognitive impairment (Rumbold *et al.*, 2017). Particularly, there have been inconsistent findings among high quality studies examining the cognitive development of children born after ICSI (Leslie *et al.*, 2003; Knoester *et al.*, 2008; Sandin *et al.*, 2013; Kissin *et al.*, 2015; Rumbold *et al.*, 2017). Furthermore, most studies have investigated younger children while studies addressing the cognitive development in later childhood and adolescence have been sparse.

The school performance was generally reassuring for both ART children as a group and also for children born after the different ART techniques ICSI, IVF and FET. A subgroup analysis, found that children born after ICSI with non-ejaculated sperm had significantly lower results in Swedish and English and a significantly higher risk of poor school performance compared to children born after ICSI with ejaculated sperm. Yet, the result was based on a subgroup analysis with few events (14 events, 131 individuals). Thus, it might have been a chance finding with needs of further exploration.

Overall, the results from Paper I and II were in line with two large population-based register studies from Denmark, in which test scores in ninth grade was compared between children born after ART (n=2 836 singletons) and SC (n=5 660 singletons) (Spangmose *et al.*, 2017) and between children born after FET (n=423 singletons) and fresh embryo transfer (n=6 072 singletons) (Spangmose *et al.*, 2019). The school performance between ICSI (n=434) and IVF (n=2 110) children was also compared in a subgroup analysis of the initial study. The strengths with Paper I and II in this thesis compared to the Danish studies was the considerably larger sample sizes in Paper I and II, inclusion of national tests in third grade (Paper II) and the subgroup analysis of children born after ICSI with non-ejaculated sperm compared to children born after ICSI with ejaculated sperm. Another important strength with Paper I and II compared to the Danish studies, but also compared to other studies on school performance in ART children (Wagenaar *et al.*, 2008; Mains *et al.*, 2010), was the low proportion of missing data (2-3% in Paper I and II, 6.6% in Spangmose *et al.*,

2017, 30.5% in Wagenaar *et al.*, 2008 and 46% in Mains *et al.*, 2010). A further strength was the possibility to analyse children with missing data and find an explanation for the absent registration in about 30-40% of the children in Paper I and II (diagnosis of CP or mental disability).

### **Type 1 diabetes in ART children**

The incidence of type 1 diabetes has increased significantly during the past decades and Sweden has one of the highest incidence rates of type 1 diabetes worldwide (Dabelea, 2009; Patterson *et al.*, 2019). Type 1 diabetes is a chronic disease that, despite advances in care, continues to be associated with considerable risks of medical complications, psychological and financial burden (DiMeglio *et al.*, 2018). The disease has most often a young debut age (peak at 5-14 years) (Rawshani *et al.*, 2014; Patterson *et al.*, 2019) making it possible to study in the relatively young ART population. ART has increased in parallel with the increase in type 1 diabetes in the past decades and different technologies, such as ICSI and FET, have improved and rapidly been implemented. Therefore, it was important to investigate whether ART children had increased risk of developing type 1 diabetes.

Compared with children born after SC, ART children did not have an increased risk of type 1 diabetes when adjustment for important confounders such as parental diabetes had been performed. However, when children born after FET were compared to children born after fresh embryo transfer and children born after SC, FET children had an increased risk of type 1 diabetes. The results about type 1 diabetes in ART children were in line with two earlier register-based cohort studies from Denmark. Hargreave *et al.* (2016) studied 110 393 children born to women with fertility problems and compared them with 1 440 126 children born to women without fertility problems. In all, 313 children born to women with fertility problems and 5 176 children born to women without fertility problems developed type 1 diabetes. After adjustment for a number of important covariates, including parental diabetes, no association with type 1 diabetes was found. However, there was no information on whether the women in the study had undergone ART or not. In the study of Kettner *et al.* (2016), 8 490 children born after IVF or ICSI, 14 985 children born after ovulation induction or intrauterine insemination and 541 641 children born after no fertility treatment were examined. A total of 29 children after IVF or ICSI, 54 children after ovulation induction or intrauterine examination and 1 928 children born after no fertility treatment developed type 1 diabetes. No association was found between IVF or ICSI and type 1 diabetes in the primary analysis. In a secondary

analysis, however, an association was found between ovulation induction or intrauterine insemination and type 1 diabetes (HR 3.22; 95% CI 1.20 to 8.64). To conclude, no increased risk of type 1 diabetes was found for ART children in general in these Nordic studies.

The subgroup analysis, where a significant association between FET and type 1 diabetes was found, was based on few events (n=45) and the results should therefore be taken with caution. The reason for such a possible association between FET and type 1 diabetes is not known. Several studies have shown an increased risk of LGA and macrosomia in children born after FET compared to children born after fresh embryo transfer and SC (Wennerholm *et al.*, 2013; Berntsen and Pinborg, 2018; Maheshwari *et al.*, 2018) and an increased risk of type 1 diabetes in children with high birth weight have been described (Haynes *et al.*, 2007; Levins *et al.*, 2007; Cardwell *et al.*, 2010). A population-based study in Australia in which perinatal risk factors for type 1 diabetes was explored, noticed that the incidence of type 1 diabetes increased with 13% for every 500g increase in birth weight (Haynes *et al.*, 2007). Similar results were found in a meta-analysis of 29 studies, in which a linear increase in type 1 diabetes risk was found when the birthweight was increasing (Cardwell *et al.*, 2010). Accordingly, one theory is that increased birth weight is a mediator between FET and type 1 diabetes.

Another possible mechanism behind the association between FET and type 1 diabetes could be epigenetic alterations. Studies have shown that epigenetic modifications are more common in individuals with autoimmune diseases such as type 1 diabetes (Wang *et al.*, 2017). Animal studies have also shown that epigenetic alterations can be induced by manipulation of oocytes, sperm and early embryos (Doherty *et al.*, 2000; Market-Velker *et al.*, 2010; Katari *et al.*, 2009; Mainigi *et al.*, 2016). Katari *et al.* (2009) suggested that IVF was associated with lower mean methylation at CpG sites in placenta but higher mean methylation at CpG sites in cord blood. Another study from Hiura *et al.* (2017) showed that specific microRNAs were reduced in term placentae derived from FET compared to fresh embryo transfer and SC. In functional analyses, signalling pathways related to positive regulation of gene expression, growth and type II diabetes were enriched. A recently published meta-analysis, of 8 825 neonates from 24 birth cohorts in the Pregnancy And Child Epigenetics Consortium, found an association between DNA methylation and birth weight (Küpers *et al.*, 2019). Both positive and negative directions of associations between methylation levels and birthweight were observed. Whether epigenetic alterations could induce high birth weight and/or diabetes or it could be the birth

weight or diabetes causing epigenetic alterations, is unclear and needs further exploration.

### **Cardiovascular disease, obesity and type 2 diabetes in ART children**

Mortality and morbidity from cardiovascular disease, obesity and type 2 diabetes represents an enormous burden to individuals and society. Some studies have raised concerns as to whether ART treatment could affect the cardiometabolic risk factors in ART children which was the reason for performing Paper IV.

We did not find any increased risk of any cardiovascular disease (ischemic heart disease, cardiomyopathy, heart failure or cerebrovascular disease) in ART children compared to their SC counterparts. Neither did we find any increased risk of type 2 diabetes. No large register-based studies on clinical outcomes have been published earlier and studies performed so far have been small cohort studies with high risk of selection bias. Further, surrogate measurements have been used instead of clinical diagnoses. In the meta-analysis of Guo *et al.* (2017), ART children were found to have minor but statistically significant differences in systolic and diastolic blood pressure as well as a suboptimal cardiac diastolic function. Five studies measuring cardiovascular morphology were included (Valenzuela *et al.*, 2013; Zhou *et al.*, 2014; Liu *et al.*, 2015; von Arx *et al.*, 2015; Scherrer *et al.*, 2012). The studies comprised 54–128 IVF/ICSI children, thus small sample sizes. In another study (Juonala *et al.*, 2020), 172 ART individuals and 78 SC individuals, aged 22 to 35 years, were compared regarding cardio-metabolic risk factors, without finding any increased risk in ART children. However, only 36% of the invited individuals born after ART and 18% of the invited individuals born after SC agreed to participate, causing a high risk of selection bias. An additional study about cardio-metabolic profiles in ART children (Cui *et al.*, 2020) was recently published. In total, 380 IVF/ICSI children and 380 SC children, at the age of 6-10 years, were examined regarding glucose metabolism, lipid profiles and vasculature structure. The results showed significantly higher fasting blood glucose and serum insulin levels in IVF/ICSI children compared to their counterparts. Moreover, an increased thickness of carotid intima-media was shown. However, the authors concluded that the long-term consequences of these findings are uncertain. In the present study, a large number of ART children were included and the risk of selection bias was low due to the population-based design. Yet, a major limitation was the short follow-up time, and particularly for type 2 diabetes there were few events.

A small but significantly increased risk of obesity was found in ART children in Paper IV. The finding should be interpreted with caution due to several methodological limitations brought up in the result section. Still, several mechanisms could potentially explain why ART children might have an increased risk of obesity. The Developmental origins of adult Health and Disease (DoHaD) describes a hypothesis that was originally presented by Barker *et al.* (1993), who found an association between foetal undernutrition and metabolic syndrome in adults (type 2 diabetes, hypertension and hyperlipidaemia). This hypothesis has been further developed through a number of epidemiological studies worldwide and has now been extended to also focus on overnutrition in foetal life, which may contribute to obesity (McMillen *et al.*, 2008). Since ART children have an increased risk of adverse perinatal outcomes, such as PTB, LBW and SGA, and children born after FET have an increased risk of macrosomia and LGA (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004, McDonald *et al.*, 2009, Pandey *et al.*, 2012; Qin *et al.*, 2017, Wennerholm *et al.*, 2013; Berntsen and Pinborg, 2018 Maheshwari *et al.*, 2018), the DoHaD hypothesis is highly relevant to explore in the ART population.

In addition, DNA methylation has been associated with obesity (Xu *et al.*, 2013; Wahl *et al.*, 2017; Mendelsen *et al.*, 2017). Already in 2013, a study from Xu *et al.* (2013) showed several CpG sites related to obesity in a young cohort and also found that the variance of DNA methylation was greater in the obese individuals than in the lean controls. Later, a much larger study, with data from over 10 000 whole blood samples, found 187 CpG sites significantly associated with BMI (Wahl *et al.*, 2017). Using Mendelian randomization, the authors showed that the alterations in DNA methylation in blood most often were the result of obesity itself but sometimes they were also the cause of the obesity, findings that were supported by Mendelson *et al.* (2017) in another study. An important finding in the study from Wahl *et al.* (2017) was that DNA methylation sites associated with obesity predicted future risk of type 2 diabetes. Interestingly, the impact of epigenetics on cardiovascular disease is also under intense examination (Prasher *et al.*, 2020).



## General discussion

While the short-term outcome of children born after ART is comprehensively examined, data on the long-term outcome are still limited. Additionally, even if the adverse obstetric and perinatal outcomes are well-known, the reasons for these findings are not fully understood and several factors probably contribute. Besides the higher rates of multiple pregnancies, two main factors exist. Firstly, the background characteristics of the infertile parents are different compared to SC parents. ART parents are generally older, have lower parity and are more well educated than SC parents (which can be seen in all four papers of this thesis), but it is also possible that they have more intercurrent diseases such as chronic hypertension, diabetes and/or genetic diseases. Epigenetic defects in the gametes may also be more common in infertile men and women (Niemitz and Feinberg, 2004). Secondly, the ART technique *per se* can potentially affect the child outcome. Technical aspects that potentially could affect the child outcome are the culture media, culture time, the cryopreservation and the invasiveness of ICSI. In addition, there are potential side effects of ovarian stimulation.

A major challenge for researchers, is to separate the contribution of the parental background characteristics from the ART technique *per se*. One way to overcome this difficulty, is to choose a control group with subfertile couples i.e. individuals with an infertility diagnosis but SC. Such control groups are however difficult to find and the most common control group in studies about ART children is SC children without infertility. Another way to disentangle the contribution of the parental background characteristics from the ART technique *per se* is to compare siblings, where the same mother has given birth to a singleton child after ART and a singleton child after SC. The major strengths with such studies are the relatively constant genetic, socio-economic and lifestyle factors. Yet, the possibility that a mother change lifestyle or partner between two pregnancies exist and it can be difficult to measure. A few sibling studies have been published (Romundstad *et al.*, 2008; Henningsen *et al.*, 2011; Pinborg *et al.*, 2013; Luke *et al.*, 2016). The first study from Norway (Romundstad *et al.*, 2008) found similar perinatal outcomes among infants born after ART and SC in the same mother, indicating that adverse perinatal outcomes in ART children are an effect of the parental background characteristics rather than the ART technique *per se*. A few years later, a larger population-based cohort study from Denmark (Henningsen *et al.*, 2011) compared the perinatal outcomes of ART and SC siblings. This study implied that also the ART technique contributes to the adverse outcome. Additionally, a sibling study from the United

States (Luke *et al.*, 2016), showed that declining fertility status, with and without ART, was associated with increased risk of adverse outcomes. Thus, both the infertility and the ART technique *per se* seems to contribute to the adverse outcomes in children born after ART.

Since the first child was born in Sweden in 1982, several changes in the ART treatment have taken place. A shift from DET to SET occurred in the early 2000s, which led to a substantial decline in multiple births and subsequently decreased perinatal and obstetric complications (Thurin *et al.*, 2004; Bergh *et al.*, 2020). More recently, there has also been a shift from cleavage stage to blastocyst culture and different culture media have been used during the study period. Furthermore, an increased use of cryopreservation has taken place, due to a shift from the slow-freezing method to vitrification. At last, the use of ICSI has increased. The effects of all these changes is difficult to predict and completely account for. Regarding culture media, no information was available in the Q-IVF, therefore it was impossible to adjust for. Some studies have shown that culture media could influence birthweight (Dumoulin *et al.*, 2010; Kleijkers *et al.*, 2016). A recent study also observed that two culture media were associated with differences in body weight, BMI and waist circumference in 9-year old ART children whereas the risk of cardiovascular development was similar between the groups (Zandstra *et al.*, 2018). Where relevant, we adjusted for FET and ICSI and we also performed subgroup analyses to explore the influence of FET and ICSI on children outcomes.

### **Strengths and limitations**

The major strengths of all four studies are the population-based design with the use of large nationwide birth cohorts and registers with high coverage rates and high validity, minimizing the risk of missing data. Data to the registers were collected for quality reasons and not for the purpose of these studies, minimizing the risk of selection and recall bias. In all studies, it was also possible to adjust for important confounders, such as parental educational level in the first two studies, parental type 1 diabetes in the third paper and maternal cardio-metabolic diseases in the fourth paper. A strength in the first two studies, about school performance, was also that not only public but also private schools were included. Furthermore, the national tests in third grade were standardized tests. Lastly, in Paper III and IV, it was possible to use combinations of health data registers and quality registers to define diabetes and obesity which made it possible to include more children with these diagnoses (for Paper IV only in Sweden).

The specific limitations of each study have been addressed in the comments in the Result-section. General limitations of the studies were the retrospective analyses, the risk of unmeasured and unknown confounders and missing data. In Paper I and II, the missing data were relatively low (2-3%) and comparable between the studied groups. With the high coverage rates in SWEDIABKIDS, NDR and NPR, the missing data were probably also low in Paper III and for cardiovascular diseases and type 2 diabetes in Paper IV. For the outcome obesity, however, the missing data were definitely a larger proportion since this condition is often not diagnosed as a disease. In addition, obesity is primarily an outpatient diagnosis and outpatient visits were not registered in the beginning of the study period.

The choice of comparison group in the studies of this thesis could be discussed. In all four studies the main comparison group consisted of children born after SC. The reason for this was that the infertility diagnoses are primarily set at outpatient visits in Sweden and the Outpatient Register did not start until 2001. Besides, the cause of the infertility is often unclear, since ART treatment frequently is initiated without any major investigation of the infertility. Consequently, the infertility diagnoses are many times poorly registered, and therefore not yet included in the Q-IVF. In our studies we did not adjust for the same mother giving birth more than once, since the studies include very large number of women and small cluster sizes (normally 1-3 children per mother). Consequently, the intra class correlation coefficient (i.e. a coefficient between 0 and 1, describing how strongly individuals in the same group resemble each other) became neglectable ( $<0.01$ ).

Another question that can be discussed is the choice to only evaluate singletons. This can be considered as both a strength and a limitation. The strength is that the IVF technique *per se* is much better assessed when ART singletons are compared to SC singletons, without having the influence of multiple births. The limitation is that no results are presented for the multiples or for the ART group overall. If we had chosen to include multiple births, we could have performed one analysis for singletons only and one analysis, in which we compared all ART children to all SC children to get an overall estimate of the influence of ART. The main problem, however, when comparing ART twins to SC twins, is the different rate of zygosity and chorionicity. About 30% of SC twins are monozygotic whereas the corresponding figure for ART twins is about 1-2%. Since two thirds of the monozygotic twins are monochorionic, with a much higher risk of many perinatal complications, it is difficult to compare ART and SC twins without taking this into consideration. One way to handle this, is

to only include twins with different gender (to be sure that they are dizygotic). However, this would exclude a substantial part of the twins in both groups.

Another important question, is the ethical aspect that needs to be considered when working with register-based studies. Ethical permission is required for register-based research in Sweden and Norway (but not in Denmark and Finland) and all studies in this thesis were approved by the Regional Ethical Committee at University of Gothenburg and the Regional Committee for Medical and Health Research Ethics in Norway, described in the Method section. However, written consent from patients for register-based studies is generally not required, which is particularly important to have in mind when rare outcomes are investigated. The Declaration of Helsinki emphasizes the importance of integrity in research work. While the anonymity of individuals must be guaranteed, important results of rare diseases are crucial to present. This was a challenge particularly in Paper IV, where some of the cardiovascular diseases were very rare. We therefore had to lump the cardiovascular events together for all countries, and we also had to avoid presenting exact values when the events were less than five, to protect the integrity of certain individuals.

# Conclusion

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Below is a summary of the results followed by conclusions:

- In the crude analyses, children born after ART performed better in school compared to children born after SC. After adjustment for important covariates, small differences were observed in favour of the SC children in a few analyses. The differences observed were small and probably not of any clinical relevance.
- Children born after ICSI had similar school performance as children born after IVF. In the ninth grade, no differences were observed between children born after ICSI and children born after SC whereas small differences were observed in the third grade in favour of children born after SC.
- *To conclude: school performance up to ninth grade is reassuring for ART children, also for specific ART techniques.*
- Children born after ART did not have an increased risk of type 1 diabetes after adjustment for important covariates. In a subgroup analysis, however, children born after FET had an increased risk of type 1 diabetes compared to children born after fresh embryo transfer and SC.
- Children born after ART did not have an increased risk of cardiovascular disease (ischemic heart disease, cardiomyopathy, heart failure or cerebrovascular disease) or type 2 diabetes after adjustment for important covariates. A small but significantly increased risk of obesity was found.
- *To conclude: the cardio-metabolic outcomes in ART children were generally reassuring. However, the number of events were limited for several diseases and small negative differences were observed in a few analyses of ART children. Since even small differences in a young population can have major consequences in the long run and previous studies with medical examinations of ART children repeatedly have suggested that there might be small differences in cardio-metabolic surrogate outcomes, there is a clear need for further studies in this area.*



# Future perspectives

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Studies on the long-term outcome of children are still scarce and there is a considerable gap in knowledge that needs to be explored in the future. Cohorts with older children are particularly needed to be able to investigate disorders such as cardiovascular disease, obesity and type 2 diabetes. Another area that needs deeper knowledge, is the neurodevelopmental health in ART children, more specifically the risk of CP, mental retardation and autism, where studies so far have shown divergent results. The cognitive development in children born after ICSI with non-ejaculated sperm also needs further research in the future as well as the reproductive function in ICSI children. For these rare outcomes, large data sets are crucial to be able to find enough cases. Health data registers and quality registers constitute a goldmine for such research.

Furthermore, the influence of other ART techniques than ICSI, such as FET, culture media, culture duration and the side effects of ovarian stimulation, needs to be examined. It has been shown that different ART techniques are associated with various adverse outcomes in the children. Thus, it is necessary to investigate each ART treatment separately.

It is difficult to disentangle the background characteristics of the infertile parents from the ART technique *per se*. Future studies should focus on sibling analyses and to a greater extent use subfertile couples as a control group, making it easier to evaluate the ART technique *per se*. The cause of infertility, and how it is related to various adverse outcomes in both mothers and children, is also essential, although challenging since this is not always examined. Further epigenetic mapping is also necessary, to extend the knowledge about epigenetic alterations, which could be possible links between genes and the environment. Lastly, life-style factors, such as nutrition and BMI, are important confounders and should be more included in the analyses in the future.

It is essential to implicate the knowledge about adverse outcomes in ART pregnancies and children into clinical practice. An important issue is whether ART pregnancies and/or ART children need extra screening and surveillance. For instance, several studies have shown reduced risks of preeclampsia and intrauterine growth restriction when low doses of aspirin have been used (Roberge *et al.*, 2017;

Roberge *et al.*, 2018; Duley *et al.*, 2019). Whether ART pregnancies in general could benefit from aspirin is one question of interest.

To conclude, the number of ART children are constantly increasing and new techniques develop steadily. Therefore, it is of outmost importance not only to continue the surveillance of ART children but also to spread the knowledge to the general population and to health care personnel. Additionally, it is important to implement the knowledge into clinical practice.



# Acknowledgements

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I am so grateful to everyone who have contributed and helped me with the work of this thesis. In particular I would like to thank:

All **national health data registers** and **quality registers** that have contributed with their data to the studies of this thesis.

Professor **Christina Bergh**, my main supervisor, for your enormous support in my research education, from the first day until this final dissertation. Without you, I would not have been able to do this research. Thank you for always being available, for the contribution and sharing of your extensive knowledge and for your enthusiasm in my project.

Associate professor **Ulla-Britt Wennerholm**, my co-supervisor, for your enormous support in this project, for always being available, no matter where you have been in the world. Thank you for your patience, your encouraging, your inspiration and for introducing me to this research field.

Professor **Max Petzold**, statistician of this project, for all the time you have spent on these studies. Also, thank you for your positive attitude and patience when new analyses have been added and when the computer has failed. Thank you for always answering my statistical questions with an educational answer.

**The CoNARTaS group** for sharing the data in the CoNARTaS database, your knowledge and your time. I am especially thankful to **Mika Gissler** for your invaluable help with Paper IV.

**All co-authors**, for contributing with your important knowledge to the papers.

**Ylva Carlsson, Gun Forsander** and **Klaus Groth** for constructive questions at my half-time seminar.

**Jan Funke** for creating the illustrations to this thesis in a very skilful way.

**Ian Milsom, Nils Crona, Lena Otterlind and Radha Korsoski**, Heads of Department of Obstetrics, at Sahlgrenska University Hospital, for giving me time off from clinical work, making it possible to perform this research project.

**Annette Nattland** for your invaluable help with the layout of the thesis.

**Anja Andersson and Heléne Nielsen** for your administrative help.

**All colleagues** at the department of Obstetrics and Gynecology, Sahlgrenska University Hospital, for giving me motivation and for doing the clinical work while I had time off for research. A special thanks to **Erica Ginström Ernstad**, for all support and for making my PhD-time much more fun and **Victoria Klerelid** for always being such a supportive colleague and friend.

All other wonderful friends. Especially thanks to my friends since childhood **Cecilia, Jennie, Ulrika and Emma**. I love our “tjejträffar” and could not have coped without them. My friends **Emma and Jenny** in Ekeback. I am so glad that I have met you.

My mother **Kerstin**, for your endless and unconditionally love. For listening, supporting and helping me in all ways. My father **Erik**, for always loving me, listening to me and giving me good advices. My siblings **Anna, Anders and Fredrik**, I am so lucky and grateful that I have you, and that we always have so much fun together. I love you all.

**Stefan**, the love of my life, for always loving, listening and supporting me. For being such a good father to our three children **Oliver, Wilhelm, Tuva**, who are the most fantastic, warm, clever and fun children I could ever wish for. I love you all so much and you mean everything to me.

All other relatives, I am so grateful to have you in my life.

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

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