Obstetric outcome after single embryo transfer

by

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"Don't tell me the moon is shining; show me the glint of light on broken glass."

"Wisdom.... comes not from age, but from education and learning."

"Даже болеть приятно, когда знаешь, что есть люди, которые ждут твоего выздоровления, как праздника."

Anton Chekhov, MD

Contents

Abstract

Background: Children born after IVF have a poorer neonatal outcome than children born after spontaneous conception, even after adjustment for confounders. In Sweden, since 2003 an increasing amount of IVF cycles are single embryo transfers (SET). This gives the opportunity to investigate and compare the outcome after SET and double embryo transfers (DET).
Aim: To assess the neonatal and maternal outcomes after IVF, particularly after SET.
Methods: *Paper I*: All IVF children born in Sweden after IVF treatment during the years 2002-2006 and their mothers were included (n=13 544 children) and compared with all children in the general population born during the same time period and their mothers (n=587 009 children) concerning neonatal and maternal outcomes. *Paper II*: All IVF singletons born after fresh IVF cycles and own oocytes were included (n= 8941) and cross-linked with the Swedish Medical Birth Registry. Four major outcomes were investigated: very preterm birth (<32 weeks), small for gestational age (SGA), placenta previa and placental abruption. Maternal characteristics (age, parity, BMI, smoking and years of infertility) and treatment-related variables (number of oocytes retrieved, number of embryo culture days, number of transferred and cryopreserved embryos and "vanishing twin") were investigated for independent association with the four selected outcomes. *Paper III*: All singletons after cryopreserved (n=2348) and fresh IVF cycles (n=8944) were included and compared with all singletons born after spontaneous conception (n=571 914). *Paper IV*: Outcomes for women (n=921) undergoing two IVF pregnancies with singletons (n=1842) were compared with women (n=991) undergoing one IVF pregnancy with twins (n=1982).
Results: *Paper I*: Children born after IVF had a poorer neonatal outcome than children in the general population. Comparing IVF singletons, irrespective of the number of embryos transferred, with singletons in the general population, significantly higher rates of preterm birth (<28 w, <37 w), low birth weight (LBW) and very low birth weight (VLBW) were found. *Paper II:* Age, primiparity, smoking, BMI, years of infertility and 'vanishing twin' were associated with an increased risk of one or both of the two selected outcomes very preterm birth and SGA. Maternal age and blastocyst transfer were associated with an increased risk of placenta previa. Smoking was significantly associated with placental abruption. *Paper III:* Singletons from cryopreserved cycles had increased rates of extreme preterm birth (<28 w) as compared with singletons from the general population. A lower rate of LBW was found for singletons after cryopreservation cycles than for singletons from fresh cycles. The rates of large for gestational age (LGA) and macrosomia (>4500g) were higher for singletons after cryopreservation cycles than for singletons in the general population and for singletons after fresh cycles. Higher rates of preeclampsia were noted for pregnancies after cryopreservation cycles versus general population and fresh cycles. *Paper IV:* Preterm birth, very preterm birth, LBW, VLBW and SGA were dramatically increased for IVF twins as compared with two IVF singletons with the same mother with adjusted odds ratios between 4 and 16. Significantly higher rates of respiratory complications, sepsis and jaundice were detected among the IVF twins. Significantly higher rates of preeclampsia, preterm premature rupture of the membranes and Cesarean section were observed for IVF twin pregnancies.
Conclusions: Children born after IVF, also singletons and irrespective of the number of embryos transferred, had a poorer neonatal outcome than singletons in the general population. In singletons born after fresh IVF, certain maternal characteristics and the number of embryos transferred, when there was a 'vanishing twin', affected the neonatal outcome negatively. Singletons born after cryopreservation as compared with fresh IVF cycles had a better neonatal outcome as regards LBW. An increased rate of placenta previa was observed after blastocyst transfer. Maternal and neonatal outcomes were dramatically better for women who had two IVF singleton pregnancies than for those with one IVF twin pregnancy. The finding of an increased rate of LGA and macrosomia after cryopreservation needs further studies. The results support SET as the main transfer strategy.

Keywords: *in-vitro fertilization/ single embryo transfer/ neonatal outcome/ maternal outcome*

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Svensk sammanfattning

Bakgrund och syfte: Sverige har en unik möjlighet att genomföra stora registerstudier angående barn födda efter IVF (provrörsbefruktning) genom korskörning mot svenska nationella hälsodataregister. Barn födda efter IVF, även barn födda i enkelbörd, har ett sämre neonatalt utfall jämfört med barn födda efter spontan konception, även när justering för "stör faktorer" (mammaålder, paritet, flerbörd, år av barnlöshet) har gjorts. I Sverige har sedan 2003 en ökande mängd IVF cykler genomförts med återföring av ett embryo (single embryo transfer, SET).
Syftet med studien var att undersöka det neonatala och maternella utfallet efter IVF, framför allt efter SET.

Metoder: *Delarbete I*: Alla barn födda i Sverige efter IVF-behandling under åren 2002-2006 och deras mödrar (n=13 544 barn) jämfördes med alla barn i populationen (n=587 009 barn) och deras mödrar avseende neonatalt och maternellt utfall. *Delarbete II*: Alla barn födda i enkelbörd efter färska IVF-cykler och med egna ägg under 2002-2006, inkluderades i studien (n=8941) och korskördes med det svenska medicinska födelseregistret. Fyra allvarliga komplikationer undersöktes: mycket för tidig födsel (<32 veckor), att födas liten för tiden (small for gestational age, SGA), föreliggande moderkaka (placenta previa) och avlossning av moderkakan. Maternella egenskaper (ålder, paritet, BMI, rökning och år av barnlöshet) och behandlingsrelaterade variabler (antal ägg, antal odlingsdagar av embryon, antal överförda och frysta embryon, förekomst av "försvinnande tvilling" (vanishing twin)) undersöktes för ett eventuellt samband med de fyra utvalda komplikationerna. *Delarbete III*: Alla barn födda i enkelbörd efter frys/tinings-cykler (n=2348) och färska cykler (n=8944) inkluderades och korskördes med det svenska medicinska födelseregistret och jämfördes med alla barn födda i enkelbörd i populationen (n=571 914). *Delarbete IV*: Det neonatala och maternella utfallet för kvinnor som genomgick två IVF graviditeter med enkelbörd jämfördes med kvinnor som genomgick en IVF tvillinggraviditet. Alla kvinnor (n=921) som födde två IVF barn i enkelbörd (n=1842) och alla kvinnor (n=991) som födde tvillingar efter IVF (n=1982) ingick.

Resultat: *Delarbete I*: Barn födda efter IVF hade ett sämre neonatalt utfall jämfört med barn i populationen. Även barn födda i enkelbörd efter IVF, oberoende av antalet återförda embryon, hade betydligt högre risk för förtidsbörd (<28 veckor, <37 veckor), låg födelsevikt (<2500g) och mycket låg födelsevikt (<1500g). *Delarbete II*: Att föda första barnet, rökning, BMI och "vanishing twin" var associerat med en ökad risk för mycket för tidig födsel (<32 veckor). Moderns ålder, att föda första barnet, rökning, BMI och år av barnlöshetet var associerat med en ökad risk för SGA. Moderns ålder och återföring av blastocyster var associerat med en ökad risk, och första barnet med en minskad risk för placenta previa. Rökning var associerat med en ökad risk för avlossning av placenta. *Delarbete III*: Enkelbörd efter frysning av embryon hade, jämfört med enkelbörd i befolkningen, en ökad risk av extremt för tidig födsel (<28 veckor). En lägre frekvens av låg födelsevikt noterades för barn efter fryscykler jämfört med färska cykler. Risken att födas "stor för tiden" (large for gestational age, LGA) och makrosomi (>4500g) var högre för barn efter fryscykler jämfört med populationen och färska IVF cykler. Högre frekvens av havandeskapsförgiftning

noterades hos graviditeter från frysförvarade embryon jämfört med populationen och färska IVF cykler. Frekvensen av placenta previa var lägre bland graviditeter efter fryscykler jämfört med graviditeter efter färska IVF cykler. *Delarbete IV*: Frekvensen för tidig födsel (<37 veckor), mycket för tidig födsel, låg födelsevikt, mycket låg födelsevikt och SGA var dramatiskt högre för IVF tvillingar jämfört med två IVF enkelbörd med samma mor med justerade oddskvoter mellan 4 och 16. Väsentligt högre risker för andningskomplikationer, sepsis och gulsot noterades bland IVF tvillingar. Betydligt högre risker för havandeskapsförgiftning, prematur för tidig hinnbristning och kejsarsnitt observerades för IVF tvillinggraviditeter jämfört med två IVF enkelbörd graviditeter med samma mor.

Slutsatser: Barn födda efter IVF, även om födda i enkelbörd, och oberoende av antalet överförda embryon, hade ett sämre neonatalt utfall jämfört med barn från populationen. Vissa maternella egenskaper och behandlingsrelaterade variabler påverkade det neonatala utfallet negativt. Barn födda efter frys/tinings-cykler hade ett bättre neonatalt utfall än barn födda efter färsk cykel avseende låg födelsevikt. En ökad frekvens av placenta previa observerades efter blastocyst överföring. Mödra-och neonatala utfallet var dramatiskt bättre för kvinnor som genomgick två IVF enkelbörd graviditeter än för dem med en IVF tvillinggraviditet. Fyndet av en ökad frekvens av LGA och makrosomi efter frys/tinings-cykler behöver studeras ytterligare. Resultaten stöder användningen av SET och indikerar att livsstilsfaktorer (rökning och avvikande BMI) är viktiga för förlossningsutfallet.

List of publications

This thesis is based on the following papers, which will be referred to by their Roman numerals in the text:

I. Sazonova A, Källen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C.
Obstetric outcome after *in-vitro* fertilization with single or double embryo transfer.
Human Reproduction 2011;26:442-450.

II. Sazonova A, Källen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C.
Factors affecting obstetric outcome of singletons born after IVF.
Human Reproduction 2011;26:2878-2886.

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

IV. Sazonova A, Källen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C.
Neonatal and maternal outcomes comparing women undergoing two *in vitro* fertilization (IVF) singleton pregnancies and women undergoing one IVF twin pregnancy.
Fertility & Sterility 2013;99:731-737.

Abbreviations and definitions

AOR	Adjusted odds ratio
ART	Assisted reproductive technology, includes IVF and intrauterine inseminations
ASRM/SART	American society of reproductive medicine/Society of assisted reproductive technology
BMI	Body mass index
CI	Confidence interval
DET	Double embryo transfer
eSET	Elective single embryo transfer
ESHRE	European society of human reproduction and embryology
ET	Embryo transfer
FSH	Follicle stimulating hormone
GEE	Generalized Estimating Equation technology
GIFT	Gamete intrafallopian transfer
GnRH	Gonadotropin releasing hormone
hCG	Human chorionic gonadotrophin
ICD	International Classification of Diseases
ICSI	Intracytoplasmic sperm injection
IVF	In-vitro fertilization
LBW	Low birth weight
LGA	Large for gestational age
MBR	Medical Birth Registry
Non-eSET	Non elective single embryo transfer
NPR	National Patient Register
OHSS	Ovarian hyperstimulation syndrome
OR	Odds ratio
PPROM	Preterm premature rupture of the membranes
PTB	Preterm birth
RR	Risk ratio
Q-IVF	National Quality Register for Assisted Reproduction, Sweden
SET	Single embryo transfer
SGA	Small for gestational age
SIR	Standardized incidence ratio
VLBW	Very low birth weight

Definitions

eSET	Fresh IVF cycle when several embryos are available, one embryo is transferred and one or more embryos are cryopreserved
Extreme PTB	Preterm birth (<28 gestational weeks)
LBW	Low birth weight (<2500g)
LGA	Large for gestational age, more than two standard deviations (SD) above the Swedish growth standard
Neonatal mortality	The sum of early and late neonatal mortality
Early neonatal mortality	The death of a live-born baby within the first seven days of life
Late neonatal mortality	The death of a live-born baby covering the time after the first seven days of life until 28 days
Non-eSET	Fresh IVF cycle when only one embryo is available, one embryo is transferred and no embryos are cryopreserved
Perinatal mortality	The number of stillbirths and neonatal deaths in the first week of life
Placenta previa	Placenta located low in the uterus and partially or completely covers the cervix
Placental abruption	Placenta has separated from the uterus
PPROM	Preterm premature rupture of membranes, rupture of membranes before 37 weeks of gestation
Preeclampsia	High blood pressure (≥140/90) and significant amounts of protein in the urine (≥300 mg in a 24-hour urine sample) in a pregnant woman after 20 weeks of gestation
PTB	Preterm birth (<37 gestational weeks)
SGA	Small for gestational age, more than two standard deviations (SD) below the Swedish growth standard
Stillbirth	Intrauterine fetal death ≥28 weeks of gestation (before 1 July 2008)
Very PTB	Preterm birth (<32 gestational weeks)
VLBW	Very low birth weight (<1500g)

Introduction

Infertility as a world-wide problem

Infertility affects 10-15% of couples of fertile age. Infertility is not a life-threatening disease, but still severely influences quality of life and contributes to psychosomatic illness (Lal, 2009; Greil *et al*., 2011). The prevalence of infertility is more or less similar all over the world and does not depend on the level of industrialization of society (Boivin *et al*., 2007). Calculations based on a study of a group of 170 000 infertile women suggest that about 72.4 million women/couples worldwide are currently affected by infertility (Boivin *et al.*, 2007). It might well be a growing problem, especially in the industrialized countries, where women today plan for childbearing later in life and give birth to their first child between 30 and 35 years of age.

The World Health Organization (WHO) has defined infertility as failure to become pregnant after one year of unprotected intercourse. According to ESHRE classi-fication 2010 (ESHRE, 2010), 20-30% of infertility cases are linked to physiological causes in men, 20-35% to physiological causes in women, and 25-40% of cases are attributable to a joint problem. In 10-20% no cause is found. Infertility is also associated with lifestyle factors such as smoking, body weight and stress.

The most effective treatment of most types of infertility is *in vitro* fertilization (IVF). The first IVF baby was born in 1978 (Steptoe and Edwards, 1978) and IVF quite soon became a well-established method of infertility treatment. The introduction of intracytoplasmic sperm injections (ICSI) has been an essential step in the treatment of male infertility (Palermo *et al.*, 1992). Assisted reproductive technologies (ART) are defined as supporting methods to achieve pregnancy when the process of intercourse is replaced either by artificial insemination or fertilization of oocytes outside the body. Some forms of ART are also performed in fertile couples for genetic reasons (preimplantation genetic diagnosis (PGD). ART is a constantly expanding field, and accounts today for 4.6% of all children born in Denmark while, a lower rate is noticed in other countries such as Turkey, 0.5% (Ferraretti *et al*., 2012). ART utiliza-tion in 2008 in Europe was highest in Belgium: 13 069 cycles per million women ages 15-45. The corresponding figure in Sweden was 9228 cycles per million women and in UK 4066 cycles per million women (Ferraretti *et al*., 2012). Infants con-ceived with ART accounted for 1.4% of the total births in the United States in 2009 and ART utilization in USA was 2361 cycles per million women (Sunderam *et al*., 2012).

In Sweden, almost 50 000 children have been born after IVF, and approximately 3500 IVF children are born each year. IVF children thereby constitute about 3.3% of all newborn children in Sweden (Statistics Sweden, 2010, National Quality Register for Assisted Reproduction, Sweden, 2010).

IVF procedure

The main steps in IVF are: (i) hormone stimulation of ovaries to develop multiple follicles; (ii) ultrasound-guided transvaginal oocyte retrieval; (iii) preparation of egg and sperm for fertilization; fertilization (iv) culture and selection of embryos and (v) embryo transfer to the uterus.

There are two main current protocols for ovarian stimulation: gonadotrophin stimulation in combination with a gonadotrophin releasing hormone (GnRH) agonist or gonadotrophin stimulation in combination with a GnRH antagonist. In GnRH agonist cycles, down-regulation of the pituitary gonadal axis is commonly performed by nasal administration of a GnRH agonist 2-4 weeks before the start of ovarian stimulation and until administration of human chorionic gonadotrophin (hCG). In the GnRH antagonist cycle, the natural menstrual cycle is used and premature ovulation is prevented with injections of a GnRH antagonist usually started on stimulating day 5-6 and performed in parallel with ovarian stimulation until ovulation induction.

Ovarian stimulation is performed by daily subcutaneous injections of follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG) and monitored by serum estradiol levels and/or vaginal ultrasound.

Final oocyte maturation is induced by injection of human chorionic gonadotropin (hCG), which works as an analog of luteinizing hormone (LH). It can also be performed by injecting GnRH agonist, which stimulates the endogenous LH surge. This can only be used, however, in GnRH antagonist cycles. Time to ovulation is estimated to be between 38-40 hours after hCG injection. However, optimal time for aspiration of oocytes is between 34-36 hours, when the eggs are fully mature.

The oocytes are aspirated from the ovaries with transvaginal ultrasound-guided puncture, usually with conscious sedation of the patient combined with local anesthesia.

In Sweden, IVF is both publicly and privately funded. Publicly funded IVF is offered to childless couples after investigation performed after at least one year of infertility and if no other treatment is considered suitable. Certain age limits exist, usually 40 years for women. Exact rules differ somewhat between different regions. Up to three subsidized cycles with fresh embryos are offered in most regions. After achieving one live birth, no more publicly funded cycles are offered.

Different IVF techniques

IVF by standard method

The oocytes are placed and incubated in a nutritional solution containing a fixed concentration of sperm (a ratio of about 75 000:1), allowing the sperm to penetrate and fertilize the egg.

ICSI

In some situations, such as a low sperm count, poor sperm motility or poor fertilization in a previous standard IVF cycle, a single sperm is injected directly into the egg by intracytoplasmic sperm injection (ICSI).

Today, around 50% or even more of IVF cycles use ICSI. ICSI is a more invasive procedure than standard IVF, where lack of natural selection of sperm and use of sperm of lower quality have been discussed as factors that might increase the risk of chromosomal and genetic abnormalities (Bonduelle *et al*., 2002; Källen *et al.,* 2005b). Complementary methods of treatment of severe male factor infertility (azoospermi) are a combination of microsurgical sperm retrieval procedures from the epididymis (PESA) or testis (TESA) followed by ICSI.

Blastocysts

Usually, embryos are grown until they reach the size of 4-8 cells (the cleavage stage) two to three days after oocyte retrieval. However, embryo transfer can also be done at the blastocyst stage five-six days after oocyte retrieval. Transfer of blastocysts is clinically routine in many countries and has shown higher pregnancy and delivery rates as compared with cleavage stage embryos (Blake *et al*., 2007). Yet no differences in the total delivery rates, including fresh and frozen cycles from the same oocyte retrieval, have been observed between the blastocyst and early cleavage-stage embryos (Guerif *et al*., 2009). Deficiencies of the blastocyst transfer concept are the following: more expensive and labor-intensive culture technology, a greater risk of failure of embryo transfer due to a risk that no embryos will survive to the blastocyst stage, fewer embryos available for freezing (Blake *et al*., 2007).

Cryopreservation

An increasing proportion of the IVF births are children born after freezing and thawing of embryos. Cryopreservation cycles constituted 18.2% of all IVF cycles in Europe in 2008 and in some countries more than 30% of all IVF cycles (Switzerland 42.4%, Finland 39.8%, Iceland 35.6% and Belgium 34.5%) (Ferraretti *et al.*, 2012). In Sweden, cryopreservation cycles constituted 32.1% of all IVF cycles in 2010 (National Quality Register for Assisted Reproduction, Sweden).

The traditional cryopreservation technique is called “slow freezing”, in which cryo preservation of embryos is performed in a stepwise procedure and embryos are then stored in liquid nitrogen at minus 196°C. ”Vitrification” is a new, ultra-rapid freezing method that is 600 times faster than conventional cryopreservation, with a very short exposure of embryos to the most dangerous temperature zones from plus 15 to minus 5°C. According to data from a systematic review and meta-analysis, significantly higher rates of post-thawing survival was detected for vitrified embryos/blastocysts as compared with slow freezing embryos/blastocysts (Loutradi *et al*., 2008) while no differences in pregnancy rates between the two techniques have been noted to date (Kuwayama *et al.,* 2005; Liebermann *et al*., 2006).

Results after IVF

In Europe in 2008, the clinical pregnancy rates per aspiration and transfer were 28.5% and 32.5%, respectively, and for ICSI the corresponding rates were 28.7% and 31.9%.

In cryopreservation cycles, the pregnancy rate per thawing was 19.3%. Delivery rate per aspiration was 21.2% for standard IVF and for ICSI 20.4% in fresh cycles (Ferraretti *et al*., 2012). In Sweden in 2010,

the clinical pregnancy rates per aspiration and per transfer were 31.8% and 36.1%, respectively, and for ICSI the corresponding rates were 31.3% and 35.4%. In cryo-preservation cycles, the pregnancy rate per transfer was 28.1%. Delivery rate per aspiration was 24.5% for standard IVF and 24.2% for ICSI in fresh cycles. Delivery rates per transfer were 27.8% for standard IVF, 27.4% for ICSI in fresh cycles and 21.2% in cryopreservation cycles (National Quality Register for Assisted Reproduction, Sweden).

Complication during IVF

Complications during the IVF procedure are rare. Ovarian hyperstimulation syndrome (OHSS) is one of the most serious complications, which was reported in 1% of all stimulated cycles in Europe in 2008 and varied in different countries from 0.2-2.2% (Ferraretti *et al*., 2012). The exact patho-physiological mechanisms of OHSS are not known, but the ovulation induction dose of hCG is considered to be a triggering factor, which increases vascular permeability resulting in transport of fluid into the third space. The resulting hemoconcentration may be complicated in severe cases with thromboembolic events or renal insufficiency and can be life-threatening with pleural effusion, multiple organ failure and disseminated intravascular coagulation. Other complications during the IVF procedure, such as severe intraabdominal hemorrhages and ovarian abscesses are rare and were reported in 0.06% and 0.003%, respectively (Aragona *et al*., 2011).

Obstetric outcome after IVF

Obstetric outcome after IVF in general versus general population.

IVF children have higher rates of low birth weight (LBW), very low birth weight (VLBW), preterm birth (PTB), very PTB, and mortality than children from the general population. Large registry studies have shown that the increased risks for IVF children are mostly attributable to the high rate of multiple births (Bergh *et al*., 1999; Klemetti *et al*., 2002, Schieve *et al*., 2002; Schieve *et al*., 2007; Helmerhorst *et al*., 2004; Wang *et al*., 2005). In the mid-1990s The Swedish Board of National Health and Welfare initiated a national IVF register including all children born after IVF since 1982. The first register study that assessed outcomes for IVF children in Sweden as compared with children from the general population was published in Lancet 1999 (Bergh *et al*., 1999) (Fig.1).

This study analyzed the obstetric outcomes in the first Swedish cohort of IVF children (n=5856) and demonstrated that the risks of PTB, VLBW and LBW were 5-6 fold higher among IVF children than children in the general population. Perinatal mortality was 1.9% for IVF children and 1.1% for children in the general population. Multiple birth rate among IVF children in this study was 26%. Although the multiple birth rate among IVF children has decreased during last years, it is still high in most countries. The latest reports indicate that the multiple birth rate in Europe was 22% (2008) and in the

USA 31% (2009) (Ferraretti *et al*., 2012; Sunderam *et al*., 2012) (Fig. 2). Ninety five per cent of all multiple births were twins and it is well recognized that maternal and neonatal morbidity and mortality are significantly increased in IVF twin pregnancies as compared with singleton pregnancies (Helmerhorst *et al.,* 2004; Ananth *et al.,* 2004).

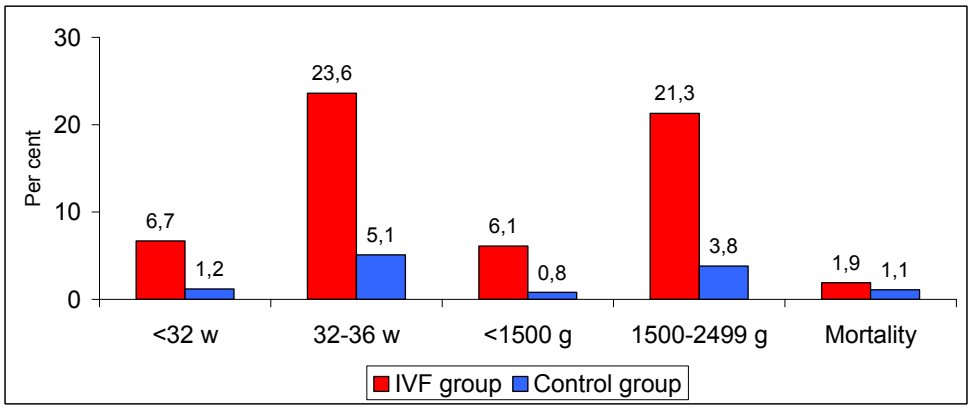

Figure 1. Obstetric outcome for children born after IVF in Sweden1982-1995 (n=5856). Multiple birth rate 26% (from Bergh *et al.,*1999).

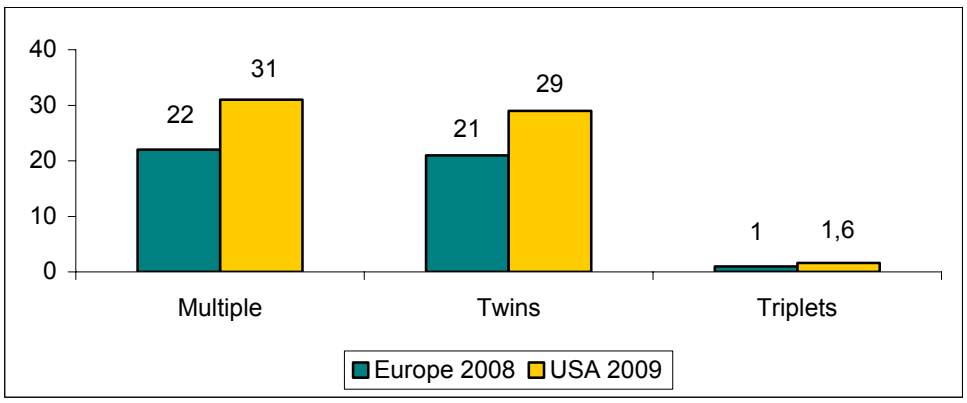

Figure 2. Multiple birth rates after IVF, Europe and USA (from Ferraretti *et al*., 2012; Sunderam *et al*., 2012).

Obstetric outcome for IVF singletons versus general population

Single embryo transfer (SET)

The best way to minimize the risk of multiple births is to reduce the number of embryos transferred. The first study, demonstrating SET as a successful option in IVF, was performed in Finland (Vilska *et al.,* 1999*)*. The Nordic countries, Sweden in particular, are the leading countries in reduction of multiple birth rate by implementing SET as the main treatment strategy. As a consequence, the multiple birth rate in Sweden decreased from 26% in 2001 to only 5-6% in 2004 with the delivery rate almost unchanged (Fig. 3). The SET rate increased from 10% in 2000 to 70-80% of all embryo transfers from fresh cycles and has stabilized in recent years. For cryo-cycles, the SET rate is currently 85%.

Obstetric outcome for IVF singletons versus general population

Results from large epidemiological studies and meta-analyses have shown that IVF singletons also have a poorer outcome with two-three times higher risks of PTB, LBW and very LBW as compared with singletons in the general population. These risks remain significant even after adjustment for maternal confounders (Jackson *et al*., 2004; Helmerhorst *et al*., 2004; McDonald *et al*., 2009; Pandey *et al.,* 2012) (Table 1). The rate of small for gestational age (SGA) is also elevated by a factor of 1.5 (Helmerhorst *et al*., 2004.)

There is no real explanation for these adverse outcomes but maternal characteristics, hormonal stimulation and culture technology are being discussed as factors that might negatively affect outcomes for IVF singletons.

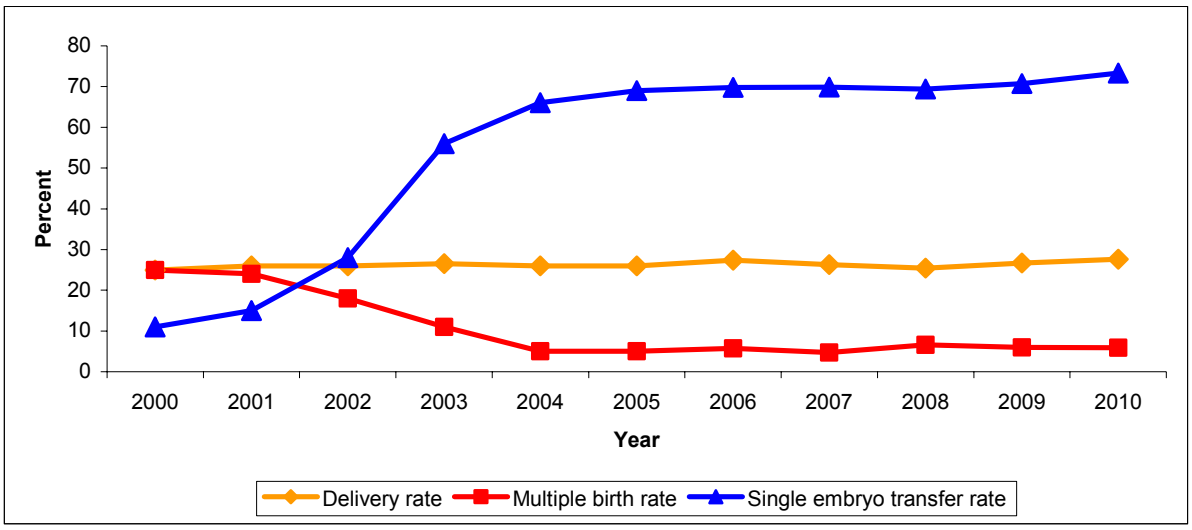

Figure 3. Single embryo transfer in Sweden between 2000 and 2010 (National Quality Register for Assisted Reproduction, Sweden).

Table 1. Obstetric outcome in singletons after IVF: Results from systematic reviews & meta-analyses.

RR or AOR (95% CI)	Helmerhorst 2004 *n*=5361	Jackson 2004 *n*=12 283	McDonald 2009 *n*=31 032	Pandey 2012 *n*=28 352
Very preterm birth, <32 w	3.3 (2.0-5.3)	3.1 (2.0-4.8)	2.3 (1.7-3.0)	1.7 (1.5-1.9)
Preterm birth, <37 w	2.0 (1.8-2.3)	2.0 (1.7-2.2)	1.8 (1.5-2.2)	1.5 (1.5-1.6)
Low birth weight, <2500 g	1.7 (1.5-1.9)	1.8 (1.4-2.2)	1.6 (1.3-2.0)	1.7 (1.6-1.8)
Very low birth weight, <1500 g	3.0 (2.1-4.4)	2.7 (2.3-3.1)	2.7 (1.8-3.8)	1.9 (1.7-2.2)

RR risk ratio, AOR adjusted odds ratio

A recent systematic review and meta-analysis (Pinborg *et al*., 2012) summarized studies of maternal characteristics and treatment related variables. Current literature suggests that both subfertility *per se* and treatment related variables contribute to a higher risk of PTB among IVF singletons. This conclusion is also supported in "sibling studies" (Romundstad *et al*., 2008; Henningsen *et al*., 2011), where obstetric outcome is compared between singletons conceived spontaneously and IVF singletons with the same mother. In the large Danish study (Henningsen *et al*., 2011) a higher PTB rate was found among ART singletons. Perinatal mortality for IVF singletons in the first Swedish IVF cohort (1982-1995) was 0.82%, as compared with 0.66% for singletons in the general population (Bergh *et al.,* 1999). The effects of IVF on perinatal mortality in IVF singletons (risk ratio RR 1.6 (1.2-2.6)) were no longer significant after stratification for year of birth, maternal age, parity and years of infertility.

An elevated odds ratio (OR 2.2, 95% CI 1.6-3.0) of perinatal mortality was also detected for IVF singletons in the meta-analysis by Jackson and co-workers (Jackson *et al*., 2004), but significance disappeared after adjustment for maternal confounders. In the later meta-analysis (McDonald *et al*., 2009) based on 17 studies and over 30 000 IVF singletons, analysis of perinatal mortality was not included.

Obstetric outcome for SET/eSET singletons versus general population and versus DET singletons

Elective single embryo transfer (eSET) was defined as transfer of one fresh embryo when at least one embryo was cryo-preserved in the same treatment cycle. Randomized trials and meta-analyses (Pandian *et al*., 2009; McLernon *et al*., 2010) comparing the pregnancy/delivery rates after SET/eSET and DET show significantly higher live birth rates after DET. However, when adding one cryo SET to the SET group no significant difference in live birth rates between SET and DET were noted. A dramatic decrease in multiple birth rate, from 33% to 0.8%, was noted in

the SET group (Thurin *et al*., 2004).

Reports about obstetric outcome in IVF singletons after SET and DET have been divergent. One Belgian study (De Sutter *et al*., 2006) demonstrated significantly higher rates of PTB and LBW rates among singletons after DET, compared with singletons after SET, while another study from Finland found no differences (Poikkeus *et al*., 2007). In a large population-based study from Australia (Wang *et al*., 2009), 13 000 SET singletons were compared with 16 000 DET singletons. Significantly higher rates of PTB and LBW were found for singletons after DET. However, that study included no adjustment for years of infertility. Studies, particularly from Denmark (Pinborg *et al*., 2007), have shown that singletons from "vanishing twin pregnancies" (singleton pregnancies when more than one sac is identified at the first trimester sonography, i.e. two embryos implanted but only one child is born), have a worse outcome than DET pregnancies with one sac at the first trimester sonography.

Other outcomes fors children born after IVF

Congenital malformations and chromosomal disorders after IVF

Three meta-analyses (Rimm *et al*., 2004; Hansen *et al*., 2005; McDonald *et al*., 2005) and the latest large Swedish registry study (Källen *et al*., 2010c) have demonstrated a significantly higher risk of malformations after IVF compared to children in the general population (OR 1.25-1.4). Similar risks of malformations were found both after IVF and ICSI and for singletons as well as multiples. In the Swedish study, the absolute risk of serious congenital malformations was 3.7% for IVF children, as compared with 3.0% in children in the general population (Källen *et al*., 2010c). The risks of certain specific malformations, such as neural tube and cardiovascular defects, oesophageal atresia and limb reduction, were significantly increased even after adjustment for maternal confounders.

In a study from Australia (Davies *et al*., 2012), the absolute risk of birth defects for all IVF children was 8.3% as compared with 5.8% for children in the general population. Separate analysis of these risks for IVF and ICSI children demonstrated 7.2% and 9.9%, respectively. The risk of birth defects was significantly increased in ICSI children even after adjustment for maternal confounders.

It should be noted that inclusion of the diagnosis "cerebral palsy" and the 5-years follow-up of all children could have had an impact on the results and contribute to higher rates of birth defects in this study as compared with results in the previous studies.

Prenatal diagnostic investigations conducted by the Belgian research group (Bonduelle *et al*., 2002) have shown slightly higher rates of inherited chromosomal abnormalities and sex chromosomal aberrations for ICSI children. In a recent large Swedish registry study no increased risk of chromosomal aberrations was detected among the 31 850 IVF children (Källen *et al*., 2010c) as compared with children in the general

population. A higher rate of Y-deletions (missing genes in the Y chromosome) after ICSI has also been found, giving similar fertility problem in the sons since they are inherited from the subfertile father (Reijo *et al*., 1995).

Neurological sequelae after IVF

Cerebral palsy is a physical disorder in childhood that is strongly associated with preterm deliveries (Hvidtjorn *et al*., 2006). Three large studies from the Nordic countries (Klemetti *et al*., 2006; Strömberg *et al*., 2002; Hvidtjorn *et al*., 2006) have reported a significantly increased risk of cerebral palsy in children born after IVF (singletons and multiples) as compared with spontaneously conceived children. Even for IVF singletons the risk of neurological disorders was significantly increased with OR 1.8 (95% CI 1.3-2.5) as compared with singletons in the general population (Hvidtjorn *et al*., 2009).

Imprinting diseases and IVF

Epigenetics refer to changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence. Imprinting diseases, associated with inadequate epigenetic modification of the genome (impairment in DNA methylation), are rare (1 in 14 000-15 000 newborns) but have severe consequences for the children. Several smaller studies (Cox *et al*., 2002; De Baun *et al*., 2003; Manipalviratn *et al*., 2009) have found a possible association between some imprinting diseases such as Beckwith-Wiedemann and Angelman's syndromes and ART. In later register studies (Källen *et al*., 2005a; Lidegaard *et al*., 2005, Källen *et al*., 2010a) no certain increase in imprinting disorders was detected. However, larger cohorts are needed to investigate this problem.

Cancer incidence after IVF

Reported data about cancer after IVF is controversial. A Dutch study reported a fivefold increased risk of retinoblastoma in children born after IVF between 1995 and 2002 (Moll *et al*., 2003). In an expanded Dutch study (Marees *et al*., 2009), including follow-up of IVF children from 2002 to 2007, no significantly increased risk of retino-blastoma was noted.

A study from Australia (Bruinsma *et al*., 2000) and a systematic review from Netherlands (Middelburg *et al*., 2008) reported no increased incidence of cancer among IVF children as compared with children in the general population.

In a large Swedish study including 16 280 IVF children born between 1982 and 2001, a similar cancer risk was noted for IVF children as for children in the general population, but an unexpectedly high incidence of histiocytosis was reported (standardized incidence ratio SIR 5.6; CI 95% 1.8-13) (Källen *et al.,* 2005a). In a widened cohort of IVF children (n= 26 692) born in Sweden between 1982 and 2007 a significantly increased total cancer risk (SIR 1.42, CI 95% 1.09-1.87) was reported. When 6 cases of histiocytotis were excluded, the OR dropped to 1.34 but still remained significant (Källen *et al*., 2010a).

Growth, physical and cognitive development during infancy after IVF

Several studies have evaluated growth in IVF children and compared them with children in the general population. No differences have been found between the two groups (Ceelen *et al*., 2008a). However, a study of IVF children 8 to 18 years old demonstrated higher blood pressure and blood glucose levels compared to age-matched controls (Ceelen *et al*., 2008b; Ceelen *et al*., 2009), and a predisposition to obesity in IVF children after ICSI was recently reported from Belgium (Belva *et al*., 2012). According to a systematic review (Middelburg *et al*., 2008), preterm delivery was associated with a certain risk of cognitive dysfunction, but no difference was found between IVF children and children after spontaneous conception.

Maternal outcomes after IVF singleton pregnancies

The large nationwide study from Sweden (Källen *et al*., 2005c) analyzed obstetric characteristics, maternal morbidity and mortality for all women who delivered after IVF in Sweden between 1982 and 2001 (n=13 261) and compared them with all women (n=2 013 633) who were reported to MBR during the same period of time. IVF women with singleton pregnancies had a significantly increased risk of preeclampsia (AOR 1.2, 95% CI 1.1-1.3), placental abrup-tion (AOR1.9, 95% CI 1.4-2.5), placenta previa (AOR 3.8, 95% CI 3.3-4.5), bleeding in association with vaginal delivery (AOR 1.2, 95% CI 1.2-1.3) and preterm premature rupture of the membranes (PPROM) (AOR 1.5, 95% CI 1.3-1.7) as compared with women in the general population.

Several studies have also demonstrated significantly higher risks for preeclampsia and placental complications for singleton pregnancies after IVF than for singleton pregnancies in the general population.

In a meta-analysis of complications related to ART and including 1910 ART pregnancies (from 36 studies), the risk of preeclampsia was significantly increased (AOR 1.6, 95% CI 1.23-1.99) and the risk of placenta previa was almost threefold higher after ART than non-ART pregnancies (AOR 2.9, 95% CI 1.54-5.37) (Jackson *et al*., 2004).

A cohort study from Norway (Romundstad *et al*., 2006) compared 7568 IVF pregnancies to 845 384 pregnancies in the general population, reported to the MBR in Norway between 1988 and 2002. The strength of this study was inclusion of 1349 women who had conceived both spontaneously and after assisted fertilization. A six fold higher risk of placenta previa (AOR 5.6, 95% CI 4.4-7.0) was found in ART pregnancies than pregnancies in the general population. Among mothers who had conceived both spontaneously and after ART, the risk of placenta previa was almost threefold higher (AOR) in the ART pregnancies.

A retrospective cohort study (1991-2004) from Australia (Healy *et al*., 2010) compared the prevalence of obstetric hemorrhages in 6730 IVF singleton preg-

nancies with 24 619 singleton pregnancies in the general population. Higher risks of antepartum hemorrhages (AOR 2.0, 95% CI 1.8-2.3), placenta previa (AOR 2.3, 95% CI 1.9-2.9), placental abruption (AOR 2.1, 95% CI 1.4-3.0), and post-partum hemorrhages (AOR 1.3, 95% CI 1.2-1.4) were found in IVF singleton pregnancies than in singleton pregnancies in the general population.

Thromboembolism in pregnancies after IVF

Thromboembolism is known as a major cause of maternal mortality during pregnancy (Clark *et al.,* 2008). In a large Swedish study (Källen *et al*., 2005c) including 13261 women who delivered after IVF in Sweden in 1982-2001, an increased risk of thromboembolic comp-lications during pregnancy was detected as compared with all women (n=2 013 633) reported to the MBR during the same period of time.

Two recent Swedish studies (Rova *et al*., 2012; Henriksson *et al*., 2013), which also included maternal BMI in the analysis, demonstrated a significantly increased risk of thromboembolic complications in IVF pregnancies as compared with pregnancies in the general population. The risk was particularly increased during the first trimester, with a hazard ratio 4.22, 95% CI 2.46-7.26) (Henriksson *et al*., 2013), OR 9.8, 95% CI 6.7-14.3 and 100-fold in the presence of OHSS (Rova *et al*., 2012) as compared with pregnancies in the general population.

Summarizing recent data, it is apparent that IVF singletons have poorer obstetric outcome, assessed as PTB and LBW, than singletons in the general population. It remains unclear which particular factors influence outcomes negatively. The assess-ment of obstetric outcomes in a large and complete cohort of IVF children, born after DET and SET, in combination with medical registries of high validity, thus provides a good opportunity to evaluate risks for IVF singletons, particularly singletons after SET.

Table 2. Maternal complications in IVF singletons. Results adapted from systematic reviews and meta-analyseis and large cohort studies.

AOR, 95% CI	Källen 2005	Jackson 2004	Romundstad 2006	Healy 2010	Pandey 2012
Pre-eclampsia	1.2 (1.1-1.3)	1.6 (1.2-2.0)			1.5 (1.4-1.6)
Placental abruption	1.9 (1.4-2.5)			2.1 (1.4-3.0)	
Placenta previa	3.8 (3.3-4.5)	2.9 (1.5-5.4)	5.6 (4.4-7.0)	2.3 (1.9-2.9)	
Ante-partum hemorrhages (placental abruption, Placenta previa, third trimester vaginal bleeding)					2.5 (2.3-2.7)

AOR adjusted odds ratio

Aims of the study

General aims

The aims of the study were to assess obstetric outcomes for IVF singletons.

Specific aims

- To assess perinatal outcomes for IVF children and maternal complications in Sweden during the years 2002-2006, when SET was widely introduced in Sweden.

- To analyze factors (maternal, IVF treatment related) which negatively affect obstetric outcomes assessed as very PTB (<32 w), SGA, placenta previa and placental abruption.

- To investigate obstetric outcomes in IVF singletons after transfer of cryo-preserved embryos.

- To compare neonatal and maternal outcomes for women undergoing two in vitro fertilization (IVF) singleton pregnancies and women undergoing one IVF twin pregnancy.

Methodological considerations

Settings and study designs

This study is a retrospective national register study based on analysis of IVF children born in Sweden from 2002 to 2006 after IVF treatment.

Data was collected from all 16 Swedish IVF clinics and cross-linked with the MBR. This cohort of IVF children covers the period when SET was widely introduced in Sweden, resulting in a high rate of IVF singletons and giving us the opportunity to analyse the obstetric and neonatal outcomes particularly of SET singletons.

The main strength of this study was inclusion of a complete and large cohort of IVF children. The use of a unique personal identification number in Sweden enables for cross-linkage of data with different medical registries. Weakness of this study was the lack of information on hormonal doses used during stimulation, infertility reason and embryo quality in data collected from IVF clinics.

The present study was divided into four papers (Figure 7). In the first paper we analysed all IVF treatments performed in Sweden during the years 2002-2006 resulting in births, separated into SET and DET.

The second paper concerned an analysis of maternal and IVF treatment-related variables for possible association with two adverse neonatal outcomes (very PTB, <32w and SGA) and two maternal outcomes (placenta previa and placental abruption) in IVF singleton pregnancies.

The increased use of SET in fresh cycles contributes to a large number of embryos available for cryopreservation. In paper III we performed a detailed assessment of neonatal outcomes of singletons born after cryopreservation of embryos. In addition to the main assessed variables (LBW, PTB and perinatal mortality) we also analyzed large for gestational age (LGA) and birth weight >4500 g, since higher rates of these outcomes had recently been reported both from Finland (Pelkonen *et al*., 2010) and Denmark (Pinborg *et al*., 2010; 2011). We also analyzed maternal outcomes. Despite overwhelming evidence that the use of SET has reduced the risks of both maternal and neonatal complications, there is still an ongoing debate as to whether twins are a desired outcome of IVF (Gleicher *et al*., 2009; Gleicher, 2013). Suggesting that most parents want two children, in the fourth paper we analyzed the neonatal and maternal outcomes for women undergoing two IVF singleton pregnancies and compared this data with data on women undergoing one IVF twin pregnancy.

In Paper I a detailed analysis of neonatal and maternal outcomes after all IVF treatments resulting in births during the years 2002-2006 was reported. All IVF children born in Sweden after IVF treat-

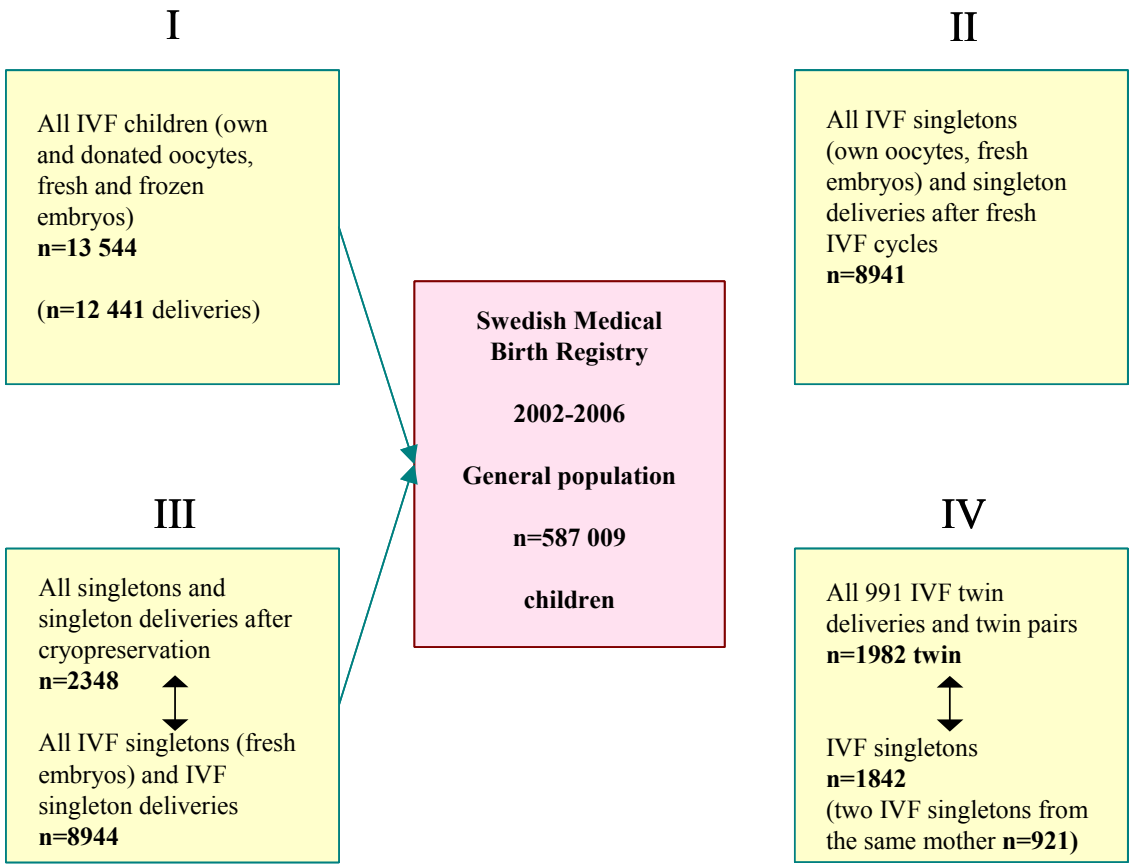

Figure 7. Study design.

ments (with own and donated oocytes, fresh and frozen embryos) were included (13 544 children) and compared with all children born during the same period in the general population (587 009 children). Data was cross-linked with the Swedish Medical Birth Register (MBR).

In Paper II an analysis of factors affecting neonatal and maternal outcomes in 8941 IVF singleton pregnancies was performed (own oocytes, fresh embryos).

Paper III focused on outcomes for IVF singletons born after cryopreservation (n=2348) compared with IVF singletons born after fresh IVF (n=8944) and all singletons in the general population (n=571 914). Maternal outcomes were also analyzed and compared.

In Paper IV we compared maternal outcomes for 921 women undergoing two IVF pregnancies with singletons with 991 women undergoing one IVF twin pregnancy.

Neonatal outcomes, including severe child morbidity, for all reported 1982 twins after IVF were compared with outcomes for 1842 IVF singletons (Table 4).

Table 4. Study settings and patients.

	Paper I	Paper II	Paper III	Paper IV
Setting	National register study in Sweden			
Study design	Retrospective register study			
Study period	2002-2006			
No. of infants in the study groups	13 544 All reported IVF infants (own and donated oocytes fresh and frozen embryos) Singletons and multiples	8941 IVF singletons (own oocytes, fresh embryos)	2348 Singletons after cryopreservation 8 944 singletons after fresh IVF cycles	1982 IVF twins after DET 1 842 IVF singletons (two singletons from the same mother)
Control group	587 009 Non IVF infants 579 450 pregnancies	-	571 914 Non IVF singletons	-
Deliveries	12 441 All IVF singleton and multiple deliveries	8941 Singleton deliveries after fresh IVF cycles	2348 Singleton deliveries after cryo-preservation 8944 singleton deliveries after fresh IVF cycles	991 twin and 1842 singleton deliveries (921 mothers)
Cross-linkage	The Swedish MBR			The Swedish MBR National Patient Register
Main outcomes	Preterm birth <28w, <32w, <37w LBW VLBW SGA Peri/neonatal mortality Maternal complications (preeclampsia, placenta previa, placental abruption, gestational diabetes, PPROM)	Very preterm (<32w) SGA Maternal complications (placenta previa, placental abruption)	Preterm birth <28w, <32w, <37w LBW VLBW SGA LGA Perinatal mortality Maternal complications (preeclampsia, placenta previa, placental abruption, gestational diabetes, PPROM)	Preterm birth <28w, <32w, <37w LBW VLBW SGA Severe neonatal morbidity and peri/neonatal mortality Maternal complications (preeclampsia, placenta previa, placental abruption, gestational diabetes, PPROM)

DET double embryo transfer, VLBW very low birth weight (<1500 g), LBW low birth weight (<2500 g), SGA small for gestational age, LGA large for gestational age, PPROM preterm premature rupture of the membranes

Data collection

Data collected from the IVF clinics included the following: Personal identification number of the women, type of IVF treatment (fresh, frozen, IVF, ICSI, ejaculated sperm, epididymal sperm and testicular sperm) donated/own oocytes, number of oocytes retrieved, cleavage stage transfer, blastocyst stage transfer, date of embryo transfer, number of embryos transferred, number of frozen embryos, number of gestational sacs at first ultrasound and date of ultrasound, date of delivery and number of children born.

Data from the IVF clinics were cross-linked with the Swedish MBR (National Board of Health and Welfare, 2003) and compared with all children born in the general population during the same time period (Papers I, III). Data about maternal age, parity, maternal smoking, maternal BMI, years of involuntary childlessness and obstetric outcomes were retrieved from the MBR (Papers I-IV). Information on severe neonatal morbidity (ICD codes) was collected from the MBR and from the National Patient Registry (Paper IV).

The Swedish MBR

The Swedish MBR was established in 1973 and includes data on practically all deliveries in Sweden. It is mandatory for every health care provider to report to the register and the information is collected from medical records from prenatal, delivery and neonatal care. The number of infants born each year varied between 86 000 and 120 000. Records for a small percentage of all infants, 0.5-3.0%, are missing completely, for some others the information is incomplete owing, for example, to missing data from antenatal care clinics and pediatric wards. Although the basic structure of the register has remained unchanged during the years, there have been major modifications to content and methods of data collection. The register quality has been evaluated three times: in 1976; in 1988 (Cnattingius *et al*., 1990) and in 2001.The quality controls consisted of analyses of data from the register, and comparisons between the original medical records and the corresponding register data. More detailed information on the quality of the register was published in 2003 under the title: "The Swedish MBR - A Summary of Content and Quality". The register has been recognized as having high validity and completeness (Research Report from Centre for Epidemiology (EpC). National Board of Health and Welfare, 2003).

Missing records

Births may not be recorded in the register when documents relating to delivery are not sent by the delivery hospital to the register. Every year, infants reported to the register are compared with infants reported to Statistics Sweden (SCB). When missing cases are discovered, a request is sent to the responsible clinic to obtain copies of the medical records, but this is not always successful. Consequently, about 1.4% of all infants born in Sweden are not recorded in the register. With regard to exposure data, information on smoking in early pregnancy is relatively good (4.2-9.0% missing). In our data, information on smoking was unknown in 10.0% for mothers in the IVF group and in 8.0% for mothers in the general

population. Figures for BMI were 14.5% and 14.1% respectively (Paper I). The missing information about years of infertility for women with IVF twin and singleton pregnancies was 29.3% in 991 twin IVF pregnancies and 22.4% in 921 women who gave birth to two IVF singletons (Paper IV). In cases of missing information on smoking and/or maternal BMI in the stratified analysis (Mantel-Haenszel Procedure) missing data were included in the separate classes (Papers I and III). In the logistic regression models (Papers II, III, IV) imputations were made so that the missing values were replaced by the overall mean among records with valid information (Paper IV).

Infant diagnoses

A complete lack of infant diagnoses was relatively rare during the period 1973-1981 (less than 0.1%). For 1982-1989, the frequency increased to 2.3%; after 1990 the rate increased further to about 10%, and in 1998 to 15%. These cases probably represent infants transferred to neonatal units with no feedback in discharge diagnoses when reported to the MBR. Moreover, these under-reported diagnoses were unevenly distributed among hospitals. For 1998, less than 10% of records in the majority of maternity hospitals lack infant diagnoses; but some hospitals lack such information for up to 79% of cases, which cannot be explained in terms of neonatal transfers.

In our study, especially in the fourth part, when analysing severe infant morbidity, we presumed that missing diagnoses were equally distributed in both groups of IVF children. Furthermore, we used the National Patient Register as an additional source of information about severe morbidity of IVF newborns.

The National Patient Register (NPR)

It is compulsory for all medical care providers to report to the NPR since 1984. All "in-patient care" records are included in NPR since 1987 and outpatient appointments including day surgery and psychiatric care since 2001. Primary care is not yet included in the NPR.

The aim of the NPR is to follow health trends in the population, to improve access to health care, to prevent diseases and to support healthcare development. The register provides data including descriptive statistics and evaluation of patient safety.

Missing reports for the last few years are estimated to be less than 1% for somatic in-patient care. The social security number is missing or incorrect for less than 1% of health care episodes in recent years, most of which are related to children or people living abroad. Main diagnoses (ICD-10 codes) are missing for about 1% of health care episodes. Variables such as hospital, clinic, sex, age, admission and discharge dates are almost complete.

The Swedish National Quality Register for Assisted Reproduction (Q-IVF)

In 2007, the Swedish National Quality Register for Assisted Reproduction was established to collect data for all IVF treatments in Sweden. All 16 Swedish IVF clinics, public as well as private, participate and send individual data to the register.

The purpose of this register is to continuously monitor outcomes and possible medical risks for both IVF children and treated patients. The register is also a good platform for research.

Ethical considerations

Ethical approval was obtained from the ethics committee of the University of Gothenburg for the whole project in June 12, 2006, Dnr: 304-06, Ad 304-06, T109-08.

Power calculation

A power analysis (power 80%, significance level 5%) revealed that with 7763 SET children and 587 009 children in the population, it is possible to detect a difference in preterm birth from 5.0% in the population to 5.8% in the SET group (risk ratio 1.15), and a difference in LBW from 3.2% in the population to 3.8% (risk ratio 1.19) (Paper I).

Statistical methods

All statistical analyses were done in collaboration with professional statistician Karin Källen, PhD, Associated Professor, Department of Reproduction Epidemiology, Tornblad Institute, Institution of Clinical Sciences, Lund University, Lund, Sweden. The logistic regression analysis and calculations of crude odds ratios (OR) and adjusted OR (AOR) with 95% confidence intervals (CIs) were performed using Gauss (GaussTM, Aptech Systems Inc., Maple Valley, WA, USA, http://www.aptech.com).

All tests were conducted at a significance level of 0.05.

We used two different statistical methods to study the obstetric and neonatal outcomes after IVF treatment. When comparing the pregnancy outcome after IVF with the general population, the Mantel-Haenszel procedure was performed to obtain ORs. In comparisons within the IVF-group, simple and multiple logistic regression analyses were committed instead. The Mantel-Haenszel procedure was introduced in 1959 by Mantel and Haenszel and is most used in population-based investigations when studying a possible association between a dichotomous exposure variable (for example, eSET) and a dichotomous outcome variable (for example, SGA), taking several possible confounders into account (for example, maternal age and BMI). A Mantel-Haenszel analysis requires a huge population-based sample, and also the data have to be divided into classes. Furthermore, the test assumes that the ORs are homogenous over the different strata (for example, a certain OR among young women does not significantly differ from the corresponding OR among older women). Simple and multiple logistic regression analyses do not require huge data sets. An old and generally accepted 'rule of the thumb' says that the number of investigated independent variables should never exceed 1/10 of the number of cases. The data set do not have to be classified; the investigated independent variables could be continuous, quasi-continuous, categorical, or dichotomous. However, when performing logistic regression analyses, efforts must be taken to

check whether the model assumption fits the data. For example, if the association between a certain independent variable and the investigated outcome is not linear, but *U*-shaped, a linear logistic regression model would yield erroneous results. We checked the fit of all our models performing Hosmer-Lemeshov goodness-of-fit-tests. If appropriate, we added second degree factors (for example, maternal age^2 and BMI^2) to our models in order to adapt to a *U*-shaped association between the investigated variables.

Statistical methods for the different papers are presented in Table 5.

Table 5. Statistical methods used in Papers I-IV.

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Descriptive statistics	Numbers and percentages	Numbers and percentages, Mean, SD SDs (z-scores)	Numbers and percentages, Mean SD,	Numbers and percentages
Analytical statistics IVF children vs. population IVF children vs. IVF children	Mantel-Haenszel procedure, Miettinen method OR and AOR (95% CI)	Kruskal –Wallis non parametric test Logistic regression analysis Hosmer – Lemeshov test OR and AOR (95% CI)	Mantel-Haenszel Procedure Miettinen method OR and AOR (95% CI) Logistic regression analysis OR and AOR (95% CI)	Univariate and multiple logistic regression analyses Generalized Estimating Equation technology OR and AOR (95% CI)
Adjustment performed for confounders	Year of birth Maternal age Parity Maternal smoking (at the first antenatal visit) BMI (at the first antenatal visit) Years of infertility (before pregnancy)	Maternal age (linear, continuous variable, or second degree polynomia). Primiparity (yes/no). (BMI) (linear, continuous/ second degree polynomial) Years of infertility (linear, continuous variable). Maternal smoking (quasi-continuous variable) Number of aspirated oocytes (linear, continuous variable). Number of embryos frozen (linear, continuous variable)	Maternal age (years) Primiparity (yes/no) Years of infertility (continuous variable) Maternal smoking (semi-continuous variable) BMI (continuous variable)	Year of birth (continuous variable) Maternal age at delivery (continuous variable) Nulliparity (yes/no) Maternal smoking (semi-continuous variable) BMI (continuous variable)

OR odds ratio, AOR adjusted odds ratio

Analyzed variables for each paper and statistical methods

Paper I

Obstetric outcome after *in vitro* fertilization with single or double embryo transfer

Main outcome measures were preterm birth (<28 w, <32 w, <37 w), VLBW (<1500 g) and LBW (<2500 g). Other outcome measures were as follows: small for gestational age (SGA, more than 2 SDs below the Swedish growth standard) (Marsal *et al.,* 1996), peri/neonatal mortality, Apgar score and rate of Cesarean section. Maternal outcomes included the following: Preeclampsia (ICD 10 codes O.14, O.15), gestational diabetes (ICD 10 code O.24.4), placenta praevia (ICD 10 code O.44), placental abruption (ICD 10 code O.45) and premature rupture of the membranes (PPROM) (ICD 10 code O.42).

Elective SET (eSET) and non-elective SET (non-eSET) only refer to transfers with fresh embryos. eSET was defined as transfer of one fresh embryo when at least one embryo was cryopreserved in the same treatment cycle. Non-eSET was defined as fresh cycles when one embryo was transferred and no embryos were cryopreserved.

Gestational age was determined from the second trimester ultrasonography for both IVF and non-IVF pregnancies to get the same dating for both IVF and spontaneous pregnancies.

When we compared obstetric and neonatal outcomes for IVF children with corresponding outcomes for children in the general population, Mantel-Haenszel procedure was used to obtain odds ratios.

Adjusted odds ratios were calculated after stratification for the year of delivery, age of the mother, parity, smoking, body mass index (BMI) and years of infertility, using the method proposed by Miettinen (Miettinen, 1974).

Paper II

Factors affecting obstetric outcome of singletons born after IVF

Dependent variables were: Very preterm birth (<32 w), small for gestational age (SGA), placenta previa (diagnosed at delivery) and placental abruption. These four specific outcomes were selected since they were regarded as important and robust. Very preterm birth and SGA, reflecting fetal growth, are associated with major neonatal morbidity and mortality and may also have long-term health consequences for the child. Gestational age was determined according to the day of embryo transfer and number of culture days. This was regarded as appropriate since all statistical comparisons in this paper were performed within the IVF cohort and the day of embryo transfer was assumed to give more precise dating of gestation than second trimester ultrasonography.

Singletons were grouped according to embryo transfer characteristics: Eelective single embryo transfer (eSET), non-eSET, double embryo transfer (DET) resulting in one sac and DET with a ”vanishing twin” (DET with more than one sac at first trimester sonography, i.e. two embryos implanted but only one child born). This

subdivision was done from a clinical perspective and on the basis of a discussion in recent years concerning a possible difference in outcome for singletons depending on the number of embryos transferred.

Comparisons between IVF singletons in various study groups were performed using logistic regression analysis. We followed a general recommendation not to consider more than n/10 variables, where *n* is the sample size, in order to avoid over-estimation of each variable's importance.

Predicting variables may be either continuous or categorical. In this paper BMI, maternal smoking and years of infertility were entered as continuous variables. Maternal age was entered into the analyses as a linear continuous variable, where a *U*-shaped association with maternal age was detected. Parity, expressed as primiparity (yes/no) and transfer of blasto-cyst (yes/no) were entered as categorical variables**.** Logistic regression analysis was performed for this analysis, but Kruskal-Wallis non-parametric test was used to compare continuous variables between the study groups. This test is commonly used to analyse three or more unmatched groups from Non-Gaussian populations.

Independent variables of the investigation were:

1. eSET, non-e-SET, DET one sac and DET with a vanishing twin.
(expressed as binary variables with eSET as the reference group).

2. Blastocyst (yes/no).

The following factors were expected to be associated with the four main outcomes as well as with the independent variables of the investigation. They were therefore regarded as possible confounders and were tested for independent prediction:

1. Maternal age (entered into the analyses as a linear, continuous variable, or for some outcomes where a *U*-shaped association with maternal age was detected, as a second degree polynomia, as specified in the results).

2. Parity (expressed as primiparity yes/no).

3. Body mass index (BMI) (expressed as a linear, continuous variable, or for some outcomes, where a *U*-shaped association with BMI was detected, as a second degree polynomia as specified in the results). In the multiple models, missing information was replaced by the overall mean BMI (=24.7).

4. Duration of infertility (no. of years) (expressed as a linear, continuous variable).

5. Maternal smoking in the first trimester (expressed as a quasi-continuous variable; 1=no smoking, 2=smoking 1-9 cigarettes per day, 3=smoking ≥10 cigarettes per day). In the multiple models, missing smoking information was replaced by the overall mean (=1.04 in the semi-continuous scale).

6. Number of aspirated oocytes (expressed as a linear, continuous variable). This variable was chosen as a proxy for

hormonal stimulation. Hormonal stimulation is one factor discussed as negatively influencing obstetric outcomes, since children born after cryopreservation of embryos mostly transferred in natural cycles without hormonal stimulation generally have a better outcome than children born after fresh IVF including hormonal stimulation (Wennerholm *et al*., 2009).

7. Number of embryos frozen (expressed as a linear, continuous variable). This variable was chosen as a proxy for embryo quality. Embryo quality has also been discussed as influencing obstetric outcomes.

In order to determine which variables should enter the final multiple logistic regression models the following steps were taken: The best logistic model for each investigated variable (linear, quadratic, or divided into designed class variables) was determined by investigating the level of significance and goodness of fit according to the Hosmer-Lemeshov test.

If a *U*-shaped relationship was identified, the variable in question was represented by one linear and one quadratic term (second grade polynomial). The resulting final univariate model for each investigated factor is described above. Vanishing twin, non-eSET, DET with one sac and blastocyst transfer were regarded as the key variables of interest, and were thus included in the final models, regardless of their significance levels.

For the other considered factors, variables with p-values below 0.20 in the final univariate models were initially included in the multivariable models, and then excluded if the p-values exceeded 0.20 in the multivariable analysis.

Paper III

Obstetric outcome in singletons after *in vitro* fertilization with cryopreserved/ thawed embryos

Main outcome measures were: PTB (<37 w) and very PTB (<32 w), extreme PTB (<28 w), LBW, VLBW. Other outcome measures were: SGA, perinatal mortality (stillbirths and neonatal deaths <7 days), Apgar score and rate of Cesarean section. LGA (more than two standard deviations above the Swedish growth standard >+22%) (Marsál *et al.,* 1996), macrosomia (>4500 g) were selected as additional main outcomes measures since higher rates of these outcomes were reported after cryopreservation (Pinborg *et al*., 2010, 2011; Pelkonen *et al*., 2010).

We also created and analyzed composite outcomes subdivided into two groups: any composite outcome (<37 weeks, <2500 g, SGA or perinatal mortality) and serious composite outcome (<32 weeks, <1500 g, or perinatal mortality), in order to optimize identification of such adverse outcomes.

Maternal outcomes included: pre-eclampsia, gestational diabetes, placenta praevia, placental abruption and PPROM. Gestational age was determined from second trimester ultrasonography for both IVF and non-IVF pregnancies.

For comparisons between IVF children and the general population, odds ratios (OR) were obtained using the Mantel-Haenszel

procedure. When specified, stratification was done for the year of birth (one-year interval), maternal age at delivery (classes: <20, 20-24, 25-29, and one-year intervals up to 45 years of age), parity (number of previous children +1), maternal smoking at the first antenatal visit (not known, none, smoking <10 cigarettes per day, smoking ≥10 cigarettes per day), maternal body mass index (BMI) at the first antenatal visit (not known, <20 kg/m^2, 20-24.9 kg/m^2, 25-29.9 kg/m^2, ≥30 kg/m^2), and number of years of involuntary childlessness before the pregnancy (0, 1-2, 3-4, 5 years or more). Approximate 95% confidence intervals (CI) were calculated using the method proposed by Miettinen (Miettinen, 1974).

Comparison between singletons from cryopreserved SET/DET and singletons from fresh SET/DET was performed using logistic regression analysis. When specified, adjustments were made for maternal age (years), primiparity (yes/no), maternal smoking (semicontinuous variable 1=no smoking, 2=smoking <10 cigarettes per day, 3=10 cigarettes per day or more), involuntary childlessness (years, continuous variable), and maternal BMI (continuous variable).

Paper IV

Neonatal and maternal outcomes after comparing women undergoing two in vitro fertilization (IVF) singleton pregnancies and women undergoing one IVF twin pregnancy

Outcome measures were: PTB (<37 w) and very PTB (<32 w), extreme PTB (<28 w), LBW, VLBW. Other outcome measures were: SGA, perinatal mortality (stillbirths after 28 completed gestational weeks and neonatal deaths the first 7 days) and Apgar score (Apgar score <7 at 5 minutes). A further outcome was severe neonatal morbidity including: intraventricular haemorrhage grade ≥3 (ICD-10 code P52.2), periventricular leucomalacia (ICD-10 code P91.2), septicaemia (ICD-10 code P36.9), necrotizing enterocolitis (ICD-10 code P77.9), retinopathy of prematurity (ICD-10 code H35.1), bronchopulmonary dysplasia (ICD-10 code P27.1), jaundice (ICD-10 code P59.0-9) and respiratory diagnoses (include infants treated with continuous positive airway pressure or mechanical ventilation), relatively severe malformations (ICD-10 code beginning with a Q, excluding infants with one or more of the following conditions: preauricular appendix, undescended testicle, tongue tie, hip (sub)luxation or unstable hip, patent ductus arteriosus in a preterm infant, single umbilical artery, and nevus), meconium aspiration (ICD-10 code P24.0), convulsions (ICD-10 code P90.9), hypoxic-ischemic encephalopathy ≥grade 3 (ICD-10 code P91.6C). In addition, a composite outcome was created including the most severe neonatal diagnoses (intraventricular haemorrhage ≥grade 3, periventricular leucomalacia, necrotizing enterocolitis, retinopathy of prematurity ≥stage 3, bronchopulmonary dysplasia, hypoxic-ischemic encephalopathy ≥grade 3, infant mortality, including stillbirth after 28 completed gestational weeks or death during the first year). Such severe neonatal complications occur relatively infrequently and the composite outcome helps to capture a group of children having at least one of the described severe complications.

Maternal outcomes included: preeclampsia, gestational diabetes, placenta praevia, placental abruption, PPROM and the rate of Cesarean section.

Selected maternal and neonatal outcomes in IVF twin pregnancies after DET versus two IVF singleton pregnancies were calculated per woman and/or per infant when appropriate. The outcomes for the children were calculated per child in both groups. Maternal outcomes for the twin group were calculated per twin mother. For the singleton group, maternal outcomes were calculated for mothers having the relevant complication in at least one of her two pregnancies. This rate was used for comparison. In addition, mothers having the relevant complication in both her pregnancies are presented. The maternal and neonatal outcome measures were analyzed with univariate and multiple logistic regression analyses, using Generalized Estimating Equation technology (GEE) to obtain robust variance estimation and compensate that one woman gave birth to two IVF singletons (dependence variables). GEE analysis is generally used in large epidemiological cohort studies because it can handle many types of unmeasured dependence between outcomes.

If not otherwise stated, adjustments were made for year of birth (continuous variable), maternal age at delivery (continuous), nulliparity (yes/no), maternal smoking at the first antenatal visit (semi-continuous variable: 1=none, 2=smoking <10 cigarettes per day, 3=smoking ≥10 cigarettes per day), maternal body mass index (BMI, kg/m^2) at the first antenatal visit (continuous variable). In cases of missing information on smoking or maternal BMI, imputations were made so that the missing values were replaced by the overall mean among records with valid information, 24.6 for BMI, and 1.03 for the semi-continuous smoking scale, respectively. For the other background characteristic, there was no loss of information.

Results and comments

Paper I

Obstetric outcome after *in vitro* fertilization with single or double embryo transfer

All IVF children born after eSET, non-eSET, DET were included and compared with children in the general population.

In total, and including fresh and frozen transfers with own oocytes, 7763 children were born after SET and 5724 children after DET. The multiple birth rates were 1.2% for SET and 21.2% for DET.

All IVF children

Descriptive statistics for all IVF children are given in Table 8.

Comparing all IVF children (SET, DET, fresh and frozen transfers, own and donated oocytes, singletons and multiples) with all non-IVF children in the general population, significantly higher rates were found in the IVF children for all main outcome measures (AOR 1.7-1.9).

Comparing all SET children (eSET, non-eSET, fresh and frozen transfers, own oocytes, singletons and multiples, n=7763) with all children in the general population (singletons and multiples), significantly higher rate of extreme PTB <28w was found for the IVF children (AOR 1.45, 95% CI 1.04-2.03) (Table 9).

Table 8. Perinatal outcome in all children born after IVF in Sweden 2002-2006.

	Fresh transfers with own oocytes			All transfers with donated oocytes	Fresh and frozen transfers with own oocytes			
	eSET	non-eSET	DET		All SET (eSET and non-eSET)	DET	All IVF	All children in the general population
Perinatal outcome	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Total infants, *n*	4304	1887	4510	57	7763	5724	13 544	587 009
Singletons	4187 (97.3)	1860 (98.6)	2897 (64.2)	55 (96.5)	7580 (97.6)	3712 (64.8)	11 347 (83.8)	571 914 (97.4)
Multiples	117 (2.7)	27 (1.4)	1613 (35.8)	2 (3.5)	183 (2.4)	2012 (35.2)	2197 (16.2)	15 095 (2.6)
<28w	22 (0.5)	16 (0.8)	39 (0.9)	1 (1.8)	50 (0.6)	51 (0.9)	102 (0.8)	1680 (0.3)
<32w	67 (1.6)	34 (1.8)	165 (3.7)	5 (8.8)	126 (1.6)	201 (3.5)	332 (2.5)	5450 (0.9)
<37w	378 (8.8)	153 (8.1)	917 (20.3)	10 (17.5)	646 (8.3)	1150 (20.1)	1806 (13.3)	35 325 (6.0)
<1500g	61 (1.4)	29 (1.5)	136 (3.0)	3 (5.3)	114 (1.5)	161 (2.8)	278 (2.1)	4426 (0.8)
<2500g	281 (6.5)	122 (6.5)	818 (18.1)	7 (12.3)	468 (6.0)	988 (17.3)	1463 (10.8)	24 398 (4.2)
SGA	172 (4.0)	76 (4.0)	344 (7.6)	3 (5.3)	287 (3.7)	416 (7.3)	706 (5.2)	15 854 (2.7)
Apgar 5'<7	53 (1.2)	34 (1.8)	97 (2.2)	1 (1.8)	116 (1.5)	128 (2.2)	245 (1.8)	7022 (1.2)
Peri/neonatal mortality	5 (0.1)	7 (0.4)	22 (0.5)	1 (1.8)	21 (0.3)	31 (0.5)	53 (0.4)	1320 (0.2)
Cesarean section	1025 (23.8)	522 (27.7)	1786 (39.6)	26 (45.6)	1966 (25.3)	2 256 (39.4)	4248 (31.4)	100 650 (17.1)

Table 9. Perinatal outcome (AOR) for all IVF singletons and multiples and all SET singletons and multiples versus all singletons and multiples in the general population.

Outcome	All IVF (fresh and frozen transfers, own and donated oocytes, SET and DET) singletons and multiples versus singletons and mutiples in the general population Adjusted OR (95% CI)	All SET (fresh and frozen transfers, own and donated oocytes, e-SET and non-eSET) singletons and multiples versus singletons and multiples in the general population Adjusted OR (95% CI)
<28 w	**1.69 (1.34-2.14)**	**1.45 (1.04-2.03)**
<32w	**1.70 (1.50-1.94)**	1.13 (0.93-1.38)
<37 w	**1.80 (1.70-1.90)**	1.06 (0.97-1.16)
<1500g	**1.72 (1.49-1.99)**	1.23 (0.99-1.51)
<2500g	**1.91 (1.79-2.04)**	0.87 (0.79-0.97)
SGA	**1.43 (1.30-1.56)**	0.98 (0.86-1.12)
Apgar 5`<7	1.14 (0.98-1.32)	0.96 (0.78-1.18)
Peri/neonatal mortality	**1.56 (1.12-2.16)**	1.11 (0.68-1.81)

eSET elective single embryo transfer, SET single embryo transfer, DET double embryo transfer, OR odds ratio, CI confidence interval
Adjusted ORs were obtained after stratification for year of birth, maternal age, parity, smoking, BMI, and years of involuntary childlessness

Singletons

Descriptive statistics for singletons are presented in Table 10.

The rates of LBW and VLBW for singletons after all SET (fresh and frozen transfers with own oocytes) were 5.0% and 1.2%, and for singletons after all DET (fresh and frozen transfers with own oocytes) 5.2% and 1.3%. Separated into eSET, non-eSET and DET singletons (only fresh transfers with own oocytes) the rates of LBW and VLBW for singletons were 5.2% and 1.1%, 5.8% and 1.3%, 5.3% and 1.3%, respectively. The rates of PTB (<37 w) and very PTB (<32 w) for all SET and DET singletons (fresh and frozen transfers with own oocytes) were 7.2% and 1.3% versus 7.5% and 1.5%. Separated for eSET, non-eSET and DET (only fresh transfers with own oocytes) the rates were 7.5% and 1.2%, 7.3% and 1.6%, 7.5% and 1.4%, respectively (Table 10).

Comparing all IVF singletons (SET, DET, fresh and frozen transfers, own and donated oocytes, n=11 347) with singletons in the general population, significantly higher rates were found of PTB <28 w, <37 w, LBW and VLBW (AOR between 1.1-1.7) (Table 11).

When comparisons were made separately for eSET, non-eSET and DET singletons (only fresh transfers with own oocytes) versus singletons in the general population, most significance disappeared except for eSET singletons for <37 w (AOR 1.15, 95% CI 1.02-1.30) and non-eSET singletons for <28 w and <2500g (AOR 2.56, 95% CI 1.47-4.46, AOR 1.26, 95% CI 1.02-1.55) (Table 11).

Table 10. Perinatal outcome in singletons born after IVF in Sweden 2002-2006.

Outcome in singletons	eSET *n* (%)	Non-eSET *n* (%)	DET *n* (%)	Donated oocytes *n* (%)	All SET *n* (%)	All DET *n* (%)	All IVF *n* (%)	Ref *n* (%)
Total infants, *n*	4187	1860	2897	55	7580	3712	11 347	571 914
<28w	16 (0.4)	16 (0.9)	16 (0.6)	1 (1.8)	42 (0.6)	21 (0.6)	64 (0.6)	1291 (0.2)
<32w	49 (1.2)	30 (1.6)	41 (1.4)	3 (5.5)	99 (1.3)	54 (1.5)	156 (1.4)	4122 (0.7)
<37w	312 (7.5)	136 (7.3)	218 (7.5)	8 (14.5)	545 (7.2)	279 (7.5)	832 (7.3)	28 643 (5.0)
<1500g	44 (1.1)	25 (1.3)	38 (1.3)	3 (5.5)	88 (1.2)	47 (1.3)	138 (1.2)	3358 (0.6)
<2500g	219 (5.2)	108 (5.8)	153 (5.3)	6 (10.9)	379 (5.0)	192 (5.2)	577 (5.1)	18 249 (3.2)

Table 11. Perinatal outcome in IVF singletons versus singletons in the general population.

Outcome in singletons	eSET Adj. OR (95% CI)	Non-eSET Adj. OR (95% CI)	DET Adj. OR (95% CI)	All IVF singletons Adj. OR (95% CI)
<28 w	1.20 (0.72-2.02)	**2.56 (1.47-4.46)**	1.34 (0.80-2.24)	**1.67 (1.24-2.22)**
<32 w	1.03 (0.76-1.39)	1.35 (0.91-2.00)	1.06 (0.76-1.48)	1.19 (0.99-1.43)
<37 w	**1.15 (1.02-1.30)**	1.12 (0.93-1.35)	1.12 (0.96-1.30)	**1.15 (1.06-1.25)**
<1500g	1.07 (0.78-1.47)	1.33 (0.87-2.03)	1.18 (0.83-1.68)	**1.25 (1.03-1.52)**
<2500g	1.13 (0.98-1.32)	**1.26 (1.02-1.55)**	1.07 (0.90-1-29)	**1.13 (1.02-1.25)**

eSET elective single embryo transfer, SET single embryo transfer, DET double embryo transfer, OR odds ratio, CI confidence interval
Adjusted ORs were obtained after stratification for year of birth, maternal age, parity, smoking, BMI, and years of involuntary childlessness

Maternal outcome

Mothers of IVF children were older (44.1% over 35 years old) than mothers in the general population (19.6% over 35 years old), 3/4 of IVF mothers were primiparous and smoking was less common in IVF mothers. No significant difference in BMI was found between groups.

We did not find any increased risk of preeclampsia, PPROM or gestational diabetes in singleton IVF pregnancies (fresh and frozen) as compared with singleton pregnancies in the general population.

Placenta previa occurred more frequently among singleton pregnancies after both SET and DET, and placental abruption occurred more frequently among SET singleton pregnancies as compared with singleton pregnancies in the general population (Table 12).

Comments

Children born after IVF had poorer obstetric outcomes than children from the general population. Singletons, irrespective of whether born after eSET, non-eSET or DET also had poorer obstetric outcomes with higher rates of PTB and LBW than singletons in the general population. More placental complications were observed in singletons after both SET and DET.

Paper II

Factors affecting obstetric outcome of singletons born after IVF

In total, 8941 singletons were born after fresh transfer with the couples' own gametes; 4194 infants after eSET, 1860 infants after non-eSET, 2715 infants after DET one sac and 175 infants after DET with vanishing twin. Obstetric outcomes for the four groups are described in Table 13.

Mean birth weight differed significantly between groups, with the lowest birth weight for singletons from "vanishing twin" pregnancies.

Factors investigated for possible association with the four main outcomes (PTB <32w, SGA, placenta previa, placental abruption) are presented in Figure 8.

Table 12. Placental complications in singleton pregnancies after SET and DET (fresh and frozen transfers, own oocytes) versus singleton pregnancies in the general population in Sweden 2002-2006.

Outcome	Singleton pregnancies after SET *n* (%)	Singleton pregnancies after DET *n* (%)	Non IVF singleton pregnancies in the general population *n* (%)	Singleton pregnancies after SET Adjusted OR (95% CI)	Singleton pregnancies after DET Adjusted OR (95% CI)
Placenta previa	117 (1.5)	50 (1.3)	1579 (0.3)	**3.38 (2.74-4.16)**	**2.40 (1.74-3.31)**
Abruptio placentae	65 (0.9)	22 (0.6)	2185 (0.4)	**1.88 (1.43-2.48)**	1.04 (0.66-1.63)

SET single embryo transfer, DET double embryo transfer

Table 13. Obstetric outcome in singletons born after IVF in Sweden 2002-2006. Fresh transfers with own oocytes.

Total number of pregnancies or infants	eSET n=4191 *n* (%)	non-eSET n=1860 *n* (%)	DET one sac n=2715 *n* (%)	DET with vanishing twin n=175 *n* (%)	Total n=8941 *n* (%)	*p-value
Gestational age	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	
Mean (SD)	39.4 (2.1)	39.4 (2.2)	39.5 (2.1)	39.2 (2.8)	39.4 (2.1)	0.34
<37 weeks	322 (7.7)	141 (7.6)	219 (8.1)	15 (8.6)	697 (7.8)	
<32 weeks	53 (1.3)	30 (1.6)	38 (1.4)	6 (3.4)	127 (1.4)	
<28 weeks	17 (0.4)	16 (0.9)	13 (0.5)	3 (1.7)	49 (0.5)	
Birth weight						
Mean (SD)	3427 (601)	3426 (619)	3460 (609)	3360 (619)	3435 (607)	0.03
<2500g	220 (5.2)	108 (5.8)	140 (5.2)	10 (5.7)	478 (5.3)	
<1500g	44 (1.0)	25 (1.3)	32 (1.2)	5 (2.9)	106 (1.2)	
z-scores, Mean (SD)	-0.11 (1.11)	-0.08 (1.11)	-0.03 (1.12)	-0.11 (1.01)	-0.08 (1.11)	0.04
SGA	136 (3.2)	63 (3.4)	80 (2.9)	4 (2.3)	283 (3.2)	
Perinatal mortality	9 (0.2)	6 (0.3)	16 (0.6)	2 (1.1)	33 (0.4)	
Maternal outcomes						
Preeclampsia	169 (4.0)	94 (5.1)	128 (4.7)	6 (3.4)	397 (4.4)	
Gestational diabetes	58 (1.4)	30 (1.6)	36 (1.3)	2 (1.1)	126 (1.4)	
Placenta praevia	76 (1.8)	30 (1.6)	44 (1.6)	1 (0.6)	151 (1.7)	
Placental abruption	35 (0.8)	22 (1.2)	13 (0.5)	3 (1.7)	73 (0.8)	
PPROM	98 (2.3)	44 (2.4)	75 (2.8)	2 (1.1)	219 (2.4)	

Data is presented as numbers (%) or mean (SD), z-scores-mean standard deviation scores
*p-value assessed by Non-Parametric Test (Kruskal-Wallis)
eSET elective single embryo transfer, SET single embryo transfer, DET double embryo transfer, SD standard deviation, SGA small for gestational age, PPROM preterm premature rupture of the membranes

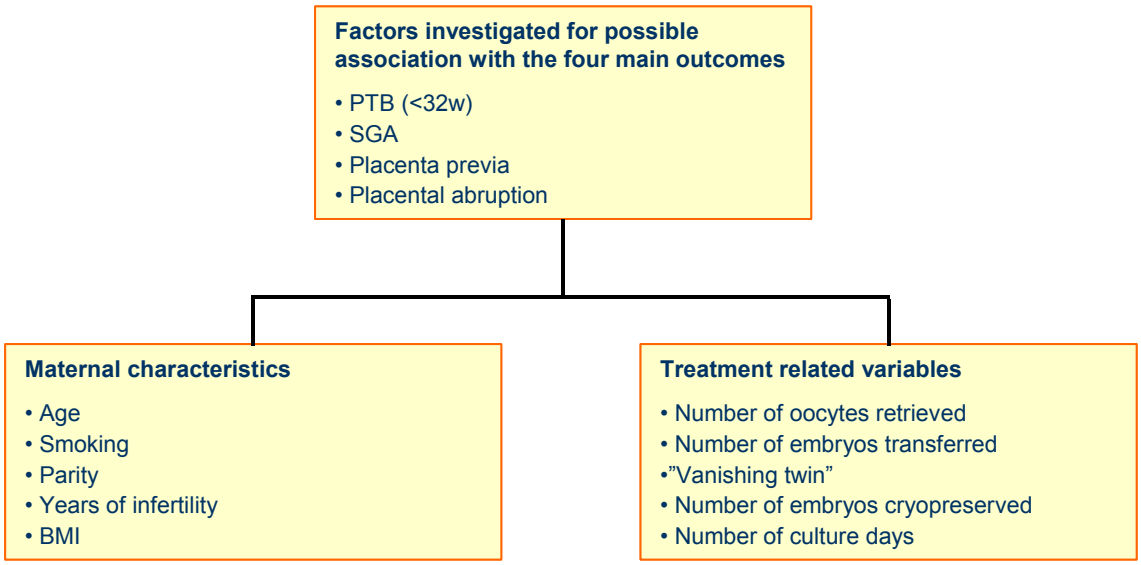

Figure 8. Factors investigated for possible association with the four main outcomes.

Very preterm birth, less than 32 weeks of gestation

Primiparity (AOR 2.52, 95% CI 1.51-4.20), maternal smoking (AOR 1.71, 95% CI 1.02-2.85), BMI (AOR 1.05, 95% CI 1.01-1.10) and "vanishing twin" (2.47, 95% CI 1.03-5.96) (with eSET as reference) were independently associated with very PTB (<32 weeks) (Table 14). No association was found between non-eSET and DET one sac (with eSET as reference) and very PTB (<32 weeks). No significant association was found for blastocyst transfer, number of eggs retrieved or number of embryos cryopreserved.

Small for gestational age (SGA)

Maternal age (AOR1.04, 95% CI 1.01-1.07), primiparity (AOR 1.99, 95% CI 1.43-2.75), years of infertility (AOR 1.07, 95% CI 1.02-1.13) and maternal smoking (AOR 1.57, 95% CI 1.06-2.34) were positively associated with SGA, while a *U*-shaped association was found between BMI and SGA (p=0.0233) (Table14). No association was found for number of oocytes retrieved, number of embryos transferred, number of embryos cryopreserved or blastocyst transfer.

Placenta previa

Maternal age (AOR 1.06, 95% CI 1.01-1.11), and blastocyst transfer (AOR 2.23, 95% CI 1.51-3.29) were positively associated, while primiparity was negatively associated (AOR 0.51, 95% CI 0.36-0.72) with placenta previa (Table 15).

Placental abruption

Smoking was the only variable significantly associated with placental abruption (AOR 1.94, 95% CI 1.02-3.67) (Table 15).

Table 14. Predictive variables for preterm birth (<32w) and SGA. Fresh IVF treatments, singletons only.

	32 w		SGA	
	AOR [c]	95% CI	AOR [c]	95% CI
Factors related to maternal characteristics				
Maternal age (years)			**1.04**	**1.01-1.07**
Linear term	0.66	Simultaneous		
Quadratic term	1.01	p-value: 0.167		
Primiparity versus multiparity	**2.52**	**1.51-4.2**	**1.99**	**1.43-2.75**
Maternal smoking [a]	**1.71**	**1.02-2.85**	**1.57**	**1.06-2.34**
Maternal BMI (kg/m^2)[b]	**1.05**	**1.01-1.1**		
Linear term			0.77	Simultaneous
Quadratic			1.005	p-value 0.0223
Years of involuntary childlessness[b]			**1.07**	**1.02-1.13**
Factors related to IVF treatment				
Non-eSET vs eSET	1.25	0.80-1.97	1.01	0.75-1.38
DET one sac vs eSET	1.04	0.67-1.62	0.82	0.61-1.11
DET with vanishing twin vs eSET	**2.47**	**1.03-5.96**	0.58	0.21-1.60
Blastocyst vs non-blastocyst	1.26	0.75-2.11	0.82	0.54-1.23

[a] Quasi-continuous variable: 1=no smoking, 2=smoking 1-9 cigarettes per day, 3=smoking ≥10 cigarettes per day
[b] Continuous variable
[c] Results from one multiple logistic regression analysis including all variables listed in the column. The multivariable model included variables with p<0,2 in the multiple model
OR=odds ratio AOR=adjusted odds ratio

Table 15. Predictive variables for placenta previa and placental abruption. Fresh IVF treatments, singleton pregnancies only.

	Placenta previa		Placental abruption	
	AOR [c]	95% CI	AOR [c]	95% CI
Factors related to maternal characteristics				
Maternal age (years)	**1.06**	**1.01-1.11**		
Primiparity versus multiparity	**0.51**	**0.36-0.72**		
Maternal smoking [a]			**1.94**	**1.02-3.67**
Maternal BMI(kg/m^2)[b]	0.96	0.92-1.01		
Years of involuntary childlessness [b]	1.05	0.98-1.14		
Factors related to IVF treatment				
Non-eSET vs eSET	0.85	0.55-1.30	1.40	0.82-2.40
DET one sac vs eSET	0.78	0.52-1.15	0.57	0.30-1.09
DET with vanishing twin vs eSET	0.27	0.04-1.97	2.04	0.62-6.70
Blastocyst vs non-blastocyst	**2.23**	**1.51-3.29**	1.31	0.68-2.40

[a] Quasi-continuous variable: 1=no smoking, 2=smoking 1-9 cigarettes per day, 3=smoking ≥10 cigarettes per day
[b] Continuous variable
[c] Results from one multiple logistic regression analysis including all variables listed in the column. The multivariable model included variables with p<0,2 in the multiple model
OR odds ratio AOR adjusted odds ratio

Comments

Certain maternal characteristics and number of transferred embryos (”vanishing twin”) affected obstetric outcome negatively. An increased rate of placenta previa was observed after blastocyst transfer. No association was found for other IVF related techniques.

We did not find that hormonal stimulation, as reflected in the number of retrieved oocytes, influenced the obstetric outcomes. A negative effect of the hormonal stimulation during IVF treatment on the obstetric outcome has been suggested to explain the poorer outcome in IVF singletons compared with singletons in the general population, particularly as singletons from cryopreserved embryos and without further hormonal stimulation have been observed as having better outcomes (Wennerholm *et al*., 2009). One might assume that if a correlation exists between hormonal stimulation and adverse outcome it is probably not the gonadotrophin dose *per se* but the effect of high estradiol levels, caused by the gonadotrophin stimulation, that may have negative effects on, for example, the endometrium. There is good correlation between estradiol levels and the number of retrieved oocytes. This explains why we included the number of oocytes in the analysis and used it as a proxy for hormonal stimulation. When considering the lack of a negative effect of hormonal stimulation found in this study it should be noted that no patient underwent IVF without hormonal stimulation, and thus IVF without hormonal stimulation could not be investigated. In recent large study from Japan (Nakashima *et al*., 2013) singletons from stimulated cycles had a significanly higher rates of LBW than singletons from natural cycles.

The results of our study support single embryo transfer in order to minimize the rate of ”vanishing twin”. The study also suggests that the lifestyle factors such as BMI and smoking are of importance. The finding of an increase rate of placenta previa after blastocyst transfer needs further investigation.

Paper III

Obstetric outcome in singletons after *in vitro* fertilization with cryopreserved/ thawed embryos

Perinatal outcomes

Maternal characteristics are described in Table 16.

In total, 2348 singleton children (1533 SET and 815 DET) born after cryopreserved IVF/ICSI cycles were compared with 8944 singletons (6047 SET and 2897 DET) from fresh IVF/ICSI cycles and 571 914 singletons from the general population. Perinatal and maternal outcomes are described in Table 17.

Table16. Maternal characteristics of singletons born after cryopreserved/thawed SET/DET and fresh SET/DET in Sweden 2002-2006

	Cryo SET/DET *n*=2348 *n* (%)	Fresh SET/DET *n*=8944 *n* (%)	General population *n*=571 914 *n* (%)
Age >35 years	1096 (46.7)	3845 (43.0)	111 367 (19.5)
Nulliparous	1323(56.3)	6475 (72.4)	255 449 (44.7)
BMI >30	307 (15.2)	1115 (14.2)	69 794 (14.2)
Smoking	77 (3.6)	293 (3.6)	46 299 (8.8)
Involuntary childlessness >5 years	465 (28.4)	1572 (23.7)	3805 (0.7)

% refer to women with known BMI, smoking and involuntary childlessness status

Table 17. Perinatal and maternal outcomes in singletons born after cryopreserved/thawed SET, cryopreserved/thawed DET, fresh SET and fresh DET in Sweden 2002-2006 (donated oocytes are excluded).

	Cryo SET *n* (%)	Cryo DET *n* (%)	Fresh SET *n* (%)	Fresh DET *n* (%)	References (all non-IVF singletons in the general population) *n* (%)
Perinatal outcome					
Total infants, *n*	1533	815	6047	2897	571 914
<28w	10 (0.7)	5 (0.6)	32 (0.5)	16 (0.6)	1291 (0.2)
<32w	20 (1.3)	13 (1.6)	79 (1.3)	41 (1.4)	4122 (0.7)
<37w	97 (6.3)	61 (7.5)	448 (7.4)	218 (7.5)	28 643 (5.0)
<1500g	19 (1.2)	9 (1.1)	69 (1.1)	38 (1.3)	3358 (0.6)
<2500g	52 (3.4)	39 (4.8)	327 (5.4)	153 (5.3)	18 249 (3.2)
>4500g	69 (4.5)	35 (4.3)	174 (2.9)	97 (3.3)	21 840 (3.8)
>5500 g	0 (0.0)	1 (0.1)	2 (0.0)	1 (0.0)	268 (0.0)
SGA	33 (2.2)	28 (3.4)	216 (3.6)	88 (3.0)	13 624 (2.4)
LGA (+2 SD)	81 (5.3)	43 (5.3)	173 (2.9)	98 (3.4)	21 505 (3.8)
LGA (+3SD)	9 (0.6)	12 (1.5)	35 (0.6)	22 (0.8)	4063 (0.7)
Apgar 5'<7	26 (1.7)	18 (2.2)	81 (1.3)	48 (1.7)	6529 (1.1)
Perinatal mortality	10 (0.7)	5 (0.6)	15 (0.2)	18 (0.6)	2464 (0.4)
Composit, any	124 (8.1)	79 (9.7)	624 (10.3)	290 (10.0)	40 777 (7.1)
Composit, serious	27 (1.8)	15 (1.8)	99 (1.6)	56 (1.9)	6364 (1.1)
Cesarean section	399 (26.0)	233 (28.6)	1462 (24.2)	818 (28.2)	92 676 (16.2)
Maternal outcome					
Preeclampsia	74 (4.8)	51 (6.3)	262 (4.3)	133 (4.6)	15 984 (2.8)
Gestational diabetes	14 (0.9)	21 (2.6)	88 (1.5)	38 (1.3)	5412 (0.9)
Placenta previa	11 (0.7)	4 (0.5)	106 (1.8)	46 (1.6)	1579 (0.3)
Placental abruption	8 (0.5)	5 (0.6)	57 (0.9)	17 (0.6)	2185 (0.4)
PPROM	31 (2.0)	18 (2.2)	141 (2.3)	77 (2.7)	8747(1.5)

SET single embryo transfer, DET double embryo transfer, SGA small for gestational age, LGA large for gestational age, Composite, any: <37 weeks, <2500 g, SGA or perinatal mortality, Composite, serious: <32 weeks, <1500 g or perinatal mortality, PPROM preterm premature rupture of the membranes

Singletons from cryopreserved SET/DET versus singletons from the general population

When comparing singletons from cryopreserved SET/DET cycles with singletons from the general population (Table 18) significantly higher rates of extreme preterm birth (<28 w) (AOR 1.92, 95% CI 1.12-3.93), macrosomia (>4500g) (AOR 1.29, 95% CI 1.04-1.59) and LGA (AOR 1.48, 95% CI 1.22-1.81) were found for singletons from cryopreserved cycles. The rate of Cesarean section was also significantly higher in cryopreserved cycles. For other outcomes (<32 w, <37 w, <1500g, Apgar score <7[5], composite outcomes), significantly increased crude ORs were noted, but they disappeared after adjustment for confounders.

Singletons from cryopreserved SET with DET excluded versus singletons from the general population

When comparing singletons from cryopreserved SET cycles versus singletons from the general population (Table 18), significantly increased rates of extreme preterm birth (<28 w) (AOR 1.99, 95% CI 1.04-3.81), macrosomia (>4500g) (AOR 1.35, 95% CI 1.05-1.74) and LGA (greater than +2 SD) (AOR 1.51, 95% CI 1.19-1.92) were found for children from cryopreserved cycles. For other outcomes (<32 w, <37 w, <1500g, Apgar score <7[5], Cesarean section, serious composite outcome), significantly increased crude ORs were noted but disappeared after adjustment for confounders.

Singletons from cryopreserved SET/DET versus singletons from fresh SET/DET

When comparing singletons from cryopreserved SET/DET cycles with singletons from fresh SET/DET cycles, significantly lower rates of low birth weight (<2500 g) (AOR 0.76, 95% CI 0.60-0.95) were found (Table 18) for the cryopreserved cycles. Significantly higher rates of macrosomia (>4500g) (AOR 1.46, 95% CI 1.15-1.85), LGA (AOR 1.59, 95% CI 1.26-1.99), low Apgar score (AOR 1.42, 95% CI 1.00-2.01) and perinatal mortality (AOR 1.90, 95% CI 1.03-3.54) were observed for infants from cryopreserved cycles. For other outcomes (<28w, SGA), significantly lower crude ORs were noted but disappeared after adjustment for confounders. When the two composite outcomes, (<37w, <2500g, SGA or perinatal mortality) and (<32 w, <1500g or perinatal mortality), respectively, were analyzed, no significant differences were found between singletons from cryopreserved versus fresh cycles.

Table 18. Singletons from cryopreserved SET/DET and from cryopreserved SET (DET excluded) versus singletons from the general population and singletons from fresh SET/DET.

	Singletons from cryopreserved SET/DET	Singletons from cryopreserved SET (DET excluded)	Singletons from cryopreserved SET/DET
	Singletons in the general population as the reference group		Singletons from fresh SET/DET as the reference group
	AOR 95% CI	AOR 95% CI	AOR 95% CI
<28 w	**1.92 (1.12-3.93)**	**1.99 (1.04-3.81)**	
LBW <2500g			**0.76 (0.60-0.95)**
Macrosomia (>4500g)	**1.29 (1.04-1.59)**	**1.35 (1.05-1.74)**	**1.46 (1.15- 1.85)**
LGA (>2SD)	**1.48 (1.22-1.81)**	**1.51 (1.19-1.92)**	**1.59 (1.26-1.99)**
Perinatal mortality			**1.90 (1.03-3.54)**

We also compared the rate of LGA (+3SD) and birth weight >5500g between singletons from cryopreserved and singletons from fresh cycles and singletons from the general population. No significant differences in these rates were found.

Maternal outcomes

Singleton pregnancies from cryopreserved SET/DET or cryopreserved SET cycles versus singleton pregnancies from the general population

Mothers of IVF singletons (both after cryopreservation/thawing and fresh cycles) were older than mothers in the general population, most of the IVF mothers were primiparous (56.3% for singletons after cryopreservation/thawing and 72.4% for IVF singletons after fresh cycles), as compared with 44.7% primiparous mothers in the general population, and smoking was less common for IVF mothers. No significant difference in BMI was found between groups (Table 16).

When comparing maternal outcomes for singleton pregnancies from cryopreserved SET/DET cycles with singleton pregnancies from the general population a significantly higher rate of preeclampsia was noted (AOR 1.25, 95% CI 1.03-1.51). For other outcomes (gestational diabetes placenta previa and PPROM), significantly increased crude ORs were noted, but they disappeared after adjustment for confounders.

When comparing singleton pregnancies from cryopreserved SET cycles with singleton pregnancies from the general population significantly increased crude ORs were noted for preeclampsia and placenta previa, but they disappeared after adjustment for confounders.

Singleton pregnancies from cryopreserved SET/DET versus fresh SET/DET cycles

When comparing singleton pregnancies from cryopreserved SET/DET versus fresh SET/DET cycles significantly higher rates of preeclampsia were noted (AOR 1.32, 95% CI 1.07-1.63) while significantly lower rates of placenta previa were found for pregnancies from cryopreserved cycles (AOR 0.32, 95% CI 0.19-0.55) (Table 19).

Table 19. Maternal complications in singleton pregnancies from cryopreserved SET/DET versus singletons in the general population and singletons from fresh SET/DET cycles.

	Cryopreserved SET/DET Singleton pregnancies in the general population as the reference group AOR (95% CI)	Cryopreserved SET/DET Singleton pregnancies from fresh SET/DET as the reference group AOR (95% CI)
Preeclampsia	**1.25 (1.03-1.51)**	**1.32 (1.07-1.63)**
Placenta previa		**0.32 (0.19-0.55)**

Comments

This large registry study on perinatal and maternal outcomes in singleton pregnancies after cryopreservation in comparison with pregnancies from the general population showed that infants after cryopreservation cycles had a higher rate of extreme preterm birth, while for most other outcomes the results were similar. In comparison with singletons from fresh cycles, singletons from cryopreservation cycles showed a lower rate of LBW but a higher rate of low Apgar score and perinatal mortality. Singletons from cryopreservation cycles also had higher rates of LGA and macrosomia than both singletons from the general population and singletons from fresh cycles. A higher rate of preeclampsia was noted for the pregnancies after cryopreservation cycles, while the rate of placenta previa was lower than in pregnancies from fresh cycles. Although most of the results are reassuring, the finding of higher rates of LGA, macrosomia and perinatal mortality could indicate increased risks for children born after cryopreservation and emphasizes the need for further follow-up of children born after different ART techniques.

Paper IV

Neonatal and maternal outcomes comparing women undergoing two in vitro fertilization (IVF) singleton pregnancies and women undergoing one IVF twin pregnancy

Mothers of IVF twins were older (46.2% >35 years) than mothers of IVF singletons (29.4% >35 years). The mothers of IVF twins were multiparous in 35.4% of cases as compared with only 6.5% of mothers of IVF singletons. Smoking, BMI and infertility duration were similarly distributed in both groups. Tables 20-21 demonstrate the neonatal and maternal outcomes in IVF twin pregnancies as compared with two IVF singleton pregnancies per woman or per infant. In total, 991 women with 1982 twins (991 pairs of twins) after DET (frozen and fresh) were compared with 1842 IVF singletons after SET (frozen and fresh) born to the 921 women with two singleton pregnancies.

Neonatal outcomes

Descriptive statistics for neonatal outcomes are presented in Table 20. When comparing neonatal outcomes in IVF twins with IVF singletons, significantly higher rates of PTB (<37w; AOR 12.67, 95% CI 9.62-16.68), very PTB (<32w; AOR 7.43, 95% CI 4.25-12.99), LBW (<2500g; AOR 16.6, 95% CI 12.28-22.42) and VLBW (<1500g; AOR 4.72, 95% CI 2.83-7.88), and SGA (AOR 7.62, 95% CI 5.14-11.29) were found for IVF twins (Table 22). There were no significant differences in incidences of perinatal mortality or Apgar score <7 at 5 minutes.

All neonatal adverse outcomes occurred more frequently in the first than in the second singleton pregnancy (for example, <1500 g 1.8% versus 0.8%, <2500 g 6.4% versus 2.8%, SGA 3.1% versus 1.5%, complete data not shown).

Neonatal morbidity

Significantly higher rates of respiratory complications (AOR 4.92, 95% CI 3.68-6.58), sepsis (AOR 2.31, 95% CI 1.29-4.13) and jaundice (AOR 5.03, 95% CI 3.77-6.70) were detected among the IVF twins. Higher rates of unadjusted composite serious morbidity were found for IVF twins (crude

OR 1.84, 95% CI 1.06-3.17), but after adjustment for maternal confounders (age, parity, BMI, smoking, years of infertility) the difference was no longer significant (AOR 1.34, 95% CI 0.70-2.57) (Table 22).

There were no significant differences in incidences of mortality during the first year or in relatively severe malformations between the two groups.

Maternal outcomes

Maternal outcomes are described in Tables 21. Significantly higher rates of preeclampsia (AOR 2.64, 95% CI 1.81-3.86) and PPROM (AOR 8.43, 95% CI 4.86-14.63) were observed for the IVF twin pregnancies. The rate of Cesarean section was also significantly higher for IVF twin pregnancies (AOR 4.19, 95% CI 3.32-5.29). Placenta praevia was observed less frequently (AOR 0.37, 95% CI 0.17-0.81) in IVF twin pregnancies than in IVF two singleton pregnancies (Table 22).

In a few mothers of singletons the maternal complications occurred in both pregnancies (Table 21). Many maternal adverse outcomes occurred more often in the first singleton pregnancy than in the second (e.g. preeclampsia 5.1% versus 2.3%, complete data not shown).

Comments

The maternal and neonatal outcomes were dramatically better for women with two IVF singleton pregnancies than for those with one IVF twin pregnancy. These results support single embryo transfer to minimize the risks associated with twin pregnancies.

Table 20. Neonatal outcomes in IVF twin pregnancies (after DET) versus two IVF singleton pregnancies. Outcome per infant.

	Twins *n* (%)	Singletons *n* (%)	Siblings[a] *n* (%)
Total, infants	1982	1842	
<37 weeks	925 (46.7)	133 (7.2)	17 (0.9)
<32 weeks	148 (7.5)	23 (1.2)	2 (0.1)
<2500 g	769 (38.8)	85 (4.6)	9 (0.5)
<1500 g	106 (5.3)	24 (1.3)	2 (0.1)
Small for gestational age	246 (12.4)	43 (2.3)	5 (0.3)
Peri/neonatal mortality	23 (1.2)	18 (1.0)	1 (0.1)
Apgar <7[5]	59 (3.0)	30 (1.6)	0 (0.0)
Severe neonatal morbidity			
Relatively severe malformations	97 (4.9)	70 (3.8)	
Respiratory disorders	322 (16.2)	83 (4.5)	
Meconium aspiration	1 (0.1)	4 (0.2)	
Bronchopulmonary dysplasia	12 (0.6)	5 (0.3)	
Intraventricular hemorrhage (≥grade 3)	4 (0.2)	1 (0.1)	
Convulsions	4 (0.2)	6 (0.3)	
Periventricular leukomalacia	4 (0.2)	0 (0.0)	
Hypoxic-ischemic encephalopathy (≥grade 3)	1 (0.1)	1 (0.1)	
Retinopathy of prematurity	13 (0.7)	2 (0.1)	
Retinopathy of prematurity (≥stage 3)	5 (0.3)	0 (0.0)	
Sepsis	45 (2.3)	20 (1.1)	
Necrotizing enterocolitis	3 (0.2)	0 (0.0)	
Jaundice	381 (19.2)	90 (4.9)	
Stillbirth and infant mortality <1 year	27 (1.4)	18 (1.0)	
Composite serious morbidity	45 (2.3)	23 (1.2)	

Values are *n* and (%), DET double embryo transfer
[a]Numbers refer to when complication occurred in both singletons
Composite serious morbidity: Bronchopulmonary dysplasia, intraventricular hemorrhage (≥grade 3), periventricular leukomalacia, hypoxic-ischemic encephalopathy (≥grade 3), retinopathy of prematurity (≥stage 3), necrotizing enterocolitis, stillbirth and infant mortality <1 year

Table 21. Maternal outcomes in IVF twin pregnancies (after DET) versus two IVF singleton pregnancies. Outcome per woman.

	Twins DET *n* (%)	Singleton x2[a] *n* (%)	Singleton x2[b] *n* (%)
Total, women	991	921	
Preeclampsia	135 (13.6)	59 (6.4)	9 (1.0)
Gestational diabetes	13 (1.3)	15 (1.6)	3 (0.3)
Placenta praevia	9 (0.9)	27 (2.9)	2 (0.2)
Placental abruption	14 (1.4)	12 (1.3)	0 (0.0)
PPROM	119 (12.0)	23 (2.5)	1 (0.1)
Cesarean section	571 (60.7)	252 (27.4)	116 (12.6)

[a]Outcome for singleton x 2 group is valid provided that at least one of the two pregnancies had been complicated with respective outcome
[b] Outcome for singleton x 2 group provided that both pregnancies had been complicated with respective outcome
Values are n and (%), DET double embryo transfer, PPROM preterm premature rupture of the membranes

Table 22. Neonatal and maternal outcomes (crude and adjusted ORs) in IVF twin pregnancies after DET versus two IVF singleton pregnancies.

Outcome	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
Neonatal outcomes		
<37 weeks	**11.24 (8.94-14.15)**	**12.67 (9.62- 16.68)**
<32 weeks	**6.38 (3.86-10.55)**	**7.43 (4.25-12.99)**
<2500g	**13.10 (10.09-17.02)**	**16.60 (12.28-22.42)**
<1500g	**4.28 (2.60-7.06)**	**4.72 (2.83-7.88)[b]**
Small for gestational age	**5.93 (4.13-8.51)**	**7.62 (5.14-11.29)**
Perinatal mortality	1.19 (0.62-2.27)	0.91 (0.43-1.92)[b]
Apgar <7[5]	1.30 (0.95-1.79)	1.52 (0.83-2.80)
Severe neonatal morbidity		
Relatively severe malformations	1.30 (0.95-1.79)	1.14 (0.81-1.62)
Respiratory complications	**4.11 (3.13-5.40)**	**4.92 (3.68-6.58)**
Sepsis	**2.12 (1.21-3.72)**	**2.31 (1.29-4.13)**
Jaundice	**4.63 (3.55-6.05)**	**5.03 (3.77-6.70)**
Stillbirth and infant mortality <1 year	1.40 (0.75-2.62)	0.98 (0.46-2.09)
Composite serious morbidity	**1.84 (1.06-3.17)**	1.34 (0.70-2.57)
Maternal outcomes		
Preeclampsia	**2.30 (1.67-3.17)**	**2.64 (1.81-3.86)**
Gestational diabetes	0.80 (0.38-1.70)	NA
Placenta praevia	0.30 (0.14-0.65)	**0.37[c] (0.17-0.81)**
Placental abruption	1.09 (0.50-2.36)	1.30[d] (0.58-2.89)
PPROM	**5.33 (3.38-8.41)**	**8.43 (4.86-14.63)**
Cesarean section	**4.01 (3.30-4.86)**	**4.19 (3.32-5.29)**

[a] Adjustment was performed for age, BMI, parity, smoking and years of infertility if not otherwise stated
[b] Adjusted for parity and age
Composite serious morbidity: Bronchopulmonary dysplasia, intraventricular hemorrhage (≥grade 3), periventricular leukomalacia, hypoxic-ischemic encephalopathy (≥grade 3), retinopathy of prematurity (≥stage 3), necrotizing enterocolitis, stillbirth and infant mortality <1 year
[c] Adjusted for parity and age
[d] Adjusted for parity
PPROM preterm premature rupture of the membranes, NA not available (not calculated due to few events)

General discussion

Increasing demand for ART has contributed to an increasing number of IVF children all over the world, and in many countries 1-4% of all children are born annually after IVF. Rapid development of new techniques in IVF necessitates continuous follow-up of obstetric and neonatal outcomes. The present national population-based study compared obstetric and neonatal outcomes in the complete cohort of IVF children born in Sweden 2002-2006 with a focus on IVF children born after SET, which has been widely introduced in Sweden since 2003. The present study was based on data reported from all IVF clinics in Sweden and data from the MBR. This registry has been found to have high validity and includes virtually all deliveries in Sweden (Cnattingius *et al.,* 1990; National Board of Health and Welfare, 2003).

Neonatal outcomes after IVF

Neonatal outcomes for IVF children versus general population

The results of our study when comparing all IVF children (SET, DET, fresh and frozen transfers, own and donated oocytes, singletons and multiples) with all children in the general population have demonstrated significantly higher rates of all main outcome measures (adjusted odds ratio (AOR) between 1.7 and 1.9) in the IVF children. Previous large registry studies have shown that the increased risks for IVF children are mostly attributable to the high rates of multiple births (Bergh *et al*., 1999; Klemetti *et al*., 2002; Schieve *et al*., 2002; Schieve *et al.,* 2007; Helmerhorst *et al*., 2004; Wang *et al.,* 2005). In the first Swedish cohort of IVF children (Bergh *et al*., 1999) the risks for PTB, VLBW and LBW were five-to-sixfold higher among IVF children, as compared with children in the general population. The multiple birth rate among IVF children in this cohort was 26%.The multiple birth rate in our cohort of IVF children was much lower (8.8%), attributed to the wide prevalence of SET (62% of the IVF pregnancies were pregnancies after SET). This dramatic decrease in multiple birth rates has lowered the risk of PTB, VLBW and LBW considerably as compared with earlier studies.

However, the multiple birth rate is still high internationally and the latest reports demonstrated that the multiple birth rate in Europe and the USA were 22% and 31% respectively, in 2009 (Ferraretti *et al*., 2012; Sunderam *et al.,* 2012). Ninety-five per cent of all multiple births were twins and it is well recognized that maternal and neonatal morbidity and mortality are significantly increased in IVF twin pregnancies as compared with singleton pregnancies (Helmerhorst *et al*., 2004; Ananth *et al*., 2004).

In summary: The obstetric outcome for all IVF children has improved considerably in recent years in Sweden owing to a dramatic lowering of the multiple birth rates after using SET as the main embryo transfer (ET)

strategy.
In many countries, however, a high multiple birth rate still results in huge problems for the children and their families.

Neonatal outcome for IVF singletons versus singletons in the general population
The main findings of the present study (Paper I) was that singletons, irrespective of whether the children were born after eSET, non-eSET or DET, had a poorer obstetric outcome, with higher rates of PTB <37w and LBW <2500g, than singletons in the general population. In the present study, however, we found considerably better outcomes for IVF singletons, measured as rates of PTB and LBW than in previous studies (Bergh *et al*., 1999; Helmerhorst *et al*., 2004; Jackson *et al*., 2004; Schieve *et al*., 1999; Källen *et al*., 2005b). In a recent systematic review and meta-analysis (Pinborg *et al*., 2012), poorer perinatal outcomes (PTB, LBW) with significantly higher risks (AOR respectively between 1.41-2.04 and 1.08-1.72) were found for IVF singletons as compared with singletons in the general population. However, in a recent large registry study from Sweden covering the years 1982-2006, trends towards better neonatal outcomes were observed for all IVF children, as well as for singletons separately (Källen *et al.,* 2010a). In our study of the younger cohort of IVF children a risk of PTB in IVF singletons was still significantly increased but with a lower AOR (1.15). The same tendency in very PTB rates was also observed (Paper I).

In summary: Singletons after IVF have a poorer obstetric outcome than singletons in the general population. However, the obstetric outcome was improved for IVF singletons as compared with previous studies.

Neonatal outcomes for IVF singletons after e-SET and singletons after non-eSET and DET
A working hypothesis before the start of the present study was that outcomes for singletons after SET/eSET might be better than outcomes for singletons after DET and comparable with outcomes for singletons in the general population.

Singletons after eSET, non-eSET and DET showed similar rates of PTB and LBW. When comparing each subgroup with singletons in the general population some significant differences with increased AORs were observed for the IVF singletons. For most variables, however, crude ORs were significantly increased, although the significance disappeared after adjustment for confounders. However, interpretation of these subgroup analyses on eSET, non-eSET and DET singletons should be made with caution since the study was not powered for subgroup analyses. Poorer outcomes may well exist for all these subgroups, similar to the main analysis. For the non-eSET singletons, poorer outcomes were noticed even after adjustment for confounders.

Poorer outcomes for the non-eSET children might reflect a more severe infertility situation, with fewer embryos of good quality. Some of the non-eSET embryos transferred was probably not high-quality embryos. Embryo quality, however, has not been found to influence neonatal outcomes, although only few studies have addressed this question thoroughly (Ebner *et al*., 2001).

Previous studies have reported conflicting results concerning perinatal outcome for singletons after SET and DET. One Belgian study (De Sutter *et al.,* 2006) demonstrated significantly higher rates of PTB and LBW rates among singletons after DET as compared with singletons after SET, while another study from Finland found no differences (Poikkeus *et al.,* 2007). In a large population-based study from Australia (Wang *et al.,* 2009), 13 000 SET singletons were compared with 16 000 DET singletons. Significantly higher rates of preterm birth and LBW were found for singletons after DET. No adjustment for years of infertility was, however, made in that study.

In summary: Singletons after IVF, irrespective of the number of embryos transferred have poorer obstetric outcomes than singletons in the general population.

Factors affecting obstetric outcome of singletons born after IVF

It is obvious that outcome for IVF singletons after eSET/SET is poorer than outcome for singletons in the general population. Maternal characteristics, hormonal stimulation and IVF technique related factors have been discussed as main factors negatively affecting outcomes for IVF singletons. Identifying of such factors is important in order to be able to prevent adverse obstetric outcomes after IVF. The second part of our study analysed which factors (maternal and IVF treatment related) affect obstetric outcomes assessed as very preterm birth, SGA, placenta previa and placental abruption negatively. These specific outcomes were selected since they were regarded as important and robust. Very preterm birth and SGA, reflecting fetal growth, are associated with major neonatal morbidity and mortality and may also have long-term health consequences for the child. It was found that the maternal characteristics (age, smoking, primiparity, BMI and duration of infertility) were significantly associated with a poor obstetric outcome for the children when assessed as one or both of the two selected obstetric outcomes, very preterm birth and SGA. Among treatment variables, DET with "vanishing twin" was associated with very preterm birth. No significant association was observed for other treatment variables such as hormonal stimulation (assessed by the number of oocytes retrieved) or embryo culture time. An increased rate of placenta previa was observed after blastocyst transfer.

Most of the identified maternal variables are well known from earlier studies to be associated with poor obstetric outcome (Bergh *et al.,* 1999; Schieve *et al.,* 2002; Helmerhorst *et al.,* 2004; Jackson *et al.,* 2004; Källen *et al.,* 2005c; Källen *et al.,* 2010a.).

Subfertility per se and duration of infertility
In a recent systematic review and meta-analysis (Pinborg *et al*., 2012), maternal characteristics and treatment related variables were summarized. Current literature suggests that both subfertility *per se* and treatment related variables contribute to a higher risk of PTB among IVF singletons. This conclusion is also supported by "sibling studies" (Romundstad *et al.*, 2008; Henningsen *et al.,* 2011) where obstetric outcome is compared between singletons conceived spontaneously and IVF singletons with the same mother. In the

large Danish study (Henningsen *et al*., 2011), higher PTB rates were found among IVF singletons. One of the strengths of our study was the possibility to adjust for years of infertility. We also found a significant positive association between duration of infertility and SGA in IVF singleton pregnancies. Etiology of infertility was not recorded in the registry and was therefore not adjusted for. In one study from Australia (Wang *et al.,* 1994), a higher incidence of SGA was found for unexplained infertility, and in other studies (Wang *et al.,* 2005; Ombelet *et al.,* 2005), higher risks of PTB and LBW were noted for female factor infertility versus male factor infertility and in IVF, as compared with ICSI (Ombelet *et al.,* 2005). Other studies, however, have not found any association between etiology of infertility and obstetric outcome (Isaksson *et al.,* 2002; Chung *et al.,* 2006). We did not adjust for IVF (mainly female infertility) and ICSI (mainly male infertility), since another Swedish study did not show any difference in obstetric outcome between IVF and ICSI (Källen *et al.,* 2005b). In a recent meta-analysis, however, a lower risk of PTB was detected for ICSI singletons than for IVF singletons (Pinborg *et al.,* 2012).

Controlled ovarian stimulation

In the present study we did not find that hormonal stimulation, as reflected in the number of retrieved oocytes, influenced the obstetric outcomes. A negative effect of the hormonal stimulation during the IVF treatment on the obstetric outcome has been suggested to explain the poorer outcome in IVF singletons as compared with singletons in the general population, particularly since singletons from cryopreserved embryos and without further hormonal stimulation have been observed to have a better outcome (Wennerholm *et al.*, 2009). If there is a correlation between hormonal stimulation and adverse outcome, it is probably not the dose of gonadotrophins *per se* that causes this effect, but more likely the high estradiol levels and their impact on, for instance, the endometrium. The number of oocytes retrieved is therefore a good indicator of that stimulatory effect, which was the reason we included the number of oocytes retrieved in this analysis. In studies from Israel (Abramov *et al.,* 1998) and the US (Chung *et al.,* 2006), including both singleton and multiple pregnancies, OHSS (with higher number of oocytes retrieved compared to non OHSS) was associated with poorer obstetric outcome assessed as preterm delivery and/or low birth weight, even after adjusting for known confounders. However, when subanalysis in the American study (Chung *et al.,* 2006) was performed for singleton pregnancies, the effect of OHSS was no longer statistically significant. A large German register study, investigating the effect of stimulation during IVF did not find any association between the total dose of gonadotropins and birthweight (Griesinger *et al.,* 2008). However, a recent large study from Japan (Nakashima *et al*., 2013) showed that singletons from stimulated cycles had a significanly higher rates of LBW than singletons from natural cycles.

Number of embryo transferred

We found a negative effect on very preterm birth after DET with "vanishing twins". This is in accordance with earlier studies (Pinborg *et al.,* 2007). The number or type of embryos transferred (eSET, non-eSET or DET when resulting in only one sac) did not affect PTB and SGA rates in our study.

Conflicting results have previously been reported concerning obstetric outcome for singletons after SET and DET; some studies showed improved outcome when only one embryo was transferred (De Sutter *et al.,* 2006; Wang *et al.,* 2009), while other found no difference (Poikkeus *et al.,* 2007).

In a large cohort study from Denmark (Pinborg *et al*., 2005), significantly increased risks of PTB, very PTB, LBW and VLBW were shown in IVF singletons with a "vanishing twin" versus singletons with only one sac. In further multiple logistic regression analysis of the same cohort (Pinborg *et al*., 2007); "vanishing twin" was detected as an independent predictor of SGA. Another large registry study from the USA (Luke *et al*., 2009) found significant association between "vanishing twin" with PTB, very PTB, LBW and VLBW. Two smaller studies, one from Austria (Shebl *et al*., 2008) and one from Israel (Almog *et al*., 2010), also detected significant associations between "vanishing twin" and adverse obstetric outcomes in singletons after IVF.

Number of embryo cryopreserved

We did not find that the number of cryopreserved embryos influenced obstetric outcome. The number of frozen embryos reflects the overall quality of the embryos, and was chosen as a proxy for embryo quality. The exact quality of the transferred embryo was not included in data collected from the IVF clinics and therefore could not be accounted for. Few studies have addressed a possible effect of embryo quality on obstetric outcome. In a study from Austria (Ebner *et al.,* 2001) in which 146 pregnancies were analysed, no effect of embryo quality was observed on gestational age or birth weight.

Embryo culture time

We did not find that embryo culture time, assessed as cleaved embryo or blastocyst transfer had any significant influence on the rates of very preterm birth or SGA. Embryo culture time has been suggested to influence obstetric outcome negatively.

Conflicting data has been detected (Pinborg *et al.,* 2012) concerning the influence of embryo culture time on perinatal outcomes. One recent Swedish study (Källen *et al.,* 2010c) found a higher preterm birth rate for singletons after blastocyst transfer than for singletons after cleavage stages transfers. This study included children both from fresh and frozen embryos while the present study only included children from fresh embryos. In an Australian study (Wang *et al.,* 2009) children from SET blastocysts had a slightly better, although not statistically significant, outcome than children from SET cleavage stages. One recent Dutch study (Dumolin *et al.,* 2010) found that type of culture medium significantly affected birth weight, even after adjusting for confounders. Singleton children born after embryo culture in one particular culture medium had a mean of almost 250 grams lower birth weight than children born after the embryos had been cultured in a different medium.

In summary: Certain maternal characteristics (age, smoking, primiparity, BMI and duration of infertility) were significantly associated with very PTB and/or SGA in IVF singletons after fresh cycles. Among treatment-related variables we found a negative effect of "vanishing twin" after DET on very PTB in singletons after fresh cycles. No association between other treatments-related variables (number of oocytes retrieved, number of embryo

cryopreserved and embryo culture time) and poor neonatal outcome was detected in our study. Current literature indicates that both maternal and treatment- related factors are responsible for poorer obstetric outcomes in IVF singleton pregnancies as compared with singleton pregnancies in the general population.

Obstetric outcome in singletons after IVF with cryopreserved/ thawed embryos

The increasing use of SET in fresh cycles results in more embryos available for freezing. Therefore, safety aspects of cryopreservation techniques used in ART are always a high priority**.** Our national registry study including a complete cohort of singletons from cryopreserved/thawed cycles showed that singletons from cryopreservation cycles (both SET/DET and only SET) had higher rates of extreme preterm birth than singletons in the general population. In comparison with singletons from fresh cycles singletons from cryopreserved/thawed cycles had lower rates of LBW but higher rates of perinatal mortality. The rates of LGA and birth weight >4500 g were significantly increased for singletons from cryopreservation cycles, both in comparison with the general population and in comparison with singletons from fresh cycles.

Two other large registry studies have investigated outcomes for singleton children after cryopreservation cycles in comparison with singletons from the general population (Pinborg *et al.,* 2010; Pelkonen *et al.,* 2010). A similar pattern for perinatal outcomes as in the present study was found in these two studies. In the Danish study (Pinborg *et al.,* 2010) significantly increased AOR was found for very preterm birth in singletons born after cryopreservation cycles, while other significances disappeared after adjustment for confounders. In the Finnish study (Pelkonen *et al,.* 2010) significantly increased risks of preterm birth and low birth weight were detected for the infants from cryopreservation cycles, while other outcomes were found to be similar. The relatively small differences in outcomes between these studies might depend on different adjustment for confounders and/or selection of controls. In the present study adjustment was performed for years of infertility, which was not done in the Danish or the Finnish study. In several earlier studies infertility length was associated with poor outcomes (Basso and Baird, 2003; Källen *et al*., 2005b, 2010a). In a recent meta-analysis, an AOR of 1.20 (95% CI 0.98-1.46) was found for PTB in singletons after cryopreservation as compared with singletons in the general population (Pinborg *et al*., 2012).

The present study showed a lower rate of LBW, in singletons from cryopreservation cycles as compared with singletons from fresh cycles. Better outcomes for singletons from cryopreservation cycles as compared with singletons from fresh cycles has been found in several studies (Källen *et al.*, 2005b; Shih *et al.*, 2008; Wang *et al.*, 2005; Pinborg *et al.*, 2010; Pelkonen *et al.*, 2010). Three recent cohort studies (Pinborg *et al.*, 2010; Pelkonen *et al.*, 2010; Kato *et al*., 2012) have demonstrated lower risks of being SGA in singletons from cryopreservation cycles as compared with singletons after fresh embryo transfer. According to recent meta-analyses (Pinborg *et al*., 2012;

Maheshwari *et al*., 2012) singletons after cryopreservation have a lower risk of PTB than singletons from fresh cycles. The exact reason for this effect is not known but has been suggested to depend on hormonal stimulation and its influence on the endometrium in fresh cycles and/or positive selection of embryos by freezing/thawing procedures in the cryopreservation cycles (Shih *et al*., 2008). We found a significantly higher rate of perinatal mortality and low Apgar scores in singletons from cryopreservation cycles in comparison with fresh cycles, while no such difference was noted between singletons from cryo-preservation cycles and singletons from the general population. Previous studies have not detected any differences in perinatal mortality between singletons from fresh and cryopreservation cycles, and therefore this finding should be interpreted with caution. It might be a random finding having to do with the fact that several statistical comparisons were performed. Furthermore, the absolute rate of perinatal mortality was low and similar to that reported in other studies (Källen *et al.,* 2005b; Pinborg *et al.,* 2010; Pelkonen *et al.,* 2010). It should be noted that in the present study, the perinatal mortality in singletons from fresh cycles was extremely low (0.3% in SET and DET together) in comparison with findings in other recent studies (Pinborg *et al.,* 2010; Pelkonen *et al.,* 2010).

The higher rate of LGA in singletons after cryopreservation cycles found in this study, both in comparison with fresh cycles and singletons from the general population are, at least partly, new findings which could not be explained by higher maternal BMI or higher rate of diabetes.

Higher rates of LGA in singletons from cryopreservation cycles in comparison with singletons from fresh cycles have recently been reported from Finland (Pelkonen *et al*., 2010) and Denmark (Pinborg *et al*., 2011). In the Danish study, the LGA rate was also significantly elevated as compared with the general population. In a recent large Japanese study, including more than 14 000 singletons from frozen/thawed cycles (Nakashima *et al*., 2013), the mean birth-weight after IVF with cryopreservation was significantly higher as compared with fresh cycles and all Japanese births. The sibling study from Denmark (Henningsen *et al*., 2011) showed that singletons born after cryopreservation cycles were heavier than their siblings born after fresh IVF cycles.

In cattle and sheep, IVF stimulation and *in vitro* culture has been found to induce large offspring syndrome (Young *et al*., 1998). The mechanism for this phenomenon is not known but epigenetic and metabolic abnormalities have been discussed (Young *et al.,* 1998). Another possible explanation for the higher rates of LGA in singletons after cryopreservation cycles could be asynchrony between the endometrium and the embryo, altered fetal growth and development (Grace and Sinclair, 2009). Yet another theory is based on the hypothesis that IVF culture is prone to *in utero* overgrowth of embryos but hormonal stimulation that changes the steroid profile in fresh IVF cycles inhibits these overgrowing tendencies. Macrosomic babies are at increased risk of adverse perinatal outcomes such as stillbirth, birth asphyxia, shoulder dystocia associated injuries, hypoglycaemia, respiratory distress and perinatal mortality (Henriksen *et al*., 2008).

Maternal complications include increased rates of perineal tears, Cesarean section and postpartum haemorrhages. Macrosomia has been reported as a risk factor for childhood cancer (Källen *et al*., 2010a) and breast cancer (McCormack *et al*., 2003) and is associated with an increased risk of development of diabetes, overweight and metabolic syndrome later in life (Boney *et al.,* 2005). In a recent Japanese study the increased risk of LGA in cryopreservation cycles had disappeared after adjustment for maternal age, BMI, parity, type of stimulation protocol, ICSI versus conventional IVF fertilization, blastocyst versus cleavage-stage embryo culture and infant sex (Kato *et al.,* 2012). We also compared the rate of LGA (+3SD) and birthweight >5500 g between singletons after cryopreservation, singletons from fresh cycles and singletons from the general population. No significant differences in these rates were found.

In summary: Obstetric outcomes in singletons after cryopreservation were poorer than for singletons in the general population (higher rate of extreme preterm birth). In comparison with fresh cycles the outcomes varied. Results from recent meta-analyses demonstrated better obstetric outcomes in singletons after cryopreservation cycles than for singletons after fresh IVF cycles. A higher rate of LGA in singletons after cryopreservation, both in comparison with singletons from fresh cycles and singletons from the general population was found. Similar observations have now been made in three large Nordic studies.

Maternal outcomes in singleton pregnancies after IVF

Preeclampsia, PPROM and gestational diabetes

We did not find any increased risk of preeclampsia, PPROM or gestational diabetes in singleton IVF pregnancies (fresh and frozen cycles, Paper I) as compared with singleton pregnancies in the general population.

According to previous studies there is an increased rate of preeclampsia in ART singleton pregnancies as compared with spontaneous pregnancies (Ochsenkuhn *et al*., 2003; Källen *et al*., 2005c; Shevell *et al*., 2005). Two meta-analyses based on eight studies (Jackson *et al*., 2004) and 15 studies (16 923 IVF singleton pregnancies) (Pandey *et al*., 2012) reported an 1.6 and 1.5 increased risk of preeclampsia in ART singleton pregnancies respectively. Significantly higher risks of preeclampsia in IVF singleton pregnancies persisted after adjustment for age and parity, in some studies also after adjustment for BMI and smoking.

There are many theories, which have tried to explain the pathophysiological aspects of this increased risk. It is well known that preeclampsia has a multifactorial origin. Besides of immune-genetic mechanisms and role of placental oxidative stress in preeclampsia (Redman *et al*., 2010) it is discussed that a possible abnormal placentation with inadequate utero-placental circulation may contribute to the ART-mediated hypertensive disorders in IVF

pregnancies (Daniel *et al*., 1999). In IVF, formation of the chorion is initiated *in vitro* and that may result in impaired implantation or placentation. The IVF procedure is also associated with an insufficiency of corpus luteum, which, for example, can lead to deficiency in relaxin, impaired vasodilation and vascular dysfunction in placenta (Sherwood *et al*., 2004).

In the third part of our study (Paper III) a higher rate of preeclampsia was noted for singleton pregnancies from cryopreservation SET/DET cycles as compared with singleton pregnancies in the general population and singletons from fresh cycles. To our knowledge, no study has compared preeclampsia in pregnancies after cryopreserved versus fresh cycles. This increased risk of preeclampsia in cryopreservation cycles when compared with fresh cycles is difficult to explain and needs to be confirmed in other studies.

Placenta previa and placental abruption

We found significantly increased rates of placenta previa in SET and DET singleton pregnancies and placental abruption in SET singleton pregnancies as compared with singleton pregnancies in the general population after adjustment for maternal variables (Paper I). Placenta previa and placental abruption are major obstetric complications. Increased rates of placenta previa in ART pregnancies, both multiple and singleton, have been reported in several earlier studies (Jackson *et al*., 2004; Källen *et al*., 2005c; Källen *et al*., 2010a; Shevell *et al*., 2005; Romundstad *et al*., 2006; Schieve *et al*., 2007; Healy *et al*., 2010) as well as in studies investigating infertility treatment in general (Welmerink *et al.,* 2010), with ORs varying between 2.3 and 6.0 as compared with the general population. The reason for the increased rates of these obstetric complications is not known.

The trans-cervical embryo transfer with low placement of embryos in the uterine cavity has been suggested as a possible cause of this increased risk (Romundstad *et al.,* 2006). Interestingly, in an Australian study, GIFT (gamete intrafallopian transfer) had a similar increased rate of placenta previa as IVF/ICSI, indicating no association with the embryo transfer procedure (Healy *et al.,* 2010).

The Australian study (Healy *et al.,* 2010), including 6730 singletons pregnancies after ART, showed significant associations between placenta previa and fresh embryo transfers in stimulated cycles, endometriosis and hormone treatments. The authors suggested that events around the time of implantation may be responsible, with suboptimal endometrial function being the critical mechanism. A systematic problem related to increased surveillance, i.e. that ART pregnancies are more prone to ultrasound investigation resulting in placenta previa being more often diagnosed cannot, however, be ruled out. Another possible mechanism may be of epigenetic origin. Epigenetics has been recognized as playing an important role in placental development and function. Nelissen and co-workers described DNA methylation as essential for normal development of extra embryonic tissues, especially for the invasive behaviour of trophoblastic cells. The authors reviewed disturbances of these mechanisms by ART and the possible consequences for the mother and offspring. They also referred to other publications that suggest that tissues of trophectodermic origin are more sensitive to preimplantation

epigenetic disturbances than embryonic tissues (Nelissen *et al.,* 2010).

In the present study we found that blastocyst transfer as compared with cleavage stage transfer was an independent risk factor for placenta previa. To our knowledge, this has not been reported earlier (Paper II). The higher risk of placenta previa after blastocyst transfer as compared with transfer of cleavage stage embryos indicates that the culture time *in vitro* may also interfere with early embryo development and implantation. One could speculate as to whether a more developed embryo such as a blastocyst has less time than a cleavage stage embryo to communicate with the endometrium in order to obtain the most optimal implantation zone in the uterus In any case, the observation of an increased rate of placenta previa after blastocyst transfer is an important finding.

We found a lower rate of placenta previa in pregnancies from cryopreservation cycles than in fresh cycles (Paper III). Few studies have compared the risk of placenta previa between cryopreservation cycles and fresh cycles (Pelkonen *et al*., 2010; Wikland *et al*., 2010; Healy *et al*., 2010). In the Australian study there was also some evidence that fresh transfers were associated with an increased risk of placenta previa (AOR 1.40, 95% CI 1.00-1.95) (Healy *et al.*, 2010). One possible mechanism is the ovarian stimulation used in fresh cycles, which may cause suboptimal endometrial function.

In summary: We did not find any increased risk of preeclampsia, PPROM or gestational diabetes in singleton IVF pregnancies (fresh and frozen cycles, Paper I) as compared with singleton pregnancies in the general population. However, a higher rate of preeclampsia was found in singleton pregnancies from cryopreservation cycles than in singleton pregnancies in the general population and in singleton pregnancies from fresh IVF cycles. In large studies and a recent meta-analysis (Pandey *et al.,* 2012) higher rates (AOR 1.49, 1.39-1.59) of hypertensive disorders were reported in IVF singleton pregnancies than in singleton pregnancies in the general population. However, separate analysis for singleton pregnancies after cryopreservation cycles was not made.

Significantly increased rates of placenta previa and placental abruption were found in SET and/or DET singleton pregnancies as compared with singleton pregnancies in the general population. Similar results have been found in recent large studies and meta-analysis (Romundstad *et al.,* 2006; Healy et al., 2010; Pandey *et al.,* 2012). Blastocyst transfer was an independent risk factor for placenta previa. A lower rate of placenta previa in pregnancies from cryopreservation cycles than in fresh cycles was also detected.

Obstetric and neonatal outcomes comparing women undergoing two IVF singleton pregnancies with women undergoing one IVF twin pregnancy

The concept of single embryo transfer in IVF has reduced the risks of both maternal and neonatal complications simply by lowering the number of multiple births. Although the supporting data are overwhelming, this is still an ongoing

subject of debate, especially in countries with private health care and/or insurance systems, i.e. whether twins are a desired outcome of IVF (Gleicher *et al*., 2009; Gleicher, 2013).

The main finding in the fourth part of our study (Paper IV) was that adverse outcomes were dramatically increased for IVF twins as compared with two IVF singletons with the same mother, with AORs ranging from 4 to 16. These risk estimates are presented per child, i.e., each twin has these increased risks as compared with a singleton child. Most importantly, the above-mentioned increased rates of adverse perinatal outcomes in IVF twins were mirrored in significantly higher rates of child morbidity. Significantly higher rates of respiratory complications, sepsis and jaundice were found among twins. For other more severe diagnoses, we created a composite outcome, because each of the individual diagnoses occurs relatively infrequently. The rate of the composite outcome, representing the most severe child morbidity, was almost doubled and significantly higher for IVF twins in the crude analysis, but significance disappeared after adjustment for confounders. Even in a large registry study such as this one, severe child morbidity is rare. Such complications increase the risk of neonatal intensive care admission and may also have long-term health consequences for the children. Similar results have been reported in other studies where considerably poorer outcomes for IVF/ ICSI twins than in singletons have been found (Pinborg *et al*, 2004; Klemetti *et al*., 2006). It is obvious that more complications, both maternal and neonatal, occurred more often in the first than in the second pregnancy in the singleton group. This might be expected, because primiparity has been found to be an independent predictor of adverse neonatal outcome in previous studies (Welmerink *et al.,* 2010; Pinborg *et al*, 2012). It was evident that the twin pregnancies carried a significantly higher risk of several maternal complications, although maternal complications were more difficult to compare statistically in a study of this kind. For the group of singleton mothers we chose to include complications occurring in at least one of the two pregnancies for comparison. When using this kind of calculation, the rate of preeclampsia was doubled in the group of twin mothers. PPROM and Cesarean section also occurred more often in the mothers with a twin pregnancy.

Placenta previa was observed less frequently in the group of mothers with one IVF twin pregnancy than in the group mothers with two IVF singleton pregnancies. Risk factors for placenta previa include previous Cesarean section, increased maternal age and parity, smoking, and infertility treatment (Rosenberg *et al*., 2011). Rates of placenta previa are significantly increased in IVF singleton and twin pregnancies three-to-sixfold as compared with spontaneous conceptions, but the rates are reported to be similar in IVF twin and singleton pregnancies (Allen *et al*., 2006). In spontaneous pregnancies, placenta previa occurs in a slightly increased rate in twin pregnancies as compared with singleton pregnancies (Ananth *et al*., 2003). Repeated Cesarean sections in women with singleton IVF pregnancies may explain the higher rate of placenta previa in the present study.

The financial consequences for both parents and society, when comparing an IVF

program with a high twin rate to one with a low twin rate, was not one of the main issues in this study, but a comment is in order. Financial consequences for the patients and differences in reimbursement systems are important aspects of IVF in different countries Although the costs per child has been shown to be somewhat higher with the SET strategy compared to DET (Thurin-Kjellberg *et al.,* 2006), that additional cost can be regarded as limited in view of the considerably better outcomes for the children in the SET group. The rate of severe neonatal complications among the children was twice as high in the DET group versus the SET group (Thurin-Kjellberg *et al.,* 2006; Bergh, 2007), some giving severe neurologic sequelae. The costs for these many times lifelong conditions are difficult to calculate, but they have been estimated to be considerable (Collins, 2007; Wolner-Hansen and Rydhstrom, 1998).

In the present cohort, national data from Sweden 2002–2006, it was not possible to estimate how many cycles were needed to achieve two singleton live births or one twin birth, because the registry where the data were obtained includes only delivery after IVF, not failed cycles. However, since 2007 Sweden has a national quality registry for IVF that includes all cycles, successful as well as failed (National Quality Register for Assisted Reproduction, Sweden). In that registry the delivery rate after SET (eSET and non-eSET combined) for women of all ages was 28% for fresh cycles and 22% for frozen-thawed SET cycles in 2010. The SET rates were 73% and 87%, respectively. To estimate the number of cycles, SET or DET, that would be needed to achieve two children, we have used data from the Swedish National Quality Register for Assisted Reproduction (National Quality Register for Assisted Reproduction, Sweden) and from randomized controlled trials (Thurin *et al*., 2004).

With the SET strategy we estimated the following:

1. One fresh SET transfer results in 0.26% children. We decreased the delivery rate from 28%, which is the actual figure from the quality registry, because that figure includes only 73% of all cycles and better-prognosis patients. We assume it would be lower if SET were applied to all women.

2. In the quality registry, the mean number of frozen embryos per SET cycle is 2.1. With 75% survival, that would give 1.6 transfers x 22% (National Quality Register for Assisted Reproduction, Sweden) deliveries=0.35% children. In total: 0.26 + 0.35=0.61 children per SET. To achieve two children: 2/0.61=3.3 SET, which means 3.3 fresh SET + 3.3 x 1.6 frozen SET cycles= 3.3 fresh SET + 5.3 frozen SET.

With the DET strategy we estimated the following:

1. One fresh DET transfer results in 0.36% deliveries. This figure is not known exactly but is estimated according to results from randomized controlled trials (Thurin *et al*., 2004). Of these deliveries, 20% are twins. This gives 0.29 singletons and 0.07 twins, which corresponds to 0.29 + 0.14=0.43 children.

2. In DET cycles a mean of 1.1 embryos are frozen (Thurin *et al*., 2004). With 75% survival, that would give 0.83 transfers x 22% deliveries=0.18 children (all singletons because only one embryo is available for

transfer). In total: 0.43 + 0.18=0.61 children per DET. To achieve two children: 2/0.61=3.3 DET, which means 3.3 fresh DET + 3.3 x 0.83 frozen=3.3 fresh DET + 2.7 frozen SET.

In summary, 3.3 fresh cycles are needed with both SET and DET strategies. When DET is applied in the fresh cycle, one-half the number of frozen transfers is needed to achieve the same number of children as compared with SET.

In summary: the maternal and neonatal outcomes were dramatically better for women who had two IVF singleton pregnancies than for those with one IVF twin pregnancy. Our results demonstrate that the use of SET lowers the risk associated with twin pregnancies without any substantial increase in the number of cycles needed to achieve children.

Strengths and limitations

The main strength of this thesis was the inclusion of a complete and large cohort of IVF children and comparing them with all children born under the same period in the general population. The use of unique personal identification number in Sweden gave the opportunity for cross-linkage of data with different high validated medical registries.

Role of bias and confounders

It has to be remembered that all data on obstetric outcome after IVF is based on observational studies with inherent risk of bias and confounders. We adjusted our results for important and known confounders, such as maternal age, parity, year of birth, smoking, BMI and years of infertility. Infertility *per se* has been found to influence obstetric outcomes negatively. Several studies (Basso *et al*., 2003; Thomson *et al*., 2005; Jaques *et al*., 2010; Pinborg *et al.,* 2012) have found higher risks of PTB and LBW in women with infertility who conceived spontaneously as compared with fertile women. We did not adjust for socioeconomic factors. Socioeconomic factors are well-known to influence obstetric outcome, particularly in countries with large socioeconomic differences. IVF treatment in Sweden, is to a large extent, publicly funded and earlier studies (Bergh *et al.*, 1999) indicated no clear socioeconomic trends in women treated with IVF in comparison to population. In addition, smoking and BMI, for which adjustment were done, might, at least partly reflect socioeconomics circum-stances. We were also unable to account for all IVF technique related factors (total dose of hormonal stimulation, type of stimulation protocol, type of culture medium etc.). This information was not available in data collected from IVF clinics.

Biases are systematic errors, impossible to correct for in the statistical analysis. Close surveillance of IVF pregnancies may lead to detection of more abnormalities i.e. malformations in the newborn babies and a higher rate of placenta previa if more ultrasound investigations are performed in IVF women. IVF women might also seek health care more frequently and doctors might be more prone to intervention in an IVF pregnancy. These factors could increase the rate of PTB and LBW. This kind of bias is difficult to quantify but is probably less in later years than in the beginning of the IVF era. While it is important to be aware of methodological limitations, there is a huge consistency in the findings concerning IVF children and pregnancies. The association between IVF and a poorer obstetric outcome, also in singletons, compared to the general population is most probably true. It has been demonstrated in different populations with many different study designs, including large registry studies and studies using sibling design. The same is true for a better outcome, in terms of PTB and LBW, for cryo children versus children from fresh IVF. Also the magnitude of the effect is quite consistent between studies. It is also obvious that maternal characteristics contribute to a large extent to these adverse outcomes while the contribution of IVF related factors is less clear.

Conclusions from this thesis

- Children born after IVF had poorer obstetric outcomes than children in the general population. Singletons after IVF, irrespective of the number of embryos transferred had poorer obstetric outcomes than singletons in the general population. More placental complications were observed in singleton pregnancies after both SET and DET.

- Certain maternal characteristics and number of transferred embryos (”vanishing twin”) affected obstetric outcomes negatively. No association between other treatments-related variables (number of oocytes retrieved, number of embryo cryopreserved and embryo culture time) and poor neonatal outcome was detected. An increased rate of placenta previa was observed after blastocyst transfer.

- The obstetric outcome in singletons after cryopreservation was poorer than for singletons in the general population (higher rate of extreme preterm birth). In comparison with fresh cycles, the outcomes for singletons after cryopreservation varied. Higher rates of LGA in singletons after cryopreservation were found, both in comparison with singletons from fresh cycles and singletons from the general population.

- The neonatal and maternal outcomes were dramatically better for women undergoing two IVF singleton pregnancies as compared with one IVF twin pregnancy after DET.

Future perspectives

The detection of higher rates of PTB and LBW in IVF children may also have long-term consequences for future health. According to the Barker hypothesis there is some suggestions that these adverse birth outcomes are due to non-optimal conditions in uteri leading to later increased risks of type 2 diabetes mellitus and cardiovascular disease (Barker *et al*., 1989; Barker *et al.,* 1995). Several studies regarding growth of children conceived by ART found no significant differences in height, weight or BMI (Bonduelle *et al*., 2005; Ceelen *et al*., 2008a). Childhood hypertension has become an important health concern since recent studies indicate that hypertension in adults is already visible in childhood. A few studies have investigated cardiometabolic risks in IVF children and found higher blood pressure and blood glucose levels (Ceelen *et al*., 2008b; Ceelen *et al*., 2009) and signs of a generalized vascular dysfunction in IVF children (Scherrer *et al.*, 2012). However, much larger studies and in older children are needed to confirm or reject these preliminary findings. Other important areas for future studies are investigation of seldom occurring diseases such as cancer and imprinting disorders.

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References

Abramov Y, Elchalal U, Schenker J. Obstetric outcome of in vitro fertilized pregnancies complicated by severe ovarian hyper-stimulation syndrome: a multicenter study. *Fertil Steril* 1998;**70**:1070-1076.

Allen VM, Wilson RD, Cheung A; Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC); Reproductive Endocrinology Infertility Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC). Pregnancy outcomes after assisted reproductive technology. *J Obstet Gynaecol Can* 2006; **3**:220-250.

Almog B, Levin I, Wagman I, Kapustiansky R, Lessing JB, Amit A, Azem F. Adverse obstetric outcome for the vanishing twin syndrome. *Reprod Biomed Online* 2010;**20**: 256-260.

Ananth CV, Demissie K, Smulian JC, Vintzileos AM. Placenta previa in singleton and twin births in the United States, 1989 through 1998: a comparison of risk factor profiles and associated conditions. *Am J Obstet Gynecol* 2003;**1**:275-281.

Ananth CV, Joseph Ks K, Smulian JC. Trends in twin neonatal mortality rates in the United States, 1989 through 1999: influence of birth registration and obstetric intervention. *Am J Obstet Gynecol* 2004;**190**:1313-1321.

Aragona C, Mohamed MA, Espinola MS, Linari A, Pecorini F, Micara G, Sbracia M. Clinical complications after transvaginal oocyte retrieval in 7 098 IVF cycles. *Fertil Steril* 2011;**95**:293-294.

Basso O, Baird DD. Infertility and preterm delivery birth weight, and Cesarean section: a study within the Danish National Birth Cohort. *Hum Reprod* 2003;**11**:2478-2484.

Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet* 1989;**2**:577-580.

Barker DJ. Fetal origins of coronary heart disease. *BMJ* 1995;**311**:171-174.

Belva F, Painter R, Bonduelle M, Roelants M, Devroey P, De Schepper J. Are ICSI adolescents at risk for increased adiposity? *Hum Reprod* 2012;**27**:257-264.

Bergh T, Ericsson A, Hillensjö T, Nygren KG, Wennerholm UB. Deliveries and children born after in-vitro fertilization in Sweden 1982-1995. A cohort study. *Lancet* 1999;**354**: 1579-1585.

Bergh C. How to promote singletons. *Reprod Biomed Online* 2007;**15**:22-27.

Blake DA, Farquhar CM, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. *Cochrane Database Syst Rev* 2007;**4**: CD002118.

Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Hum Reprod* 2007;**22**:1506-1512.

Bonduelle M, Van Assche E, Joris H, Keymolen K, Devroey P, Van Steirteghem A, Liebaers I. Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. *Hum Reprod* 2002;**17**: 2600-2614.

Bonduelle M, Wennerholm UB, Loft A, Tarlatzis BC, Peters C, Henriet S, Mau C, Victorin-Cederquist A, Van Steirteghem A, Balaska A, Emberson JR, Sutcliffe AG. A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. *Hum Reprod* 2005;**20**:413-419.

Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005;**115**:290-296.

Bruinsma F, Venn A, Lancaster P, Speirs A, Healy D. Incidence of cancer in children born after in-vitro fertilization. *Hum Reprod* 2000; **15**:604-607.

Ceelen M, vanWeissenbruch MM, Vermeiden JP, van Leeuwen FE, Delemarre-van de Waal HA. Growth and development of children born after in vitro fertilization. *Fertil Steril* 2008a; **90**:1662-1673.

Ceelen M, van Weissenbruch MM, Vermeiden JP, van Leeuwen FE, Delemarre-van de Waal HA. Cardiometabolic differences in children born after in vitro fertilization: follow-up study. *J Clin Endocrinol Metab* 2008b;**93**:1682-1688.

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

Chung K, Coutifaris C, Chalian R, Lin K, Ratcliffe SJ, Castelbaum AJ, Freedman MF, Barnhart KT. Factors influencing adverse perinatal outcomes in pregnancies achieved through use of in vitro fertilization. *Fertil Steril* 2006;**86**:1634-41.

Clark SL, Belfort MA, Dildy GA, Herbst MA, Meyers JA, Hankins GD. Maternal death in the 21st century: causes, prevention, and relationship to Cesarean delivery. *Am J Obstet Gynecol* 2008;**199**:36.

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

Collins J. Cost efficiency of reducing multiple births. *Reprod Biomed Online* 2007;**15**:35- 39.

Cox GF, Bürger J, Lip V, Mau UA, Sperling K, Wu BL, Horsthemke B. Intracytoplasmic sperm injection may increase the risk of imprinting defects. *Am J Hum Genet* 2002;**71**: 162-164.

Daniel Y, Schreiber L, Geva E, Amit A, Pausner D, Kupferminc MJ, Lessing JB. Do placentae of term singleton pregnancies obtained by assisted reproductive technologies differ from those of spontaneously conceived pregnancies? *Hum Reprod* 1999;**14**:1107-1110.

Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, Haan EA, Chan A. Reproductive technologies and the risk of birth defects. *N Engl J Med* 2012;**10**:1803-1813.

De Baun MR, Niemitz EL & Feinberg AP. Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am J Hum Genet* 2003;**72**:156-160.

De Sutter P, Delbaere I, Gerris J, Verstraelen H, Goetgeluk S, Van der Elst J, Temmerman M, Dhont M. Birthweight of singletons after assisted reproduction is higher after single- than after double-embryo transfer. *Hum Reprod* 2006;**21**:2633-2637.

Dumolin JC, Land JA, Van Montfoort AP; Nelissen EC, Coonen E, Derhaag JG, Schreurs IL, Dunselman GA, Kester AD,

Geraedts JP,Evers JL. Effcet of in vitro culture of human embryos on birthweight of newborns. *Hum Reprod* 2010;**25**:605-612.

Ebner T, Yaman C, Moser M, Sommergruber M, Pölz W, Tews G. Embryo fragmentation in vitro and its impact on treatment and pregnancy outcome. *Fertil Steril* 2001;**76**: 282-285.

ESHRE ARTfact sheet 2010: http://www.eshre.eu/ESHRE/English/Guidelines-Legal/ART-fact-sheet/page.aspx/1061.

Ferraretti AP, Goossens V, de Mouzon J, Bhattacharya S, Castilla JA, Korsak V, Kupka M, Nygren KG, Nyboe Andersen A; European IVF-monitoring (EIM); Consortium for European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europé, 2008: Results generated from European registers by ESHRE. *Hum Reprod* 2012; **27**:2571-2584.

Grace KS, Sinclair KD. Assisted reproductive technology, epigenetics, and long-term health: a developmental time bomb still ticking. *Semin Reprod Med* 2009;**27**:409-416.

Greil AL, Shreffler KM, Schmidt L, McQuillan J. Variation in distress among women with infertility: evidence from a population-based sample. *Hum Reprod* 2011; **26**:2101-2112.

Griesinger G, Kolibianakis EM, Diedrich K, Ludwig M. Ovarian stimulation for IVF has no quantitative association with birthweight: a registry study. *Hum Reprod* 2008;**23**:2549-2554.

Guerif F, Lemseffer M, Bidault R, Gasnier O, Saussereau MH, Cadoret V, Jamet C, Royere D. Single Day 2 embryo versus blastocyst-stage transfer: a prospective study integrating fresh and frozen embryo transfers. *Hum Reprod* 2009;**24**:1051-1058.

Gleicher N, Barad D. Twin pregnancy, contrary to consensus, is a desirable outcome in infertility. *Fertil Steril* 2009;**91**:2426-2431.

Gleicher N. The irrational attraction of elective single-embryo transfer (eSET). *Hum Reprod* 2013;**28**:294-297.

Hansen M, Bower C, Milne E, de Klerk N, Kurinczuk J. Assisted reproductive technologies and the risk of birth defects - a systematic review. *Hum Reprod* 2005; **20**:328-338.

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

Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ* 2004;**328**:261-265.

Henningsen AKA, Pinborg A, Lidegaard O, Vestergaard C, Forman JL, Andersen AN. Perinatal outcome of singleton siblings born after assisted reproductive technology and spontaneous conception: Danish national sibling-cohort study. *Fertil Steril* 201;**95**:959-963.

Henriksen T. The macrosomic fetus: a challenge in current obstetrics. *Acta Obstet Gynecol Scand* 2008,**87**:134-145.

Henriksson P, Westerlund E, Wallén H, Brandt L, Hovatta O, Ekbom A. Incidence of pulmonary and venous thromboembolism in pregnancies after in vitro fertilisation: cross sectional study. *BMJ* 2013;**15**:346:e8632.

Hvidtjorn D, Grove J, Schendel DE, Vaeth M, Ernst E, Nielesen LF, Thorsen P. Cerebral palsy among children born after in vitro fertilization: the role of preterm delivery - a population-based, cohort study. *Pediatrics* 2006;**118**:475-482.

Hvidtjørn D, Schieve L, Schendel D Jacobsson B, Svaerke C, Thorsen P. Cerebral palsy, autism spectrum disorders, and developmental delay in children born after assisted conception: a systematic review and meta-analysis. *Arch Pediatr Adolesc Med* 2009;**163**:72-83.

Isaksson R, Gissler M, Tiitinen A. Obstetric outcome among women with unexplained infertility after IVF: a matched case control study. *Hum Reprod* 2002;**17**:1755-61.

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

Jaques AM, Amor DJ, Baker HW, Healy DL, Ukoumunne OC, Breheny S, Garrett C, Halliday JL. Adverse obstetric and perinatal outcomes in subfertile women conceiving without assisted reproductive technologies. *Fertil Steril* 2010;**94**:2674-2679.

Kato O, Kawasaki N, Bodri D, Kuroda T, Kawachiya S, Kato K, Takehara Y. Neonatal outcome and birth defects in 6623 singletons born following minimal ovarian stimulation and vitrified versus fresh fresh single embryo transfer. *Eur J Obstet Gynecol Reprod Biol* 2012; **161**;46-50.

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

Klemetti R, Gissler M, Hemminki E. Comparison of perinatal health of children born from IVF in Finland in the early and late 1990s. *Hum Reprod* 2002;**17**:2192-2198.

Klemetti R, Sevón T, Gissler M, Hemminki E. Health of children born as a result of in vitro fertilization. *Pediatrics* 2006;**118**:1819-1827.

Kjellberg AT, Carlsson P, Bergh C. Randomized single versus double embryo transfer: obstetric and paediatric outcome and a cost-effectiveness analysis. *Hum Reprod* 2006;**21**:210-216.

Källen B, Finnström O, Nygren KG, Olausson P. In vitro fertilization in Sweden: child morbidity including cancer risk. *Fertil Steril* 2005a; **84**:605-610.

Källen B, Finnström O, Nygren KG, Olausson P. In vitro fertilization (IVF) in Sweden: infant outcome after different IVF methods. *Fertil Steril* 2005b;**84**:611-617.

Källen B, Finnström O, Nygren KG, Olausson PO, Wennerholm UB. In vitro fertilization (IVF) in Sweden: obstetric characteristics, maternal morbidity & mortality. *Br J Obstet Gynecol* 2005c;**112:**1529-1535.

Källén B, Finnström O, Lindam A,Nilsson E, Nygren KG, Otterblad Olausson P. Trends in delivery and neonatal outcome after in vitro fertilization in Sweden: data for 25 years. *Hum Reprod* 2010a; **25**:1026-1034.

Källén B, Finnström O, Lindam A, Nilsson E, Nygren KG, Olausson PO. Cancer risk in children and young adults conceived by in vitro fertilization. *Pediatrics* 2010b;**126**:270-276.

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

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

Lal M. Psychosomatic approaches to obstetrics, gynaecology and andrology - a review. *J Obstet Gynaecol* 2009;**29**:1-12.

Lidegaard O, Pinborg A, Andersen AN. Imprinting diseases and IVF: Danish National IVF cohort study. *Hum Reprod* 2005;**20**:950-954.

Liebermann J, Tucker MJ. Comparison of vitrification and conventional cryopreservation of day 5 and day 6 blastocysts during clinical application. *Fertil Steril* 2006;**86**:20-26.

Loutradi KE, Kolibianakis EM, Venetis CA, Papanikolaou EG, Pados G, Bontis I, Tarlatzis BC. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. *Fertil Steril* 2008;**90**:186-193.

Luke B, Brown MB, Grainger DA, Stern JE, Klein N, Cedars MI. The effect of early fetal losses on singleton assisted-conception pregnancy outcomes. *Fertil Steril* 2009;**91**: 2578-2585.

Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* 2012;**98**:368-377.

Manipalviratn S, DeCherney A & Segars J. Imprinting disorders and assisted reproductive technology. *Fertil Steril* 2009; **91**:305-315.

Marees T, Dommering CJ, Imhof SM, Kors WA, Ringens PJ, van Leeuwen FE, Moll AC. Incidence of retinoblastoma in Dutch children conceived by IVF: an expanded study. *Hum Reprod* 2009;**24:**3220-3224.

Marsál K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr* 1996;**85**:843-848.

McCormack VA, dos Santos Silva I, De Stavola BL, Mohsen R, Leon DA, Lithell HO. Fetal growth and subsequent risk of breast cancer: results from long term follow up of Swedish cohort. *BMJ* 2003;**326**:248-254.

McDonald SD, Murphy K, Beyene J, Ohlsson A. Perinatel outcomes of singleton pregnancies achieved by in vitro fertilization: a systematic review and meta-analysis. *J Obstet Gynaecol Can* 2005;**27**:449-459.

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

McLernon DJ, Harrild K, Bergh C, Davies MJ, de Neubourg D, Dumoulin JC, Gerris JK Remer JA, Martikainen H, Mol BW, Norman RJ, Thurin-Kjellberg A, Tiitinen A, van Montfoort AP, van Peperstraten AM, Van Royen E, Bhattacharya S. Clinical effectiveness of elective single versus double embryo transfer: meta-analysis of individual patient data from randomised trials. *BMJ* 2010;**341**:6945.

Middelburg KJ, Heineman MJ, Bos AF, Hadders-Algra M. Neuromotor, cognitive, language and behavioural outcome in children born following IVF or ICSI-a systematic review. *Hum Reprod Update* 2008;**14**:219-231.

Miettinen OS. Simple interval estimation of risk ratio. *Am J Epidemiol* 1974;**100**:515-516.

Moll AC, Imhof SM, Cruysberg JR, Schouten-van Meeteren AY, Boers M, van Leeuwen FE. Incidence of retinoblastoma in children born after in-vitro fertilisation. *Lancet* 2003;**361**:309-310.

Nakashima A, Araki R, Tani H, Ishihara O, Kuwahara A, Irahara M, Yoshimura Y, Kuramoto T, Saito H, Nakaza A, Sakumoto T. Implications of assisted reproductive technologies on term singleton birth weight: an analysis of 25 777 children in the national assisted reproduction registry of Japan. *Fertil Steril* 2013;**99**:450-455.

National Board of Health and Welfare, Centre for Epidemiology.

National Quality Register for Assisted Reproduction, Sweden: www.ucr.uu.se/qivf/

Nelissen ECM, van Montfoort APA, Dumolin JCM, Evers JLH. Epigenetics and the placenta. *Hum Reprod Update* 2011;**17**:397-417.

Ochsenkühn R, Strowitzki T, Gurtner M, Strauss A, Schulze A, Hepp H, Hillemanns P. Pregnancy complications, obstetric risks, and neonatal outcome in singleton and twin pregnancies after GIFT and IVF. *Arch Gynecol Obstet* 2003;**268**:256-261.

Ombelet W, Cadron I, Gerris J, De Sutter P, Bosmans E, Martens G, Ruyssinck G, Defoort P, Molenberghs G, Gyselaers W. Obstetric and perinatal outcome of 1655 ICSI and 3974 IVF singleton and 1102 and 2901 IVF twin births: a comparative analysis. *Reprod Biomed Online* 2005;**11**:76-85.

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

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

Pandian Z, Bhattacharya S, Ozturk O, Serour G, Templeton A. Number of embryos for transfer following in-vitro fertilisation or intra-cytoplasmic sperm injection. *Cochrane Database Syst Rev* 2009;**2**:D003416.

Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari AM, Hyden-Granskog C, Martikainen H, Tiitinen A, Hartikainen AL. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995-2006. *Hum Reprod* 2010; **25**:914-923.

Pinborg A, Loft A, Nyboe Andersen A. Neonatal outcome in a Danish national cohort of 8602 children born after in vitro fertilization or intracytoplasmic sperm injection: the role of twin pregnancy. *Acta Obstet Gynecol Scand* 2004;**11**:1071-1078.

Pinborg A, Lidegaard O, la Cour Freiesleben N, Nyboe Andersen A. Consequences of vanishing twins in IVF/ICSI pregnancies. *Hum Reprod* 2005;**20**:2821-2829.

Pinborg A, Lidegaard O, la Cour Freisenleben N, Nyboe Andersen A. Vanishing twin: a predictor of small-for gestational age in IVF singletons. *Hum Reprod* 2007; **22**:2707-2714.

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

Pinborg A, Loft A, Noergaard L , Henningsen AA, Rasmussen S, Nyboe Andersen A. Singletons born after frozen embryo transfer (FET) have an increased risk of being large for gestational age in Danish National controlled cohort study of 15 078 singletons. O-230 Abstract of the 27th Annual Meeting of ESHRE, Stockholm, Sweden, 3-6 July 2011.

Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Söderström-Anttila V, Nygren KG, Hazekamp J, Bergh C. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. *Hum Reprod Update* 2013;**19**: 87-104.

Poikkeus P, Gissler M, Unkila-Kallio L, Hyden-Granskog C, Tiitinen A.Obstetric and neonatal outcome after single embryo transfer. *Hum Reprod* 2007;**22**:1073-1079.

Redman CWG, Sargent IL. Immunology of pre-eclampsia. *Am J Reprod Immunol* 2010; **63**:534-543.

Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, Rozen S, Jaffe T, Straus D, Hovatta O, *et al.* Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nat Genet* 1995;**10**:383-393.

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

Rimm AA, Katayama AC, Diaz M, Katayama KP. A meta-analysis of controlled studies comparing major malformation rates in IVF and ICSI infants with naturally conceived children. *J Assist Reprod Genet* 2004;**21**:437-443.

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

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

Rosenberg T, Pariente G, Sergienko R, Wiznitzer A, Sheiner E. Critical analysis of risk factors and outcome of placenta previa. *Arch Gynecol Obstet* 2011;**284**:47-51.

Rova K, Passmark H, Lindqvist PG. Venous thromboembolism in relation to in vitro fertilization: an approach to determining the incidence and increase in risk in successful cycles. *Fertil Steril* 2012;**97**:95-100.

Scherrer U, Rimoldi SF, Rexhaj E, Stuber T, Duplain H, Garcin S, de Marchi SF, Nicod P, Germond M, Allemann Y, Sartori C. Systemic and pulmonary vascular dysfunction in children conceived by assisted reproductive technologies. *Circulation* 2012;**125**:1890-1896.

Schieve LA, Peterson HB, Meikle SF, Jeng G, Danel I, Burnett NM, Wilcox LS. Live-birth rates and multiple-birth risk using in vitro fertilization. *JAMA* 1999;**282**:1832-1838.

Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med* 2002;**346**:731-737.

Schieve LA, Cohen B, Nannini A, Ferre C, Reynolds MA, Zhang Z, Jeng G, Macaluso M, Wright VC. A population-based study of maternal and perinatal outcomes associated with assisted reproductive technology in Massachusetts. *Maternal Child Health J* 2007;**11**:517-525.

Shebl O, Ebner T, Sommergruber M, Sir A, Tews G. Birth weight is lower for survivors of the vanishing twin syndrome: a case-control study. *Fertil Steril* 2008;90:310-314.

Shevell T, Malcone FD, Vidaver J, Porter TF, Luthy DA, Comstock CH, Hankins GD, Eddleman K, Dolan S, Dugoff L, Craigo S, Timor IE, Carr SR, Wolfe HM, Bianchi DW, D'Alton ME. Assisted reproductive technology and pregnancy outcome. *Obstet Gynecol* 2005;**106**:517-525.

Sherwood OD. Relaxin's physiological role and other diverse actions. *Endocr Rev* 2004; **25**:205-234.

Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, Baker HW. Factors affecting low birth weight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. *Hum Reprod* 2008; **23**:1644-1653.

Statistics Sweden, www.scb.se

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

Strömberg B, Dahlquist G, Ericson A, Finnström O, Köster M, Stjenquist K. Neurological sequelae in children born after in-vitro fertilization: a population-based study. *Lancet* 2002; **359**:461-465.

Sunderam S, Kissin DM, Flowers L, Anderson JE, Folger SG, Jamieson DJ, Barfield WD; Division of Reproductive Health. Assisted reproductive technology surveillance - United States, 2009. *MMWR Surveill Summ* 2012; **61**:1-23.

The Swedish Medical Birth Registry - a Summary of Content and Quality, 2003; available at: http://www.socialstyrelsen.se/publikationer//2003/2003-112-3.

Thomson F, Shanbhag S, Templeton A, Bhattacharya S Obstetric outcome in women with subfertility. *BJOG* 2005;**112**:632-637.

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

Thurin-Kjellberg A, Carlsson P, Bergh C. Randomized single versus double embryo transfer: obstetric and paediatric outcome and a cost-effectiveness analysis. *Hum Reprod* 2006;**21**:210-216.

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

Wang JX, Clark AM, Kirby CA, Philipsson G, Petrucco O, Anderson G, Matthews CD. The obstetric outcome of singleton pregnancies following *in-vitro* fertilization/ gamete intra–Fallopian transfer. *Hum Reprod* 1994;**9**:141-146.

Wang YA, Sullivan EA, Black D, Dean J, Bryant J, Chapman M. Preterm birth and low birth weight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. *Fertil Steril* 2005; **83**:1650-1658.

Wang YA, Sullivan EA, Healy DL, Black DA. Perinatal outcomes after assisted reproductive technology treatment in Australia and New Zeeland: single versus double embryo transfer. *The Medical Journal of Australia* 2009;**190**:234-237.

Welmerink DB, Voigt LP, Daling JR, Mueller PH. Infertility treatment use in relation to selected adverse birth outcome. *Fertil Steril* 2010;**94**:2580-2586.

Wennerholm UB, Soderstrom-Anttila V, Bergh C, Aittomaki K, Hazekamp J, Nygren KG, *et al.* Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. *Hum Reprod* 2009;**20:**2158-2172.

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

Wolner-Hansen P, Rydhstrom H. Cost-effectiveness analysis of in vitro fertilization: estimated costs per successful pregnancy after transfer of one or two embryos. *Hum Reprod* 1998;1**3**:88-94.

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