IN VITRO FERTILIZATION IN NORTHERN FINLAND 1990-1995

Prenatal and early childhood outcome until three years of age

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Abstract

The aim of this population-based cohort study was to evaluate prenatal and child outcome and costs resulting from prenatal and neonatal care after in vitro fertilization (IVF) in comparison to those after natural conception using a cohort of 304 IVF exposed children born between 1990–1995 in Northern Finland, and two cohorts of unexposed control children (I: n = 569, representing general population in proportion of multiple births; II: n = 103, matched for plurality). The control children were randomly chosen from the Finnish Medical Birth Register (FMBR) and matched for sex, year of birth, area of residence, parity, maternal age and socioeconomic status. Analyses were performed by comparing the whole IVF population with controls representing general population as well as stratifying by singleton or twin status.

IVF mothers carried a higher risk for vaginal bleeding, threatened preterm birth and intrahepatic cholestasis of pregnancy than control mothers, and they used specialized antenatal care more than others. Neonatal outcome was also poorer after IVF in terms of gestational age, birthweight, morbidity and intensive care treatment. The prevalence of congenital heart malformations (septal defects) was 4-fold for IVF children in comparison to controls. The three year follow-up showed delayed growth and increased morbidity for IVF children, but their psychomotor development was similar to that of the control children. Health care costs were 1.3-fold for IVF singletons in comparison to control singletons, but for twins the costs were equal. Multiple births increased the costs ~3-fold when compared to singleton births.

IVF increased the health risks for the pregnancies and the offspring, seen mostly in the comparison between the whole IVF population and controls representing natural proportion of multiple births, indicating that multiple birth is the strongest determinant of medical outcome after IVF. The effects of fertility therapy and maternal characteristics related to infertility cannot be ruled out at this point. The increased health care costs after IVF were mostly due to the high proportion of multiple births. In order to improve the outcomes and to reduce the health care costs after IVF, the amount of multiple births should be limited to a minimum by using single embryo transfer when possible.

Keywords: abnormalities, child development, fertilization in vitro, health care costs

To my family

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Oulu, April 11th , 2005 Sari Koivurova

Abbreviations

ART	Assisted reproductive technology
ASD	Atrial septal defect
CC	Clomiphene citrate
CI	Confidence interval
CNS	Central nervous system
CWC	Child welfare clinic
DRG	Diagnosis-Related Groups
DZ	Dizygotic
FET	Frozen embryo thaw
FMBR	Finnish Medical Birth Register
FSH	Follicle stimulating hormone
GDM	Gestational diabetes mellitus
GP	General practitioner
ICP	Intrahepatic cholestasis of pregnancy
ICSI	Intracytoplasmic sperm injection
IUI	Intrauterine insemination
IUGR	Intrauterine growth restriction
IVF	In vitro fertilization
LH	Luteinizing hormone
MHC	Maternal health centre
MZ	Monozygotic
NC	Natural conception
NICU	Neonatal intensive care unit
OR	Odds ratio
OS	Ovarian stimulation
PCOS	Polycystic ovary syndrome
PGD	Preimplantation genetic diagnosis
PIH	Pregnancy induced hypertension
PNMR	Perinatal mortality rate
PPROM	Preterm premature rupture of membranes
RDS	Respiratory distress syndrome

RR	Risk ratio
SD	Standard deviation
SGA	Small for gestational age
S	Singleton
T	Twin
VSD	Ventricular septal defect

List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I Koivurova S, Hartikainen A-L, Karinen L, Gissler M, Hemminki E, Martikainen H, Tuomivaara L & Järvelin M-R (2002) The course of pregnancy and delivery and the use of maternal healthcare services after standard IVF in Northern Finland 1990-1995. Hum Reprod 17: 2897-2903.

II Koivurova S, Hartikainen A-L, Gissler M, Hemminki E, Sovio U & Järvelin M-R (2002) Neonatal outcome and congenital malformations in children born after in-vitro fertilization. Hum Reprod 17: 1391-1398.

III Koivurova S, Hartikainen A-L, Sovio U, Gissler M, Hemminki E & Järvelin M-R (2003) Growth, psychomotor development and morbidity up to 3 years of age in children born after IVF. Hum Reprod 18: 2328-2336.

IV Koivurova S, Hartikainen A-L, Gissler M, Hemminki E, Klemetti R & Järvelin M-R (2004) Health care costs resulting from IVF: prenatal and neonatal periods. Hum Reprod 19: 2798-2805.

Contents

1 Introduction

Approximately 10-15% of couples suffer from infertility at some point during their lives (Healy *et al*. 1994, Evers 2002). The incidence of infertility will probably increase in the future due to socioeconomic trends of postponing pregnancy and advanced female ageing (Evers 2002). Therefore, there is an increasing need for treatment of infertility with assisted reproductive techniques (ART).

The development of modern infertility treatments, IVF (in vitro fertilization) and ICSI (intracytoplasmic sperm injection), has given many infertile couples an opportunity to procreate. Infertility treatments are widely used; by the year 2000 more than 500 000 children had been born after ART across the world (Edwards 2002). It is reported that assisted reproduction carries increased pre- and neonatal risks in form of preterm birth, low birth weight and smallness for gestational age (Friedler *et al.* 1992, Balen *et al.* 1993, Gissler *et al.* 1995, Bergh *et al.* 1999, Klemetti *et al.* 2002). Infertility treatments have increased the amount of multiple births globally, and mostly, but not entirely, the adverse effects on obstetric outcome after ART could be mediated through multiple births. Additionally, factors related to infertility itself may predispose to adverse perinatal effects (Basso & Baird 2003). The role of IVF technology on the outcome is also uncertain. These aspects have raised concern over the long-term health of ART offspring as well as over the high expenses resulting from the health care of these children.

The present population-based study was aimed to investigate the prenatal and early childhood outcomes of conventional IVF pregnancies and children in Northern Finland compared to carefully matched spontaneously conceived control children randomly chosen from the Finnish Medical Birth Registry (FMBR). The unique study design was formed so as to be able to explore separately the effects of multiple birth and IVF technology on the outcomes. Furthermore, the costs after IVF and natural births were compared by plurality.

2 Review of the literature

2.1 General aspects of infertility

2.1.1 Epidemiology

Infertility is defined as failure to conceive after one year of unprotected intercourse. People with primary infertility have never conceived, whereas people with secondary infertility have been able to conceive in the past. (Morell 1997.) According to the United Nations, reproductive health is a "state of complete physical, mental and social well-being, and not merely the absence of disease or infirmity in all matters relating to the reproductive system, and to its functions and processes" (Templeton 2000). For many people, involuntary childlessness is a life-crisis.

Difficulties in conceiving, or conceiving the number of children they desire affect 10-15% of couples at some point during their reproductive lives in developed countries (Healy *et al*. 1994, Evers 2002). This situation was the same even in the 19th century, when one marriage in 6.5 was found to be unproductive (Evers 2002). According to some estimates infertility has increased in the past decades (Notkola 1995, Morell 1997, Speroff *et al*. 1999a) concluded from the rise in the number of infertility related medical visits (Healy *et al*. 1994, Morell 1997). The western trend of postponing marriage and pregnancy along with female aging will probably increase the number of infertile people in the future (Evers 2002). There is also some evidence that sperm counts may have declined in some parts of the world, affecting the prevalence of infertility (Templeton 2000). Signs of declining sperm counts have recently been noticed in Finland as well (Jørgensen *et al.* 2002).

2.1.2 Etiology

The causes of infertility are variable; it is suggested that approximately one-third of infertility problems are attributed to female factors, one-third to male factors and one-third to a combination of the two (Fuortes *et al*. 1996). The etiology of infertility is commonly divided into the following categories: tubal and pelvic pathology (35%), male factor (35%), ovulatory dysfunction (15%), unexplained infertility (10%) and others (5%) (Speroff *et al.* 1999a). In many cases infertility has multiple etiologies (Jaffe & Jewelewicz 1991).

The underlying risk factors are also multiple. Fecundity in women decreases after the age of 30 years and and infertility accelerates after 35 years (Healy *et al*. 1994, Speroff 1994). A history of sexually transmitted diseases (especially Chlamydia trachomatis), pelvic inflammatory diseases, endometriosis, extrauterine pregnancies or abdominal surgery may predispose to tubal infertility (Jaffe & Jewelewicz 1991, Thonneau *et al*. 1993). Lifestyle factors such as obesity may have a harmful effect on ovulation (Templeton 2000), smoking (Chandra & Gray 1991, Jaffe & Jewelewicz 1991, Healy *et* al. 1994, Sundby & Schei 1996) and even moderate alcohol consumption (Grodstein *et* al. 1994) have also been linked to decreased fecundability. Increasing interest, particularly regarding male infertility, has been focused on genetic, environmental and occupational factors (Templeton 2000). A history of varicocele, male genital infections or testis damage increases the risk of male infertility (Thonneau *et al.* 1992).

2.1.3 The characteristics of an infertile woman

There are features that characterize women suffering from infertility. They are often older and of lower parity than women with normal reproductive abilities. Their time to pregnancy is also longer than that of other women. These characteristics have a major role in determining the likelihood of a spontaneous pregnancy or the outcome of possible treatment, especially regarding unexplained infertility (Templeton 2000).

2.2 Treatment of infertility

The idea of extracorporeal conception has existed for thousands of years, seen in the ancient Greek legends and in the Bible. Thereafter, a tremendous amount of work with mammalian IVF has preceded the development of IVF in humans. The first mammalian IVF with subsequent birth was reported in 1930 (Pincus 1930). Five years later the first human egg was successfully fertilized in vitro (Menkin & Rock 1935). Ten years after the beginning of their pioneering collaboration, Steptoe and Edwards reported the birth of the first human, Louise Brown, in 1978 (Steptoe & Edwards 1978). Thereafter, the use of IVF spread rapidly worldwide, bringing relief to numerous infertile couples. The

introduction of ICSI in 1992 was the second significant advance in the field of human reproduction (Palermo *et al.* 1992). The most important milestones in the development of assisted reproduction are presented in Table 1.

Table 1. Milestones in the development of human assisted reproduction.

Year	Assisted reproductive technique	Study group
1960	Clomifene citrate in clinical use	
1962	First birth after gonadotropin stimulation	Lunenfeld *et al.*
1978	First birth after IVF	Steptoe & Edwards
1983	First pregnancies after cryopreservation of embryos	Trounson & Mohr
1984	First birth after ovum donation and IVF	Lutjen *et al.*
1990	Preimplantation genetic diagnosis (PGD) first described	Handyside *et al.*
1992	First births after ICSI	Palermo *et al.*

2.2.1 Surgical treatments

Gynecologic surgery has largely been taken over by novel assisted reproductive techniques in the treatment of infertility. However, in case of large, rapidly growing or multiple uterine myomas, endometrial polyps, uterine cavity abnormalities, endometriosis or PCOS (polycystic ovary syndrome) surgery can improve the chance of a spontaneous pregnancy or the outcome of infertility treatment as well as relieve possible clinical symptoms.

2.2.2 Ovulation induction

2.2.2.1 Clomiphene citrate

Clomiphene citrate (CC) is a non-steroidal analog of estradiol that was taken into clinical use in the 1960s. It is still widely used for ovulation induction in anovulatory women. CC enhances the release of pituitary gonadotropins, resulting in follicular recruitment, selection, assertion of dominance and rupture by reducing the negative feedback of endogenous estrogens. (Shoham 2002.) The usual dose is 50-250 mg for five days in the follicular phase. Therapy should be carefully monitored to detect ovulation and should be continued for no longer than 6 months. In properly selected patients, 80% ovulate and approximately 40% become pregnant. Multiple pregnancy rate is about 10%. (Speroff *et al*. 1999b.) Good results have also been noted in treating women with unexplained infertility (Shoham 2002).

2.2.2.2 Gonadotropins

Gonadotropins have been in clinical practise for 40 years; the first child conveived by gonadotropin treatment was born in Israel in 1962 (Shoham 2002). Recombinant gonadotropins have displaced human menopausal gonadotropins allowing subcutaneous administration and diminishing side effects. Gonadotropin therapy should be performed by infertility specialists, since it requires dosage adjusting and careful monitoring of follicular growth (Speroff *et al.* 1999b).

Dominant follicle development is usually achieved by 7-14 days of continuous individual dose administration of recombinant FSH (follicle stimulating hormone). For ovulatory stimulus HCG (human chorionic gonadotropin) is given as a single dose intramuscularly at the point of follicular diameter 15-18 mm. (Speroff *et al.* 1999b.)

The results after gonadotropin induction are similar to CC induction with a similarly elevated risk of multiple pregnancy. A three-fold risk for monozycotic twinning after ovulation induction has been reported. (Speroff *et al.* 1999b).

Ovarian hyperstimulation syndrome (OHSS) is a severe complication of ovulation induction with an incidence of 1-10% (Inki & Anttila 1997). In mild cases the syndrome includes ovarian enlargement, abdominal distension and weight gain. In severe cases (1-2%) the situation can be life-threatening; ascites, pleural effusion, electrolyte imbalance and hypovolemia can lead to adult respiratory distress syndrome with a 50% mortality rate. Treatment is conservative and empiric. Women with PCOS are at greatest risk for OHSS. (Speroff *et al.* 1999b.)

After six unsuccessful gonadotropin cycles, ART is recommended (Speroff *et al.* 1999b).

2.2.2.3 Bromocriptine

Bromocriptine is a dopamine antagonist that directly inhibits pituitary secretion of prolactin. It is used to induce ovulation in cases of hyperprolactinemia or galactorrea. Once the patients reach normoprolactinemia ovulatory menses and pregnancy are achieved in 80% of the cases. Bromocriptine is administered daily until pregnancy is detected. (Speroff *et al*. 1999b.) In cases of pituitary macroadenoma the use of bromocriptine should be considered throughout pregnancy (Tiitinen & Hovatta 2004).

2.2.2.4 Metformin

Metformin is a biguanide antihyperglycemic drug used to treat noninsulin-dependent diabetes mellitus to lower blood glucose. It improves insulin sensitivity and reduces gluconeogenesis without stimulating insulin secretion. It has been used for overweight PCOS patients with anovulatory cycles to regulate menstruation and hence to improve fertility. A significant number of these women ovulate and become pregnant. Metformin may also enhance ovarial sensitivity to CC treatment. (Speroff *et al.* 1999b).

2.2.3 Intrauterine insemination

Intrauterine insemination (IUI) is a technique where washed sperm is injected in the uterine cavity during detected ovulation. IUI can be performed during natural or stimulated cycles; ovulation induction enhances the pregnancy rates. IUI has mostly been used in cases unexplained infertility. (Speroff *et al.* 1999b.) Pregnancy rates per cycle are 15-30% using ovulation induction combined with IUI and the risk for multiple pregnancy is greater than for IVF (Hamberger & Janson 1997).

2.2.4 Conventional in vitro fertilization, IVF

IVF is a more advanced method that is nowadays the treatment of choice for many types of infertility (Macklon *et al.* 2002). IVF is usually performed during stimulated cycles. To induce controlled multiple follicular development in IVF stimulation regimens GnRH (gonadotropin releasing hormone) agonist or antagonist is often added to gonadotropin stimulation for ovarian downregulation to prevent premature ovulation (Hamberger & Janson 1997, Speroff *et al.* 1999b). Luteal support is needed after GnRH agonist treatment due to pituitary LH suppression (Speroff *et al.* 1999b). Oocyte retrieval is performed approximately 34-35 hours after the HCG injection using vaginal ultrasonic guidance. The eggs are collected on petri dishes and washed sperm is placed on the dishes in couple of hours for fertilization. Next day the eggs are examined to detect fertilization (the presence of two pronuclei) and abnormal embryos are discarded. (Speroff *et al.* 1999c.) The embryo or embryos are transferred into the uterus on 2^{nd} or 3^{rd} day after retrieval at 4-8-cell stage (Braude & Rowell 2003). According to current Finnish IVF practise, one embryo will usually be transferred at a time to decrease the change of multiple pregnancy (Tiitinen & Hovatta 2004), but worldwide the transfer of two to three or more embryos is still common. Extra embryos can be cryopreserved at pronuclear or cleavage stage (Speroff *et al.* 1999c).

The delivery rates per cycle after IVF are approximately 35% (Speroff *et al.* 1999c). A major factor influencing IVF results is the low fecundity of humans; only 20-30% of normal women under 35 years of age attempting pregnancy in a given cycle are successful (Speroff *et al.* 1999c, Evers 2002). It has been noted that female (ovarian) aging, parity and duration of infertility are more important in determining the outcome of treatment than the clinical diagnosis itself. After 36 years of age pregnancy and live birth rates fall considerably and a longer duration of infertility results in a reduction of live birth rates as well. On the other hand, occurrence of any pregnancy, and especially previous live birth, enhances the outcome of IVF. (Templeton 2000.)

The increased risk of multiple pregnancies after IVF is well documented, but it can be diminished significantly or even eliminated by restricting the number of embryos transferred. In countries with liberal IVF protocols the multiple pregnancy rate is about 35% (Speroff *et al.* 1999c). In Finland the change towards single embryo transfer (SET) has resulted in a significant reduction of multiple births (from 27% in 1992 to 14% in 2002) in the last decade (Stakes 2004), and even lower multiple birth rates (7%) have been achieved in ongoing pregnancies (Martikainen *et al.* 2004).

2.2.5 Intracytoplasmic sperm injection

The development of the micromanipulation technique ICSI has revolutionized the treatment of severe male factor infertility, where conventional IVF has deficiencies. It is the treatment of choice for azoospermia and severe oligozoospermia, for cases with antisperm antibodies or poor fertilization with normal sperm, and for unexplained infertility when other treatments have failed (Speroff *et al.* 1999c). ICSI is also indicated if only a low number of oocytes are retrieved and if oocytes are considered for preimplantation genetic diagnosis (PGD, see chapter 2.2.9) (Palermo *et al.* 2002).

The induction of superovulation and scheduled oocyte retrieval are similar to conventional IVF protocol. Unlike in conventional IVF, cumulus cells that surround the retrieved oocytes are removed before microinjection. Thus, reassurement of oocyte maturity at the second round of meiosis required for ICSI is possible. (Granot & Dekel 2002.) Sperm from the ejaculate, epididymis or testis can be used for injection (Speroff *et al.* 1999c). Immediately after cumulus cell removal the single selected immobilized sperm is injected into the ooplasm bypassing zona pellucida. Similarly to IVF at 48-72 hours after microinjection the embryo or embryos are transferred into the uterine cavity.

Success and multiple pregnancy rates are similar to IVF (Stakes 2004).

2.2.6 Assisted hatching

Assisted hatching is a method where the zona pellucida of the embryo is drilled either by pipette, acid Tyrode's solution or laser at 6-8 cell stage in order to improve the ability of the embryo to get out from the zona and to implantate. It has been shown that zona drilling increases the pregnancy/implantation rate in patients with previous implantation failures. On the other hand, it has been linked to monozygotic (MZ) twinning. (Veiga & Boiso 2002.)

2.2.7 Cryopreservation of embryos

Superovulation in IVF and ICSI protocols often results in multiple oocytes for fertilization. The excess embryos can be frozen for future purposes. Cryopreservation is also safer for the woman because frozen embryos are transferred during natural cycles eliminating the risk of hyperstimulation. Furthermore, total costs can be reduced when one superovulation and oocyte retrieval can lead to many embryo transfers increasing the probability of pregnancy. Ovum donation protocol also requires cryopreservation of embryos in cases where donor and recipient cycles cannot be syncronized. Additionally, cryopreservation enables single embryo transfer protocols. (Tiitinen & Hydén-Granskog 1998.)

2.2.8 Ovum donation

Ovum donation is an option for women suffering from total ovarian infertility such as primary or secondary premature ovarian failure or gonadal dysgenesis. Other indications are repetitive IVF failure, natural menopause (though controversial) and inheritable disorders. (Sauer & Cohen 2002.) The first live birth following oocyte donation and IVF took place in 1984 (Lutjen *et al.* 1984). Infertility treatment with ovum donation follows the principles of IVF. It is shown that women with advanced reproductive age have a much better success with donated oocytes than with their own. (Sauer & Cohen 2002.)

2.2.9 Novel methods

The new methods in assisted reproduction include oocyte cryopreservation, ovarian tissue cryopreservation and transplantation, oocyte in vitro maturation (IVM) and PGD. The cryopreservation techniques are mainly focused on treating women with iatrogenic sterility after cancer treatments. The principle is to harvest oocytes or ovarian tissue prior to cancer treatments and freeze them until pregnancy is desired. The frozen thawed oocytes are fertilized by ICSI technique. Furthermore, cryopreserved ovarian tissue can be autografted either to the remaining ovarian site or to an ectopic site; the oocytes retrieved can be fertilized by IVF. (Pickering & Braude 2003.) IVM technique allows the retrieval of immature oocytes and their maturation in vitro before fertilization by ICSI. This technique reduces the amount of pharmacological intervention, costs and time required to monitor oocytes, along with complications (Söderström-Anttila *et al.* 2005). These techniques are still experimental, although live births have been achieved.

PGD offers an early alternative to prenatal diagnosis by amniocentesis or chorionic villous sampling to detect genetic defects (Yaron *et al.* 2002). Biopsy can be taken periconceptionally from both polar bodies or at preimplantation stage by removing one or two cells from the embryo or multiple cells from the blastocyst (Handyside 2002). Thereafter, only unaffected embryos will be transferred (Pickering & Braude 2003). Only a small number of children have been born after PGD, but no observable detrimental effects of PGD by polar body removal were found in them (Verlinsky *et al.* 1996*,* Strom *et al.* 2000).

2.3 Multiplicity as a phenomenon

Spontaneous multiple pregnancies are rare in general population. The prevalence of natural twin pregnancies varies in different parts of the globe; the lowest prevalence is in Japan, 0.2-0.7%, and the highest in Nigeria, 4.0-5.0%. (Little & Thompson 1988, Raudaskoski & Hartikainen 2004.) In Finland, the prevalence of twinning is 1.2%. (Raudaskoski & Hartikainen 2004.) The geographical variation in twinning rates is due to variation in dizycotic (DZ) twinning rates; MZ twinning rates are considered remarkably constant (0.35%-0.40%) around the world (Little & Thompson 1988, Blickstein 2002).

The predisposing factors of DZ twinning are well known: advanced maternal age, high parity, black race, family history of twinning and nutrition (Raudaskoski & Hartikainen 2004, Schachter *et al.* 2001). In contrast, the factors contributing to MZ twinning are poorly characterized (Schahcter *et al.* 2001). MZ twinning is thought to be a random embryological event with no environmental influences and with little or no genetic influence (Tong *et al.* 1997). On the other hand, maternal age has also been liked to MZ twinning that covers 33% of naturally conceived twins (Raudaskoski & Hartikainen 2004).

The use of infertility treatments has increased multiple birth rates globally. A 20-fold increase in the twinning rate and a 50-fold increase in the triplet rate have been observed after infertility treatments (Blickstein 2002). An increased incidence of MZ twinning (about 1.5%) has been noted among iatrogenic pregnancies (Alikani *et al.* 2003) being 3-4-fold to that observed in untreated population. The underlying causes are still unclear; zona pellucida manipulation (Blickstein 2002, Alikani *et al.* 2003), gonadotropin treatment (Schahcter *et al.* 2001) and blastocyst transfer (Milki *et al.* 2003) have been linked to MZ twinning.

Multiple pregnancies, especially monozygotic ones, increase the risks of adverse prenatal and neonatal events (Campbell & MacGillivray 1988) and are therefore of great clinical and economical importance within the framework of assisted reproduction.

2.4 The outcome of IVF pregnancies

2.4.1 Spontaneous abortions

It is generally estimated that the incidence of first trimester abortion after natural conception is 10-20%. However, tht true incidence is difficult to establish because many early abortions remain unrecognized (Tummers *et al.* 2003). It has been claimed that the spontaneous abortion rate after IVF is slightly elevated (Schenker & Ezra 1994), varying generally between 10-30% by different techniques. (Schröder & Ludwig 2002a.) Studies suggest that the higher incidence of abortions after IVF can be attributed to advanced maternal age and thus to a higher risk for chromosomal aberrations, higher rate of multiple pregnancies with increased pregnancy loss, and the early recognition of IVF pregnancies and abortions due to close monitoring (Schenker & Ezra 1994). An association between subclinical endometrial infection or inflammation and spontaneous abortion after IVF has recently been suggested (Romero *et al.* 2004). However, if we consider the infertility characteristics and the undetected abortions after natural conception the abortion risk is not higher for IVF pregnancies in comparison to general population (Tummers *et al.* 2003).

2.4.2 Ectopic pregnancies

Ectopic pregnancies occur more often in IVF pregnancies (2-8%) than in spontaneous ones (1%), while ICSI pregnancies carry a much lower risk for ectopic site gestation (1-2%) than IVF pregnancies. This can be explained by more common fallopian tube damage among IVF than among ICSI patients. (Schröder & Ludwig 2002a.) Furthermore, large volumes of transfer medium and fundal placement have been suggested as technique related causes of ectopic pregnancies (Schenker & Ezra 1994).

2.4.2.1 Heterotopic pregnancies

The incidence of heterotopic pregnancies, a combination of intrauterine and ectopic implantation, has increased dramatically along with infertility treatments (Tal *et al.* 1996). Heterotopic pregnancies occur in 1% of IVF pregnancies compared to 0.003% of spontaneous ones (Schröder & Ludwig 2002a). The main reasons for the development of heterotopic pregnancies are tubal and pelvic diseases, multiple ovulation and transfer of multiple embryos (Tal *et al.* 1996). Heterotopic pregnancy is no longer a rarity, but a condition one should be aware of while treating infertility patients.

2.4.3 The course of pregnancies

Assisted reproduction exposes pregnancies to many factors that could potentially be harmful for the pregnancy and the fetus: previous hormonal treatment and its potential teratogenic effects, potential mechanical damage to the oocyte caused by retrieval, the potential teratogenic substances in culture mediums or the passing on of genetic defects responsible for sperm impairment (Huber & Ludwig 2002). In addition to fetal and children's health the possible maternal risks have to be considered as well. Early descriptive studies have demonstrated a high risk for vaginal bleeding (24%) during pregnancy (Wennerholm *et al.* 1991), preterm rupture of membranes (13%) (Wennerholm *et al.* 1991), preterm delivery (13-30%) (Rizk *et al.* 1991, Wennerholm *et al.* 1991, Doyle *et al.* 1992, Friedler *et al.* 1992, FIVNAT 1995), pregnancy induced hypertension (PIH) (14%) (Wennerholm *et al.* 1991) and a high Cesarean section rate (45.0-47.3%) (Yuzpe *et al*. 1989, FIVNAT 1995) among IVF pregnancies. These adverse outcomes were mainly due to the high frequency of multiple births (24-27%) after ART (Friedler *et al.* 1992, FIVNAT 1995), but also singleton pregnancies seemed to be at an increased risk (Wennerholm *et al.* 1991, FIVNAT 1995).

2.4.3.1 Review of the controlled studies on singleton IVF pregnancies

The existing controlled studies are mainly retrospective cohort studies, a few register-based studies have been conducted on the course of pregnancy. Controlling for

confounding factors varies markedly between the studies, but almost all were matched for maternal age and parity, the most important confounders on pregnancy outcome. In the following presentation only pregnancies conceived by one of the IVF techniques and controlled with spontaneously conceived pregnancies have been reviewed; studies with other ART pregnancies have been excluded.

Several studies have detected an increased incidence of vaginal bleeding during pregnancy after IVF (Tan *et al.* 1992, Westergaard *et al.* 1999, Koudstaal *et al.* 2000b, Ochsenkühn *et al.* 2003, Jackson *et al.* 2004). Luteal insufficiency, vanishing fetus syndrome and ovarian stimulation have been presented as causes for blood loss during IVF pregnancy (Goldman *et al.* 1988). An increased incidence of placenta previa among IVF pregnancies has been reported (Tan *et al.* 1992, Tanbo *et al.* 1995, Jackson *et al.* 2004) that may partly explain the increased incidence of vaginal bleeding (Koudstaal *et al.* 2000b). It has been suggested that placenta previa after IVF can be related to embryo transfer technique and placement of the embryos in the lower part of the uterus or to uterine contractions induced by the catheter (Tan *et al.* 1992). Additionally, a higher incidence of velamentous and marginal cord insertions has been reported (Englert *et al.* 1987, Daniel *et al.* 1999, Schachter *et al.* 2002).

An increased incidence of gestational hypertension and/or preeclampsia has been linked to IVF pregnancies even after matching for maternal age (Tan *et al.* 1992, Tanbo *et al.* 1995, Tallo *et al.* 1995, Maman *et al.* 1998, Ochsenkühn *et al.* 2003, Jackson *et al.* 2004). It has been speculated that PIH after IVF could be related to the relatively high prevalence of PCOS among women undergoing IVF (Maman *et al.* 1998), since patients with PCOS are shown to be at an increased risk for cardiovascular diseases (Taponen *et al.* 2004). Also other pre-existing metabolic-vascular state of patients (Pouta *et al.* 2004) or the hormonal milieu induced during IVF may predispose to PIH (Maman *et al.* 1998). Tanbo *et al*. (1995) suggested that assisted reproduction or infertility itself might also predispose to PIH in some way. The Israeli research team of Maman *et al*. (1998) also noted an increased incidence of gestational diabetes mellitus (GDM) among IVF singleton pregnancies and explained that this phenomenon could arise from the same predisposing factors as PIH.

A more rarely reported complication of IVF singleton pregnancies is intrauterine growth restriction (IUGR). In a British study the relative risk for IUGR was 1.4-fold to that of spontaneous controls, which was suggested to be related to the underlying characteristics regarding infertility (Tan *et al.* 1992). The use of human menopausal gonadotropins has been associated with increases in insulin-like growth factor-binding protein 1, which has been linked to IUGR. In ART pregnancies altered levels of other endometrial proteins or structural abnormalities of the placenta may also contribute to IUGR (Schieve *et al.* 2002). One factor that may be involved in the increased incidence of IUGR among IVF pregnancies is maternal hypertension (Schieve *et al.* 2002) related to advanced age or pre-existing metabolic disturbances of the mothers.

Most of the studies comparing IVF singleton pregnancies to spontaneous ones have reported a significantly increased risk after IVF for threatened preterm birth, premature rupture of membranes or preterm birth that occurs in 10-23% of singleton IVF pregnancies in comparison to a 1-10% incidence among natural singleton pregnancies (MRC Working Party 1990, Tan *et al.* 1992, Olivennes *et al.* 1993, Wang *et al.* 1994, Tallo *et al.* 1995, Tanbo *et al.* 1995, Verlaenen *et al.* 1995, Koudstaal *et al.* 2000b, Tough

et al. 2000, Wang *et al.* 2002, Zádori *et al.* 2003, Helmerhorst *et al.* 2004, Jackson *et al.* 2004, McGovern *et al.* 2004). The underlying reasons for this phenomenon are unclear. An association between bleeding during pregnancy, a common complication of IVF pregnancies, and preterm birth has been noted earlier (MRC Working Party 1990, Doyle *et al.* 1992, Sipilä *et al.* 1992). Also maternal hypertension probably acts as a risk factor for preterm birth (MRC Working Party 1990, Doyle *et al.* 1992). In an Australian study, primiparae, especially those <30 years old, were at a very high risk of preterm birth compared to multiparae and to normal obstetric population (25.0% vs. 6.2%) (Wang *et al.* 1994). Infertility itself has been linked to preterm birth (Ghazi *et al.* 1991, Basso & Baird 2003) and according to Wang *et al*. (1994) especially women with unexplained infertility seem to be at the highest risk. However, the latter was not confirmed later in a Finnish study (Isaksson *et al.* 2002). Some researchers have suggested that ovarian stimulation may play some role in preterm birth after IVF by elevating serum relaxin concentrations (Olivennes *et al.* 1993, Koudstaal *et al.* 2000b, McGovern *et al.* 2004), which in turn increases the collagen breakdown needed for cervical dilatation (McGovern *et al.* 2004), but this remains unclear at the moment. An American study noted that singletons and twins born following vanishing embryo syndrome are born earlier than children from unreduced singleton and twin births, suggesting that the principal reason for preterm birth after IVF is the transfer of multiple embryos (Dickey *et al.* 2002). Quite recently, it was suggested that subclinical endometrial infection or inflammation may play a role in preterm birth after IVF via trophoblast apoptosis caused by microbial products and host-inflammatory mediators such as cytokines and chemokines, since a higher prevalence of bacterial vaginosis (25%) has been detected in patients undergoing IVF (Romero *et al.* 2004). Furthermore, IVF pregnancies carry an increased risk for labour induction (Tallo *et al.* 1995, Dhont *et al.* 1997, Westergaard *et al.* 1999) and Cesarean delivery (Helmerhorst *et al.* 2004), and therefore the gestational length of the pregnancy may also be influenced by iatrogenic factors. The problem with preterm birth is of major importance, since it is a prominent determinant of neo- and postnatal outcome. According to recent meta-analyses on singleton perinatal outcome after IVF, assisted reproduction doubles the risk of preterm birth in comparison to natural conception (Helmerhorst *et al.* 2004, Jackson *et al.* 2004, McGovern *et al.* 2004). Therefore, assisted reproduction can be used as a predictor for preterm birth (Helmerhorst *et al.* 2004).

2.4.3.2 Review of the controlled studies on multiple IVF pregnancies

The studies on multiple pregnancy outcome after ART are mainly conducted on twins and show variable results. To our knowledge, four twin studies have not found any significant outcome differences between ART and natural twin pregnancies (Olivennes *et al.* 1996a, Ágústsson *et al.* 1997, Putterman *et al.* 2003, Pinborg *et al.* 2004b), one study stratifying for zygosity and others not. However, five studies have reported adverse IVF twin pregnancy outcomes in comparison to spontaneous ones: increased incidence of bleeding during pregnancy (Daniel *et al.* 2000, Koudstaal *et al.* 2000a), PIH, premature contractions and IUGR (Daniel *et al.* 2000) and preterm birth (Dhont *et al.* 1997, Moise *et al.* 1998, Manoura *et al.* 2004). The study by Moise *et al.* (1998) was conducted on

dizygotic twin pregnancies and the studies by Dhont *et al.* (1997) and Koudstaal *et al.* (2000a) were matched for zygosity, whereas the studies by Daniel *et al*. (2000) and Manoura *et al.* (2004) were non-matched in this respect.

Studies on triplet or higher order IVF pregnancy outcomes are scarce and non-controlled, obviously due to lack of proper control groups. A higher incidence of antenatal complications such as preterm labour, PIH and GDM has been found among triplet and quadruplet IVF pregnancies in comparison to IVF twin pregnancies (Seoud *et al.* 1992).

The differences between the twin studies can mostly be explained by the variations in the matching criteria, four of the studies being even non-matched. The studies by Dhont *et al.* (1997) and Pinborg *et al.* (2004b) used a population-based study design while others had hospital-based control groups possibly serving as a source of selection bias. The comparison of IVF and spontaneous multiple pregnancies is further complicated by the obvious influence of zygosity-chorionicity on the pregnancy outcome. IVF technique with possible microinjection affects the zygosity rate in ART pregnancies, but this effect is two-sided. IVF twin pregnancies are more often dizygotic than spontaneous ones (Minakami *et al.* 1998, Koudstaal *et al.* 2000a) due to the transfer of separate multiple embryos. On the other hand, it has been noted that ART augments zygotic splitting by a mechanism that is yet unknown (Blickstein 2002), thus increasing the incidence of MZ twinning among ART pregnancies in comparison to general population. These phenomena interfere with the interpretation of the twin pregnancy outcome results on adequate research.

2.4.4 Delivery

Numerous studies have shown an elevated incidence of Cesarean births after ART singleton or twin pregnancies when compared to control pregnancies (Tan *et al.* 1992, Olivennes *et al.* 1993, Tanbo *et al.* 1995, Verlaenen *et al.* 1995, Ágústsson *et al.* 1997, Bernasko *et al.* 1997, Reubinoff *et al.* 1997, Maman *et al.* 1998, Westergaard *et al.* 1999, Daniel *et al.* 2000, Helmerhorst *et al.* 2004, Jackson *et al.* 2004). The authors explained this by the increased anxiety on the part of physicians and patients surrounding the management of these "premium pregnancies" (Tan *et al.* 1992, Tanbo *et al.* 1995, Reubinoff *et al.* 1997, Maman *et al.* 1998). Only one study has reported intrapartum events; cephalopelvic disproportion, prolonged labor and prolonged second stage of labor were reported to occur significantly more often in singleton IVF deliveries than in control ones (Zádori *et al.* 2003).

2.5 Neonatal outcome after IVF

2.5.1 Singletons

2.5.1.1 Mortality

In the present literature the stillbirth rates among IVF children vary between 4.3-39.7/1000 births (MRC Working Party 1990, Rizk *et al.* 1991, Friedler *et al.* 1992, Tan *et al.* 1992, Balen *et al.* 1993, Rufat *et al.* 1994, FIVNAT 1995, Tanbo *et al.* 1995, Westergaard *et al.* 1999).

The perinatal mortality rates among IVF children are reported to be substantially higher than in the general population (MRC Working Party 1990, Rizk *et al.* 1991, Friedler *et al.* 1992, FIVNAT 1995) and they vary between 8.2-38.2/1000 births (MRC Working Party 1990, Rizk *et al.* 1991, Friedler *et al.* 1992, Tan *et al.* 1992, Olivennes *et al.* 1993, Rufat *et al.* 1994, Gissler *et al.* 1995, Tanbo *et al.* 1995, Ágústsson *et al.* 1997, Bergh *et al.* 1999, Koudstaal *et al.* 2000a, Koudstaal *et al.* 2000b, Klemetti *et al.* 2002). In two recent meta-analyses the risk for perinatal mortality was 1.68-2.19-fold with statistical significance for IVF singletons in comparison to spontaneously conceived controls (Helmerhorst *et al.* 2004, Jackson *et al.* 2004). Reversely, for twins, 40% lower perinatal mortality was seen after assisted compared natural conception (Helmerhorst *et al.* 2004).

According to present literature the neonatal mortality rates of IVF neonates vary between 14.1-19.2/1000 births (MRC Working Party 1990, Rizk 1991, Rufat *et al.* 1994, FIVNAT 1995). Danish researchers have recently shown that the risk of neonatal death increases with increasing time to pregnancy, suggesting that infertility is an independent risk factor of neonatal death (Basso & Olsen 2005).

The stillbirth rates and perinatal and neonatal mortality rates vary by country due to differences in the definitions of birth used in the mortality calculations. These mortality rates after IVF are generally 2-3-fold compared to those reported for general populations (MRC Working Party 1990, Rizk *et al.* 1991, FIVNAT 1995), but they mainly reflect the high proportion of multiple and premature IVF births that are closely correlated with especially perinatal mortality (MRC Working Party 1990, FIVNAT 1995).

2.5.1.2 Birth weight

In the following presentation only controlled studies are presented. Numerous studies have shown that IVF children are born smaller, in terms of mean birth weight, than children born after natural conception (Tallo *et al.* 1995, Verlaenen *et al.* 1995, D'Souza *et al.* 1997, Westergaard *et al.* 1999, Ochsenkühn *et al.* 2003). This is a natural consequence of the increased incidence of preterm birth among IVF pregnancies in comparison to spontaneous ones. Clinically more importantly, IVF singletons carry an

increased risk for low birth weight (< 2500 g) (Tan *et al.* 1992, Olivennes *et al.* 1993, Gissler *et al.* 1995, Tanbo *et al.* 1995, Westergaard *et al.* 1999, Tough *et al.* 2000, Schieve *et al.* 2002) and very low birth weight (< 1500 g) (Gissler *et al.* 1995, Bergh *et al.* 1999, Tough *et al.* 2000). Recent meta-analyses also support these results: the risks for low birth weight after IVF was 1.70-1.77-fold and for very low birth weight 2.70-3.00-fold for IVF singletons (Helmerhorst *et al.* 2004, Jackson *et al.* 2004). The underlying reasons for low birth weight adjusted for gestational age after IVF are mostly similar to those contributing to premature birth after IVF described earlier. An association that was independent of multiple births has been noted between fertility therapy or subfertility without treatment and very low birth weight. The increased risk of very low birth weight in the subfertility group suggests an association between infertility history and a lessened capacity to maintain a healthy pregnancy. (McElrath & Wise 1997.)

2.5.1.3 Smallness for gestational age

In addition to the increased risk for gestational age adjusted low birth weight also increased risk for smallness for gestational age (SGA) has been noted among IVF singletons (Tan *et al.* 1992, Olivennes *et al.* 1993, Wang *et al.* 1994, Koudstaal *et al.* 2000b, Schieve *et al.* 2002, Helmerhorst *et al.* 2004, Jackson *et al.* 2004) which is in accordance with the increased incidence of IUGR after IVF. The reasons for this remain unclear. However, it has been suggested to be associated with the underlying condition of the infertile women rather than ART itself (Schieve *et al.* 2002). Such factors could be unexplained infertility (Wang *et al.* 1994), male infertility, hypertension and bleeding during pregnancy (Doyle *et al.* 1992). It has also been speculated that culture media related factors may play a role (Koudstaal *et al.* 2000b). Some studies have shown that the placental:fetal weight ratio is significantly higher in the IVF pregnancies than in control pregnancies, suggesting that the increased incidence of SGA is not due to placental insufficiency (Daniel *et al.* 1999, Koudstaal *et al.* 2000b). Fortunately, the incidence of term low birth weight after singleton IVF pregnancies has shown a 64% decline in the United States from 1996 to 2000 (Schieve *et al.* 2004).

2.5.1.4 Neonatal morbidity

According to five studies we were able to find, singleton neonates born after IVF need significantly more often neonatal intensive care (NICU) treatment than singletons conceived naturally (Gissler *et al.* 1995, Tanbo *et al.* 1995, Koudstaal *et al.* 2000b, Helmerhorst *et al.* 2004, Jackson *et al.* 2004), indicating that neonatal condition is worse after IVF. In a Finnish study singleton IVF newborns had significantly more often low 1 min Apgar score (0-6) than other newborns (Gissler *et al.* 1995). Another study showed a significantly higher rate of neonatal morbidity defined as severe prematurity, difficulty in feeding, respiratory problems, use of antibiotics and/or severe hyperbilirubinemia after IVF than after spontaneous conception (Yeh *et al.* 1990). Four studies, however, have shown comparable perinatal outcome in terms of morbidity and intensive care admissions

(Verlaenen *et al.* 1995, Dhont *et al.* 1997, Reubinoff *et al.* 1997, Ochsenkühn *et al.* 2003). Three of these latter studies used hospital-based control groups which may have caused some bias and diminished the differences between the comparison groups.

A Finnish study compared the perinatal health of IVF children in the early and late 1990s. The perinatal health of IVF children, in terms of preterm birth, birthweight and perinatal mortality, improved over time due to a decrease in high order multiple births, but still remained poorer than that of other children. (Klemetti *et al.* 2002.)

Generally, well-designed and controlled studies are few, and we summarize the neonatal outcomes after IVF in Table 2.

Table 2. Literature summary table of main neonatal outcomes after IVF in comparison to neonates born after natural conception.

Outcome	Observation	Comment
Mortality	The perinatal and neonatal mortality rates are 2- to 3-fold compred to those for general populations.	Reflects the high proportion of multiple pregnancies and consequent phenomena after IVF, but may also reflect the effect of infertility itself.
Gestational age	IVF neonates have an increased risk for preterm birth.	Mostly a result of multiplicity, but factors related to infertility or IVF technique may also be partly responsible.
Birth weight	IVF neonates carry an increased risk for low and very low birth weight.	A result of the increased incidence of preterm birth after IVF.
SGA	The risk of SGA is increased for the IVF neonates.	The reasons are unclear, but maternal factors associated with infertility, such as advanced age or PCOS resulting in adverse metabolic conditions, may have an impact on this matter.
Morbidity	IVF neonates need more intensive care treatment indicating that their perinatal health is worse than that of other neonates.	Mostly a result of multiplicity.

2.5.2 Twins

The numerous controlled studies on neonatal twin outcome after IVF have shown conflicting results similarly to the data on the course of twin pregnancy itself. Six studies have noted a poorer outcome for IVF twins: a lower mean birth weight (Moise *et al.* 1998, Koudstaal *et al.* 2000a, Manoura *et al.* 2004) or an increased incidence of low (Bernasko *et al.* 1997) and very low birth weight (Moise *et al.* 1998) for IVF twins in comparison to control twins. An increased discordance rate has been reported by some authors as well (Bernasko *et al.* 1997, Daniel *et al.* 2000, Koudstaal *et al.* 2000a, Pinborg *et al.* 2004a). The risk of discordant birth weight seems to be greater for twins of opposite sex (Bernasko *et al.* 1997, Pinborg *et al.* 2004a). In a Greek study IVF twins were more often treated in intensive care units with significantly longer durations of hospitalization.

Furthermore, among preterm IVF twins the incidence of intraventricular hemorrhage, which predicts neurological sequelae, was significantly higher than in control twins. (Manoura *et al.* 2004.) Moise *et al.* (1998) also showed a higher general morbidity as well as a higher perinatal mortality for IVF twins than for controls.

Equally, six studies have reported a comparable neonatal outcome between IVF and naturally conceived twins (Olivennes *et al.* 1996a, Ágústsson *et al.* 1997, Pinborg *et al.* 2003, Putterman *et al.* 2003, Pinborg *et al.* 2004a, Pinborg *et al.* 2004b). Most of these studies were adjusted for maternal age and parity. Furthermore, an even lower risk for adverse neonatal outcome has been described in induced twinning, and this has been explained with the lower frequency of MZ twinning after ART (Fitzsimmons *et al.* 1998, Minakami *et al.* 1998).

As discussed earlier, the comparison of different twin studies is difficult due to great variation in matching. Regarding neonatal outcome, only studies by Pinborg *et al.* (2004a,b) had sufficient power to detect differences between the groups. Additionally, with the exception of studies by Dhont *et al.* (1997) and Pinborg *et al.* (2004a,b), the control groups of the twin studies are recruited from hospitals, causing selection bias and probably diminishing the differences between the groups.

In conclusion, the neonatal IVF twin outcome seems to be comparable to that after natural conception, suggesting major influence of multiplicity and zygosity-chorionicity on the outcome. In comparison to IVF singletons, IVF twins have shown a considerably poorer neonatal outcome (Pinborg *et al.* 2004c).

2.6 Congenital malformations after IVF

After the early report by Lancaster (1987) of the increased incidence of spina bifida and transposition of great vessels after IVF, there has been concern over the safety of ART regarding congenital malformations. Theoretically, IVF technology may carry an increased risk for congenital malformations due to induction of chromosomal aberrations, increase in fertilization rate by abnormal semen or due to possible physical or chemical teratogens (Schröder & Ludwig 2002c). However, most studies on the subject have demonstrated low, roughly 2.6%, malformation rates after IVF that are comparable to the rates in general populations (MRC Working Party 1990, Rizk *et al.* 1991, Friedler *et al.* 1992, Rufat *et al.* 1994, FIVNAT 1995, Tanbo *et al.* 1995, Koudstaal *et al.* 2000a, Koudstaal *et al.* 2000b, Anthony *et al.* 2002). Others have reported higher rates varying between 4.6-9.0% (Yeh *et al.* 1990, Verlaenen *et al.* 1995, Palermo *et al.* 1996, D'Souza *et al.* 1997, Bergh *et al.* 1999, Westergaard *et al.* 1999, Hansen *et al.* 2002, Klemetti *et al.* in press). In most of the studies mentioned above, the diagnoses of malformations were set neonatally and only a few have taken into account possible therapeutic abortions due to malformations.

Similarly, most controlled studies have failed to show any significant differences in the incidence of congenital malformations between IVF and control children (Tanbo *et al.* 1995, D'Souza *et al.* 1997, Westergaard *et al.* 1999, Koudstaal *et al.* 2000a, Koudstaal *et al.* 2000b). However, also opposite results have recently been presented. Swedish register-based studies with large study groups and population-based control groups have

shown an increased risk for neural tube defects, gastrointestinal atresias (Bergh *et al.* 1999, Ericson & Källén 2001, Källén *et al.* 2005) and omphalocele (Ericson & Källén 2001) after IVF. An Australian population-based register study presented a two-fold risk for a major birth defect after IVF in comparison to natural conception with an excess of cardiovascular, musculoskeletal and urogenital defects in the IVF group (Hansen *et al.* 2002). A Dutch study noted a slightly increased overall risk for congenital malformations in IVF children (OR 1.20, CI 95% 1.01-1.43), which disappeared after controlling for maternal age and parity. The risk for IVF children did, however, appear higher for cardiovascular defects. (Anthony *et al.* 2002.) In a Finnish unpublished data especially IVF boys were found to be at an increased risk for a major congenital malformation (Klemetti *et al.* in press). Furthermore, Silver *et al.* (1999) noted a 5-fold risk for hypospadias in IVF boys in comparison to control boys, but this has not been repeated by others when standard IVF is considered (Ericson & Källén 2001). A recent meta-analysis pooled the results of seven adequate malformation studies showing a significantly increased 30-40% risk for malformations after ART (OR 1.40, 95% CI 1.28-1.53) (Hansen *et al.* 2005).

The factors contributing to the elevated risk for congenital malformations after IVF seen in the recent studies are difficult to ascertain. The general excess risk may be attributable to parental characteristics (Ericson & Källén 2001, Anthony *et al.* 2002, Källén *et al.* 2005) and to the underlying causes of infertility (Hansen *et al.* 2002). Multiple birth may associate with some of the increased risk since an excess of malformations has been found in twins compared to singletons (Mastroiacovo *et al.* 1999). The use of ovulation induction agents has been discussed in the context of congenital malformations after IVF (Klemetti *et al.* in press), and the excess of hypospadias among IVF boys has been linked to maternal progesterone administration or other underlying endocrine abnormalities (Silver *et al.* 1999). The excess of neural tube defects may partly be explained by the possible differences in the attitudes toward therapeutic abortion between infertile and other people (Bergh *et al.* 1999, Ericson & Källén 2001). Gastrointestinal atresias may be associated with the same type of disturbance which increases the risk for MZ twinning after IVF, since there is a connection between gastrointestinal atresia and MZ twinning (Ericson & Källén 2001). Ericson and Källén (2001) concluded that the latter may be a direct effect of the IVF procedure, though others have stated that IVF itself is not to blame for the excess of congenital malformations after IVF (Anthony *et al.* 2002).

One has to be cautious in interpreting the results of malformation studies since the malformations are defined very variably in different countries, and studies do mostly concern only the early neonatal period.

2.7 Genetic disturbances after IVF

The debate on the possibly increased incidence of spontaneous abortions after IVF has led to the theory that there might be an increased risk of chromosomal abnormalities in these embryos. The rate of chromosomal abnormalities in live births is 0.6%, while in

spontaneous abortions it is 60%, and after IVF procedure the rate has been reported to be 69-75%. (Schröder & Ludwig 2002b.)

A recent controlled study found a significant increase in the incidence of chromosomal abnormalities in IVF infants when compared to naturally conceived infants (Hansen *et al.* 2002). No differences were found between IVF and ICSI infants (Bonduelle *et al.* 2002, Hansen *et al.* 2002). The chromosomal abnormalities in IVF infants were mostly related to advanced maternal age (Bonduelle *et al.* 2002). Couples entering an IVF programme have been reported to have an increased incidence (2.4%) of chromosomal abnormalities naturally contributing to the risk of transmitted chromosomal abnormalities. Furthermore, the role of ovulation induction on this matter has been discussed, but no evidence is available. (Schröder & Ludwig 2002b.)

Recently, concern has been raised about the possible increased incidence of genetic syndromes such as Beckwith-Wiedemann and Angelman syndromes due to defects in genomic imprinting after assisted reproduction. A preliminary Australian case-control study stated that the risk of Beckwith-Wiedemann syndrome after IVF was 9-fold (1/4000) in comparison to general population (1/35580) (Halliday *et al.* 2004), but a large cohort study with 6052 IVF singletons found no increased risk for imprinting diseases after IVF (Lidegaard *et al.* 2005). Genomic imprinting defects are inherited either from the maternal or the paternal side, depending on the syndrome in question. Experimental reports in mice have raised the question that some of the steps involved in ART, such as ovarian hyperstimulation or certain culture media, might be harmful to the formation of genomic imprints, but this needs to be evaluated in further studies (Paoloni-Giagobino & Chaillet 2004).

2.8 Early childhood outcome after IVF

2.8.1 Infant mortality

Infant mortality rates among IVF children have been reported by only four studies. In the present literature it varies between 11.3-23.7/1000 births (MRC Working Party 1990, Rizk *et al.* 1991, Rufat *et al.* 1994, Westergaard *et al.* 1999). MRC Working Party (1990) and Rizk *et al.* (1991) reported figures 2- to 3-fold compared to those of national average (23.7/1000 and 21.1/1000, respectively). The excess infant mortality was accounted for by the high proportion of multiple births among IVF population. (MRC Working Party 1990, Rizk *et al.* 1991.) In the Danish register study mortality was lower, and similar among IVF and control children that represented a Danish birth cohort in 1995 (Westergaard *et al.* 1999).

2.8.2 Growth

Normal growth has been detected in children born after IVF in the four studies focusing on the topic. A French study without a control group showed no major pathological features in the physical growth of IVF children aged 6-13 years; only 2.2% of them were below 2 SD for weight and 0.3% for height in general growth charts. The SGA children tended to be shorter than others. (Olivennes *et al.* 1997.) Controlled studies with control for counfounding also show reassuring results. Brandes *et al.* (1997) show similar growth between IVF children and their matched controls in a follow-up study of 12-15 months. Both IVF and control twins and triplets had significantly lower physical indices than singletons. (Brandes *et al.* 1997.) Saunders *et al.* (1996) showed normal growth for both groups during the 2- year follow-up; IVF children were even taller than the controls. Wennerholm *et al.* (1998) from Sweden assessed postnatal growth (18 months) between children born after cryopreservation IVF, standard IVF and spontaneous conception, and found similar growth outcomes in the three groups. A recent multi-center cohort study comparing growth at 5 years of age between ICSI, IVF and NC (natural conception) children showed similar growth parameters for all groups (Bonduelle *et al.* 2005). (Table 3.)

2.8.3 Psychomotor development

The fact that IVF increases the risk of adverse obstetric outcomes has raised concern over the long-term psychomotor development of the IVF offspring. However, studies have failed to show any unfavorable results, although they can often be criticized for small sample sizes and short follow-up time.

The earliest study on the subject was conducted on 33 IVF children aged 12-37 months whose mental and psychomotor development tested by Bayley scales was within the normal range (Mushin *et al.* 1986). In a contemporary study the first 20 Australian IVF children showed, on average, faster development than standard population at their first birthday. However, a control group with relevant control for confounding was lacking and no further conclusions on advanced development could be drawn. (Yovich *et al.* 1986.) A controlled American study showed significantly higher Bayley Psychomotor Development Index scores for IVF children aged 12-30 months compared to controls, while mental development was similar between the groups. The authors concluded that the high achievement probably resulted from parental motivation and high social status. (Morin *et al.* 1989.) Others have found normal psychomotor development for IVF children aged 1-3 years without any significant differences in comparison to control children (Raoul-Duval *et al.* 1994, Ron-El *et al.* 1994, Gibson *et al.* 1998, Wennerholm *et al.* 1998). (Table 3.)

2.8.4 Morbidity

There is little data available on the morbidity of children born after IVF. Wennerholm *et al.* (1998) noted no differences in the prevalence of chronic diseases between children born after cryopreservation (18.0%), standard IVF (15.3%) and spontaneous conception (16.7%) during an 18 month follow-up period in a retrospective Swedish cohort study. However, Strömberg *et al.* (2002) also from Sweden, showed an almost 4-fold risk for cerebral palsy in IVF children aged 18 months-14 years in comparison to a population-based control group. The risk was also elevated in analyses stratified for plurality as far as singletons were concerned, but twin analyses showed no significant differences. Suspected developmental delay was also increased 4-fold in IVF children. They concluded that the elevated risks are largely due to high frequency of twin pregnancies after IVF. (Strömberg *et al.* 2002.) This was further supported by Lidegaard *et al.* (2005) who stated an 80% increased risk of cerebral palsy in IVF singletons. A recent study on neurological sequelae in IVF/ICSI twins showed a similar risk between IVF/ICSI twins and naturally conceived twins as well as IVF/ICSI singletons during the 2-7 year follow-up (Pinborg *et al.* 2004d). The same group has previously presented data on long-term (3-4 years) morbidity of IVF/ICSI twins with no significant differences in comparison to non-IVF/ICSI twins. The physical health of IVF/ICSI twins, in terms of disabilities, allergic and chronic disorders and cancer was, however, poorer than that of IVF/ICSI singletons (Pinborg *et al.* 2003). A higher general morbidity at the age of 5 years has been noted for IVF children in comparison to naturally conceived children in a recent multi-center study. They were also more likely to have had surgery for some reason (Bonduelle *et al.* 2005). A Dutch study has suggested an increased risk for retinoblastoma in children born after IVF when compared to general population. This finding is preliminary and needs to be confirmed in a population-based study. (Moll *et al.* 2003.) Others, however, have failed to find any increased risk for childhood cancer after IVF (Doyle *et al.* 1998, Bergh *et al.* 1999, Bruinsma *et al.* 2000, Klip *et al.* 2001). (Table 3 presents the studies with control groups and controlling for confounding, focusing on growth, psychomotor development and morbidity after IVF.)

Table 3. Comparison of the controlled studies with controlling for confounding focusing on growth, psychomotor development and morbidity of IVF children.

Year	Study group	Sample size	Control group	Study design	Matching criteria	Child outcome	Follow-up time
1989	Morin *et al.* (USA)	83 IVF	93 general population	Prospective population based cohort study	age, plurality, sex, race, maternal age, parental education and income	Mental development similar between the groups. The psychomotor development index score was higher in the IVF group	12-30 months
1992	Brandes *et al.* (Israel)	116 IVF	116 hospital-based	Prospective cohort study	birth weight, gestational age, birth order, mode of delivery, sex, age, maternal age and education	IVF children grow and develop similarly to their non-IVF controls.	12-45 months
1994	Ron-El *et al.* (Israel)	30 IVF	30 hospital-based	Prospective cohort study	age, gestational age	Physical, neurological and mental development similar between the groups.	28- 36 months
1994	Raoul-Duval *et al.* (France)	33 IVF 33 OS	33	Prospective cohort study	parity, social class, mothers age, plurality	Satisfactory development for the IVF children.	3 years
1996	Saunders *et al.* (Australia)	314 IVF	150 general population	Prospective population-based cohort study	plurality, gestation, date of birth	Growth of IVF children was normal at 2 years of age.	2 years
1998	Gibson *et al.* (Australia)	65 IVF	63 hospital-based	Prospective cohort study	primiparity, singleton, maternal age >28 years, living with the father	Appropriate general development at 1 year for the IVF children.	1 year
1998	Wennerholm *et al.* (Sweden)	255 FET 255 IVF	252 hospital-based	Retrospective cohort study	maternal age, parity, single or twin pregnancy, date of delivery	Growth, development and chronic illness similar between the groups.	18 months
1999	Bergh *et al.* (Sweden)	5856 IVF	1 505 724 birth cohort 1982-1995	Retrospective register- and population-based cohort study	Stratified by: maternal age, parity, previous subfertility, year of birth, plurality	No increase in childhood cancer after IVF.	Cancers detected at 3 months-2 years of age

FET = Frozen embryo thaw, OS = Ovarian stimulation

Table 3. Continued.

Year	Study group	Sample size	Control group	Study design	Matching criteria	Child outcome	Follow-up time
2001	Klip *et al.* (Netherlands)	9484 IVF	7532 general population	Retrospective population-based register/questionnaire cohort study	Stratified by: maternal age, gestation, birth weight, number of siblings, plurality	No greatly increased risk for childhood cancer after IVF.	6 years on average
2002	Strömberg *et al.* (Sweden)	5680 IVF	11 360 general population	Retrospective register- and population-based cohort study	sex, year of birth, birth hospital	IVF children carry an increased risk of neurological disability, impairment or handicap, especially cerebral palsy.	18 months-14 years (age distribution)
2003	Pinborg *et al.* (Denmark)	472 IVF t 634 IVF s	1132 t general population	Retrospective register- and population-based questionnaire cohort study	Stratified by: maternal age, parity, birth weight	Physical health of IVF/ICSI twins is comparable to control twins but worse than that of IVF/ICSI singletons.	3-4 years
2004 (d)	Pinborg *et al.* (Denmark)	3393 IVF t 5130 IVF s	10 239 t general population	Retrospective register- and population-based cohort study	Stratified by: maternal age, sex, year of birth, IVF or ICSI, low birth weight, low gestational age	IVF twins have similar risk of neurological sequelae as control twins and IVF singletons.	2-7 years
2004 (b)	Bonduelle *et al.* (multi-centre)	437 s 540 ICSI s	538 s school or nursery / general population	Prospective cohort study	age, sex, maternal education, parental socio-economic status	IVF and ICSI children were more likely to have had a significant childhood illness, surgery or medical therapy than NC children. Growth was similar between groups.	5 years
2005	Lidegaard *et al.* (Denmark)	6052 IVF s	442 349 s general population	Retrospective register- and population-based cohort study		No increased risk for imprinting diseases after IVF, but an 80% increased risk for cerebral palsy.	Mean 4.1-4.5 years

FET = Frozen embryo thaw, OS = Ovarian stimulation, NC = Natural conception, s = singleton, t = twin

2.9 The outcome after intracytoplasmic sperm injection

ICSI is a treatment of choice used to overcome male infertility, though it is also widely used to treat other types of infertility as well. For example, in Belgium ICSI is used in 60% of IVF procedures (Sutcliffe 2002). ICSI technology carries some extra causes of concern over the outcome of children conceived in comparison to standard IVF due to different technique and additional paternal genetic disturbances. In the ICSI technique, a single sperm, often severely abnormal, is injected to the oocyte bypassing natural selection and causing mechanical trauma to the oocyte. Infertile men seeking for ICSI often carry genetic anomalies such as congenital bilateral absence of vas deferens (mutation in the gene complex controlling cystic fibrosis), chromosome Y microdeletions or other sex chromosomal or autosomal aberrations that are related to or causes of their infertility problems. All these aspects can theoretically harm the outcome of ICSI offspring. (Sutcliffe 2002.)

The risk of pregnancy loss after ICSI has been found to be similar to that after standard IVF (Wisanto *et al.* 1995, Coulam *et al.* 1996). The studies comparing ICSI pregnancies and neonates with controls from natural conception have shown increased risk for preterm delivery and low birth weight after ICSI (Aytoz *et al.* 1998, Katalinic *et al.* 2004, Peeraer *et al.* 2004, abstract), although this was not seen in the twin analysis where the outcome was similar between IVF/ICSI and non-IVF/ICSI twins (Pinborg *et al.* 2004a). The comparison to IVF pregnancies and neonates has not revealed any significant differences between the two IVF methods as far as obstetric outcome is concerned (Govaerts *et al.* 1998, Van Golde *et al.* 1999, Wennerholm *et al.* 2000b, Bonduelle *et al.* 2002).

Congenital malformation rates after ICSI vary between 2.6-8.6% (Bonduelle *et al.* 1996a, Palermo *et al.* 1996, Wennerholm *et al.* 2000a, Hansen *et al.* 2002, Ludwig & Katalinic 2002, Bonduelle *et al.* 2005). An increased risk for congenital malformations has been suggested after ICSI in comparison to natural conception (Wennerholm *et al.* 2000a, Hansen *et al.* 2002, Ludwig & Katalinic 2002, Bonduelle *et al.* 2005), but when compared to standard IVF the risk was equal (Hansen *et al.* 2002). An excess of hypospadias in ICSI boys has been noted in Sweden and this was suggested to be related to paternal subfertility with a genetic background (Wennerholm *et al.* 2000a, Ericson & Källén 2001). An Australian re-analysis of a Belgian study on birth defects after ICSI found a 4-fold excess of major cardiovascular defects in ICSI children when compared to children born in Western Australia, but this re-analysis was considered to overestimate major defects by the original authors (Kurinczuk & Bower 1997).

The incidence of chromosome anomalies among ICSI children varies between 2.4-2.9% (Bonduelle *et al.* 1996b, Wennerholm *et al.* 2000a, Bonduelle *et al.* 2002) being higher than in general population (0.5%) (Bonduelle *et al.* 2002). A few controlled studies have noted a significantly increased risk for chromosomal aberrations after ICSI when compared to natural conception (Hansen *et al.* 2002, Ludwig & Katalinic 2002). Abnormal fetal karyotypes were found in 2.9% of the prenatally tested ICSI fetuses in Belgium: 1.56% were de novo aberrations and 1.18% were inherited. This higher rate of

chromosomal anomalies in ICSI children could be related to the male infertility itself and to the higher aneuploidy rate in the sperm of the fathers. (Tarlatzis & Bili 1998, Bonduelle *et al.* 2002.) Therefore, it is recommended that karyotyping is performed for the male partners in order to detect pre-existing aberrations (Tarlatzis & Bili 1998) and that couples are informed of the higher risk of transmitted and de novo aberrations (mainly sex-chromosomal) as well as the risk of transmitting fertility problems to the offspring (Bonduelle *et al.* 1998).

An early report on the development of ICSI children from Australia showed an increased risk for mild delays in mental development (memory, problem solving ability and language skills) for ICSI boys in particular at one year when compared with IVF or naturally conceived children. Psychomotor development, on the other hand, showed no significant differences between the groups. According to the authors the mild delays may have been due to chromosomal abnormalities transmitting via ICSI. (Bowen *et al.* 1998.) Later studies, however, have shown normal and comparable mental and psychomotor development for ICSI children aged 1-8 years in comparison to IVF or naturally conceived children (Sutcliffe *et al.* 2001, Bonduelle *et al.* 2003, Leslie *et al.* 2003, Leunens *et al.* 2004, abstract, Place & Englert 2003, Papaligoura *et al.* 2004).

Only six studies have focused on the growth and long-term morbidity of ICSI children. Most of the existing studies have shown normal growth and somatic health after ICSI in children aged 1-8 years when compared to IVF or naturally conceived children (Sutcliffe *et al.* 2001, Pinborg *et al.* 2003, Place & Englert 2003, Belva *et al.* 2004, abstract, Bonduelle *et al.* 2004). However, a recent multi-center study with a higher power than in the other studies showed an increased morbidity after ICSI and IVF in comparison to natural conception at the age of 5 years (Bonduelle *et al.* 2005).

2.10 The outcome after cryopreservation of embryos

Cryopreservation of embryos provides several clinical advantages presented earlier in this review. However, freezing raises concerns over the possible risks for the embryo as a result of the freezing-thawing process. In freezing a reversible cessation of the embryo development is achieved which theoretically may also be harmful in terms of later outcomes. Additionally, many legal, ethical and social questions are raised by human embryo freezing. (Wennerholm 2000c.) On the other hand, some advantages may be achieved by cryopreservation, namely higher parity and natural cycle without the need for ovarian stimulation (Tarlatzis & Grimbizis 1999).

The pregnancies and children conceived after cryopreservation are prone to the well known risks connected to IVF technology in general (Wennerholm 2000c). The risk of pregnancy loss is similar after cryopreservation and fresh IVF or ICSI (Aytoz *et al.* 1999). The studies comparing the prenatal and neonatal outcomes after cryopreservation, fresh embryo IVF and spontaneous conception have not found any adverse outcomes linked to cryopreservation (Wada *et al.* 1994, Wennerholm *et al.* 1997); a Belgian study noted a decreased risk for low birth weight after cryopreservation in comparison to fresh IVF or ICSI (Aytoz *et al.* 1999).

The congenital malformation rates after cryopreservation vary between 1.0-3.3% (Wada *et al.* 1994, Sutcliffe *et al.* 1995b, Wennerholm *et al.* 1997, Aytoz *et al.* 1999), which is comparable to the malformation rates after fresh IVF (Wada *et al.* 1994). Wada *et al*. (1994) suggested that the lower incidence of malformations after freezing could be related to the loss of some unfit embryos during the freezing-thawing process.

The few studies focusing on the postnatal health and development of children born after cryopreservation have not found any pathological features relating to the freezing-thawing process (Sutcliffe *et al.* 1995a, Olivennes *et al.* 1996b, Wennerholm *et al.* 1998).

2.11 The outcome after IVF surrogacy and oocyte donation

The amount of data on IVF surrogacy in which the pregnancy is carried by a non-biological volunteer is scarce at the moment. The outcome of IVF surrogacy pregnancies were compared to those resulting from standard IVF by an American study with reassuring results for the surrogacy group: the occurrence of pregnancy complications (PIH, third trimester bleeding) was 4-5 times lower in the IVF surrogates than in the standard IVF group, and this was independent of multiplicity. Furthermore, singletons born to surrogates had a significantly lower risk for low birth weight than standard IVF singletons. (Parkinson *et al.* 1998.) In a study by Schieve *et al.* (2002) with a small subgroup of gestational carriers, no increased risk for low or very low birth weight was noted after surrogacy when compared to controls after natural conception, indicating an infertility-related rather than treatment-related risk. The incidence of congenital malformations after IVF surrogacy has been found to be within the expected range for spontaneous conceptions. Furthermore, normal speech and motor development was noted at two years of age in children born after IVF surrogacy. The favorable outcome of IVF surrogacy may imply that a gestational carrier provides potential environmental benefits for the infant (Serafini 2001.) supporting the theory of "fetal plasticity" which is defined as "the ability of a single genotype to produce more than one alternative form of morphology, physiological state, and/or behaviour in response to environmental conditions" (Lucas 1991).

Ovum donation offers an opportunity of parenthood for women with ovarian failure or dysfunction. So far, only a limited amount of data is available on the outcome after oocyte donation. A small American study comparing ovum donation pregnancies to standard IVF pregnancies showed a similar rate of maternal complications, such as PIH, GDM and puerperal complications. Preterm birth was, however, significantly decreased and consequently mean birth weight was increased in the ovum recipient group suggesting that transfer of donor oocytes overcomes the poor outcome often seen in infertility patients. The decreased incidence of preterm birth was hypothesized to be a result of decreased serum relaxin levels seen in patients with suppressed ovarian function. (Friedman *et al.* 1996.) The sample size of this study was, however, insufficient to draw any firm conclusions. Poorer perinatal outcomes have also been presented: a Finnish study noted an increased incidence of first trimester vaginal bleeding and PIH after oocyte donation in comparison to standard IVF. Neonates of the ovum recipients were more often hospitalized and more likely to be in hospital 7 days after birth than controls.

These findings could, at least partly, be due to pre-existing subclinical conditions or underlying diseases. (Söderström-Anttila *et al.* 1998a.) Despite the pregnancy complications, the growth, development and general health of children born after oocyte donation is comparable to IVF children (Söderström-Anttila *et al.* 1998b, Seelig & Ludwig 2002).

2.12 Utilization of health care services

As a consequence of the increased incidence of perinatal adverse outcomes after ART the use of health care services is at an elevated level, as shown by the seven studies we were able to find in this field. In a Finnish register study IVF mothers started antenatal care earlier than others, they had more antenatal visits than other mothers and more than 50% were hospitalized during pregnancy (Gissler *et al.* 1995). In another Finnish study IVF mothers were found to have had significantly more antenatal visits than mothers in the IUI or natural conception groups (Nuojua-Huttunen *et al.* 1999). A Danish study on twin pregnancies showed a higher frequency of sick leave and hospitalizations among IVF/ICSI twin pregnancies in comparison to non-IVF/ICSI twin pregnancies (Pinborg *et al.* 2004b). The increased use of health care services during the neonatal period after ART is also well documented as seen in two recent meta-analyses (Helmerhorst *et al.* 2004, Jackson *et al.* 2004).

According to a large register study from Sweden the significantly increased hospitalization for IVF children seen during the neonatal period continued to be significantly increased, although to a lesser extent, to the age of 6 years compared to non-IVF children (Ericson *et al.* 2002). This is supported by a multi-center study showing that IVF (n=437) and ICSI children (n=540) were more likely to be admitted to hospital at the age of 5 years than NC children (n=538) (Bonduelle *et al.* 2005). A smaller study from Australia stated that IVF children were less likely to visit general practitioners or other health care workers during infancy, although they were more likely to utilize the resources of neonatal intensive care units (Leslie *et al.* 1998). A Danish twin study showed equal use of hospital care resources among IVF/ICSI twins and control twins, but in comparison to IVF/ICSI singletons the hospital care resources were over-utilized by IVF/ICSI twins (Pinborg *et al.* 2004e) suggesting the major influence of multiple birth on both long-term outcome of the children as well as on the resulting utilization of health care services after IVF.

The increased use health care services is a result of the adverse perinatal outcomes after IVF, but it may also to some extent reflect the extra anxiety surrounding the management of IVF mothers and offspring.

2.13 Health care costs after IVF

IVF itself is a costly treatment because of the need for highly trained personnel, expensive equipment and medication (Collins 2002). The cost of an IVF cycle varies markedly between countries (Mor-Yosef 1995) due to variable prices, differences in the

calculation methods and funding policies, making international cost comparisons difficult. According to literature the cost of an IVF delivery varies between $19,267-$211,940 (Neumann *et al.* 1994, Stern *et al.* 1995, Hidlebaugh *et al.* 1997, Granberg *et al.* 1998). It has been stated, however, that the costs are generally underestimated (Mor-Yosef 1995). The cost of an IVF cycle includes direct and indirect costs. Direct costs arise from medical consultation and visits, drugs, laboratory charges, ultrasound procedures, IVF procedures and hospital charges. Indirect costs include costs resulting from lost working days and traveling. (Collins 2002.) The cost of a "take home healthy baby" naturally also includes the excess costs arising from prenatal and neonatal care as well as the costs of failed cycles needed to achieve a pregnancy (Mor-Yosef 1995, Stern *et al*. 1995). In a Finnish study the health care costs of one IVF newborn from induction of pregnancy until the age of 7 days were 5.4-fold compared to other newborns (Gissler *et al.* 1995).

The major contributor to the costs resulting from IVF has been suggested to be multiple births, especially high order multiple births (Callahan *et al.* 1994, Goldfarb *et al.* 1996). In a hypothetical calculation study from Sweden it was shown that single embryo transfer may be more cost-efficient than two embryo transfer, since the reduced costs of hospital treatment resulting from the reduction of twin pregnancy rate may compensate the costs of extra cycles needed to achieve the same number of successful pregnancies (Wølner-Hanssen & Rydhstroem 1998). This was confirmed by a Belgian study that showed that the transfer of single top embryo is equally as effective as, but substantially cheaper than double embryo transfer due to significantly higher neonatal cost in the double embryo transfer group (Gerris *et al.* 2004). In another recent study the medical cost per twin pregnancy was more than €10,000 higher than per singleton pregnancy (€13,469 vs. €2,550) (Lukassen *et al.* 2004).

Overall, the studies comparing the health care costs after IVF and natural conception are very few, to our knowledge only Gissler *et al.* (1995) have conducted such a study.

2.14 Summary of the literature review

There is an increasing need for infertility treatments. In the early days of assisted reproduction the main focus was to achieve as high a pregnancy rate as possible. While striving towards this goal infertility treatments have developed and improved offering relief for numerous couples suffering from involuntary childlessness. To date, the IVF procedure is the most widely used method for treating infertility and it seems that it will continue to be so in the future as well. During the past decade evidence has shown that pregnancies conceived by IVF are high-risk ones, the risk of preterm birth being the most prominent adverse event, with increased health risks for the child to be born as well. Multiple birth seems to be the major factor in determining the outcome of IVF children. Therefore the focus of assisted reproduction has been changing towards the "take home healthy baby" rate by reducing the number of iatrogenic multiple births with the development of single embryo transfer protocols.

The poorer neonatal outcome of IVF children has raised concern over their longer-term outcome as well as over the societal costs resulting from the health care of these

children. The few existing follow-up studies are often limited in terms of follow-up time and with the exception of large register studies, they have suffered from insufficient power to detect differences between groups. Studies focusing on health care costs after IVF are even more scarce and usually without control groups. The concern for the health of the IVF offspring and the obvious lack of appropriate data on this topic has been the inspiration for starting this project.

3 Aims and hypotheses of the present study

An increased risk of adverse prenatal and neonatal events such as preterm birth and low birth weight following IVF has previously been documented. There is, however, lack of information on neonatal morbidity and whether the neonatal problems will have an effect on the longer-term outcome of IVF children in terms of growth, psychomotor development and morbidity coming from well-designed controlled studies with controlling for confounders and sufficient power. The hypotheses were that, compared to naturally conceived control children, IVF children have poorer perinatal outcome, grow more slowly, reach developmental milestones later and have a higher morbidity during the first three years of life, because they are more often born from multiple pregnancies than naturally conceived children. The specific aims of the study are:

1. To describe the background characteristics of IVF mothers.
2. To compare the prenatal and neonatal outcome after IVF and natural conception.
3. To find out whether the health of IVF children is similar to control children conceived naturally by the age of three years in terms of growth, psychomotor development and morbidity.
4. To compare the costs of prenatal and neonatal care after IVF and natural conception.
5. To study whether the possible differences are due to the increased incidence of multiple births and related phenomena among IVF children or due to the IVF technology itself.

4 Material and Methods

4.1 Power calculation

To study the outcomes (prenatal and early childhood) and costs of an IVF child, the pre-study sample size calculations were based on clinical developmental outcomes; the approximation of a frequency of, on average, 15% for developmental disorders including neurological signs (gross and fine motor, speech and other disabilities) among unexposed population (Hadders-Algra & Touwen 1990). For 80% power, 0.05 alpha-error, a risk ratio of 1.6 for the outcome between the groups and with a ratio of 1 (exposed):2 (unexposed), a sample size of ≥238 exposed and ≥476 unexposed children was required for comparison between IVF and general population cohorts. For twins, corresponding sample size calculations for 80% power, 0.05 alpha-error and a risk ratio of 1.6 for the outcome between the groups with a ratio of 1:1 assuming a frequency of developmental disorders among unexposed multiples of an average 30%, a sample size of ≥125 per group was required.

For prenatal analyses the number of pregnancies indicated that at least a 1.8 risk ratio was required to detect a difference between the IVF and unexposed singleton groups with a power of 80% at a 95% confidence level, assuming 6-10% occurrence of main pregnancy complications (e.g. preterm birth) in the unexposed population of this age range. Among the mothers of twins, the risk ratio should be at least 2.8 to detect a statistically significant difference between the twin groups.

4.2 Study design and study population

The study design is a retrospective matched cohort study with a cohort of IVF exposed children and two cohorts of unexposed children. The IVF exposed children born in 1990-1995 were recruited from the registers of the infertility outpatient clinics of the University Hospital of Oulu and the Family Federation of Finland in Oulu that cover all

IVF treatments in Northern Finland (i.e. the provinces of Oulu and Lapland, Fig. 1). The study population consists of all IVF children born during the study period.

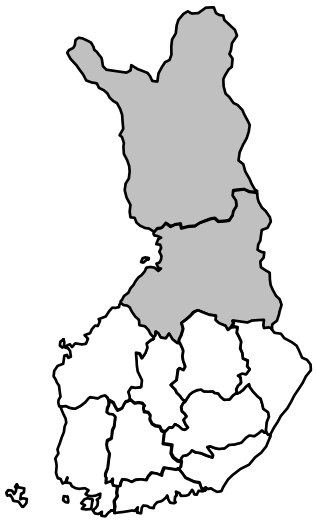

Fig. 1. The catchment area of this study.

There were 306 liveborn (154 singletons, 123 twins, 25 triplets and four quadruplets) and three stillborn (one twin and two triplets) IVF exposed children and fetuses in the study group. One singleton was conceived by ICSI, the rest of the children were born after conventional fresh embryo IVF.

As is known, a frequent outcome of IVF treatment is multiple pregnancy. To explore the effect of multiple birth on the outcome and to examine neonatal outcome, growth, psychomotor development and morbidity two separate unexposed liveborn control groups were randomly chosen from the Finnish Medical Birth Register:

1. 618 live controls (2:1, 2 x 309, at this point we were unaware of the three stillborn fetuses among the IVF population) were chosen at random for all exposed children (full sample control group) and matched in the following order: sex, year of birth, area of residence (i.e. the provinces of Oulu and Lapland), maternal parity, age and socioeconomic status defined by the occupation of the mother [upper white collar, lower white collar, blue collar, entrepreneurs (small business) and farmers, students, housewives and unknown]. This control group represents general population in proportion of multiple births.
2. 152 live controls (1:1) from multiple births for exposed children from multiple births were chosen at random and matched also for plurality in addition to the other matching criteria (for the analyses stratified for plurality).

Additionally, 308 live singleton controls (2:1) for the IVF singletons were derived from the full sample control group for the analyses stratified for plurality.

The number of mothers (pregnancies) is a result of the number of children in the study and control groups. Consequently, there were 225 IVF mothers (153 singleton, 62 twin, 9 triplet and one quadruplet pregnancy) after excluding one ICSI singleton pregnancy and 671 control mothers (580 singleton, 82 twin and 9 triplet pregnancies). The twin control group includes some solitary twins (the other twin of the pair was not chosen as a control to the study) with their mothers included in the prenatal analyses. Additionally, if a child was excluded from the appropriate analyses for some reason, the mother was still included in the prenatal analyses. Therefore, the control mothers are overrepresented in the control group of twin pregnancies. The mothers were matched as a group representing a similar area of residence, parity, age and socioeconomic status. The study design and study population is presented in Fig. 2.

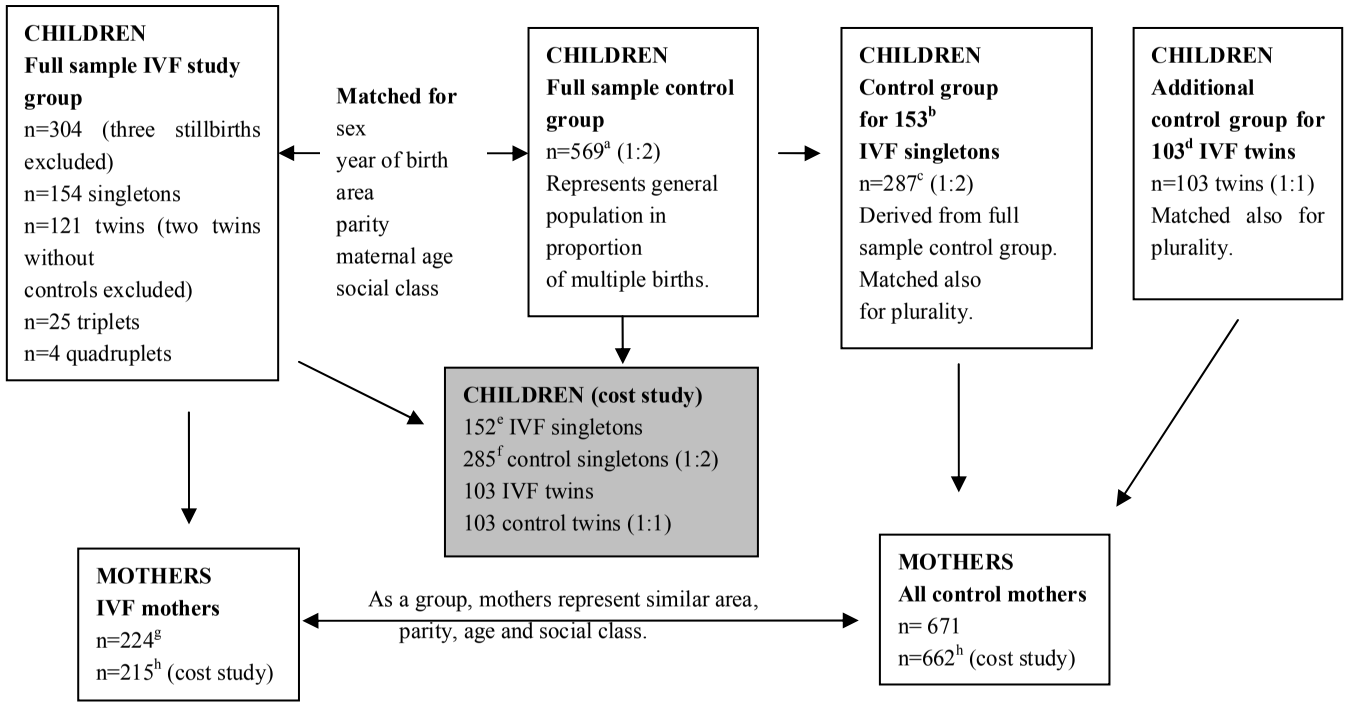

Fig. 2. The study design and study population. Shaded presentation for the cost study. [a]39 IVF children missing second control. [b] One IVF singleton missing plurality matched control. [c]19 IVF children missing second control. [d]18 IVF twins missing plurality matched control. [e]One ICSI singleton excluded. [f]Two controls of the ICSI singleton excluded. [g]One ICSI pregnancy and one quadruplet pregnancy excluded. [h]Ten mothers (IVF) and nine mothers (controls) with high-order births excluded.

4.3 IVF protocol

Ovarian stimulation was performed after pituitary downregulation with buserelin or nafarelin for at least 10 days followed by daily injections of human menopausal gonadotropin. Oocyte retrieval was performed 34-36 h after the injection of 5000-10 000 IU of human chorionic gonadotropin. One to four embryos, usually two or three, were transferred into the uterus 46-50 h after oocyte retrieval. Progesterone or chorionic gonadotropin was given for luteal support for 14 days.

4.4 Pilot study

A pilot study (30 IVF exposed and 20 unexposed controls), conducted to find out the availability and coverage of the health records, showed that data on developmental milestones were missing in an average of 10-15% of cases due to incomplete notes made by the doctors or nurses. However, the missing information was randomly distributed and was similar between the IVF and control groups.

4.5 Data collection and outcome measures

Medical outcome measures and corresponding data sources are shown in Table 4. Prenatal and neonatal data were used in the cost calculations. The mean unit prices (year 2001) of prenatal and neonatal health care services were collected from the data of the National Research and Development Center for Welfare and Health (Stakes) (Hujanen 2003). The costs of IVF treatment during 2003 (oocyte retrieval, embryo transfer, related visits to the infertility clinic) were collected from the infertility outpatient clinic of Oulu University Hospital (unpublished data). The costs of medication for ovulation induction (years 1996-1998) were received from research data of the Social Insurance Institution of Finland (KELA). Mean costs of sickness allowance due to IVF treatment were received from age- and sex-stratified statistics of KELA (Lindroos & Kuusisto 1994). The costs of unsuccessful cycles were estimated using statistics compiled by Stakes showing one successful cycle out of 5.26 cycles in total in Finland in 1996-1998, and selected parts of the IVF costs listed in Table 17 (i.e. those repeated in succeeding treatments: medication, IVF treatment and three day sick leave) were multiplied by 4.26.

Table 4. Prenatal and early childhood outcome measures and their data sources.

Outcome measure	Data source
Prenatal outcome	
Obstetric history	Hospital records including copies of maternal health
Pregnancy complications	center follow-up cards
Mode of delivery	
Use of maternal health care services	
Neonatal outcome	
Gestational age	Hospital records
Apgar score	
Birth weight and height	
Length of hospitalization (ward or NICU)	
Morbidity	
Congenital malformations	
Early childhood up to 3 years of age	
Weight and height gain	Standardized health cards of child welfare clinics
Psychomotor development	(CWC)
Morbidity	Hospital records

NICU = neonatal intensive care unit

4.6 Definitions

Definitions of different variables and age-related psychomotor milestones are shown in Tables 5-6.

Table 5. Prenatal and early childhood variables and their definitions.

Variable	Definition
Threatened preterm birth	Uterine contractions with dilated or non-dilated cervix needing specialized care in hospital.
Pregnancy induced hypertension	Gestational hypertension or pre-eclampsia. Systolic blood pressure ≥140 mmHg or elevation ≥30 mmHg, and diastolic blood pressure ≥90 mmHg or elevation ≥15 mmHg during pregnancy. The diagnosis of pre-eclampsia required proteinuria >0.3 g/day with or without subjective symptoms.
Gestational diabetes mellitus	Altered glucose metabolism requiring dietary or insulin treatment detected by 2 h oral 75g glucose tolerance test according to the recommendations of the Finnish Diabetes Association as well as international recommendations (Metzger & Coustan 1998).
Intrahepatic cholestasis of pregnancy	Itching with elevated hepatic transaminases and/or elevated serum bile acids.
Birth	Birth after completion of the 22nd gestational week or with birth weight ≥500g.
Neonatal morbidity	One or more of the following diagnoses based on ICD-10 codes: neonatal infections, hypoglycemia, hyperbilirubinemia needing phototherapy, RDS, bronchopulmonary dysplasia, patent ductus arteriosus, apnea and intracranial hemorrhage.
Ponderal index	A neonatal measure of thinnes, reflects intrauterine growth (kg/m^3)
Congenital malformation	Defined according to the definition of the Finnish Register of Congenital Malformations and Birth Defects (ICD-9).
Stillbirth	Registered from the completed 22nd gestational week onwards or if birth weight is ≥500g.
Early neonatal mortality rate	Neonatal deaths<7 days from birth /1000 live births.
Perinatal mortality rate	Stillbirths with early neonatal deaths / 1000 live births.
Late neonatal mortality rate	Neonatal deaths during 7-27 days from birth /1000 live births.
Total neonatal mortality rate	Early and late neonatal deaths / 1000 live births.
Infant mortality rate	Deaths during the first year of live / 1000 live births.
Growth	Weight and height at 1, 2 and 3 years of age; relative height for age and weight percentage (deviation from ideal body weight for height)
Psychomotor development	Psychomotor abilities of the child at 1, 2, 3, 4, 6, 7, 8, 9, 12 and 18 months, and 2 and 3 years of age (Table 6). Summary variables of 1-3 months, 4-9 months, 12 months, 18 months, 2 years and 3 years were used to simplify the analyses.
Low weight and height at 1-3 years	The lowest quartile of this study population.
Morbidity	All diagnoses found in the hospital records focusing on chronic illnesses.

Table 6. Age-related psychomotor developmental milestones collected at the child welfare clinics in the nationally standardized health card.

Developmental milestones	
1 month (nurse)	9 months (nurse)
Lies on stomach, raises head occasionally	Crawls (knees on the ground)
Fists mainly closed	Stands up leaning to support
Incidental sounds	Understands the meaning of some words
2 months (nurse)	Imitates clapping of hands
Lies on stomach, head up	12 months (GP and nurse)
Fists mainly open	Walks with or without support
Utters sounds	Throws objects
Response on a smile	Says a meaningful word
3 months (GP and nurse)	Imitates simple action
Keeps head up when lifted from the hands	Plays give and take
Babbles	18 months (nurse)
Selective smile	Walks
4 months (nurse)	Builds a tower with 2-3 bricks
Lifts head and trunk when leaning to elbows	Points to a named object from a picture
Reaches for a handed object	Fetches objects at request
Reciprocal babble	Plays hide and seek
Delight at the sight of mother	2 years (GP and nurse)
6 months (nurse)	Runs
Raises head and trunk with straightened arms	Kicks a big ball without falling
Takes support with legs when lifted to a standing position	Builds a tower with several bricks
Grips an object with the fist	Copies a vertical line
Imitates sounds, laughs out aloud	Expresses own will; uses words No, I
7 months (nurse)	Names familiar objects from a picture
Crawls or tries to crawl	Obeys simple instructions and advice
Removes a toy from one hand to another and to mouth	3 years (GP and nurse)
Babbles syllables	Jumps with both feet
Is shy of strangers	Walks on toes
8 months (nurse)	Undresses
Lifts oneself to a sitting position	Copies a circle
Thumb-index hold	Speaks sentences
Recognizes own name	Distinguishes sizes
Plays reciprocally	Obeys two-part instructions
Likes soft toys	Can handle a short absence of the mother
	Can wait for own turn for a little while

Observations are made by general practitioners (GP) or nurses. All items answered either yes or no.

4.7 Statistical analysis

4.7.1 Prenatal data (I)

Risk ratios (RR) with 95% confidence intervals (CI) were used for categorical variables and proportions of fourths (quartiles as cut-offs) to describe the data for continuous variables with Mann-Whitney *U*-tests. The main analyses were stratified for plurality. Whenever all mothers were presented together (background analysis), the analyses were adjusted for the number of fetuses. Triplet and quadruplet pregnancies were excluded from the analyses stratified for plurality due to small numbers (nine and one, respectively) and lack of eligible multiple control pregnancies, but triplet pregnancies were included in the maternal background analysis. We were unable to trace pregnancy data on two IVF mothers; however, other appropriate data were available for them. The data on specific variables were missing on average in 2% of the mothers, and consequently the numbers vary slightly, variable by variable. Maximum numbers are given in the tables. The final prenatal data included 224 IVF mothers (after excluding one ICSI and one quadruplet pregnancy) and 671 control mothers (153 singleton, 82 twin and nine triplet pregnancies) (Fig. 2).

4.7.2 Neonatal and early childhood data (II-III)

Conditional logistic regression for matched sets was used to calculate the odds ratios (OR) with 95% confidence intervals for categorical and categorized variables. The percentages were calculated using matched sets; the denominator varies slightly due to some missing information among variables. The aim of having a control cohort drawn from the general population was to compare the whole group of IVF children (full sample) with the average population to be able to estimate the effect of multiple birth on the child outcome. The analyses stratified for plurality (singletons and twins) enabled us to control the confounding caused by multiple birth and to evaluate the effect of IVF technology on the outcome. As there were not enough eligible naturally conceived children from high order pregnancies, we had to expand the area of residence. Even with the expanded area we were not able to find matched controls for IVF triplets and quadruplets; hence they were excluded from the analyses stratified for plurality but included in the full sample analyses.

Consequently, the neonatal data included 304 liveborn IVF children (two twins without controls excluded) and three stillbirths for the mortality calculations. The full sample analyses included 304 IVF children and 569 control children; the analyses stratified for plurality included 103 IVF twins and 103 control twins and 153 IVF singletons and 287 control singletons. (Fig. 2.)

Four IVF children who died during infancy and one ICSI child as well as their matched controls were excluded from the childhood data. The control children who died were excluded likewise, but their matched cases were excluded only if there was no other

control left in the set. As a result, there were 299 IVF children in the final study group and 558 control children in the full sample control group, 100 IVF twins and 100 control twins, and 150 IVF singletons and 280 control singletons for the analyses stratified for plurality. (Fig. 2.)

The SPSS software for Windows, the SAS System Release 8.02 (TSO2MO) for Windows and SAS/STAT software were used for statistical analyses.

4.7.3 Cost analysis (IV)

The mean numbers of visits to maternal health care centers (MHC) and hospital outpatient clinics, and the mean number of days of hospitalization during pregnancy and after birth were calculated for the mothers. Modes of deliveries were classified. Children were divided into five categories according to their neonatal status: (1) healthy full-term neonates; (2) full-term neonates with neonatal morbidity (excluding RDS); (3) healthy preterm neonates (birth <37 weeks of gestation); (4) preterm neonates with neonatal morbidity (birth <37 weeks of gestation, excluding RDS); (5) neonates with RDS. This DRG-based (Diagnosis-Related Groups) categorization was made to follow the national categorization of the costs resulting from neonatal hospitalization by Stakes (Hujanen 2003). The percentages of children in different categories were calculated. For the children the mean numbers of days of hospitalization in neonatal wards and intensive care units were calculated in these categories.

The cost calculations were performed using the known mean unit prices of different health care services in Finland (Hujanen 2003) to calculate the actual costs for one day, and then multiplying by the mean number of visits to MCH and outpatient clinics or days in the hospital. To simplify the MCH cost calculations, all mothers were regarded as primiparas although a minority were giving subsequent births. The statistical significance tests were computed for the differences between the means or proportions but not for the total costs between IVF and control groups, because the total prices constituted the sum of the means of all available cases. We used all available mothers in the prenatal comparisons (mothers of singletons and twins in the full sample control group and the additional control group of mothers of twins), and hence the matching proportions 1:2 and 1:1 for the children cannot be seen in the number of mothers.

The costs were inflated to correspond to the prices during 2003 using the consumer price index. Sensitivity analysis was performed by calculating the health care costs at 25^{th} and 75^{th} percentiles for IVF and control groups to indicate the interquartile range of the health care costs in these groups and also by calculating the health care costs applying 10.0% frequency of multiple births among the IVF population as recommended by the ESHRE Campus Course Report (2001) in comparison with: (1) the multiple birth frequency of 46.2% in our IVF population 1990-1995; and (2) 12.0% as it was in 2002 in the population of IVF children in Northern Finland.

The cost calculations included 215 IVF mothers (153 singleton and 62 twin pregnancies) and 662 control mothers (580 singleton and 82 twin pregnancies) and 255 IVF neonates (152 singletons and 103 twins) with 388 control neonates (285 singletons

and 103 twins) for the comparisons stratified for plurality. The only ICSI case was excluded from the calculations. (Fig. 2.)

4.7.4 Ethical considerations

The study proposal was approved by the Ethics Committee of the University of Oulu and by the Ministry of Public Health and Social Affairs. No major ethical problems were expected; the persons were not contacted as was required by the Ministry of Public Health and Social Affairs. Confidentiality was strictly followed in collecting and storing the data.

5 Results and comments

5.1 Prenatal outcome (I)

5.1.1 Maternal background characteristics

The maternal characteristics are presented in Table 7 by fetal plurality. The majority of the women were primiparous. There was one previous IVF-delivery in both groups. Arterial hypertension was the most common pre-existing disease among both IVF and control mothers. After adjusting for fetal plurality spontaneous abortions were not more common among IVF women (RR 1.3, 95% CI 0.9-1.7) while ectopic pregnancies were clearly so in comparison to the controls (RR 11.3, 95% CI 6.2-20.4). The etiology of infertility varied: tubal occlusion 41%, unexplained infertility 25%, male factor 16% and others 17% (endometriosis, mixed etiology, hormonal).

Table 7. Background characteristics (n,%) of IVF and control mothers by fetal plurality.

Variable	Singletons[a]		Multiples[a]	
	IVF mothers (N = 153) n (%)	Control mothers (N = 580) n (%)	IVF mothers (N = 71) n (%)	Control mothers (N = 91) n (%)
Primiparous	119 (77.8)	449 (78.6)	59 (83.1)	59 (65.6)
Arterial hypertension	3 (2.0)	11 (1.9)	0	3 (3.4)
Spontaneous abortions	30 (19.9)	101 (17.9)	17 (23.9)	10 (11.1)
Ectopic pregnancies	32 (21.2)	12 (2.1)	17 (23.9)	1 (1.1)

[a]Numbers may vary slightly variable by variable due to up to 2% of missing values, maximum numbers are given.

Comment. The similarity in the number of previous spontaneous abortions between IVF and control women indicates that some spontaneous reproductive capability had been

present for every fifth IVF mother. The excess of ectopic pregnancies probably arises from impaired tubal function as a cause of infertility. The mothers were reasonably healthy; only a few women with pre-existing morbidity were present in either the IVF or the control group. Therefore, no firm conclusions can be drawn regarding pre-existing morbidity between IVF and control mothers.

5.1.2 Pregnancy complications

First trimester bleeding was significantly more common in IVF than in control pregnancies, regardless of plurality. The same phenomenon continued throughout the rest of the pregnancy, although to a lesser extent. After excluding the cases of vanishing embryos (six in singletons and three in twins) from the analyses, the differences remained significant (RR 3.2, 95% CI 1.9-5.5 for singletons; RR 6.0, 95% CI 2.1-16.7 for twins). The risk of threatened preterm birth needing specialized hospital care was significantly increased for singleton but not for twin IVF pregnancies (RR 1.8, 95% CI 1.1-2.9; RR 0.8, 95% CI 0.6-1.3, respectively). The risk of ICP was nearly 4-fold for IVF singleton pregnancies, and the same trend was seen among IVF twin pregnancies, although not nominally significantly. There were only three IVF cases with placental complications that were all placenta previas (one singleton pregnancy and two twin pregnancies). One abruption occurred in the entire control group. (Table 8.)

In different infertility groups, women with hormonal infertility seemed to be more predisposed to GDM than women with tubal infertility (RR 5.8, 95% CI 1.3-27.0), but no other significant differences were found (data not shown in the tables).

The mean (SD) gestational ages at birth did not differ between the IVF and control pregnancies among either singletons [38.9 (2.6) vs. 39.3 (1.9) weeks] or twins [36.2 (2.1) vs. 36.0 (2.8) weeks]. The risk of preterm birth before the beginning of week 32 of gestation was non-significantly increased for IVF singleton pregnancies (RR 1.9, 95% CI 0.5-7.4). In twin pregnancies the situation was reversed, but due to small numbers the estimate was imprecise (RR 0.2, 95% CI 0.02-1.5). Gestational lengths at delivery were also analyzed at 32-36, 37-42 and >42 weeks strata, but no statistically significant differences were found in any of the comparisons (data not shown).

Table 8. Pregnancy complications in singleton and twin IVF and control pregnancies (n,%).

Variable	Singletons[a]			Twins[a]		
	IVF (n=153)	Controls (n=580)	RR (95%CI)	IVF (n=62)	Controls (n= 82)	RR (95%CI)
1st trim. bleeding	16 (10.7)	9 (1.6)	6.7 (3.0-15.0)	10 (16.1)	1 (1.3)	13.2 (1.7-100.6)
2nd and 3rd trim. bleeding	13 (8.7)	18 (3.2)	2.7 (1.4-5.5)	11 (17.7)	3 (3.8)	4.9 (1.4-16.6)
Threatened preterm birth	22 (14.7)	47 (8.4)	1.8 (1.1-2.9)	23 (37.7)	36 (45.0)	0.8 (0.6-1.3)
ICP	4 (2.7)	4 (0.7)	3.8 (1.0-15.0)	6 (9.7)	4 (5.0)	2.0 (0.6-6.7)

[a]Numbers may vary slightly variable by variable due to up to 2% of missing values, maximum numbers are given.

Comment. Due to matching the mothers in this study represent a more advanced age distribution which is not comparable to childbearing women in the general population. Advanced age is a common character among infertile women and it is known that the risk of pregnancy complications increases with maternal aging. Advanced age (>35 years) of the mother has also been shown to relate to poor neonatal outcome (Sipilä *et al.* 1994). However, in this study, we wished to eliminate the known potential confounding of maternal age in order to explore the age-independent differences between IVF and control mothers.

The most prominent finding regarding pregnancy complications in this study was the increased risk for vaginal bleeding throughout pregnancy after IVF, even after excluding the cases of vanishing embryos. Similar results have been reported previously (Tan *et al.* 1992, Westergaard *et al.* 1999, Koudstaal *et al.* 2000b, Ochsenkühn *et al.* 2003, Jackson *et al.* 2004). We believe that this difference is not due to overreporting in the IVF group, since any bleeding during pregnancy usually leads to referral to specialized health care unit. The increased incidence of low-lying placentas after IVF (Tan *et al.*1992, Tanbo *et al.* 1995, Jackson *et al.* 2004) may partly explain vaginal bleeding during pregnancy, but also the more detailed early antenatal surveillance focused on IVF pregnancies as well as the increased incidence of preterm uterine contractions after IVF probably contribute to the matter (Koudstaal *et al.* 2000b). This is supported by our results on preterm uterine contractions. One might also speculate as to the role of IVF technology on this matter in form of impaired placentation that might increase the incidence of low-lying placentas. Placenta previa after IVF has been suggested to be a treatment-related phenomenon resulting from the placement of the embryos in the lower part of the uterus (Tan *et al.* 1992). The theory of suboptimal placentation after IVF may be further supported by the fact that serum HCG at mid-trimester has been shown to be elevated after IVF (Heinonen *et al.* 1996, Perheentupa *et al.* 2002).

Previous studies have demonstrated a clearly increased risk for preterm birth in singleton IVF pregnancies (MRC Working Party 1990, Tan *et al.* 1992, Olivennes *et al.* 1993, Wang *et al.* 1994, Tallo *et al.* 1995, Tanbo *et al.* 1995, Verlaenen *et al.* 1995, Koudstaal *et al.* 2000b, Tough *et al.* 2000, Wang *et al.* 2002, Zádori *et al.* 2003, Helmerhorst *et al.* 2004, Jackson *et al.* 2004, McGovern *et al.* 2004). This is, to some

extent, supported by our results showing a nearly doubled threat of preterm birth in singleton IVF pregnancies. The fact that no increased risk of actual preterm birth was found in this study, correspondingly to Reubinoff *et al.* (1997), is probably due to the more thorough controlling for maternal confounding (area of recidence, social class) in the present study in comparison to others, indicating that factors other than IVF technology might be more important determinants of preterm birth in IVF pregnancies.. The factors behind the increased risk of threatened or actual preterm birth and vaginal bleeding after IVF may partly arise from the same origins, as a relationship between second trimester bleeding and preterm birth has been observed previously (Sipilä *et al.* 1992). This is also supported by our results. Chorionamnionitis is an important predisposing factor for preterm birth, but our data showed no increased incidence of intrauterine infections among IVF pregnancies.

The incidence of PIH was similar between the IVF and control groups as reported earlier (Verlaenen *et al.* 1995, Reubinoff *et al.* 1997, Koudstaal *et al.* 2000b). The results regarding the association between PIH and IVF are, however, inconsistent, others showing an increased risk for PIH after IVF (Tan *et al.* 1992, Tallo *et al.* 1995, Tanbo *et al.* 1995, Maman *et al.* 1998, Ochsenkühn *et al.* 2003, Jackson *et al.* 2004). Since all of the studies mentioned above were matched for maternal age and all but Tan *et al.* (1992) and Tallo *et al.* (1995) were matched for parity, the discrepancy probably reflects differences in sample sizes. The studies showing an increased risk for PIH after IVF have larger sample sizes than the present study, with a higher power to detect the increased risk for PIH. It is, however, probable that PIH as such is not related to IVF technology itself, but rather to other risk factors represented by infertile women.

We found an increased risk for ICP in our IVF population; the difference was significant for singleton IVF pregnancies but non-significant for twin IVF pregnancies. In spite of the imprecise estimates, we find this phenomenon clinically important, since the risk was multiple and significantly higher compared to controls even in this relatively small sample of IVF pregnancies. Previous studies have not reported such a pregnancy complication, possibly due to the rarity of ICP in most countries, with an incidence ranging from 1/1000 to 1/10 000 deliveries (Davidson 1998). For reasons unknown, the incidence of this severe clinical problem with risk of fetal death is far more frequent (nearly 2%) in Finland and Sweden (Reyes & Simon 1993). The causes behind the association of ICP and IVF remain unclear; it can only be speculated that they could be related to some metabolic disturbances related to the infertile status of the women.

Apart from vaginal bleeding, the course of twin pregnancies was comparable between IVF and control groups. Vaginal bleeding after IVF has also been reported by two other twin studies (Daniel *et al.* 2000, Koudstaal *et al.* 2000a). Otherwise, our results support five studies that have not found any differences between twin IVF and natural pregnancies (Olivennes *et al.* 1996, Ágústsson *et al.* 1997, Fiztsimmons *et al.* 1998, Putterman *et al.* 2003, Pinborg *et al.* 2004b). However, adverse IVF twin pregnancy outcomes have previously been reported: PIH, premature contractions and IUGR (Daniel *et al.* 2000) as well as preterm birth (Moise *et al.* 1998, Lambalk & van Hooff 2001, Manoura *et al.* 2004). The inconsistent results reported by the twin studies are mainly due to variations in the matching criteria and differences in the controlling for zygosity-chorionicity between the studies.

As a novel observation, to summarize, we found an increased risk for ICP after IVF. Other results confirm those reported earlier, showing that IVF pregnancy is a high-risk pregnancy with a need for careful surveillance.

5.1.3 Delivery

There were no statistically significant differences in the mode of delivery between IVF and control groups. For IVF singletons (vs. controls), normal delivery rate was 63% (vs. 59%), Cesarean section rate 25% (vs. 25%) and vacuum extraction/breech delivery rate was 12% (vs. 15%). For twins, the rates were 40% vs. 40%, 52% vs. 45% and 8% vs. 15%, respectively.

Comment. The absence of any differences in the mode of delivery between IVF and control mothers probably reflects the matching for maternal characteristics, namely age and parity. The effect of advanced age and low parity on the delivery mode can be seen in the high Cesarean delivery rate of singleton control mothers. The rate for singleton mothers in the general population during the same time period was considerably lower, 13%. However, for primiparous women >30 years of age the rate was 27% correspondingly to our results. (FMBR, unpublished data). An elevated incidence of Cesarean deliveries after ART compared to general female population at delivery age has also been noted by others (Tan *et al.* 1992, Olivennes *et al.* 1993, Tanbo *et al.* 1995, Verlaenen *et al.* 1995, Ágústsson *et al.* 1997, Bernasko *et al.* 1997, Reubinoff *et al.* 1997, Maman *et al.* 1998, Westergaard *et al.* 1999, Daniel *et al.* 2000, Helmerhorst *et al.* 2004, Jackson *et al.* 2004). It is not likely that the risk of Cesarean delivery is increased by IVF itself, but by other factors such as being an old primipara or having a multiple pregnancy as suggested by our results. Additionally, it is probable that the decision of the delivery mode is to some extent influenced by the preciousness of IVF pregnancies as experienced both by obstetricians and the parents to be.

5.2 Neonatal outcome (II)

5.2.1 Mortality

Three stillbirths occurred in the IVF group (a twin and two triplets). Two IVF neonates (a twin and a triplet) and three control neonates (a singleton and two twins) died during the neonatal period. (Table 9.) The causes of neonatal death in the IVF group were pulmonary hypoplacy for one case and multiple anomalies (amniotic band sequence) for the other case. Both were born preterm. For two controls the cause of death was intraventricular hemorrhage, the other case also suffered from sepsis. Data were missing for the third case. All three controls were born preterm.

Table 9. The mortality rates in the IVF group and in the general population from FMBR in Northern Finland in 1990-1995.

Mortality rate	IVF deaths n	Mortality in the IVF population/1000 births (n = 309)	Mortality in the general population/1000 births (n = 55 195)
Stillbirth rate	3	9.7	4.3
Perinatal mortality rate		16.2	7.5
Early neonatal mortality	2	6.5	2.8
Late neonatal mortality	0	0	1.2
Total neonatal mortality	2	6.5	4.0
Infant mortality	4	13.1	5.2

Comment. Nearly all mortality rates in the IVF population were doubled in comparison to general population. The power of this study in this respect was too small to make statistical inferences. Our IVF stillbirth rate is comparable to other studies reporting rates ranging between 4.3-39.7/1000 births (MRC Working Party 1990, Rizk *et al.* 1991, Friedler *et al.* 1992, Tan *et al.* 1992, Balen *et al.* 1993, Rufat *et al.* 1994, FIVNAT 1995, Tanbo *et al.* 1995, Westergaard *et al.* 1999). Two recent meta-analyses reported a 1.68-2.19-fold risk of perinatal death for IVF singletons in comparison to naturally conceived ones (Helmerhorst *et al.* 2004, Jackson *et al.* 2004), which is also in accordance to our results. On the contrary, IVF twins seem to have better perinatal outcome than naturally conceived ones as far as mortality is concerned, since a 40% lower perinatal mortality for IVF twins was detected in comparison to twins after natural conception (Helmerhorst *et al.* 2004). Total neonatal mortality rate in our IVF population was lower than those reported by others (14.1-19.2/1000 births) (MRC Working Party 1990, Rizk *et al.* 1991, Rufat *et al.* 1994, FIVNAT 1995), but being still somewhat higher than in the general population.

The higher of mortality of IVF children during the perinatal and neonatal period mainly reflects the high proportion of multifetal pregnancies and preterm birth among IVF offspring (MRC Working Party 1990, FIVNAT 1995), but also maternal characteristics related to infertility, especially advanced age, may affect the matter.

5.2.2 Neonatal characteristics

The risk of preterm birth was nearly six-fold for IVF children in the full sample analysis where the whole IVF population was compared to controls representing general population with respect to multiple births. The risk of preterm birth <32 weeks after IVF was even more prominent (OR 7.5, 95% CI 2.6-21.5), and the risk of birth at 32-36 weeks was over four-fold in the IVF group. The mean birth weights were lower for IVF children in the full sample and singleton comparisons. The risk of low birth weight (1 500-2 499 g) was ten-fold and the risk of very low birth weight (<1 500 g) was six-fold for IVF children compared to controls. The IVF children were also thinner at birth, as low ponderal index (kg/m^3) was 2.6 times more prevalent among them (95% CI 1.8-3.8). The mean birth weights were: full sample comparison [2 917 g (SD 746) vs. 3 453 g (SD

586)], singletons [3 364 g (SD 596) vs. 3 483 g (SD 570)] and twins [2 594 g (SD 528) vs. 2 547 g (SD 602)]. The risk of neonatal morbidity and hospitalization were 2-3-fold for the IVF children in the full sample comparisons, and IVF children were more likely to be intubated after birth, but no differences were found in the occurrence of low Apgar scores. (Table 10-11.)

Analyses stratified by plurality showed no significant differences regarding any of the variables studied.

Comment. Our results showed a generally poorer neonatal outcome for IVF children in comparison to control children in general population, which is in accordance with previous publications (MRC Working Party 1990, Friedler *et al.* 1992, Gissler *et al.* 1995, Tallo *et al.* 1995, D'Souza *et al.* 1997, Bergh *et al.* 1999). When stratified for plurality, no significant differences were seen, contradicting the results of previous studies that have shown a poorer neonatal outcome for IVF singletons when compared to singletons conceived naturally (Tan *et al.* 1992, Olivennes *et al.* 1993, Gissler *et al.* 1995, Tanbo *et al.* 1995, Bergh *et al.*1999, Westergaard *et al.* 1999, Tough *et al.* 2000, Schieve *et al.* 2002, Helmerhorst *et al.* 2004, Jackson *et al.* 2004, McGovern *et al.* 2004). This discrepancy probably reflects differences in the study designs, especially variations in the matching criteria used. The comparable outcomes between IVF twins and control twins in this study support others (Olivennes *et al.* 1996, Ágústsson *et al.* 1997, Pinborg *et al.* 2003, Putterman *et al.* 2003, Pinborg *et al.* 2004a, Pinborg *et al.* 2004b). Generally, the results on twin outcome after ART have been conflicting: others have found adverse outcomes after ART (Bernasko *et al.* 1997, Moise *et al.* 1998, Daniel *et al.*2000, Koudstaal *et al.* 2000a, Lambalk & van Hooff 2001, Manoura *et al.* 2004) while others have found better outcomes after ART (Fitzsimmons *et al.* 1998, Minakami *et al.* 1998). A possible explanation behind this controversy is the variation of matching between the studies; only three have taken zygosity into account and six have not used matching at all, the rest having matched for maternal age and parity.

Based on our results, we can conclude that multiple birth and related complications such as preterm birth and low birth weight are the strongest determinants of neonatal outcome for IVF children. The small and non-significant differences in the singleton analysis may, however, reflect the effects of infertility itself on the outcome as was recently presented by Danish authors (Basso & Baird 2003). The equal outcome for twins probably reflects the intermediating effect of zygosity-chorionicity as well as the decreased power in the twin analyses.

Table 10. Demographic parameters (n, %; mean birthweight (SD)) of IVF and control neonates. (The denominator varies slightly varable by variable due to some missing values.)

Variable	Full sample[a]			Singletons[b]			Twins[b]		
	IVF N = 304	Controls N = 569[d]	OR (95% CI)	IVF N = 153	Controls N = 287[e]	OR (95% CI)	IVF N = 103	Controls N = 103	(OR 95% CI)
Preterm birth <37 weeks	95 (31.5)	44 (7.8)	5.6 (3.7-8.6)	13 (8.6)	16 (5.6)	1.5 (0.7-3.2)	45 (43.7)	45 (43.7)	1.0 (0.6-1.8)
Birth <32 weeks	16 (5.3)	6 (1.1)	7.5 (2.6-21.5)	3 (2.0)	3 (1.1)	2.0 (0.4-9.8)	2 (1.9)	11 (10.8)	[c]
Birth at 32-36 week	81 (26.9)	38 (6.8)	4.4 (2.8-7.0)	11 (7.3)	14 (5.0)	1.2 (0.5-2.9)	44 (42.7)	34 (33.3)	1.3 (0.7-2.3)
Mean birth weight g (SD)	2917 (746)	3453 (586)		3364 (596)	3483 (570)		2594 (528)	2547 (602)	
Birth weight <1500g	11 (3.6)	5 (0.9)	6.2 (2.0-19.0)	3 (2.0)	2 (0.7)	3.0 (0.5-18.0)	1 (1.0)	5 (4.9)	0.2 (0.02-1.8)
Birth weight 1500-2499 g	79 (26.1)	21 (3.7)	9.8 (5.6-17.3)	6 (4.0)	7 (2.5)	1.5 (0.5- 4.7)	46 (44.7)	42 (40.8)	1.1 (0.6-1.9)
Ponderal index <25 kg/m^3	82 (27.4)	70 (12.3)	2.6 (1.8-3.8)	24 (15.9)	37 (12.9)	1.2 (0.7-2.1)	29 (28.7)	36 (36.0)	0.7 (0.4-1.3)

[a]The whole IVF group was compared with matched controls representing average population in proportion of multiple births. [b]Analyses stratified for plurality.
[c]n too small to calculate OR and CI. [d]39 IVF children had only one control child. [e]19 IVF children had only one control child.

Table 11. Neonatal parameters and congenital malformations (n,%) in matched IVF and control groups. (The denominator varies slightly variable by variable due to some missing values.)

Variable	Full sample[a]			Singletons[b]			Twins[b]		
	IVF N = 304	Controls N = 569[c]	OR (95% CI)	IVF N = 153	Controls N = 287[d]	OR (95% CI)	IVF N = 103	Controls N = 103	(OR 95% CI)
Neonatal morbidity	85 (28.6)	82 (14.6)	2.4 (1.7-3.4)	27 (18.2)	43 (15.1)	1.3 (0.7-2.2)	31 (30.1)	35 (34.7)	0.8 (0.5-1.5)
Neonatal hospitalization	89 (30.0)	67 (11.9)	3.2 (2.2-4.6)	20 (13.4)	30 (10.6)	1.3 (0.7-2.4)	39 (38.2)	46 (44.7)	0.8 (0.4-1.4)
Intubation	42 (14.1)	26 (4.6)	3.3 (2.0-5.5)	7 (4.7)	15 (5.3)	0.9 (0.4-2.2)	17 (16.7)	19 (18.8)	0.9 (0.4-1.8)
Heart malformations	10 (3.3)	5 (0.9)	4.0 (1.4-11.7)	3 (2.0)	2 (0.7)	3.0 (0.5-18.0)	4 (3.9)	5 (5.0)	0.8 (0.2-3.4)
Other malformations	11 (3.7)	20 (3.5)	1.1 (0.5-2.4)	3 (2.0)	8 (2.8)	0.8 (0.2-3.1)	4 (3.9)	4 (4.0)	1.0 (0.3-4.0)

[a]The whole IVF group was compared with matched controls representing average population in proportion of multiple births. [b]Analyses stratified for plurality. [c]39 IVF children had only one control child. [d]19 IVF children had only one control child.

5.2.3 Congenital malformations

Minor or major congenital malformations/syndromes affected 20 IVF children (11 males and 9 females) giving a malformation rate of 6.6%. Six IVF singletons, eight twins and six triplets had malformations. Fourteen IVF children out of 20 with malformations were born preterm. One case of trisomy 21 occurred in the IVF group, while no malformations occurred in stillbirths.

Among controls representing general population, 25 children had a malformation; 24 singletons and one twin, giving a malformation rate of 4.4%. The majority of the children (23) with malformations were born at term. The prevalence of malformations in the control group was strongly associated with male sex (21 males vs. 4 females) even if urogenital malformations were excluded.

Specific malformations are listed in Table 12. A number of inguinal (IVF vs. controls, 9 vs. 8) and umbilical hernias (10 vs. 3), unstable hips (2 vs. 7), undescended testes (2 vs. 5) and hydroceles (1 vs. 2) occurred in both groups, but were not considered as malformations.

When all heart malformations listed in Table 12 were analyzed together, there was a four-fold increase in their prevalence in the IVF group compared with full sample controls (OR 4.0, 95% CI 1.4-11.7). A similar non-significant trend was found in the singleton analyses, but twins showed no difference in this respect. For other malformations no differences were found in any of the comparisons. (Table 11.)

Table 12. Comparison of the prevalence of children with congenital malformations/syndromes in the liveborn full sample IVF (n = 304) and control groups (n = 569[a], represents general population in proportion of multiple births).

Malformation	IVF		Controls	
	n	(%)	n	(%)
Heart malformations				
ASD (atrial septal defect)	2	(0.7)	1	(0.2)
VSD (ventricular septal defect)	4	(1.3)	2	(0.4)
ASD and VSD	2	(0.7)	0	(0.0)
Aortic coarctation	0	(0.0)	2	(0.4)
Urological malformations				
Hypospadia	0	(0.0)	1	(0.2)
Pelveourethral stenosis	0	(0.0)	1	(0.2)
Gastroentrological malformations				
Perianal fistula	1	(0.3)	0	(0.0)
Esophagus atresia	0	(0.0)	1	(0.2)
Duodenal stenosis	0	(0.0)	1	(0.2)
Other				
Limb anomalies	4	(1.3)	7	(1.2)
Cleft palate	1	(0.3)	2	(0.4)
CNS malformations	0	(0.0)	3	(0.5)
Auricular atresia	1	(0.3)	0	(0.0)
Hemangioma	1	(0.3)	0	(0.0)
Pectoral muscle aplasia	0	(0.0)	1	(0.2)
Pulmonary hypoplasia	1	(0.3)	0	(0.0)
Congenital hypothyroidism	0	(0.0)	2	(0.4)
Syndromes/multiple malformations				
Amniotic band sequence[b]	1	(0.3)	0	(0.0)
Goldenhar syndrome	1	(0.3)	0	(0.0)
Trisomy 21[c]	1	(0.3)	0	(0.0)
Hemifacial microsomia	0	(0.0)	1	(0.2)
Total	20	(6.6)	25	(4.4)

[a]39 children had only one control child. [b]Includes a VSD and other malformations, which are not separately seen in this table, but are included in the OR calculations. [c]Includes an ASD and a VSD, which are not separately seen in this table, but are included in the OR calculations.

Comment. The malformation rate in our population of IVF children was quite high compared to the rates reported by others in general. Only three studies have reported a higher rate than ours: 8.6% in an American study on only 29 IVF children (Yeh *et al.* 1990), 9.0% in a larger Australian study on 837 IVF children (Hansen *et al.* 2002), and 8.3% in a recent and so far the largest study from Sweden on 16 280 IVF children (Källén *et al.* 2005). The somewhat higher rate seen in this study can possibly be explained by the fact that we were able to obtain information on these children up to the age of three years, making it possible to include malformations detected after the neonatal period. In 15

previous studies the diagnosis of malformation was set at the neonatal period, while only five studies continued the follow-up postnatally. Another factor influencing on the malformation rate is the question of therapeutic abortions due to congenital malformations. Unfortunately, we do not have such data for the whole study period, but no therapeutic abortions were performed for IVF mothers during 1992-1995 in our catchment area. There is no knowledge concerning the years 1990-1991. Additionally, it would have been impossible for us to obtain similar information on therapeutic abortions in the control population. Nine of the 21 studies on congenital malformations after IVF we were able to trace had taken therapeutic abortions into account with no notable increase in the malformation rates compared to those excluding therapeutic abortions. The information on spontaneous abortions with congenital malformations is practically impossible to obtain. The starting point of this study was to choose IVF pregnancies ending in a birth and thus all abortions were excluded. Furthermore, the control children were chosen among live births, which was the reason why comparisons were made between liveborn children. The malformation rate in the control group representing general birth population (of older mothers) was lower than in IVF population, and was comparable to that in the general Finnish population (The Finnish Register of Congenital Malformations and Birth Defects, unpublished data).

The prevalence of heart malformations, namely septal defects, was increased four-fold in IVF children seen in the comparison to controls representing general population. The increased risk for heart malformation after IVF has been supported by two recent studies; Anthony *et al.* (2002) showed a 1.56-fold risk for cardiovascular malformations for IVF children (95% CI 1.10-2.22) in a large study of over 4 000 subjects. All specific cardiovascular malformations were more frequently reported for IVF children, but only the difference in occurrence of single umbilical artery reached statistical significance (OR 1.93, 95% CI 1.11-3.35). Furthermore, Hansen *et al.* (2002) also noted a significantly greater prevalence of cardiovascular malformations after IVF in a study population of 837 IVF children and their controls. An Australian re-analysis of a Belgian study on birth defects with 420 Belgian ICSI children and a Western Australian control group of 100 454 liveborn infants, also found a four-fold excess of major cardiovascular defects among ICSI children (Kurinczuk & Bower 1997). This re-analysis was, however, quite controversial and criticized by the original authors as overestimating the number of cardiac malformations. Furthermore, the comparison to an Australian control group can also be criticized because of the probable differences in the ethnic backgrounds and health care systems between the two countries. The underlying reasons for the increased prevalence of cardiac malformations after IVF are difficult to ascertain. Congenital heart malformations are due to complex multifactorial genetic and environmental causes, and fewer than 10% of all cardiac malformations arise from recognized chromosomal aberrations and mutations of single genes (Friedman & Child 1998). In this study, no significant excess of septal defects was found for IVF children in the analyses stratified for plurality, suggesting that the effect of IVF technology itself on this matter is small. On the other hand, the power regarding malformations is decreased in the analyses stratified by plurality probably diminishing the differences between the groups. Twinning has been linked to an elevated risk of heart malformations as well as other malformations (Mastroiacovo *et al.* 1999), which coinsides with our finding. An association between mothers with poor reproductive history and VSDs in the offspring has been noted

previously (Sands *et al.* 1999), indicating that maternal characteristics regarding infertility may partly be responsible for the development of cardiac malformations. This might also be supported by our results, since an increase in septal defects, though non-significant, was present for IVF singletons as well.

As far as other malformations are concerned, we found no significant differences in any of the analyses. It must be noted, that the numbers for other malformation were quite small to show any robust results. Five quite recent studies with large register-based study and control groups have shown a generally elevated risk for congenital malformations after IVF. The ORs for the difference between the groups range between 1.2-2.0 with confidence intervals showing statistical significance (Bergh *et al.* 1999, Ericson & Källén 2001, Anthony *et al.* 2002, Hansen *et al.* 2002, Klemetti *et al.* in press). The differences do, however, decrease after adjusting for maternal characteristics (Ericson & Källén 2001, Anthony *et al.* 2002), indicating rather the effect of factors related to infertility on the outcome in question. Increased risk for neural tube defects, gastrointestinal atresias (Bergh *et al.* 1999, Ericson & Källén 2001, Källén *et al.* 2005) and omphalocele (Ericson & Källén 2001), as noted by Swedish research groups, was not found in our study population. One might speculate that the higher prevalence of congenital malformations seen in IVF children is a result of a more thorough investigation of these children by the phycicians. However, we believe that no major ascertainment bias was present due to the very uniform health care system both at specialized level and in child welfare clinics where the GPs are not necessarily aware of the IVF treatment. Studies showing comparable malformation outcome between IVF and control groups have mainly been conducted on small study populations with insufficient power.

The comparison of the different malformation studies is complicated by the fact that malformations are defined very variably in different countries. The malformation rates are affected by the definitions: whether the minor malformations are included or not and whether conditions related to preterm birth such as hernias or patent ductus arteriosus are included or not. Our study, even with a relatively small sample size in this respect, showed a multiple risk for heart malformations after IVF, which was supported by two larger contemporary studies (Anthony *et al.* 2002, Hansen *et al.* 2002). Furthermore, a significantly increased risk for malformations after ART has been shown in a recent meta-analysis (Hansen *et al.* 2005) indicating that special attention should be focused on IVF children. Meanwhile, it is of importance that large population-based, preferably register studies with standardized criteria for malformations, are conducted to explore this topic further.

5.3 Early childhood outcome (III)

5.3.1 Infant mortality

Four of IVF children died during infancy (two of them postnatally) resulting in an infant mortality rate of 13.1/1000 live births. The corresponding figure in the general population in Northern Finland in 1990-1995 was 5.2/1000 live births. (Table 9.) The causes of death

among IVF infants who died postnatally were RDS (singleton; death at 28 days of age) and severe asphyxia during labour with prematurity (singleton, death at 3 months of age).

Comment. The infant mortality rate of IVF children was about 2-fold to that of the general population during the study period. This is in accordance with the other IVF mortality rates in this study. Our infant mortality is comparable to a Danish figure of 11.3/1000 live births (Westergaard *et al.* 1999). Even higher figures from 20.8 to 23.7/1000 live births have been reported (MRC Working Party 1990, Rizk *et al.* 1991, Rufat *et al.* 1994). These figures are substantially higher than those of general populations reflecting the high proportion of multifetal pregnancies after IVF (MRC Working Party 1990, Rizk *et al.* 1991).

5.3.2 Growth

IVF children were significantly shorter and lighter in weight than their controls representing general population during the whole follow-up period to three years of age, although the difference was most prominent at birth. At one and two years of age IVF singletons were also significantly lighter in weight than control singletons, but height was similar in the singleton case/control groups. For twins no significant differences were found with the exception of mean of the difference in height at one year of age in favour of IVF twins. (Fig. 3-4.)

The risks of low weight and height, defined as the lowest quartile of this study population, at one (OR 1.5 95% CI 1.1-2.2, OR 1.6 95% CI 1.1-2.4, respectively) and two years of age (OR 1.6 95% CI 1.1-2.4, OR 1.7 95% CI 1.2-2.5, respectively) were significantly higher and the risk of low height at three years of age marginally higher (OR 1.4 95% CI 1.0-2.1) for IVF children in the full sample analyses. Analyses stratified by plurality showed no significant differences apart from the two-fold risk of low height at two years of age for IVF singletons (OR 1.9 95% CI 1.1-3.2). (Table 13.)

Height at ages 0, 1, 2 and 3 for IVF and control children as groups (means of groups used, full sample analysis) was converted to standard deviation scores of height for chronological age and body weight correspondingly to percentage of ideal body weight for height. The scores and percentages are presented in Table 14. On standard growth charts the IVF children's height was at –1.4 SD level at birth but as a group both boys (to –0.5 SD score) and girls (to ±0 SD score) caught up in height during the first year of life.

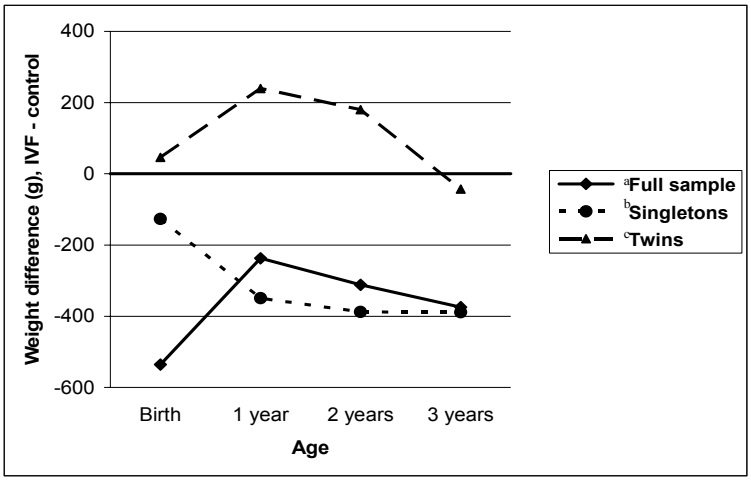

Fig. 3. Mean of the differences in weight between IVF and control children in matched sets at birth, 1, 2 and 3 years of age. Full sample IVF n = 299, controls n = 558; singletons IVF n = 150, controls n = 280; twins IVF n = 100, controls n = 100. The dotted lines should be compared with the 0-reference line. [a]Full sample analysis: p<0.0001, 0.018, 0.011, 0.041. [b]Singleton analysis: p=0.127, 0.007, 0.017, 0.104. [c]Twin analysis: p=0.514, 0.187, 0.437, 0.884.

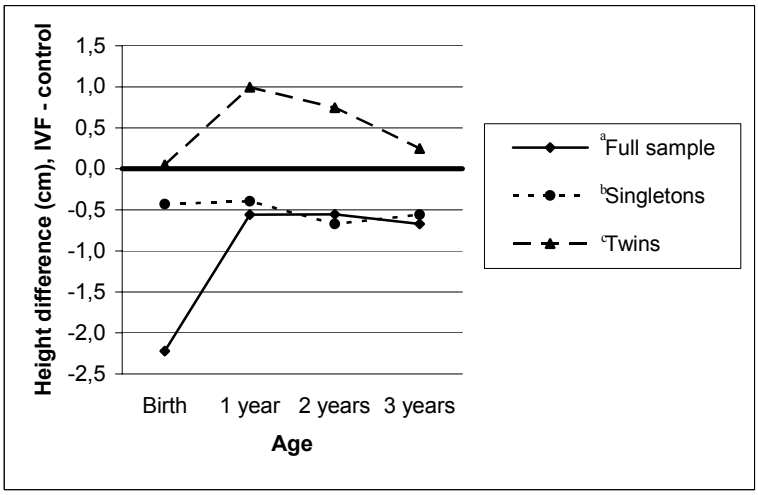

Fig. 4. Mean of the differences in height between IVF and control children inside matched sets at birth, 1, 2 and 3 years of age. Full sample IVF n = 299, controls n = 558; singletons IVF n = 150, controls n = 280; twins IVF n = 100, controls n = 100. The dotted lines should be compared with the 0-reference line. [a]Full sample analysis: p<0.0001, 0.014, 0.021, 0.026. [b]Singleton analysis: p=0.178, 0.242, 0.069,0.251. [c]Twin analysis: p=0.539, 0.047, 0.125, 0.679.

Table 13. Mean (SD) weights and heights and the percentage of low weight and low height, defined as the lowest quartile threshold point in this study population, at 1, 2 and 3 years of age in IVF and control children.

Variable	Full sample[a]			Singletons[b]			Twins[b]		
	IVF N = 299	Control N = 558	OR (95% CI)	IVF N = 150	Control N = 280	OR (95% CI)	IVF N = 100	Control N = 100	OR (95% CI)
Mean weight (kg) (SD)									
Birth	2.9 (0.7)	3.5 (0.6)		3.4 (0.5)	3.5 (0.5)		2.6 (0.5)	2.6 (0.6)	
1 year	9.8 (1.2)	10.0 (1.2)		9.8 (1.0)	10.1 (1.3)		9.7 (1.3)	9.5 (1.2)	
2 years	12.6 (1.6)	12.9 (1.6)		12.6 (1.3)	13.0 (1.5)		12.5 (1.7)	12.4 (1.7)	
3 years	14.8 (2.0)	15.2 (2.0)		14.9 (1.8)	15.3 (1.9)		14.6 (1.9)	14.6 (2.0)	
Low weight n (%)									
1 year, <9.0 kg	67 (23.7)	92 (17.5)	1.5 (1.1-2.2)	23 (16.8)	40 (15.3)	1.3 (0.7-2.3)	33 (33.3)	34 (34.7)	1.0 (0.5-1.7)
2 years, <11.5 kg	61 (21.7)	77 (14.6)	1.6 (1.1-2.4)	24 (17.4)	36 (13.6)	1.3 (0.7-2.2)	26 (27.1)	25 (25.5)	1.0 (0.5-1.9)
3 years, <13.5 kg	58 (21.6)	87 (18.0)	1.2 (0.8-1.8)	18 (14.1)	40 (16.3)	0.8 (0.4-1.5)	26 (27.4)	19 (21.8)	1.3 (0.6-2.7)
Mean height (cm) (SD)									
Birth	47.8 (3.4)	49.9 (2.5)		49.7 (2.4)	50.1 (2.4)		46.4 (3.0)	46.0 (3.0)	
1 year	75.8 (2.7)	76.4 (2.9)		76.3 (2.5)	76.6 (2.9)		75.5 (2.9)	74.5 (3.3)	
2 years	87.9 (3.1)	88.4 (3.3)		88.1 (3.0)	88.6 (3.3)		87.7 (3.4)	87.0 (3.1)	
3 years	96.1 (3.5)	96.8 (3.7)		96.5 (3.4)	97.1 (3.6)		95.6 (3.8)	95.3 (3.9)	
Low height n (%)									
1 year, <74 cm	65 (23.0)	80 (15.3)	1.6 (1.1-2.4)	22 (16.1)	39 (14.9)	1.1 (0.6-2.0)	30 (30.3)	35 (35.7)	0.8 (0.4-1.4)
2 years, <86 cm	80 (28.6)	101 (19.2)	1.7 (1.2-2.5)	37 (27.0)	46 (17.4)	1.9 (1.1-3.2)	27 (28.1)	30 (30.9)	0.9 (0.5-1.5)
3 years, <94 cm	70 (26.2)	96 (19.8)	1.4 (0.95-2.1)	26 (20.5)	43 (17.6)	1.2 (0.7-2.2)	31 (32.6)	31 (36.1)	0.9 (0.5-1.6)

[a]The whole IVF population was compared with controls representing general population in proportion of multiple births. [b]Analyses stratified for plurality.

Table 14. The sex stratified relative height for age (SD score, SDs) and percentage deviation from ideal body weight for height of IVF and control children in the full sample analysis.

Variable	Girls		Boys	
	IVF n=144	Controls n=262	IVF n=154	Controls n=296
Birth				
Weight	± 0%	+ 6%	– 1%	+ 3%
Height	– 1.4 SDs	– 0.3 SDs	– 1.4 SDs	– 0.3 SDs
1 year				
Weight	± 0%	+ 3%	+ 1%	± 0%
Height	± 0 SDs	+ 0.1 SDs	– 0.5 SDs	– 0.1 SDs
2 years				
Weight	– 2%	+ 2%	± 0%	± 0%
Height	+ 0.2 SDs	+ 0.3 SDs	– 0.1 SDs	+ 0.1 SDs
3 years				
Weight	– 1%	+ 3%	+ 1%	± 0%
Height	± 0 SDs	+ 0.3 SDs	– 0.1 SDs	+ 0.1 SDs

Comment. Our results showed that the growth of IVF children in the full sample group was behind that of control children representing general population during the whole follow-up period of three years. The difference between the groups was most prominent at birth and a catch-up growth was seen during the first year of life. IVF singletons also grew more slowly after infancy than their singleton controls. However, their growth was within normal range on standardized growth charts.

Previous reports on the growth of IVF children have shown more reassuring results (Table 3). Brandes et al. (1992) showed similar growth between IVF and non-IVF children in a series of 116 IVF children that were followed for 12 to 45 months. Saunders et al. (1996) also found normal growth for 314 IVF children followed for two years in comparison with a smaller control group. In a Swedish study, similar growth was found for 255 IVF children in comparison to 255 FET children and 252 spontaneously conceived children during a follow-up period of 18 months (Wennerholm et al. 1998). Similarly, no differences were found in the growth parameters of 5-year-old IVF, ICSI and NC children (Bonduelle *et al.* 2005).

The difference in the results between our study and those of others can be explained by the differences in the study designs. Other studies focusing on investigating the effects of IVF technology on the outcome have used plurality or gestation as matching criteria making it impossible to find out outcomes that are strongly related to multiple or preterm birth. With a comparison to controls representing general population (full sample analysis) in our study the differences were apparent. The power of this study is also higher than that of the other studies with the exception of the study by Bonduelle *et al.* (2005). However, the results seen in this study indicate that further studies with longer follow-up periods are needed to evaluate the growth of IVF children.

5.3.3 Psychomotor development

Regarding psychomotor development at the age of three years no significant differences were found either in the full sample analysis or in the analyses stratified for plurality (Table 15).

Table 15. Failure (n, %), defined as inability to perform at least one of the developmental tests, in psychomotor developmental tests at three years of age in IVF and control children.

Variable	IVF n (%)	Control n (%)	OR (95% CI)
Full sample[a] (n=299/n=558)	15 (10.2)	27(8.6)	1.4 (0.6-3.1)
Singletons (n=150/n=280)	8 (10.4)	17 (11.0)	0.8 (0.2-2.4)
Twins (n=100/n=100)	6 (11.1)	6 (12.2)	0.8 (0.2-3.4)

[a]The whole IVF population is compared with controls representing general population in proportion of multiple births. [b]Analyses stratified by plurality.

Comment. The psychomotor development of IVF children was normal and similar to that of control children in spite of the different growth pattern after IVF seen in the full sample and singleton analyses. The data regarding psychomotor development collected from the health records of CWCs were not complete: 10-15% of observations regarding specific developmental milestones (Table 4) were missing. However, the missing data appeared randomly in the whole population, and the personnel at the CWCs do not necessarily know the origin of the child, and consequently we assume that no major selection or information bias was present. Additionally, this is supported by the fact that practically all Finnish children use CWC services to get their scheduled vaccinations. At the time of vaccination, children also have a medical examination. Furthermore, selection bias is reduces by the fact that the two infertility clinics cover and keep the register of all infertility treatments in our target area.

Our results are in accordance with the six previous studies on the subject we were able to trace, showing no pathological features in the development of IVF children (Table 3). An early American study with 83 IVF children showed a significantly higher psychomotor achievement for IVF children aged 12 to 30 months in comparison to children conceived naturally possibly resulting from parental motivation and high social status (Morin *et al.* 1989). Two Israeli studies showed similar psychomotor development between IVF and control children during a three year follow-up period (Brandes *et al.* 1992, Ron-El *et al.* 1994). Others have also found normal/satisfactory development for IVF children when compared to naturally conceived controls (Raoul-Duval *et al.* 1994, Gibson *et al.* 1998, Wennerholm *et al.* 1998). However, a common feature for these studies is the insufficient power to detect developmental delays. Furthermore, the follow-up periods are short, three years in maximum, as is also the case for the present study. This makes it difficult to detect all developmental delays, especially with this large scale screening method used in this study. The deficiencies seen in these studies concerning

psychomotor development after IVF reflect the challenges in conducting research which is both time- and money-consuming involving difficulties in the formation of adequate control groups.

5.3.4 Morbidity

Of the respiratory diseases, pneumonia and obstructive bronchitis were significantly more common in the full sample of IVF children in comparison to controls representing general population. In the singleton comparison obstructive bronchitis showed significance as well. All in all, the cumulative incidence of respiratory diseases was significantly higher among IVF children in the full sample comparison (OR 3.5 95% CI 1.9-6.5) and marginally higher in the singleton comparison (OR 3.1 95% CI 1.0-9.4). The incidence of diarrhea needing hospital treatment was also significantly higher for IVF children in the full sample (OR 3.7 95% CI 2.2-6.2) and singleton comparisons (OR 5.7 95% CI 2.6-12.7). Neurological signs (eight febrile convulsions, eight with muscular hypotonia, two cases of delayed motor development and one mental retardation) occurred two times more often for IVF children in the full sample analysis (OR 2.2 95% CI 1.1-4.5). The risk of any diagnosed illness was two-fold for IVF children in the full sample and singleton comparisons. Twin analysis showed no significant differences in any of the variables regarding early childhood morbidity. (Table 16.) Generally, boys predominated slightly over girls in the cumulative incidence of illnesses (data not shown).

Table 16. Cumulative incidence of different diseases[a] in IVF and control children up to three years of age.

Variable	Full sample[b]			Singletons[c]			Twins[c]		
	IVF n = 299 n (%)	Control n = 558 n (%)	OR (95% CI)	IVF n = 150 n (%)	Control n = 280 n (%)	OR (95% CI)	IVF n = 100 n (%)	Control n = 100 n (%)	OR (95% CI)
Respiratory diseases	30(10.3)	18 (3.3)	3.5 (1.9-6.5)	9 (6.2)	7 (2.5)	3.1 (1.0-9.4)	15 (15.2)	8 (8.0)	2.6 (0.9-7.3)
Pneumonia	6 (2.1)	2 (0.4)	5.6 (1.1-27.8)	1 (0.7)	0 (0.0)		3 (3.0)	1 (1.0)	3.0 (0.3-28.8)
Obstructive bronchitis	24 (8.2)	10 (1.8)	6.0 (2.6-14.1)	9 (6.1)	5 (1.8)	5.1 (1.3-19.1)	11 (11.1)	7 (7.0)	1.8 (0.7-5.0)
Asthma	6 (2.1)	7 (1.3)	1.5 (0.5-4.6)	0 (0.0)	3 (1.1)		5 (5.1)	2 (2.0)	2.5 (0.5-12.9)
Diarrhea	44 (15.0)	25 (4.5)	3.7 (2.2-6.2)	24 (16.3)	9 (3.2)	5.7 (2.6-12.7)	12 (12.1)	13 (13.0)	1.0 (0.4-2.4)
Neurological signs[d]	19 (6.5)	20 (3.6)	2.2 (1.1-4.5)	4 (2.7)	12 (4.3)	0.7 (0.2-2.5)	10 (10.2)	4 (4.0)	3.0 (0.8-11.1)
Any illness[e]	95 (32.7)	94 (17.0)	2.3 (1.7-3.2)	45 (30.8)	48 (17.3)	2.1 (1.3-3.3)	34 (34.7)	27 (27.0)	1.5 (0.8-2.9)

[a]A child is counted only once in the incidence, repeated events are not taken into account. [b]The whole IVF population was compared with controls representing general population in proportion of multiple births. [c]Analyses stratified for plurality. [d]Includes diagnoses of febrile convulsions, muscular hypotension, delayed motor development, mental retardation and delayed speech development. [e]Includes any diagnosed illness or neurological sign.

Comment. During the first three years of childhood the IVF children had an increased cumulative incidence of different illnesses needing hospital inpatient or outpatient care, especially regarding respiratory diseases and diarrhea. The cumulative incidence of any illness was high among IVF population (33%) being two-fold compared to that of control children representing general population. Lower prevalence of chronic illness among IVF children (15.3%) was reported by a Swedish cohort study on 255 IVF children, being of the same magnitude among children born after cryopreservation and natural conception (Wennerholm *et al.* 1998).

The increased cumulative morbidity seen in our IVF offspring has been recently supported by Bonduelle *et al.* (2005) with a larger sample size than ours. Others have failed to show any differences in the childhood health between IVF and natural conception (Wennerholm *et al.* 1998, Pinborg *et al.* 2003). As discussed earlier, this is in also this case probably due to differences in the study designs. Other studies on this topic have matched for plurality to bring out the effects of IVF technology on the outcome that diminishes the differences between comparison groups regarding outcomes that are strongly related with multiple or preterm birth. IVF children are more often born preterm than naturally conceived children and consequently they may be more susceptible than others to common infections, such as respiratory or gastrointestinal infections. For example, preterm birth is a known predisposing factor to respiratory syncytial virus infections (Law *et al.* 2002) or chronic lung disease (Kurkinen-Räty *et al.* 2000). This might explain the significantly higher incidence of pneumonia detected in the full sample of IVF children. Furthermore, it is possible that the increased incidence of neonatal morbidity seen among IVF children and possible treatments with ventilators may predispose them to respiratory infections later in life. The cumulative morbidity was significantly increased after IVF also in the singleton comparison. This probably reflects the common nature of these conditions in the early childhood as well as sufficient power in this respect.

Similarly to other outcomes measured earlier in this study, twin comparisons failed to reveal any differences between IVF and natural conception. This is probably due to the effect of zygosity-chorionicity on the outcome as discussed earlier as well as due to decreased power in the twin analyses.

A recent Swedish large population-based study noted an increased risk of developing neurological problems, especially cerebral palsy, after IVF. The authors concluded that this was largely a result of the high frequency of twin pregnancies, low birth weight, and prematurity after IVF (Strömberg *et al.* 2002.) This was not the case in a Danish study that stated a similar risk for neurological sequelae between IVF/ICSI twins and IVF/ICSI singletons during a follow-up time of 2-7 years. The discrepancy between these studies was explained by the differences in the definitions of neurological sequelae as well as by the fact that the Swedish study comprised an earlier cohort of children born 1982-1995, while the Danish children were born 1995-2000, benefiting from the improved prenatal and neonatal care during that time. (Pinborg *et al.* 2004d.) However, also IVF singletons have been found to be at an increased risk for cerebral palsy, possibly due to vanishing twin phenomenon, which carries an increased risk for cerebral palsy for the surviving twin (Lidegaard *et* al 2005). In our study, no children with cerebral palsy were present in any of the comparison groups reflecting a sample size that was too small in this respect. Different neurological signs were, however, more common for IVF children in the full

sample comparison, probably as a result of multiplicity and consequent phenomena such as preterm birth and low birth weight.

The diagnoses regarding morbidity were collected from hospital records on inpatient and outpatient care at pediatric wards in central or regional hospitals around Finland, indicating specialized pediatric care and reliable diagnoses from the data collection point of view. The diagnoses set at the hospitals and appearing in the Finnish Hospital Discharge Register have been found to be of high quality (Keskimäki & Aro 1991). Similar conditions may, to some extent, be also treated in health centers by GPs or by private pediatricians, though eventually these diagnoses usually lead to referral to specialized care. One might speculate that IVF children may be more easily referred to specialized care by their GPs while other children may be more often treated by their own GPs. If this is the case, it may serve as a source of bias in our results. Furthermore, it is possible that parents of IVF children may seek medical help more easily than parents of naturally conceived children, leading to more diagnosed conditions for the IVF offspring, and burdening the health care services as well as increasing the resulting costs.

In conclusion, our results show that the health problems of IVF children continue even after the neonatal period reflecting multiple births and consequent morbidity, and indicating that long-term follow-up of the health of these children is needed.

5.4 Health care costs (I, IV)

5.4.1 The costs of IVF procedure

The costing structure of an IVF procedure is presented in Table 17.

Table 17. The cost of one successful IVF treatment in Oulu University Hospital in 2003 (€).

Cost variables	€
1. First visit to infertility outpatient clinic (couple)	282.0
2. IVF treatment	1 432.0
three visits to infertility outpatient clinic	
oocyte retrieval and embryo transfer	
costs of equipment	
costs of trained staff	
3. Visit to infertility outpatient clinic (gestational week 7)	120.0
4. Medication (mean price)[a]	1 347.6
5. Three-day sick leave[a,b]	109.1
Total	3 290.7

[a]The prices have been inflated to correspond to the year 2003. [b]€31.4/day for women aged 25-34 years in 1993 (Lindroos & Kuusisto 1994).

5.4.2 The costs of prenatal and neonatal care

5.4.2.1 Utilization of maternal health care services (I)

The number of MHC visits between IVF and control groups was similar [mean (SD), 12.6 (3.3) vs. 13.1 (3.4) for singletons; 9.2 (2.9) vs. 9.1 (3.3) for twins], but both singleton and twin IVF mothers had significantly more contact with hospital outpatient clinics during pregnancy than controls [5.7 (3.7) vs. 2.4 (2.6) for singletons; 10.6 (3.5) vs. 6.8 (2.9) for twins]. The length of antenatal hospitalization was significantly longer for singleton IVF mothers when compared to controls [3.5 (6.7) vs. 2.4 (5.4) days], but the length of postpartum hospitalization was similar [7.0 (2.0) vs. 6.8 (1.9) days]. For twin mothers, there were no significant differences in the length of antenatal hospitalization [14.1 (21.9) vs. 10.1 (14.0) days], but the length of postpartum hospitalization was, in turn, longer for IVF twin mothers than for controls [10.1 (2.7) vs. 9.1 (2.7) days]. (I.)
Comment. The utilization of specialized antenatal care was significantly increased after IVF with the exception of IVF mothers of twins. No differences, however, were found in the use of MHCs, MHC being the primary place of antenatal health care. This suggests that IVF mothers may be more easily referred to specialized care by GPs than mothers with naturally conceived pregnancies, and it may also partly reflect parental wishes regarding pregnancy surveillance. The length of antenatal hospitalization was significantly longer for IVF singleton mothers than for controls. This probably reflects the maternal characteristics related to infertility and the excess of pregnancy complications after IVF seen in this study; namely vaginal bleeding, ICP and threatened preterm birth. The perception of IVF pregnancy being a high-risk one probably also contributes to the matter. Due to the very uniform MHC system in Finland, we assume that there was no major bias in referring the mothers to specialized care from different MHCs. IVF twin mothers stayed longer in the hospital after birth than control twin mothers, probably reflecting the excess anxiety around ART twins, since no medical differences existed between the twin groups. No differences were found in the singleton analyses, most likely due to similar delivery events between the groups.

Previous literature has reported similar results. According to Gissler *et al.* (1995), Finnish IVF mothers started antenatal care earlier, had more antenatal visits than others, and more than 50% of them were hospitalized during pregnancy. IVF mothers have also been noted to have significantly more antenatal visits than IUI mothers or mothers with naturally conceived pregnancies (Nuojua-Huttunen *et al.* 1999). Danish IVF/ICSI twin mothers have been found to spend more days on sick leave and in hospital during pregnancy than non-IVF/ICSI twin mothers (Pinborg *et al.* 2004b).

The increased use of antenatal care by IVF mothers suggests that induced pregnancies are high-risk ones with higher maternal morbidity, but at the same time it probably reflects the preciousness of these pregnancies as experienced by the parents and phycicians.

5.4.2.2 Prenatal and neonatal costs

The mean numbers of the utilization and unit prices of prenatal and neonatal health care services, on which the cost calculations are based, are given in the original publication (IV). The use of prenatal care and mode of delivery are presented and discussed earlier in chapters 5.4.2.1 and 5.1.3. Regarding neonatal care, IVF singletons were treated for RDS more often than control singletons. Control twins, in turn, had more days in the hospital than IVF twins, mostly in the RDS group. Apart from prenatal hospital outpatient and ward service utilization, no significant differences were found in the utilization of other health care services. (IV.)

Prenatal costs were higher in IVF groups compared to control groups; 1.2-fold in the singleton comparisons and 1.3-fold in the twin comparisons. The neonatal costs for IVF singletons were 1.5-fold those for control singletons, but for twins the situation was reversed; 1.4-fold for controls compared to IVF twins. When prenatal and neonatal costs were calculated together, the costs were 1.3-fold in the singleton comparisons and 1.1-fold in the twin comparisons for IVF groups compared to controls. Prenatal costs were 2.4-2.5-fold, neonatal costs 3.7-7.8-fold and total health care costs 2.7-3.2-fold for twins compared to singletons. (Table 18.)

An example of the health care cost calculations (IVF neonates with RDS): divide the unit price (€) 24 405.8 by 22.55 (to obtain the cost of one day treatment), multiply this by 27.0 (to obtain the cost of 27 day treatment) and multiply this result by 0.0204 (proportion of neonates in the IVF RDS group) to obtain the cost of RDS treatment in the IVF group (€596.1).

Table 18. The different health care costs (€) per woman or child in the singleton and twin comparisons. The prices have been inflated to correspond to the year 2003.

Health care variables	Singletons		Twins	
	IVF	Controls	IVF	Controls
MHC visits	349.9	362.6	288.4	277.8
Visits to outpatient clinics	808.6	346.6	1 501.8	981.9
Prenatal hospitalization	1 559.4	1 086.8	6 899.0	4 867.1
Postpartum hospitalization	2 020.5	1 996.4	3 030.8	2 808.5
Prenatal costs	4 738.4	3 792.4	11 720.0	8 935.3
Neonatal hospitalization				
1. Healthy full-term neonate	9.8	33.7	207.7	239.9
2. Full-term neonate with neonatal morbidity[a]	79.7	215.5	65.3	109.2
3. Healthy preterm neonate	6.7	50.7	562.1	418.7
4. Preterm neonate with neonatal morbidity[a]	347.4	225.0	1 402.8	1 048.0
5. Neonate with RDS	596.1	178.3	1 621.6	3 696.6
Neonatal costs	1 039.7	703.2	3 859.5	5 512.4
Total health care costs	5 778.1	4 495.6	15 579.5	14 447.7

[a]Does not include respiratory distress syndrome (RDS). MHC = maternal health centre

Sensitivity analysis at 25th and 75th percentiles of the resource use showed that the interquartile range of the total health care costs would be €3 230-€6 790 for IVF singletons, €1 950-€5 280 for control singletons, €6 360-€18 180 for IVF twins and €7 000-€17 380 for control twins. The total prenatal and neonatal costs for the whole IVF population (n=303, one ICSI case excluded, 46.2% multiple births) were about €3 100 000. By decreasing the frequency of multiple births to 10.0% the costs would be reduced to €2 000 000. With a frequency of 12.0% of multiple births in 2002 among IVF population in Northern Finland (Stakes, unpublished data) the costs would be €2 100 000.

Comment. To our knowledge, only Gissler *et al.* (1995) have compared the cost of an IVF birth with the cost of a spontaneous birth. International comparisons of the costs are difficult, because medical practise, definitions and prices vary widely among countries (Lukassen *et al.* 2004).

The prenatal costs were higher for the IVF children in both comparisons, which is understandable due to the increased maternal morbidity among IVF pregnancies presented earlier. Delivery costs were equal and high among all comparison groups reflecting the high Cesarean section rate seen both in IVF and control groups. Costs of neonatal care among IVF singletons exceeded those of control singletons, but for twins the situation was reversed, probably reflecting the higher prevalence of MZ twinning among control twins. Furthermore, it can be speculated that mothers of IVF twins received prophylactic corticosteroid treatment for lung maturation when staying in hospital more often than control mothers with a possible favorable effect on the child outcome. The increased health care costs of IVF singletons may be due to the maternal characteristics of infertile women, since infertility has been linked with adverse birth outcomes even without infertility treatments (Basso & Baird 2003).

When singleton costs were compared to twin costs the difference was multiple for the twins indicating the high price of multiple pregnancies as reported earlier (Callahan *et al.* 1994, Goldfarb *et al.* 1996, Lukassen *et al.* 2004). A recent Belgian study showed that the cost difference between IVF/ICSI singletons and twins is mainly due to the higher neonatal costs of twins (Gerris *et al.* 2004) in contrast to our study, where all the different health care costs were clearly higher for twins than for singletons. Lucassen *et al.* (2004) showed recently that prenatal and neonatal costs of IVF twins (€13 469) were €10 000 higher than those of IVF singletons (€2 550) being in accordance with our results. The total costs presented by Lucassen *et al.* (2004) from the Netherlands were somewhat lower than in this study reflecting the differences of health care systems between the two countries as well as differences in the cost calculations; the unit costs of antenatal care in the Dutch study were based on hospital charges resulting in a much lower prenatal cost compared to ours, while our calculations were solely based on societal costs. Delivery costs were also markedly lower in the Netherlands compared to Finland.

Our study is the first to describe that the health care costs were €1 100-€1 300 higher for an IVF child than for a control child of the same plurality, indicating poorer perinatal health and increased use of health care after IVF. Multiple births, however, increase the costs by almost €10 000 per child, reflecting the major contribution of multiplicity on the health care costs after IVF.

5.4.2.3 Additional costs of IVF technology and 1st trimester pregnancy loss

After adding the costs of a successful IVF treatment to the health care costs the costs were 2.0-fold for singletons and 1.3-fold for twins compared to controls, respectively. The costs of unsuccessful cycles were estimated to be between €5 740-€12 306 per IVF pregnancy - the lower limit including only the costs of medication, and the upper limit also including the costs of IVF treatment and a three day sickness allowance. Furthermore, there were on average 0.3 spontaneous abortions and ectopic pregnancies in the obstetric history of IVF women, with control women having had on average 0.1-0.2 spontaneous abortions and 0.01-0.02 ectopic pregnancies. After adding the costs of unsuccessful cycles and 1st trimester pregnancy loss the costs of IVF neonates were 4.7-4.8-fold in the singleton comparison and 2.2-fold in the twin comparison. (Table 19.)

Table 19. Total costs (€) with the costs of IVF and additional costs from the unsuccessful cycles and 1st trimester pregnancy loss added.

Costs per woman	Singletons		Twins	
	IVF	Controls	IVF	Controls
Health care costs	5 778.1	4 495.6	15 579.5	14 447.7
Cost of successful IVF cycle	3 290.7	-	3 290.7	-
Cost of unsuccessful cycles	12 305.9	-	12 305.9	-
Cost of prior 1st trimester pregnancy loss	1 196.6	269.8	1 270.0	151.2
Total costs	22 571.3	4 765.4	32 446.1	14 598.9

[a]The estimated costs of unsuccessful cycles varies between €5 740.8 and €12 305.9 (lower limit: the cost of medication; upper limit: the cost of medication, IVF treatment and 3 day sick leave). The maximum sum is used in the table. [b]The unit price of treatment for spontaneous abortion €880.3/1.09 days and ectopic pregnancy €3 167/1.86 days (Hujanen 2003).

Comment. The cost of an IVF child also includes the costs resulting from IVF technology and from probable unsuccessful cycles. In the present study IVF technology increased the costs 2-5-fold. This is consistent with another Finnish study where the health care costs for one IVF newborn from induction of pregnancy until the age of 7 days were 5.4-fold compared to other newborns (Gissler *et al*. 1995). Similarly, unsuccessful first trimester pregnancies that commonly characterize the obstetric history of infertile women tend to increase costs. In our maternal study population especially ectopic pregnancies were more common among IVF mothers than among control mothers (21-24% vs. 1-2%).

Unfortunately, we did not have data on the extent of loss of working days after the IVF treatment or on the traveling costs, which is why the societal costs after IVF and spontaneous conception are somewhat underestimated in the present study. Therefore, the actual cost difference between IVF and control groups reported here is probably reduced by the lack of these data.

The additional costs resulting from unsuccessful IVF cycles and early pregnancy loss increase the costs of an IVF child by up to €13 500 for which unsuccessful cycles are mostly responsible. This reflects the expensiveness of IVF technology that often exceeds the expenses resulting from other health care.

6 General discussion

The concern over the health of IVF children is global and of current interest; there is, however, little relevant data on the topic at the moment. This study was conducted inspired by this lack of knowledge and to fill in the gap in the literature. With respect to previous studies, the present study design is unique; it enables us to evaluate simultaneously the effect of multiple birth and IVF technology on the outcome. Multiple pregnancy is a well-known and common outcome after IVF, and therefore we wished to analyze the effects of multiplicity on the outcome. The controls are drawn from the general delivery population and not from the hospital populations, reducing selection bias. The careful matching reduces the confounding effects of the most important factors influencing pregnancy outcome such as maternal age, parity and socioeconomic standing. On the other hand, overmatching has been prevented by handling gestational age and zygosity-chorionicity as intermediate factors on the causal pathway from IVF to child outcome contrary to some other studies. The matching criterion of area of residence was the only one we were not able to follow due to lack of multiple control pregnancies in Northern Finland. Power calculation was based on clinical developmental outcomes (15% for developmental disorders among unexposed population) and the sample size was large enough to detect significant differences regarding most of the outcomes studied. As far as rare events such as malformations affecting certain organ systems, cerebral palsy or childhood cancer are concerned the power was, however, insufficient. For the twin comparisons the power was lower and did not allow us to find small differences between the groups. The problem of missing data (10-15%) was faced regarding developmental milestones. However, data were missing randomly in both IVF and control groups and consequently we assume that no major (differential) selection or information bias existed. As a weakness in this study concerning psychomotor development, a follow-up period of three years is quite short, especially with the rather grand-scale routine screening method we used in this study due to the large sample size. The screening method in question is, however, the one used for screening of developmental disorders and significant diseases in the general population and therefore relevant in this respect as well. To further investigate the psychomotor development after IVF, prospective studies with clinical neurological examination are recommended. Based on the aspects mentioned, we believe

that the differences we observed between the IVF and control groups are not random or due to bias or confounding, and that they will have clinical importance and implications.

The main prenatal results of this study show that IVF pregnancies are more often complicated by vaginal bleeding, threat of preterm birth and ICP, the latter being described for the first time. The main results concerning child outcome indicate that IVF children are at an increased risk for cardiac malformations, mild delays in growth and chronic illnesses during early childhood.

The adverse child outcomes during neonatal period and early childhood after IVF were mostly seen in the full sample comparisons where the whole IVF population was compared with controls representing general population with respect to multiple births (around 1%). This strongly suggests that most of the adverse events after IVF are a result of multiple births. The design of our study allowed us to show this in a unique way. The excess of septal defects after IVF cannot, however, be solely explained by multiplicity, but by other complex mechanism with a probable influence by the infertility status of the mother. Nevertheless, multiplicity is commonly accepted as the most important complication of IVF treatment as well as a source of excess health care costs shown also by this study, and therefore ESHRE Campus Course Report (2001) has recommended that the multiplicity rate after ART should be globally reduced to 10% by favoring single embryo transfer if possible. This recommendation has been achieved in parts of Northern Europe (Hamberger *et al.* 2005), but for example in the United States of America the overall multiple birth rates still often exceed 40% (Reynolds *et al.* 2003). It has been reported that acceptable pregnancy rates can be achieved by elective single embryo transfer, while dizygotic twins will be avoided (Martikainen *et al.* 2001, Tiitinen *et al.* 2003). In ongoing IVF/ICSI pregnancies in Finland the twin rate is ~7% (Martikainen *et al.* 2004, Suikkari 2005, personal communication) indicating that the health of IVF children has improved and economic savings have already been gained, referring to our sensitivity analysis where the multiple birth rate of the study population was 46%.

The effect of IVF technology on the outcome has been neither proved nor eliminated by previous studies or the present study. The analyses stratified for plurality were designed for that purpose, but the effect of maternal characteristics related to infertility not measured in this study serve as a residual confounder in this respect. With the exception of vaginal bleeding during pregnancy, threat of preterm birth, ICP, respiratory diseases and diarrhea, analyses stratified for plurality showed only small non-significant differences, making us believe that the effect of IVF itself on prenatal and child outcome is in general small. The role of IVF technology, however, can be speculated regarding vaginal bleeding during pregnancy, since IVF has been noted to increase the incidence of low-lying placentas (Jackson *et al.* 2004). The phenomenon of vanishing embryos that has been linked to increased risk of preterm birth (Dickey *et al.* 2002) can also be regarded as an IVF treatment-related complication in the form of multiple embryo transfer.

Infertility itself has been noted to be an independent risk for adverse birth outcomes (Basso & Baird 2003, Basso & Olsen 2005) and although its effects on the outcomes cannot be seen directly in this study, it is most likely that the differences between IVF and control groups are partly due to factors related to the infertility status of the mothers.

The twin outcome was generally similar between the IVF and control groups, although decreased power probably diminished the differences to some extent. Therefore, it can be

concluded that multiplicity is a stronger determinant of perinatal outcome than IVF technique or infertility. On the other hand, IVF affects zygosity-chrionicity distribution in two ways: it increases the incidence of DZ twinning by multiple embryo transfer, but at the same time the technique may also increase the risk MZ twinning. However, most IVF twin pregnancies are dizycotic and therefore one might expect that the outcome would be better for IVF twins. This was not, however, confirmed by this study.

The results presented in this study mostly confirm the a priori hypotheses of adverse prenatal, neonatal and early childhood outcome as well as higher health care costs after IVF. The specific hypothesis on delayed psychomotor development after IVF was, however, not confirmed.

Practical implications and future perspectives. To reduce the health problems and related health care costs after IVF the proportion of multiple births should be reduced to a minimum by using single embryo transfer whenever possible. Additionally, thorough consideration is suggested in treating severely ill, premenopausal or even postmenopausal women. Legislation around these extremities of infertility treatment would probably be beneficial. According to other literature, the safety of ICSI to the offspring has not been thoroughly clarified at this point, suggesting that IVF is the treatment of choice for infertility while ICSI should be saved for cases with IVF failure and male infertility (Ola *et al.* 2001). The present finding that IVF children have growth delays and increased morbidity during their early childhood in comparison to naturally conceived children with similar backgrounds indicates that studies with longer follow-up periods and larger sample sizes are needed to further investigate the long-term outcome after IVF. In conducting such studies national IVF registries would be of use. It is also important that couples entering an IVF program are aware of the possible adverse outcomes after IVF in order to be able to make an informed decision.

7 Conclusions

1. IVF pregnancies are more prone to obstetric problems, such as vaginal bleeding, threat of preterm birth and intrahepatic cholestasis of pregnancy, than spontaneous pregnancies. Consequently, IVF mothers use more specialized antenatal care than mothers with naturally conceived pregnancies. The increased prenatal risks after IVF are probably due to maternal characteristics regarding infertility.
2. Neonatal outcome after IVF is poorer than after natural conception in terms of preterm birth, low birth weight and morbidity, the increased risks being mostly related to the high proportion of multiple pregnancies after IVF. In order to improve neonatal outcome after IVF, the number of multiple pregnancies should be limited to a minimum.
3. IVF children have a higher risk for heart malformations, especially septal defects. This cannot be explained by multiplicity, but possibly by other complex environmental or genetic mechanisms.
4. IVF children are at an increased risk for postnatal growth restriction in comparison to control children; however, their psychomotor development was similar. Their somatic health until the age of three years was poorer than that of control children, probably reflecting the excess problems in the neonatal period.
5. The health care costs resulting from prenatal and neonatal care are higher for IVF singletons than for control singletons, probably reflecting the effects of infertility on pregnancy outcome. For twins the costs were equal. Health care costs after IVF are markedly increased by multiple births; the cost of an IVF twin is €10 000 higher than the cost of an IVF singleton. Therefore, the reduction of multiple pregnancies is the most effective way to reduce the health care costs resulting from IVF.

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