

Outcome of Singleton Pregnancy after Assisted Reproductive Treatment

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Academic Dissertation

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Supported by the
Clinical Graduate School in Paediatrics and Obstetrics/Gynaecology
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To be publicly discussed with the permission of the Faculty of Medicine
of the University of Helsinki, in Lecture Hall 3, at Biomedicum Helsinki,
on 9th of March, 2007, at 12 noon.

Helsinki 2007

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ISBN 978-952-92-1662-8 (paperback)

ISBN 978-952-10-3747-4 (pdf)

<http://ethesis.helsinki.fi>

University Printing House

Helsinki 2007

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

- I Poikkeus P, Hiilesmaa V, Tiitinen A. Serum hCG 12 days after embryo transfer in predicting pregnancy outcome. *Hum Reprod* 17: 1901-1905, 2002.
- II Poikkeus P, Unkila-Kallio L, Vilksa S, Repokari L, Punamäki R-L, Aitokallio-Tallberg A, Sinkkonen J, Almqvist F, Tulppala M, Tiitinen A. Impact of infertility characteristics and treatment modalities on singleton pregnancies after assisted reproduction. *Reproductive Biomed Online* 13: 135-144, 2006.
- III Poikkeus P, Gissler M, Unkila-Kallio L, Hyden-Granskog C, Tiitinen A. Obstetric and neonatal outcome after single embryo transfer. *Hum. Reprod. Advanced Access* published January 24, 2007, doi:10.1093/humrep/del492.
- IV Poikkeus P, Saisto T, Unkila-Kallio L, Punamäki R-L, Repokari L, Vilksa S, Tiitinen A, Tulppala M. Fear of childbirth and pregnancy-related anxiety in women conceiving with assisted reproduction. *Obstet Gynecol* 108: 70-76, 2006.

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ABBREVIATIONS

ART	assisted reproductive treatment
BMI	body mass index
CI	confidence interval
COS	controlled ovarian stimulation
CS	Caesarean section
D1	day one after insemination
D2	day two after insemination
D5	day five after insemination
DET	double embryo transfer
E2	estradiol
eSET	elective single embryo transfer
FET	frozen embryo transfer
FSH	follicle-stimulating hormone
GnRH	gonadotrophin-releasing hormone
hCG	human chorionic gonadotrophin
HDR	Hospital Discharge Register
ICSI	intracytoplasmic sperm injection
IUI	intrauterine insemination
IVF	<i>in vitro</i> fertilization
LBW	low birth weight
LH	luteinizing hormone
MBR	Finnish Medical Birth Register
NICU	neonatal intensive care unit
NPV	negative predictive value
OHSS	ovarian hyperstimulation syndrome
OR	odds ratio
OS	ovarian stimulation without GnRH agonist
PAS	pregnancy anxiety scale
PNMR	perinatal mortality rate
PPV	positive predictive value
RCM	Register of Congenital Malformations
rFDQ	revised version of the fear-of-childbirth questionnaire
rFSH	recombinant follicle-stimulating hormone
SES	socioeconomical status
SET	single embryo transfer
SGA	small for gestational age
TVS	transvaginal sonography
VLBW	very low birth weight

ABSTRACT

Singleton pregnancies achieved by means of assisted reproductive treatment (ART) are associated with increased obstetric and neonatal risks in comparison with spontaneously conceived singleton pregnancies. The impact of infertility- and treatment-related factors on these risks is not properly understood. In addition, the psychological effects of infertility and its treatment on the experience of pregnancy have scarcely been studied. Thus, the aim of the present study was to evaluate the importance of infertility- and treatment-related factors on prediction of pregnancy outcome, obstetric and neonatal risks, fear-of-childbirth and pregnancy-related anxiety.

The subjects consisted of three different cohorts of infertile women who achieved a singleton pregnancy by means of *in vitro* fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) at the Infertility Clinics of Helsinki University Central Hospital, The Family Federation of Finland and The Deaconess Institute. The control groups comprised spontaneously conceiving women with singleton gestations. Early pregnancy outcome was assessed by means of assay of serum human chorionic gonadotrophin (hCG) in single samples. Other outcome data were collected from patient records, national Health Registers and via prospective questionnaire surveys.

Viable pregnancies were associated with significantly higher serum hCG levels 12 days after embryo transfer than non-viable pregnancies. Among singleton pregnancies, aetiological subgroup, treatment type or the number of transferred embryos did not impair the predictive value of single hCG assessment.

According to the register-based data, age-, parity- and socioeconomic status- adjusted

risks of gestational hypertension, preterm contractions and placenta praevia were more frequent in the ART pregnancies than in the control pregnancies. Significantly higher rates of induction of delivery and Caesarean section occurred in the ART group than in the control group. The risks of preterm birth and low birth weight (LBW) were increased after ART pregnancy. Duration or aetiology of infertility, treatment type (fresh or frozen IVF or ICSI) or rank of treatment did not contribute to the risks of preterm birth or LBW. In addition, the risks of preterm birth and LBW remained elevated regardless of the number of transferred embryos. Although mean duration of pregnancy was shorter and mean birth weight lower in the ART pregnancies than in the control pregnancies, these differences were hardly of clinical significance.

Fear-of-childbirth and pregnancy-related anxiety were equally common to women conceiving by means of ART, or spontaneously. Partnership of five to ten years appeared to be protective as regards severe fear-of-childbirth, whereas long preceding infertility (\geq seven years) had the opposite effect.

In conclusion, no specific infertility- or patient-related factor that would alter the predictive value of an early hCG assessment could be identified. Neither did we recognise any infertility- or patient-related factors that would expose infertile women to increased obstetric or neonatal risks. However, a long period of infertility was associated with severe fear-of-childbirth. These findings suggest that the reasons for increased obstetric and neonatal risks in singleton gestation after ART should be sought among underlying factors other than the clinical factors involved in this study.

INTRODUCTION

One in six couples suffers from infertility some time during their life. Infertility is considered a major life-crisis and feelings of grief, anxiety and depression are common.

Infertility can be efficiently treated by means of ART. Since the introduction of IVF in 1978 approximately two million infants worldwide have been born with the aid of different ART modalities. In 2004, 2.7% of all Finnish infants were born after ART.

As early as in the first pregnancy outcome reports in the 1980s, significantly more obstetric and neonatal complications in ART pregnancies than in spontaneously conceived control pregnancies were documented. To a great extent this was the result of a nearly 20-fold higher rate of multiple gestations (~25%) in ART pregnancy cohorts than in cohorts following spontaneous conception. Although the high rate of multiple pregnancies can be prevented with single embryo transfer, all the observed adverse

pregnancy outcomes may not. Indeed, singleton pregnancies after ART are associated with increased risks of ectopic pregnancy, abnormal placentation, Caesarean section, preterm birth, neonatal mortality and congenital malformations compared with age- and parity-matched control pregnancies.

Better understanding of the underlying mechanisms behind adverse pregnancy outcomes in singleton pregnancies after ART is crucial for patient counselling, performing ART and for clinically following these pregnancies. Furthermore, more pronounced anxiety over loss of pregnancy after ART may negatively affect the experience of pregnancy and lay grounds for fear-of-childbirth. The present study was designed to evaluate infertility- and treatment-related factors in relation to early pregnancy monitoring, obstetric and neonatal complications and prevalence of fear-of-childbirth and pregnancy-related anxiety.

REVIEW OF THE LITERATURE

Infertility

The failure to conceive after a year of unprotected timed intercourse is referred to as infertility in medical literature (Tietze 1968; Evers 2002). The incidence of infertility is highly dependent on female age and varies from six per cent in the age group of women under 25 years to 15–35% in women after their thirties (Menken et al. 1986; Baird et al. 2005). The lifetime incidence of infertility is estimated to lie between 10 to 17% (Hull et al. 1985; Snick et al. 1997). Also, according to a recent Finnish health survey, 14.4% of all female participants and 17.6% of women aged 20–54 years had experienced a period of involuntary childlessness sometime during their lives (Health 2000 2004).

Possible changes in the incidence of infertility over time are difficult to assess because of contraceptive use, delay in the attempt to become pregnant, and timing of pregnancies. However, population-based surveys indicate that infertility has decreased rather than increased in the past four decades (Akre et al. 1999; Joffe 2000; Stephen and Chandra 2006).

Aetiology of infertility

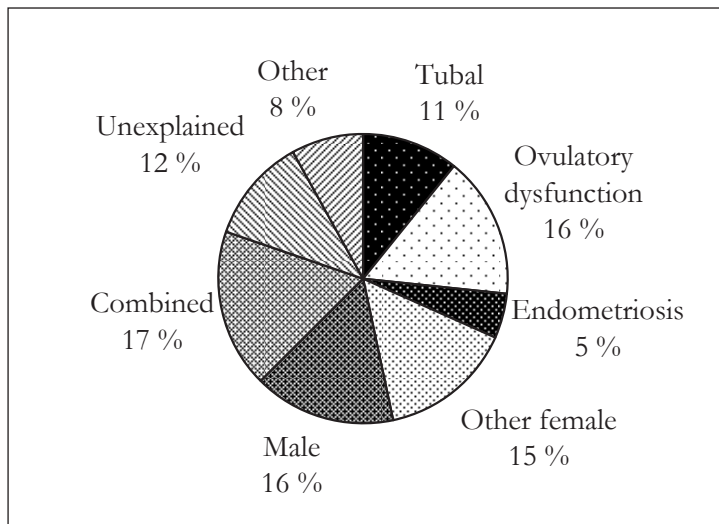
The aetiology of infertility is categorised as female in 25–47% of cases, male in 16–26%, combined in 18% and unexplained in 12–30% (Hull et al. 1985; Evers 2002; Wright et al. 2006). The main subgroups of female infertility can be further defined as tubal factor (24% of all female causes), ovulatory dysfunction (34%), endometriosis (11%) and other (31%) (Figure 1) (Wright et al. 2006). The distribution of different aetiological subgroups in cases of

female infertility shows demographic and global variation and corresponds to the conditions that lead to infertility. In the developed world, an overweight condition, smoking and increased female age at the time of pregnancy attempts are related to a higher incidence of ovulatory dysfunction, whereas in parts of the developing world pelvic inflammatory disease is relatively common and increases the rate of tubal factor infertility (Cates et al. 1985).

The aetiology of male infertility is less well understood. In nearly 50% of infertile men no single cause of infertility can be identified (Iammarrone et al. 2003). In the rest, impaired sperm production and function may be related to hypogonadotrophic hypogonadism, chromosomal or genetic disorders, cryptorchidism (failure of the testes to descend), cancer and its treatment, drugs and environmental toxins (Skakkebaek et al. 1994). In addition, sperm transport can be disturbed as a result of testicular obstruction (following vasectomy, infections or absence of the vas deferens), retrograde ejaculation or impotence.

The classification of different forms of male factor infertility is based on sperm concentration and morphology. Semen quality has been suggested to have deteriorated in the past century (Carlsen et al. 1992; Andersen et al. 2000; Aitken et al. 2004), which might be one manifestation of disturbed fetal testicular development and testicular dysgenesis syndrome (Bay et al. 2006). Nevertheless, abnormal semen values do not easily discriminate an infertile man from a fertile one (Guzick et al. 2001).

Figure 1. Percentage distribution of different aetiological subgroups among 74 296 infertile couples who received fresh embryo transfers in the USA in 2003 (Wright et al. 2006).



The subgroup “other female” includes cases with uterine factor or multiple, female-related factors. The subgroup “other” includes cases with immunological problems or chromosomal abnormalities, cancer chemotherapy, or serious illnesses.

Assisted reproductive treatment (ART)

The first infant following transfer of an oocyte fertilized *in vitro* was born in 1978 (Stephoe and Edwards 1978). Initially, IVF was developed to overcome tubal factor infertility. Later on, achievements in treatment of other female-related infertility subgroups and the introduction of ICSI for male factor infertility in 1992 (Palermo et al. 1992) have broadened the indications for ART.

Controlled ovarian stimulation

Assisted reproductive treatment was initially performed in a natural cycle with single embryo transfer (Stephoe and Edwards 1978). Transfer of a single embryo yielded only moderate results and therefore controlled ovarian stimulation (COS) was developed in order to achieve numerous oocytes to be fertilized and transferred. The use of gonadotrophin-releasing hormone (GnRH) agonists for reversible pituitary down-regulation in combination with exogenous gonadotrophin stimulation was introduced in the 1980s (Fleming et al. 1982; Porter et al. 1984) and has remained the most widely used COS protocol. Antagonists of GnRH have also been an option in COS since the late 1990s (Borm and Mannaerts 2000).

Treatment with GnRH agonists usually starts in the mid-luteal phase of the preceding menstrual cycle, with leuprolide acetate, nafarelin acetate, buserelin or triptorelin (Simberg et al. 1998). Most commonly, recombinant follicle-stimulating hormone (rFSH) is used as an exogenous gonadotrophin (Tulppala et al. 1999). Ovarian stimulation is monitored by means of (serial) serum estradiol (E2) measurements and transvaginal sonography (TVS). When at least two follicles reach a diameter of 17 mm in TVS, exogenous hCG is administered to complete follicular maturation. Oocyte retrieval is performed (with TVS guidance) 34–36 hours after hCG administration.

Semen collection and fertilization of oocytes

In conventional IVF, semen is collected by means of masturbation. In cases of severe male factor infertility semen can be obtained by epididymal or testicular sperm aspiration or from the urinary bladder. The semen sample is subjected to a “swim-up” procedure and centrifugation in order to obtain motile sperm for insemination. The retrieved oocytes are inseminated with sperm approximately four hours after retrieval. An oocyte can also be fertilized with a single sperm, as in ICSI. This is the treatment of choice in male factor infertility, in cases of surgically retrieved sperm or in cases of previous fertilization failure in conventional IVF cycles.

Approximately 50–70% of inseminated/injected oocytes are fertilized in an IVF/ICSI cycle. Fertilization is assessed after approximately 18 hours of incubation (i.e. on the first day after retrieval). Fertilization has occurred when two polar bodies and two (female and male) pronuclei can be visualized under a light microscope. After fertilization the pronuclear membranes break down, a diploid number of chromosomes is achieved and the first cell division is possible.

Embryo selection and transfer

Morphological criteria

The development of an embryo follows a distinct pattern of events: the zygotes start to divide approximately 25–27 hours after insemination, i.e. on day one (D1) (Balakier et al. 1993), a four cell stage can be reached on day 2 (D2) and a blastocyst stage on day five (D5).

Numerous morphological features of cleavage-stage embryos have been used to distinguish the best implanting embryos. The early cleavage rate (Shoukir et al. 1997; Sakkas et al. 1998; Lundin et al. 2001; Salumets et al. 2003a), number of blastomeres as regards the day of embryo development (Van Royen et al. 1999), blastomere fragmentation (Antczak and Van Blerkom 1999), and presence of

multinucleated blastomeres have all been found to be predictive of both implantation and pregnancy rates (Jackson et al. 1998; Hardarson et al. 2001). Ideally, a four-cell embryo on D2 or an eight-cell embryo on D3 with less than 20% fragmentation, equal blastomere size and without multinucleated blastomeres is transferred.

Number of transferred embryos

For as long as the recognition of embryos with the best implantation potential had not been settled, the only option to improve pregnancy rates was to increase the number of transferred embryos (Testart et al. 1986). Transfer of three to four embryos at the same time became standard, but at the price of increasing the numbers of triplet and quadruplet pregnancies. However, by the mid 1990s it had become evident that limiting the number of transferred embryos to two instead of three maintained the pregnancy rates but significantly decreased the rate of triplet and higher-order pregnancies (Staessen et al. 1993; Templeton and Morris 1998). At the end of the 1990s elective single embryo transfer (eSET) was introduced (Gerris et al. 1999; Vilska et al. 1999). Elective single embryo transfer refers to a procedure where a single embryo is selected from a pool of embryos and the rest are frozen. Currently, 46% of Finnish ART cycles are performed with single embryo transfer (SET) (STAKES 2006). The corresponding figure for the whole of Europe in 2002 was 13.7% (The European IVF-monitoring programme (EIM), 2006) and in the United States in 2003 it was 10.2% (Wright et al. 2006).

Cryopreservation and thawing of embryos

The first human pregnancy after cryopreservation and thawing of an embryo was reported in 1983 (Trounson and Mohr 1983). Thereafter, cryopreservation became an essential part of ART. The main advantages of embryo cryopreservation are the possibility to perform embryo transfer with fewer embryos at a time but with several embryos altogether, the prevention of exacerbation of ovarian hyperstimulation syndrome (OHSS) by postponing embryo transfer to a later cycle, and possible preservation of fertility for future purposes.

Human embryos can be frozen and thawed from the pronuclear to the blastocyst stage (Michelmann and Nayudu 2006). In general, freezing and thawing is associated with a 70–80% survival rate of blastomeres (Edgar et al. 2000). Frozen embryo transfer (FET) can be performed in natural or hormonally substituted cycles. Regardless of the type of cycle, synchronisation of the stages of development of frozen-thawed embryos and the endometrium is important. The timing of embryo transfer is carried out by assessing the luteinizing hormone (LH) surge in a natural cycle and by evaluating the appearance of the endometrium in TVS in a hormonally substituted cycle.

Recently, the concept of elective transfer of single embryos has also been applied in frozen-thawed ART cycles (Hyden-Granskog et al. 2005).

Treatment outcomes after ART

In Europe in 2002, the clinical pregnancy rates per embryo transfer were 29.5% (IVF), 29.4% (ICSI) and 18.4% (FET) (The European IVF-monitoring programme (EIM), 2006). The pregnancy rate remains similar in the first three ART cycles (Templeton et al. 1996) but the cumulative pregnancy rate plateaus by the sixth cycle to a level of 55% to 66% (Dor et al. 1996; Stolwijk et al. 2000; Hoveyda et al. 2002; Olivius et al. 2002; Witsenburg et al. 2005). In Finland in 1992–2004, the clinical pregnancy rates per embryo transfer were 28.4% (IVF), 28.1% (ICSI) and 19.3% (FET) (STAKES 2006).

The pregnancy rates after ART are dependent on several subject- and treatment-related characteristics. Therefore, great variation both in national and international figures exists and comparison of pregnancy rates without clinical details gives only a restricted picture. The most important prognostic factors, such as female age, weight, smoking, and number and quality of embryos influence the rate of both non-viable and viable pregnancies – understandably in opposite directions.

Prognostic factors

The most important prognostic factor of an ongoing pregnancy after ART is female age: women under 36 are most likely to achieve a pregnancy after ART (Templeton et al. 1996; Stolwijk et al. 2000; Olivius et al. 2002; Sharma et al. 2002; Witsenburg et al. 2005). On the other hand, increasing female age elevates the probability of miscarriage – most markedly after the age of 35 years (Gray and Wu 2000; Nybo Andersen et al. 2000; Schieve et al. 2003; Spandorfer et al. 2004a). The impact of paternal age is less evident, but male age ≥ 40 years has been suggested to decrease the ongoing pregnancy rates and to increase the rate of miscarriages (de la Rochebrochard and Thonneau 2002; de la Rochebrochard et al. 2006).

A woman's exposure to tobacco smoke sometime during her life (Klonoff-Cohen et al. 2001), and especially in association with ART, decreases her chances of achieving an ongoing pregnancy (Augood et al. 1998) and delivering an infant (Lintsen et al. 2005). In addition, her partner's smoking and passive smoking are deleterious as regards female fertility (Lintsen et al. 2005) and success in ART (Zitzmann et al. 2003; Neal et al. 2005). Fewer retrieved (El-Nemr et al. 1998; Klonoff-Cohen et al. 2001; Zitzmann et al. 2003), mature (Zenzes et al. 1997) and fertilized oocytes in female smokers than in non-smoking women undergoing ART are observed. Although male smoking is associated with worse morphology of sperm and reduction in sperm motility (Kunzle et al. 2003) the impact of male smoking on pregnancy rates after ART is less evident than that of female smoking and may be mediated through passive smoking of the female spouse.

The results of studies on the effect of alcohol consumption on fertility are heterogeneous. Whereas some state that increasing female alcohol consumption decreases the odds of achieving a pregnancy (Jensen et al. 1998), others have not found an association (Juhl et al. 2001). As regards alcohol consumption and pregnancy rates after ART, both female and male alcohol consumption close to ART seem to decrease the chance of achieving a pregnancy (Klonoff-Cohen et al. 2003).

Both underweight and overweight may impair fertility in otherwise healthy women. In ART, overweight and obese women (body mass index [BMI] > 25 kg/m² and > 30 kg/m², respectively) require higher gonadotrophin doses (Dodson et al. 2006; Dokras et al. 2006) and produce fewer oocytes (Spandorfer et al. 2004b) than women of normal weight. Their cancellation rate per cycle is higher (Dokras et al. 2006) and miscarriages more frequent (Wang et al. 2002a; Fedorcsak et al. 2004) than in lean women. Consequently, pregnancy and delivery rates are lower in overweight and obese women than in women of normal weight (Lintsen et al. 2005; Dokras et al. 2006). Increased male BMI has been suggested to correlating negatively with number of motile sperm (Kort et al. 2006). Both low (< 20 kg/m²) and high (> 25 kg/m²) BMI correlate with a reduction in sperm concentration (Jensen et al. 2004; Fejes et al. 2006). Nevertheless, the impact of male body weight on pregnancy rates after ART is not documented.

Duration of infertility plays a marginal role in pregnancy rates after ART and is usually confounded by increased female age. Each additional year of infertility decreases the odds of a birth by two per cent after maternal age, aetiology of infertility, number of previous ART attempts and number of fertilized and transferred embryos have been taken into account (Templeton et al. 1996). Couples with less than four years of infertility have been proposed to stand a better chance of achieving a pregnancy after ART (Templeton et al. 1996), but this was not confirmed in another, although smaller, study (Stolwijk et al. 2000). A prolonged time to achieve a pregnancy (i.e. 12 months or more) has been associated with a moderately increased risk of miscarriage (Joffe and Li 1994; Gray and Wu 2000; Axmon and Hagmar 2005), but the impact of duration of infertility on miscarriage rates after ART has not been studied.

The significance of different aetiological infertility subgroups on pregnancy rates is controversial. According to the largest published study, women with tubal factor infertility stand a decreased chance (by 30%) of achieving a viable pregnancy compared with women with other aetiologies of infertility (Templeton et al.

1996). Women with tubal factor infertility also have a higher incidence of ectopic pregnancies (Dubuisson et al. 1991; Karande et al. 1991; Ribic-Pucelj et al. 1995; Strandell et al. 1999). Women with diminished ovarian reserves also tend to have lower than average pregnancy rates (Wright et al. 2006). In respect to the risk of miscarriage after ART, male factor infertility and endometriosis-related infertility are suggested to lower this risk (Schieve et al. 2003; Lintsen et al. 2005), whereas increased risks in women with polycystic ovary syndrome (Balen et al. 1993; Wang et al. 2001) and unexplained infertility (Balen et al. 1993; Wang et al. 2001; Omland et al. 2005) have been proposed.

Previous pregnancy and live birth – especially if achieved by means of ART – increases the possibility of achieving a pregnancy after ART (Tan et al. 1994; Alsalili et al. 1995; Templeton et al. 1996; Stolwijk et al. 2000).

Increased numbers of retrieved (≥ 5 ; [Sharma et al. 2002]) and fertilized (≥ 4 ; [Templeton et al. 1996]) oocytes improve the pregnancy rates, as does an increased number of available embryos (≥ 5) for transfer (Hoveyda et al. 2002).

The quality of transferred embryos is also of great importance as regards pregnancy rates. Transfer of an early cleaving embryo (corresponding to a two-cell stage 25–27 hours after insemination) results in a nearly twofold higher pregnancy rate than transfer of a later cleaving embryo (Shoukir et al. 1997; Sakkas et al. 1998; Lundin et al. 2001). Early cleaving has a similar impact both in multiple embryo (Shoukir et al. 1997; Sakkas et al. 1998; Lundin et al. 2001) and in single embryo transfers (Salumets et al. 2003a). Nevertheless, it seems that early cleavage might not bear an independent positive impact on pregnancy rate; rather, it coexists with more significant predictors – female age and the total number of good quality embryos (Lundin et al. 2001).

Embryo cleavage during the subsequent days in embryo culture also plays a role. Four

blastomeres on D2 and eight on D3 are associated with better ongoing pregnancy rates than other numbers of blastomeres per embryo (Van Royen et al. 1999). Further, embryos with no or less than 20% fragmentation (Giorgetti et al. 1995; Ziebe et al. 1997; Van Royen et al. 1999), with equal-sized blastomeres and without multinucleated blastomeres (Jackson et al. 1998; Hardarson et al. 2001) are associated with improved ongoing pregnancy rates. The implantation and clinical pregnancy rates do not seem to correlate strongly with the developmental stage of frozen-thawed embryos (Salumets et al. 2003b). However, the number of intact blastomeres ($> 50\%$) and the presence of embryo cleavage are associated with improved implantation and clinical pregnancy rates (Guerif et al. 2002; Tang et al. 2006).

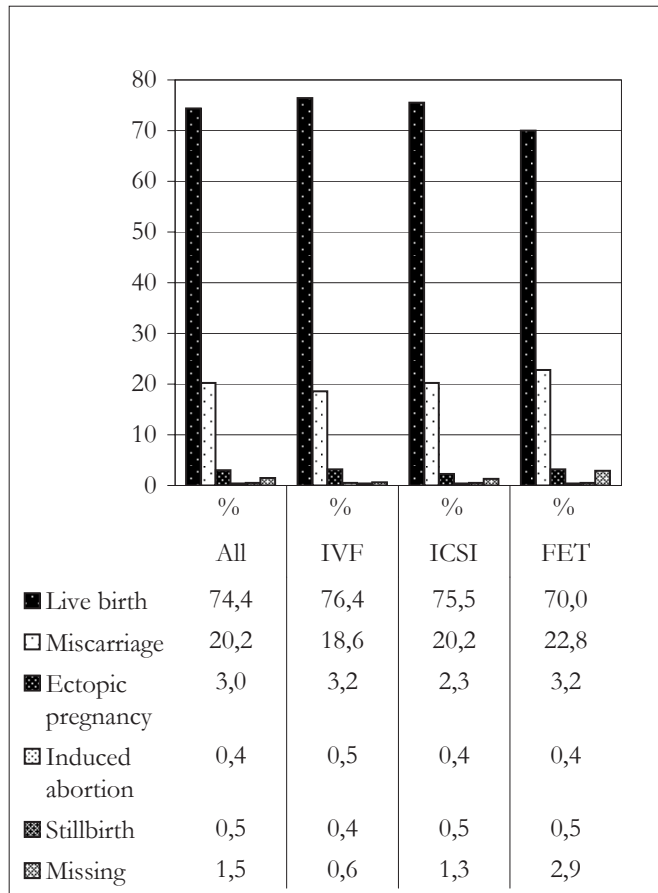
No difference in clinical pregnancy rate between transfers of D2 versus D3 cleavage stage embryos is evident (Blake et al. 2005). Both improved (Papanikolaou et al. 2006) and similar (Blake et al. 2005) pregnancy rates after transfer of blastocyst stage versus cleavage stage embryos have been reported. Transfer of three or more embryos at a time does not increase the chance of pregnancy over the transfer of two embryos, but it increases the number of multiple gestations (Templeton and Morris 1998). Double embryo transfer (DET) results in higher pregnancy and live birth rates than SET (Pandian et al. 2005). Nevertheless, when fresh SET is followed by a subsequent frozen embryo transfer cycle, similar live birth rates between SET and DET have been observed (Thurin et al. 2004; Pandian et al. 2005).

In vitro fertilization and ICSI are associated with similar pregnancy and live birth rates, but FET is associated with lower pregnancy and live birth rates than fresh embryo transfer cycles (La Sala et al. 2004; STAKES 2006; The European IVF-monitoring programme (EIM) 2006; Wright et al. 2006). The lower live birth rate after FET is partly a consequence of a higher miscarriage rate after FET than after fresh embryo transfer (STAKES 2006).

Outcome of clinical pregnancies

According to Finnish statistics, approximately one third of embryo transfers results in a clinical pregnancy (STAKES, 2006). Out of these clinically confirmed pregnancies 74.4% lead to live birth, 20.2% to miscarriage, 3% to ectopic pregnancy, 0.4% to induced abortion and 0.5% to stillbirth (Figure 2).

Figure 2. Outcomes of clinical pregnancies ($n=17\ 834$) after ART in Finland, 1992–2004 (STAKES 2006).



Miscarriage

Miscarriage is fairly common in human gestation. Depending on the criteria and study methodology 10–20% of all clinical pregnancies end in miscarriage (Abma et al. 1997; Nybo Andersen et al. 2000; Speroff and Fritz 2005).

The incidence of miscarriage after clinically confirmed ART pregnancy is similar (i.e. 17–20%) to that after spontaneous conception and is higher in singleton than in multiple gestations (Schieve et al. 2003; Tummers et al. 2003; Spandorfer et al. 2004a; The European IVF-monitoring programme (EIM), 2006).

The frequency of miscarriages in singleton ART pregnancies achieved by means of eSET varies from 10.9% to 20.5% in fresh cycles (Gerris et al. 2002; Thurin et al. 2004; STAKES 2006).

Ectopic pregnancy

In an ectopic pregnancy a gestational sac lies outside the uterine cavity, most often in the ampullary part of the Fallopian tubes. In cases in which both extrauterine and intrauterine gestations exist, the pregnancy is defined as heterotopic. Both ectopic and heterotopic pregnancies can cause serious morbidity and further impairment of fertility.

The worldwide incidence of ectopic pregnancy is 1–2%. In Finland the incidence of ectopic pregnancy after ART is around 3% (Figure 2) and a twofold increase in the risk of ectopic pregnancy in international studies has been reported (Cohen et al. 1986; FIVNAT [French In Vitro National] 1995; Marcus and Brinsden 1995a; Strandell et al. 1999). However, the incidence of ectopic pregnancy rises from 5.4% to 11.1% in women with tubal factor infertility (Dubuisson et al. 1991; Ribic-Pucelj et al. 1995; Strandell et al. 1999).

The incidence of heterotopic pregnancy after ART has been approximately 1 per 100 (Svare et al. 1993; Marcus et al. 1995b; Tal et al. 1996) but it has decreased in the past decade. Controlled ovarian stimulation, multiple embryo transfer and pre-existing tubal damage predispose women to heterotopic pregnancies.

Human chorionic gonadotrophin (hCG) monitoring

Monitoring the outcome of a clinically confirmed pregnancy after ART by means of TVS is common. The purpose is to confirm the location and viability of pregnancy. In cases of multiple gestation, early TVS also confirms the number of gestational sacs and determines the zygosity. A gestational sac can be visualized at the fifth week of gestation and fetal heart beat at the seventh in viable pregnancies. However, the possible complications of ectopic pregnancy develop in earlier gestational weeks. In order to detect an ectopic pregnancy before diagnosis by means of TVS is feasible, other monitoring methods are required.

Human chorionic gonadotrophin is a glycoprotein that consists of non-covalently linked α and β subunits. During pregnancy, placental hCG is synthesized in syncytiotrophoblasts. The production and secretion of hCG in syncytiotrophoblasts is regulated by GnRH, growth factors, sex steroids and cytokines (Speroff and Fritz 2005). The function of placental hCG is to take part in the maintenance of pregnancy by stimulating progesterone production in the corpus luteum in early gestation.

In vitro, transcription of the hCG gene can first be detected in embryos at the eight-cell stage (Bonduelle et al. 1988). In a culture media, hCG can be detected seven to eight days after fertilization (Hay and Lopata 1988; Lopata and Hay 1989). *In vivo*, hCG first appears in maternal serum seven to ten days after fertilization (Marshall et al. 1968; Braunstein et al. 1973; Lenton et al. 1982; Stenman et al. 1997). After COS, maternal serum hCG is first detectable seven to nine days after embryo transfer (Lopata et al. 1982; Alftan et al. 1988; Hsu et al. 1998). Exogenous hCG used for oocyte maturation before oocyte retrieval results in a peak of serum hCG one day after injection, with baseline values again reached within eight to thirteen days (Stenman et al. 1997).

Maternal serum concentrations of hCG rise exponentially in early pregnancy. The doubling time of hCG in viable pregnancies increases with increasing gestational age

(Pittaway et al. 1985; Check et al. 1992). The doubling times in viable pregnancies following spontaneous conception and ART are similar.

Measurement of maternal urinary or serum hCG has proven to be a reliable tool for early confirmation of pregnancy. With most current pregnancy test kits, 98% of samples will be positive seven days after implantation, i.e. at the time of missed menses (Chard 1992). A single serum hCG measurement has also shown the best discriminatory potential among commonly used biochemical markers in predicting the outcome of ART (Lower and Yovich 1992) and has been widely accepted for monitoring early pregnancy after ART (Table 1). Prediction of pregnancy after ART by means of a single hCG assessment can be done with sufficient reliability eleven to twelve days after ET (Lenton et al. 1982; Lopata et al. 1982; Legro et al. 1995). Generally, viable pregnancies are associated with higher hCG levels and a shorter doubling time than non-viable pregnancies (Confino et al. 1986; Okamoto et al. 1987; Schmidt et al. 1994; Bjercke et al. 1999; Chung et al. 2006). Further, multiple gestations show higher levels of maternal serum hCG than singleton gestations. In non-viable pregnancies, the lowest hCG levels are shown in biochemical pregnancies. In first trimester miscarriages and in ectopic pregnancies, initial hCG levels are lower than in viable pregnancies, but significant overlapping with the hCG levels of viable pregnancies may occur. In ectopic pregnancies, implantation of a blastocyst occurs later than in intrauterine pregnancies, thus resulting in a later rise of hCG than in viable pregnancies (Korhonen et al. 1996; Sugantha et al. 2000). Nevertheless, the doubling time of hCG concentrations in ectopic pregnancies is

similar to that in viable singleton pregnancies (Korhonen et al. 1996; Sugantha et al. 2000) and serial hCG sampling may be needed.

Different ART protocols may have a different impact on a single hCG level. Treatment with GnRH agonists has been suggested to be associated with a slower initial rise but with a similar doubling time of serum hCG concentrations compared with ovarian stimulation without GnRH agonists (Tur-Kaspa et al. 1990; Ertzeid et al. 2000). In addition, the treatment type and the aetiology of infertility may be related to differences in “early” hCG levels in viable ART pregnancies. Gold and colleagues (2000) reported that hCG levels were higher in cases with tubal factor infertility than in those with male factor and unexplained infertility. Further, in a small subgroup of women with unexplained infertility, lower hCG values were observed 16 days after embryo transfer in pregnancies following ICSI than in pregnancies following IVF (Gold et al. 2000).

The impact of the number of transferred embryos on the discriminatory value of early hCG levels in singleton ART pregnancies has not been thoroughly studied. A single study involved assay of hCG values 12 days after SET and it was found that an hCG- β cut-off value of ≥ 45 IU/L had 75.6% sensitivity and 100% specificity for predicting viable pregnancy (De Neubourg et al. 2004).

Serum hCG concentrations are different in ART pregnancies than in spontaneous pregnancies at the time of maternal serum screening for Down’s syndrome. Higher hCG levels after ART (fresh and frozen IVF or ICSI) than after spontaneous conception are usual (Perheentupa et al. 2002; Rätty et al. 2002).

Table 1. Predictive value of serum hCG concentrations (IU/L) for the outcome of ART.

Reference	<i>n</i>	Assay	Time*	Predicted outcome	hCG cut-off level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Okamoto, 1987	161	hCG- β	12	ectopic pregnancy	30.6	100	68	17	100
Schmidt, 1994	390	hCG- β	14/16	viable pregnancy	100.0	91	71	83	83
Fridström, 1995	329	hCG	13	viable pregnancy	150.0	79	78	45	89
Legro, 1995	77	hCG- β	9	viable pregnancy	10.0	91	75	91	91
Glatstein, 1995	351	hCG (intact)	14	viable pregnancy	135.0	93	94	93	94
Bjercke, 1999	417	hCG (intact)	14	viable pregnancy	55.0	91	61	60	90
Homan, 2000	662	hCG	16**	ongoing pregnancy	500.0	95			
Sugantha, 2000	429	hCG (total)	12	non-viable pregnancy	50.0	85	79	66	92
De Neubourg, 2004	180	hCG- β	12	viable pregnancy	45.0	76	100		

* refers to days after embryo transfer or ovulation**

NPV = negative predictive value

PPV = positive predictive value

Elective single embryo transfer

Multiple pregnancy occurs in 24.5–33.6% of gestations achieved via ART (The European IVF-monitoring programme (EIM) 2006; Wright et al. 2006). This means a more than twenty-fold increase in multiple pregnancies compared with spontaneous conception and is a consequence of transferring more than one embryo at a time. The European Society for Human Reproduction and Endocrinology (EHSRE) has recommended halving the multiple pregnancy rate by increasing the number of SETs (ESHRE Campus Course Report 2001). In clinics where SET represents 50% or more of all embryo transfers a twinning rate of 10% or less is achieved (Tiitinen and Gissler 2004).

Great diversity in the number of transferred embryos exists among European countries and overall SET still represents a minority of all embryo transfers (The European IVF-monitoring programme (EIM) 2006). The great variation in implementing active eSET policies depends on several factors (Gerris 2005). In some countries embryo selection has to be made at the oocyte stage, all cultured embryos may have to be transferred, or no embryo freezing is allowed. In other countries, eSET is encouraged by a reimbursement system (Belgium) or nationwide regulation (Sweden). In Finland the initiative to implement eSET was started in 1997 and driven by infertility professionals (Vilksa et al. 1999). At first the motive to perform eSET was to reduce the obstetric risks related to multiple pregnancies in women with a chronic illness, uterine malformation or previous preterm birth (Vilksa et al. 1999). During the past decade the indications have broadened, but as yet no national recommendations exist. Currently, 46% of Finnish embryo transfers are performed with a single embryo and 78% of these are performed as eSET (STAKES IVF-statistics, unpublished data).

The first convincing results after eSET were published by Vilksa and colleagues in 1999 (Vilksa et al. 1999). In their one-year cohort the clinical pregnancy rate per transfer was similar in the eSET and DET groups (29.7% vs. 29.4%) but the twin pregnancy rate was significantly lower after eSET than after DET (0 vs. 23.9%)

(Vilksa et al. 1999). Thereafter, the results of both randomized studies and retrospective observational studies have confirmed a total twinning rate after eSET comparable to that after spontaneous conception (Gerris et al. 1999; Martikainen et al. 2001; Tiitinen et al. 2001; Tiitinen et al. 2003; Thurin et al. 2004). However, the rate of monozygous twinning is higher (~1–2%) after SET than after spontaneous conception (Alikani et al. 2003).

The challenge of active eSET policy, on the other hand, is to maintain acceptable overall pregnancy rates. According to the results of some randomized works and all retrospective observational studies with selected subjects, pregnancy and live birth rates comparable to those after DET can be achieved with elective transfer of single embryos (Vilksa et al. 1999; Martikainen et al. 2001; Tiitinen et al. 2001; Gerris et al. 2002; De Sutter et al. 2003; Tiitinen et al. 2003; Lukassen et al. 2005). In contrast to previous results significantly lower pregnancy and live birth rates after one eSET than after one DET were found in the only existing prospective, randomized study (Thurin et al. 2004). However, if supplementary transfer of a frozen-thawed single embryo was combined with previous fresh eSET, pregnancy and live birth rates similar to those after one DET were achieved (Thurin et al. 2004).

Pregnancy rates after eSET are largely influenced by the subjects' characteristics. In the majority of studies eSET is used among women with a high chance of conceiving, i.e. younger women (age less than 35–37 years) having their first or second fresh IVF or ICSI treatment. However, according to a recent review only ~35% of all infertile women belong to this highly selected subgroup, with good pregnancy and live birth rates (Gerris 2005). Indeed, when applying eSET to unselected women (i.e. no selection as regards woman's age or embryo quality), pregnancy rates have been reported to be significantly lower than after DET: 21.4% vs. 40.3% (van Montfoort et al. 2006). Nevertheless, also similar pregnancy and live birth rates after eSET and DET in an observational study with women aged 36–39 years have also been found (Veleva et al. 2006).

Obstetric and neonatal outcome after ART

The first published studies on obstetric and neonatal outcome in pregnancies achieved by means of ART in the 1980s suggested that an excess of certain maternal complications, and preterm births, were characteristic of these pregnancies (Australian *in vitro* fertilisation collaborative group 1985; Frydman et al. 1986; Steptoe et al. 1986). During the past twenty years many of the initial discoveries have been verified in numerous studies on obstetric and neonatal outcome of ART singletons.

Course of pregnancy

The frequencies of specific maternal complications are presented in Table 2. According to age- and parity-matched studies, maternal vaginal bleeding in the first trimester (OR 4.1; 95% CI 3.6–4.8) and in the later trimesters (OR 2.7; 95% CI 1.4–5.5) is more typical of singleton ART pregnancies than of spontaneous singleton pregnancies (Koudstaal et al. 2000; Koivurova et al. 2002a; Källén et al. 2005a). First trimester vaginal bleeding may be related to the 10% vanishing twin survivor cohort in singleton ART pregnancies following DET (Pinborg et al. 2005; De Sutter et al. 2006a) but it is also frequent after single embryo transfer. First trimester bleeding increases the risk of vaginal bleeding that occurs later in singleton ART pregnancies (De Sutter et al. 2006a).

Vaginal bleeding in the second and third trimesters can be a sign of abnormal placentation.

According to the results of two large cohort studies, placenta praevia was diagnosed in 1.3%–1.6% of singleton ART pregnancies, thus resulting in four- to fivefold age- and parity-adjusted risks of placenta praevia (Källén et al. 2005a; Romundstad et al. 2006). Somewhat lower age- and parity-adjusted increased risks of placenta praevia (OR 2.9; 95% CI 1.5–5.4) were noticed in a review of fifteen case-control studies concerning singleton ART pregnancies (Jackson et al. 2004). Along with more frequent vaginal bleeding and placenta praevia, increased risks of placental abruption (OR 1.9; 95% CI 1.4–2.5) and bleeding in association with vaginal delivery (OR 1.2; 95% CI 1.2–1.3) have been reported (Källén et al. 2005a).

In some studies pre-eclampsia (OR 1.2; 95% CI 1.1–1.3) and gestational diabetes (OR 2.0; 95% CI 1.4–3.0) have been reported to be more common in singleton ART pregnancies than in spontaneous singleton pregnancies (Jackson et al. 2004; Källén et al. 2005a). Premature rupture of the membranes (adjusted OR 1.5; 95% CI 1.3–1.7) occurs more often in singleton ART pregnancies than in spontaneous singleton pregnancies (Källén et al. 2005a).

Mothers with singleton pregnancies after ART use outpatient care more frequently and are more often hospitalised during the pregnancy than mothers conceiving spontaneously (Koudstaal et al. 2000; Klemetti et al. 2002; Koivurova et al. 2002a; Källén et al. 2005a).

Table 2. Frequencies of maternal complications in singleton pregnancies following ART.

Reference	Tan, 1992	FIVNAT, 1995	Reubinoff, 1997	Maman, 1998	Koudstaal, 2000	Koivurova, 2002a	Källén, 2005a*	De Neubourg, 2006**	Kjellberg, 2006**
Study period	1978-87	1986-90	1983-93	1984-94	-1992	1990-5	1982-2002	1998-2003	2000-3
Study place	England	France	Israel	Israel	Netherlands	Finland	Sweden	Belgium	Scandinavia
Data source	Obstetric register	National registry	Patient records	Patient records	Patient records	MBR	HDR	Questionnaire, Birth Register	Questionnaires
<i>n</i> (ART)	494	3889	260	169	307	153	10 087	251	128†
<i>n</i> (controls)	978	-	260	469	307	580	2 013 633	59 353	-
Vaginal bleeding	17% (↑)		3.1% (↔)	12.5% (↑)	8.7% (↑)‡	1.8% (↑)			
Placenta praevia	3% (↑)	1.5%	0.4% (↔)	2.3% (↔)	1.4% (↑)				
Placental abruption		0.5%		↔	0.5% (↑)				
PIH	14% (↑)		11.2% (↔)	9.5% (↑)	13.7% (↔)	12.6% (↔)	7.6% (↑)		7.0%
Pre-eclampsia		4.9%							
GDM		0.8%	6.2% (↔)	21.3% (↑)	↔	5.3% (↔)			
Preterm contractions			12.3% (↑)			14.7% (↑)		14.3%	
PROM		3.1%			↔		2.5% (↑)		0

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 ** pregnancies achieved with SET
 † includes one twin pregnancy
 ‡ 2nd and 3rd trimester bleeding
 ↑ increased frequency in ART vs. control group
 ↔ similar frequency in ART and control group
 GDM = gestational diabetes mellitus
 HDR = Hospital Discharge Register
 MBR = Medical Birth Register
 PIH = pregnancy-induced hypertension (gestational hypertension and pre-eclampsia)
 PROM = premature rupture of membranes

Delivery

Induction of delivery is more typical of singleton ART pregnancies than spontaneous singleton pregnancies (parity- and age- adjusted OR 1.4; 95% CI 1.3–1.5) (Källén et al. 2005a). A similar rate of vacuum extractions (Källén et al. 2005a) but a significantly higher rate of Caesarean sections (on average ~25%) than in spontaneous singleton pregnancies have consistently been observed (Klemetti et al. 2002; Helmerhorst et al. 2004; Jackson et al. 2004; Källén et al. 2005a).

Postpartum complications

Relatively few studies have been focused on the prevalence of maternal postpartum complications. In a Finnish sample maternal hospitalisation seven days after delivery was more frequent in the ART cohort than in the spontaneously conceiving mothers (Klemetti et al. 2002). Longer hospitalisation after delivery is probably associated with more frequent Caesarean sections and prolonged healing. In addition, more frequent maternal complications may require time to settle and longer hospitalisation is needed. According to the results of a Swedish Register-based study, re-hospitalisation within two months after delivery, especially because of bleeding, is more common in the ART cohort than in the spontaneous conception cohort (Källén et al. 2005a).

Gestational age at birth, birth weight and neonatal complications

The frequency of preterm births (< 37 weeks) in singleton pregnancies after ART has varied from 8.7% in a Finnish two-year cohort (Klemetti et al. 2002) to 14.1% in a one-year cohort in the United States (Schieve et al. 2004). The corresponding figure in singleton births following spontaneous conception was 4.8% in Finland in 1998–9. Thus in Finland the age-, parity- and smoking-adjusted risk of preterm birth was 1.8 (95% CI 1.5–2.1) (Klemetti et al. 2002). In international studies an approximately twofold risk of preterm birth in ART singletons has been indicated (Helmerhorst et al. 2004;

Jackson et al. 2004). Very preterm birth (< 32 weeks) also occurs more frequently in singleton ART gestations (~2%) than in singleton gestations following spontaneous conception (~1%) (Gissler et al. 1995a; Koivurova et al. 2002b; Källén et al. 2005b). The age- and parity-adjusted risk of very preterm birth has been found to be significantly increased (two- to threefold) (Helmerhorst et al. 2004).

Given that preterm birth contributes most to other neonatal outcomes it is not surprising that the rate of infants with LBW (i.e. birth weight < 2500 g) is also relatively high (5.5% in Finland, 7.3% in Sweden, and 13.2% in the US) and the adjusted risk of LBW lies between 1.5 to 2 (Klemetti et al. 2002; Schieve et al. 2002; Helmerhorst et al. 2004; Källén et al. 2005b). Furthermore, the proportion of infants with very low birth weight (VLBW: < 1500 g) after singleton ART pregnancy corresponds to the proportion of infants born very preterm: i.e. 1.7% to 2.6% (Koivurova et al. 2002b; Schieve et al. 2002; Schieve et al. 2004; Källén et al. 2005b). Again in line with the age- and parity-adjusted risk of very preterm birth, the rate of VLBW is increased two- to threefold compared with that of singleton infants from spontaneous conceptions (Koivurova et al. 2002b; Schieve et al. 2002; Helmerhorst et al. 2004; Schieve et al. 2004). Gestational age-adjusted birth weight is reported only in some studies and the incidence of small-for-gestational age (SGA) infants varies from 5% to 16% (Tan et al. 1992; Reubinoff et al. 1997; Koudstaal et al. 2000; Källén et al. 2005b). Based on a meta-analysis, the adjusted risk of SGA in singleton ART infants is 1.4 (95% CI 1.2–1.7) (Helmerhorst et al. 2004).

Due to increased rate of preterm births low Apgar scores (< 7) at one minute of age (5.7% vs. 4.3% [Klemetti et al. 2002]) and at five minutes (2.6% vs. 1.3% [Källén et al. 2005b]) are significantly more common in ART singletons than in spontaneously conceived singletons. Singletons from ART pregnancies are also admitted to a neonatal intensive care unit (NICU) more often than other singleton infants (adjusted OR 1.3; 95% CI 1.2–1.4) (Helmerhorst et al. 2004). Among neonatal complications the risk of respiratory problems (OR 1.26; 95% CI 1.15–1.43) is increased (Källén et al. 2005b).

The perinatal mortality rate (PNMR) refers to stillbirths and deaths during the first week of life per 1000 births. Some discrepancy exists in the inclusion criteria as regards perinatal mortality figures in the medical literature, as some record stillbirths from the 20th gestational weeks onwards whereas others start at the 28th gestational week. In most of the studies all stillbirths of infants weighing at least 500 grams are recorded. Despite the differences, higher PNMRs (OR 1.7; 95% CI 1.1–2.6) in ART singleton births than in singleton births after spontaneous conception are reported in most studies (Helmerhorst et al. 2004). However, the significantly increased risk of perinatal mortality among singleton ART infants could not be confirmed in a Finnish sample (Klemetti et al. 2002).

Congenital malformations

The frequency of congenital malformations in singleton ART infants is estimated to be eight to nine per cent (Hansen et al. 2002; Källén et al. 2005c). Approximately half of these are classified as major congenital malformations (a major structural anomaly, chromosomal defect, or congenital hypothyroidism) (Klemetti et al. 2005). Based on a meta-analysis, ART singleton infants are regarded to have a 30–40% increased risk of congenital anomalies when compared with singleton infants from spontaneous conceptions (Hansen et al. 2005). Additional risks of neural tube defects, choanal atresia and alimentary tract atresia have been suggested, according to a Swedish register-based study (Källén et al. 2005c). A higher rate of cardiovascular anomalies has also been suggested in some studies (Hansen et al. 2002; Koivurova et al. 2002b), whereas no increase in the rate of cardiovascular defects was found in larger samples (Klemetti et al. 2005; Källén et al. 2005c).

Increased rates of urogenital and musculoskeletal anomalies (Hansen et al. 2002; Klemetti et al. 2005), especially in singleton ART boys, has been noticed (Klemetti et al. 2005). Among urogenital anomalies, hypospadias is more frequent after ART than after spontaneous conception (Ericson and Källén 2001; Klemetti

et al. 2005) and may be associated with male factor infertility (Ericson and Källén 2001).

Recently a possible association between genetic imprinting (i.e. genetic silencing of either maternal or paternal alleles of genes) and ART has been suggested. Both Angelman syndrome (Cox et al. 2002; Orstavik et al. 2003) and Beckwith-Wiedemann syndrome (DeBaun et al. 2003; Gicquel et al. 2003; Maher et al. 2003; Sutcliffe et al. 2006) have been linked to ART. In contrast, no excess of imprinting disorders in a Danish National IVF cohort (1995–2001) was found (Lidegaard et al. 2005).

Infertility- and treatment-related factors and obstetric and neonatal risks

The increased risks of vaginal bleeding, placenta praevia, placental abruption, pregnancy induced hypertension, Caesarean section and preterm birth remain increased in singleton ART gestations even after controlling for known risk factors such as increased female age, parity and smoking (Cleary-Goldman et al. 2005; Duckitt and Harrington 2005). Obesity, (a well-established risk-factor as regards gestational diabetes, gestational hypertension, pre-eclampsia and Caesarean delivery [Weiss et al. 2004a; Raatikainen et al. 2006; Villamor and Cnattingius 2006]), on the other hand, is not frequently controlled for. However, obesity is not over-presented among women conceiving with ART. Thus the observed increase in obstetric risks in ART singleton pregnancies imply that other and possibly infertility- or treatment-related factors may be involved in the development of the above-mentioned obstetric complications.

In theory, one possible explanation for the increased obstetric risks in singleton ART gestations could be inadequate invasion of cytotrophoblasts and associated abnormal placentation. Inadequate invasion of cytotrophoblasts to the spiral arteries has been implicated in the pathophysiology of pre-eclampsia (Kim et al. 2003; Redman and Sargent 2005) and preterm labour (Kim et al. 2003). Further, the clinical manifestation of inadequate cytotrophoblast invasion – first trimester vaginal bleeding – is relatively common in singleton

ART gestation. First trimester bleeding, in turn, increases the risks of pregnancy-induced hypertension, placental abruption (Nagy et al. 2003; Weiss et al. 2004b) and preterm birth (Weiss et al. 2004b). In conclusion, many of the over-presented obstetric complications associated with ART may be mediated through inadequate placentation. In ART, altered hormonal, endometrial and embryological factors compared with spontaneous conception may contribute to the process of implantation, but they are difficult if not ethically impossible to study.

In practice, infertility- and treatment-related reasons for increased obstetric and neonatal risks have to be studied in terms of clinically assessable measures. However, evaluation of patient- and treatment-related factors as regards obstetric and neonatal outcomes after ART is difficult, as these factors coexist in women conceiving by means of ART. In addition, very little prospectively collected data on the backgrounds of infertile couples exists and registers usually lack information on the background of infertility, or previous treatment attempts.

Duration and aetiology of infertility

One approach in the study of reasons for the increased obstetric and neonatal risks is to compare the outcomes of subfertile women who conceive spontaneously with those of women who conceive with the aid of ART. The results of population-based studies suggest that a prolonged time to pregnancy (> 12 months) increases the risks of preterm birth (Henriksen et al. 1997; Basso and Baird 2003) and perinatal (Draper et al. 1999) and neonatal mortality (Basso and Olsen 2005). In the above-mentioned studies the increased risks were more pronounced in couples achieving pregnancy spontaneously than in couples conceiving by means of infertility treatment. In contrast, in a recent Dutch population-based study it was claimed that the risks of preterm birth and low birth weight are significantly more increased in singletons following ART than in singletons born to subfertile but spontaneously conceiving women (Kapiteijn et al. 2006).

A prolonged time to pregnancy also increases the risk of congenital malformations, irrespective of ART (Zhu et al. 2006). However, an excess risk of genital anomalies in singletons born to couples who received infertility treatment over those conceiving spontaneously has been observed (Zhu et al. 2006). The impact of duration of infertility on obstetric and neonatal outcome in couples achieving pregnancy after ART has not been studied in detail.

Based on data from two nation-wide cohorts of singleton ART pregnancies, couples with female factor infertility have increased risks of preterm birth and having an infant with LBW compared with couples with male factor infertility (Schieve et al. 2004; Wang et al. 2005). Among female factor-related infertility subgroups, women with tubal factor infertility may be at an increased risk of preterm birth versus women with unexplained infertility (Omland et al. 2005).

Hormonal stimulation

The adverse obstetric and neonatal outcomes in singleton pregnancies after ART might also be related to hormonal stimulation, or IVF and ICSI methods rather than to underlying infertility. This has been studied by comparing obstetric and neonatal outcomes in pregnancies achieved by means of ovarian stimulation without gonadotrophin agonist down-regulation (OS), and intrauterine insemination (IUI), pregnancies achieved by means of IVF or ICSI and pregnancies after spontaneous conception. Data on the prevalence of obstetric complications in such groups is scarce. An increased risk of placental abruption and foetal loss after the 24th gestational week in the OS/IUI group compared with the spontaneously conceiving controls has been noticed but no comparison between the OS/IUI and the IVF/ICSI groups has been carried out (Shevell et al. 2005). Preterm and very preterm birth (Ombelet et al. 2006), and LBW infants (Nuojua-Huttunen et al. 1999; Gaudoin et al. 2003; Ombelet et al. 2006) occur more frequently in the OS/IUI subgroup than in the spontaneous conception group. However, in the OS/IUI subgroup, neonatal outcome similar to that in the IVF/ICSI group has been confirmed

in two studies (Nuojua-Huttunen et al. 1999; De Sutter et al. 2005), whereas more preterm births after IVF/ICSI than after OS/IUI were found in another study (Wang et al. 2002b). To conclude, the comparable neonatal outcomes in the OS/IUI and IVF/ICSI groups indicate that either the patients' background or the hormonal stimulation but not the IVF or ICSI methods themselves seem to be responsible for the higher risks compared with controls.

Type of treatment of infertility

Only limited information exists in respect to different ART modalities (IVF vs. ICSI, fresh vs. frozen embryo transfer). The risks of preterm birth and LBW are similar in ICSI and IVF subgroups (Bonduelle et al. 2002; Schieve et al. 2004). However, singleton gestations achieved by means of FET are linked to decreased risks of preterm birth (Källén et al. 2005b; Wang et al. 2005) and LBW (Aytoz et al. 1999; Källén et al. 2005b; Wang et al. 2005; Wright et al. 2006) compared with singletons achieved by means of fresh embryo transfer.

Number of transferred embryos

Keeping in mind the fact that an active eSET policy began a little less than a decade ago, it is not surprising that only a few studies on its influence on obstetric and neonatal outcome are published.

To date, three studies, involving a total of 782 singleton pregnancies after SET and resulting in a live birth have been presented (De Neubourg et al. 2006; De Sutter et al. 2006b; Kjellberg et al. 2006). In all these studies good-prognosis patients receiving a good quality embryo were included and data were received from the clinics' own databases or from self-submitted questionnaires.

Pregnancy induced hypertension is more frequent after SET than after spontaneous singleton conception (7.6% vs. 4.6%, $p = 0.02$) (De Neubourg et al. 2006). Vaginal bleeding throughout the pregnancy and preterm contractions were reported in 14.3% (both) of SET pregnancies (De Neubourg et al. 2006), but no comparison with the controls could

be carried out. The Caesarean section rate in the SET groups ranged from 20 to 23% (De Neubourg et al. 2006; Kjellberg et al. 2006).

According to the study by De Sutter and colleagues the mean gestational duration at birth was longer (39.4 vs. 39.1 weeks, $p < 0.001$) and the mean birth weight was greater (3324 g vs. 3204 g, $p < 0.01$) than in singleton pregnancies following DET (De Sutter et al. 2006b). In contrast, no difference in median gestational duration (40 vs. 40.3 weeks, $p = 0.26$) or in mean birth weight (3550 g vs. 3405 g, $p = 0.20$) between SET and DET singleton groups in a randomised controlled study was noticed (Kjellberg et al. 2006). Further, similar gestational durations (38.7 vs. 38.9 weeks, $p = 0.06$) and mean birth weights (3322 g vs. 3330 g, $p = 0.82$) in comparison with spontaneous singleton pregnancies have also been suggested (De Neubourg et al. 2006).

The incidence of preterm births in the SET groups was 6.2% according to De Sutter and colleagues (De Sutter et al. 2006b) but close to 10% in the other two studies (De Neubourg et al. 2006; Kjellberg et al. 2006). The incidence of LBW after SET was 4.2% to 7.0% in these studies. Because neonatal complications were reported differently and the number of neonatal complications was relatively low in the above-mentioned studies, no conclusion regarding the impact of SET on neonatal health can be drawn.

Ten to eighteen percent of singletons born after DET are survivors of an original twin gestation – i.e. vanishing twin survivors (Pinborg et al. 2005; Lambers et al. 2006; La Sala et al. 2006). Cessation of development of the co-twin can have an adverse impact on pregnancy outcome, especially if it occurs after eight weeks of gestation (Pinborg et al. 2005).

A vanishing twin pregnancy may become manifest with first trimester bleeding (De Sutter et al. 2006a). Pre-eclampsia is suggested to be more common in vanishing twin pregnancies than in singleton pregnancies (Chasen et al. 2006a). The mean length of pregnancy is shorter (Dickey et al. 2002; Schieve et al. 2004; Pinborg et al. 2005), and the frequency of preterm and very preterm births is higher (Pinborg et al. 2005) in the vanishing twin survivor cohort than in

the singleton cohort following implantation of a single embryo. Consequently, the mean birth weight is lower (Dickey et al. 2002; Schieve et al. 2004; Pinborg et al. 2005) and the proportions of infants with LBW and VLBW are elevated in vanishing twin pregnancies versus singletons originating from a single gestational sac. The cessation of embryonal/foetal development of a co-twin can also have life-long consequences on the survivor's health: according to two population-based cohort studies the prevalence of cerebral palsy (Pharoah and Adi 2000; Hvidtjorn et al. 2005) and other forms of cerebral impairment (Pharoah and Adi 2000) are increased.

The poorer neonatal outcome of the vanishing twin cohort following DET may confound the overall neonatal results of ART singletons, because the majority of the published studies on the outcome of singleton pregnancies after ART concern singleton pregnancies following DET.

Psychological features of pregnancy

Pregnancy is a period of intense emotional processing and preparation for a permanent life change, particularly for first-time parents. During pregnancy both parents-to-be work through their experiences and expectations of parenthood and create an emotional bond with the infant (Broden 2006). Ambivalent feelings towards the pregnancy and the infant are part of this process and optimally these feelings diminish as the pregnancy proceeds. For a father-to-be the physiological process that occurs in a pregnant woman may raise feelings of being outside. Integrating the concepts of a mother and a spouse into one person may also be demanding for an expectant father (Haukkamaa 2000). Optimally, the expectant couple can establish an attachment to the foetus together and support one another with the physical and psychological demands of pregnancy.

Pregnancy after ART

Achieving an ongoing pregnancy after ART usually follows years of uncertainty regarding a couple's possibility to ever become parents.

For many couples infertility, the associated investigations and ART raise feelings of stress, anxiety and depression (Beaurepaire et al. 1994; Visser et al. 1994; Brighenti et al. 1997; Lukse and Vacc 1999; Souter et al. 2002; Verhaak et al. 2005; Verhaak et al. 2006). Anxiety and stress associated with ART are also among the most important reasons for discontinuing further treatments (Olivius et al. 2004; Rajkhowa et al. 2006). In general, women experience more ART-related distress than their spouses (Wright et al. 1991; Slade et al. 1997; Hjelmstedt et al. 1999; Holter et al. 2006). The impact of the number of previous unsuccessful ART cycles and the duration of infertility on ART-related anxiety is controversial: some state that stress, depressiveness and somatic symptoms increase after cumulative ART attempts or a long duration of infertility (Abbey et al. 1992; Domar et al. 1992; Boivin et al. 1995; Kee et al. 2000), whereas others have not found an association (Lukse and Vacc 1999; Verhaak et al. 2005). The impact of the aetiology of infertility on psychological distress has scarcely been studied. Before the time of ICSI, couples with male factor infertility regarded their distress as being more severe than that among couples with other aetiological backgrounds (Connolly et al. 1992). Thereafter, no difference in psychological variables in infertile couples has been confirmed (Wischmann et al. 2001).

Although anxiety and depressive symptoms affect 20–30% of infertile women during ART (Souter et al. 2002; Verhaak et al. 2005), these symptoms usually diminish or become resolved when the long-awaited pregnancy starts (Wright et al. 1991; Verhaak et al. 2005). With the exception of one study (van Balen et al. 1996), the majority of investigators have confirmed similar levels of general anxiety, depressiveness and low self-esteem during pregnancy among women conceiving by means of ART and those conceiving spontaneously (McMahon et al. 1997; Klock and Greenfeld 2000; Hjelmstedt et al. 2003). However, assessment of general anxiety may not be sensitive enough when considering pregnancy-specific anxiety (Huizink et al. 2004). Indeed, higher levels of pregnancy-specific anxiety in pregnant ART women than in control pregnant women

have been found in some studies (McMahon et al. 1997; Hjelmstedt et al. 2003). Women conceiving by means of ART tend to be more anxious over loss of pregnancy and are more concerned about the infant's health and possible injury during delivery than control women (McMahon et al. 1997; Hjelmstedt et al. 2003). Worry about the threat of losing the pregnancy and not having a healthy child is suggested to be related to the number of treatment cycles and infertility-related distress (McMahon et al. 1997; Hjelmstedt et al. 2003).

Fear-of-childbirth and pregnancy-related anxiety

Mild to moderate fear-of-childbirth and pregnancy-related anxiety are common (Areskog et al. 1981; Melender 2002) and can be considered as normal dimensions of a woman's emotional maturation regarding her new role as a mother. Nevertheless, for 6–13% of pregnant women fear-of-childbirth becomes a serious problem negatively affecting their psychosocial well-being and social relationships (Areskog et al. 1981; Söderquist et al. 2004). Fear-of-childbirth can be considered a multifactorial anxiety disorder which correlates with pregnancy-related anxiety (Saisto et al. 2001a). Previous studies have shown that women with fear-of-childbirth more frequently suffer from nausea and vomiting (Swallow et al. 2004), are on sick-leave, visit their obstetricians (Andersson et al. 2004) and request Caesarean section in comparison with women without such fear. According to Areskog and colleagues, women who complain of fear-of-childbirth are more likely to experience delivery less favourably than other parturients (Areskog et al. 1983). More recently, labour pain and operative delivery rather than preceding fear-of-childbirth have most accurately been proven to predict disappointment with delivery (Saisto et al. 2001b). Fear-of-childbirth may occupy a woman's mind and building an attachment to the

child may become difficult (Areskog et al. 1984). Further, fear-of-childbirth may be a reason for not becoming pregnant again (Bahl et al. 2004).

Several psychosocial and obstetric factors contribute to the probability of being afraid of delivery or experiencing pregnancy-related anxiety (Table 3). In general, nulliparous women experience moderate fear-of-childbirth and pregnancy-related anxiety more often than parous women (Areskog et al. 1983; Wijma et al. 1997; Alehagen et al. 2001; Saisto et al. 2001a; Johnson and Slade 2002; Söderquist et al. 2004). Severe fear-of-childbirth and post-traumatic stress disorder, on the other hand, are more typical of parous parturients than of nulliparous parturients (Söderquist et al. 2004). In parous parturients previous complicated or operative delivery is the most important underlying reason for fear-of childbirth (Ryding et al. 1997; Sjögren 1997; Saisto et al. 1999). In addition, negative life experiences (Areskog et al. 1983), psychiatric disorders (Andersson et al. 2003; Söderquist et al. 2004), general anxiety (Saisto et al. 2001a; Söderquist et al. 2004), lack of social support (Saisto et al. 2001a; Söderquist et al. 2004) and unemployment (Saisto et al. 2001a) are common risk factors for fear-of-childbirth and pregnancy-related anxiety.

Fear-of-childbirth and pregnancy-related anxiety in women undergoing ART have been assessed only in four studies. Previous infertility has not been found to be an independent predictor of fear-of-childbirth in subsequent pregnancy (Saisto et al. 1999). Because 70–80% of women conceiving via ART are expecting their first child, more frequent moderate fear-of-childbirth than in the general pregnant population would be expected. In addition, more pronounced anxiety over possible loss of pregnancy and over the infant's health in women conceiving by means of ART than in spontaneously conceiving women (McMahon et al. 1997; Hjelmstedt et al. 2003) might be a sign of underlying fear-of-childbirth.

Assessing fear-of-childbirth and pregnancy-related anxiety

Defining fear-of-childbirth and pregnancy-related anxiety is difficult because they both entail many different features associated with pregnancy and giving birth. They both share aspects of being afraid or anxious about pain, tearing and maternal death during delivery, losing self-control and having an injured or malformed infant. To date, no internationally accepted definitions of fear-of-childbirth or pregnancy-related anxiety exist.

Measuring fear-of-childbirth and pregnancy-related anxiety has not been settled either. The first questionnaire used to assess fear-of-childbirth was generated by Areskog and colleagues in the late seventies (Areskog et al. 1982). They interviewed a sample of 139 pregnant women and analysed how well their self-expressed fear-of-childbirth and the scores of their 19-item fear-of-childbirth questionnaire

(FDQ) correlated at the 31st to 33rd gestational week (Areskog et al. 1982). The original fear-of-childbirth questionnaire was revised in subsequent studies in order to suit the studied population (Di Renzo et al. 1984; Wijma et al. 1998). In the late nineties Saisto et al. applied a revised ten-item version of the fear-of-childbirth questionnaire in an unselected Finnish pregnant population and it proved to be a reliable and valid tool for measuring fear-of-childbirth in Finnish samples (Saisto et al. 2001a).

Similarly, inconsistency in measuring pregnancy-related anxiety characterises the studies in this area. In 1991, Levin revised the pregnancy anxiety scale (PAS) originally created in 1974 (Burstein et al. 1974; Levin 1991). The PAS has ten questions that cover three dimensions of anxiety: anxiety about being pregnant, anxiety about childbirth and anxiety about hospitalisation. Saisto et al. have also validated the PAS to suit unselected pregnant Finnish women (Saisto et al. 2001a).

Table 3. Predisposing factors regarding fear-of-childbirth.

Predisposing factor	Reference	n	Study setting	Assessment time	Criteria for fear-of-childbirth
<i>Obstetric</i>					
Nulliparity	Areskog, 1983	176	cross-sectional	h31-33	FDQ \geq 6
	Di Renzo, 1984	810	cross-sectional	h38-41	rFDQ (20)
	Wijma, 1997	1640	cross-sectional, retrospective	1-13 months postpartum	W-DEQ \geq 85
	Alehagen, 2001	74	cross-sectional	at delivery	DFS
	Johnson, 2002	443	cross-sectional	h32	W-DEQ \geq 85
	Söderquist, 2004	995	prospective	h32	W-DEQ \geq 85
Previous Miscarriage	Saisto, 1999	300	cross-sectional, retrospective	subsequent pregnancy	a request for CS
Previous operative delivery	Ryding, 1997	25	retrospective	1 week and 1-2 months postpartum	DSM-III-R
	Sjögren, 1997	100	retrospective	h20-35	self-expressed extreme fear of childbirth
	Jolly, 1999	480	retrospective	5 years after delivery	postal questionnaire
	Saisto, 1999	300	cross-sectional, retrospective	subsequent pregnancy	a request for CS
<i>Psychosocial</i>					
Psychiatric disorders	Anderson, 2003	1556	cross-sectional	h16-17	PRIME-MD
	Söderquist, 2004	995	prospective	h32	W-DEQ \geq 85
General anxiety	Saisto, 2001a	278	cross-sectional	h30	rFDQ (10) \geq 6
	Söderquist, 2004	995	prospective	h32	W-DEQ \geq 85
Negative life experiences	Areskog, 1983	176	cross-sectional	h31-33	FDQ \geq 6
Lack of social support	Saisto, 2001a	278	cross-sectional	h30	rFDQ (10) \geq 6
	Söderquist, 2004	995	prospective	h32	W-DEQ \geq 85
Unemployment	Saisto, 2001a	278	cross-sectional	h30	rFDQ (10) \geq 6
Dissatisfaction with partnership	Saisto, 2001a	278	cross-sectional	h30	rFDQ (10) \geq 6

CS = Caesarean section

DFS = Delivery Fear Scale, range 1–10

DSM-III-R = Diagnostic and statistical manual of mental disorders, third revised version

FDQ = fear-of-childbirth questionnaire, original 19-item version

h = gestational weeks

rFDQ() = revised version of FDQ, number in parenthesis indicates the number of included items

PRIME-MD = Primary Care Evaluation of Mental Disorders

W-DEQ = Wijma Delivery Expectancy/Experience Questionnaire, range 0–165

AIMS OF THE STUDY

The present study was undertaken to investigate the outcome of singleton ART pregnancy. The specific aims were:

1. to evaluate the predictive value of a single hCG measurement in determining the outcome of ART
2. to analyse the impact of infertility- and treatment-related characteristics on obstetric and neonatal outcome
3. to compare the obstetric and neonatal outcome of singleton pregnancies following SET, DET and spontaneous conception
4. to study the prevalence and contributing factors of fear-of-childbirth and pregnancy-related anxiety in women conceiving by means of ART

SUBJECTS AND METHODS

These studies involved infertile patients from the Infertility Clinics of Helsinki University Central Hospital, the Family Federation of Finland (Helsinki, Oulu and Turku) and the Deaconess Institute (Helsinki). Studies I and III were approved by the Administrative Head of the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital. In Study III the National Research and Development Centre for Welfare and Health (STAKES) gave permission to use register data and to perform data linkages between different health registers. Studies II and IV were approved by the Ethics Committee of the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, the Family Federation of Finland and the Deaconess Institute and the subjects gave their informed consent before participation.

Subjects

Detailed characteristics of the subjects according to each study are given in Table 4.

Study I

The study subjects consisted of a retrospective cohort of 650 women with 774 ART cycles. They received fresh or frozen IVF or ICSI treatment at the infertility clinic of Helsinki University Central Hospital in 1994–1999.

Studies II & IV

The subjects comprised female participants with singleton pregnancies from a prospective controlled follow-up study on somatic and mental health after ART. The women in the ART group were recruited after a viable pregnancy was confirmed at the infertility clinics of Helsinki University Central Hospital, the Family Federation of Finland (Helsinki, Oulu and Turku) and the Deaconess Institute (Helsinki) in 1999. Pregnancies achieved with the couples' own gametes and with fresh or frozen IVF or ICSI treatment were included.

Consecutive, spontaneously conceiving control women were recruited from screening ultrasonographic scan appointments at gestational weeks 16–18 at the Maternity Clinic of Helsinki University Central Hospital in 1999. The exclusion criteria for the control women were previous infertility or infertility treatment, age less than 25 years and difficulties in Finnish comprehension. A total of 367 (92.4%) out of 397 initially recruited women in the ART group and 379 (81.7%) out of 464 control women participated in the first questionnaire assessment at the 20th gestational week ($p < 0.001$). At the second questionnaire assessment eight weeks postpartum 324 (88.3%) women in the ART group and 304 (80.2%) women in the control group took part ($p = 0.03$).

Study III

Women having a viable singleton pregnancy after fresh SET ($n = 270$) or DET ($n = 231$) at the infertility clinic of Helsinki University Central Hospital in 1997–2003 were identified retrospectively from the patient records. The identified pregnancies and the infants born were linked to the Finnish Medical Birth Register (MBR), the Hospital Discharge Register (HDR) and the Register of Congenital Malformations (RCM) according to the maternal and infant identification numbers. Two of these 501 pregnancies ended in selective termination in the second trimester and the remaining 499 pregnancies, 269 after SET and 230 after DET, formed the final ART group. The control group ($n = 15\ 037$) was randomly taken from the MBR. The controls represent a ten per cent population-based sample of women with spontaneous singleton pregnancies and infants born matched for the year of delivery and mother's place of residence.

ART treatment protocols

Ninety-eight per cent of fresh IVF and ICSI cycles were started with long luteal phase pituitary down-regulation with nafarelin or

buserelin. After two weeks of down-regulation rFSH was added for ovarian stimulation. When two or more follicles reached a diameter of > 17 mm in TVS, exogenous hCG was administered. Oocyte retrieval took place 34–36 h after hCG administration. Embryo transfers were performed two to three days after insemination. Micronized vaginal progesterone (600 mg) was used for luteal support for two weeks.

In FET cycles, embryo transfers were completed both in natural and substituted cycles. In natural FET cycles embryo transfer took place three days after the LH surge was measured with a home test kit. Luteal support was provided

with micronized vaginal progesterone (200 mg) for two weeks. In substituted FET cycles oral oestrogen (4–6 mg daily) was started in the first days of the menstrual cycle. When the endometrium had reached a minimum thickness of 7 mm in TVS, micronized vaginal progesterone at a daily dose of 600 mg was added to the hormonal substitution. Embryo transfer was carried out usually three to four days after initiation of progesterone treatment. Both types of medication were continued until the 10th gestational week in viable pregnancies.

Treatment outcome was monitored with serum hCG sampling and TVS.

Table 4. Subject characteristics in Studies I–IV.

	<i>n</i>	Age (y)	Nulli- parous	Aetiology of infertility				Treatment type		
				Female	Male	Combined	Un- explained	IVF	ICSI	FET
		mean (SD)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)
I										
ART	774*	32 (4)	-	387 (50)	128 (17)	56 (7)	203 (26)	518 (67)	119 (15)	137 (18)
II										
ART	324	33 (4)	227 (70)	107 (33)	88 (27)	61 (19)	68 (21)	128 (40)	59 (18)	137 (42)
Controls	304	33 (3)	104 (34)							
III										
SET	269	33 (4)	215 (80)	142 (53)	51 (19)	19 (7)	57 (21)	201 (75)	68 (25)	-
DET	230	34 (4)	187 (81)	98 (43)	53 (23)	13 (6)	66 (29)	167 (73)	63 (27)	-
Controls	15037	30 (5)	6588 (44)							
IV										
ART	367	33 (4)	260 (71)	122 (33)	95 (26)	71 (19)	79 (22)	146 (40)	70 (19)	151 (41)
Controls	379	33 (3)	135 (36)							

* number of studied treatment cycles

Table 5. Study design according to the original publications.

Study	I	II	III	IV
Setting	retrospective	prospective, controlled	retrospective	prospective, controlled
Data source	patient records	questionnaire survey, patient records	MBR, HDR, RCM	questionnaire survey, patient records
End-point of data collection	sixth week postpartum or after cessation of pregnancy	eighth week postpartum	sixth week postpartum	20 th gestational week
Main assessment variable(s)	serum hCG 12 days after ET	aetiology and duration of infertility, treatment type	number of transferred embryos (i.e. SET vs. DET)	rFDQ, PAS
Main study question	prediction of ART outcome	evaluation of risk of adverse pregnancy outcomes with patient characteristics	impact of the number of transferred embryos on obstetric and neonatal outcome	prevalence of fear-of-childbirth and pregnancy-related anxiety
Hypothesis	patient-related factors alter the predictive value of hCG	patient characteristics relate with adverse pregnancy outcomes	SET associates with improved obstetric and neonatal outcome	fear-of-childbirth and pregnancy-related anxiety are more frequent after ART

ART = assisted reproductive treatment

DET = double embryo transfer

ET = embryo transfer

hCG = human chorionic gonadotrophin

HDR = Hospital Discharge Register

MBR = Medical Birth Register

PAS = pregnancy anxiety scale

rFDQ = revised version of the fear-of-childbirth questionnaire

SET = single embryo transfer

Methods

The data was collected from patient records (I–IV), structured questionnaires (II and IV) and from national Health Registers (III). Detailed description of the data sources and assessed variables is given below. The study designs are summarised in Table 5.

Assay of hCG (I)

Serum intact hCG was measured twelve days after embryo transfer by solid phase, two-site fluoroimmunoassay (AutoDELFIA®; Wallac, Turku, Finland). The intra-assay variation was 2.7–5.1% and inter-assay variation 1.6–3.9% at the studied hCG levels. The standard had been calibrated against the WHO Third International standard of hCG for immunoassay (code 75/537).

Questionnaire survey (II and IV)

The first questionnaire was given to the participants at recruitment and it was returned at a mean (\pm SD) of 20 (\pm 3.2) gestational weeks. The first questionnaire included multiple choice questions concerning social background (education, employment and partnership), general health (presence of a chronic illness and need of continuous medication), tobacco smoking (0, <5, 5–10, >10 cigarettes per day), alcohol consumption (0, <5, \geq 5 units per week) and somatic problems (hyperemesis, vaginal bleeding and other not specified) in the studied pregnancy.

The second questionnaire was mailed to the participants after the research nurse had contacted them after delivery. This questionnaire was returned at a mean (\pm SD) of eight (\pm 3.4) weeks postpartum. In the second questionnaire the participants were asked to report whether they had had any pregnancy complications (preterm contractions, vaginal bleeding, gestational hypertension, pre-eclampsia, gestational diabetes or cholestasis in pregnancy) and related hospitalisation after the 20th gestational week. The onset (spontaneous or induced) and the type (vaginal, vacuum extraction, Caesarean section) of delivery were addressed in multiple choice

questions. The health of the newborns was assessed via questions concerning gestational age at birth, birth weight and height, Apgar scores, general health, and need of admittance to a NICU and length of stay there. Perinatal mortality rate referred to all stillbirths and deaths of newborns (weighing \geq 500 g) occurring from the 22nd completed gestational week to the age of six days.

In addition to the two above-mentioned questionnaires, data on age, BMI, obstetric history and detailed information concerning previous and current ART were collected at recruitment. Mean gestational age, mean birth weight and Apgar scores of infants were checked from the clinics' patient records.

Register data (III)

The Finnish Medical Birth Register (MBR)

The MBR includes data on maternal characteristics, and obstetric and neonatal outcomes of all live births and stillbirths of infants/foetuses born after the 22nd gestational week or weighing at least 500 g. The data is submitted by the delivery hospitals and completed by data from the Population Register Centre and Cause-of-Death Register at Statistics Finland. The Coverage of the MBR is practically 100% (Gissler et al. 1995b).

The Hospital Discharge Register (HDR)

The HDR receives yearly data on outpatient and inpatient care from hospitals. The data is collected according to the instructions of The National Research and Development Centre of Welfare and Health and categorised according to the 10th version of the international Classification of Diseases (ICD 10) and to the national version of the Nordic Classification of Surgical Procedures.

The Register of Congenital Malformations (RCM)

The RCM contains data on congenital anomalies diagnosed during the child's first year of life from hospitals, health-care professionals, cytogenetic laboratories and health registers of STAKES (National Research and Development Centre for Health and Welfare) and Statistics Finland. The coverage and validity of RCM

data is good after multi-source data collection. A congenital anomaly is defined as a structural anomaly, a chromosomal defect or congenital hypothyroidism in live births, stillbirths and in selective terminations of pregnancy. Congenital anomalies are registered and included in the RCM according to instructions from European Surveillance of Congenital Anomalies.

The included maternal variables from the MBR, HDR and RCM were age, socio-economic status (SES), number and type of previous pregnancies and deliveries, smoking, number of outpatient visits, obstetric complications (ICD 10 codes O10–O99), prevalence of maternal hospitalisation during the pregnancy, onset and type of delivery, and surgical procedures. The neonatal variables included were gestational age at birth (defined according to the best clinical estimation), birth weight, SGA (according to national sex-specific standards [Pihkala et al. 1989]), low one-minute Apgar scores (0–6), admission to a NICU, perinatal deaths (stillbirths and neonatal deaths during the first week of life) and presence of congenital malformations.

Revised version of the Fear-of-Childbirth Questionnaire and the Pregnancy Anxiety Scale (IV)

Fear-of-childbirth was assessed with the revised version of the fear-of-childbirth questionnaire (rFDQ). The rFDQ consisted of 11 dichotomous questions giving a possible range of sum scores from 0 to 11. Affirmative answers indicated fear-of-childbirth. The sum score of the rFDQ was non-normally distributed and therefore medians were used. The reliability of the rFDQ was 0.72 (Cronbach's alpha).

Pregnancy-related anxiety was assessed by means of the Pregnancy Anxiety Scale (PAS). This consisted of ten five-scale questions giving a possible "range" of sum scores from ten to

fifty. High scores in the individual questions and in the sum score indicated pregnancy-related anxiety. The PAS sum scores and scores in three sub-dimensions were non-normally distributed and medians are given. The reliability of the PAS in our sample was 0.80 (Cronbach's alpha). The contents of rFDQ and PAS are given in Appendix.

Severe fear-of-childbirth and severe pregnancy-related anxiety were regarded as sum scores equal to or greater than the 90th percentile of sum scores (i.e. rFDQ scores ≥ 6 and PAS scores ≥ 30 , respectively). The prevalence of severe fear-of-childbirth and severe pregnancy-related anxiety was studied first between the ART and the control groups and then separately among nulliparous and parous participants.

Statistical analyses

In cases of normally distributed continuous variables Student's *t*-test (I–IV) and one-way analysis of variance (ANOVA) were used (I–III). In study I an exponential transformation of hCG values in viable pregnancies gave a good fit. With non-normally distributed continuous variables the Mann–Whitney *U*-test (I, II) and Kruskal–Wallis analysis of variance (I) were run. For comparison of proportions the χ^2 -test or Fisher's exact test were performed when applicable (I–IV). In study I a receiver operating characteristic curve (ROC) was utilised to define the most suitable hCG cut-off level to distinguish between viable and non-viable pregnancies. In studies II–IV adjusted multiple logistic regression analyses were carried out.

A probability level of < 0.05 was regarded as statistically significant. Statistical analyses were performed by using NCSS 2000 (NCSS Inc., Kaysville, Utah), SPSS version 12.0.1 (SPSS Inc., Chicago, IL) and the eighth version of SAS (SAS Institute, Cary, NC) software.

RESULTS

Serum hCG in predicting the outcome of ART (I)

The median hCG concentration was higher in viable than in non-viable pregnancies (126 IU/L vs. 31 IU/L, $p < 0.0001$). A cut-off value of 76 IU/L predicted viable pregnancies with 80% sensitivity, 82% specificity, and had 87% PPV and 74% NPV.

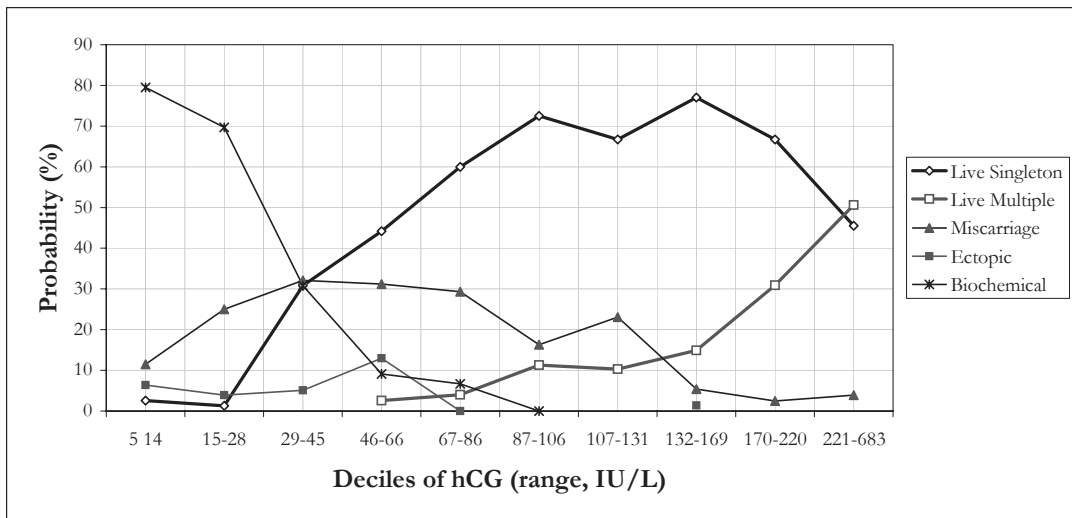
Probability of various pregnancy outcomes according to deciles of serum hCG concentrations are given in Figure 3.

Among viable singleton pregnancies, male factor infertility and ICSI treatment were

associated with the lowest median hCG values (83 IU/L vs. 120 IU/L, $p = 0.001$). Median hCG concentrations were similar in fresh and frozen embryo transfer groups (114 IU/L vs. 115 IU/L, NS). Further, the median hCG level did not differ in singleton pregnancies following transfer of either one or two embryos (104 IU/L vs. 119 IU/L, NS).

We also studied whether hCG concentrations and the gestational age- and sex-adjusted weights of singleton infants were correlated, but no significant correlation was found.

Figure 3. Probability of various pregnancy outcomes according to deciles of serum hCG concentration 12 days after embryo transfer.



Infertility- and treatment-related factors and obstetric and neonatal outcome (II)

In general, the prevalence of maternal pregnancy complications was similar in the ART and the control group, but hospitalisation before delivery was more typical of the mothers in the ART group than those in the control group (18.8% vs. 11.2% respectively, $p = 0.006$). Spontaneous preterm delivery (4.9% vs. 1.6%, $p = 0.003$), medical induction of delivery (27.7% vs. 18.5%, $p = 0.01$), vacuum extractions (10.2% vs. 4.9%, $p = 0.02$) and Caesarean sections (26.2% vs. 19.1%, $p = 0.02$) were more common to the ART group than to the control group. The mean (\pm SD) duration of pregnancy at birth was shorter (39.6 ± 1.7 vs. 39.9 ± 1.4 weeks, $p = 0.01$) and the mean birth weight lower (3490 ± 554 vs. 3630 ± 500 g, $p = 0.001$) in the ART group than in the control group. These differences remained

similar even if only the eSET subgroup was compared with the control group. The frequency of preterm births (7.4% vs. 3.9%, $p = 0.07$) and LBW infants (4.0% vs. 1.3%, $p = 0.06$) tended to be higher in the ART than in the control group. Nevertheless, no difference in the NICU admissions (3.7% vs. 1.6%, $p = 0.11$) or in the PNMR (8‰ vs. 3‰, $p = 0.63$) between the ART and the control group existed.

The impact of aetiology and duration of infertility, number of previous ART cycles and the current treatment type on the risks of preterm birth and LBW were evaluated in age- and BMI-adjusted multiple logistic regression analysis (Table 6). None of the infertility- and treatment-related patient characteristics played an independent role in the risks of preterm birth or LBW. However, a history of induced abortion increased the risk of preterm birth (OR 4.5; 95% CI 1.2–17.1).

Table 6. Age- and BMI-adjusted multiple logistic regression. Odds ratios (ORs) and 95% confidence intervals (CIs) for preterm birth and low birth weight in the ART singletons are shown.

	Preterm birth (< h 37)			Low birth weight (< 2500 g)		
	<i>n</i>	OR	95% CI	<i>n</i>	OR	95% CI
Total	309			310		
Previous non-viable pregnancies						
None*	193	1	-	193	1	-
Miscarriage	61	2.8	0.9-8.9	61	1.7	0.3-8.8
Induced abortion	29	4.5	1.2-17.1	29	3.3	0.6-19.2
Ectopic pregnancy	26	2.7	0.6-11.2	26	2.7	0.4-16.8
Nulliparity	218	0.9	0.3-2.3	219	1.4	0.3-6.2
Aetiology of infertility						
Female*	91	1	-	91	1	-
Male	84	0.3	0.1-1.5	84	1.0	0.1-7.3
Combined	60	0.8	0.2-3.0	60	2.1	0.3-12.3
Unexplained	74	0.8	0.2-3.0	75	1.2	0.2-7.9
Duration of infertility (years)						
< 2*	143	1	-	143	1	-
2-5	55	0.0	0.0-	56	0.0	0.0-
> 5	111	0.7	0.2-2.0	111	1.4	0.4-5.4
Number of treatments						
1-2*	161	1	-	162	1	-
3-4	83	0.5	0.1-1.6	83	0.2	0.0-1.7
≥5	65	1.1	0.3-3.0	56	0.6	0.1-3.7
Treatment type						
IVF*	120	1	-	121	1	-
ICSI	56	1.6	0.4-5.7	56	0.6	0.1-7.2
FET	133	0.5	0.2-1.6	133	1.6	0.4-7.1

h = gestational weeks

* = reference group

Number of transferred embryos and obstetric and neonatal outcome (III)

The mothers in the SET group were younger than the mothers in the DET group but older than the control mothers (Table 4; $p < 0.001$). The SES of the mothers and the proportion of nulliparous women were similar in the SET and DET groups. In comparison with the controls, SET mothers were of higher SES, smoked less frequently during the pregnancy (10.0% vs. 15.7%, $p = 0.04$) and were more often nulliparous (79.9% vs. 43.8%, $p < 0.0001$).

ART characteristics

The frequencies of the different infertility aetiologies and ART types did not differ between the SET and DET groups (Table 4). A total of 224 (83.3%) pregnancies in the SET group followed eSET. Twenty-five (10.9%) out of the 230 pregnancies in the DET group originated from a vanishing twin pregnancy. Cessation of embryonal/foetal development was diagnosed by eight weeks of gestation in 48% of the cases.

Single embryo transfer versus double embryo transfer

The prevalence of maternal pregnancy complications and hospitalisation pre- and postpartum was equally common in the SET and DET groups. The proportions of induced deliveries (13.8% vs. 18.7%, $p = 0.13$) and Caesarean sections (26.8% vs. 25.7%, $p = 0.77$) were comparable in the SET and DET groups.

The mean gestational age at birth, mean birth weight, numbers of SGA infants and NICU admissions, and the PNMR were alike in the SET and DET groups (Table 7). No difference in the prevalence of major congenital anomalies between the SET (555/10 000) and DET groups (476/10 000) existed ($p = 0.84$).

Single embryo transfer versus spontaneous conception

The age-, parity- and SES-adjusted risks of gestational hypertension (OR 2.35; 95% CI 1.57–3.53), deep venous thrombosis (OR 27.7; 1.49–513.8), maternal care because of congenital malformation of the uterus (OR 5.55; 1.14–27.0) and cervical incompetence (OR 7.58; 2.81–21.9), placenta praevia (OR 4.75; 2.57–8.78) and preterm contractions (OR 2.70; 1.83–3.98) were increased in the SET mothers. Also, Caesarean sections (adjusted OR 1.54; 1.18–2.00) and manual removal of the placenta (adjusted OR 2.50; 1.50–4.16) were more typical of the SET group than the control group. The mean gestational age at birth was less and the mean birth weight was lower in the SET than in the control group (Table 7). The age-, parity- and SES-adjusted risks of preterm birth (OR 2.58; 1.96–4.16), LBW (OR 2.01; 1.19–3.99) and low Apgar scores (OR 1.96; 1.01–3.04) were elevated in the SET group.

When comparing the DET group with the control group, increased risks of preterm birth (OR 2.63; 1.73–4.00), LBW (OR 3.46; 2.20–5.46), low Apgar scores (OR 1.75; 1.01–3.04) and NICU admission (OR 2.23; 1.08–4.58) were observed.

Table 7. Neonatal outcome after SET, DET and spontaneous conception.

	SET	DET	Controls	<i>p</i>	<i>p</i>
				SET vs. DET	SET vs. CONTROL
Total	269	230	15037		
Gestational weeks at birth					
mean (SD)	38.7 (2.2)	38.7 (2.5)	39.4 (1.8)	1.00	<0.001
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)		
≥ 37	236 (87.7)	204 (88.7)	14302 (95.1)	}0.90	} <0.001
32–36	30 (11.2)	23 (10.0)	558 (3.7)		
< 32	3 (1.1)	3 (1.3)	108 (0.7)		
Birth weight (g)					
mean (SD)	3364 (621)	3340 (668)	3541 (547)	0.34	<0.001
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)		
≥ 2500	253 (94.1)	208 (90.4)	14554 (96.8)	}0.16	} 0.04
1500–2499	12 (4.5)	20 (8.7)	355 (2.4)		
< 1500	3 (1.1)	2 (0.9)	86 (0.6)		
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)		
SGA	10 (3.7)	10 (4.4)	314 (2.1)	0.72	0.07
Apgar scores 0–6 at 1 min	16 (5.9)	14 (6.1)	555 (3.7)	0.95	0.05
NICU admission	8 (3.0)	8 (3.5)	227 (1.5)	0.75	0.07
PNMR (all)	3 (1.1)	3 (1.3)	68 (0.45)	0.85	0.11
Stillbirths	3 (1.1)	2 (0.9)	47 (0.31)	0.78	0.02
Neonatal deaths	0	1 (0.4)	21 (0.14)	0.28	0.54

Fear-of-childbirth and pregnancy-related anxiety (IV)

The mean (\pm SD) age of the participants was similar in the ART and control groups (Table 4; $p = 0.18$). Nulliparity was more typical of the ART mothers than the controls (Table 4; $p < 0.001$). The duration of partnership (mean \pm SD) was longer in the ART than in the control group (9.7 ± 4.4 vs. 7.7 ± 4.4 years, $p < 0.001$).

ART versus control group

The median scores in the rFDQ (2.0) and PAS (20.0) were similar in the two groups. No differences in the median scores of pregnancy-, delivery- and hospitalisation-specific PAS questions existed. Also, severe fear-of-childbirth and pregnancy-related anxiety was equally common to the ART and the control groups (Table 8). According to the results of adjusted multiple logistic regression analysis in nulliparous participants, partnership of five years or more protected women from severe fear of childbirth (OR 0.3; 0.2–0.7), whereas maternal age, education, previous nonviable pregnancies or somatic problems in the present pregnancy did not contribute to the risk of

either severe fear-of-childbirth or pregnancy-related anxiety.

Nulliparous versus parous participants

Severe fear-of-childbirth was more frequent among the nulliparous women than among the parous women (13.4% vs. 8.3%, $p = 0.03$). Also, severe pregnancy-related anxiety was more typical of nulliparous than parous participants (14.4% vs. 7.7%, $p = 0.004$). However, when comparing the impact of parity separately in the ART and control groups, only the nulliparous control women showed severe pregnancy-related anxiety more frequently than the parous controls (Table 8).

Impact of infertility and treatment-related characteristics

The aetiology or treatment type did not influence the risks of severe fear-of-childbirth or pregnancy-related anxiety. A long period of infertility (seven or more years) increased the risk of severe fear-of-childbirth (OR 4.4; 1.2–16.9), whereas more than four previous IVF treatments decreased the risk of severe fear-of-childbirth (OR 0.06; 0.005–0.7).

Table 8. Prevalence of severe fear-of-childbirth and pregnancy-related anxiety (total scores equal to or more than the 90th percentile in rFDQ or in PAS) according to group and parity.

	Group		<i>p</i>
	ART <i>n</i> (%)	CONTROL <i>n</i> (%)	
Total	367	379	
rFDQ	42 (11.4)	40 (10.6)	0.56
PAS	46 (12.5)	38 (10.0)	0.16
Nulliparous	260	135	
rFDQ	34 (13.1)	19 (14.1)	1.00
PAS	37 (14.2)	20 (14.8)*	1.00
Parous	107	244	
rFDQ	8 (7.5)	21 (8.6)	1.00
PAS	9 (8.4)	18 (7.4)*	0.51

* = $p < 0.05$ between the nulliparous and parous participants in the control group

PAS = pregnancy anxiety scale

rFDQ = revised version of the fear-of-childbirth questionnaire

DISCUSSION

Assisted reproductive treatment has proven an efficient treatment for infertile couples since its introduction in 1978. The numbers of initiated cycles and children born is still on the increase in Finland (STAKES 2006), in Europe as a whole (The European IVF-monitoring programme (EIM) 2006) and in other parts of the world (International Committee for Monitoring Assisted Reproductive Technology 2006). Although ART is efficient it entails increased risks of ectopic pregnancy, certain obstetric complications, operative delivery and preterm birth, even in singleton gestations. These elevated risks may all be of significance as regards the short- and long-term somatic and mental health of women, their infants and the family. Therefore, better understanding of the possible underlying mechanisms behind the elevated risks is important.

The aim of the present study was to evaluate the associations between infertility- and treatment-related characteristics and early pregnancy monitoring, obstetric and neonatal outcome and prevalence of fear-of-childbirth and pregnancy-related anxiety in singleton pregnancies after ART. These associations were studied among representative samples of women receiving ART: the response rate was nearly ninety per cent as regards the longitudinal material (II, IV) and whole patient cohorts were used in the cross-sectional studies (I, III). The aetiological and treatment subgroups in the study's ART group correspond well to the nationally reported distribution of aetiological and treatment subgroups (Gissler and Tiitinen 2001; STAKES 2006). The used data was collected both prospectively from a longitudinal, self-reported questionnaire survey (II, IV) and retrospectively from patient- and national health registers (I, III). These approaches to obtain data complement one another. Although the information provided by the national health registers is accurate and the coverage is nearly 100%, individual patient and treatment characteristics are not recorded in these registers. Further, evaluation of the psychological aspects of pregnancy – such

as the prevalence of fear-of-childbirth and pregnancy-related anxiety after ART – requires sensitive and valid assessment tools not present as part of the patient records.

The use of self-reported data is suitable for the assessment of subjective well-being. Subjective interpretation of one's health may be of more importance to an individual's psychological well-being than objectively measured health. On the other hand, self-reported perception of the somatic course of pregnancy (although interpreted via structured questions) may differ from the perception of medical professionals and should therefore be interpreted carefully. The most critical neonatal outcome measures (gestational age at birth and birth weight) in Study II were also checked from the clinics' patient records.

The limitation of these studies is in the statistical power. Although the sample sizes can be considered sufficient for finding differences in various comparisons, much larger samples would have been needed to confirm non-significant findings with high power. However, because two of these studies (II, III) are concerned with a relatively new ART option – i.e. eSET – much larger samples were not achievable within the study period.

The predictive value of single hCG measurement

In line with earlier results, women with viable pregnancies had significantly higher median serum hCG levels than those with non-viable pregnancies (Schmidt et al. 1994; Bjercke et al. 1999; Sugantha et al. 2000). The chosen cut-off level of hCG (76 IU/L) assessed 12 days after ET predicted viable pregnancies with 80% sensitivity, 82% specificity, and had 87% PPV and 74% NPV. These figures mean that 87% of women whose serum hCG concentration was 76 IU/L or higher had a viable pregnancy, whereas 74% of women with an hCG level under 76 IU/L had a non-viable pregnancy. The cut-off level for prediction of pregnancy outcome is always a compromise between sensitivity and specificity.

Both lower and higher cut-off levels than ours have been suggested (Okamoto et al. 1987; Fridström et al. 1995; Sugantha et al. 2000). The heterogeneity of results in respect to the cut-off levels could be a consequence of differences in hCG assays, assessment times and definitions of viable and non-viable pregnancies between the various studies. Therefore, every infertility clinic should preferably define the optimal hCG cut-off level to use as regards their individual setting.

The most valuable information as regards hCG was that a single serum hCG level determination appears to suit all viable singleton pregnancies irrespective of aetiological and treatment subgroups. The observed similar median hCG levels in singleton pregnancies achieved by means of single embryo transfer and double embryo transfer is also of clinical importance. Nearly 50% of Finnish embryo transfers are currently performed with SET, but hCG values in singleton pregnancies following SET had not been published before this study. In 2004, single hCG measurement was confirmed as a valuable tool in pregnancies following SET (De Neubourg et al. 2004). The estimated ten per cent cohort of vanishing twin pregnancies after DET might be associated with higher initial hCG levels than in singleton pregnancies achieved with SET. Although this assumption has not been confirmed by early hCG assay, higher hCG- β values later in the first trimester in vanishing twin pregnancies than in singleton pregnancies have been noticed (Chasen et al. 2006b). Probably, the effect of the number of transferred embryos on initial hCG rise should be studied in larger samples.

The lowest hCG value among viable singleton pregnancies was observed in the male factor subgroup, treated by means of ICSI. However, hCG levels in the viable singleton pregnancies in this subgroup were above the chosen cut-off level, suggesting that misclassification of these pregnancies to non-viable pregnancies is unlikely. Lower hCG levels after ICSI than after IVF have also been reported before (Gold et al. 2000). The reason for a lower initial hCG concentration could be related to later implantation and different trophoblast invasion after ICSI than after IVF.

However, in ICSI many steps of fertilization are by-passed and embryonic development together with the hCG rise might be expected to begin earlier rather than later compared with IVF.

Early recognition of an ectopic pregnancy offers a clinical challenge. In our sample most women with ectopic pregnancies (i.e. 19 out of 20) had an hCG level lower than 66 IU/L. As miscarriages and biochemical pregnancies are characterised by hCG levels similar to those observed in ectopic pregnancies, repetitive sampling is advisable. Repetitive sampling is especially advisable for patients with low serum hCG concentrations and underlying tubal factor infertility.

We found no association between serum hCG levels 12 days after ET and adjusted birth weight of singleton infants. As desirable as an early biochemical marker for the evaluation of successful placentation and neonatal outcome may be, placentation is far too complicated a process to be predicted by means of a single biomarker.

Impact of infertility- and treatment-related factors on obstetric and neonatal outcome

The association between a prolonged time to achieve a pregnancy and increased risks of preterm birth, LBW and neonatal mortality is well documented (Henriksen et al. 1997; Basso and Baird 2003; Basso and Olsen 2005). The impact of duration of infertility preceding successful ART, however, has not been studied. In this study we classified the duration of infertility into three subgroups (less than two years, two to five years and over five years) and analysed its contribution to neonatal risks. No association between increasing duration of infertility and risk of preterm birth or LBW was observed. This observation may be confounded by the fact that the women with the worst prognosis and longest duration of infertility might have dropped out because of total fertilization failure or a low number of retrieved oocytes. Furthermore, the duration of infertility is correlated to female age. As the participants in this study were 40 years of age at

most, evaluation of the impact of the duration of infertility on adverse neonatal outcomes may be limited. The number of preceding treatments did not contribute to the risk of preterm birth or LBW either.

The aetiological and treatment subgroups did not differ in respect to the risks of preterm birth and LBW. In larger samples significantly decreased risks of preterm birth and LBW after singleton ART gestation in couples with male factor versus female factor infertility and after frozen versus fresh embryo transfer have been noticed (Schieve et al. 2004; Källén et al. 2005b; Wang et al. 2005). The lower risks of preterm birth and LBW in cases of male factor infertility probably reflect normal female fecundity. The lower risks of preterm birth and LBW after frozen embryo transfer might also in part reflect only moderately impaired fertility of these couples, as conception rates are generally lower after FET than after fresh embryo transfer. Further, hormonal stimulation is considerably more “gentle” in FET cycles than in fresh embryo transfer cycles, which may be related to the decreased neonatal risks.

Even though high-risk patients are difficult to identify by means of patient- and treatment-related factors, our results support the current treatment policy: Carrying out ART among women with a relatively long duration of infertility, or with a history of numerous previous ART cycles, does not increase the risk of adverse neonatal outcomes.

The only identified risk factor for preterm birth among the background characteristics of women conceiving after ART was a history of previous induced abortion. A history of previous induced abortion has been considered a risk factor for subsequent preterm birth in an international multi-centre case-control study (Ancel et al. 2004). Whereas a history of a single induced abortion is not considered to increase the risk of preterm birth, multiple induced abortions may predispose a woman to preterm birth via destruction of the cervical canal and endometrium. In the present analysis a history of induced abortion was coded dichotomously (no induced abortion or one/more induced abortion) and therefore the inclusion of possible repetitive induced abortions as an explanation

for the observed increased risk of preterm birth cannot be excluded. However, because a history of induced abortion is present in a minority of women undergoing ART (9% in our sample), the absolute increase in preterm births as a result of previous induced abortion is probably small.

Number of transferred embryos

If an active single embryo transfer policy is not adhered to, approximately 90% of embryo transfers are carried out with replacement of two or more embryos into the uterine cavity. Apart from the high (25–30%) twinning rate and associated obstetric and neonatal complications, DET can have a negative influence on singleton outcomes via the worse neonatal outcome of vanishing twin survivors.

Our hypothesis was that obstetric and neonatal outcome of singleton pregnancies following SET would be better than that of singleton pregnancies following DET. However, we did not find differences in obstetric and neonatal outcome between SET and DET groups in our seven-year cohort. In addition, in age-, parity- and SES-adjusted comparison with spontaneously conceiving controls the SET group had increased risks of gestational hypertension, preterm contractions, placenta praevia, Caesarean section, preterm birth and LBW. Similarly, increased risks of preterm birth and LBW were also observed when adjusted comparisons between the DET and the spontaneously conceiving control group were carried out.

The results of many observational and register-based studies have confirmed more frequent pregnancy-induced hypertension, placenta praevia and Caesarean section in singleton pregnancies after ART than after spontaneous conception (Jackson et al. 2004). Here, these risks were also observed in singleton pregnancies following SET. The results remained essentially same even if only pregnancies following eSET were analysed. Our results imply that the vanishing twin phenomenon is not the only mechanism behind these increased risks. Further, the similar obstetric risk after transfer

of good quality embryos in eSET cycles (i.e. embryos with less than 20% fragmentation and without multinucleated blastomeres) suggests that other patient- and probably female-related reasons for increased obstetric risks exist in the ART population. One possible mechanism behind more frequent hypertensive disorders and placenta praevia after ART may be abnormal placentation, which on the other hand can be related to hormonal stimulation, embryo culture and the embryo transfer technique rather than to the number of transferred embryos. The increased risk of emergency Caesarean sections after SET is probably explained by the more frequent obstetric complications.

Very few studies have addressed the impact of the number of transferred embryos on singleton outcomes after ART (De Sutter et al. 2003; De Neubourg et al. 2006; De Sutter et al. 2006b; Kjellberg et al. 2006). Inclusion of only good-prognosis patients (i.e. women aged less than 35–37 years, having their first or second treatment cycle, and receiving a good-quality embryo) is typical of other SET studies. In contrast, in our sample, SET was initially carried out among women with known obstetric risks (Vilksa et al. 1999). For the last three study years SET has been the preferred treatment in the majority of cases and DET took place only if no special contraindication for a twin pregnancy was recognised, the couple had gone through two unsuccessful IVF cycles, or only poor quality embryos were obtained (Tiitinen et al. 2003).

Despite the differences in patient characteristics between previously published studies and this study, the mean gestational age at birth and the mean birth weight in our SET sample were similar to those reported before in observational studies (De Sutter et al. 2003; De Neubourg et al. 2006). In contrast, the duration of pregnancy was longer and the mean birth weight was higher in the SET singletons in a randomized study (Kjellberg et al. 2006) than in our study. The observed rate of preterm births (12.3%) in our sample was higher than that in the other SET studies (6.2% to 11.6%) (De Neubourg et al. 2006; De Sutter et al. 2006b; Kjellberg et al. 2006). This probably reflects the inclusion of women with congenital

malformation of the uterus and a history of cervical incompetence in our sample. On the other hand, the rate of preterm births in our study is similar to that of 8–14% in national and international register-based studies which do not take the number of transferred embryos into account (Klemetti et al. 2002; Schieve et al. 2004; Källén et al. 2005a).

The increased risks of LBW and low Apgar scores in our SET sample compared with the controls reflects the higher incidence of preterm births. The non-significantly increased risk of SGA, on the other hand, implies that the shorter duration of pregnancy rather than growth retardation results in a lower birth weight. We could not compare the incidence of congenital anomalies between the SET and the control group. However, the observed incidence of congenital anomalies after SET seemed similar to that in a larger cohort of Finnish infants after ART and higher than in spontaneously conceived infants (Klemetti et al. 2005).

Prevalence and predicting factors of fear-of-childbirth and pregnancy-related anxiety

In contrast to our expectations we found a similar prevalence of severe fear-of-childbirth and pregnancy-related anxiety in the ART and control groups in our study. This opposes the hypothesis that the psychologically demanding treatment of infertility and possible cumulative disappointments after unsuccessful treatment cycles would increase formerly infertile women's vulnerability to the emotional demands of pregnancy. Our finding further disagrees with previous results suggesting more pronounced pregnancy-specific anxiety after ART (McMahon et al. 1997; Hjelmstedt et al. 2003).

The observed similar prevalence of severe fear-of-childbirth and pregnancy-related anxiety may be a result of selection of patients with good psychological coping strategies and satisfactory relationships in our ART group. Generally, couples seeking infertility treatment do not have an excess of psychological disorders.

However, unsuccessful ART enhances levels of anxiety and depression (Newton et al. 1990; Verhaak et al. 2005). The psychological demands of repeated ART after previous failure represent a common reason for discontinuing further treatment (Olivius et al. 2004). Therefore, the decision to continue ART despite unsuccessful treatment can be a sign of well-functioning coping strategies and adequate social support from the spouse (Peterson et al. 2003; Schmidt et al. 2005) or other social network. Interestingly, for women, an increasing number of previous unsuccessful treatment attempts correlates with good marital satisfaction, even during the child's first year of life (Repokari et al., in press^a). In our sample, 70% of the participants had a history of previous ART attempts.

Several investigators have shown that after successful ART the prevalence of depression in women is similar to (Reading et al. 1989; Gibson et al. 2000; Klock and Greenfeld 2000) or less than (Repokari et al. 2005) that in controls. Levels of general anxiety decrease after successful ART (Verhaak et al. 2005) and come close to the norm (Repokari et al. 2005). The data suggest that the negative emotional burden of ART primarily results from the threat of remaining childless and it is not because of the ART method itself. In addition, the results of short- and long-term longitudinal studies on parenthood after ART indicate that there is no excess of parenting stress or mental problems in formerly infertile couples (Abbey et al. 1992; Gibson et al. 2000; Greenfeld and Caruso Klock 2001; Colpin and Soenen 2002; Repokari et al. 2005). Instead, ART mothers tend to experience motherhood more positively than spontaneously conceiving mothers regardless of more frequent obstetric and neonatal complications (Repokari et al. in press^b). In conclusion, couples who conceive after ART are probably psychologically well-adjusted for the new emotional demands of parenthood. Against this background our result of similar prevalence of severe fear-of-childbirth in the ART and control groups is not surprising.

We tried to identify the predictive factors of severe fear-of-childbirth and pregnancy-related anxiety in nulliparous women. We decided to limit the multiple logistic regression analysis to

nulliparous women, as we were unable to control for the impact of previous delivery. We restricted the evaluation of predictive factors of severe fear-of-childbirth and pregnancy-related anxiety to demographic-, obstetric- and infertility-related factors. This decision is justified, as these factors are easily assessable during clinical follow-up. Identification of a patient group at an increased risk, using such factors, would be of clinical use. In contrast to earlier studies, neither somatic symptoms such as hyperemesis (Swallow et al. 2004), nor previous non-viable pregnancy (Saisto et al. 1999) influenced the risk of severe fear-of-childbirth. Instead, a five- to ten-year partnership protected women from severe fear-of-childbirth. Previously, dissatisfaction with the partnership has been verified among the most significant predictors of fear-of-childbirth (Saisto et al. 2001a). It is difficult to say whether a long partnership is also a satisfactory one, but the protective role of a five- to ten-year partnership could be mediated through partnership satisfaction. The observed protective role of a relatively long partnership could also explain the less evident impact of parity on severe fear-of-childbirth and pregnancy-related anxiety in the ART group than in the control group, as the partnerships of both the nulliparous and parous participants had lasted longer in the ART group than in the control group.

We did not study simultaneous prevalence of depression and general anxiety in association with fear-of-childbirth and pregnancy-related anxiety. However, mental health was compared between the groups separately (Repokari et al. 2005). The women in the ART group had fewer depressive symptoms but equal levels of general anxiety compared with the control women at the time when fear-of-childbirth and pregnancy-related anxiety were assessed (i.e. gestational week 20). Because underlying anxiety rather than depression is an independent psychological predictor of fear-of-childbirth (Saisto et al. 2001a), the difference in level of depression does not explain the similarly expressed fear-of-childbirth in the groups. More frequent depression could, on the other hand, predispose the control women to more frequent pregnancy-related anxiety (Saisto et al. 2001a). However,

women expecting their first child were less depressed than the parous participants, although they reported severe pregnancy-related anxiety more frequently.

Among infertility- and treatment-related characteristics only a long duration of preceding infertility (seven or more years) increased the risk

of severe fear-of-childbirth. Even though this finding is based on a relatively small subgroup of patients it fits our original hypothesis well. Women with a long duration of infertility may be especially vulnerable to the potential threat of losing the pregnancy or the infant, which could be expressed as intense fear-of-childbirth.

SUMMARY AND CONCLUSIONS

The aim of this study was to evaluate the impact of infertility- and treatment-related factors on prediction of pregnancy outcome, obstetric and neonatal risks and experience of pregnancy in singleton pregnancy after ART.

Our results indicate that single serum hCG sampling as early as 12 days after embryo transfer is a reliable tool for predicting viable pregnancy. The aetiology of infertility, treatment type or the number of transferred embryos did not affect the predictive value of the serum concentration of hCG. If a woman with tubal factor infertility has a serum hCG level below the suggested cut-off level, repetitive hCG sampling is advisable. Our findings help in follow-up of early pregnancy and may relieve the psychological distress related to the uncertainty of pregnancy outcome experienced by couples undergoing ART.

The risks of preterm birth and LBW were increased in singleton ART pregnancies regardless of the aetiology of infertility or treatment type. In addition, the risks of preterm birth and LBW remained elevated compared with those in the controls in our preliminary cohort of singleton ART pregnancies achieved after SET. This suggests that even the most preferable treatment procedure – SET – does

not moderate all the commonly recognised risks associated with ART. Nevertheless, SET is definitely a better treatment for a woman with obstetric risks than DET. Larger samples with different subject characteristics will probably complement our results.

The similar prevalence of fear-of-childbirth and pregnancy-related anxiety in the ART group compared with the spontaneously conceiving controls is a relieving finding. It implies that after successful ART previous infertility does not have a long-standing negative effect on a woman's psychological well-being. It further implies that fear-of-childbirth can hardly be an explanation for more common Caesarean sections in singleton ART gestations. Altogether, the similar prevalence of fear-of-childbirth and pregnancy-related anxiety suggests that women conceiving by means of ART are psychologically well adjusted and they probably receive adequate support from their partners. Nevertheless, women with a long duration of preceding infertility seem to be at an increased risk of fear-of-childbirth and may need psychosocial support during the pregnancy. The challenge for infertility professionals and obstetricians remains to recognise and respond to patients' needs on an individual basis.

ACKNOWLEDGEMENTS

This work was carried out at the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, during 1999–2006. I wish to express my deep gratitude to the Head of Department, Professor Olavi Ylikorkala, and the Administrative Head of Department, Professor Maija Haukkamaa, for providing me with such good research facilities. I also owe my sincere gratitude to Professors Jorma Paavonen and Markku Heikinheimo, Heads of the Clinical Graduate School in Paediatrics and Obstetrics/Gynaecology, for offering wonderful research settings and an inspiring scientific atmosphere.

My warmest gratitude is addressed to my supervisors:

Professor Aila Tiitinen, for her admirable skill in combining scientific and clinical work. Her expertise, practicality, guidance, and open-minded support have been endless during these years filled with scientific, clinical and parental goals.

Docent Maija Tulppala, especially for teaching me the very basis of psychological aspects related to infertility and its treatment. I wish to thank her for her patient encouragement, support and analytical thinking during these years.

I wish to express my gratitude to the reviewers of this thesis, Docents Anneli Pouta and Niklas Simberg, for valuable criticism and expert revision.

I also wish to thank many talented persons who have made this thesis possible:

The members of my thesis committee at the Clinical Graduate School: Dr, Ph.D., Leila Unkila-Kallio, for continuous interest, enormous helpfulness in every aspect of scientific work and an ability to create a positive atmosphere whenever needed; Professor Raija-Leena Punamäki, for her inspiring and creative conversations and practical help in preparing the manuscripts; Docent Niklas Simberg for his constructive criticism and kind interest in this thesis.

My co-authors: Dr Sirpa Vilksa, for an ambitious amount of data validation and improvement of manuscripts; Dr Leena Repokari, for sharing the very first and often challenging steps in the scientific world; Docent Vilho Hiilesmaa, for his valuable introduction and help in statistics; Docent Mika Gissler, for his enthusiastic way of working and expert interpretation of statistics; Dr, Ph.D. Terhi Saisto, for her experienced insight into fear-of-childbirth; Christel Hyden-Granskog for her precious work with and understanding of cell biology; Dr, Ph.D. Ansa Aitokallio-Tallberg, for her perinatalogical contribution and Dr Jari Sinkkonen and Professor Fredrik Almqvist for their collaboration.

Riitta Luhtio, for her valuable, patient and thorough work in recruiting subjects and in data collection.

The personnel at the Infertility Clinics of Helsinki University Central Hospital, the Family Federation in Helsinki, Turku and Oulu, and the Deaconess Institute and especially Dr, Ph.D. Rita Sieberg, Dr, Ph.D. Tuula Penttilä, Docent Leena Anttila, Dr, Ph.D. Leena Tuomivaara, Dr, Ph.D. Sinikka Nuojua-Huttunen and Dr, Ph.D. Kari Ratsula for taking part in the longitudinal questionnaire survey by recruiting the subjects, and collecting and completing the data. The personnel at the Maternity Clinic of Helsinki University Central Hospital for providing good facilities when recruiting the control subjects.

Participants in the questionnaire survey for making this work possible.

Nina Hedkrok, Laila Selkinen, Teija Karkkulainen, Kirsi-Marja Äyräväinen and Leena Vaara, for their practical help during these years.

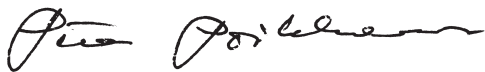
Nick Bolton, Ph.D., for skilful revision of the language.

All colleagues and friends at Helsinki University Central Hospital and the South Karelian Central Hospital, for their interest regarding this thesis during these years.

I'm deeply grateful to my dear friends: Aino, Heini, and Ilona, especially for sharing the strenuous moments of this thesis and offering encouragement and valuable advices. Also Jonna and Nelli are thanked for fun and relaxing moments outside this study.

I wish to thank my dear parents, Raija and Pekka, for their love, support and encouragement throughout my life; my brother Pasi and his family, and my sister Niina, for their support; my parents-in-law, Marjatta and Esko, for their interest in this thesis.

Helsinki, February 2007

A handwritten signature in cursive script, appearing to read "Jari Parkkinen".

Finally, my husband Perttu and our dear son, Eero, are warmly thanked for bringing true joy, laughter, and love into my life.

This study was financially supported by the Clinical Graduate School in Paediatrics and Obstetrics/Gynaecology, University of Helsinki, and by research Funds from Helsinki University Central Hospital and the South Karelian Central Hospital, the Sohlberg Foundation, Organon, Finland, Serono, Finland and the Finnish Foundation of Gynaecology and Obstetrics.

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APPENDIX

Table 9. Contents of the revised version of the Fear-of-Childbirth Questionnaire (modified from Saisto et al. 2001b).

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1. Do you have difficulties relaxing because you are thinking of the delivery?
 2. Are you afraid of being seized with panic at the delivery?
 3. Are you afraid that the nurses won't take care of you during the delivery?
 4. Have you always been afraid of giving birth?
 5. Are you afraid that the baby will not be healthy?
 6. Have you had nightmares about the delivery?
 7. Are you afraid of rupturing during the delivery?
 8. Are you afraid of painful injections during the delivery?
 9. Are you afraid of losing control of yourself at the delivery?
 10. Do you prefer Caesarean section to an ordinary delivery?
 11. Are you afraid of death when thinking of the delivery?
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each question was asked to be answered dichotomously (i.e. either yes=1 or no=0)

Table 10. Contents of the Pregnancy Anxiety Scale (Saisto et al. 2001b).

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1. Has anyone frightened you about having a baby?
 2. Have you read anything that frightened you about having a baby?
 3. Do you fear that you will fall and hurt your baby?
 4. Are you afraid of the labour pain?
 5. Are you going to ask for pain medicine in delivery?
 6. Do you fear about being cut when the baby is born?
 7. Are you afraid your baby would not be normal?
 8. Are you afraid you will be alone in the hospital?
 9. Are you worried that the doctors may not be friendly?
 10. Are you worried that the nurses may not be friendly?
-

each question was asked to be answered on five-point scale (i.e. not at all=1, little=2, some=3, yes=4, a lot =5)