

Cumulative Live Birth Rates after In Vitro Fertilization

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2009

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Front cover: "Sperm & Egg", design by James Steidl. Purchased at www.istockphoto.com.

ISBN: 978-91-628-7914-3

*Triumf att leva, triumf att andas, triumf att finnas till!
Triumf att känna tiden iskall rinna genom sina ådror
och höra nattens tysta flod
och stå på berget under solen.*

...

*Solen fyller upp mitt bröst med ljuvlig honung upp till
randen
och hon säger: en gång slockna alla stjärnor, men de lysa
alltid utan skräck.*

(Ur *Triumf att finnas till*, Edith Södergran 1916)

*A triumph to live, a triumph to breathe, a triumph to exist!
A triumph to feel time run icy cold through your veins
and hear the silent river of night
and stand on the mountains under the sun.*

...

*The sun fills up my breast up to the brim with sweet
honey
and she says: one day all stars will die, but they always
shine fearlessly.*

(From *A triumph to exist*, Edith Södergran 1916, translation by Martin Allwood)

Abstract

Background: In vitro fertilization (IVF) has become increasingly common, today representing about 3% of all live births in some countries. Most patients have to undergo more than one treatment in order to achieve a live birth. Thus cumulative live birth rates are highly interesting to the patients. The most important health problem in IVF is the high rate of multiple births, leading to increased risks for preterm birth and perinatal morbidity. Therefore, single embryo transfer (SET) has become more frequently used.

Aims: The aims of this thesis were to assess cumulative live birth rates after IVF and to investigate factors affecting the live birth rates.

Methods: *Paper I:* Cumulative live birth rates after a treatment programme consisting of three fresh IVF cycles and subsequent frozen-thawed cycles were investigated in 974 patients. Life table analysis with and without taking dropouts into account gave three estimates; "pessimistic", "realistic" and "optimistic". *Paper II:* Many of the patients in Paper I discontinued the treatment. The reasons for this were investigated in Paper II, by scrutinizing medical records and using questionnaires. *Paper III:* Maternal and embryonic factors were analyzed in 371 patients for possible prediction of live birth in frozen-thawed SET, using multiple logistic regression. *Paper IV:* A follow up of a previous randomized controlled trial (RCT), comparing single and double embryo transfer (DET) in 661 patients. Data on all additional frozen-thawed cycles were collected in order to present cumulative live birth rates.

Results: *Paper I:* The cumulative live birth rate after three fresh IVF cycles, mostly DETs, including subsequent frozen-thawed cycles was 63% with a "realistic" approach. *Paper II:* Of the couples in Paper I who did not achieve a live birth, 54% discontinued the treatment programme. The most important reasons were psychological stress and poor prognosis. The most frequent comment was "needed more information about the treatment". *Paper III:* Positive predictors for live birth in frozen-thawed SET were blastomere survival rate, number of previous fresh cycles and conventional IVF as compared with intracytoplasmic sperm injection (ICSI). Number of embryos needing to be thawed in order to perform one transfer was negatively associated with pregnancy. *Paper IV:* The cumulative live birth rates after one fresh SET or DET and subsequent frozen-thawed cycles, with one or two embryos transferred according to the patient's wish, were 44% in the SET group and 51% in the DET group ($p=0.08$). The multiple birth rates were 2% in the SET group and 28% in the DET group ($p<0.001$).

Conclusions: There is a good chance of achieving a live birth through a treatment programme of three IVF cycles. Implementation of SET is an effective way to decrease multiple birth rates. The cumulative live birth rate after one SET, including frozen-thawed transfers, was not significantly lower than after DET. The frozen-thawed cycles contribute significantly to the cumulative live births, and the knowledge of predictive factors for live birth in frozen-thawed cycles is valuable when deciding whether to perform SET or DET. The dropout rate from the treatment programme was high. The knowledge that many patients perceive IVF treatment as psychologically stressful and feel a need of more information can be useful in patient consultations and when organizing the care at the IVF clinics.

Key words: In vitro fertilization, cumulative live birth, single embryo transfer, frozen-thawed cycle, discontinuation

ISBN: 978-91-628-7914-3

Sammanfattning på svenska

Provrörsbefruktning (in vitro fertilization, IVF) ger upphov till omkring 3% av alla födslar i Sverige idag. De flesta patienter behöver genomgå mer än en IVF-behandling för att få barn. Kumulativa födelsetal, dvs andelen par som får barn efter ett antal behandlingscykler, är därför av stort intresse för patienterna. Det största hälsoproblemet med IVF är den höga flerbördsfrekvensen. Flerbörd är förknippat med ökad risk för förtidig födsel och komplikationer för barnen. För att minska flerbördsfrekvensen tillämpas i allt större utsträckning återförande av endast ett befruktat ägg per behandling (single embryo transfer, SET).

Artikel I: Kumulativa födelsetal analyserades i en grupp av 974 IVF-patienter. Sannolikheten för att få barn efter det behandlingsprogram som erbjuds i regionens regi om tre färsk IVF-cykler med efterföljande fryscykler var 63%. Sannolikheten var högre för kvinnor under 35 år (67%) än för kvinnor 35-40 år (52.5%), men det var ingen skillnad för patienter med olika infertilitetsdiagnoser.

Artikel II: Skälen till varför 54% av de patienter som inte fick barn i Artikel I hade avbrutit behandlingsprogrammet i förtid undersöktes genom journalstudier och enkäter. De vanligast förekommande skälen var psykisk stress och dålig prognos.

Artikel III: Faktorer förknippade med födsel vid återförande av frysta och tinade befruktade ägg undersöktes hos 371 kvinnor. Cellöverlevnaden hos det tinade befruktade ägget, antalet färsk behandlingscyklar patienten hade genomgått, och standard-IVF som befruktningssätt jämfört med mikroinjicering av spermien i ägget (intracytoplasmic sperm injection, ICSI) var faktorer förknippade med en ökad chans till födsel. Antalet befruktade ägg som behövde tinas för att åstadkomma ett äggåterförande var förknippat med minskad chans till graviditet.

Artikel IV: Uppföljning gjordes av en tidigare studie där 661 patienter genom slumpmässig tilldelning genomgått antingen SET eller återförande av två befruktade ägg (double embryo transfer, DET). Kumulativ födelsefrekvens efter färsk SET eller DET inklusive efterföljande fryscykler, i vilka ett eller två befruktade ägg hade återförts oberoende av den inledande slumningen, var 44% i SET-gruppen och 51% i DET-gruppen (ej statistiskt signifikant skillnad). Flerbördsfrekvensen var 2% i SET-gruppen och 28% i DET-gruppen.

Slutsats: Sannolikheten är god att få barn efter tre IVF-behandlingar. SET är en effektiv metod för att minska flerbördsfrekvensen vid IVF. Kumulativa födelsetal efter SET med efterföljande fryscykler var inte signifikant lägre än efter DET. Kunskapen om vilka faktorer som påverkar prognosen vid fryscykler är viktig när man avgör om ett eller två befruktade ägg ska återföras. En stor andel av patienterna som inte fick barn avbröt behandlingsprogrammet i förtid. Medvetenhet om att många patienter upplever IVF-behandlingen som psykiskt påfrestande kan vara av värde i patientkonsultationer och när man organiserar vårdens utförande.

List of publications

- I. Olivius C, Fridén B, Lundin K and Bergh C.
Cumulative probability of live birth after three in vitro fertilization/ intracytoplasmic sperm injection cycles.
Fertility and Sterility 2002;77:505-510.

- II. Olivius C, Fridén B, Borg G and Bergh C.
Why do couples discontinue in vitro fertilization treatment? A cohort study.
Fertility and Sterility 2004;81:258-261.

Comment:
Olivius C, Fridén B, Borg G and Bergh C.
Psychological aspects of discontinuation of in vitro fertilization treatment.
Fertility and Sterility 2004;81:276.

- III. Olivius C, Lundin K and Bergh C.
Predictive factors for live birth in cryopreservation single embryo transfer cycles.
Reproductive Biomedicine Online 2008;17:676-683.

- IV. Thurin-Kjellberg A, Olivius C and Bergh C.
Cumulative Live-Birth Rates after Single-Embryo versus Double-Embryo Transfer.
Accepted for publication.

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Abbreviations and definitions

AH	Assisted hatching
AIH	Artificial insemination husband
ART	Assisted reproductive technology, includes IVF and intrauterine insemination
CI	Confidence interval
Completed cycle	IVF cycle that achieved embryo transfer
Conventional IVF	Also called “standard IVF”
DET	Double embryo transfer
ESHRE	European Society of Human Reproduction and Embryology
FET	Frozen-thawed embryo transfer
FSH	Follicle-stimulating hormone
GEE	Generalized estimation equation
GnRH	Gonadotropin-releasing hormone
GQE	Good quality embryos
hCG	Human chorionic gonadotropin
hMG	Human menopausal gonadotropin
ICMART	International Committee for Monitoring Assisted Reproductive Technology
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilization. Refers to the entire treatment. Includes both cycles with fertilization with standard IVF and with ICSI
LH	Luteinizing hormone
Low birth weight	<2500 g
OHSS	Ovarian hyperstimulation syndrome
Optimistic estimate	Cumulative live birth rate estimate where all patient dropouts are given the same chance of a live birth as the continuers (standard life table analysis)
Pessimistic estimate	Cumulative live birth rate estimate where all patient dropouts were given no chance of live birth (i.e. real, observed rate)
PGD	Preimplantation genetic diagnosis
PGS	Preimplantation genetic screening
Preterm birth	<37 gestational weeks
RCT	Randomized controlled trial
Realistic estimate	Cumulative live birth rate estimate; the patients who dropped out owing to poor prognosis were given no chance of a live birth, while the other dropouts were given the same chance as the continuers (modified life table analysis)
SET	Single embryo transfer
SD	Standard deviation
Started cycle	IVF cycle where ovarian stimulation was initiated

Introduction

Historical background of assisted reproduction

The mechanism of reproduction has historically been a matter of great interest. An important step away from superstition and folklore beliefs took place when one of the first microscopists, Leeuwenhoek (1632-1723), made the first observations of human spermatozoa. The scientists initially believed that the spermatozoa were parasites, hence the ending -zoa. A widely discussed theory was that a homunculus, a miniature human, was encapsulated in the sperm head, ready for implantation in the female uterus.

The first example of assisted reproductive technology (ART) was when the Italian scientist Spallanzani successfully inseminated a spaniel bitch in 1783. In 1827, von Baer and van Beneden made the first observation of a mammalian oocyte. Human artificial insemination was introduced in 1838 by Dr Girault in France, but acceptance amongst the medical establishment took a long time, and artificial insemination remained controversial in many countries until mid twentieth century (Clarke, 2006). From the late nineteenth century, several scientists made attempts to fertilize both human and animal oocytes in vitro but without success (Schenk, 1878; Pincus and Enzmann, 1935). It was not until the discovery by Austin and Chang in 1951 of sperm capacitation, a sperm maturation process that occurs after entrance into the female reproductive tract and is necessary

for penetration into the egg, that fertilization in vitro succeeded (Austin, 1952). Rabbit oocytes were fertilized in vitro in 1954 (Dauzier et al., 1954), and in 1959 the first in vitro fertilization leading to a live birth was achieved, in a rabbit (Chang 1959). The first evidence of fertilization of a human oocyte in vitro was seen in 1969 (Edwards et al., 1969), but it took years before the IVF technology led to a human live birth.

The first human IVF resulting in a live birth took place in 1978 (Steptoe and Edwards, 1978). During the early 1980s, the cryopreservation techniques for in vitro fertilized oocytes were developed, resulting in the first pregnancy after a frozen-thawed embryo transfer in 1983 (Trounson and Mohr, 1983). Intracytoplasmic sperm injection (ICSI) has been an important addition to the treatment arsenal of male infertility since 1992, when the first human live birth after ICSI was achieved (Palermo et al., 1992). IVF has increased steadily as a treatment for infertility since its beginnings in 1978, and has until today resulted in about 4 million live births (personal communication 2009, Karl Nygren, ICMART Chair).

Infertility and ART today

Infertility is defined by the World Health Organization as failure to become pregnant after one year of unprotected intercourse, and affects 10-15% of couples of reproductive age. The causes of infertility are female in approximately one third, male

in one third, and multifactorial or unexplained in one third. Causes of female infertility include tubal pathology, polycystic ovary syndrome, other ovulatory dysfunction, diminished ovarian reserves, endometriosis, and uterine factors. Male infertility is attributable to poor sperm quality, mainly idiopathic oligozoospermia, astenozoospermia or teratozoospermia. The poor sperm quality is often idiopathic, but in some cases infections, chromosomal abnormalities, systemic diseases and hormonal disorders are underlying causes (Irvine, 1998).

ART includes IVF and intrauterine insemination. IVF includes cycles with fertilization using standard IVF or ICSI, using own eggs or donor eggs, own sperm or donor sperm, in fresh cycles and frozen-thawed cycles. Table 1 shows the quantity of ART treatments in Europe in 2005. The European countries perform the largest number of IVF treatments followed by the USA. In 2002 241,000 cycles reaching egg aspiration were performed in Europe, 74,000 in the USA, 45,000 in Asia, 29,000 in the Middle East, 18,000 in New Zealand/Australia and 20,000 in other countries (ICMART, 2009). In 2005, IVF treatment represented 2-4% of all live births

in the European countries and 2.9% of all live births in Sweden (Nyboe Andersen et al., 2009).

Sperm donation has, during the last two decades, been available for couples with severe male infertility, used by insemination or by IVF. Sperm donation is in some countries also offered to lesbian couples and single women. Oocyte donation, where the donor's oocytes are fertilized in vitro with sperm from the partner of the recipient, after which the embryos are transferred to the female recipient, has become an alternative for couples where the woman has poor oocyte quality or poor oocyte reserves. In Sweden oocyte donation is only permitted for women of reproductive age, while in some other countries it is also available for women with normal age-related infertility. Gestational surrogacy is an option where, after fertilization in vitro, embryos are transferred to a gestational carrier. Gestational surrogacy was developed primarily for women with good egg quality but uterine pathology. It is also available for male homosexuals in some countries. In cancer patients, cryopreservation of sperm for later use in IVF is a successful method, while cryopreservation of oocytes or ovarian tissue is still under development.

Table 1. ART treatments performed in Europe in 2005 (Nyboe Andersen et al., 2009)

	No. of cycles
Fresh standard IVF cycles	118.074
Fresh ICSI cycles	203.329
Frozen-thawed cycles (IVF and ICSI)	79.140
Oocyte donation cycles	11.475
Intrauterine insemination (husband)	128.908
Intrauterine insemination (donor)	20.568

The legislation concerning ART differs greatly between different countries. Some countries have hardly any legislation at all, while others have very strict rules. The legislations of many European countries are documented at www.eshre.com. The Swedish legislation and guidelines include crude age restrictions, restrictions on egg and sperm donation, assessment of social factors and rules for infection screening. For example, anonymous gamete donation is not allowed; each child has the right to know his/her genetic origin after having reached maturity. The woman should not have reached the age when fertility is normally in sharp decline when IVF with donated eggs is initiated. The guidelines from 2003 also declare that only one embryo should normally be transferred. Two embryos might, however, be transferred when the risk of a twin pregnancy is considered limited. Lesbian couples are offered IVF with donor sperm. In Sweden, treatment of single women with sperm donation or of male homosexual couples with gestational surrogacy is currently not allowed.

According to the local rules at

Sahlgrenska University Hospital, the treated woman must not be over 40 years of age and her partner under 55 years of age when the IVF treatment is initiated, and the couple must have a stable relationship, defined as being married or having lived together for at least two years. Drug abuse, severe criminality, life threatening disease or psychiatric or social circumstances that make it impossible to take care of a child, are considered as relative or absolute contraindications for IVF.

IVF procedures

IVF can be performed either in natural cycles or in hormonally stimulated cycles. Natural cycle IVF was abandoned early owing to much lower pregnancy rates, but has been discussed again lately in accordance with the trend of transferring fewer embryos and using milder stimulation (Nargund and Frydman, 2007). In hormonally stimulated IVF cycles, down-regulation of the pituitary gonadal axis is commonly performed by nasal administration of a gonadotropin-releasing hormone (GnRH) agonist for 2-4 weeks (Figure 1).

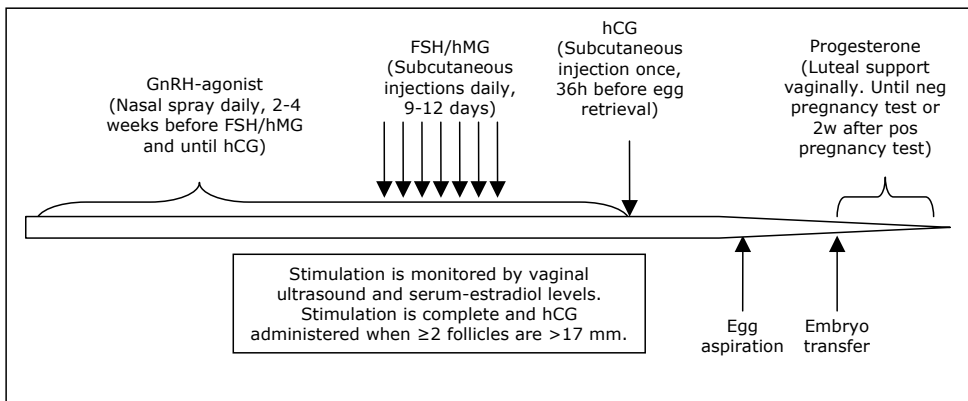


Figure 1. Example of a hormone stimulation protocol using GnRH agonist.

A shorter protocol using a GnRH antagonist is also possible. Ovarian stimulation is initiated by daily subcutaneous injections of follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG), and monitored by serum-estradiol levels and vaginal ultrasound. The stimulation is complete when at least two ovarian follicles measures >17 mm, and human chorionic gonadotrophin (hCG) is injected for final oocyte maturation. The oocytes are aspirated 36 h after the hCG injection, using transvaginal ultrasonographically guided puncture, usually with conscious sedation in combination with local anesthetics.

Fertilization is performed using conventional IVF or intracytoplasmic sperm injection (ICSI; Figure 2 and 3). The embryos are examined daily and graded by degree of fragmentation, number of cells, cell size and presence of multinucleation. Embryos are transferred either in the cleavage stage, on day 2-3, or in the blastocyst stage, on day 5-6. Embryo transfer is performed by depositing the embryo in the uterus through a catheter, guided by abdominal ultrasound.

Since about ten oocytes are aspirated in an average IVF cycle and only one or two embryos are transferred, there are often supernumerary embryos available for freezing. In cryopreservation, the embryos are exposed to a cryoprotectant agent, e.g. 1,2-propanediol, which replaces the intracellular water and reduces the intracellular formation of ice crystals. In the traditional cryopreservation technique called “slow freezing”, the embryos are slowly cooled to around -100°C before transfer to and storage in liquid nitrogen in -196°C. A new ultra-rapid freezing technique called “vitrification” that may have some advantages over slow freezing, has recently

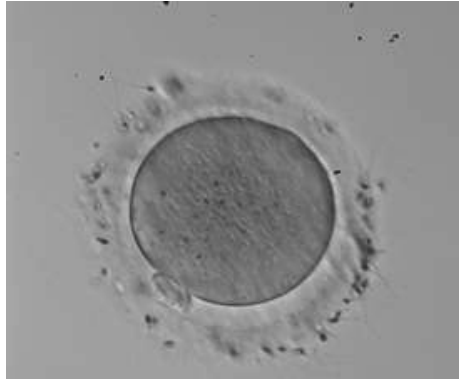


Figure 2. Conventional ("standard") IVF. The oocyte is placed in a nutritional solution containing a fixed concentration of sperm, allowing the sperm to penetrate and fertilize the egg.



Figure 3. ICSI. One sperm is injected into a mature oocyte by use of a micropipette.

been introduced. Frozen-thawed cycles are performed in either natural menstrual cycles or, for anovulatory patients, hormone stimulated cycles, usually with oral estrogen and vaginal progesterone.

Ovarian hyperstimulation syndrome (OHSS) is the most serious complication for

the woman in IVF. OHSS is an iatrogenic condition with enlarged ovaries, abdominal pain, ascites, nausea, and can, in severe cases, be life threatening with pleural effusion, multiple organ failure and disseminated intravascular coagulation. High estradiol levels and a high number of large follicles are known risk factors for developing OHSS (Delvigne et al., 2002, Kahnberg et al., 2009). Possible actions to prevent OHSS in patients at risk include reduction of FSH dosages during stimulation, “coasting” of cycles, i.e. stopping the gonadotropin administration and delaying hCG until estradiol levels decrease, and cryopreservation of all embryos instead of fresh embryo transfer (Delvigne et al., 2002). Other complications in IVF such as bleeding and infections are rare. Apart from the immediate medical complications associated with treatment, the high incidence of multiple births is considered the main adverse outcome in IVF (Bergh et al., 1999).

Live birth rates after IVF

Live birth rates after IVF have improved steadily over the years. The most recent live birth rates are listed below (Table 2). The live birth rates per embryo transfer are higher in the USA than in Sweden and Europe (Table 2). This can at least partly be explained by the higher number of embryos per transfer, also reflected in the fact that there are more multiple births in the USA than in Europe. When calculating the live birth rate per embryo transferred this was, however, shown to be higher in Sweden than in the USA during 1995-2003 (Karlström and Bergh 2007). The most important factor influencing the multiple birth rate is the number of embryos transferred. Worldwide, there has been a

steady decrease in the number of embryos transferred per cycle; however most embryo transfers still take place with two or more embryos in most countries. The highest rates of single embryo transfer (SET) are in the Scandinavian countries, Belgium, Holland and Australia/New Zealand (ICMART 2009).

The live birth rates after ICSI are slightly lower than after conventional IVF (Table 2). This is probably attributable to the fact that ICSI is used not only for male factor infertility but also in cases of poor fertilization, which is a poor prognostic factor; ICSI for male infertility has similar live birth rates as other diagnostic subgroups (Lintsen et al., 2007).

The live birth rates after frozen-thawed cycles are lower than after fresh cycles (Table 2). However, frozen-thawed cycles are an important complement to the fresh cycles and contribute to a considerable share of the live births after IVF: 23.7% in Sweden, 15.0% in Europe, and 16.3% in the USA in 2005 (The Swedish National Board of Health and Welfare 2008, Nyboe Andersen et al., 2009, Wright et al., 2008). Frozen-thawed cycles have some advantages, as they are safer, cheaper and more comfortable for the woman, as compared with fresh cycles.

Transfer of blastocysts (cultured to day 5/6) has shown superior live birth rates as compared with cleavage stage embryos (cultured to day 2/3), and in a Cochrane systematic review the live birth rate per couple was 36.0% with blastocysts and 29.4% with cleavage stage embryos (Blake et al., 2007). However, there are some disadvantages with blastocyst transfer: it is a more expensive culturing technique, a greater risk of failure of transferring any embryos at all in a particular cycle, and

Table 2. Live birth rates, number of embryos transferred and multiple birth rates in 2005.

	Sweden	Europe*	USA*
Live birth rate per embryo transfer (%)			
-Fresh, total	25.5	19.4	34.3
-Fresh IVF	27.0	21.3	-
-Fresh ICSI	23.8	18.4	-
-Frozen-thawed	18.6	13.5	28.0
Number of embryos transferred (% of fresh transfers)			
-One	69.4	20.0	7.0
-Two	30.6	56.1	58.8
-Three	0	21.5	26.7
-Four or more	0	2.3	7.6
Multiple birth rate (%)	6.7	21.8	30.5

(The Swedish National Board of Health and Welfare, Nyboe Andersen et al., 2009, Wright et al., 2008).

*Including Sweden.

*Results according to fertilization method (IVF/ICSI) were not accessible. The number of embryos transferred refers only to women below 35.

yields fewer embryos available for freezing (Blake et al., 2007). In addition, the survival of embryos after cryopreservation of blastocysts has not been as good as for cleavage stage embryos, but with the introduction of vitrification that might change (Loutradi et al., 2008).

Factors affecting the live birth rates

The woman's age is the most important factor influencing the live birth rate. The chances of a live birth after IVF begin to decline after the age of 35, and the decline is substantial after 40 (Wright et al., 2008, The Swedish National Board of Health and Welfare 2009, Templeton et al., 1996). Somewhat surprisingly the live birth rates were slightly lower in women under 25 in two large studies (Templeton et al., 1996, Lintsen et al., 2007). This is contradicted by the most recent Swedish data where the live birth rates are similar for women under 25 as for women 25-34 years of age (The Swedish National Board of Health and

Welfare 2009).

The live birth rates for couples with different reasons for infertility do not differ to any major extent (Templeton et al., 1996, Stolwijk et al., 2000, Lintsen et al., 2007). Unexplained infertility has a slightly better prognosis (Omland et al., 2005), and tubal pathology and endometriosis have slightly poorer prognoses in some publications (Omland et al., 2005, Wright et al., 2008). Since there is a rather high rate of spontaneous pregnancy in couples with unexplained infertility up to three years before it levels off, these couples are in many countries advised to wait for up to three years before starting IVF treatment (Pandian et al., 2005). In a Dutch study on patients on waiting lists for IVF it was found that 9.1% of the couples achieved a spontaneous ongoing pregnancy within a year. The chances of a spontaneous pregnancy were higher for couples where the woman was relatively younger, had shorter duration of infertility, secondary

compared with primary infertility, and for couples with unexplained, male or immunological reasons for infertility (Eijkemans et al., 2008).

Longer duration of infertility has been shown to negatively affect live birth rates, as well as the number of previous failed IVF cycles (Templeton et al., 1996, Lintsen et al., 2007). Previous pregnancy and live birth are positive predictors for ongoing pregnancy and live birth (Templeton et al., 1996, Stolwijk et al., 2000). Overweight (BMI>30) was found to have a negative effect on the live birth rate (Fedorcsak et al., 2004). In a study from our own centre it was shown that undergoing the first IVF cycle, IVF as fertilization method as compared with ICSI, transfer of a 4-cell embryo and ovarian sensitivity correlated independently to ongoing implantation (Thurin et al., 2005).

The question as to whether anxiety and depression can cause infertility or lower the chances of a live birth has been investigated in many studies, and conflicting data have been presented. In a recent Dutch study (Lintsen et al., 2009) of 783 patients, the level of anxiety and depression showed no association to the chance of a pregnancy, or to the drop-out rate. Other studies with fewer patients have shown significant correlations between stress, anxiety and lower pregnancy rates after IVF (Klonoff-Cohen et al., 2001, Smeenk et al., 2001). Somewhat surprising results were shown in another study, in which the women who were more negative before treatment had a greater chance of a pregnancy than those who were less negative (de Klerk et al., 2008).

In frozen-thawed cycles, blastomere survival rate after thawing has been shown to be positively associated with pregnancy

(Salumets et al., 2006; Tang et al., 2006; Edgar et al., 2007). Pregnancy in fresh IVF/ICSI cycle from which the frozen embryos originated (El-Toukhy et al., 2003; Urman et al., 2007), and the number of embryos transferred have also been shown to be predictive factors for pregnancy in frozen-thawed cycles (Lahav-Baratz et al., 2003; Salumets et al., 2006; Edgar et al., 2007).

Cumulative live birth rates

When an infertile couple presents at the IVF clinic, their most important question is how great their chances are of having a child after the treatment, the “take home baby-rate”. Cumulative live birth rates answer the question of the probability of live birth after a series of fresh IVF cycles with or without subsequent frozen-thawed cycles, or after one fresh cycle including its subsequent frozen-thawed embryo transfers. The usually provided statistics presented at a national level or by separate clinics are live birth rates per cycle (The Swedish National Board of Health and Welfare 2008, Nyboe Andersen et al., 2009, Wright et al., 2008). Cumulative live birth rates are more complex to analyze. Cumulative live birth rates are affected by, in addition to the factors affecting the live birth rates per cycle mentioned above, the patient dropout rate, the utilization of surplus cryopreserved embryos, and the decline in live birth rate with each failed cycle. The pioneer in calculating cumulative success rates after IVF was Dr HW Jones, who used a parametric statistical model and presented cumulative pregnancy rates in 1986 (Guzick et al., 1986).

The statistical method most frequently used when calculating cumulative live birth rates is the life table analysis, also called the

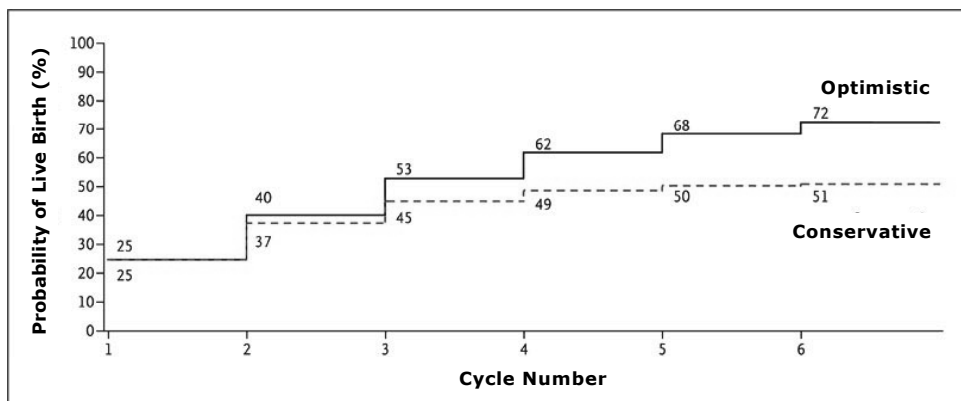


Figure 4. Malizia et al., 2009. Cumulative live birth rates after in-vitro fertilization. "Optimistic" (life table analysis) and "conservative" (observed) cumulative live birth rates among 6,164 women. (Published with permission from N Engl J Med).

Kaplan-Meier Curve. Since all patients who drop out of treatment are assumed to have the same probability of live birth as women who continue in life table analysis, this method tends to overestimate the live birth rates (Stolwijk et al., 1996., Land et al., 1997). Thus, to compensate for the overestimation, a modified life table analysis has been introduced producing one "pessimistic", one "optimistic" and one "realistic" estimate that differ in terms of the way the drop-out group is dealt with (Stolwijk et al., 1996, see Methodological considerations, statistics).

A former study of cumulative live birth rates performed at our centre included 398 couples undergoing IVF treatment during 1990-1992. The cumulative live birth rate was 50.0% after three available IVF cycles ("conservative estimate", Bergh et al., 1995). A recent large American study presented "optimistic" and "conservative" cumulative live birth rates as illustrated in Figure 4 (Malizia et al., 2009).

Outcome in children born following IVF

Numerous publications have reported increased risks for perinatal mortality, preterm birth, low birth weight, congenital malformations and neurological complications for IVF children as compared with children born after spontaneous conception (Bergh et al., 1999; Strömberg et al., 2002; Helmerhorst et al., 2004; Jackson et al., 2004). Most of the increased risks can be explained by the high multiple birth rate (Nygren et al 2007). However, even for IVF singletons as compared with spontaneously conceived singletons, and for IVF twins as compared with spontaneously conceived twins of unlike sex, an increased risk for preterm birth and low birth weight has been found (Bergh et al., 1999, Nygren et al., 2007, Hansen et al., 2009). The risk decreases to a large extent when adjusting for parental characteristics, including their subfertility status, but remains significant (Bergh et al., 1999, Nygren et al., 2007).

Cross-linkage between the Swedish IVF registry and other population-based registries in Sweden has been performed on three occasions. In the first report from these cross-linkages, which compared almost 6,000 IVF children with 1,500,000 spontaneously conceived children, the rates in IVF children versus spontaneously conceived children for preterm birth were 30.3% and 6.3%, low gestational weight 27.4% and 4.6% and perinatal mortality 1.9% and 1% respectively. Most of the higher risk for the IVF children could be explained by multiple births (Bergh et al., 1999).

The risk of neurological complications was investigated in a Swedish cohort study, where the IVF children were found to have OR 3.7 (2.0-6.6) for cerebral palsy as compared with matched controls, and a four-fold higher risk for suspected developmental delay. The risk elevation was mainly attributable to the increased incidence of preterm birth and multiple births (Strömberg

et al., 2002). A recent Dutch meta-analysis found no evidence of increased risk of mental retardation and cerebral palsy from the IVF/ICSI treatment per se, and the higher risk for neurological complications could be completely explained by the preterm birth rate and other risk factors associated with IVF. However, there is limited research on these children beyond pre-school age (Middelburg et al., 2008).

Concerning congenital malformations, controlled studies and meta-analyses have shown a slight increase in malformation rates among IVF/ICSI children as compared with children born after spontaneous conception (Rimm et al., 2004; Hansen et al., 2005; Mc Donald et al., 2005). In the Swedish registry study including 16,000 IVF children, which differs from the other studies in that it is the only one that adjusted for years of childlessness, the crude OR for congenital malformations among infants born after IVF as compared with all infants was 1.42. However, when adjustments for

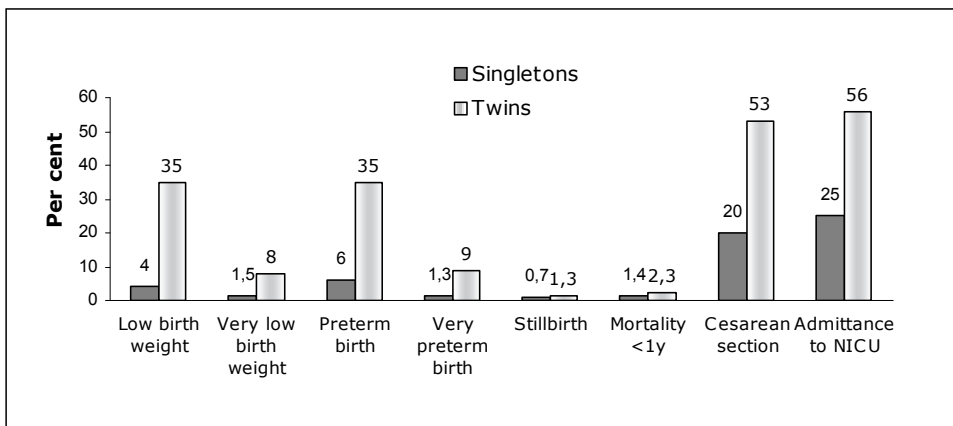


Figure 5. Obstetric outcome in IVF/ICSI twins versus IVF/ICSI singletons. Results from original study by Pinborg et al., 2004, including 3,438 twins and 5,164 singletons. All differences in variables between singletons and twins were significant.

maternal age, parity, plurality and years of childlessness were made, the OR decreased below 1.0 and was no longer significant. (Källén et al., 2005a).

No increase in childhood cancer has been found (Bergh et al., 1999; Källén et al., 2005b).

Outcome in multiple births

The increased perinatal risks for IVF twins as compared with IVF singletons are illustrated in Figure 5. Maternal risks associated with multiple gestations include placental abruption, pre-eclampsia and eclampsia, antenatal venous thromboembolism and postpartum haemorrhage (Campbell and Templeton, 2003). In early comparisons of IVF twins with spontaneously conceived twins, IVF twins were found to have the same or better outcome in terms of preterm births and perinatal mortality (Schieve et al., 2002; Helmerhorst et al., 2004). This was, however, probably due to the fact that the vast majority of IVF twins, unlike the spontaneously conceived twins, are dizygotic and have lower risks as a group than monozygotic twins. In later studies, IVF twins have instead been compared with spontaneously conceived twins of unlike sex to avoid using monozygotic twins as controls. In those studies, IVF twins were found to have increased risks for preterm birth, low birth weight and perinatal mortality as compared with controls (Pinborg et al., 2004; Hansen et al., 2009).

Outcome in singleton births

For IVF singletons too, an approximately two-fold higher risk has been shown for perinatal mortality, preterm birth and low birth weight as compared with spontaneously conceived singletons (Bergh et al.,

1999; Helmerhorst et al., 2004; Jackson et al., 2004; Schieve et al., 2002; McGovern et al., 2004). The risk of cerebral palsy was found to be almost three times higher (OR 2.8, 1.3-5.8) in IVF singletons as compared with matched spontaneously conceived controls, mostly owing to the increased incidence of preterm birth and low birth weight among IVF singletons (Strömberg et al., 2002).

The increased risk of adverse events in IVF singleton births can partly be explained in terms of maternal characteristics such as maternal age and parity, as well as the subfertility itself (Källén et al., 2005ab). Why the number of years of involuntary childlessness is a risk factor for adverse events is not fully understood, but after adjusting for years of subfertility the risk decreases. Women on waiting list for IVF who have become spontaneously pregnant have been found to have a similar increased risk of preterm birth as women who become pregnant following IVF treatment (Basso and Baird, 2003). Women seeking infertility treatment are generally older and more often primiparous than spontaneously conceiving women. In a Swedish study the IVF patients were also less often smokers, had higher levels of education and higher BMI (Källén et al., 2005d). In a study of women who had conceived both spontaneously and after assisted fertilization, it was shown that birth weight, gestational age, small for gestational age, and preterm delivery did not differ significantly between infants born to the same woman (Romundstad et al., 2008).

One study with a limited number of patients reported higher risks for preterm birth and low birth weight among IVF fresh singletons born after fresh DET than after SET (De Sutter et al., 2006). Today there is no clear explanation for the increased risk

among IVF singletons for adverse obstetric events, but hormonal stimulation and embryo culture have both been discussed as potential risk factors. An interesting finding is that children born after cryopreservation and without hormonal stimulation were shown to have the same or lower incidence of preterm birth and low birth weight as compared with children born after fresh cycles in a recent systematic review (Wennerholm et al., 2009).

SET

In the last ten years, SET has been introduced with the aim of avoiding multiple pregnancies. The much lower multiple birth rates after SET than DET can be seen in Table 3. The SET rates in different countries are shown in Figure 6. Sweden has been one of the leading countries when it comes to reducing the number of embryos transferred per cycle. Historically in Sweden, triple embryo transfer was used in a majority of cycles until 1993, after which DET was most common. Since 2003 when recommendations from The Swedish National Board of Health and Welfare stated that SET

should be the primary choice, a majority of the fresh cycles have been SETs. In spite of this, the birth rate has remained on about the same level since 1993, about 26% per transfer, while the multiple birth rate has gone down from 35% in 1991 to 5% in 2004 (Karlström and Bergh 2007).

Several randomized controlled trials (RCT) comparing SET with DET have been performed, showing satisfactory delivery rates after SET in good prognosis patients. The delivery rates are, however, significantly higher after fresh DET than SET (Table 3). Importantly, the multiple birth rates are dramatically reduced with SET. The delivery rates after SET might be restored to similar levels as in DET when adding a frozen-thawed cycle to the fresh SET (Thurin et al., 2004). Observational studies have shown similar pregnancy and live birth rates after elective SET as after DET, particularly in women under 35, with more than one good quality embryo, and when adding subsequent frozen-thawed cycles (Vilksa et al., 1999, Lundin et al., 2007, Veleva et al., 2009).

In a study for selection of patients

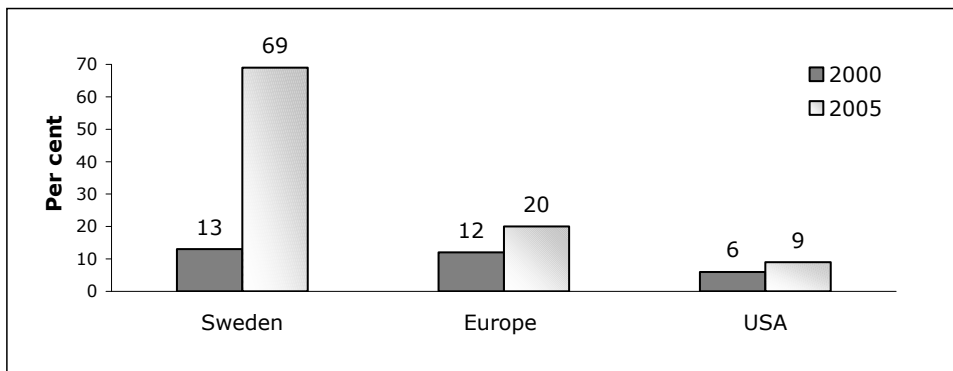


Figure 6. SET rates in fresh embryo transfers in 2000 and 2005. (The Swedish National Board of Health and Welfare, Nyboe Andersen et al., 2004 and 2009, Wright et al., 2003 and 2008).

Table 3. Randomized SET-DET studies.

	No. of patients	Age (years)	<u>SET</u>		<u>DET</u>	
			Live birth rate	Multiple birth rate	Live birth rate	Multiple birth rate
Gerris et al., 1999	53	<34	38.5 ^{ab}	10.0 ^a	74.1 ^{ab}	30.0 ^a
Martikainen et al., 2001	144	21-40	29.7	4.2 ^b	40.0	39.3 ^b
Thurin et al., 2004						
Fresh+frozen SET versus fresh DET:	661	<36	38.8	0.8 ^b	42.9	33.1 ^b
Fresh SET versus fresh DET:	661	<36	27.6 ^b	1.1 ^b	42.9 ^b	33.1 ^b
Lukassen et al., 2005	107	<35	25.9 ^c	0.0 ^b	35.8	36.8 ^b
Van Montfoort et al., 2006	308	No age limit	21.4 ^{ab}	0.0 ^{ab}	40.3 ^{ab}	21.0 ^{ab}

Rates are in percentages.

^a (Multiple) ongoing pregnancy rate.

^bP<0.05.

^c The first SET of two in this study.

suitable for SET performed at our centre, it was shown that the woman's age and the number of good quality embryos transferred were independent predictive factors for multiple births. The authors also developed a prediction model for selecting patients for SET, based on age, cycle number and presence of tubal infertility (Strandell et al., 2000). Another prediction model for selection of patients suitable for SET in mild stimulation found the most important factors to be body mass index, the total gonadotropin dose needed, number of oocytes retrieved, and the availability of at least one top-quality embryo (Verberg et al., 2008b). An embryo scoring system for prediction of implantation potential of day 2 embryos was developed by another research group. The number of blastomeres, mononuclearity in the blastomeres, and the blastomere size variation was found to be

independent predictive factors for implantation (Holte et al., 2007).

The delivery rate after transfer of a single blastocyst has been shown to be higher than after transfer of a single cleavage stage embryo, 32.0% versus 21.6% in an RCT including women of less than 36 years of age (Papanicolau et al., 2006). In an observational study, the delivery rate was 36.7% for single blastocyst transfer and 25.1% for single cleavage stage embryo transfer (Guerif et al., 2009). However, owing to smaller number of embryos available for freezing and lower survival rates after thawing in the blastocyst group, the cumulative delivery rates were similar in the two groups, 37.9% and 34.2%, respectively (Guerif et al., 2009).

The SET rate has also increased in frozen-thawed cycles in Sweden, from 22% in 2000 to 65% in 2004. Despite this, the

delivery rate per frozen-thawed embryo transfer in total rose from 16% in 2000 to 21% in 2004, and per frozen-thawed SET from 9% in 2000 to 17% in 2004 (The Swedish National Board of Health and Welfare).

In a systematic review of articles about economic considerations, DET was shown to be more expensive but also to result in significantly higher live birth rates than SET. SET was more cost-effective than DET only when performed in good prognosis patients and when frozen-thawed cycles were included (Fiddlers et al., 2007). In a study on cost-effectiveness including the same randomized study population as in Paper IV, SET was found to be more cost-effective than DET when the number of deliveries with at least one live-born child, incremental cost-effectiveness ratio and maternal and paediatric complications were taken into consideration (Thurin-Kjellberg et al., 2006).

Despite the availability of SET for more than ten years, implementation has been limited outside the Northern countries, the Netherlands and Belgium (Table 2, Nyboe Andersen et al., 2009). The reason for this is mainly the lower success rates in SET as compared with DET. In the USA, IVF treatments are usually given at private clinics, which are highly competitive, leading to stronger emphasis on high live birth rates in the USA than in the publically funded clinics in Europe. An investigation among professionals in The Netherlands to determine why elective SET is not implemented more often showed that poor live birth rates in frozen-thawed cycles, not seeing twin pregnancies as a complication, and lack of a SET protocol were the main reasons, and that professionals with university hospital background were more

willing to perform elective SET than others (Van Peperstraten et al., 2008). Attitudes towards multiple birth and SET were recently investigated among Nordic IVF doctors. It was shown that almost all doctors thought that a singleton pregnancy was more favourable than a twin pregnancy, and a twin rate above 10% was acceptable for 5% of Swedish doctors, 21% of Finnish doctors, and 35% of Danish and Norwegian doctors (Bergh et al., 2007). For women under 36, performing their first cycle and with two good quality embryos, almost all doctors would recommend SET, while for women over 36 in a similar situation, only Swedish and Finnish doctors would recommend SET (Bergh et al., 2007).

In a study where IVF patients were asked about reasons for choosing SET, it was shown that positive predictive factors were if the patient had confidence in the possibility of a pregnancy with SET, was of a younger age and was undergoing her first treatment, while sense of time urgency was a negative predictive factor (De Lacey et al., 2007). The doctor's attitude toward SET was also an important predictive factor (De Lacey et al., 2007).

Patients dropping out of IVF treatment

In Sweden and some other countries, treatment programmes consisting of several IVF treatment cycles or up to one live birth are offered, fully subsidised, to infertile couples. In countries without reimbursement the patients are free to perform any number of treatments but have to pay for them out of their own pockets. In spite of reimbursement, a large number of patients drop out of treatment before having achieved a live birth or gone through the full treatment program. The dropout rates reported in studies varies;

64% of the patients with no live birth after the first cycle in a British study (Sharma et al., 2002), 39.9% after the first cycle and 62.2% after the fourth cycle in a German study (Schroder et al., 2004) and 62.4% after three cycles in a Dutch study (Land et al., 1997). In countries with no reimbursement of IVF treatments, inability to pay for further treatments is a common reason for discontinuing treatments (Rajkhowa et al., 2006).

The most important reason for dropping out of treatment has been found in several studies to be psychological distress (Rajkhowa et al., 2006, Verberg et al., 2008a). “Active censoring”, i.e. discouraging the patient from further treatment due to poor prognosis, is a reason for dropping out

for some patients. Women who drop out of treatment in advance have been shown to have a poorer prognosis than those who continue (Land et al., 1997, Sharma et al., 2002, Malizia et al., 2009). Other reasons for patient dropout are divorce, moving, adoption or ethical objections to ICSI treatment after failed IVF (Verberg et al., 2008a).

High dropout rates have a negative impact on the cumulative live birth rates. When calculating cumulative rates with life table analysis the reasons for dropping out are important, since if a large proportion of the dropouts are attributable to poor prognosis, this leads to overestimation of the live birth rates (Stolwijk et al., 1996).

Aims of the study

General aims

The general aims of the study were to investigate cumulative live birth rates after IVF and factors affecting the live birth rates.

Specific aims

The specific aims of the study were to investigate:

- a. Cumulative live birth rates in a treatment programme consisting of three fresh IVF cycles and subsequent frozen-thawed cycles.
- b. The reasons why couples chose to discontinue the treatment programme in advance, before having achieved a live birth.
- c. Predictive factors for achieving a live birth in frozen-thawed SET.
- d. Cumulative live birth rates after fresh SET and DET respectively, including subsequent frozen-thawed cycles. Follow up from a Scandinavian randomized controlled trial (Thurin et al., 2004).

Methodological considerations

Settings and study designs

All four studies were performed at the University of Gothenburg, Sweden with patients from the Centre for Reproductive Medicine, Sahlgrenska University Hospital. Paper IV was a multicentre study including patients from eleven clinics in Sweden, Denmark and Norway, both public and private, and was based on an earlier trial from this unit (Thurin et al., 2004). The fact

that three IVF treatments are subsidised in this region of Sweden, makes an appropriate setting for studies on cumulative live birth rates (Paper I). The subsidy of IVF treatment is an important factor when studying reasons for dropping out of IVF treatment (Paper II), since financial constraints seldom is the main cause for that in countries with reimbursed IVF treatment. SET cycles have constituted a large

Table 4. Study settings and patients

	<i>Paper I</i>	<i>Paper II</i>	<i>Paper III</i>	<i>Paper IV</i>
Setting	Sahlgrenska University Hospital		Scandinavian multi-centre	
Study design	Retrospective observational	Prospective observational	Retrospective observational	Retrospective observational
Study period	1996-1997		2003-2006	2000-2008
No. of women	974		371	661
No. of fresh and frozen-thawed cycles	1914		622	1261
Age (years) [§]	32.5 (21-40)		32.2 (22-40)	30.9 (21-35)
Infertility diagnoses, (%)*				
-Male factor	33.5		46.4	51.9
-Tubal pathology	22.9		9.7	19.7
-Other female factors	13.6		21.8	18.2
-Multifactorial	10.7		-	-
-Unexplained infertility	19.3		22.1	19.1

[§]Age at beginning of first treatment cycle.

*Other female infertility factors include hormonal factors, endometriosis, and polycystic ovary syndrome. In Paper IV, some patients had several diagnoses.

proportion of the IVF treatments in Sweden since 2003, leading to good prerequisites for performing SET studies (Papers III, IV).

Papers I, III and IV were retrospective observational studies. Cumulative live birth rates were calculated in both Papers I and IV, but in Paper IV as opposed to Paper I, the patients were randomized to two groups, having undergone either SET or DET in a previous RCT. Paper II was a prospective observational study, since most of the data were not available but had to be collected through questionnaires.

Patients

Paper I: To assess the cumulative live birth rates in paper I, around one thousand patients were considered a sufficiently large number. The collection of data took place during late 2000 and 2001. All consecutive patients who had begun their first IVF treatment cycle between January 1996 and December 1997 were included, a total of 974 patients. The relatively short inclusion time period of two years and the fact that all treatments were performed at the same clinic implied that there were no major variations in the treatment methods. There were no major changes in methods or staff during that time period. Some other cumulative studies have included larger number of patients, for example 6,164 patients with treatments over six years (Malizia et al., 2009) and 2,130 patients with treatments over seven years (Schroder et al., 2004). Such long inclusion periods might be disadvantageous since the IVF technology and treatment results have developed and changed rapidly over time.

Paper II: Based on the same patient cohort as in Paper I and including the 450 out of 974 patients who did not achieve a live birth. The reasons were investigated as

to why 288 out of the 450 patients who did not achieve a live birth discontinued their IVF treatment before having completed their three subsidized IVF cycles. Inclusion of consecutive patients as used in Paper II was also the inclusion method in other studies on reasons for dropping out (Rajkhowa et al., 2006, Smeenk et al., 2004). The data were collected during 2002 and 2003.

Paper III: The aim in paper III was to study predictive factors for live birth in frozen-thawed SET cycles. The data were collected in 2007. All consecutive patients performing fresh IVF/ICSI cycles in 2003 and 2004 with at least one additional frozen-thawed SET were included. SET had, during this time period, become increasingly common in both fresh and frozen-thawed cycles. The choice to study only SET cycles was also due to the fact that it is more useful to study embryonic variables in SET cycles since in case of a pregnancy it is clear which embryo implanted. A majority of previously published studies on predictive factors for live birth in frozen-thawed cycles have included cycles with two or more embryos transferred (Salumets et al., 2006, Edgar et al., 2007). The inclusion time period was relatively short and the patients were all treated at one centre, which ensured similar treatment methods. Both variables associated with the fresh cycle, such as ovarian stimulation, number of egg retrieved and fertilization method, as well as variables associated with thawing, were included. Of the 922 women treated at our centre during 2003 and 2004, 371 (40.2%) could be included in the study since their fresh cycles resulted in one or several frozen-thawed SETs. The 371 women performed 410 out of a total of 1,276 fresh cycles (32.1%) during that time period, resulting in 622 frozen-thawed SETs. The frozen-thawed SETs

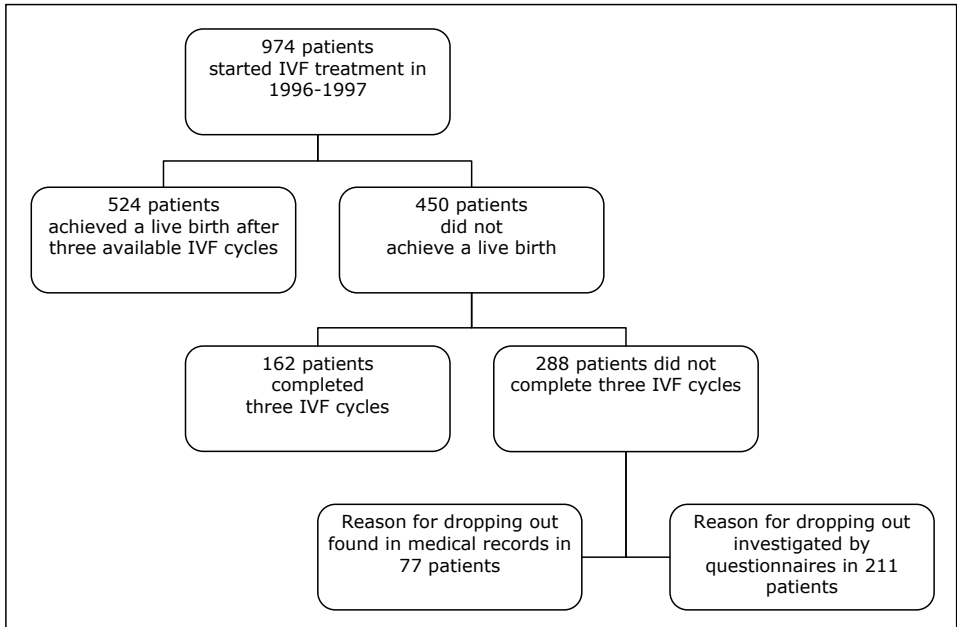


Figure 7. Flow chart of the women participating in Papers I and II.

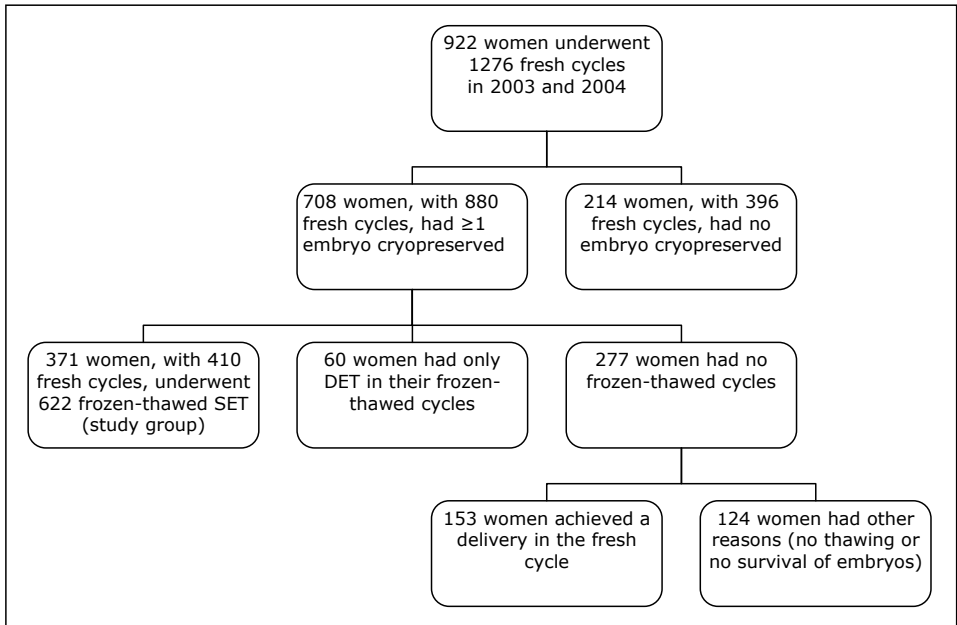


Figure 8. Flow chart of the women participating in Paper III.

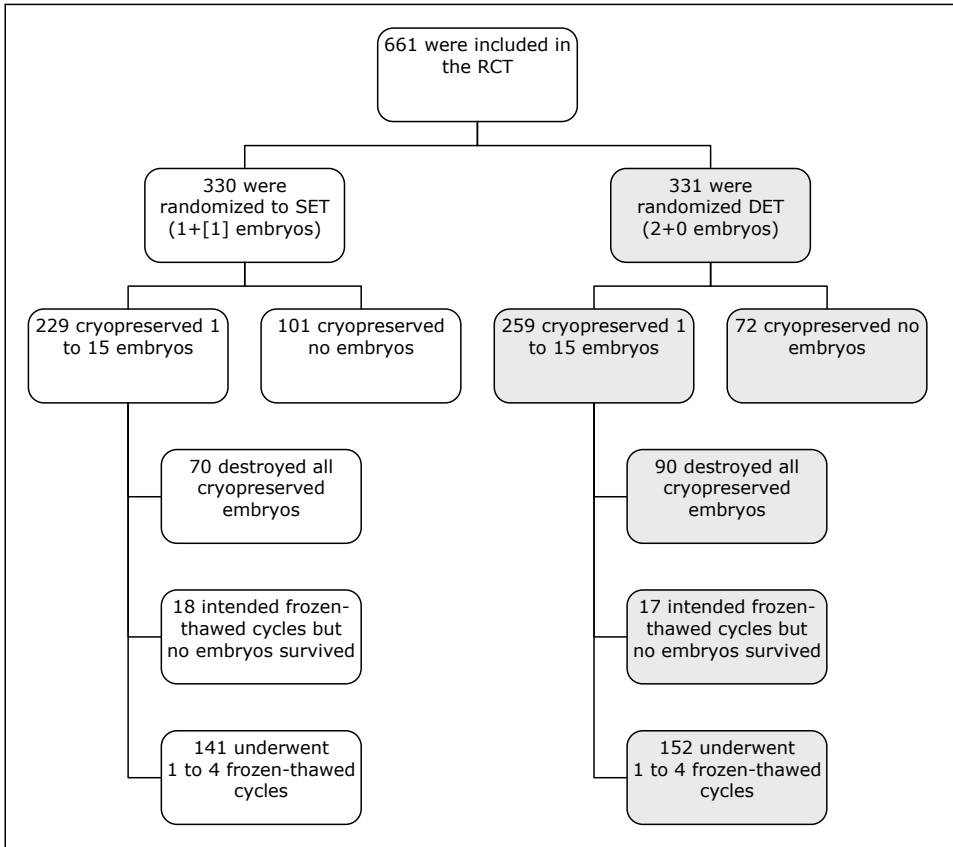


Figure 9. Flow chart of the women participating in Paper IV.

were performed from March 2003 to October 2006, and during that time 1,333/1,797 (74%) of all frozen-thawed cycles were SETs.

Paper IV: A follow up of an RCT in which 661 women were randomized, as being below 36 years of age at the time of the transfer of the fresh embryo, undergoing their first or second IVF cycle, and with at least two embryos of good quality available for transfer or freezing. The recruitment took place from May 2000 to October 2003 at eleven clinics in Sweden, Norway and Denmark. In the RCT, 330 were

randomized to one fresh SET, and if this did not result in a live birth, one frozen-thawed SET was performed. Three hundred and thirty-one women were randomized to one fresh DET. Demographics of the two groups did not differ in predicting variables. Surplus embryos of good quality (see definition below) were cryopreserved. After completion of the RCT, the patients could use their cryopreserved embryos in additional frozen-thawed cycles, in which one, two and sometimes three embryos were transferred according to the patient's wish. The results from the additional frozen-

thawed cycles were added to the results from the RCT, to investigate cumulative live birth rates.

The patients included in all four Papers were below 40 years of age when treatment started. The reason for this is the reimbursement rules; when these studies took place, women had to be below 38 years of age when referred to be offered reimbursement IVF treatment at our clinic. A non-negligible proportion of the IVF patients treated with IVF in society are, however, older than our study population. In 2005, 11.0% of all fresh embryo transfers were performed on IVF women over 40 years in Sweden (The Swedish National Board of Health and Welfare).

Ethical considerations

Paper I was a retrospective register study, and with the legislation at that time there was no need for ethics committee approval. Papers II, III and IV were approved by the local ethics committee.

Sample size calculation

In Papers I and II no power calculation was performed since these were descriptive studies with no comparisons of interventions or different groups of patients. In paper III a post-hoc power calculation was performed, showing that with the number of patients included in the study and with a live birth rate of 17% in the first cycle it was possible to identify a difference of at least 9% in live birth rate between the first and second frozen-thawed SET from the same egg retrieval procedure (80% power, $\alpha = 0.05$, two-tailed test). A power calculation was made in the original RCT of which Paper IV was a follow up, assuming that with a true live birth rate in the two groups of 0.30, the upper limit of the 95% CI of

the observed difference in live birth rates between the groups would not exceed 0.10 (Thurin et al., 2004, 80% power, $\alpha = 0.05$, two-tailed test).

IVF procedures

Down-regulation of the gonadal axis was performed using a GnRH agonist in a long protocol. Ovarian stimulation was performed using recombinant FSH or urine-derived human menopausal gonadotropin. Stimulation was monitored by vaginal ultrasound and serum estradiol measurements. Oocytes were retrieved 36-38 hours after hCG injection using ultrasonographically guided puncture. Fertilization was achieved by standard IVF or by ICSI using standard protocols. Embryo transfer or freezing of good quality embryos (GQEs, see below, terms and definitions) were performed on day two or three (in some patients in Paper IV on day five). Luteal support was given with vaginal or intramuscular progesterone until the day of a negative pregnancy test or two weeks after a positive pregnancy test.

GQEs not used for fresh transfer were cryopreserved, using a propanediol-based slow freezing protocol. Usually embryos were frozen and thawed one by one, particularly when the fresh transfer was a SET. After thawing, embryos were transferred on the same day. Usually only embryos with >50% survival of cells were transferred. Embryo transfers were performed mostly in natural cycles that were monitored by vaginal ultrasound and urinary LH, with the embryos being transferred on day three after the natural LH surge. For anovulatory patients, hormone replacement therapy with oral estrogen and vaginal progesterone was given, and if a pregnancy ensued, the treatment continued until week 7.

Data collection

In Papers I and III, data on patients and IVF treatments were collected from local IVF databases and medical records. After coding, data were established in a research database. In Paper IV, data on the cryopreserved embryos and additional frozen-thawed cycles were collected from all participating clinics. At some clinics a nurse, embryologist or the doctor responsible for the study filled out the data on a special data sheet and sent it to the study group, while we ourselves visited the other clinics to collect data.

Questionnaires

In Paper II all patients' medical records were primarily screened. In most cases it was not obvious why the patient discontinued treatment in advance. To the 211 patients whose reason for dropping out was not evident from their medical records, a questionnaire was sent out (Figure 10). If there was no response, the questionnaires were sent to the patients a total of three times. The questionnaire was drawn up by our own research group, since there was no pre-existing validated questionnaire for this question.

Some other studies have also, in addition to using their own questionnaires to get answers to the specific question of reason for dropping out, assessed the patients using standardized psychological questionnaires such as State and Trait Anxiety Inventory, Becks Depression Inventory, Maudsley Marital Questionnaire, Hospital Anxiety and Depression scale (Smeenk et al., 2004, Verberg et al., 2008a). Questionnaires used in other studies on IVF patients' psychological well-being and coping behaviour include the validated Psychological General Well-Being index

(Anderheim et al., 2005, Holter et al., 2006), Ways of Coping Questionnaire (Peterson et al., 2006), SF-36 (Thurin-Kjellberg et al., 2006), Fertility Problem Inventory (Boivin et al 2005, Peterson et al 2006), and Daily Record Keeping Chart (De Klerk et al., 2008). The reason for not using any of these standardized psychological questionnaires in our study was that our aim was to investigate the reasons for dropping out and not primarily the patients' psychological well-being.

The questionnaire used in Paper II was designed with tick-box alternatives (Figure 10). The advantage of statements with tick-box alternatives is that they are easily understood, quickly to complete, and measure general attitudes (Boynton, 2004a). Using pre-formulated tick-box alternatives is referred to as a closed-ended design, and makes it easy to produce data quickly, although it can give rise to frustration in the responders since their opportunity to express opinions and emotions is limited. For that reason, an open-ended design was also used. Free text boxes were inserted under each category of reasons, and the patients were also given free text rows for expressing general points of view and making suggestions as to how to improve treatment. The data analysis of the open-ended questions and free text alternatives is, however, somewhat more complicated as compared with the closed-ended questions (Boynton, 2004ab). In Paper II we chose to present the patients' quotations after having divided them into different categories such as emotional and stressful reactions attributable to the infertility situation, organizational problems, the staff having a poor ability to handle patients in psychological distress, lack of autonomy during treatment and satisfaction with the



Questionnaire to couples who discontinued IVF treatment

Our reason for discontinuing IVF treatment was (put an X in the relevant box. You may mark one or several boxes):

Financial reasons

- We were not offered more treatments by our county council
- Private payment, could not afford more treatments
- Other reason, specify

Medical reasons

- Our IVF doctor discouraged us from further treatment owing to a poor prognosis
- Other medical reasons, such as severe illness
- The treatment was too physically demanding
- The treatment was too psychologically demanding
- Other reason, specify

Social reasons

- Separation and relationship problems
- Moved from the region
- Other reason, specify

View of the treatment

- Good
- Less than good
- Poor

What can we do to improve the treatment?

Other opinions?

Name

Personal identity number

Figure 10. Questionnaire in Paper II.

care. The free-text comments also contributed in some cases to the decision as to which reason for dropping out was most important, since some patients had ticked several boxes and only one main reason per patient was registered. One common reason for low response rates is that the responder cannot follow or understand the questionnaire, so the questions should generally be short and to the point, twelve words or less (Boynton, 2004a), with which the questionnaire in Paper II is well in accord. The questionnaires were only sent out in Swedish language, since we assumed that patients with poor understanding of Swedish could get help with reading it from relatives or friends. This could be a possible weakness in our methodology. A considerable proportion of the patients today do not have Swedish mother tongue, and many need a professional interpreter at appointments. The woman's name in each couple was written on the envelope, but the questionnaire itself was addressed to the couple. The fact that only the woman's name was written on the envelope may have been perceived as an exclusion of the man in the couple, and could be another weakness. The couples were allowed to tick several boxes in the questionnaire, giving more than one reason for discontinuing treatment. However, when we summarized the results only one reason per couple was registered. In the cases where more than one reason was given, the absolute reasons such as having moved from the area, not being offered more treatments from the county council, discouragement from further treatment by the doctor, or divorce, were chosen over more relative reasons such as psychological or physical strain. In unclear cases the members of the research group reached a common decision. A better design

of the questionnaire would have been to explicitly ask for the main reason, and invite the couples to make further comments on other contributing reasons.

Terms and definitions

Poor prognosis: In Papers I and II, the medical records of patients who dropped out of treatment were screened. The couples were interpreted as having discontinued treatment because of a poor prognosis if it was found that they had very poor embryo quality, poor ovarian response on stimulation or recurrent pregnancy loss.

"Troublesome treatment": In Paper I, patients were labelled as having discontinued treatment because of "troublesome treatment" if there were comments in the medical records concerning exceptional psychological or physical strain or if the couple had explicitly informed their doctor they wanted to quit treatment because it was too troublesome.

Started and completed cycles: In Paper I, the live birth and pregnancy rates were reported per started and per completed cycle. Started cycle describes all cycles where ovarian stimulation was initiated with gonadotropins, and completed cycles describes all cycles that reached embryo transfer. Live births achieved in subsequent frozen-thawed cycles were included in the fresh cycle from which the cryopreserved embryos had originated. Most other studies on cumulative live birth rates have reported outcome only per started cycle (Alsaili et al., 1995, Engmann et al., 1999, Witsenburg et al., 2005, Malizia et al., 2009). The previous report on cumulative live birth rates from our centre, however, also presented results both per started and per completed cycle (Bergh et al., 1995). The reason for presenting rates per completed

cycle, and including results from frozen-thawed cycles in each fresh cycle, was that that was in accord with our reimbursement system.

GQE: In Papers III and IV, GQEs were defined as being of grade I or II, having 4-6 cells on day two or 6-10 cells on day three, with less than 20% fragmentation and no multinucleation. In Paper III embryo grade was one of the variables analyzed: Grade I embryos were GQEs with no fragmentation and even-sized cells, Grade II embryos were all other GQEs.

Statistics

For descriptive data, sum, percentage, mean, median, standard deviation (SD) and range were used. Comparisons of categorical variables between groups were done using Fisher's exact test and the Chi-square test. For continuous variables, Student's t-test was used for parametric data and Mann-Whitney U-test for non-parametric data. A p-value of less than 0.05 was considered significant and 95% confidence intervals (CI) were used. All significance tests were two-sided. The statistical analyses were done in collaboration with professional statisticians, and SPSS (version 13.0 and 16.0) and SAS (version 8.2) were used as statistical computer software.

Analyzed variables

Paper I: Cumulative pregnancy and cumulative live birth rates after three completed and started IVF cycles, respectively. Pregnancy and live birth rates per started and per completed cycle. The variables were analyzed both in the total patient group and according to the woman's age and the couple's reason for infertility.

Paper III: Univariate analyses of pregnancy and live birth were performed for:

woman's age, tubal factor, fertilization method, number of fresh and cryopreservation cycles in patient's history, number of failed fresh and failed cryopreservation cycles in patient's history, total and failed number of cryopreservation cycles from the same fresh cycle, pregnancy or live birth in the fresh cycle, pregnancy or live birth in any previous cryopreservation cycle from the same fresh cycle, surgically retrieved sperm, blastomere survival rate, number of cells in the thawed embryo, embryo quality grade, number of embryos needed to be thawed in order to obtain one embryo transfer, hormone stimulated cryopreservation cycle, number of oocytes aspirated in the fresh cycle, number of GQEs in the fresh cycle, FSH/hMG-dose per aspirated oocyte. Variables associated to pregnancy or live birth with $p < 0.10$ were included in the multivariate analysis.

Paper IV: The SET and DET groups were analyzed according to the intention-to-treat principle. Cumulative live birth rates and multiple birth rates were analyzed as main variables. Mean number of live births, mean number of live-born children, mean number of additional cryopreserved embryos, mean number of additional frozen-thawed cycles, number of embryos transferred per additional frozen-thawed cycle, mean number of pregnancies, mean number of miscarriages, intrauterine foetal death rates, ectopic pregnancy rates, mean gestational age and preterm birth rates were analyzed as secondary outcomes.

Life table analysis

When calculating cumulative success rates, life table analysis is the most frequently used statistical method (Stolwijk et al., 2000, Engmann et al., 1999, Malizia et al., 2009). The life table analysis, also called the

Kaplan-Meier survival curve, is otherwise typically used in clinical studies to estimate the probability of survival during a given length of time, for example after cancer treatment. The total length of time is divided into many smaller intervals, for example days or weeks. The probability of one-year survival can be expressed as a multiplication of the probability of surviving each week, each week having a different p-value, since it is on the condition that the object survived the previous weeks:

$$p_1 \times p_2 \times p_3 \times \dots \times p_n = p_{\text{total}}$$

In cumulative IVF studies, the time unit is translated to treatment cycles, and the probability of achieving a live birth after a certain number of treatment cycles is assessed. The model used in Paper I is called the Kaplan-Meier product-limit estimate, where the cumulative probability of achieving a live birth after x number of cycles, in which p_x is the probability of achieving live birth in cycle x , is:

$$[1 - (1 - p_1)(1 - p_2)\dots(1 - p_x)] \times 100\%$$

Patients who discontinued the treatment programme, despite not having achieved a live birth, are referred to in the life table analysis as *censored*, and are given the same probability of the endpoint, in this case pregnancy or live birth, as those who continued. This is generally believed to cause an overestimation of the cumulative live birth rates, since the patients who discontinued the treatment program include women who were actively discouraged from further treatment owing to a poor prognosis. Some studies have shown that women who discontinue treatment have a poorer prognosis as a group than those who

continue (Sharma et al., 2002, Malizia et al., 2009). Other studies, however, found no prognostic differences between women who dropped out of treatment and those who continued (Roest et al., 1998, De Vries et al., 1999).

To solve the problem of suspected overestimation, a modified life table analysis was introduced in 1996 yielding three estimates: one “*pessimistic*” where the dropout group was given no chance of pregnancy (observed, real rates); one “*optimistic*” where the dropouts were given the same chance of live birth as those who continued treatment (standard life table analysis); and one “*realistic*” where women who discontinued treatment because of a medical condition were given no chance of live birth while the others were given the same chance as those who continued (Stolwijk et al., 1996). The realistic estimate is believed to be the most accurate (Stolwijk et al., 1996). In recent cumulative studies, the authors have chosen to present only the pessimistic and the optimistic estimates (Schroder et al., 2004, Malizia et al., 2009). One reason for this could be that the realistic estimate requires extra effort, since the reasons for dropping out have to be established. In Paper I, we used life table analysis with these three different ways of handling dropouts producing one “*pessimistic*”, one “*realistic*”, and one “*optimistic*” estimate.

Log-rank test

The most frequently used method for comparing survival curves of independent groups is the log-rank test, which tests the null hypothesis that the groups are samples from the same population. In paper I, the log-rank test was used to test differences between the age groups and infertility

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

	Paper I	Paper II	Paper III	Paper IV
Significance level	0.05, two-sided tests			
Descriptive statistics	Mean, SD, range	Sum, percent	Mean, SD, Median, Mean, SD, range range	
Analytical statistics	Fisher's exact test Chi-square test Life table analysis Log rank test	Fisher's exact test Student's t-test	Fisher's exact test Mann-Whitney U-test Univariate analysis Multiple regression analysis	Fisher's exact test Chi-square test Mann-Whitney U-test

diagnosis groups.

Multiple regression analysis

In Paper III we used multiple regression to analyze how the different maternal and embryonic variables influenced live birth rate and pregnancy rate. Multiple regression is used to investigate the way one dependent variable is influenced by several other variables, referred to as independent variables. Multiple linear regression is the method used when the dependent variable is continuous, while if the dependent variable is categorical, the method is referred to as multiple logistic regression, or just logistic regression. One pitfall with multiple regression analysis is if too many variables are used on too small a sample, because this may lead to an overestimation of each variable's importance. There is a general recommendation not to look at more than $n/10$ variables, where n is the sample size. In Paper III we investigated 24 variables in 371 patients, which was correct according to the recommendation above. However, the large number of variables studied in Paper III increased the risk of random findings. The analysis in Paper III was initiated with a

univariate analysis, investigating the correlation between each independent variable and the dependent variable. All independent variables that correlated with the dependent variable with a p-value of less than 0.10 were then included in the multiple regression analysis.

To estimate the predictive value of the regression model, the c-value (which is the same as area under curve) of the prediction model in Paper III was calculated, and was found to be 0.60 (1.0=very good, 0.5=poor). The relatively low c-value means that the predictive capacity for the end point (live birth or pregnancy), of the variables that were found to be significant in the multiple regression analysis, was not very high.

In Paper III, the generalized estimation equation (GEE) method was used to adjust for the dependence within each woman, since more than one cryopreservation cycle per woman was included. The GEE method is an extension of the generalized linear model, developed to better suit the analysis of longitudinal or clustered data and to avoid random findings when unknown correlations are present (Ghisletta et al., 2004).

Results and comments

PAPER I: Cumulative live birth rates after three IVF/ICSI cycles

All cycles performed by the patients during the study time period are presented in Table 6. To complete three IVF cycles, the patients in the study underwent up to six started cycles, since in some cases several cycles were cancelled before embryo transfer. In spite of the fact that no more than three completed cycles were offered,

sixteen patients in the study population underwent four completed cycles. The reasons for this were, in some cases, repeated extra-uterine pregnancies or spontaneous miscarriages. The fourth completed cycles in the sixteen cases were not included in the analyses. All results presented below are based strictly on the patients' first three completed cycles and their corresponding started cycles.

The 974 women started 1,985 IVF cycles

Table 6. Cumulative live birth rates per started and completed cycle. The extra cycles are in parenthesis and are not included in the cumulative analyses.

	Cycle no.	Fresh cycles	Frozen-thawed cycles	Live births*	Cumulative live birth rate, % (95% CI)		
					Pessimistic estimate	Realistic estimate	Optimistic estimate
Started cycles	1	974	203	296 (30.4%)	30.4 (27.5-33.3)	30.4 (27.5-33.3)	30.4 (27.5-33.4)
	2	588	96	140 (23.8%)	44.8 (41.6-47.9)	46.7 (43.5-50.0)	47.0 (43.7-50.2)
	3	314	23	60 (19.1%)	50.9 (47.8-54.1)	56.3 (52.9-59.8)	57.1 (53.6-60.6)
	4	103	5	29 (28.2%)	54.7 (51.5-57.9)	65.2 (61.2-69.2)	69.2 (64.7-73.7)
	(5)	(19)	(1)	(2) (10.5%)	-	-	-
	(6)	(3)	(-)	(1) (33.3%)	-	-	-
Completed cycles	1	944	175	331 (35.1%)	35.1 (32.0-38.1)	35.1 (32.0-38.1)	35.1 (32.0-38.1)
	2	491	73	135 (27.5%)	49.4 (46.2-52.6)	52.1 (48.8-55.5)	52.9 (49.5-56.3)
	3	217	12	58 (26.7%)	55.5 (52.4-58.7)	63.1 (59.6-66.7)	65.5 (61.8-69.2)
	(4)	(16)	(2)	(4) (25.0%)	-	-	-

*Live birth rates per fresh cycle, births from frozen-thawed cycles included.

of which 1,652 cycles from 944 patients achieved fresh embryo transfer (“completed cycles”). In 28 cycles all embryos were cryopreserved owing to impending ovarian hyperstimulation syndrome (OHSS) and used later in frozen-thawed embryo transfers, these were also counted as completed cycles.

A total of 260 frozen-thawed embryo transfers were performed with embryos originating from the fresh cycles. The live births from the frozen-thawed cycles were included into the results of the started/completed cycle from which the embryos originated. The majority of fresh and frozen-thawed embryo transfers were with two embryos. Standard IVF was used as the fertilization technique in the first IVF cycle in 47%, ICSI in 51%, and both techniques were used in 2% (split cycles).

The 1,912 fresh and frozen-thawed embryo transfers resulted in 524 live births. One singleton was stillborn, and in four twin pregnancies one twin was stillborn. There were a total of 678 pregnancies of which 142 miscarried spontaneously, two were legally aborted owing to foetal polycystic

kidney disease and trisomy 3, and nine were extrauterine pregnancies. The twin birth rate was 23% of all live births, no multiple birth of higher order occurred.

Cumulative live birth rates

The cumulative live birth rate for all patients after three completed cycles was 63.1% in the “realistic” estimate. The “pessimistic” outcome was 55.5% and the “optimistic” outcome was 65.5% (Figure 11). The corresponding rates per three and four started cycles can be seen in Table 6. The live birth rate per completed cycle was 35.1% in cycle 1, 27.5% in cycle 2, and 26.7% in cycle 3 ($p=0.004$ cycle 1 versus cycle 2, $p=0.02$ cycle 1 versus cycle 3). The 260 frozen-thawed cycles resulted in 52 live births, representing 10% of all live births in the study. The live birth rate per frozen-thawed embryo transfer was thus 20.0%. In 2001, when the last data was collected, 72 patients still had embryos cryopreserved from the fresh study cycle. A follow up in 2009 showed that no more live births from frozen-thawed cycles had been achieved. Six of the patients had undergone additional

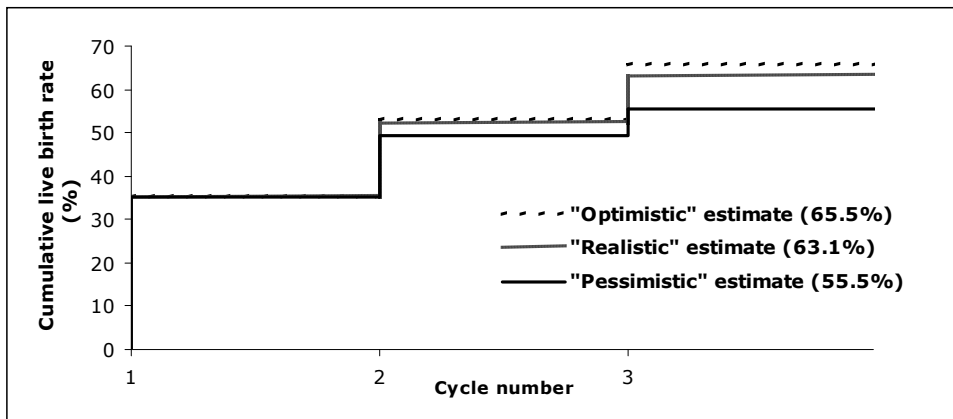


Figure 11. Cumulative live birth rates after three completed IVF cycles.

frozen-thawed cycles, of which one resulted in a pregnancy that miscarried before week 6. The other patients had had their cryopreserved embryos destroyed.

Thirty-seven of the patients dropped out of the treatment owing to a spontaneous pregnancy. These live births were not included into the original analyses, but if they had been, the cumulative “pessimistic” live birth rate after three completed cycles would have been 59.4% (561/944).

Seven of the patients included in the study were treated with artificial insemination (artificial insemination husband, AIH) which, in these cycles, had been transformed to IVF due to a high number of follicles. In retrospect, these patients should not have been included in the study. The seven patients did not achieve any live births in their IVF cycles. When followed up in 2009, two of them had achieved live births in later AIH cycles, not included in our analyses. If these seven AIH patients were excluded and the thirty-seven spontaneous pregnancies

were included, the cumulative “pessimistic” live birth rate after three completed cycles would have been 59.9% (561/937).

Age, infertility diagnoses and cumulative live birth rates

The patients were divided into three age groups depending on the age of the woman when the treatment was initiated. The cumulative live birth rates after three completed cycles are illustrated in Figure 12, and were higher in the two younger age groups as compared with the relatively older age group ($p=0.018$ for 20-29 years versus 35-40 years, $p=0.0021$ for 30-34 years versus 35-40 years). Women under 30 delivered twins significantly more often than did women over 30, reflecting higher implantation rates (twin rate 34%, 19% and 18% respectively; $p=0.0008$ and 0.0025). The cumulative live birth rates were also compared for different infertility diagnosis subgroups (Figure 13). The differences were not statistically significant.

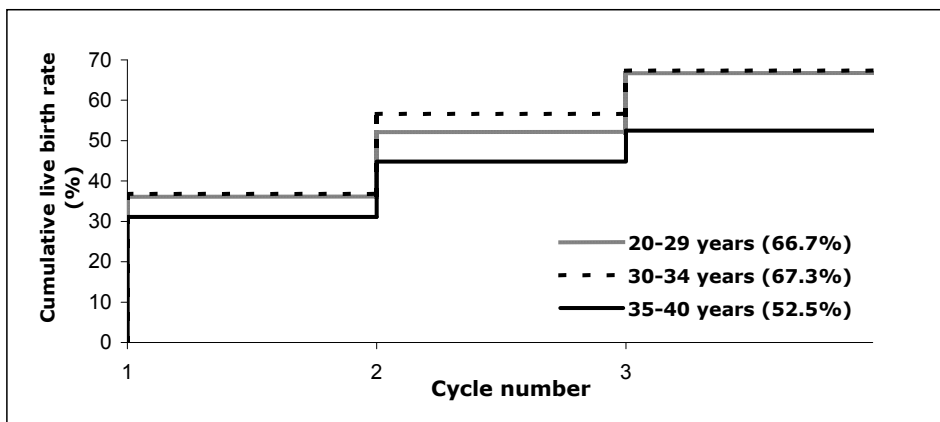


Figure 12. Cumulative live birth rates after three completed IVF cycles for different age groups, “realistic” estimate.

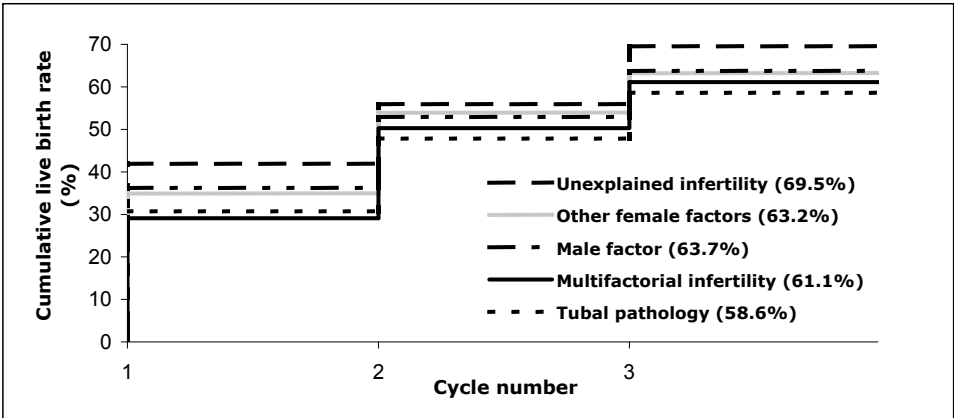


Figure 13. Cumulative live birth rates after three completed IVF cycles according to infertility diagnosis, “realistic” estimate. (In the original publication, the curve was based on started cycles, why it looks slightly different).

Patients who discontinued treatment

Of the 450 patients who did not achieve a live birth, 290 (see below why this number differs along the text) left the treatment programme before having completed three cycles. Thirty patients did not undergo any embryo transfer, 121/613 (19.7%) dropped out after one completed cycle, and 139/356 (39.0%) after two completed cycles. The

medical records of these 290 patients were searched to determine their reasons for dropping out. Poor prognosis was the reason for 50 patients. In the “realistic estimate”, these 50 patients with a poor prognosis were given no chance of a live birth, whereas the other dropouts were given the same chance of a live birth as those who completed their cycles.

PAPER II: Why do couples discontinue IVF treatment? A cohort study.

The reasons as to why a large proportion of the couples in Paper I discontinued their treatment was investigated in this study. Of the 290 patients reported as having discontinued treatment in Paper I, one completed three cycles with no live birth after Paper I was published, and another was an AIH patient, and therefore they were no longer considered dropouts. Thus, 288 of the 450 patients who did not achieve a live birth (64%) discontinued before completing three IVF cycles. The medical records of the 288 couples were scrutinized. The reason for discontinuation was obvious in 77 cases. A questionnaire was sent out to the remaining 211 couples. Of the 211 couples who received the questionnaire, 162 (77%) responded, of which one had sent back the questionnaire blank, without having answered the questions. No statistically significant differences were found between

responders and non-responders concerning age of the women and or reason for infertility. The questionnaires showed that 46 of the couples were only offered two cycles by their county council. Thereby, of the 288 couples who did not complete three IVF cycles, 242 discontinued for reasons unrelated to the reimbursement system. In 192 (79%) of the 242 cases, the reasons for not undergoing more treatments could be identified either from medical records (n=77) or questionnaires (n=115).

Reasons for discontinuing IVF treatment

The main reason for discontinuation was the psychological stress in 26%, poor prognosis in 25%, spontaneous pregnancy in 19%, physical factors in 6%, serious disease in 2%, and other reasons such as having adopted or moved in 7% (Figure 15). Psychological stress was caused by, for example, several previous failed treatments for infertility, late miscarriage, legal abortion owing to foetal chromosomal

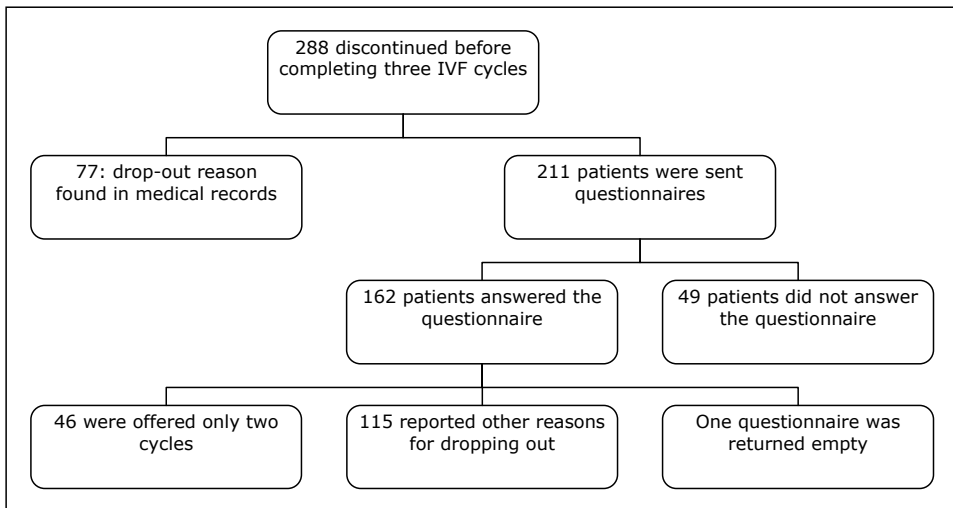


Figure 14. Flow chart over the participants and distribution of questionnaires in Paper II.

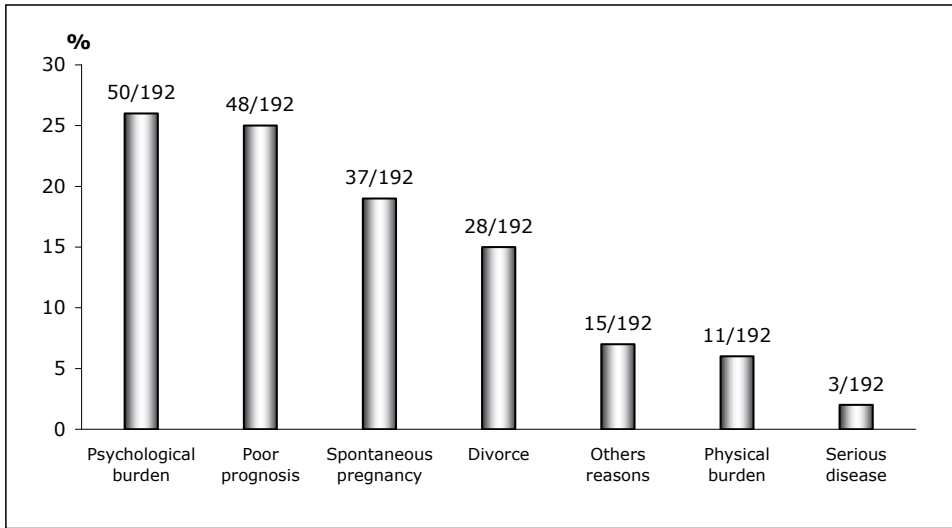


Figure 15. Reasons for discontinuing IVF treatment.

abnormalities, seeing different doctors at each appointment, and feeling pressure to succeed with the treatment. Physical factors included, for example, severe gynaecological or abdominal infection, ovarian hyperstimulation syndrome, and pain from injections. The number of patients having discontinued treatment due to a poor prognosis was assessed to be 50 after the scrutiny of the medical records in Paper I, but changed to 48 after having investigated it also by questionnaires in Paper II.

Free text comments and rating of the care

One hundred forty-three couples shared one or more free text comments. The comments were divided into five major categories, as presented in Table 7. Comments concerning lack of autonomy were the most frequent ones. The patients reported experiencing stressful “assembly-line” treatment, and a need for more inform-

ation about alternatives to the treatment such as adoption, and about the treatment itself. The next most frequent comments concerned experiencing not being listened to, and not being met with empathy, i.e. shortcomings in the psychological aspects of the care. Several of the patients reported that they felt a need for psychological counselling. Another frequent remark was that the clinic had organizational problems, and that the patients wished to have more continuity in terms of the number of different caregivers they had to meet. On the other hand, a substantial number of patients reported positive experiences and a feeling of being well taken care of. It should be born in mind that these comments derive from a negatively selected group, who did not achieve a live birth and who dropped out of treatment, and do not represent the average IVF patients. In the questionnaire the patients were invited to grade the care at the centre as “good”, “less good” or “poor”.

Table 7. Free text comments from 143 patients on the care at the IVF clinic.

Type of comment	Example	n*
Emotional and stressful reaction due to the infertility situation	"We needed to talk to a psychologist"	22
	"Couldn't cope with more treatment"	3
Organizational problems	"Poor organization at the clinic"	23
	"Insufficient care of the man"	5
	"Never the same people from appointment to appointment"	9
Poor ability to handle patients in psychological distress	"The doctors and nurses didn't listen to me"	18
	"I wasn't met with empathy"	21
	"The doctors/nurses didn't treat me nicely"	4
Lack of autonomy during treatment	"Stressful, assembly-line treatment"	29
	"Needed more information concerning the treatment and alternatives"	31
Good care	"Great commitment and professionalism despite high stress level, very well cared for"	23

* Some patients made more than one comment.

Of the 152 patients who graded the care, 100 (66%) depicted it as "good", 35 (23%) as "less good" and 17 (11%) as "poor". The ratings between those who discontinued of

their own volition and those who were forced to discontinue since they were offered only two reimbursed cycles by their county council did not differ significantly.

PAPER III: Predictive factors for live birth in frozen-thawed SET

In this study, predictive factors for live birth in frozen-thawed SET were analysed in 371 patients with fresh cycles during 2003-2004 resulting in at least one frozen-thawed SET. The patients underwent 622 frozen-thawed SETs during 2003-2006, resulting in 97 live births (16%). One twin and 96 singleton live births occurred. The pregnancy rate was 22%. The corresponding live birth and pregnancy rates for cryopreservation DET were 19% and 28%.

Univariate analyses

In the univariate analysis female age, IVF as fertilization method, and blastomere survival rate differed significantly between patients with and without a live birth (Table 8). For pregnancy, blastomere survival rate and the number of embryos thawed to obtain this transfer were significantly correlated. All variables with a p-value of less than 0.10 in the univariate analysis were included in the multivariate analysis.

Multivariate analysis

The factors that were found to correlate independently to pregnancy and live birth are shown in Table 9. In the multivariate analysis on predictors of live birth IVF as fertilization method, blastomere survival rate and number of fresh cycles in the patient's history were found to be independent predictors. When the endpoint was pregnancy, blastomere survival rate, number of fresh cycles in the patient's history and number of embryos thawed to obtain this ET were found to be independent predictors. That blastomere survival rate, conventional IVF as fertilization method as compared with ICSI were positive

predictors, and the number of embryos thawed to obtain one transfer was a negative predictor, was not surprising since it is in accord with findings in other studies or can be explained by existing theories (see Discussion). It was an unexpected finding that the number of fresh cycles in the patient's history was an independent predictor of live birth, but it might be explained by the fact that both previous successful and failed cycles were included in the variable. When analysing the number of failed fresh cycles in the patient's history separately, this variable was not shown to affect the live birth rate. This can be seen as encouraging for couples who have undergone several previous failed fresh cycles. However, the mean number of previous fresh cycles in the patients' history in this study was low, 1.3 and 1.2 in the groups with a live birth and with no live birth, respectively. In the group with a live birth in the frozen-thawed cycle, 16.5% had a live birth after the fresh cycle, as compared with 12.2% in the group with no live birth after the fresh cycle, the difference not being significant. It was somewhat surprising that the woman's age was not shown to be an independent predictor in the present study. Age is a well known predictor of live birth in IVF. The reason for this may be the homogeneity in age in our study population.

Success rates according to cycle number with eggs from the same egg retrieval

The success rates in the first, second and third or more frozen-thawed cycles, when all earlier frozen-thawed cycles from the same egg retrieval had failed, were compared (Figure 16). There was no statistical difference in pregnancy or live birth rate irrespectively whether the patients

Table 8. Univariate analysis of maternal and embryological factors investigated for influence on pregnancy and live birth rates.

	Preg- nancy (n=138)	No pregnancy (n=484)	P (preg- nancy)	Live birth (n=97)	No live birth (n=525)	P (live birth)
Age	32.7 (3.4)	32.1 (3.7)	0.060	32.9 (3.3)	32.1 (3.7)	0.039
Tubal factor, n (%)	14 (10.1)	56 (11.6)	0.651	8 (8.2)	62 (11.8)	0.294
Fertilization method, standard IVF, n (%)	79 (57.2)	245 (50.6)	0.175	60 (61.9)	264 (50.3)	0.039
Number of fresh cycles in patient's history	1.3 (0.7)	1.2 (0.7)	0.061	1.3 (0.7)	1.2 (0.7)	0.070
Number of cryo-cycles in patient's history	1.8 (1.1)	1.8 (1.0)	0.663	1.7 (1.1)	1.8 (1.0)	0.403
Number of cryo-cycles from the same fresh cycle	1.6 (0.9)	1.5 (0.8)	0.828	1.5 (0.8)	1.6 (0.8)	0.532
Number of failed fresh cycles in the patient's history	1.1 (0.7)	1.0 (0.8)	0.160	1.1 (0.7)	1.0 (0.8)	0.409
Number of failed cryo-cycles in the patient's history	0.8 (1.1)	0.7 (1.0)	0.556	0.7 (1.1)	0.8 (1.0)	0.473
Number of failed cryo-cycles from the same fresh cycle	0.5 (0.8)	0.5 (0.8)	0.743	0.5 (0.8)	0.5 (0.8)	0.620
Pregnancy in the fresh cycle, n (%)	37 (26.8)	114 (23.6)	0.455	26 (26.8)	125 (23.8)	0.535
Pregnancy in previous cryo-cycle from the same fresh cycle, n (%)	12 (8.7)	27 (5.6)	0.165	6 (6.2)	33 (6.3)	0.938
Either of the two previous variables, n (%)	44 (31.9)	137 (28.3)	0.444	30 (30.9)	151 (28.8)	0.682
Live birth in the fresh cycle, n (%)	19 (13.8)	61 (12.6)	0.728	16 (16.5)	64 (12.2)	0.257
Live birth in previous cryo-cycle from the same fresh cycle, n (%)	4 (2.9)	16 (3.3)	0.761	2 (2.1)	18 (3.4)	0.376
Either of the two previous variables, n (%)	23 (16.7)	77 (15.9)	0.865	18 (18.6)	82 (15.6)	0.484
Surgically retrieved sperm, n (%)	10 (7.2)	29 (6.0)	0.579	4 (4.1)	35 (6.7)	0.333
Blastomere survival rate, in percent	95.4 (10.3)	91.3 (14.7)	0.002	95.5 (10.2)	91.6 (14.5)	0.008
No of cells in the thawed embryo	4.0 (0.6)	3.9 (0.8)	0.462	4.0 (0.6)	3.9 (0.8)	0.358
Embryo grade	1.8 (0.4)	1.7 (0.4)	0.423	1.7 (0.4)	1.8 (0.4)	0.861
Number of embryos thawed to obtain this transfer	1.4 (0.7)	1.6 (0.9)	0.015	1.4 (0.7)	1.6 (0.9)	0.130
Stimulated cryo-cycle, n (%)	28 (20.3)	107 (22.1)	0.642	16 (16.5)	119 (22.7)	0.184
Number of oocytes aspirated in the fresh cycle	15.0 (6.7)	15.8 (7.1)	0.228	15.1 (7.0)	15.7 (7.1)	0.436
Number of GQEs in the fresh cycle	6.3 (3.6)	6.2 (3.3)	0.611	6.5 (3.7)	6.1 (3.3)	0.223
FSH/HMG dose per aspirated oocyte, in the fresh cycle	182.8 (160.5)	184.0 (215.0)	0.889	185.4 (173.4)	183.4 (209.4)	0.938

Numbers are expressed as mean (SD) unless otherwise indicated.

Table 9. Multivariate analysis of maternal and embryological factors investigated for independent relation to pregnancy and live birth.

	Pregnancy			Live birth		
	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>
Blastomere survival rate	1.025	1.008-1.042	0.003	1.026	1.007-1.045	0.007
Number of fresh cycles in the patient's history	1.312	1.014-1.670	0.039	1.372	1.031-1.826	0.030
Fertilization method, standard IVF	-	-	-	1.607	1.022-2.526	0.040
Number of embryos thawed to obtain this transfer	0.772	0.602-0.990	0.042	-	-	-

OR=odds ratio. CI=confidence interval.

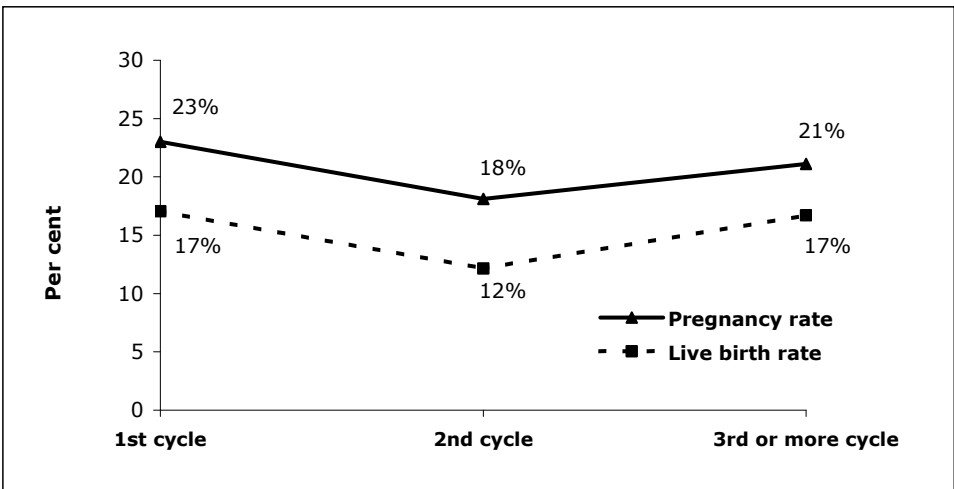


Figure 16. Live birth rate and pregnancy rate, calculated by cycle number of frozen-thawed SET, derived from the same oocyte retrieval, when all previous frozen-thawed SET from the same cohort of eggs had failed. The differences were not significant.

had undergone one, two or three or more failed cryopreservation cycles from the same egg retrieval. However, the number of women performing a third or more frozen-thawed cycle from the same egg retrieval when the previous ones had failed was limited (n=66), giving a low power to detect a difference in live birth rates between cycles. The fact that the number of thawed

embryos required to perform one transfer was a significant negative predictor of pregnancy in the multivariate analysis may, however, indicate that if there is a poor embryo quality in some of the eggs from a particular cohort, the chances of a live birth might be decreased when using other eggs from that cohort. This theory is called “cohort homogeneity” (see Discussion).

PAPER IV: Cumulative Live-Birth Rates after Single-Embryo versus Double-Embryo Transfer

In this follow up study, all subsequent frozen-thawed cycles with embryos originating from the fresh cycles in the RCT by Thurin et al. were traced, generating cumulative data. A major strength of the study was that no patients were lost to follow up and all embryos were accounted for. Of the 661 patients included in the RCT, 141 patients in the SET randomization group and 152 patients in the DET randomization group underwent additional frozen-thawed cycles. Table 10 shows the characteristics of the frozen-thawed cycles. A surprisingly large group of the patients did not use their cryopreserved embryos; 30.6% in the SET group and 34.7% in the DET group had all their embryos destroyed

(Figure 9 in Methodological considerations). Of these 31.4 % in the SET group and 33.3% in the DET group had not achieved a live birth in the RCT. Of the patients who did not achieve a live birth either in the original RCT or in the addition frozen-thawed cycles, 17.8% in the SET group and 22.2% in the DET group had at least one cryopreserved embryo destroyed. The patients' reasons for not using their cryopreserved embryos are not completely known. Achieving a live birth in the RCT could be one reason; 108/270 (40.0%) of the patients with a live birth in the RCT and 43/47 (91.5%) of the patients with a multiple birth in the RCT had all their surplus embryos destroyed. Some couples chose to continue with stimulated fresh cycles instead of frozen-thawed cycles. However, the local policy at our clinic is to use the cryopreserved embryos before

Table 10. Characteristics of the additional frozen-thawed embryo transfers (FETs; after completion of the RCT). Numbers are expressed in mean±SD (range). Rates are expressed in % (no.).

	SET group (n=330)	DET group (n=331)	P
Number of additional cryopreserved embryos			
-total	848	873	0.37
-mean±SD (range)	2.57±2.64(0-15)	2.64±2.48(0-15)	
Number of embryos destroyed			
-no (%)	344 (40.6%)	349 (40.0%)	-
Number of additional FETs			
-total	233	189	0.61
-mean±SD (range)	0.71±0.98(0-4)	0.57±0.72(0-4)	
Number of embryos transferred per additional FET	1.35±0.44(1-3)	1.51±0.52(1-3)	0.011
SET rate in additional FETs	66.5 (155/233)	51.9 (98/189)	0.003
DET rate in additional FETs	32.2 (75/233)	46.0 (87/189)	0.005
Triple embryo transfer rate in additional FETs	1.3 (3/233)	2.1 (4/189)	0.71

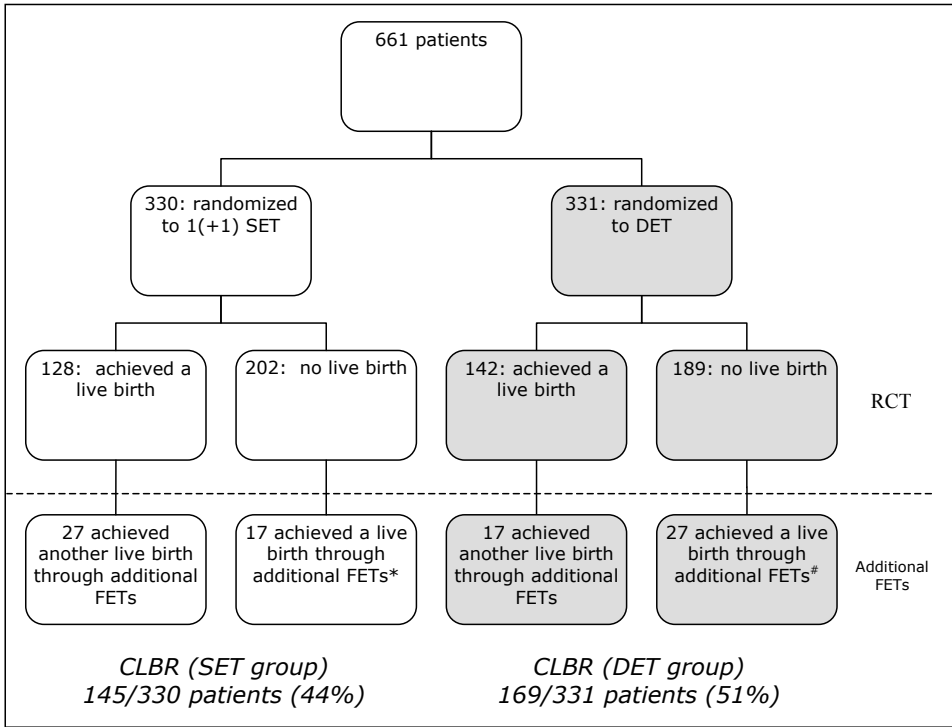


Figure 17. Flow chart of the participants, with cumulative live birth rates.

CLBR= cumulative live birth rate.

*Two patients achieved two live births in the additional FETs.

#One patient achieved two and one three live births in the additional FETs.

proceeding with more fresh cycles. A spontaneous pregnancy could be a reason for not using the extra embryos in some cases. It should also be born in mind that five years is quite a long time, and changes in life circumstances such as severe disease and divorce might also explain the waste of the extra embryos.

The number of embryos transferred in the additional frozen-thawed cycles was unrelated to which randomization group the patient belonged to. One, two or in a few cases three embryos were transferred per frozen-thawed cycle, according to the

patient's wishes and routines at each IVF clinic (Table 10). The SET rate in the frozen-thawed cycles was, however, found to be significantly higher in the SET group than in the DET group, 66.5% and 51.9% respectively. Also among the patients with no live birth in the RCT, there was a higher SET rate in the additional frozen-thawed cycles in the SET group (76.3%) than in the DET group (50.0%). However, in some cases the couple had no other choice than SET since they only had one cryopreserved embryo. This was true for 17.0% of the transfers in the SET group and 29.8% in the

DET-group. The reason the patients in the SET group chose more often SET in their frozen-thawed cycles is not clear, but may reflect a psychological effect that couples who previously failed with SET are more prone to accept one more SET than patients who previously failed with DET.

Cumulative live birth rates

The cumulative live birth rate, defined as the proportion of patients having achieved at least one live birth, was lower in the SET group (43.9%) than in the DET group (51.1%), but not significantly so ($p=0.080$, Table 11). The absolute difference in cumulative live birth rates was 7.1% (95% CI -0.5 to 14.7). Some patients achieved more than one live birth, and the total number of live births was 174 in the SET group and 189 in the DET group. Forty-seven of the patients achieved two live births each, and one patient achieved three live births (Figure 17). Owing to the higher number of multiple births, the number of live born children was higher in the DET

group ($n=239$), than in the SET group ($n=178$, $p=0.009$). The “implantation rate” expressed as the number of live born children per embryo transferred, was 0.22 in the SET group and 0.25 in the DET group ($p=0.84$). In the SET group there were 45 miscarriages and three ectopic pregnancies. In the DET group there were 40 miscarriages and two ectopic pregnancies. None of the differences in pregnancy outcome between the SET and DET groups was significant.

A larger proportion of the patients in the original study with no live birth, 47.6%, as compared with the patients with a live birth, 39.6%, continued with additional frozen-thawed transfers. Among the women who continued with additional frozen-thawed cycles, a smaller proportion of the patients who did not achieve a live birth in the original study, 44/186 (23.7%), succeeded in achieving a live birth in the additional frozen-thawed cycles as compared with the patients who achieved a live birth in the original study, 44/107 (41.1%, $p=0.002$).

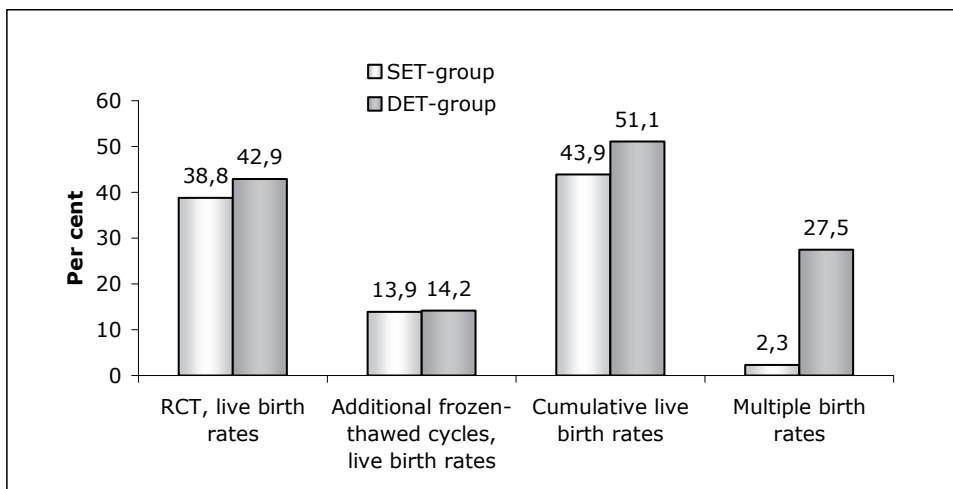


Figure 18. Cumulative live birth and multiple birth rates

Table 11: Cumulative live births and obstetric outcomes.

	SET group (n=330)	DET group (n=331)	P
<i>Cumulative live birth rates</i> [#]	43.9 (145/330)	51.1 (169/331)	0.080
Number of live births*			
-total	174	189	0.20
-in the original study	128	142	0.28
-in the additional FETs	46	47	0.99
Number of live born children			
-total	178	239	0.009
-in the original study	129	189	0.016
-in the additional FETs	49	50	0.90
Live born children per embryo transferred			
-total	0.22 (178/825)	0.25 (239/944)	0.84
-in the original study	0.25 (129/511)	0.29 (189/660)	0.078
-in the additional FETs	0.16 (49/314)	0.18 (50/284)	0.73
Multiple birth rates			
-total	2.3 (4/174)	27.5 (52/189)	<0.001
-in the original study	0.8 (1/128)	34.5 (49/142)	<0.001
-in the additional FETs	6.5 (3/46)	6.4 (3/47)	1.00
Preterm birth rates			
-total	11.8 (21/178)	25.5 (61/239)	<0.001
-in the original study	11.6 (15/129)	28.0 (53/189)	<0.001
-in the additional FETs	12.2 (6/49)	16.0 (8/50)	0.81
Very preterm birth rates			
-total	2.2 (4/178)	5.9 (14/239)	0.11
-in the original study	2.3 (3/129)	7.4 (14/189)	0.076
-in the additional FETs	2.0 (1/49)	- (0/50)	0.99
Gestational age, days			
-total	275.5±17.0 (200-298)	267.4±24.6 (168-304)	0.001
-in the original study	276.2±16.7 (200-298)	265.2±26.0 (168-304)	<0.001
-in the additional FETs	273.9±17.9 (200-298)	275.7±16.0 (231-301)	0.58

Rates are expressed as % (no), gestational age as mean±SD (range).

FETs=frozen-thawed embryo transfers.

[#]Number of patients with at least one live birth.

*Some patients achieved more than one live birth.

This may be an expression of a better prognosis in the group with than without a live birth in the original study.

Multiple births

The multiple birth rate was dramatically lower in the SET group than in the DET group (Table 11, Figure 18). In the SET group there were four twin live births. In the DET group there were 48 twin live births and one triplet live birth.

Obstetrical outcome

The preterm birth rates (<37 gestational weeks) were significantly higher in the DET group, as a consequence of the higher multiple birth rates, than in the SET group (Table 11). Two intrauterine foetal deaths occurred. In three of the twin pregnancies in the DET group, one twin died in utero before gestational week 28.

General discussion

Cumulative live birth rates

Cumulative live birth rates answer the question of how great the chances are of a live birth after a series of IVF treatments, the “take-home-baby-rate”. In paper I the cumulative live birth rates after three IVF cycles were presented. With the “realistic” estimate, 63% of the couples achieved a live birth after the three reimbursed fresh IVF/ICSI cycles, including the following frozen-thawed transfers. The use of cumulative live birth rates instead of live birth rates per cycle provides the couple with a more accurate prognosis for achieving a live birth after IVF/ICSI treatment. The patients in the study underwent their fresh IVF/ICSI cycles

between 1996 and 2000. However, since the birth rates per cycle have remained about the same (annual results of 24.6% to 26.6% per fresh embryo transfer during 1996-2000, as compared with 27.3% in 2006 in Sweden, The Swedish National Board of Health and Welfare) despite the fact that the average number of embryos transferred has decreased, the cumulative live birth rates presented in the study are probably also valid for the patients in treatment today. This is supported by the fact that a later observational study from our centre including the patients’ first two fresh cycles with subsequent frozen-thawed transfers showed similar cumulative live birth rates as in Paper I (Figure 19; Lundin and Bergh

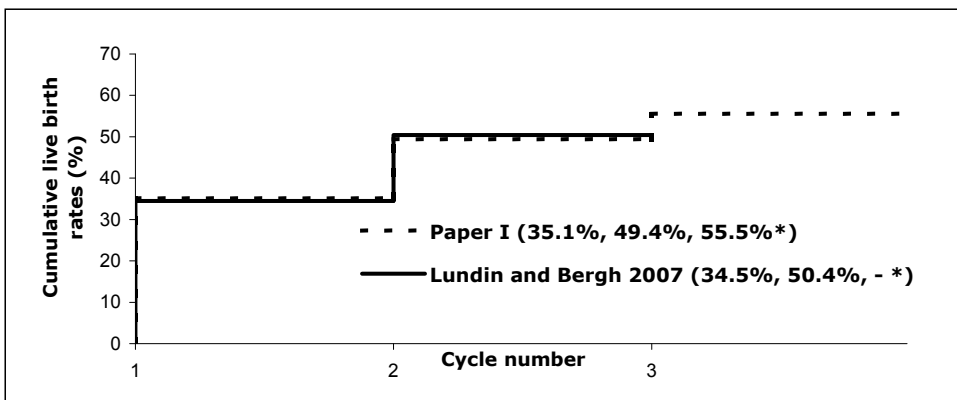


Figure 19. Cumulative live birth rates in Paper I as compared with a later published study from Sahlgrenska University Hospital (Lundin and Bergh 2007).

*Cumulative live birth rates after cycles 1, 2 and 3, “conservative” estimates.

2007). The patients included in that study underwent their first fresh cycles during 2003 and 2004. The majority of transfers in Paper I were DETs, while in Lundin and Bergh the majority of transfers were SETs.

In a recently published article from the USA, the cumulative live birth rates after three/six started cycles were 45%/51% in the conservative estimate and 53%/72% in the optimistic estimate (life table analysis; Malizia et al., 2009). However, in that study, frozen-thawed cycles were calculated as separate cycles, in contrary to Paper I. In another study, the cumulative pregnancy rates after three/four fresh started IVF cycles, without frozen-thawed cycles included, were 30%/31% in the conservative estimate and 45%/53% in the life table analysis (same as the optimistic estimate; Schroder et al., 2004).

When comparing cumulative live birth rates in different studies it is important to be aware of whether the results are presented per completed cycle or, as in most other studies, per started cycle (Alsaili et al., 1995; Engmann et al., 1999; Schroder et al., 2004; Witsenburg et al., 2005; Malizia et al., 2009). Other details to observe are whether the frozen-thawed cycles are calculated as separate cycles (Malizia et al., 2009), not included at all (Tan et al., 1992; Schroder et al., 2004) or included in the results of each fresh cycle (Paper I; Witsenburg et al., 2005). The reason in Paper I for presenting the cumulative results of three completed cycles or up to one live birth, and including the frozen-thawed cycles in each fresh cycle, was that the reimbursement system allowed that number of treatments.

How many cycles are worth performing?

In studies presenting cumulative pregnancy/live birth rates for up to six

consecutive cycles per woman, a steady increase in the cumulative pregnancy/ live birth rates with each additional cycle has been seen (Guzick et al., 1986; Alsaili et al., 1995, Schroder et al., 2004; Malizia et al., 2009). However, the live birth rate per cycle has been shown to decrease with each cycle, with the highest probability of a live birth in the first cycle (Templeton et al., 1996). In Paper I there was a decline in the live birth rates from 35.1% in the first cycle to 26.7% in the third ($p=0.02$). The live birth rates in the fifth and sixth cycles in a recent large study were 17.3% and 13.0% respectively, as compared with 24.5% in the first cycle (Malizia et al., 2009). The decrease in live birth rates with later cycles is probably explained by the fact that the patients with a good prognosis achieve a live birth before patients with poorer prognosis.

It can be concluded from the literature that the cumulative live birth rates continue to rise at least up to six cycles, however the chance of a live birth per cycle decreases with each cycle.

Contribution to the cumulative live births from the frozen-thawed cycles

The frozen-thawed cycles provides an important contribution to the cumulative live births after IVF (see Introduction, p15). In Paper I, the contribution of the frozen-thawed cycles to the live births was 20.0%. The frozen-thawed cycles are safer and more convenient for the woman since there is no need of ovarian stimulation, however the live birth rates per embryo transfer are lower after frozen-thawed than after fresh transfers (Table 2, introduction). A recent systematic review found better or as good obstetric outcome in children born after embryo cryopreservation, measured as

preterm birth and low birth weight, when compared with children born after fresh embryo transfer. The neonatal data is mainly based on slow freezing of cleavage stage embryos; there are few reports on neonatal outcome after slow freezing of blastocysts and vitrification of cleavage-stage embryos, oocytes and blastocysts (Wennerholm et al., 2009).

In Paper IV, 40.3% of all cryopreserved embryos were destroyed according to the patients' own wish or because of the legislated time limit, and 33.1% of the patients had at least one cryopreserved embryo destroyed. A more efficient usage of the cryopreserved embryos would lead to improved cumulative live birth rates. Studies show that the couples' decision-making about what to do with surplus embryos is difficult and emotional. The decision-making is influenced by life circumstances, embryo quality/quantity, personal values, embryo conceptualization and clinic information. The decision-making can be described in terms of a step-wise process; whether or not to use the embryos for further conception attempts, whether or not to keep the embryos in cryopreservation, and whether or not to donate the embryos to other infertile couples or to research (Nachtigall et al 2009; Provoost et al., 2009; however, embryo donation is currently not allowed in Sweden). The patients' conceptualization of their frozen embryos varies from seeing them in a medical-technical way, to seeing them as genetic "insurances", a symbol of the relationship or "virtual" children having interests that must be considered and protected (Nachtigall et al 2005; Provoost et al 2009). Studies suggest that patients can benefit from more information about their alternatives, and opportunities to talk to others in similar

situations (Fuscaldo et al., 2007). In addition to this, a well-functioning cryopreservation program with a high survival rate of the thawed embryos and satisfactory live birth rates in frozen-thawed cycles is important. Local policies at each clinic that encourages the couples not to proceed with another fresh cycle before having used their frozen embryos, as well as patient information about the advantages of cryopreservation cycles such as safety and convenience, might also contribute.

Paper III showed that high blastomere survival rates, IVF compared to ICSI as fertilization method, and a high number of fresh cycles in the patient's history were positive predictive factors for live birth in frozen-thawed SET. The need to thaw a large number of embryos in order to achieve one transfer was a negative predictor for pregnancy. That blastomere survival rate is a predictor for live birth/pregnancy in cryopreservation cycles has also been shown in several other studies (Guerif et al., 2002; Pal et al., 2004; Salumets et al., 2006; Tang et al., 2006; Edgar et al., 2007). The fact that IVF as fertilization method is a positive predictor as compared with ICSI is in good accord with national Swedish (The Swedish National Board of health and welfare) and European results (Nyboe Andersen et al., 2009) in both fresh and frozen-thawed cycles. The finding that the number of embryos thawed to obtain one transfer was a negative predictor of pregnancy has not to our knowledge been shown previously. It is in accord with the theory of "cohort homogeneity", i.e. that the embryos originating from the same egg retrieval ("egg cohort") are of similar quality (Trounson et al., 1986). The finding in other studies that previous pregnancy/live birth in cycles from the same egg retrieval are

positive predictive variables also supports the theory of cohort homogeneity (El-Toukhy et al., 2003; Urman et al., 2007). The reason a large number of fresh cycles in the patient's history was a positive predictor of live birth is probably that the variable included both failed and successful previous cycles. When only previous failed cycles were studied, they were not found to impact on the outcome. Other studies have shown that previous failed cycles were a negative predicting variable (Thurin et al., 2005).

The newly introduced cryopreservation technique of vitrification has shown better survival rates after thawing for both cleavage stage embryos and blastocysts as compared with slow freezing (Loutradi et al., 2008). There is at present no evidence of better clinical pregnancy rates after vitrification as compared with slow freezing, but the data published so far are limited (Kolibianakis et al., 2009).

Improving the live birth rates; new techniques

Transfer of blastocyst stage embryos, which are cultured to day 5/6, has been shown to result in higher live birth rates per couple than transfer of cleavage stage embryos, which are cultured to day 2/3, 36.0% versus 29.4% in a Cochrane review article (Blake et al., 2007). However, in the blastocyst group as compared with the cleavage stage embryo group, there were fewer surplus embryos to cryopreserve, and failure to transfer any embryos at all was more frequent (Blake et al., 2007). In the good prognosis group of patients, defined as having a high number of eight cell embryos on day three, there was no difference in cycle cancellation rate and the authors concluded that single blastocyst transfer may be applicable in that patient group

(Blake et al 2007). The delivery rates were similar after single cleavage-stage embryo transfer including frozen-thawed cycles, and single blastocyst transfer including frozen-thawed cycles, 34.2% and 37.9% respectively in an observational study (Guerif et al., 2009). Blastocyst culture is also being used increasingly after cryopreservation, i.e. embryos that were cryopreserved on day 2/3 can be cultivated for another 2-3 days after thawing. This procedure might be an option for selecting an embryo with a high survival and implantation potential, especially for patients with a large number of cryopreserved embryos.

Preimplantation genetic diagnosis (PGD) is a laboratory technique where one or two cells from the embryo are biopsied and genetically analysed prior to transfer. Preimplantation genetic screening (PGS) is a special type of PGD that has been used in conjunction with IVF in order to screen the embryos for aneuploidy, i.e. chromosomal numerical errors. The purpose of such screening is to improve pregnancy and live birth rates, especially in women of advanced maternal age. Even if a large number of observational studies have shown encouraging results after PGS, later randomized trials failed to confirm this. In fact, a meta-analysis concluded that PGS for aneuploidy was associated with lower rates of ongoing pregnancies and live births than standard IVF/ICSI without PGS (Checa et al., 2009). The explanation for this might be that human embryos have a high rate of mosaicism, leading to poor diagnostic values when only one or two cells are examined (Baart et al., 2006). Another reason might be a detrimental effect of the biopsy procedure itself on embryonic development (Hardarson et al., 2008).

It has been hypothesized that failure of the blastocyst to implant could be caused by inability of the blastocyst to escape from the zona pellucida, a process called “hatching”. Assisted hatching (AH), is a technique where a hole is made in the zona pellucida, chemically or by laser. A recent Cochrane review article showed an increased clinical pregnancy rate and also a higher multiple birth rate in patients randomised to AH as compared with controls (Das et al., 2009).

The “omics” are a number of techniques where the genomic constitution and/or the metabolism of the embryo is analysed. It can be either invasive, when cells from the embryo or blastocyst are biopsied and analysed for genetic markers (genomics), or non-invasive when proteins or metabolites are analysed in the spent embryo culture medium (proteomics, metabolomics). Metabolomic and proteomic profiling of human embryos have been shown in some studies to correlate to implantation or live

birth (Brison et al., 2004; Estes et al., 2008; Seli et al., 2009; Sturmeijer et al., 2009).

SET for preventing multiple births

IVF and health aspects for the children

The long-term impact on general health of the higher multiple birth rates and the higher preterm birth rates that have been a consequence of the increasing use of IVF, cannot be fully evaluated today, since only relatively few IVF children have reached adulthood. However, small size at birth has been found to be associated with cardiovascular disease and mortality in adult life, according to the Barker theory (Barker et al., 1995). A recent study on IVF children of about twelve years of age showed significantly higher blood pressure and higher fasting glucose levels in the IVF children as compared with age and gender matched controls who were offspring to subfertile couples. The differences could not

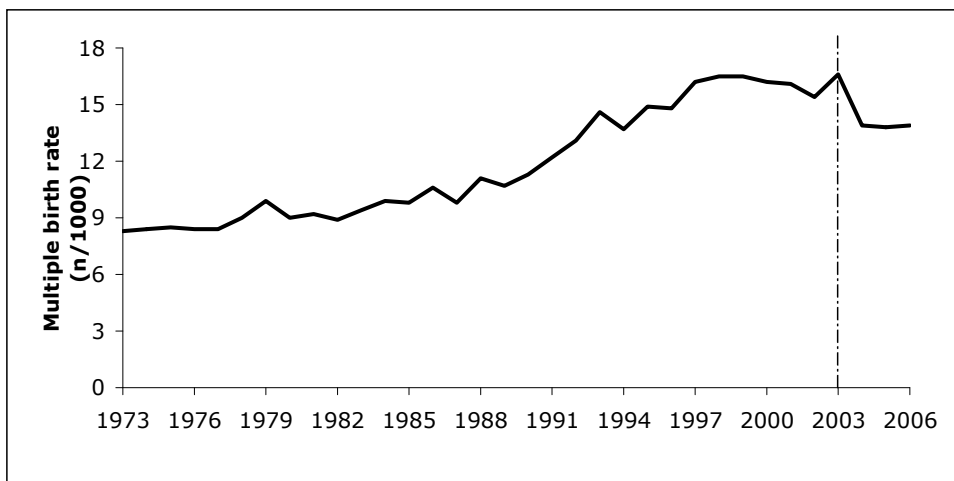


Figure 20. Multiple birth rates in Sweden 1973-2005, of all births (The Swedish National Board of Health and Welfare).

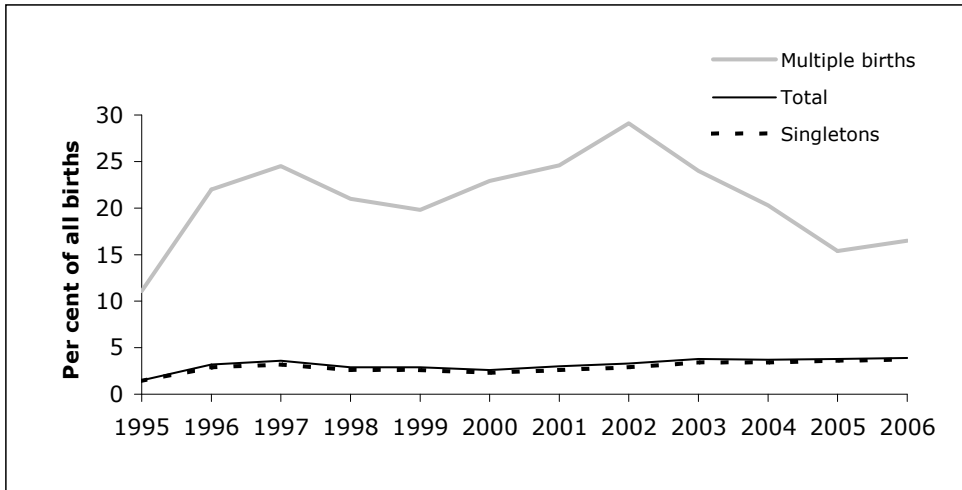


Figure 21. Contribution of ART to multiple births, singletons births and all births in Sweden 1995-2006 (data from The Swedish National Board of Health and Welfare).

be explained by current body size, birth weight, early life factors, or parental characteristics such as cause of subfertility. The subfertile couples and their controls were part of a large Dutch cohort, registered at IVF clinics between 1980 and 1995, where some received IVF treatment and some did not (Ceelen et al., 2008). Cerebral palsy was found to be 3.7 times more common in IVF-children than in controls (95% CI 2.0-6.6) and 2% of the IVF children as compared with 1% of the controls were in contact with a childhood disability centre in a Swedish population study (Strömberg et al., 2002). Although it can be concluded from the literature that IVF singletons also have an increased risk for adverse events and more research should be performed to find the reasons for that, a large share of the adverse events can be Healthcare Utilization Registry, the Malformation Registry and the Cause of Death Registry. Previous cross-linkages between the former IVF registry which, in

prevented by reducing the multiple birth rates.

As can be seen in Figure 20, the trend toward increasing multiple birth rates was broken in Sweden in 2003, and the contribution of IVF to all multiple births has been decreasing since 2002 (Figure 21). This is in agreement with the major increase in use of SET during the same time period. The Swedish National Board of Health and Welfare has presented annual reports on ART treatments since 1991, containing aggregated data. A Swedish nationwide Quality Registry for IVF, collecting data on all the IVF treatments on an individual level, was established in 2007 (data are reported on the web at <http://www.ucr.uu.se/qivf>). This will enable cross-linkages with other population registries such as the Medical Birth Registry, the Cancer Registry, the contrast to the new IVF registry contained data only on the IVF treatments resulting in a birth, and the population based registries have resulted in important knowledge,

particularly on safety aspects for the children (Bergh et al., 1999; Ericson and Källén, 2001; Ericson et al., 2002; Källén et al., 2005abc).

Cumulative live birth rates after SET as compared with DET

The most important measure for decreasing high multiple birth rates is to implement SET to a greater extent. SET has become increasingly implemented in Sweden and some other Scandinavian and European countries during recent years. However, worldwide, SET cycles represent a minority of all IVF cycles (see Introduction, Figure 6).

In Paper IV, the cumulative live birth rates after SET were not significantly lower than after DET in a randomized study population. The cumulative number of live born children was, however, higher in the DET group than in the SET group owing to a substantially higher multiple birth rate in the DET group. A previously published Finnish randomized study, smaller than ours, reported cumulative live birth rates after only one fresh SET or one fresh DET including subsequent frozen-thawed cycles of 39% and 51% respectively, the difference not being significant. Most of the frozen-thawed transfers in that study were, however, with two or more embryos (Martikainen et al., 2001). A Dutch RCT with 404 patients included compared mild stimulation protocol and SET, with conventional stimulation and DET. Cumulative live birth rates including frozen-thawed transfers were 43.4% in the mild-SET group and 44.7% in the conventional-DET group, the difference was not statistically significant. The subsequent frozen-thawed transfers were either SET or DET in both groups according to the

patients' preferences, in similarity to Paper IV (Heijnen et al., 2007).

An observational study looking at the patients' first two fresh cycles including subsequent frozen-thawed cycles showed similar cumulative live birth rates of 33.5% and 32.3% after fresh DET and 34.8% and 32.2% after fresh SET, although a larger number of frozen-thawed transfers were needed in the SET group (Lundin and Bergh, 2007). A study comparing success rates from a time period with a higher rate of DET to a time period with a wider use of elective SET, showed significantly higher cumulative live birth rates in the SET period than in the DET period, 41.7% versus 36.6% (Veleva et al., 2009). Interestingly, the risks of low birth weight and preterm birth have been shown to be significantly higher after DET than SET when comparing only singletons (De Sutter et al., 2006). In a systematic review comparing SET with DET from a cost-effectiveness point of view, taking into account total cost per live birth, a health care perspective and a societal perspective, it was concluded that elective SET may be preferable to DET in patients with a good prognosis and when frozen-thawed cycles are included (Fidderers et al., 2007).

In conclusion, the live birth rate per fresh transfer is higher after DET than SET, whereas the cumulative live birth rates when including subsequent frozen-thawed transfers have not shown to differ significantly in three RCTs. There were, however, numerically higher cumulative live birth rates in the DET group in two of the RCTs (Martikainen et al., 2001; Paper IV), not reaching statistical significance.

“Mild” IVF

The decrease in the number of embryos transferred per cycle that has taken place over the last decade has led to a growing interest in milder ovarian stimulation protocols, with the aim of creating a safer, more patient-friendly and cost-effective regimen. The mild stimulation protocols often include low-dose gonadotropin administration combined with GnRH antagonist later during the stimulation, in contrast to the conventional long stimulation protocol with initial GnRH-agonist and higher dosages of gonadotropins (Verberg et al., 2009). The mild stimulation result in fewer mature oocytes per cycle, but is considered to have the advantages of a lower risk of ovarian hyperstimulation syndrome (OHSS), lower costs, lower dropout rates, and possibly less patient discomfort (Heijnen et al., 2007; Verberg et al., 2008a; Aboulgar 2009). One interesting finding was the occurrence of less embryo aneuploidy following mild stimulation than after conventional stimulation (Baart et al., 2007).

A randomized non-inferiority trial compared mild stimulation combined with SET with conventional stimulation combined with DET, in a cohort of women under 38 years of age (Heijnen et al., 2007). The cumulative live birth rates after one year were similar in both groups (see previous page), but there was a significantly lower multiple birth rate (0.5% versus 13.1%) in the mild SET group as compared with the conventional DET group. Although there was a larger number of started cycles in the mild stimulation group (mean 2.3 versus 1.7, $p < 0.0001$), owing to a higher cancellation rate the number of embryo transfers per group was similar (mean 1.5 versus 1.4, $p = 0.5$). The levels of patient

discomfort were approximately the same in the two groups (Heijnen et al., 2007). A cost-effectiveness analysis on these patients showed lower costs over a 12 months period for the mild SET group than the conventional DET group, mainly due to higher obstetric and postnatal costs connected to the multiple births in the latter group (Polinder et al., 2008).

A milder stimulation protocol yields fewer embryos per cycle for cryopreservation, which could lead to lower cumulative live birth rates. However, in Paper IV a large number of the cryopreserved embryos were not used by the patients, an important observation that needs further analysis.

It seems reasonable to change the ovarian stimulation protocols in a milder and safer direction, producing somewhat fewer embryos per cycle than today but with the advantage of less discomfort for the patient and a lower risk of OHSS. However, to evaluate milder stimulation per se, both for efficacy and safety, well designed RCTs are needed.

Patients' experiences of IVF treatment

Many IVF-patients, not surprisingly, perceive the treatment as emotionally stressful, particularly when they do not succeed in achieving a live birth (Holter et al., 2006; Verhaak et al., 2007). Couples entering IVF treatment have, however, been shown to be in general psychologically well adjusted (Eugster et al., 1999).

The infertility problem encompasses many aspects of life: male/female identity, existential questions and societal pressure. These can be difficult issues to deal with. However, encouraging results were shown in a Chinese small follow-up study of couples that had experienced unsuccessful

IVF treatment. Some remained childless, some had adopted, and some had conceived naturally. The women and men in the study all reported valuable gains through experiencing their infertility problem: on a personal level, in their relationships, and in some cases also spiritual growth (Lee et al., 2009). Another study that assessed men and women before, during and 6 months after infertility treatment found more discouraging data: after unsuccessful treatment the women showed increased anxiety and depression, and at follow-up, >20% of the women showed remaining subclinical forms of anxiety and/or depression. Personality characteristics, meaning of the fertility problems, and social support determined the course of psychological response. The men in the study showed no significant changes in anxiety or depression levels (Verhaak et al., 2005). The marital relationship has been reported in several studies to either be strengthened during the infertility treatment or not affected at all (Sydsjö et al., 2005; Holter et al., 2006). In a Swedish study on gender differences in psychological reactions to infertility, the women reacted more strongly to the infertility problem than the men, and the most important factor for the women of having children was summarized as “the major focus of life”. The most important factor for the men of having children was “The male role and social pressure” (Hjelmstedt et al., 1999).

What the patients experience as most stressful during the treatment, besides an unsuccessful treatment, is the waiting time after embryo transfer until the pregnancy test (Eugster et al., 1999). After successful IVF treatments, IVF patients experience more stress during pregnancy than parents who conceived spontaneously (Eugster et al., 1999). Mothers of IVF children have

been shown to experience a higher quality in the parent-child relationship than mothers who naturally conceived (Eugster et al., 1999). In studies on coping behaviour in IVF couples, avoidance behaviour (e.g. avoiding being with pregnant women or children, working to take one’s mind off things) and accepting responsibility (e.g. criticizing one-self, feeling responsible for having brought the problem on oneself) were related to increased infertility stress. Seeking social support, problem solving, distancing and meaning-based coping (e.g. trying to find a meaning in what is happening) were related to less infertility stress (Schmidt et al., 2005; Peterson et al., 2006; Peterson et al., 2009).

Do psychological factors affect the live birth rates?

The questions of whether stress, depression, anxiety and other psychological factors have negative impacts on the live birth rates, and whether psychological interventions can improve live birth rates, have been matters for a great deal of research. No scientific consensus has been reached, and there are plenty of contradicting studies. Although two systematic reviews found no evidence for a relation between psychological stress and decreased pregnancy rates, the studies in this field are heterogeneous and difficult to compare (Klonoff-Cohen et al., 2005; Homan et al., 2007).

A study conducted at our centre showed no association between psychological well-being before treatment measured using questionnaires and the chance of pregnancy (Anderheim et al., 2005). In another study from our centre, the patients were offered extended encounters with a midwife before, during and after the treatment, at which the

patients were encouraged to talk about their feelings and what was important to them. The extended encounters did not result in any significant effects on the patients' well-being or on the pregnancy rates. However, the patients who did not achieve a pregnancy and who had extended midwife encounters expressed greater satisfaction with the care than the controls (Anderheim et al., 2007).

In a systematic review of the effects of psychosocial interventions in infertility treatment, pregnancy rates were not found to be affected by the interventions. In terms of increase the psychological well-being, group interventions that emphasized training and skills (e.g. relaxation training) were more effective than interventions that emphasized emotional expression (e.g. discussing feelings and thoughts, Boivin, 2003).

Reasons for discontinuing IVF treatment

An unexpectedly high proportion of the couples in Paper I discontinued their IVF treatment, 54% of all couples who did not achieve a live birth. A small group (3.1%) had only cancelled cycles and performed no embryo transfers at all. The dropout rates for patients who did not achieve a live birth after the first and second completed cycles were 19.9% and 39.0%, respectively. Similar or higher dropout rates have been reported in other studies. In a study from Germany, where the insurance companies cover the cost of the first four treatments, it was shown that 39.3% of the non-pregnant patients dropped out after one started cycle and 44.1% after two started cycles (Schroder et al., 2004). In a Dutch study where there is a similar reimbursement system as in Sweden, 43% of the patients dropped out following the first cycle and 57% following the second cycle (Verberg et

al., 2008a). In a British study, with a high rate of self-funded patients, there was a dropout rate after one cycle of 58.5% (Sharma et al., 2002).

The most frequently reported reasons for discontinuing treatment given in Paper II were psychological stress (26%) and poor prognosis (25%). A recent Dutch study on reasons for dropout showed similar results (Verberg et al., 2008a). Physical and psychological factors were the most important reasons (28%). This study also showed that mild stimulation can result in lower dropout rates (Verberg et al., 2008a). In a British study, psychological stress was cited as reason for dropping out by 36% of the patients, and lack of success by 23%; these two reasons were strongly correlated (Rajkhowa et al., 2006). The fact that treatment was not reimbursed was stated by 23% of the patients as the reason for dropping out, and changes in personal circumstances by 30% (Rajkhowa et al., 2006). Pre-treatment psychological characteristics were related to the dropout rate in the group who stopped for psychological reasons in one study (Smeenk et al., 2004), while another study found no influence on the dropout rates of pre-existing anxiety or depression (Lintsen et al., 2009). In-depth interviews with women who decided to drop out showed that the women felt they had started treatment with unrealistic expectations. The women felt vulnerable to the pressures from society and media. Although the decision to cease IVF treatment was a way out of the distress it also led to a sense of confrontation with issues they had previously avoided. Couples who had adopted a child reported feeling less societal pressure than those who remained childless (Peddie et al., 2005). Several studies have shown correlations

between poor prognosis and dropping out of treatment (Sharma et al., 2002, Malizia et al., 2009), but some studies have also shown no difference in prognostic factors among those who continued and those who dropped out (De Vries et al., 1999).

What can be done to lower the dropout rates?

In countries with no reimbursement system covering infertility treatments, lack of funding is an important reason for not continuing treatment (Rajkhowa et al., 2006). In these countries, subsidized IVF treatment would be an important political step in order to increase nativity rates.

A considerable proportion of the patients in Paper II reported organizational shortcomings: “never the same people from one appointment to the next”, “stressful assembly-line treatment”, “we needed more information on treatment and alternatives”. Taking these comments into consideration,

organizational and educational improvements can be made to create a more “patient-friendly” environment at IVF clinics. A recent American article on dropout reasons among insured patients, showed that stress was the most important factor, and the top-rated suggestions from the patients for patient support, were written information on how to deal with psychological stress and easy access to a psychologist or social worker (Domar et al., 2009).

In summary, IVF treatment is often experienced as emotionally demanding for the patients. There is no evidence of a negative effect of psychological stress on the live birth rates. Supporting the couples to cope with infertility-related stress is not only valuable to their well-being, but might also decrease the dropout rates, which in turn could lead to improved cumulative live birth rates.

Conclusions

The following conclusions can be drawn from this thesis:

- There is a good chance of achieving a live birth through a treatment programme of three fresh IVF/ICSI cycles including subsequent frozen-thawed cycles. The cumulative live birth rates after one fresh SET including subsequent frozen-thawed cycles was not significantly lower than after DET, in a randomized patient population. The advantage of SET is the dramatically reduced multiple birth rate.
- Frozen-thawed embryo transfers constitute an important contribution to the cumulative live births. The knowledge of which factors are predictive for live birth in frozen-thawed cycles is of value when deciding whether to perform SET or DET in frozen-thawed cycles.
- A large proportion of the patients discontinued the treatment programme before achieving a live birth. The most common reasons were psychological stress and poor prognosis. A frequent comment in the questionnaires was the need of more information about the treatment. The knowledge that many patients perceive IVF treatment as emotionally demanding and feel a need of more information can be useful in patient consultations and when organizing care at the clinics.

Acknowledgements

I am grateful to many persons for their valuable support. In particular I wish to thank:

- All IVF patients who contributed to these studies.
- Professor *Christina Bergh*, my tutor. Thank you for great support and for always encouraging me and believing in my abilities. You are a role model in combining being a warm, feeling woman, with being a successful researcher and physician.
- Dr *Ann Thurin*, MD, PhD, my co-tutor. For valuable support and for nice and interesting conversations.
- Associate professor *Kersti Lundin*, my co-tutor. An impressive woman of skills, professionalism and warmth, for being very supportive.
- Professor *Gudmundur Johannsson*, head of the Endocrinology section, Department of Medicine. For always supporting and encouraging me to do research, and for appreciating my interest in the reproductive endocrinology field.
- Co-author *Barbro Fridén*, MD, PhD for nice and fruitful collaboration.
- Co-author *Gunilla Borg*, midwife, for helpful collaboration.
- Professor *Ian Milsom*, Head of the institute of Clinical Sciences, Department of Obstetrics and Gynaecology. For making it possible to perform this study.
- Professor emeritus *PO Jansson*, for having encouraged me on many occasions.
- Associate professor *Inger Bryman*, Head of the Clinical Department of Gynaecology and Reproductive medicine. Provided good facilities and atmosphere for the study.
- All colleagues and midwives at the Clinical Department of Reproductive Medicine and the Clinical Department of Medicine at Sahlgrenska University Hospital.
- *Nils-Gunnar Pehrson, Mattias Molin, Oskar Röntfors, Gunnar Ekeröth*, for statistical assistance.
- The staff at the IVF laboratory, with whom I shared room during my research, thank you for inspiring me.
- *Annette Nattland* for professional secretary work and for being amazingly kind and helpful when making this book.
- *Ann-Louise Gejervall*, midwife, PhD, for helping me and supporting me during the work with this doctoral thesis.
- Friends and family. All persons in my proximity who stood by my side for better and for worse. My father for love and support and for courageous fighting against his severe illness. Jonas, Olle and Sanna Gillmor for making my life much more beautiful.

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