Zdravka Veleva

FACTORS AFFECTING THE OUTCOME OF IVF/ICSI
FACTORS AFFECTING
THE OUTCOME OF IVF/ICSI

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Abstract

Fertility declines with advancing age and the number of couples seeking infertility treatment at an older age is constantly increasing. A top quality embryo is believed to have the highest potential for implantation and development into a child. A better understanding of the relative importance of patient and treatment characteristics and of embryo quality could help to optimise the existing therapeutic schemes and the safety of in vitro fertilisation/intracytoplasmic sperm injection (IVF/ICSI).

In this work, databases of five Finnish infertility clinics were studied retrospectively. Data on treatments performed in the years 1994–2005 were collected. A total of 19,000 treatment cycles were analysed. Special attention was paid to the relative significance of the transfer of top quality embryos with regards to pregnancy, miscarriage, live birth and cost of treatment in the general IVF/ICSI patient population and in groups with expected poor outcome.

The results showed that the transfer of a top quality embryo is associated with a better chance of pregnancy and live birth. However, it does not diminish the probability of miscarriage. Both low and high BMI increase the miscarriage rate. Advancing age and a positive history of previous miscarriages are also associated with a higher probability of miscarriage. In addition, the need for hormonal substitution in cases of frozen-embryo transfer is a risk factor of miscarriage, probably because of suboptimal endometrial function.

Since the transfer of several embryos leads to multiple pregnancies, which are associated with a high risk of maternal and fetal complications, elective single embryo transfer (eSET) of a top quality embryo allows all additional good quality embryos to be frozen and transferred later in frozen-thawed embryo transfer cycles. The present work demonstrates that eSET is a safe treatment strategy at least until the age of 40. However, it might not be performed in women with fewer than four collected oocytes, since the prognosis might remain poor even if the response is improved in a following cycle.

When eSET is applied routinely and on a large scale, it diminishes treatment costs while increasing the number of deliveries occurring at term, making IVF/ICSI at the same time safer and more affordable even to patients without access to reimbursed IVF treatment.

Keywords: cost effectiveness, elective single embryo transfer, fertilisation in vitro, frozen-thawed embryo transfer, low response, obesity, spontaneous abortion, top quality embryo
Acknowledgements

The idea for this work originates back in the spring of 2002 when Juha S. Tapanainen asked me whether I would be interested in doing research in Oulu. He was bold enough to say the PhD would take only a couple of years and I was enthusiastic enough to believe it. However, in the six years that the thesis took to form I have had the pleasure of taking part in an exciting project with not only one, but two supervisors and an amazing amount of unanalysed data.

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Oulu, 25 August 2008

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ach</td>
<td>acetylcholine</td>
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<tr>
<td>AMH</td>
<td>anti-Müllerian hormone</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CC</td>
<td>clomiphene citrate</td>
</tr>
<tr>
<td>CEA</td>
<td>cost-effectiveness analysis</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>eSET</td>
<td>compulsory single embryo transfer</td>
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<td>DET</td>
<td>double embryo transfer</td>
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<tr>
<td>ET</td>
<td>embryo transfer</td>
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<tr>
<td>eSBT</td>
<td>elective single blastocyst transfer</td>
</tr>
<tr>
<td>eSET</td>
<td>elective single top quality embryo transfer</td>
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<td>FET</td>
<td>frozen-thawed embryo transfer</td>
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<tr>
<td>FISH</td>
<td>fluorescent <em>in situ</em> hybridisation</td>
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<tr>
<td>FSH</td>
<td>follicle-stimulating hormone</td>
</tr>
<tr>
<td>GH</td>
<td>growth hormone</td>
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<tr>
<td>GnRH</td>
<td>gonadotropin-releasing hormone</td>
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<tr>
<td>hCG</td>
<td>human chorionic gonadotropin</td>
</tr>
<tr>
<td>HMG</td>
<td>human menopausal gonadotropin</td>
</tr>
<tr>
<td>HR</td>
<td>high ovarian response</td>
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<tr>
<td>ICER</td>
<td>incremental cost-effectiveness ratio</td>
</tr>
<tr>
<td>ICSI</td>
<td>intracytoplasmic sperm injection</td>
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<tr>
<td>IGFBP-1</td>
<td>insulin-like growth factor-binding protein-1</td>
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<tr>
<td>IR</td>
<td>implantation rate</td>
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<tr>
<td>IVF</td>
<td><em>in vitro</em> fertilisation</td>
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<tr>
<td>LBR</td>
<td>live birth rate</td>
</tr>
<tr>
<td>LH</td>
<td>luteinising hormone</td>
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<tr>
<td>LR</td>
<td>low response</td>
</tr>
<tr>
<td>LR→NR</td>
<td>low response, followed by a normal response in a consecutive cycle</td>
</tr>
<tr>
<td>MBR</td>
<td>multiple live birth rate</td>
</tr>
<tr>
<td>MR</td>
<td>miscarriage rate</td>
</tr>
<tr>
<td>NR</td>
<td>normal response</td>
</tr>
<tr>
<td>NR→LR</td>
<td>normal response, followed by a low response in a consecutive cycle</td>
</tr>
<tr>
<td>nt-eSET</td>
<td>elective single embryo transfer of a non-top quality embryo</td>
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<tr>
<td>OD</td>
<td>oocyte donation</td>
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<tr>
<td>OHSS</td>
<td>ovarian hyperstimulation syndrome</td>
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</table>
OPU  ovum pick-up
PCOS  polycystic ovary syndrome
PCR  polymerase chain reaction
PGS  prenatal genetic aneuploidy screening
PR  pregnancy rate
ROS  reactive oxygen species
SD  standard deviation
tLBR  term live birth rate
WHO  World Health Organisation
List of original articles

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


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1 Introduction

About 15% of all couples are involuntarily infertile [1] and require fertility treatment. Thirty years after the birth of the first infant after IVF, the number of children born worldwide as a result of IVF/ICSI already exceeds three million [2]. Clinical and laboratory procedures have been constantly improving and nowadays pregnancy rates of about 30% per transfer are routinely reported [3]. IVF/ICSI is therefore an important method for correcting unfavourable demographic indices despite associated costs, being as high as one quarter of annual household expenditure in some countries [4].

The biggest challenge for present-day IVF/ICSI is the high proportion of multiple pregnancies, which significantly affect even nationwide demographics [5]. Multiple pregnancies are associated with a worsened obstetric outcome as a result of prematurity [6]. Consequently, the infants have a significantly elevated risk of life-long complications such as cerebral palsy [7], language development delay and behavioural problems [6]. Because of this, the parents of multiples often suffer from exhaustion and have an elevated risk of depression [6].

The cause of multiple pregnancies is the transfer of several embryos at a time. In Europe, more than one embryo is transferred in 84% of IVF/ICSI cycles [3], while in the United States this number is even higher (~99%) [8]. The transfer of one embryo is the only efficient way to minimise the number of multiple pregnancies. There is accumulating evidence that if several embryos are created and one of them is selected for transfer on the basis of good morphology, PR and LBR are high [9-11]. All other good quality embryos can be frozen and transferred later. This strategy results in a cumulative PR of up to 60% in young women [12, 13].

However, the eSET strategy is subject to criticism, as it is widely believed that if only one embryo is transferred, success rates will diminish and the price of treatment will rise [14-16]. Part of this scepticism is based on the fact that embryo morphology does not absolutely correlate with its pregnancy potential [17]. The zygote receives most of its cytoplasm from the oocyte and during the first five or six days embryo development is under the control of factors synthesised in the ooplasm [18]. This means that before embryos are transferred into the uterus, their potential cannot be fully predicted even if they have top quality morphology.

Finland is one of the pioneer countries as regards eSET. Experience with eSET at Oulu University Hospital started as early as in 1996. In the present study, the relative importance of the transfer of a top quality embryo on the outcome of
IVF/ICSI treatment was studied by using the computerised databases of five Finnish infertility clinics. Pregnancy, miscarriage and live birth rates were studied in women approaching the end of their fertile period, in fresh IVF/ICSI and in FET cycles. Most importantly, the overall effect of eSET on the outcome of the whole IVF/ICSI programme and on the costs of treatment was evaluated.
2 Review of the literature

2.1 History of IVF/ICSI

The milestones of IVF/ICSI are presented in Fig. 1. Initially, IVF was performed in a natural cycle. Since only one oocyte was collected at a time, the success rate was very low, with a LBR of only 9.6% [19]. Treatment was also limited only to women with spontaneous ovulation. The introduction of ovarian stimulation in 1981, first using clomiphene citrate [20] and later gonadotropins [21] and GnRH agonists [22] increased the number of collected oocytes and embryos available for transfer. At the same time, indications for IVF treatment broadened to anovulation of different aetiologies. Later on, ICSI allowed severe male reproductive disorders to be treated as well [23].

Initially, the simultaneous transfer of several embryos was favoured because of the low chance of implantation. In 1991, three embryos were transferred in 40.5% and more than 3 embryos in 24.6% of cycles, but the overall LBR per started cycle was only 12% [24]. Despite the low success rate, 25% of the deliveries were multiple births. After the identification of embryo quality characteristics it became clear that the transfer of several good quality embryos dramatically increased the multiple pregnancy rate, to 31–32% if three embryos were transferred [25, 26]. The proportion of triplet and higher-order multiple pregnancies also increased and was as high as 18% if three good quality embryos were transferred [27].

The high multiple pregnancy rate has exposed a large number of children to health risks. Compared with singleton births, perinatal mortality rates are at least four-fold higher for twins and at least six-fold higher for triplets [6]. Twins born after IVF have almost a ten-fold higher risk of being born prematurely than IVF singletons, and because of this a 3.8-fold increased risk of admittance to a neonatal intensive care unit [28]. These risks are even higher in children from higher-order pregnancies [6] who are born before the 37th gestational week with few exceptions (1%) [29]. Multiples also suffer from long-term medical and developmental problems. The risks of prematurity-associated neurological sequelae such as cerebral palsy are 3–7 times higher in twins and over 10 times higher in triplets, compared with singletons [6].
The unacceptably high number of triplet and higher-order pregnancies prompted a restriction on the number of embryos transferred. The transition from triple embryo transfer to DET was started in 1993 [26, 27, 30] and was adopted early on in Europe, but not in the United States [31, 32]. More than five embryos were transferred in a total of 4350 cycles (about 10% of all cycles) in the United States during one calendar year [33].

As already observed in 1993 [27], the transfer of no more than two embryos at a time almost eliminated the triplet and higher-order multiple pregnancies, but not twin gestations and as a result the overall MBR remained high (25–40%) [31, 33-38]. Following publication of the morphological criteria for a top quality embryo [39, 40], the transition from DET to eSET was started. In eSET, one top quality embryo is transferred after ovum pickup. All other good quality embryos are frozen and can be transferred in a FET cycle. eSET was first described in women with medical contraindications to multiple gestation [9]. Since then, a
multitude of trials have shown the effectiveness of eSET in different selected groups of subjects, typically in their first IVF/ICSI cycle and aged less than 36 years [10, 41-45].

Currently, eSET is the recommended strategy in both Europe [35] and the United States [46], but clinical practice shows little adherence to the guidelines outside the Nordic countries [3, 5]. In 2003, there were 37,000 multiple births after IVF in Europe [3], the United States [5] and Canada [47]. More IVF cycles are performed each year and the total number of multiple births, especially twin births, is constantly increasing [3, 5, 8, 36, 37, 48] so that between 1998 and 2003, the number of twin births in the whole population of the United States grew by 17% [5]. eSET is practiced on a large scale in Finland, Belgium and Sweden, where it is regulated by law (in Belgium and Sweden) or by consent of IVF units (Finland) [3]. Finnish data from the years 1996–2006 show that the number of multiple births after IVF/ICSI decreased from 24% to 11%, while the LBR has remained stable [49]. Similarly, Swedish statistics also show that LBR was maintained at around 26% while the MBR decreased dramatically, from about 35% to around 5% in the period 1991–2004 [38].

2.2 Measuring the success of IVF/ICSI

Traditionally, IVF outcome has been expressed in terms of biochemical or clinical pregnancies and total live births. However, these parameters also include multiple gestations and births, which are considered to be complications of IVF because of the dimensions of their prematurity-related problems [6, 35]. This is why a lengthy discussion on the definition of the main outcome of IVF treatment ended without reaching consensus [50]. Competition between IVF clinics has resulted in outcome being expressed for selected groups of patients (biochemical PR/ET, for example). This can be illustrated with data from a study of BMI, in which the biochemical pregnancy rate/ET among women with normal BMI was 34.2% (1033/3018) while for the same group the LBR/cycle was “only” 20.8% (718/3457) [51]. In addition, the outcome of a stimulation cycle can be fully evaluated only if the outcome of FET is also taken into account in cumulative PR or LBR [52]. In 2003, the LBR after all fresh cycles performed in Finland was 21.5% while the cumulative LBR/OPU after one or several FET cycles per woman was much higher (31.0%) [3]. This can be explained by the policy of extensive use of cryopreservation in Finland. A drawback of cumulative outcome is that sufficient time should be allowed for all FET cycles to take place. This is
why the design of randomized studies almost never reflects general practice. For example, in a study of eSET, half of the patients had between four and 16 good quality embryos, but the study involved evaluation of the outcomes of the transfer of two embryos only [42]. In addition to several FET cycles, IVF/ICSI patients frequently undergo more than one stimulation cycle as well. While cumulative outcome from several stimulations has been reported [51, 53, 54], the full effect of treatment per subject has not been evaluated until now.

It is difficult to decide whether singleton pregnancies are the desired outcome of treatment (singleton LBR), as even singletons after IVF are often born prematurely [55]. However, with more than three million children born as a result of IVF/ICSI, the technology is not a novelty treatment anymore [2]. Criteria already in force for evaluation of spontaneous pregnancies should be used for the outcome of IVF/ICSI gestations as well. According to the WHO, the pregnancies with the best outcome are usually delivered between 38 and 42 weeks of gestation (term live birth). Therefore, the tLBR is the most logical measure of the clinical outcome of IVF/ICSI, with both singletons and twins included, since twins born at term generally have a better prognosis than premature ones. Up to now, the tLBR has been used only in a few investigations [56, 57].

2.3 Embryo quality

2.3.1 Morphological

The assessment of embryo quality in IVF/ICSI is essential and it determines the number of embryos to be transferred and frozen. Cleavage speed and degree of fragmentation were the first parameters to be assessed [58]. Other morphological aspects found to be related to embryo quality were cytoplasm appearance, blastomere irregularity and degree of fragmentation [59]. Together with cleavage rate, these factors were evaluated in the first embryo quality scoring method (the cumulative embryo score) which was aimed at optimising PR while avoiding triplet and other higher-order pregnancies [59].

At present, morphological evaluation of the embryo routinely includes zona pellucida assessment, as a thick zona pellucida has been found to affect fertilisation negatively [60]. Multinucleation of blastomeres is another factor that diminishes the chance of pregnancy [61]. Embryo quality is expressed not only in terms of morphology and cleavage speed two or three days after fertilisation but
also as regards the time of the first mitotic division [62, 63]. Embryos which complete the first mitotic division within 25–27 h after insemination have been associated with higher PR (40.5% vs. 31.3%), compared with late-cleaving ones [63].

The first grading score of individual embryos was published in 1995 after analysis of single embryo transfers on day 2; the best embryos had 4 regular cells and lacked anucleated fragments [39]. The criteria were further extended so that the definition of a top quality embryo included no multinucleated blastomeres, ≤20% anucleated fragments and four or five blastomeres on day 2, or seven or more blastomeres on day 3 [40]. The results of an analysis by Van Royen and colleagues suggested that eSET might be considered if a top quality embryo is available [40]. In a study of eSET, transfer of a single top quality embryo resulted in a PR of 44%, while after the transfer of one non-top embryo the PR was only 19% [64]. This grading system, with slight modifications, is used in most centres that perform eSET.

2.3.2 Developmental stage

Most often embryos are transferred on day 2 or 3 after fertilisation in order to allow selection of the most suitable ones for transfer. Cultures supporting later embryo development have been used since the late 1990s [65]. Embryo implantation into the endometrium takes place on day 5 and therefore the transfer of blastocysts on that day is aimed at improving the synchronicity of endometrial and embryonic development. Good quality blastocysts are also supposed to have higher pregnancy potential compared with cleavage stage (day 2–3) embryos, since not all embryos survive until the blastocyst stage.

There have been conflicting reports on the advantages of blastocyst transfer. In a recent meta-analysis the outcomes of prospective randomized trials were reviewed and no difference in PR per couple in good prognosis patients was found (day 2–3 38.8% vs. blastocyst 40.3%) [66]. Furthermore, there was no difference in MR per couple between the two groups. The rate of embryo freezing per couple was over two times higher in cleavage-stage transfers, while in the blastocyst group there were more cases in which no embryos were available for transfer (OR 3.21). To date, there is insufficient data for a comparison of the outcome of FET cycles among cleavage-stage and blastocyst groups.
2.3.3 Genetics

In the early embryo, cell cycle control is performed by factors synthesised in the oocyte before fertilisation. Expression of the embryonic genome increases gradually from the 8-cell stage onwards [18] and genetically abnormal embryos are gradually eliminated by natural selection. Due to maternal control of early divisions, a significant proportion of morphologically normal IVF/ICSI embryos have genetic abnormalities [67]. Consequently, it has been speculated that genetic analysis of the embryo might help in selection of those with normal composition and might improve the LBR.

In prenatal genetic analysis, a single cell is removed from the embryo. Usually, this happens on day 3 although it is also possible in the blastocyst stage when several cells can be biopsied at the same time with more reliable results [68]. Aneuploidy screening by means of FISH (PGS) is then performed on the biopsied cell(s) [69]. Genetic analysis by means of PCR can also be performed if there is risk of transmission of monogenetic diseases [70, 71]. In Finland, genetic analysis has been performed on embryos of carriers of translocations and of infantile neuronal ceroid lipofuscinosis [72].

Since PGS is an expensive procedure, it is currently performed in cases with an expected high risk of chromosomal abnormalities, such as advanced female age, low ovarian response and recurrent implantation failure [68, 73]. The major drawback of this procedure is that results are difficult to interpret because of embryo mosaicism, i.e. different genetic composition of the cells of the same embryo (Fig. 2). Mosaicism occurs because of mitotic errors in the cleaving embryo, and has been found in up to 70% of biopsied embryos [68]. Because of mosaicism, the risk of misdiagnosis is as high as 60% of cases with one biopsied cell [74]. In line with this fact, two recent meta-analyses revealed insufficient evidence for improved PRs after PGS [68, 75].

Mosaicism further complicates the results of genetic analysis, since the prognosis after transfer of a mosaic embryo might depend on the number of aneuploid cells. If this number is low (<40%), the embryo may have a relatively high chance of developing to the blastocyst stage (78%), while a higher number of abnormal cells may be associated with a lower survival rate (33%) [76]. This finding can be explained by activation of the embryonic genome during the morula stage (8–64 cells) [18] and the following mitotic arrest of abnormal cells, leading to death of either individual cells or of the entire embryo. The loss of abnormal embryos continues after implantation into the uterus, so that the
majority of the remaining abnormal embryos are lost before the 12th gestational week [18].

Fig. 2. Proportions of cytogenetically normal, abnormal and mosaic embryos at different stages of embryonic development. Modified, with permission, from Los et al. [18].

2.3.4 Metabolism

Because neither morphological criteria nor PGS can fully predict the potential of an embryo to develop into a healthy child, new non-invasive methods are currently being investigated. Analysis of embryo culture medium reveals the components of the medium used or released by the embryo, such as glucose, pyruvate and ammonium [77].

Since embryo metabolism is thought to be a critical determinant of viability, investigation methods at the level of the metabolite network (metabolomics) are currently being developed. It is considered that a viable human embryo possesses a unique metabolic fingerprint, which is expressed in culture medium as a metabolic footprint. The entire spectrum of interactions between embryo and culture medium is consequently studied using novel spectroscopic methods which show distinctly different spectra of embryos in cases of no implantation, and biochemical and clinical pregnancy [78].
2.3.5 Quality of frozen-thawed embryos

To date, there is no generally accepted grading system for frozen-thawed embryos, even though FET cycles have been performed successfully for about 15 years [79]. Various factors have been reported to affect the outcome after freezing and thawing, including cleavage stage [12, 80], pre-freeze morphological appearance [81, 82], the ovarian stimulation procedure used before oocyte collection [83] and the outcome of the fresh embryo transfer cycle [84, 85].

Not all embryo cells may survive after thawing. An embryo is considered to have survived cryopreservation if at least half of the initial blastomeres remain intact [86]. Survival rates are 50–80% [87, 88]. They are higher in morphologically normal embryos with no fragments and equally-sized blastomeres [89, 90] and in embryos with a relatively low cell number at freezing [83], as shown in studies on the outcome of day 2 and day 3 embryos [86, 91].

Reports concerning embryo survival and the transfer of damaged embryos are conflicting [81-83]. Overnight culture of thawed embryos allows embryos to be chosen for transfer if cleaving has restarted after thawing [92]. The implantation rate is higher in cleaved embryos compared with uncleaved ones (20% vs. 3%) [93]. Accordingly, a high rate of aneuploidy (75–80%) has been observed in morphologically good embryos which do not cleave after overnight culture [94, 95].

2.3.6 Male and female factors and embryo quality

Embryo grading cannot give an accurate prediction of pregnancy. This is why the evaluation of clinical factors helps predict the outcome of IVF/ICSI. The majority of these factors are characteristics of the female rather than the male patient. This can be explained by the fact that although the zygote contains equal amounts of chromosomal DNA of maternal and paternal origin, most of the cytoplasm and all the mitochondria originate from the oocyte. The results of several studies have indicated a relationship between oocyte and embryo quality [96-98], but the importance for early embryo development of factors related to spermatozoa is less clear. An analysis of cycles with shared oocytes in an OD programme revealed that embryo morphology (blastomere uniformity and fragmentation) is determined by oocyte quality, while the cleavage rate is affected by both oocyte and spermatozoa quality [99].
The outcome of clinical pregnancy is also affected more by maternal rather than paternal factors. A large multicentre European study of spontaneous pregnancies revealed that although both paternal and maternal age implied an increased risk of miscarriage with increasing age, advanced maternal age plays a more significant role (Fig. 3) [100]. In line with observations in spontaneous pregnancies, a significant increase in pregnancy loss (41.5% vs. 24.4%), a decrease in LBR (41.3% vs. 56.0%), and a decrease in blastocyst formation rate in an OD programme have been noted in men aged more than 50 years, compared with younger men [101]. These results have also been confirmed in IVF/ICSI patients using their own gametes [102].

### 2.4 Endometrial receptivity

It has been estimated that uterine receptivity accounts for about 31–64% of implantation [103]. A blastocyst can implant into the endometrium only during a short period of time called the window of implantation. It is believed that it lasts about 48 hours, beginning 6–10 days after the LH surge in a spontaneous cycle [104]. The window is advanced in clomiphene citrate- or gonadotropin-stimulated cycles [105-107] and can be delayed in steroid hormone replacement cycles for donor recipients [108].
Embryo implantation is regulated by a multitude of factors (Fig. 4). Glycodelin is the major component of endometrial secretion and its expression is regulated by progesterone. Glycodelin has immunosuppressive properties and contributes to the maintenance of pregnancy during the first trimester [109]. It is believed that the appearance of glycodelin secretion reflects endometrial maturation, which is essential for embryo implantation [110]. Accordingly, concentrations of serum glycodelin have been found to be decreased in women with early pregnancy loss [111]. It is believed that the outcome of the first few days after implantation is determined by embryo morphology but that the continuation of pregnancy beyond 6 weeks is more dependent on the combination of embryonic and uterine factors [112]. In humans, there is accumulating evidence of a molecular dialogue between the developing embryo and the maternal endometrial epithelium [113-118]. This crosstalk involves, among other things, nutrition markers such as leptin [115, 119] as well as factors regulated by insulin, such as IGFBP-1 and αβ3 integrin [120-122].

It is assumed that in cases in which the ovarian response is good and several high quality embryos are created, the uterine milieu is supportive of a pregnancy. Hormonal manipulation of the endometrium has recently been reviewed [123]. It is assumed that the asynchronous development of the endometrium during stimulation with GnRH agonists and gonadotropins is normalised with progesterone or hCG supplementation in the late luteal phase, so that stimulation has no major impact on actual endometrial receptivity. However, stimulation with
gonadotropins and GnRH antagonists may be associated with impaired endometrial receptivity, compared with stimulation using a GnRH agonist, as shown in a recent analysis of top quality embryo cycles [124].

In IVF/ICSI, endometrial thickness is the only routinely assessed “marker” of uterine receptivity. Oocyte donation cycles are a good model for studying endometrial receptivity. In a study of OD cycles, lower PRs and LBRs were found in subjects with an endometrial thickness less than 8 mm before the start of progesterone administration, compared with those with a thicker endometrium [125]. However, endometrial thickness is a weak predictor of pregnancy [126, 127], since successful pregnancy has been established even in cycles with a maximal endometrial thickness of 4 mm [128, 129]. Other non-invasive methods of evaluation include Doppler and 3-D ultrasonography. However, they are not routinely performed and the results are conflicting [104].

While the window of implantation is relatively narrow in a spontaneous cycle, in an artificial cycle it can be extended to up to five weeks using fixed doses of estradiol. This has been extensively studied in cycles of recipients of donated oocytes [108, 130–133]. In an analysis of 3089 OD cycles, decreasing IRs and PRs were observed only after the 7th week of estrogen replacement [134]. Progesterone administration seems to allow normal endometrial maturation regardless of the length of estradiol therapy. However, a short duration of estradiol replacement (<10–14 days) is associated with a high MR (up to 53%) [130, 132]. Recently, it has been suggested that it is appropriate to start progesterone administration as soon as the endometrium is developed sufficiently, i.e. when it is at least 8 mm in thickness with a trilaminar ultrasonographic appearance, and to perform embryo transfer not before 3–4 days of progesterone treatment [135].

Uterine receptivity remains constant until the end of the fourth decade of life, as shown in a study of 17,300 OD cycles. Beyond the age of 38 years, implantation, pregnancy and live birth rates decreased while the frequency of miscarriages increased [136]. This observation has been supported by the results of other studies involving transfers of embryos created by the patients’ own gametes [137, 138].
2.5 Female age

Female age is a major determinant of the success rate of infertility treatment and was the first recognised prognostic factor in IVF/ICSI. Women are tending to delay childbirth until the third decade of life in growing proportions. In Finland, the percentage of women >35 years old giving birth for the first time increased from 6% in 1987 to 18% in 2006 [49]. Statistics from the United States show that the birth rate (per 1000) among women aged 35–39 years almost doubled in the period 1976–1998 (from 19.0 to 37.4) [139, 140]. Since fertility decreases rapidly with age [1], the number of women seeking infertility treatment because of advancing age is increasing.

2.5.1 Ageing and the ovarian pool

It is believed that women are born with a fixed number of oocytes arrested in the first meiotic division, and the numbers subsequently decline during their lives [141]. The number of germ cells reaches a maximum at mid-gestation during fetal life, and declines continuously thereafter. This trend is accentuated at about the age of 37–38, when there is an acceleration of the loss of oogonia [142]. Exhaustion of the oocyte pool occurs around the age of 50 years, leading to menopause.

Recently, this theory has been challenged by the discovery of germ cells that can differentiate into oocytes and follicles in mouse ovaries after birth [143]. These germ cells originate in the bone marrow and reach the ovaries via peripheral blood [144]. In a recent study of adult human ovaries it has been shown that progenitor cells from the tunica albuginea and cells from ovarian surface epithelium can differentiate into granulosa cells and oocytes [145, 146]. These data indicate that the pool of primary follicles in adult human ovaries might not be static, as previously believed, and that a cessation of follicular renewal, rather than a depletion of the static ovarian pool, might take place with advancement of age [147]. These new findings and their interpretations are the subjects of considerable scrutiny and criticism at the present time [148].
2.5.2 Ageing and oocyte quality

Neither the classical nor the new ovarian pool theory challenges the fact that oocyte numbers and quality decline with age. It has long been known that in spontaneous pregnancies the risk of chromosomal abnormalities increases with maternal age [149, 150]. Likewise, studies of human oocytes have revealed a growing number of chromosomal abnormalities in the oocytes of older women [151, 152]. As explained above, oocyte abnormalities are the main cause of embryonic chromosomal defects [152–154].

Causes of chromosomal abnormalities have been reviewed in a recent article [155] and are summarised in Fig. 5. The occurrence of aneuploidy has been classically attributed to chromosomal non-disjunction during either meiosis I or II [156]. However, premature separation of chromatids during meiosis might be the main factor contributing to the formation of oocytes with abnormal chromosomal complements [157]. This is caused by the gradual but constant age-related degradation of cohesins and other factors holding the four chromatids together during metaphase I.

Fig. 5. Causes of chromosomal abnormalities of human oocytes, leading to non-disjunction or to premature disjunction of chromatids during meiosis I. Based on Djahanbakhch et al. [155].

Human oocytes enter the first stage of meiosis during fetal life but are selected for further development years later in adult life. Until then, the oocyte’s mitochondria are exposed to ROS, which damage the mitochondrial DNA. This leads to abnormal oxidative metabolism and production of adenosine triphosphate, which might be responsible for abnormalities in the meiotic spindle [158].
The decline in fertility in older women can also be explained by telomere shortening due to the combined effects of prolonged exposure to ROS and telomerase deficiency [159]. Telomeres are repetitive DNA sequences at chromosomal ends. Adequate telomere length is essential for the proper alignment of chromosomes during metaphase, but this length might be diminished in oocytes from older women because of damage caused by ROS. The enzyme telomerase protects against telomere shortening. Its activity in female germ cells is significant during early fetal life but is limited in later-stage oocytes.

The increased incidence of oocyte abnormalities with maternal age can also be explained by hormonal imbalances, abnormalities in follicular development due to ageing of the somatic cells surrounding the oocyte and impaired perifollicular microcirculation [155].

### 2.5.3 Female age and outcome of IVF/ICSI

The deleterious effect of increasing age on the outcome of IVF/ICSI is indisputable. Lower numbers of oocytes are collected and fewer embryos are created, leading to lower PRs and LBRs and increased MRs. It is generally considered that this decline starts after the age of 35 years and the effect is more pronounced after the age of 40. Accordingly, female age is either studied in groups or in linear regression models. Linear regression analysis of 1101 IVF cycles with ovum pickup showed a significant negative linear correlation between age and ongoing pregnancy rate [160]. The ongoing PR/ET declined from 26% in patients younger than 30 years of age to 9% in those aged 37 years, whereas the MR increased from 29% in women under the age of 40 to 50% in those ≥40 years old.

If a large sample of cases is studied, more complex interactions appear between age and the outcome of IVF/ICSI. A polynomial (cubic) relationship between age and LBR was found in an analysis of almost 37,000 IVF cycles [161] (Fig. 6), with a peak at about 30 years of age, after a number of confounding factors were studied simultaneously (previous live births, diagnosis, duration of infertility, number of previous unsuccessful IVF treatments). To date, this relationship has not been explained but it is possible that the determining factor will be identified in the future.
Despite the increased number of genetically abnormal embryos and resulting lower PRs, women over the age of 35 years who conceive after IVF/ICSI are still at risk of multiple pregnancy. According to US national surveys, multiple births in women aged 35–39 constitute 12–21% of all live births in this age group if two embryos are transferred [8, 32, 33]. If the number of embryos in this age group is increased from three to ≥7, the percentage of triplet and higher-order gestations increases from 4% to 22.3%, respectively, and the MBR to over 40% [32]. Multiple pregnancies are especially risky in older women, since the frequencies of complications such as gestational diabetes and pre-eclampsia are higher in these subjects [162, 163].

### 2.5.4 Individual ageing rates

The mechanisms that determine individual speeds of reproductive ageing are not fully understood. The importance of environmental and lifestyle factors such as oral contraceptive use, parity and smoking [164], chemotherapy, radiotherapy, pelvic surgery [165, 166], pelvic infection or tubal disease [167, 168] is well known. However, these factors do not explain all variations in the length of the fertile period. An investigation into early ovarian ageing, based on epidemiological data on variation of age at menopause, revealed that about 10% of women might be affected by an accelerated decline of fertility as early as at the age of 32 years [169].
Current knowledge about the genetics of reproductive ageing has recently been reviewed [170]. Estimates of heritability of age at menopause range from 30 to 85% and reproductive ageing is most likely determined by the interplay of many genes. Genes that have been identified so far include those that determine factors regulating the transition from primordial follicles into early growing follicles, blood circulation factors (coagulation factors V and VII and apolipoprotein E), steroid hormone conversion and apoptosis factors.

2.6 Low ovarian response

LR is observed when the outcome of ovarian stimulation is suboptimal, leading to a low chance of pregnancy and birth after IVF/ICSI. LR is one of the significant problems of IVF/ICSI because it occurs in up to 24% of cases [171]. The diagnosis, prognostic factors and treatment options for women with LR have been the subject of several reviews [170-176]. Although much is known about the nature of LR, numerous definitions exist because of the different diagnostic tools used in evaluation of the infertile patient.

They include the following:

- Low peak estradiol level during stimulation (<300 to <500 pg/ml).
- Estradiol level <200 pg/ml on day 5 of stimulation.
- Elevated day 3 FSH (≥7 to ≥15 mIU/ml).
- Low number of collected oocytes (<3 to <5).
- Low number of growing follicles (from <3 to <5 on day of hCG).
- Female age ≥40 years.
- Lately, the definition of poor response also includes poor embryo quality [177].

It is believed that women with LR are in the early stages of ovarian ageing, even if they have regular menstrual cycles. This is illustrated by a study in which a lower number of antral follicles and higher FSH and progesterone levels were found in women with LR and regular menstrual cycles [178]. Furthermore, a LR in first IVF treatment is associated with an increased risk of early menopause [179]. Because of individual variations, LR can occur even in women of young calendar age [169, 180].

PGS studies of embryos of low responders have shown that 54–64% of the embryos in patients with poor prognosis and/or LR have chromosomal abnormalities [17, 73, 181, 182], although they look morphologically normal.
This may explain low PRs (3.2% per started cycle) [183] and the high MR (up to 90%) observed in women with LR [184-188].

### 2.6.1 Prognostic factors of LR

As a reflection of the different characteristics of ovarian reserve, several prognostic factors of LR have been proposed. Although women of ≥40 years have a five times greater risk of LR, compared with younger women [189], age has a low predictive power [186, 190-195]. The basal FSH concentration was the first widely used endocrine marker of ovarian reserve that had better potential than age for predicting decreased ovarian function and diminished success rates after IVF [190, 191]. Elevations of basal (day 3) FSH may reflect declining ovarian reserve despite regular menstruation, as serum FSH levels in the early follicular phase begin to rise 5–6 years before menopause [196]. In a study of 758 stimulations, women with moderately elevated (15–24.9 mIU/ml) and elevated FSH levels (>25 mIU/ml) had lower ongoing PRs, compared with women with normal basal FSH of <15 mIU/ml (9.3% vs. 3.6% vs. 17%, respectively) [191]. Basal FSH is still widely used in the evaluation of infertility. However, high intercycle variability means that it does not accurately predict the response to gonadotropin stimulation [197].

Hormonal markers of low ovarian reserve used predominantly in the past include a high basal estradiol level [198], an elevated day 3 FSH/LH ratio [199] and a low day 3 LH level [200]. A low day 3 serum inhibin-B (<45 pg/ml) is associated with fewer collected oocytes after ovarian stimulation [201]. This can be explained by lower follicle numbers and respectively fewer granulosa cells in low responders. A previous LR or a low number of collected oocytes in previous treatment may also lead to a repeated low response in a subsequent stimulation [176].

The number of antral follicles (2–5 mm) visible in ultrasonography is a reflection of the actual resting oocyte pool [202–204]. The number of antral follicles decreases by about 60% between 22 and 42 years of age [204], at a rate of 5% per year before the age of 37 years and 17% per year thereafter [202]. The number of antral follicles before gonadotropin stimulation has been found to correlate with the number of collected oocytes [192] and no pregnancy was observed if fewer than four antral follicles were visible before stimulation in a study of 149 treatment cycles [205]. To date, the antral follicle count provides the best estimation of ovarian response, compared with age, BMI and hormonal
markers and consequently it is currently considered to be the best single tool in the prediction of LR [186, 192-195, 206].

The predictive value of AMH is also gaining popularity. AMH is a member of the transforming growth factor superfamily involved in the regulation of follicular growth and development [207]. In the ovary AMH is produced by the granulosa cells of early developing follicles. Compared with other hormonal tests, AMH seems to be the best marker reflecting the decline of reproductive age [207]. It can also be used to predict the outcome of IVF stimulation, since one study revealed antral follicle count and AMH to be equally important in predicting ovarian response [208]. Furthermore, IVF failure has been associated with AMH levels less than 1.1 ng/ml [209].

2.6.2 Management of LR

Long protocol with a GnRH agonist is considered the standard regimen in most IVF centres. However, only two studies have concerned investigation of the outcome of low responders in consecutive long protocol cycles with an increased gonadotropin dose. In the first study [210] the daily gonadotropin dose was increased to 450–600 IU, but the PR in previous low responders (6%) was much lower than that in women with a normal response (29%). In the second study [183], the PR among previous low responders remained as low as 3% despite an increase of the gonadotropin dose to 450 IU/day.

A multitude of other approaches have been attempted to overcome the low success rate associated with LR [171-175]. They are summarised in Table 1.

Table 1. Protocols used for treatment of low responders.

<table>
<thead>
<tr>
<th>Changes of gonadotropin and/or GnRH analogue administration:</th>
<th>Use of adjuvants or laboratory methods:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased gonadotropin dose with or without change in protocol</td>
<td>Glucocorticoids (immunosuppression)</td>
</tr>
<tr>
<td>‘Milder’ pituitary suppression: gonadotropins alone, CC+HMG/FSH, short and ultrashort protocols</td>
<td>Use of GH, GH-releasing factor, pyridostigmine (increases GH secretion)</td>
</tr>
<tr>
<td>Replacing purified FSH with recombinant FSH</td>
<td>L-arginine (donor of nitric oxide)</td>
</tr>
<tr>
<td>Luteal initiation of gonadotropins</td>
<td>Low dose aspirin (improved microcirculation)</td>
</tr>
<tr>
<td>Luteal initiation of GnRH agonist (stop-Lupron)</td>
<td>Routine use of ICSI</td>
</tr>
<tr>
<td>Pre-treatment with oral contraceptive pills or progestogens</td>
<td>Assisted hatching</td>
</tr>
<tr>
<td>Natural cycles</td>
<td></td>
</tr>
</tbody>
</table>
Unfortunately, no stimulation protocol has been proved to be beneficial for low responders. A meta-analysis of the outcome with a GnRH agonist long protocol vs. short protocol vs. GnRH antagonist protocol revealed similar results with each protocol [211]. An ongoing randomised study of long vs. short vs. antagonist protocol may provide an answer to the problem of LR management [212].

2.7 BMI as an outcome predictor of IVF/ICSI

The BMI, calculated as the ratio between a subject’s weight and squared height, is a measure that can be used to evaluate energy storage levels in the body. It is modifiable and depends on genetic as well as lifestyle characteristics.

2.7.1 Overweight, obese and insulin resistant women

The prevalence of overweight (BMI 25–29.9 kg/m²) and obese adults (BMI ≥30 kg/m²) is rising around the world. In Western countries the prevalence of obesity is increasing at a rate of 1–6% per year [213]; this rate is now referred to as the obesity epidemic.

Adipose tissue is a complex metabolic and endocrine organ that responds to signals from circulating hormones, as adipocytes express receptors for pituitary hormones and hypothalamic peptides, including FSH and LH [214]. It also modifies steroid hormones and produces adipokines [215].

Leptin is the most studied adipokine. Its serum levels are in direct proportion to adipose tissue mass as well as to nutritional status in pregnant [216] and non-pregnant women [217] and during ovarian stimulation [218]. Leptin initiates pubertal development, accelerates GnRH pulsatility and stimulates pituitary release of LH and FSH [219]. In addition, leptin and its receptor are expressed in reproductive tissues, including the secretory endometrium [115, 220-222] in which they may regulate uterine angiogenesis [223]. They are also expressed in the blastocyst and participate in embryo implantation [115, 222].

Obesity is characterised by high leptin levels that are related to insulin resistance through altered fatty acid metabolism in skeletal muscle [224, 225] and a pro-inflammatory shift of the immune system [226, 227]. The inflammatory molecules TNF-α and IL-6 have been shown to alter insulin sensitivity by affecting different key steps in the insulin signalling pathway. Their serum concentrations are positively correlated with obesity, impaired glucose tolerance
and insulin resistance [213]. In addition, abnormal levels of TNF-α have been shown to be involved in the pathogenesis of miscarriage and recurrent miscarriage through resorption of the fetus [228].

Insulin resistance may lead to miscarriage through diminished endometrial production of IGFBP-1 and uterine αβ3 integrin [229–231]. In addition, lower serum glycodelin levels have been described in both obese subjects and in women with miscarriage [232]. Changes in endometrial receptivity caused by insulin resistance are shown in Fig. 7.

Other adipokines with effects on reproductive functions include ghrelin, adiponectin and resistin. Ghrelin is another adipokine, the secretion of which is inversely proportional to BMI [219]. Ghrelin possibly acts via hypothalamic and pituitary receptors, and is known to inhibit embryo development and implantation in vitro [233]. Adiponectin and resistin modulate glucose and fat homeostasis, affect insulin action and thus may be another link between obesity, insulin resistance and fertility [234].

Data from large epidemiological studies indicate that chronic sleep deprivation is also related to obesity and insulin resistance. The mechanism has been recently reviewed [235]. Sleep deprivation worsens glucose metabolism, increases appetite, provides more time for eating and possibly decreases energy expenditure though tiredness. These in turn lead to insulin resistance and obesity. There are also indications that too much sleep (over 9 hours/day) can also lead to insulin resistance, although the exact mechanism remains unknown.

![Fig. 7. Changes in the uterine milieu which contribute to increased MR in women with insulin resistance. Lower expression of IGFBP-1 and αβ3 integrin lead to diminished adhesion, and lower secretion of glycodelin together with a higher concentration of TNF-α contribute to the resorption of the conceptus.](image)
Stein and Leventhal (1934) were the first to recognize the relationship between obesity and reproductive disturbances. The association between lifestyle characteristics, obesity and reproductive events is summarised in Fig. 8. Leptin resistance, low-grade inflammation and impaired glucose tolerance lead to early menarche, menstrual disorders, low fertility and various complications in pregnancy. Abdominal obesity particularly worsens the clinical features of menstrual irregularity and infertility because of increased hormonal disturbances [213]. The strength of the association between high BMI and anovulatory infertility found in several reports [236-238] has been evaluated in the Nurses’ Health Study [239]: the risk of anovulatory infertility increased from 1.3 in the group with a normal BMI to 2.7 in women with a BMI >32 kg/m². Obesity has
also been found to be an independent cause of miscarriage [240], with MR between 17% [241] and 27% [242] in spontaneous pregnancies.

Obesity is an infertility factor that can be modified through changes in lifestyle. Even moderate weight reduction before infertility treatment might be beneficial [243, 244], since physical activity has been shown to improve insulin sensitivity [245].

**Obesity and IVF/ICSI**

Even though the consequences of obesity in women are well known, in IVF/ICSI there is controversy regarding the effect of obesity on the outcome of treatment.

Obese women require higher gonadotropin doses for ovarian stimulation [51, 247, 248], despite which, fewer oocytes are collected [51, 248]. However, similar numbers have also been reported [241]. In a study of 3600 IVF/ICSI cycles, an elevated BMI was found to be associated with decreased cumulative PR after controlling for multiple confounding factors [54]. Overweight women had only an 80% chance, obese only a 70% chance and very obese women only a 50% chance of pregnancy compared with women of normal weight, even after several IVF/ICSI cycles. Obesity also diminishes cumulative LBR. It was estimated that because of a lower success rate at every step of IVF/ICSI, only 17% of obese patients have a live birth, compared with 21% of normal-weight women [51].

Despite this evidence, it is difficult to draw final conclusions about the effect of obesity on the outcome of IVF/ICSI because of the contradictory results of miscarriage studies (Table 2). Results from different reports cannot be compared easily, as the definitions of obesity, scope of analysis, stimulation protocols and confounding factors studied differ significantly. However, the statistical methods used have been similar. In most analyses, outcomes among overweight and obese patients have been compared with the outcome of a reference group. Linear regression has also been used in one study [51]. Several studies have revealed a U-shaped relationship between BMI and PR or MR but a mathematical model explaining the relationship has not been developed [54, 242, 249].

In obesity studies, controversy regarding embryo quality and endometrial function exists as well. Obesity may affect the quality of embryos, as suggested in an article in which the mean grade of embryos of women with a BMI of ≥30 kg/m² was lower than that in normal-weight and overweight women [250]. However, the results of other studies have shown no significant effect of obesity on embryo quality [51, 54, 247]. Reports that obesity increases the MR in OD
cycles indicate that obesity might affect the outcome of IVF/ICSI through diminished endometrial receptivity [251, 252]. This effect has not been observed consistently in all studies [253, 254].

Table 2. Summary of findings of previous studies on BMI and pregnancy loss before 12 weeks of gestation.

<table>
<thead>
<tr>
<th>Study</th>
<th>Groups</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>No effect of BMI on MR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lashen et al. 1999 [241]</td>
<td>&lt;19; ≥27.9 kg/m²</td>
<td>333 started cycles</td>
</tr>
<tr>
<td>Winter et al. 2002 [249]</td>
<td>WHO classification</td>
<td>1196 pregnancies</td>
</tr>
<tr>
<td>Roth et al. 2003 [255]</td>
<td>&lt;20; 20–26.9; ≥27 kg/m²</td>
<td>494 pregnancies</td>
</tr>
<tr>
<td>Dechaud et al. 2006 [247]</td>
<td>&lt;20; 20–24.9; 25–29.9; ≥30</td>
<td>789 started cycles</td>
</tr>
<tr>
<td>MR increases along with BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fedorcsak et al. 2000 [248]</td>
<td>&lt;25; ≥25 kg/m²</td>
<td>383 pregnancies</td>
</tr>
<tr>
<td>Wang et al. 2001 [256]</td>
<td>&lt;20; 20–24.9; 25–29.9; 30–34.9; ≥35 kg/m²</td>
<td>1018 pregnancies</td>
</tr>
<tr>
<td>Wang et al. 2002 [242]</td>
<td>WHO classification</td>
<td>2349 pregnancies</td>
</tr>
<tr>
<td>Fedorcsak et al. 2004 [51]</td>
<td>WHO classification</td>
<td>5019 started cycles</td>
</tr>
</tbody>
</table>

* BMI obesity groups according to WHO: underweight, <18 kg/m²; normal weight, 18–24.9 kg/m²; overweight, 25–29.9 kg/m²; obese, 30–34.9 kg/m²; very obese, ≥35 kg/m².

2.7.2 Underweight women

Similarly to obesity, a low body mass is also associated with menstrual disturbances and ovulatory infertility [257-262]. Furthermore, underweight women also have an increased risk of miscarriage [263, 264], intrauterine growth retardation [265] and preterm birth [266].

Not much is known about uterine function in women with low BMI. Low plasma leptin levels have been associated with early [216] and recurrent miscarriage [216, 267], possibly through suboptimal uterine angiogenesis [223], which may explain the reproductive failures observed in underweight women. However, it is not clear whether low BMI predicts a low chance of conception after IVF/ICSI. The IVF study with the highest number of underweight women had 441 subjects (12.3% of studied cycles) [54]. These women tended to have a reduced cumulative chance of pregnancy (OR 0.8, 95% CI 0.65–1.01), compared with normal-weight women. In a later smaller study from the same group, the MR also tended to be lower (OR 0.5, 95% CI 0.26–1.00) [256]. However, in the analysis with the highest proportion of underweight subjects (22%, 87 women) a similar LBR was observed in comparison with normal-weight subjects (20.8% vs. 39%).
15.2%). In other studies, women with a low BMI have constituted a much smaller proportion of the study population (2.7–3.0%) [51, 242, 249].

2.8 Main infertility diagnosis

2.8.1 PCOS

PCOS is the most common endocrine disorder in women of reproductive age. According to the Rotterdam consensus [268], it is diagnosed when two of the three following criteria are present: oligo/anovulation, clinical and/or biochemical signs of hyperandrogenemia, and polycystic ovaries (≥12 follicles measuring 2–9 mm in each ovary). The aetiology of PCOS is unknown. Insulin resistance is a main feature of the pathogenesis of the syndrome [269]. Obesity, especially the abdominal type, is another characteristic of PCOS. Obesity exacerbates endocrine disturbances of PCOS but even lean women with PCOS have fertility problems similar to those occurring in obese women without the syndrome [238, 270, 271] (Figs. 7 and 8).

As in obese women, weight reduction of even as little as 5% of body weight improves menstrual function and hormonal levels of obese patients with PCOS [272]. Treatment with metformin has been shown to improve insulin sensitivity, to restore ovulation [273-275] and to improve pregnancy outcome [276]. However, metformin therapy was recently found to be inferior to ovulation induction compared with clomiphene citrate [277].

In IVF/ICSI, PCOS is a known risk factor of OHSS [278], a rare [279] but serious complication of ovarian stimulation. More oocytes are usually collected from women with PCOS compared with subjects without the syndrome [270]. However, PCOS has been associated with lower cumulative PR after IVF/ICSI after controlling for obesity, age and other factors [54]. Earlier studies revealed an increased MR in these patients [280]. However, more recent research has showed that PCOS loses its independent effect on miscarriage after controlling for obesity and a number of other factors [242, 248, 256]. Furthermore, it has been demonstrated that insulin resistance increases the risk of miscarriage independently of BMI or PCOS status [281].
2.8.2 Other diagnoses

The main subgroups of female infertility can be defined as tubal factor (24% of all female causes), ovulatory dysfunction (34%), endometriosis (11%) and other (31%), including uterine factor or multiple, female-related factors [360]. Analyses of the effects of the main infertility diagnoses on outcome of IVF/ICSI have given inconsistent conclusions. Women with tubal factor infertility have been found to have lower LBR [30, 161, 282] and lower cumulative LBR [53]. Male factor infertility [282] and endometriosis [283] have also been associated with a low chance of live birth. Other factors with an effect on the outcome of IVF/ICSI are smoking, primary infertility, a long duration of infertility [282] and a high number of previous IVF/ICSI treatments [284].

2.9 FET

FET allows all good quality embryos to be stored and to cancel ET if the patient is at risk of OHSS [285]. According to data from 2003, Finland is the country with the highest number of FET cycles performed relative to the number of OPUs (56%) [3]. In other European countries, FET is performed to a much smaller extent (mean 20.5%, 1.0–39.9%).

The PR after FET is lower than that in fresh cycles (18% vs. 26%) [3]. This can be explained by embryo damage during the freezing and thawing procedure. An additional factor may be the fact that the best embryo(s) are always used in fresh cycles. Factors affecting the outcome of FET include infertility aetiology [286], age at the time of the fresh cycle [91, 286], type of ovarian stimulation protocol used in the fresh cycle [287], outcome of the fresh cycle [84, 85, 286], freezing method [288], as well as the embryo factors described above. In a study where embryos of similar grade were transferred, no difference was observed between the outcomes of spontaneous vs. hormonally substituted FET cycles (LBR 11% vs. 9.8%) [289].

Despite the lower PR, the risk of multiple pregnancy is elevated even in FET. In 2003, there were 993 multiple births after FET in Europe (MBR 15.1%) [3]. This can be explained by the number of embryos transferred in FET. A retrospective study revealed significantly higher LBR after the transfer of two embryos rather than one in FET SET (25.7% vs. 19.2%) but the MBR was ten times higher in the cycles with two (21.9%) compared with those with one embryo transferred (2.0%) [290].
2.10 Number of transferred embryos and pregnancy outcome

The chance of pregnancy increases along with the number of embryos transferred [32, 81, 290]. In a mathematical analysis it was found that the number of embryos implanted depends on the number of embryos transferred, so that the implantation probability of a given embryo is increased by 22% for each additionally implanted embryo [291]. This can be explained by the dialogue between the embryo and the endometrium before implantation [291] or by the increased placental mass in early multiple implantations, which produces more hCG and progesterone than singleton placentas [292, 293] and might affect important factors in implantation [119].

Embryo synergism continues after implantation so that the loss of a single embryo is lower in pregnancies with two or three gestational sacs, compared with singleton pregnancies [112, 292-294]. However, the benefits of multiple implantation are lost in later pregnancy. As mentioned earlier, multiple pregnancies often result in the premature births of infants with low birth weight. Even if up to 36% of twin gestations undergo a spontaneous reduction of one gestational sac [295–298], the outcomes of the resulting singleton pregnancies are not as good as in gestations with a single implanting embryo. The probability of low and very low birth weight are twofold higher in vanishing twin pregnancies, compared with single implantations [296]. A negative effect on the outcome may also be exerted by the number of embryos transferred but not developing into pregnancies visible in ultrasonography. Singletons born after DET have been shown to have higher ORs for preterm birth (1.8) and for low birth weight (3.4), compared with singletons after SET [295].

2.11 eSET with FET

During the nine years after the first study of eSET [9], considerable evidence has accumulated about the safety of this treatment option. Results from randomised and retrospective trials are shown in Table 3. In general, eSET is associated with a considerably lower multiple pregnancy rate compared with DET. However, PRs in randomised studies are lower in cases of eSET than in DET. In retrospective studies, similar PRs are observed with either transfer strategy.
Table 3. Results from eSET studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Design</th>
<th>PR</th>
<th>Cumulative MPR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>eSET vs. DET</td>
<td>eSET vs. DET</td>
</tr>
<tr>
<td>Randomised</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gerris et al. 1999 [41]</td>
<td>53 women &lt;34 years, first cycle, ≥2 top quality embryos</td>
<td>1 eSET vs. 1 DET</td>
<td>38.5 vs. 74%</td>
<td>3.9 vs. 30%</td>
</tr>
<tr>
<td>Martikainen et al. 2001 [10]</td>
<td>144 women, ≥4 good quality embryos</td>
<td>Cumulative outcome after 1 eSET + FET vs. 1 DET + FET</td>
<td>47.3 vs. 58.6%</td>
<td>5.4 vs. 26.2%</td>
</tr>
<tr>
<td>Thurin et al. 2004 [42]</td>
<td>631 women &lt;36 years, ≥2 good embryos</td>
<td>2 cycles eSET + FET vs. 1 cycle DET</td>
<td>47.9 vs. 52.6%</td>
<td>0.8 vs. 33.1% a</td>
</tr>
<tr>
<td>Lukassen et al. 2005 [45]</td>
<td>107 women &lt;35 years, first cycle, ≥1 good quality embryo</td>
<td>2 cycles eSET vs. 1 cycle DET</td>
<td>41 vs. 36%</td>
<td>0 vs. 37%</td>
</tr>
<tr>
<td>van Montfoort et al. 2006 [299]</td>
<td>308 unselected women</td>
<td>1 eSET vs. 1 DET</td>
<td>21.4 vs. 40.3%</td>
<td>0 vs. 21.0%</td>
</tr>
<tr>
<td>Retrospective</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vilska et al. 1999 [9]</td>
<td>910 transfers</td>
<td>eSET if risk of OHSS, patient preference or medical indication</td>
<td>29.7 vs. 29.4%</td>
<td>0 vs. 23.9%</td>
</tr>
<tr>
<td>Tiitinen et al. 2001 [12]</td>
<td>All transfers in 1998–1999, 708 cycles</td>
<td>eSET performed if patient preference or medical indication</td>
<td>38.6 vs. 40.0%</td>
<td>2.9 vs. 26.2% a</td>
</tr>
<tr>
<td>Gerris et al. 2004 [43]</td>
<td>367 women &lt;38 years, first cycle or previous delivery, ≥1 top quality embryo b</td>
<td>1 eSET vs. 1 DET</td>
<td>49.0 vs. 54.0%</td>
<td>2.5 vs. 25.4%</td>
</tr>
<tr>
<td>van Montfoort et al. 2005 [300]</td>
<td>326 women &lt;38 years, ≥1 top quality embryo</td>
<td>Cumulative outcome after three fresh treatment cycles + FET</td>
<td>41 vs. 30%</td>
<td>0 vs. 33%</td>
</tr>
</tbody>
</table>

a multiple live birth rate, b patients chose between eSET or DET prior to treatment. In the eSET group, eSET was carried out if ≥1 top quality embryo was available, otherwise DET was performed. Results shown are for groups based on patient wishes.
Currently, eSET is practised on a large scale only in Finland, Sweden and Belgium [3]. The Finnish national birth registry has shown a steady decrease in the proportion of multiple births after IVF/ICSI from 24% in 1996, before the implementation of eSET, to 14% in 2002, while the LBR has remained unchanged [52]. Similar results have been reported from Belgium [301, 302] and also from Sweden [38, 64] where the proportion of cases of eSET reached 67.4% in 2004. Consequently, in the same year the Swedish national MBR was as low as 5.7% [38].

The eSET and multiple embryo transfer strategies have been compared using Swedish national data as an example of an eSET strategy and national data of the United States as a model of multiple ET [38]. Throughout the years since 1991, a continuous increase in LBR/ET was observed in the United States, whereas in Sweden it remained stable after 1993. A reduction by half in the number of embryos transferred took place in Sweden after the introduction of the law on eSET, followed by a considerable reduction in MBR. In the United States, the reduction in the number of embryos transferred was less pronounced (30%) and consequently the MBR decreased only slightly. Finally, the analysis showed that the LBR/embryo transferred was higher with eSET than with the transfer of several embryos (19% in Sweden and about 13% in the United States).

2.11.1 Physicians’ considerations

Countries in which eSET is practised on a large scale have state funding for IVF/ICSI [3]. State insurance has been found to affect embryo transfer practices. In a study of state-mandated insurance coverage for IVF/ICSI in the United States, a smaller proportion of transfers of at least 3 embryos was observed in states having insurance for IVF, compared with the states without insurance [303]. Utilisation of IVF services and treatment outcome were also studied in 360 fertility clinics in the United States, of which 31 clinics worked under complete insurance coverage [304]. State-mandated insurance for IVF was associated with increased use of treatment, fewer embryos transferred per cycle and a lower percentage of high order gestations. However, a lower PR/cycle was also observed in the clinics with insurance coverage. This may be a reason why infertility practitioners in places without infertility insurance are reluctant to decrease the number of embryos transferred and to consider eSET.

Before the latest nationwide data became available [38, 301, 302], several investigators had voiced the fear that if eSET was applied to a larger extent in
everyday practice, PRs per transfer would diminish considerably [14-16, 305]. Other concerns expressed were overall effectiveness and cost of treatment for the patient, since eSET might result in an increased cost of successful treatment and a longer time to achieve pregnancy.

2.11.2 Patients’ preferences

Patients’ preferences regarding the plurality of desired pregnancy have been subject to extensive investigation. Between 14% and 90% of IVF patients prefer twins rather than singletons [306-312]. In a Danish survey, only 23% of patients preferring twins expressed a positive attitude towards twins as the primary reason for their desire [313]. Many more patients wanted to maximise the chance of their child having siblings or to have as few IVF treatments as possible. Patients preferring the transfer of more than one embryo have also been concerned about the price of treatment [306, 308]. Many couples view the possibility of no pregnancy after treatment and the possibility of a multiple pregnancy as the only possible outcomes after IVF.

It is known that patients’ attitudes depend on the way health information is presented to them [359]. In addition, infertility patients seem to be unaffected by perceptions of the high risk that is associated with twins [306]. However, if success rates and prices are similar, 82% of patients would prefer eSET to DET [311]. Physician motivation also plays a role, as illustrated by a study in which 64% of couples chose to have eSET after careful explanation of the risks of multiple pregnancies [44].

2.12 Cost-effectiveness analysis

CEA is one of the techniques of economic evaluation designed to compare the costs and benefits of healthcare intervention in order to assess whether it is worth executing. The aim of CEA is to maximise the level of health effects relative to the resources available. It can be based on different types of data. With data from randomized studies, CEA tests the extent to which a form of intervention does more good than harm under ideal circumstances (efficacy, “Can it work?”). For a better estimation, data from healthcare practice under usual circumstances are studied instead (effectiveness, “Does it work in practice?”) [314].

In CEA, the total costs and total effects of a new and an old therapy are compared. Total costs can be grouped in four categories [315]:
- health care resources: costs of organising and operating a health care programme, including resources necessary to dealing with adverse effects caused by the programme;
- patient and family resources: out-of-pocket expenses of the patient or family members as well as the value of any resources that they contribute to the treatment process, such as formal care;
- productivity losses: patients or family members lose time from work while seeking treatment or participating in a health programme;
- other losses: social worker visits etc.

Depending on the desired endpoint, any of the cost categories or all of them can be evaluated.

The effects of health care interventions are quoted as numerical variables, such as number of births, years of life gained, millimetres of blood pressure lowered. In IVF, the outcomes most often used in economic analyses are live births or live-born children [316], although, as already mentioned, term live births have also recently been used [56, 57].

Total costs and total health effects are separately calculated for the new and old therapies. Possible results are presented in Fig. 9. Dominance is not common in economic evaluations, because in most cases more effective treatments are also more costly [317, 318]. The most common outcome of CEA is a new therapy which is more effective but also more costly, compared with the reference therapy. Whether it is an acceptable (cost-effective) option depends on the maximum amount of money that society is prepared to pay for the gain in effectiveness [315].

<table>
<thead>
<tr>
<th>New therapy…</th>
<th>… less effective</th>
<th>… as effective</th>
<th>… more effective</th>
</tr>
</thead>
<tbody>
<tr>
<td>… less costly</td>
<td>Non-dominance</td>
<td>Weak dominance</td>
<td>New therapy dominates old</td>
</tr>
<tr>
<td></td>
<td>Necessary to save money?</td>
<td>Accept new therapy?</td>
<td>Implement new therapy</td>
</tr>
<tr>
<td>… as costly</td>
<td>Weakly dominated</td>
<td>Non-dominance</td>
<td>Weak dominance</td>
</tr>
<tr>
<td></td>
<td>Reject new therapy?</td>
<td>Other considerations?</td>
<td>Accept new therapy?</td>
</tr>
<tr>
<td>… more costly</td>
<td>New therapy is dominated</td>
<td>Weakly dominated</td>
<td>Non-dominance</td>
</tr>
<tr>
<td></td>
<td>Reject new therapy</td>
<td>Reject new therapy?</td>
<td>Additional funds available?</td>
</tr>
</tbody>
</table>

Fig. 9. Interpretation of the outcome of cost-effectiveness analyses.
Incremental cost-effectiveness ratios are useful tools when comparing two or more treatment strategies. They show the change in price for one unit of effect. The following formula is used in the calculation of an ICER:

\[
\text{ICER} = \frac{\Delta \text{costs}}{\Delta \text{effects}} = \frac{\text{total costs of old therapy} - \text{total costs of new therapy}}{\text{number of health effects with old therapy} - \text{number of health effects with new therapy}}
\]

It is never possible to estimate completely accurately the cost of healthcare. Likewise, some uncertainty always remains about the effects of different treatment strategies. A sensitivity analysis, which is an essential part of CEA, tests all the assumptions used in the model.

### 2.12.1 Cost of IVF/ICSI treatment

IVF/ICSI treatment is very costly, with prices differing considerably between countries. There is only one comparative study including data from European countries and the United States [4]. In 2002, the average price of a single IVF cycle was about €2700 in Finland, but about €3450 in other European countries (ranging from €1942 in the Netherlands to €4115 in Italy) and about €10,000 in the United States.

Depending on the country, treatment costs can be as high as 25% of annual household expenditure [4]. Use of treatment is therefore low throughout the world, with only 22% of infertile patients receiving IVF/ICSI, even in developed countries [319].

The actual cost of IVF/ICSI is even higher because of the cost of multiple pregnancies. Multiple pregnancies are associated with an increased risk of maternal complications necessitating absence from work and hospital stay, while twins and children born after higher-order multiple pregnancies frequently require hospitalisation [6, 28, 34, 320]. The family costs per multiple live birth are approximately 4-, 11- and 18-fold greater for twins, triplets and higher-order deliveries, respectively, compared with singleton deliveries [6]. Similarly, a study carried out in Oulu, Finland, has shown that the costs of maternal and pediatric care up to the end of the neonatal period are €5780 for an IVF singleton and €15,582 for an IVF twin pair [321].

In the United Kingdom, although multiple pregnancies after IVF/ICSI account for 27% of live births after treatment, they incur more than half of the direct cost of IVF/ICSI pregnancies up to the end of the first year after delivery.
In the same study it was estimated that if eSET is implemented, savings in comparison with multiple pregnancies and births could allow the state funding of an additional 10,124 IVF cycles per year.

### 2.12.2 Cost-effectiveness of eSET

Up to now, the financial impact of eSET has been studied insufficiently [323]. Most cost-effectiveness analyses have been based on clinical data from randomised studies [43, 45, 324-327]. As explained above, this means that their conclusions are valid only in the setting of a randomised study, i.e. highly selected patients and a limited number of treatment cycles [315]. The results are shown in Table 4. The effect of FET cycles after eSET was evaluated only in two studies, and not to its full extent because of time limitations or assumptions overestimating the success rate [325, 326].

Because of different endpoints and costs evaluated, the outcomes of these studies have been re-analysed in a review [328]. eSET might be associated with smaller costs, compared with DET, if IVF treatment, pregnancy and paediatric care are evaluated together, but not if only the costs of IVF are analysed. The difference in effectiveness between eSET and DET was found to decrease substantially when eSET with FET was performed to patients with a good prognosis.

### 2.13 Alternatives to eSET

There are two current alternatives to eSET: eSBT and mild stimulation. The former is favoured by some clinics because of the high PR in fresh cycles following blastocyst transfer. Like eSET, eSBT has been shown to lower the MBR while preserving PR and LBR within an acceptable range with or without FET [329-332]. Two years of experience with eSBT has shown a decrease in MBR with no change in LBR [333], similarly to eSET [38, 52, 302]. However, with eSBT the MBR remains higher than with eSET (17% vs. 5%) [38, 333].
Table 4. Summary of findings of previous cost-effectiveness studies of eSET.

<table>
<thead>
<tr>
<th>Study</th>
<th>Subject data</th>
<th>Design</th>
<th>Costs of IVF</th>
<th>Outcome of IVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Sutter et al. 2002 [325]</td>
<td>144 women, &gt;4 good embryos</td>
<td>&lt;3 eSET + FET vs. &lt;3 DET + FET</td>
<td>Lower</td>
<td>Worse</td>
</tr>
<tr>
<td>Gerris et al. 2004 [43]</td>
<td>367 women, &lt; 38 yrs, first IVF or prev. delivery</td>
<td>1 eSET vs. 1 DET</td>
<td>Lower</td>
<td>Better</td>
</tr>
<tr>
<td>Lukassen et al. 2005 [45]</td>
<td>107 women, &lt; 35 yrs, &gt;2 good embryos</td>
<td>2 eSET vs. 1 DET</td>
<td>Similar</td>
<td>Similar</td>
</tr>
<tr>
<td>Thurin Kjellberg et al. 2006 [326]</td>
<td>661 women, &lt; 36 yrs, &gt;2 good embryos</td>
<td>eSET + eSET/FET vs. 1 DET</td>
<td>Lower</td>
<td>Better</td>
</tr>
<tr>
<td>Fiddelers et al. 2006 [327]</td>
<td>308 women, first IVF, &gt;2 embryos</td>
<td>1 eSET vs. 1 DET</td>
<td>Lower</td>
<td>Worse</td>
</tr>
</tbody>
</table>

To date, the outcomes of eSET and eSBT have not been compared in a single study. The cumulative outcome with cleavage-stage embryos and blastocysts in other trials has been reviewed recently [66]. In two of the three identified studies, there was a higher cryopreservation rate as regards the cleavage-stage embryos and a higher implantation rate of the fresh blastocyst transfers, but the cumulative outcome was similar. In the third study there was a cumulative PR of 46% in the cleavage-stage group vs. only 27% in the blastocyst group [334].

The mild stimulation protocol is aimed at minimising the adverse effects of ovarian stimulation (down-regulation symptoms and multiple births) [335]. Gonadotropin administration is initiated on day 5 of the cycle and a GnRH antagonist is started on the day when at least one follicle has a diameter of ≥ 14 mm [336]. A larger proportion of euploid embryos has been observed after mild stimulation than after long protocol (51% vs. 35%), but the average number of embryos was the same (1.8), regardless of the protocol [337]. In addition, mild stimulation is associated with a high cancellation rate of 17–22.4% [336, 338], compared with stimulation using the long protocol (cancellation of about 1% of cycles performed at Oulu University Hospital).
A cost-effectiveness study has been carried out in which the outcomes of mild stimulation and of a long protocol with DET were compared [56]. Patients were randomised to one year of treatment, and FET was performed if cryopreserved embryos were available. The cumulative tLBRs/woman were similar: 43.4% with mild stimulation and 44.7% with the long protocol and DET. Mild stimulation was associated with lower total costs up to six weeks after expected delivery, but overall the costs of IVF treatment were similar in the two treatment groups.
3 Aim

At present, only 50% of women have a child following IVF or ICSI. There is insufficient knowledge about the interplay between characteristics of the patient, of the treatment and especially of embryo transfer. The overall aim of this work was to study the relative importance of the transfer of a top quality embryo with respect to features of the female patient and of laboratory data. The groups studied included the general IVF/ICSI patient population as well as treatment groups with expected worse outcome such as women with LR to ovarian stimulation, obese and underweight subjects as well as those of advanced reproductive age. In addition, the work involved assessment of the economic viability of the elective transfer of a single top quality embryo.

The specific aims were as follows:

1. To investigate the incidence of LR in subjects stimulated more than once and the outcomes of cycles with (a) top quality embryo(s).
2. To study factors that might affect first trimester miscarriage after fresh IVF/ICSI, spontaneous and hormonally substituted FET. To this end, we performed a multivariate analysis including embryo morphology and other baseline and treatment characteristics that have previously been shown to have an effect on miscarriage. Specific attention was paid to states of insulin resistance such as obesity and anovulation.
3. To verify the applicability of eSET of a top quality embryo in older women aged 36–39 years, by comparing the outcomes of eSET with those of DET in subjects of the same age.
4. To determine whether the overall outcome, costs of IVF/ICSI treatment and time to live birth are affected by the implementation of eSET in everyday clinical practice.
4 Materials and Methods

**Study I**
*Low responders*
2237 women, 3846 fresh cycles

**Study II**
*Miscarriage*
8981 fresh, 7015 FET cycles

**Study III**
*Older women*
Women 36–39 years old
1224 fresh, 824 FET cycles

**Study IV**
*Overall outcome*
1510 women
2469 fresh, 1851 FET cycles

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*Fig. 10. Design of the studies included in this work.*
4.1 Study population

The IVF Units of Oulu University Hospital and Helsinki University Central Hospital, the Oulu and Helsinki infertility clinics of the Family Federation of Finland, and the AVA-Clinic, Fertility Centre, Tampere, provided detailed data about IVF/ICSI and FET procedures carried out during the years 1994–2005. A total of 18,815 cycles have been analysed.

The design of the studies is illustrated in Fig. 10. Study I was based on data from the IVF Unit of Oulu University Hospital and the Family Federation of Finland, Oulu, from 1994–2002. Cycles of women stimulated <3 times or without LR (3473 cycles), as well as data on women who had undergone ovarian surgery (133 cycles) were excluded from the analysis.

Study II included the first clinical pregnancy cycles of women who were treated at the Infertility Clinics of Oulu and Helsinki University Hospitals and the Family Federation of Finland in Oulu and Helsinki, and the AVA-Clinic in Tampere during 1999–2004. Cycles with no pregnancy (11,946 cycles) and data on 701 non-first pregnancies were excluded from the analysis.

Study III involved analysis of the treatment outcomes of women aged 36–39 years who had undergone IVF/ICSI during the period 2000–2003 and FET during 2000–2004. Data for this study were collected from the Infertility Clinics of Oulu and Helsinki University Hospitals, and the Family Federation of Finland in Oulu and Helsinki.

Study IV was based on treatment cycles from Oulu University Hospital only: IVF/ICSI cycles performed in 1995–2004 and FET cycles up to 2005. All consecutive cycles of each woman were analysed. In cases with a live birth, subsequent cycles were excluded (738 fresh and 537 FET cycles).

4.2 Treatment protocols

IVF/ICSI protocols

Ovarian stimulation was performed using the long GnRH agonist protocol or the GnRH antagonist protocol [9, 12, 192, 339]. In a small proportion of cycles, other stimulation protocols were used (short and ultra-short protocols) (Study II). The starting gonadotropin dose was determined according to the patient’s age, BMI, antral follicular count in the baseline ultrasonographic scan and the outcome of previous infertility treatments. The cycle was cancelled if less than three growing...
follicles are observed on day 10 of stimulation, resulting in a yearly cancellation rate of about 1%. Oocytes/embryos were cultured as previously described [290, 339]. A LR cycle was defined as yielding ≤3 oocytes, a NR cycle 4–14 oocytes, and a HR cycle ≥15 oocytes. Embryo transfer was carried out on day 2 or 3 after oocyte retrieval, depending on the day of the week – on Monday if oocytes were collected on Friday and 2 days after ovum pickup in all other cases. Such a strategy has been adopted to minimize work during the weekends. A top quality embryo had 4–5 evenly sized cells and <20% fragmentation when cultured for 2 days, or ≥8 cells and <20% fragmentation when cultured for 3 days [40]. Extra embryos were frozen on the day of embryo transfer, using a slow freezing protocol [290].

**FET cycles**

In spontaneous FET, ET was performed after spontaneous ovulation. The urinary LH surge was measured by means of a home test kit. Depending on the day of freezing of the embryo, ET was carried out four (2–5) days after a positive result. In some spontaneous FET cycles, luteal support with 200 mg Lugesteron/day (Leiras, Helsinki, Finland) was started on the day of ET and continued for two weeks.

In hormonally substituted FET cycles, estradiol valerate or 17β-estradiol (Estrofem, Novo Nordisk, Bagsværd, Denmark; Merimono, Novartis, Basel, Switzerland; Progynova, Schering, Berlin, Germany; Zumenon, Solvay Pharmaceuticals, Hannover, Germany) was administered at a daily dose of 4–6 mg. Vaginal micronized progesterone (400–600 mg Lugesteron/day) or Crinone vaginal gel (8%), 1.125 g twice a day (Serono, Geneva, Switzerland) was started when the endometrial thickness was ≥6 mm on cycle day 11–13 and treatment was continued until 9–12 gestational weeks after a positive pregnancy test result. In cases of a thinner endometrium, the estradiol dose was increased to 6–8 mg/day and progesterone administration was delayed until a thickness of ≥6 mm was reached. A GnRH agonist (Suprecur, 450 mg/day, Sanofi-Aventis, Paris, France, or Synarel, 400 mg/day, Pfizer, New York City, U.S.A.) was used in some cycles for pituitary down-regulation before starting endometrial preparation in order to prevent spontaneous ovulation and to control the timing of ET. Thawed embryos were graded using the same criteria as in the fresh cycles, although at present there is no consensus of opinion on the definition of a top quality embryo in FET cycles.
Pregnancy was detected by means of urinary hCG test (in Oulu) or by serum hCG measurement (in Helsinki and Tampere). Clinical pregnancies were diagnosed by transvaginal ultrasonography at gestational week 6–7 by visualisation of a gestational sac and cardiac activity.

4.3 Cost of IVF/ICSI

A “payer perspective” analysis of total treatment charges and medication costs up to the time of the pregnancy test was carried out on the two study periods in Study IV. Financial calculations were based on the pricelist of the IVF Unit of Oulu University Hospital for the year 2008 (Table 5). A 3% discounting rate was used. At Oulu University Hospital, the charge for freezing and cryostorage of supernumerary embryos is included in the charge of the fresh cycle. Costs that resulted from complications of ovarian stimulation (OHSS, bleeding, infections) were not taken into consideration.

Table 5. IVF/ICSI costs at the IVF Unit of Oulu University Hospital for the year 2008.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Unit price, €</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh cycles</td>
<td></td>
</tr>
<tr>
<td>Stimulationa</td>
<td>1495</td>
</tr>
<tr>
<td>IVF</td>
<td>1542</td>
</tr>
<tr>
<td>ICSI</td>
<td>2158</td>
</tr>
<tr>
<td>Progesterone 14 days</td>
<td>34</td>
</tr>
<tr>
<td>FET cycles</td>
<td>600</td>
</tr>
<tr>
<td>Hormonal supportb</td>
<td>66</td>
</tr>
</tbody>
</table>

a includes GnRH agonist for long protocol, 2400 IU of recombinant FSH, 5000 IU of hCG. b includes LH test and 14 days of vaginal progesterone (spontaneous FET) or 28 days of estradiol valerate and 14 days of vaginal progesterone (hormonally substituted FET).

4.4 Statistical analyses of clinical data

For linear variables, log-transformation was used, if necessary, to normalise distributions before statistical testing by means of Student’s two-tailed t-test or one-way ANOVA with the Tukey multiple comparisons test as a post hoc analysis. Where distributions continued to be skewed after log-transformation, the non-parametric Mann–Whitney U or Kruskall–Wallis tests were used. For categorical variables, statistical significances in frequency differences between groups were evaluated by using the chi-square test. Statistical analysis was
performed with SPSS software, versions 10.1–16.0 (SPSS Inc., Chicago, IL, USA), with $P<0.05$ as the limit of significance. Unless otherwise indicated, results are expressed as mean ± SD.

In Study I, the primary outcome measure was the PR/cycle. Baseline and treatment characteristics, as well as the treatment outcome in the study groups were compared, using the overall mean PR/cycle during the study period in all cycles and in cycles with at least one top quality embryo transferred.

The primary outcome measure in Study II was the MR/cycle. Miscarriage was defined as pregnancy loss before 12 full weeks of gestation. Logistic regression was used to study the effect on MR of characteristics of the study subjects and the treatment cycles (see Table 6). Univariate logistic regression was first performed as regards all factors, after which the variables showing independent effects in these analyses were studied by means of multivariate logistic regression. Variables showing a non-significant effect were removed from the model in a stepwise manner. The fit of the final logistic model was assessed by means of the Hosmer–Lemeshow test.

In Study III, the PR/ET and the cumulative PR/OPU were the primary outcome measures. Baseline and treatment characteristics of the studied groups were also compared. In Study IV, the cumulative tLBR/woman was the clinical primary outcome measure. The analysis was not limited only to comparison of characteristics of the treatment groups. In order to assess the independent effect of the treatment period on clinical outcome, logistic regression with backward elimination after multicollinearity examination was performed on the cumulative LBR data. The independent clinical effect of the change in embryo transfer policy on the cumulative tLBR was evaluated by using the predicted probability for term live birth for each woman in the DET period. For the same subjects, the probabilities of term live birth with the regression coefficient of the eSET period were calculated. The difference between the two probabilities showed the change in clinical outcome from DET to eSET policy.
Table 6. Characteristics of treatment cycles examined by multivariate regression analysis in Study IV.

<table>
<thead>
<tr>
<th>Type of factor</th>
<th>Factors studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient characteristics</strong></td>
<td>Age (categorical, linear, cubic)</td>
</tr>
<tr>
<td><em>(all cycles)</em></td>
<td>BMI (four different models)</td>
</tr>
<tr>
<td></td>
<td>Categorical: obese vs. non-obese&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Categorical: WHO groups&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Linear: exact BMI values</td>
</tr>
<tr>
<td></td>
<td>Quadratic: BMI and BMI&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Main infertility diagnosis</td>
</tr>
<tr>
<td></td>
<td>Primary vs. secondary infertility</td>
</tr>
<tr>
<td></td>
<td>Previous miscarriages: yes vs. no</td>
</tr>
<tr>
<td><strong>Treatment type</strong></td>
<td>Fresh, spontaneous or hormonally substituted FET</td>
</tr>
<tr>
<td><strong>Embryo characteristics</strong></td>
<td>Origin of the embryo: IVF vs. ICSI</td>
</tr>
<tr>
<td><em>(all cycles)</em></td>
<td>Number of embryos transferred</td>
</tr>
<tr>
<td></td>
<td>Transfer of ≥1 top quality embryo</td>
</tr>
<tr>
<td><strong>Fresh cycles only</strong></td>
<td>Stimulation protocol: GnRH agonist vs. GnRH antagonist</td>
</tr>
<tr>
<td></td>
<td>Gonadotropin dose</td>
</tr>
<tr>
<td></td>
<td>Endometrial thickness at end of stimulation</td>
</tr>
<tr>
<td></td>
<td>Number of oocytes:</td>
</tr>
<tr>
<td></td>
<td>Collected</td>
</tr>
<tr>
<td></td>
<td>Fertilised</td>
</tr>
<tr>
<td></td>
<td>Cleaved</td>
</tr>
<tr>
<td></td>
<td>Ovarian response: LR vs. NR vs. HR</td>
</tr>
<tr>
<td></td>
<td>Day 2 vs. day 3 fresh ET</td>
</tr>
<tr>
<td></td>
<td>eSET</td>
</tr>
<tr>
<td></td>
<td>Number of embryos frozen</td>
</tr>
<tr>
<td><strong>Spontaneous FET only</strong></td>
<td>Use of luteal phase progesterone: yes vs. no</td>
</tr>
<tr>
<td><strong>Hormonally substituted FET only</strong></td>
<td>Use of GnRH agonist: yes vs. no</td>
</tr>
</tbody>
</table>

<sup>a</sup> Obesity was defined as BMI ≥27 kg/m<sup>2</sup>, as this is the threshold value above which insulin sensitivity decreases significantly [340].

<sup>b</sup> BMI groups according to WHO: underweight, <18 kg/m<sup>2</sup>; normal weight, 18–24.9 kg/m<sup>2</sup>; overweight, 25–29.9 kg/m<sup>2</sup>; obese, 30–34.9 kg/m<sup>2</sup>; very obese, ≥35 kg/m<sup>2</sup>.

<sup>c</sup> BMI was centred at 23 kg/m<sup>2</sup> (which approximates the mean BMI) in order to prevent possible collinearity between the BMI variables.

### 4.5 Cost-effectiveness analysis

Because of the multimodal distribution of the total treatment cost per woman, the treatment costs of the fresh and FET cycles per woman were analysed in a multivariate general linear model after log-transformation. The independent
effects of the treatment period, and clinical and laboratory characteristics were assessed after multicollinearity examination. Variables without an independent effect were removed from the model. The contrast estimate of the eSET period was used to calculate the change of the total treatment cost for the women from the DET period from a DET to an eSET policy.

Total costs and total term live births in the study periods were compared. The ICER was calculated using the following formula:

\[
\text{ICER} = \frac{\Delta \text{costs}}{\Delta \text{effects}} = \frac{\text{total costs of DET period} - \text{total costs of eSET period}}{\text{number of term live births during DET period} - \text{number of term live births during eSET period}}
\]

Sensitivity analysis was performed using the 95% CI of the number of fresh and FET cycles and of the tLBRs in the fresh and in the FET cycles. As the FSH dose required for stimulation varies substantially between subjects, the SD of the FSH dose was used in price calculations for the sensitivity analysis instead of the 95% CI. Finally, the range of the change in the predicted probabilities of term live birth and the range of the change in the total cost from the general linear model were used in estimating the independent effect of the change from DET to eSET policy.

In the year 2002, the cost of an IVF cycle in Finland was about 1.5-fold lower than in other European countries and 3.7-fold lower than in the United States [4]. Assuming similar inflation of health care costs in Finland, in the other European countries and in the United States, the ICER and its upper and lower confidence limits were multiplied by 1.5 and by 3.7 in order to facilitate interpretation of the results.

4.6 Data protection

Since all data were collected retrospectively, Ethics Committee permission was not sought. The personal identification code (henkilötunnus) of each female patient was linked to a working identification code at the time of data extraction. The files containing the original and the working codes were kept in each clinic according to its own data protection rules. The working databases contain only the working codes. Partners’ personal identification codes, as well as any names, addresses and phone numbers were not collected.
5 Results and discussion

5.1 Value of a top quality embryo in LR

Study I showed that LR cycles constituted 11.5% (441/3846) of all IVF/ICSI cycles performed from 1994 to 2002, which is consistent with previous reports [179, 341]. Of the 80 subjects with LR cycles who were stimulated three times, only two (2.5%) had a low ovarian response in all cycles. Therefore, results for recurrent low responders are not shown. The finding that LR is not a recurrent problem in a patient population consisting mostly of women of <40 years of age confirms a previous report showing that the majority of young poor responders with normal FSH values have normal responses in their second and third cycles [342].

The proportion of LR cycles in the groups with an initial LR and with an initial NR was similar (Table 7). The proportion of women with inactive ovaries at the beginning of ovarian stimulation tended to be higher in the LR→NR group (42.4%, 53/125) than in the NR→LR group (27.5%, 28/102, \( P=0.06 \)). However, it was higher in both groups than in the general IVF/ICSI population (10.2%) [192]. These results confirm previous observations that a low antral follicle count is associated with lower numbers of retrieved oocytes [192, 205].

Stimulation characteristics were similar in the groups with an initial LR and with an initial NR. In LR→NR subjects, after LR in the first cycle the mean gonadotropin dose was increased from 2349.1 ± 685.3 to 2977.2 ± 977.8 IU (\( P<0.0001 \)), which resulted in a higher number of collected oocytes (2.1 ± 0.9 vs. 6.7 ± 2.7, \( P<0.0001 \)). PRs per cycle were similar between the study groups but were significantly lower (initial LR: \( P<0.0001 \); initial NR: \( P=0.01 \)) when compared with the mean overall PR/cycle during the study period (27.2%). The fact that increasing the gonadotropin dose after a LR cycle results in an improved response with a low PR in the subsequent cycle is supported by the results of other studies [183, 210, 343-345].

As expected, the proportion of cycles with \( \geq 1 \) top quality embryo transferred was lower than that observed in the general IVF/ICSI population (64.6% at the Oulu University Hospital in 2004). In these cycles, the total numbers of transferred embryos, especially the numbers of top quality embryos, did not differ between the two groups with LR cycles. However, following the transfer of top quality embryos, women with an initial LR had a significantly lower PR (17.8%...
vs. 41.2%, \( P=0.03 \) and LBR (11.1% vs. 32.4%, \( P=0.03 \)) than subjects with a NR in the first cycle. The PR and the LBR in the group with an initial LR were also lower than the overall mean PR (37.2%, \( P=0.007 \)) and LBR (28.4%, \( P=0.01 \)) associated with cycles with top quality embryos, but the outcomes among women with an initial NR were similar to the overall mean outcomes (\( P>0.6 \)).

Table 7. General outcome of women with LR and outcome in cycles with top quality embryos.

<table>
<thead>
<tr>
<th>Factor</th>
<th>NR→LR</th>
<th>LR→NR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n or mean</td>
<td>SD or %</td>
<td>n or mean</td>
</tr>
<tr>
<td>All cycles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of LR cycles</td>
<td>42/105</td>
<td>40.0</td>
<td>61/129</td>
</tr>
<tr>
<td>Age, years</td>
<td>34.2</td>
<td>5.5</td>
<td>34.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.4</td>
<td>5.0</td>
<td>24.9</td>
</tr>
<tr>
<td>Total gonadotropin dose, IU</td>
<td>2522.4</td>
<td>861.5</td>
<td>2690.8</td>
</tr>
<tr>
<td>Oocytes</td>
<td>5.1</td>
<td>3.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Fertilisation rate</td>
<td>61.4</td>
<td>31.0</td>
<td>59.2</td>
</tr>
<tr>
<td>Number of embryos transferred</td>
<td>1.7</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Frozen</td>
<td>0.6</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>PR/cycle</td>
<td>17/105</td>
<td>16.2</td>
<td>13/129</td>
</tr>
<tr>
<td>LBR/cycle</td>
<td>11/105</td>
<td>10.5</td>
<td>8/129</td>
</tr>
<tr>
<td>≥1 top quality embryo transferred</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cycles</td>
<td>34/105</td>
<td>32.4</td>
<td>45/129</td>
</tr>
<tr>
<td>Number of top quality embryos</td>
<td>1.38</td>
<td>0.49</td>
<td>1.33</td>
</tr>
<tr>
<td>Total number of transferred embryos</td>
<td>2.0</td>
<td>0.6</td>
<td>1.9</td>
</tr>
<tr>
<td>PR/cycle</td>
<td>14/34</td>
<td>41.2</td>
<td>8/45</td>
</tr>
<tr>
<td>LBR/cycle</td>
<td>11/34</td>
<td>32.4</td>
<td>5/45</td>
</tr>
</tbody>
</table>

Reference values:

- Mean overall PR/cycle: 1046/3846, 27.2
- Mean overall PR/cycle, ≥1 top quality embryo transferred: 724/1946, 37.2
- Mean overall LBR/cycle, ≥1 top quality embryo transferred: 553/1946, 28.4

The poor outcome after an initial LR cycle, even in top quality embryo cycles, suggests that embryos of good morphological quality may still have a low pregnancy potential in this particular group of patients. Up to 64% of the embryos in patients with poor prognosis and/or low response have been found to have chromosomal abnormalities despite normal morphology [17, 73, 181, 182]. This can explain the low PR in the group with an initial LR. In a previous study in which LR and NR cycles were compared, a similar PR was found when the same number of top quality embryos was transferred in women aged <37 years, but the definition of
LR was not the one used in Study I (<5 oocytes compared with <4 in Study I) [346].

5.2 Value of a top quality embryo in fresh and FET cycles

At present, there is no consensus of opinion on the characteristics of a frozen-thawed top quality embryo. In the FET cycles in Study II, embryos were graded with the same criteria used for fresh embryos. The outcomes of the fresh cycles and of the spontaneous FET cycles analysed in Study II were similar in cycles with one top quality embryo transferred (Table 8).

Table 8. Outcome of fresh and spontaneous FET cycles after the transfer of one top quality embryo. Data from cycles collected for Study II.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Fresh cycles</th>
<th>Spont. FET cycles</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR/ET, all cycles</td>
<td>745/2128</td>
<td>94/255</td>
<td>0.6</td>
</tr>
<tr>
<td>MR of cycles analysed in</td>
<td>39/167</td>
<td>24/169</td>
<td>1.0</td>
</tr>
<tr>
<td>Study IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBR, all cycles</td>
<td>610/2128</td>
<td>74/255</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Top quality grading [40] takes into account several characteristics of the cleaving embryo: cleavage speed, regularity of blastomeres, absence of multinuclearity and degree of fragmentation. These factors have been found to be of importance for the outcome of frozen-thawed embryos [12, 80, 83, 93] and therefore it is not surprising that results in FET are similar to those of the fresh cycles.

5.3 Top quality embryos and miscarriage

In Study I, the MR in women with LR (36.7%, 11/30, \(P=0.005\)) was more than twofold higher than the overall mean MR during the study period (15.5%, 162/1046). An increase in miscarriage was also observed in the cycles with top quality embryos among subjects with LR (27.3%, 6/22, \(P=0.07\)), although as a result of low numbers the difference did not reach statistical significance. Analysis of the effect of embryo quality on miscarriage was extended by Study II. In the fresh cycles in Study II, the MR was 22.3% in LR (25/112), 13.9% (188/1353) in NR and 12.2% (87/713) in HR cycles (\(P=0.02\), also for linear association). However, the proportions of cycles with top quality embryos
transferred were similar in the fresh cycles with and without miscarriage (58.2% vs. 61.4%, \( P=0.3 \)) (Table 9).

In fact, the multivariate analysis carried out in Study II revealed that embryo morphology did not determine the MR after IVF/ICSI or FET. Factors with an independent effect on MR are shown in Table 10. The finding that embryo morphology does not affect the MR supports the results of a previous analysis in which it was concluded that embryo morphology determines implantation but that continuation of pregnancy also depends on uterine factors [112]. Likewise, embryo morphology at the time of freezing has not been found to affect the MR after FET [81]. These findings can be explained by research showing that during the 2–3 days before transfer the embryonic genome does not have full control over cell function [18]. Consequently, the genetic composition of an embryo cannot be predicted by its morphology.

### 5.3.1 Factors affecting MR: age

The higher MR in women with LR (Studies I and II) can be explained by the effect of ovarian ageing. In Study I, 35.7% (81/227) of the subjects with at least one LR cycle had inactive ovaries with few antral follicles, while a much lower incidence of inactive ovaries has been observed previously in unselected IVF/ICSI cycles (10.2%) [192]. The multivariate analysis carried out in Study II revealed that age and not ovarian response is a stronger factor in predicting the probability of miscarriage in fresh cycles. The detrimental effect of female calendar age on pregnancy loss is well documented [17, 73, 182, 284]. The final model in Study II included different age categories, since from a clinical point of view age as a categorical variable is more informative than age as a continuous one. Age <35 years was used as a reference. If the age of the woman was 35–39 years, the MR increased 1.5-fold, and if the age was \( \geq 40 \) years, the MR increased 2.6-fold. Fig. 11 shows the modelled probability of miscarriage in different age groups. Although a cubic effect of age on MR has been previously described [161], such was not found in the multivariate analysis in Study II. A relatively low number of cases in the present analysis might explain this difference, even though to date this is the largest study of miscarriage.
Table 9. Characteristics of the study groups in Study II.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fresh cycles</th>
<th></th>
<th>Spontaneous FET</th>
<th></th>
<th>Hormonally substituted FET</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Miscarriage</td>
<td>No miscarriage</td>
<td>Miscarriage</td>
<td>No miscarriage</td>
<td>Miscarriage</td>
<td>No miscarriage</td>
</tr>
<tr>
<td></td>
<td>n or mean</td>
<td>% or mean</td>
<td>n or mean</td>
<td>% or mean</td>
<td>n or mean</td>
<td>% or mean</td>
</tr>
<tr>
<td>n</td>
<td>303</td>
<td>13.8</td>
<td>1895</td>
<td>86.2</td>
<td>76</td>
<td>11.4</td>
</tr>
<tr>
<td>Age, years</td>
<td>34.0</td>
<td>4.9</td>
<td>32.6</td>
<td>4.2b</td>
<td>34.5</td>
<td>3.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.6</td>
<td>4.3</td>
<td>23.2</td>
<td>3.6</td>
<td>22.9</td>
<td>3.6</td>
</tr>
<tr>
<td>Obesity (BMI ≥27 kg/m²)</td>
<td>53</td>
<td>17.5</td>
<td>245</td>
<td>12.9c</td>
<td>7</td>
<td>9.2</td>
</tr>
<tr>
<td>Dysovulation</td>
<td>39</td>
<td>13.1</td>
<td>205</td>
<td>11.1</td>
<td>5</td>
<td>6.6</td>
</tr>
<tr>
<td>Secondary infertility</td>
<td>152/298</td>
<td>51.0</td>
<td>945/1845</td>
<td>51.2</td>
<td>56/76</td>
<td>73.7</td>
</tr>
<tr>
<td>Previous miscarriage</td>
<td>59/217</td>
<td>27.2</td>
<td>316/1468</td>
<td>21.5</td>
<td>21/63</td>
<td>33.3</td>
</tr>
<tr>
<td>Number of embryos transferred ≥1 top quality embryo transferred</td>
<td>167/287</td>
<td>58.2</td>
<td>1051/1711</td>
<td>61.4</td>
<td>16/73</td>
<td>21.9</td>
</tr>
<tr>
<td>eSET</td>
<td>113/303</td>
<td>37.3</td>
<td>745/1895</td>
<td>39.3</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

a P<0.0001, compared with the other two treatment types. b P<0.0001, c P=0.04, d P =0.003, e P=0.002, f P=0.002, g P=0.03, compared with the miscarriage group.
Table 10. Multivariate analysis of miscarriage <12 weeks.

<table>
<thead>
<tr>
<th>Factor</th>
<th>P value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>0.3</td>
<td>0.98</td>
<td>0.94–1.02</td>
</tr>
<tr>
<td>BMI squared</td>
<td>0.013</td>
<td>1.01</td>
<td>1.002–1.011</td>
</tr>
<tr>
<td>Age &lt;35 years</td>
<td>—</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Age 35–39 years</td>
<td>0.004</td>
<td>1.45</td>
<td>1.13–1.86</td>
</tr>
<tr>
<td>Age ≥40 years</td>
<td>&lt;0.0001</td>
<td>2.64</td>
<td>1.83–3.82</td>
</tr>
<tr>
<td>Previous miscarriage no</td>
<td>—</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Previous miscarriage yes</td>
<td>0.002</td>
<td>1.51</td>
<td>1.17–1.95</td>
</tr>
<tr>
<td>Type of cycle Fresh cycle</td>
<td>—</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Type of cycle Spontaneous FET</td>
<td>0.3</td>
<td>0.85</td>
<td>0.63–1.15</td>
</tr>
<tr>
<td>Type of cycle Hormonally subst. FET</td>
<td>0.002</td>
<td>1.65</td>
<td>1.21–2.27</td>
</tr>
</tbody>
</table>

* Predicted probability of miscarriage is $b/(1+b)$, where $b = \text{Exp} (-2.308 - 0.024\times(BMI-23) + 0.006\times(BMI-23)^2 + 0.371 \times \text{age is 35–39 years (yes=1, no=0)} + 0.972 \times \text{age is ≥40 years (yes=1, no=0)} + 0.411 \times \text{previous miscarriage (yes=1, no=0)} - 0.162 \times \text{spontaneous FET (yes=1, no=0)} + 0.502 \times \text{hormonally substituted FET (yes=1, no=0)})$.

(3330 first pregnancy cycles). In the multivariate analysis, ovarian response had no independent effect on miscarriage because of collinearity with age when only one cycle per subject was analysed (Pearson’s correlation coefficient −0.12, $P<0.0001$). This underlines the association between LR and ovarian ageing [184–188]. Collinearity with age can also explain why in the fresh cycles the number of collected oocytes and the performance of eSET did not affect independently the probability of miscarriage.

The interaction between advancing age, eSET and miscarriage was studied more extensively in Study III. The MR of eSET patients aged 36–39 years was 18.9%. This value was slightly higher than previously reported MRs after eSET in younger women (15.3%–16.8%) [13, 42]. Another reflection of reproductive ageing was the fact that eSET was performed in 27% of women aged 36–39 years, while in the same time period 53% of women aged ≥35 years underwent eSET. However, the MR of the older eSET patients was considerably lower than the MR in the general population aged 35–39 years (25%) [347], indicating that women eligible for eSET in this age group may have a greater ovarian reserve compared with that of the general population of the same age.
5.3.2 Factors affecting MR: BMI

In Study II no effect of BMI on MR in the categorical (two and five groups) and linear multivariate regression models was found. A cubic component also did not reach significance. However, in the quadratic regression model, BMI independently affected the MR. Both underweight and obese women have an increased risk of miscarriage, since the relationship between BMI and the MR after IVF/ICSI or FET is not linear but U-shaped (Fig. 11).

This analysis clarifies the long-lived controversy concerning the effect of BMI on MR after IVF/ICSI [261]. In previous studies, underweight and obese women have been found to have lower PRs [54] and higher rates of early pregnancy loss [249] and miscarriage [51] but the results of these studies did not reach statistical significance as a result of the linear or categorical regression models used. In addition, Study II included a relatively high number of underweight subjects (517 treatment cycles of women with BMI <20 kg/m² and 113 cycles of women with BMI <18.5 kg/m²). An insufficient number of underweight subjects and/or the statistical methods used might be the reason(s) for the controversial results in previous studies [51, 241, 242, 247, 248, 256].

The higher MR in subjects with low and high BMI may be explained by the action of leptin. Leptin participates in the regulation of uterine angiogenesis [223] and embryo implantation [115, 222]. Low plasma leptin levels leading to insufficient trophoblast support may explain why underweight women have an increased MR. On the other hand, obesity is characterised by high leptin levels [225, 348] and insulin resistance [246]. These factors may be involved in miscarriage through deficient cross-talk between the embryo and the endometrium because of lower expression of the adhesion factors IGFBP-1 and uterine αvβ3 integrin [229-231]. A more hostile endometrial milieu reflected in higher levels of inflammatory markers such as TNF-α [228] and by lower levels of the immunosuppressor glycodelin [230, 232, 349] may also contribute to the higher MR observed in overweight and obese subjects.

5.3.3 Factors affecting MR: type of cycle

In Study II, a hormonally substituted FET cycle was found to increase the probability of miscarriage 1.65-fold (95% CI 1.21–2.27), while spontaneous FET did not affect MR significantly, compared with the fresh cycles. This finding cannot be explained by insufficient hormonal preparation of the endometrium. In
FET, endometrial thickness was greater in hormonally substituted \( (n=157) \) than in spontaneous FET cycles \( (n=106) \) \( (10.8 \pm 2.3 \text{ vs. } 9.6 \pm 1.7 \text{ mm, } P<0.0001) \) on the day of embryo transfer (mean: day 17.4 of the cycle for both groups). It is difficult to compare this observation with earlier ones owing to the small study groups in previous analyses of spontaneous and hormonally substituted FET cycles [289, 350–352]. In addition, no international guidelines on ultrasonographic monitoring of the endometrium in FET exist at the moment.

![Fig. 11. Modelled probability of miscarriage <12 weeks.](image)

The higher MR in the hormonally substituted cycles can be explained by the fact that hormonal substitution was used in women with ovulatory problems, the most likely aetiology of which was hyperinsulinemia and insulin resistance. Insulin resistance increases the risk of miscarriage independently of BMI or PCOS status [281] and can lead to a less receptive endometrial environment in hormonally
substituted cycles, characterised by a relative deficiency of adhesion factors and glycodelin. The serum levels of glycodelin and estradiol correlate during ovarian stimulation [353] and it is possible that in hormonally substituted FET cycles endometrial estradiol levels, as well as those of glycodelin, remain lower than those in fresh cycles after ovarian stimulation. This is supported by the finding that in the fresh cycles, dysovulation was not associated with a higher MR, compared with other diagnoses.

5.3.4 Other factors affecting MR

Study II revealed that previous miscarriages also affected the MR. Women who had previously had ≥1 miscarriage were at a 1.6- to twofold greater risk of miscarriage after FET. A similar impact of previous miscarriages on the MR after IVF/ICSI has been observed previously [256], with the risk of miscarriage increasing slightly with the number of miscarriages [284]. This observation can also be explained by changes in glycodelin levels. Lower glycodelin levels have recently been observed in women with recurrent miscarriages [349].

The main infertility diagnosis had no effect on the MR. In this respect, the results of Study II are in accord with those of previous analyses, in which no independent effect of PCOS on MR after controlling for obesity [242, 248, 256] has been observed. The differences among studies in which lower success rates in cases of tubal factor infertility [30, 161, 282], male factor infertility [282] or endometriosis [283] have been found might be explained by different treatment strategies.

In summary, the transfer of a top quality embryo is not associated with a lower MR in either fresh or FET cycles. Both underweight and obese women have an elevated risk of miscarriage. PCOS does not have an independent effect, but women who need hormonally substituted FET because of anovulation have a higher MR. Age, ovarian reserve and previous miscarriages are the other factors that affect the probability of miscarriage during the first trimester of pregnancy.

5.4 eSET and age

In Study III the outcome of IVF/ICSI in women aged 36–39 years was analysed. The main outcome according to the type of embryo transfer is shown in Table 11. In the fresh cycles, the PR/ET (33.1 vs. 29.9%, \(P=0.3\)) and the LBR/ET (26.0 vs. 21.9%, \(P=0.2\)) did not differ significantly between the eSET and DET groups.
However, women in the eSET group had a higher cumulative PR (54.0% vs. 35.0%) and a higher cumulative LBR (41.8% vs. 26.7%, \(P<0.0001\)) compared with those in the DET group. The cumulative multiple birth rate in the eSET group was 1.7%, while in the DET group it was 16.6% (\(P<0.0001\)).

Table 11. Clinical outcome after eSET and DET in women aged 35–39 years.

<table>
<thead>
<tr>
<th>Factor</th>
<th>eSET</th>
<th>DET</th>
<th>n=1-eSET</th>
<th>cSET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Fresh cycles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR/ET</td>
<td>111/335</td>
<td>33.1</td>
<td>175/585</td>
<td>29.9</td>
</tr>
<tr>
<td>LBR/ET</td>
<td>87/335</td>
<td>26.0</td>
<td>128/585</td>
<td>21.9</td>
</tr>
<tr>
<td>MR</td>
<td>0/111</td>
<td>0</td>
<td>31/175</td>
<td>17.7(^a)</td>
</tr>
<tr>
<td>Cumulative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR/OPU</td>
<td>181/335</td>
<td>54.0</td>
<td>205/585</td>
<td>35.0(^a)</td>
</tr>
<tr>
<td>LBR/OPU</td>
<td>140/335</td>
<td>41.8</td>
<td>156/585</td>
<td>26.7(^a)</td>
</tr>
<tr>
<td>MR</td>
<td>3/181</td>
<td>1.7</td>
<td>34/205</td>
<td>16.6(^a)</td>
</tr>
</tbody>
</table>

\(^a\)\(P<0.0001\), \(^b\)\(P<0.001\), \(^c\)\(P<0.05\), \(^d\)\(P<0.01\), compared with the eSET group.

Similar PRs/ET (30.8–34.5%) [10, 33, 354] and LBRs/ET (27.2–29.7%) [10, 13, 42, 354] in the fresh cycle after eSET have been found in previous studies on eSET in younger women. The finding is in contrast to that in a previous analysis of unselected IVF/ICSI cases, in which the chance of live birth among women aged \(\geq 35\) years was estimated to be 35% lower than in younger women [355]. This shows that embryo morphology is a stronger determinant of pregnancy than calendar age and suggests that patients should be selected for eSET on the basis of embryo criteria, rather than age.

The cumulative PR after one or two FET cycles in patients aged 36–39 years was 54%, with a live birth rate of over 40%. In younger women even higher values of up to 60% have been reported [12, 13]. The difference can be explained by the results of Study IV, in which higher numbers of collected oocytes (14.4 ± 6.7 vs. 11.9 ± 6.7, \(P=0.007\)) and viable embryos (6.9 ± 3.5 vs. 5.9 ± 3.2, \(P=0.045\)) were observed in eSET women \(\leq 35\) years old than in those aged 36–39 years. These results, however, show that eSET can be safely carried out in all women under the age of 40 years. The outcome after eSET in older women is very acceptable, especially when compared with the cumulative PR in unselected IVF/ICSI subjects with a mean age of 35 years (33.7%) [356].
5.4.1 Value of a non-top quality embryo in eSET

In Study III, eSET was also performed in a smaller group of subjects with only non-top quality embryos. As expected, the PR in fresh cycles was relatively low and was similar to that reported previously (18–19%) [12, 64]. However, through several FET cycles the cumulative PR almost doubled and was similar to the cumulative PR after DET. At the same time the MBR was only 2.8%, which is much lower than that observed in the DET group with two non-top quality embryos transferred (18.6%). These results show that eSET of a non-top quality embryo can be beneficial to women who have an increased risk of obstetric complications.

5.5 Embryo transfer policy in cycles with top quality embryo(s)

5.5.1 Overall outcome in the years 1995–2004

The dynamics of eSET and the cumulative outcome of IVF/ICSI at Oulu University Hospital during the years 1995–2004 were analysed in Study IV and are shown in Fig 12. eSET was started in 1996 and during the study period up to 2004 the proportion of eSET cycles increased from 4.2% to 46.6% ($P$<0.0001). At the same time, the cumulative MBR decreased from 17.1% in 1996 to 4.8% in the 2004 ($P$<0.0001).

![Fig. 12. Dynamics of eSET and cumulative outcome of IVF/ICSI at Oulu University Hospital during the period 1995–2004.](image-url)
Study IV showed that in their starting cycles, women in the eSET and DET periods were comparable in respect of age, BMI, percentage of cases of ICSI and number of viable embryos (numbers not shown). Subjects in the eSET period had fewer fresh cycles (1.5 ± 0.8 vs. 1.6 ± 0.9, \( P=0.002 \)) and more FET cycles (1.0 ± 1.3 vs. 0.8 ± 1.1, \( P=0.0001 \)) than those in the DET period. The total number of cycles/woman (2.5 ± 1.7 vs. 2.4 ± 1.6, \( P=0.06 \)) was similar in the two periods, as was the duration of treatment (eSET 8.8 ± 10.1 vs. DET 9.5 ± 11.8 months, \( P=0.77 \)).

In contrast to fear of decreased effectiveness as a result of the widespread use of eSET [14, 15], the cumulative pregnancy, live birth and term live birth rates per OPU were higher in the eSET period than in the DET period (Table 12). However, the cumulative MBR/OPU was significantly lower in the eSET period (9.5% vs. 19.6%, \( P<0.0001 \)). At the same time, the PR in FET cycles increased from 17% in the DET period to 24% in the eSET period, reflecting better embryo implantation potential. This can be explained by the fact that if two or more embryos are routinely transferred in a fresh cycle, only a few patients would have good quality embryo(s) to be frozen. Consequently, the chance of pregnancy in subsequent FET cycles would remain low.

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcome factor</strong></td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Per OPU</td>
</tr>
<tr>
<td>Cumulative PR (%)</td>
</tr>
<tr>
<td>Cumulative LBR (%)</td>
</tr>
<tr>
<td>Cumulative MBR (%)</td>
</tr>
<tr>
<td>Cumulative tLBR (%)</td>
</tr>
<tr>
<td>Per woman</td>
</tr>
<tr>
<td>Cumulative PR (%)</td>
</tr>
<tr>
<td>Cumulative LBR (%)</td>
</tr>
<tr>
<td>Cumulative MBR (%)</td>
</tr>
<tr>
<td>Cumulative tLBR (%)</td>
</tr>
</tbody>
</table>

Taking into account all the fresh and FET treatments of each patient, the cumulative pregnancy and live birth rates per woman were similar in the two periods. The main clinical outcome measure of Study IV was the tLBR, which increased (41.7% vs. 36.6%, \( P=0.04 \)) as a result of the decreased number of multiple births in the eSET period compared with the DET period (8.4% vs.
19.6%, P<0.0001). This finding is in agreement with Finnish, Belgian and Swedish national registry data showing a steady decrease in the proportion of multiple births after IVF/ICSI but no change in the LBR [38, 301, 357]. Similar results have also been shown in a clinic in the UK in which eSBT was used instead of eSET [333].

Logistic regression revealed that the single-embryo period independently increased the odds of term live birth (odds ratios 1.37 and 1.43 per woman and per OPU, respectively) (Tables 13 and 14).

Table 13. Results of logistic regression analysis of the odds of term live birth after all fresh and thawed-embryo cycles (taking into account the cumulative tLBR per woman)*

<table>
<thead>
<tr>
<th>Factor</th>
<th>P value</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eSET</td>
<td>0.01</td>
<td>1.37 (1.07–1.75)</td>
</tr>
<tr>
<td>DET Reference</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>Years of follow-up</td>
<td>0.052</td>
<td>0.92 (0.85–1.001)</td>
</tr>
<tr>
<td>Age</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>&lt;30 years Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30–34 years</td>
<td>0.22</td>
<td>1.18 (0.81–1.52)</td>
</tr>
<tr>
<td>≥35 years</td>
<td>0.23</td>
<td>0.82 (0.59–1.13)</td>
</tr>
<tr>
<td>BMI²</td>
<td>0.001</td>
<td>0.993 (0.989–0.997)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Endometriosis Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysovulation</td>
<td>0.71</td>
<td>0.91 (0.56–1.48)</td>
</tr>
<tr>
<td>Male factor</td>
<td>0.53</td>
<td>1.11 (0.80–1.55)</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>0.01</td>
<td>0.62 (0.42–0.91)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>0.03</td>
<td>0.65 (0.44–0.96)</td>
</tr>
<tr>
<td>Number of top quality embryos created</td>
<td>&lt;0.0001</td>
<td>1.15 (1.08–1.22)</td>
</tr>
</tbody>
</table>

*Values in starting model: period (eSET vs. DET), years of follow-up, age (<30 years, 30–34 years, ≥35 years), BMI² (centred at 24 kg/m²), diagnosis (excluding cases with multiple diagnoses), method of fertilisation (IVF vs. ICSI), number of top quality embryos created.
Table 14. Results of logistic regression analysis of the odds of term live birth after one fresh and all consequent thawed-embryo cycles (taking into account the cumulative tLBR per OPU).

<table>
<thead>
<tr>
<th>Factor</th>
<th>P value</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eSET</td>
<td>0.01</td>
<td>1.43 (1.09–1.88)</td>
</tr>
<tr>
<td>DET Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years of follow-up</td>
<td>0.08</td>
<td>1.09 (0.99–1.19)</td>
</tr>
<tr>
<td>Age</td>
<td>0.01</td>
<td>0.993 (0.988–0.998)</td>
</tr>
<tr>
<td>&lt;30 years</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>30–34 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥35 years</td>
<td>0.28</td>
<td>0.74 (0.42–1.28)</td>
</tr>
<tr>
<td>BMI²</td>
<td>0.10</td>
<td>0.70 (0.46–1.07)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>0.006</td>
<td>0.54 (0.35–0.84)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>0.08</td>
<td>0.68 (0.44–1.04)</td>
</tr>
<tr>
<td>Dysovulation</td>
<td>0.09</td>
<td>1.35 (0.96–1.90)</td>
</tr>
<tr>
<td>Male factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubal factor</td>
<td>&lt;0.0001</td>
<td>1.26 (1.18–1.34)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>0.08</td>
<td>0.68 (0.44–1.04)</td>
</tr>
<tr>
<td>ICSI</td>
<td>0.09</td>
<td>1.35 (0.96–1.90)</td>
</tr>
<tr>
<td>IVF Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of top quality embryos created</td>
<td>&lt;0.0001</td>
<td>1.26 (1.18–1.34)</td>
</tr>
</tbody>
</table>

* Values in starting model: see Table 13.

The probabilities of term live birth among subjects in the double-embryo period were higher in the “eSET” than in the “DET” estimation (mean ± SD of the difference 0.060 ± 0.007, 95% CI 0.059–0.060, range 0.011–0.070).

More than 90% of the live births in Study IV occurred within four consecutive cycles (fresh or FET): 90.7% (302/333) of the live births in the eSET period and 93.3% (348/373) of the live births in the DET period (P=0.2). An evaluation of time until delivery for the patients in the study periods was also made. Even though the LBR was lower after FET than after fresh transfer, the time until delivery was similar in the DET and the eSET periods (6.7 ± 9.1 vs. 7.4 ± 9.3 months, P=0.2). This suggests that the time to delivery with eSET is not longer than with DET, as has been thought [307].
5.5.2 Cost-effectiveness of eSET policy

Cost

The multivariate general linear model showed that the eSET period was associated with a diminished cost of fresh cycles (OR 0.95, \( P<0.0001 \)) but not the cost of FET cycles (\( P=0.07 \)) (Table 15). The total treatment costs per woman decreased by €275 (range −€1184 to −€164). This was 4.7% (€275/5890) of the total treatment price per woman in the DET period.

Cost-effectiveness results

Before discounting, the total costs during the eSET period were €3,837,964 while in the DET period they were €4,865,304 (Table 16). After 3% discounting, the total costs per woman were €4584 in the DET period, lower than those in the DET period (€4942). The discounted number of term live births per woman was higher in the eSET period (0.261) than in the DET period (0.243), i.e. the eSET period dominated the DET period. The ICER was equal to −€19,889, meaning that €19,889 was saved per term live birth in the eSET period compared with the DET period.

Sensitivity analysis

Even when uncertainty in clinical parameters was added to the cost-effectiveness model, the eSET period was associated with more adjusted term live births and with lower adjusted total costs, compared with the DET period (Table 17). The confidence limits of the ICER were from −€38,602 to −€852.

By multiplying by 1.5, the “European” ICER became −€29,834 (confidence limits −€75,023 to −€1278). When a multiplication factor of 3.7 was used, the “American” ICER was −€73,589 per term live birth (confidence limits −€185,056 to −€3152).
Table 15. Results of the multivariate general linear model of treatment costs per woman in fresh and in thawed-embryo cycles.

<table>
<thead>
<tr>
<th>Factor</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Period of treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eSET</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost of fresh cycles</td>
<td>&lt;0.0001</td>
<td>0.95 (0.91–0.97)</td>
</tr>
<tr>
<td>Cost of FET cycles</td>
<td>0.07</td>
<td>1.03 (0.997–1.07)</td>
</tr>
<tr>
<td>DET</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td><strong>Years of follow-up</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost of fresh cycles</td>
<td>&lt;0.0001</td>
<td>0.96 (0.94–0.97)</td>
</tr>
<tr>
<td>Cost of FET cycles</td>
<td>0.4</td>
<td>0.995 (0.98–1.01)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost of fresh cycles</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>&lt;30 years</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>30–34 years</td>
<td>0.73</td>
<td>1.01 (0.97–1.04)</td>
</tr>
<tr>
<td>≥35 years</td>
<td>0.37</td>
<td>0.97 (0.94–1.01)</td>
</tr>
<tr>
<td>Cost of FET cycles</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>&lt;30 years</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>30–34 years</td>
<td>0.13</td>
<td>0.98 (0.94–1.02)</td>
</tr>
<tr>
<td>≥35 years</td>
<td>0.008</td>
<td>0.94 (0.90–0.98)</td>
</tr>
<tr>
<td><strong>Method of fertilisation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICSI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost of fresh cycles</td>
<td>&lt;0.0001</td>
<td>1.07 (1.04–1.11)</td>
</tr>
<tr>
<td>Cost of FET cycles</td>
<td>0.02</td>
<td>0.96 (0.92–0.99)</td>
</tr>
<tr>
<td>IVF</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td><strong>Number of top quality embryos</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>created</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost of fresh cycles</td>
<td>&lt;0.0001</td>
<td>0.978 (0.971–0.984)</td>
</tr>
<tr>
<td>Cost of FET cycles</td>
<td>0.03</td>
<td>1.01 (1.001–1.02)</td>
</tr>
</tbody>
</table>

*Values in starting model: see Table 13.

Table 16. Raw and adjusted costs (€) and term live births in the eSET and DET periods.

<table>
<thead>
<tr>
<th>Cost factor</th>
<th>eSET</th>
<th>DET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total costs</td>
<td>3,837,964</td>
<td>4,865,304</td>
</tr>
<tr>
<td>Costs of fresh cycles</td>
<td>3,383,250</td>
<td>4,473,172</td>
</tr>
<tr>
<td>Costs of FET cycles</td>
<td>454,714</td>
<td>392,133</td>
</tr>
<tr>
<td>Total costs per woman</td>
<td>5611</td>
<td>5890</td>
</tr>
<tr>
<td>After 3% discounting:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total costs per woman</td>
<td>4584</td>
<td>4942</td>
</tr>
<tr>
<td>Term live births per woman</td>
<td>0.261</td>
<td>0.243</td>
</tr>
</tbody>
</table>
Table 17. Summary of variables used in sensitivity analysis and incremental cost-effectiveness ratio in selected sensitivity analyses (€ / term live birth).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline estimate</th>
<th>Range used</th>
<th>Confidence interval of ICER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Baseline ICER</td>
<td>−19,889</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of fresh cycles in period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eSET period</td>
<td>1027</td>
<td>986.9–1071.0 ^a</td>
<td>−18,645</td>
</tr>
<tr>
<td>DET period</td>
<td>1359</td>
<td>1307.1–1409.0 ^a</td>
<td></td>
</tr>
<tr>
<td>Number of FET cycles in period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eSET period</td>
<td>683</td>
<td>610.1–747.9 ^a</td>
<td>−50,015</td>
</tr>
<tr>
<td>DET period</td>
<td>589</td>
<td>325.3–650.0 ^a</td>
<td></td>
</tr>
<tr>
<td>Term live birth rate, fresh cycle (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eSET period</td>
<td>16.4</td>
<td>14.1–18.6 ^a</td>
<td>−23,723</td>
</tr>
<tr>
<td>DET period</td>
<td>17.7</td>
<td>15.6–19.7 ^a</td>
<td></td>
</tr>
<tr>
<td>Term live birth rate, FET cycle (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eSET period</td>
<td>17.1</td>
<td>14.3–20.0 ^a</td>
<td>−24,883</td>
</tr>
<tr>
<td>DET period</td>
<td>10.5</td>
<td>8.0–13.0 ^a</td>
<td></td>
</tr>
<tr>
<td>Gonadotropin cost</td>
<td>1329</td>
<td>839–1735 ^b</td>
<td>−22,865</td>
</tr>
<tr>
<td>Discounting rate (%)</td>
<td>3</td>
<td>0–5</td>
<td>−19,561</td>
</tr>
<tr>
<td>Regression model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tLBR improvement in eSET period</td>
<td>0.178 ^c</td>
<td>0.031–0.193 ^d</td>
<td></td>
</tr>
<tr>
<td>Improvement in total treatment cost per woman in eSET period</td>
<td>−275.05 ^c</td>
<td>−1183.53 to</td>
<td></td>
</tr>
<tr>
<td>Mean ICER from regression model</td>
<td>−1545</td>
<td>−38,602</td>
<td>−852</td>
</tr>
</tbody>
</table>

^a 95% confidence intervals. ^b Standard deviation of the cost of the mean gonadotropin dose used. ^c Mean from regression model. ^d Range of the difference.

Cost-effectiveness discussion

Study IV is the first evaluation of the overall cost-effectiveness of eSET policy in everyday clinical practice. By contrast, previous cost-effectiveness analyses have involved use of highly selected patients and predetermined treatment protocols, which have limited the validity of their results only to the settings of a randomised controlled trial [315]. When evaluating only costs of fresh cycles, these studies have revealed that eSET is inferior to DET either because of lower LBR [43, 45, 325, 327, 358] or because of higher costs [326].
The discrepancy with the present analysis can be explained by the high number of FET cycles during the eSET period, which are much cheaper than fresh cycles. In the eSET period, a higher proportion of all deliveries resulted from FET cycles, compared with the DET period (49% vs. 35%). This underlines the value of cryopreservation [10, 12], which until now has been underestimated.

Concerns about the economic impact of eSET policy [14, 15] are refuted by the present results. Study IV covered only the costs of IVF treatment, but it is expected that the long-term financial benefits of eSET will be significant. At Oulu University Hospital, if the costs of maternal and paediatric care up to the end of the neonatal period [321] were to have been included in the present analysis, the ICER would have increased about 1.9-fold to about €36,889 per term live birth in favour of eSET. Accordingly, earlier cost-effectiveness analyses have revealed lower overall costs with eSET if expenses for pregnancy, neonatal and paediatric care are also taken into account [43, 326, 327].

The fact that with eSET followed by cryopreservation a term live birth is more likely and less expensive can make IVF/ICSI treatment affordable for more patients. Even in developed countries only one fifth of infertile patients receive IVF/ICSI treatment [319], most likely because of the treatment costs, which can be as high as 25% of the annual household expenditure [4]. Therefore, eSET could also be beneficial in countries where patients pay for IVF/ICSI themselves. It can be estimated that if the price of IVF/ICSI decreases by 5% with eSET, as shown in the present study, a 15% increase in utilisation can be expected owing to the high price elasticity of IVF/ICSI treatment (3.0) [4].

In all countries in which eSET is practised on a large scale, a decreased number of multiple births has been reported, while the pregnancy rate has remained unchanged [3, 38, 52, 301], suggesting that the analysis made in Finland would also be valid in other countries. The results of Study IV support the conclusions made by Ledger et al. that many more IVF/ICSI cycles could be funded through savings made after the adoption of an eSET policy [322].

Mild ovarian stimulation together with eSET is another treatment strategy aimed at diminishing the costs of treatment and multiple births [56]. However, the outcome of mild stimulation seems to be inferior to the outcome of eSET after stimulation by means of the long protocol. eSET with the standard long protocol decreases the costs of IVF treatment and increases the tLBR, whereas with mild stimulation, the tLBR and costs of treatment remain similar to those associated with DET.
In summary, Study IV showed that the eSET policy dominates the policy of DET in women aged less than 40 years. More term live births are observed and substantial financial savings in IVF treatment itself are made without compromising the speed of treatment.

5.5.3 Limitations of the study

Data for the studies included in this thesis were collected retrospectively. As elsewhere in the Nordic countries, ovarian stimulation was monitored by ultrasonographic examination only. Hormonal analysis was not routinely performed, meaning that the analyses had to be limited only to anthropometric, ultrasonographic and laboratory (oocytes and embryos) variables. In Study I, analysis of ovarian activity was based on data of three groups (inactive, normal or polycystic-like ovaries, depending on the number of antral follicles) while the exact antral follicle counts were not recorded. Because of this, precise evaluation of the relationship between the number of antral follicles and ovarian response in three consecutive cycles was not possible. In Study II, despite consistency of data from the five participating clinics, numbers of gestational sacs were not available for all cycles analysed and could not be included in the multivariate analysis. However, this does not undermine the conclusions in Study II, since embryo morphology and age affect the MR independently of the number of gestational sacs [282]. Finally, an analysis of the importance of male factors as regards the outcome of top quality embryo transfer was found to be beyond the scope of the present thesis.
6 Conclusions

Transfer of a top quality embryo results in higher pregnancy and live birth rates, as shown in all studies included in the present thesis. However, if a previous cycle has resulted in less than four collected oocytes, the prognosis is poor even if the ovarian response is improved in the subsequent cycles and top quality embryos are created. On the other hand, when the ovarian response is diminished, the prognosis is good if a previous stimulation has resulted in at least four collected oocytes. These findings are important, since a low ovarian response seldom recurs (2.5%) and the majority of LR patients have both normal and low response cycles. In addition, the poor pregnancy and live birth rates after the transfer of a top quality embryo in subjects with an initial low response indicate that eSET might not be routinely performed among such women.

Transfer of a top quality embryo is also associated with better outcome in frozen-thawed cycles. The results show that an embryo with top quality characteristics before FET might have the same potential as a fresh top quality embryo. However, in contrast to these findings, the transfer of a top quality embryo does not diminish the probability of miscarriage after a fresh or a frozen-thawed embryo transfer. Multivariate analysis showed that the MR increases along with age and is elevated in cycles of women with low response as a result of a diminished ovarian reserve. Both extremes of BMI (underweight and obese) are also characterised by an increased probability of miscarriage, probably caused by altered endometrial function. Subjects with decreased glucose tolerance, such as obese women and those who need hormonal substitution for FET because of anovulation, have a suboptimal endometrium and a high MR. Miscarriages occur through defective embryo-endometrial crosstalk and through an endometrial milieu with increased inflammatory and decreased immunosuppressive factors. Finally, a previous miscarriage is another factor associated with higher probability of miscarriage in the treatment cycle.

Embryo morphology is a stronger determinant of pregnancy than calendar age. This is why elective transfer of a single top quality embryo is a safe treatment strategy regardless of the woman’s age, at least up to the age of 40 years. In this age group, eSET results in cumulative pregnancy rates of 50–60%, while multiple gestations occur in only about 2% of pregnancies. However, eSET is affected by age, since the proportion of suitable patients decreases with age. This is illustrated by the fact that during the time period analysed, eSET was
performed in 56% of the cycles of women of ≤35 years and in only 27% of the cycles of those aged 36–39 years.

The present thesis provides sufficient evidence that eSET is an effective treatment option. The proportion of eSET cycles increased from 4% in 1996 to 47% in 2004. At the same time, the cumulative multiple birth rate per ovum pickup decreased from 17% to 5%, while the cumulative pregnancy and live birth rates per woman remained stable. The increased use of eSET raised the proportion of pregnancies delivered at term as a result of the smaller number of multiple gestations. Contrary to predictions, the time required for treatment does not increase with eSET. More than 90% of births occur within four treatment cycles, but if eSET is performed, patients have relatively fewer fresh and more frozen-thawed cycles. Because frozen-thawed cycles cost less than fresh cycles, costs of IVF/ICSI treatment are smaller with eSET such that the implementation of eSET diminishes treatment costs at a rate of €19,825 per term live birth.

Future perspectives

This work resolved debates about the applicability of eSET and about the effect of obesity in IVF/ICSI, but many more questions remain unanswered. How much does the cumulative outcome vary with respect to the total number of top quality embryos? How can the outcome in hormonally substituted FET be improved? What is the effect of male factors on the outcome of top quality embryo transfer?

This work showed that use of a top quality embryo in FET has the same potential as a fresh top quality embryo. However, since ≈80% of embryos transferred in FET do not have top quality morphology, more detailed evaluation of frozen-thawed embryos is necessary in order to perform eSET in cryopreserved cycles and to decrease the multiple pregnancy rate further.
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in obese infertile women results in improvement in reproductive outcome for all forms 
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leptin levels and worsening insulin resistance independently of adiposity. J Appl 
Obesity does not adversely affect results in patients who are undergoing in vitro 


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FACTORS AFFECTING THE OUTCOME OF IVF/ICSI