Novel paradigms in successful IVF treatment

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1. Introduction

In July, 1978, Louise Brown, the world's first baby to be conceived outside the human body, was born in Britain (1). This historic event was the result of years of research. The first successful attempt to fertilise a human oocyte in vitro had been made in 1973, but the embryo did not implant into the wall of the uterus thus resulting in an early embryo death. Since the pioneering work of Edwards and Steptoe and others (2), in vitro fertilization (IVF) technology has been further refined. Twenty eight years on, IVF has become a central part of infertility treatment with 500.000 to a million in vitro fertilization cycles being performed worldwide every year and presumably way over 1 million IVF children born so far. However, IVF and pregnancies that follow do not come without a price, not only in financial terms but also in terms of medical risks and complications. Generally speaking, over the past 20 years, attention has been mainly focussed on how to improve pregnancy rates while the appropriate balance between success, risks and costs has been inadequately addressed. More attention should be paid to how to define success in IVF also considering risks like multiple pregnancies, the ovarian hyperstimulation syndrome, costs related to treatment and patient discomfort. The ability to identify treatment cycles at risk for multiple pregnancies is also of importance.

1.1 Complications associated with IVF

1.1.1 Multiple pregnancy

It is now widely recognised that the most important complication of IVF is multiple pregnancy (3). The developed world has witnessed a staggering increase in prevalence of multiple births since the introduction of IVF along with large-scale use of ovarian hyperstimulation. In the USA twin birth rates rose by 75% between 1980 and 2000, representing around 3% of total births (4). Similar trends have been reported for European countries (5). The rate of triplet and higher order multiple pregnancy has risen four-fold over the same period, which can be attributed almost entirely to infertility treatments (6). Available data suggest that 40% of twin births are related to infertility treatments. Up to 80% of higher order multiple births are attributable to ovarian hyperstimulation and ARTs. Multiple pregnancies are related to maternal, fetal and neonatal difficulties.

Maternal complications include mortality and morbidity. There is little information concerning maternal death associated with multiple pregnancy in the developed world. One publication describes a twofold increase in mortality associated with multiple pregnancies

compared with singleton pregnancies (7). Maternal death is caused mainly by eclampsia or excessive blood loss (7). Women carrying multiple pregnancies are at increased risk of requiring long periods of bed rest, hospitalisation, administration of medication to prevent preterm labour and increased risk for surgical procedures (caesarean section, cerclage). Multiple pregnancies have been shown to be an independent risk factor for woman to be admitted to an intensive care unit (8). Hypertension is one of the major maternal complications associated with multiple pregnancy (9). Severe hypertension is 2-3 times more common in twin than in singleton pregnancies. Pre-eclampsia occurs about three times more often in twin than in singleton pregnancies with an incidence of 10-20% (10). Iron and folate deficiency anaemia are more often seen in multiple pregnancies, bleeding at some time during pregnancy is also more frequent in multiple pregnancies compared with singleton pregnancies (11).

Perinatal mortality rates (including stillbirths, early neonatal, late neonatal and infant mortality) are higher in multiple pregnancies compared to singletons, and the rates increase with the number of fetuses (12). Twins are at approximately 5-fold increased risk of fetal death and 7-fold increased risk of neonatal death, compared with singletons (13).

Preterm delivery and low birth weight are the major causes of mortality and morbidity in multiple pregnancies. Gardner et al found that 54% of twins were preterm compared with 9.6% of singletons, and that birth at <32 weeks of gestation occurs in 15-17% of twin and 1-2% of singleton pregnancies (14). Martin et al found that 10.2% of twins had a birth weight below 1500 grams and 54.9% had a birth weight below 2500 grams. This compares with respective frequencies for singletons of 1.1% and 6% singletons (15). The majority of excess morbidity in multiple births is attributable to low birth weight and preterm delivery. As a result of these problems many multiples require treatment and extended care in neonatal intensive care units (NICU). According to one study 15% of singletons, 48% of twins and 78% of triplets were admitted to the NICU (16). Multiple births have been recognised as a risk factor for cerebral palsy (17). A consistent finding in the literature is that the risk of cerebral palsy increases with plurality. Multiples may also suffer long-term medical and developmental problems. The major morbidity is neurological impairment and varies from clinical neurological impairment to minor and probably sub-clinical abnormalities.

In addition to the medical risks of multiple pregnancies there are psychological consequences for the children themselves, the siblings and the parents (18). Twins have been extensively studied (19). It has been shown that they are frequently slower learners in language and in other school subjects. Multiples begin to speak later than singletons, owing to

less individual attention or because they learn to communicate in another way with each other. Parents of multiples are affected socially and psychologically (20). These parents are more likely to be exhausted, depressed or anxious after birth (21). Increased rate of depression far beyond the infancy period has been reported in mothers of twins (22). The burden of raising multiples may be further increased for the parents if the children are physically or mentally disabled (23). Social isolation and little time for themselves may place a great deal of stress on the marital relationship.

1.1.2 Ovarian Hyperstimulation Syndrome

Another serious complication in IVF is the ovarian hyperstimulation syndrome (OHSS). Although rare (24), it entails potentially serious and even life-threatening medical damage. Prevention of OHSS is possible by identifying known risk factors such as polycystic ovaries (25), by an appropriate choice and application of drugs during treatment i.e. using a GnRH-antagonist instead of a GnRH agonist to prevent a LH surge (26) or by administering a lower dose of gonadotropins, cancelling the cycle, coasting (27), elective cryopreservation of all embryos or prolonging the use of the GnRH antagonist (28) in case of a too high ovarian response.

1.1.3 Other complications associated with IVF

Only few patients experience side effects with the use of fertility drugs. Side effects of fertility drugs include local reactions e.g. mild bruises and soreness at the site of injections. Research has shown that, pituitary down-regulation with GnRH agonist was associated with elevated levels of symptoms of depression (29) and headache (30). In another study women undergoing pituitary downregulation with a GnRH agonist reported more frequent headache, lower back pain and muscle pain then control patients (31).

Apart from health risks, standard IVF treatment can be an emotional burden to patients. According to a study by Olivius at al (32), psychological distress is the main reason why many patients drop out of IVF treatment. The authors reported a cumulative drop out rate of 54% after two cycles. Many couples have to face treatment failure, which seems to be related to an increased prevalence of subclinical anxiety and depression in women (33). Furthermore, IVF treatment itself, with its daily injections, ultrasounds and invasive procedures, such as oocyte retrieval, might be a cause of psychological distress.

Bleeding and infection after oocyte pick up are also complications of the IVF treatment but these complications are rare. Furthermore, research to investigate the long term

risks of ovarian stimulation is ongoing and may lead to the discovery of additional adverse events.

1.2 Alternative Approaches in IVF

1.2.1 Reducing the number of embryos to transfer

Many clinics, especially in Europe, now offer transfer of one embryo as routine clinical care in selected patient groups (34,35,36,37,38). Improved quality assessments of embryos enhances the effectiveness of single embryo transfer (36,39). Although comparative trials have persistently shown a decrease in pregnancy rates for elective single embryo transfer (40,41,42,43,44,45), single embryo transfer applied in centres with good laboratory performance and in selected patients, birth rates are comparable following the transfer of one or two embryos. These results should encourage other centres to offer single embryo transfer in selected patients. Table 1 shows the results from randomised controlled trials comparing single versus dual embryo transfer (40,41,42,43,44,45). Table 2 shows the results from the observational studies (46,47,37,42,48,38,49,50).

Table 1. Results form randomised controlled trial concerning elective single embryo transfer versus dual embryo transfer

Author, year	**N**	**Pregnancy Rate 1 ET**	**Delivery Rate 1 ET**	**Twin Pregn (%)**	**Pregnancy Rate 2 ET**	**Delivery Rate 2 ET**	**Twin Pregn (%)**
Gerris, 1999	53	38.5	na	0.1	74	na	30
Martikainen, 2001	144	32.4	29.7	0.04	47.1	40	39.3
Gardner, 2004	48	60.9	na	0	76	na	47.4
Thurin, 2004	661	28.5	27.6	0.01	43.8	42.4	33.1
Lukassen, 2005	107	37	26	0	47	36	37
Montfoort, 2006	308	21.4	na	0	40.3	Na	21
Total	**1321**	**29.5**	**27.7**	**0.01**	**46**	**41.6**	**31.7**

Table 2. Results from observational studies concerning elective single embryo transfer versus dual embryo transfer

Author, year	**N**	**Pregnancy**	**Delivery**	**Twin**	**Pregnancy**	**Delivery**	**Twin**

		Rate 1 ET	Rate 1 ET	Pregn (%)	Rate 2 ET	Rate 2 ET	Pregn (%)
Vilska, 1999	816	29.7	24.3	0	29.4	na	23.9
Tiitinen, 2003	1494	34.4	27.2	1.6	36.7	26.9	27.6
Gerris, 2003	1152	35.1	na	0.9	36.2	na	35.3
De Sutter, 2003	2898	28.2	na	0.6	31.7	na	30.4
Gerris, 2004	367	40.3	37.4	0	40.4	36.6	30.8
Martikainen, 2004	1111	34.7	27.9	0.9	31.8	na	na
Montfoort, 2005	521	35.1	31.5	0	34.6	29	23
Saldeen, 2005	340	45.5	na	0	34.7	na	19.5
Total	**8699**	**34.5**	**29.4**	**0.7**	**33.3**	**28.4**	**29.3**

Only one randomised controlled trial was conducted comparing the transfer of one or two embryos in an unselected group of patients (i.e. irrespective of the woman's age and embryo quality) (44). However the mean age in this trial was still young (32.7 years in the SET-group and 32.4 years in the DET-group). No randomised controlled trial have been performed in women above 38 years only. Because implantation will considerably decrease with age most clinicians agree that single embryo transfer is not advisable in women of 38 years and older (51). Many clinics advise the transfer of three embryos in this age group. Little is known on the feasibility of transferring two instead of three embryos in women of this age in order to decrease the incidence of multiple gestation. Large but retrospective studies did not find a difference in pregnancy rates per cycle performing the transfer of 2 embryos compared to the transfer of three embryos. Obviously such a study approach lacks the insight into the accumulation of pregnancies in subsequent cycles (52,53).

Despite the high costs involved, detailed cost studies of the IVF treatment have received little attention. Mathematical models indicate that single embryo transfer might be more cost effective than dual embryo transfer (35), but well designed prospective studies are needed to confirm this possibility. The studies comparing costs of single and dual embryo transfer were not randomised controlled trials, but all used theoretical extrapolations or decision-analysis calculations (35,54,55). De Sutter and colleagues used a health-economic decision-analyis model to compare dual embryo transfer with single embryo transfer. The model calculated treatment, pregnancy and neonatal care costs. They found that the cost per child born was the same for single as for dual embryo transfer. When costs are calculated per term live birth

instead of child born (and a twin should be calculated as one instead of two) costs for dual embryo transfer would be higher than for SET, which can be explained by the four fold higher cost of pregnancy of a twin instead of a singleton that they used in their calculations. In a study of Lukassen (56) it was shown that medical cost per twin pregnancy was much higher than for a singleton pregnancy. An earlier study (35) showed that, irrespective of the level of costs and irrespective of the level of performance of an IVF centre, the cost per child born from a SET policy is comparable with the costs per child in the dual embryo transfer policy. This was explained by the fact that higher pre- and neonatal cost due to the twin pregnancies arising after dual embryo transfer is balanced by higher cost for more SET cycles needed to obtain the same number of children (41).

When implementing single embryo transfer at large counselling is of great importance (57). A change in practice can only be achieved if those seeking treatment can be convinced as well as those responsible for delivering it. Couples on the threshold of IVF treatment may find it difficult to see beyond the short term gains of a pregnancy, and focus on the longer term benefits of a healthy singleton child. To many, having twins appears to offer a cost-effective way of completing their family and may represent a willingness to take risks in order to achieve pregnancy.

1.2.2 Mild ovarian stimulation

For around 15 years profound ovarian stimulation using a GnRH-agonist to prevent premature luteinization has dominated treatment in IVF. This approach in ovarian stimulation that aims at achieving multiple dominant follicles, is costly, takes many weeks with frequent injections and possibly implies high burden on patients in terms of risk and side effects.

The introduction of GnRH antagonists into clinical practice has enabled shorter treatment protocols to be applied, since, in contrast to GnRH agonists, treatment can be limited to the days in the mid-to-late follicular phase at risk of a premature LH rise (58,59). Moreover since this approach enables the endogenous inter-cycle FSH rise to be utilized rather than suppressed, it has opened the way to the development of mild ovarian stimulation protocols in which exogenous FSH administration is limited to the mid-late follicular phase (60,61,62,63).

Mild ovarian stimulation protocols may reduce drop-outs from IVF and therefore increase the overall number of cycles per patient, resulting in increased overall birth rates per started treatment. Furthermore patient-friendly stimulation protocols may increase efficiency, enabling more cycles to be carried out in a given period than is possible with conventional

stimulation protocols. Current attitudes to profound ovarian stimulation should change certainly with the growing tendency currently towards the transfer of a reduced number of embryos to reduce multiple pregnancies (43).

1.3 From embryo to patient: Determinants of IVF outcome

While much progress has been made in improving ovarian stimulation regimens to optimise embryo selection for transfer (64) it is becoming increasingly clear that patient related factors may be just as, or more important in determining the chance of success of treatment. The ability to identify those treatment cycles at particular risk of leading to multiple pregnancy and for which SET would not reduce the chance of achieving a singleton pregnancy may encourage the adoption of SET into clinical practice. A number of prognostic factors have been identified which enable the patient to be appropriately counselled.

The most important factor is age. A Swedish study showed that women under 35 years of age with at least two good-quality embryos available for transfer were at high risk for multiple birth. A decline in birth rate occurred 1 year later. They concluded that 36 years can be recommended as an age limit for single embryo transfer. The initial dose of FSH, the total dose of FSH, the number of oocytes (65), oocyte quality (66), fertilization rates (67,68) and number of embryos (69,70,71) are all related to age and have therefore little additional predictive value. Another very important factor for predicting a multiple pregnancy is the developmental stage and the morphology score of the two best embryos available (72). Other studies showed the importance of the cycle number. A decrease in the chance of a live birth in the third cycle was noticed (73) suggesting that SET should only be performed in the first and second cycle. Subjects who have had a previous pregnancy have an increased chance on delivering a live birth after IVF. If a patient has had a live birth after IVF the chance on delivering again a live birth after IVF is even bigger (74). The chance on success is decreasing with increasing duration of infertility (75).

The extent to which the underlying pathology itself can impact on the chance of success has been the subject of considerable study. A meta-analysis comparing pregnancy rates after IVF in women suffering from endometriosis and tubal factor controls showed a significantly lower fertilization, implantation and pregnancy rate in the first group. Tubal disease is not associated with poor outcome in IVF. However patients with tubal disease associated with hydrosalpinges have a lower chance on success in IVF (76). If the indication for IVF is male factor results of IVF are determined by age of the woman, sperm motility and sperm morphology. Chronic anovulation is a common cause of infertility. Normogonadotropic

anovulatory infertility (World Health Organization (WHO) group II) (77,78) can be identified in 18-25% of the couples presenting with infertility (79). Polycystic ovary syndrome (PCOS) represents the most common diagnosis within this patient group (80). Classic induction of ovulation (including clomiphene citrate as first line and exogenous gonadotropins as second line treatment) results in cumulative singleton live birth rates of up to 71% in 2 years. Patients not conceiving with classical ovulation induction or poor prognosis PCOS women will continue with IVF. Studies comparing IVF treatment outcome in PCOS versus controls have shown that more oocytes could be retrieved, but with a reduced proportion of oocytes fertilized (81,82,83). Despite reduced overall fertilization, IVF pregnancy rates in PCOS patients appear to be comparable to normo-ovulatory women (81,82,83). With improved outcome and the more frequent use of single embryo transfer, eliminating chances for multiple pregnancies, IVF has become a serious treatment option in women suffering from anovulatory infertility.

In general it can be concluded that important factors when selecting patients for single embryo transfer are female age (<35-37 years, previous pregnancy, IVF cycle number (1st or 2nd), number of good-quality embryos available (≥ 2) and absence of hydrosalpinges or endometriosis. It is important to consider this factors when advising patients about the number of embryos to be transferred.

1.4 Defining success in IVF

To compare different treatment strategies the numerator and denominator of results in IVF treatments have to be consistent (84). The rationale for the use of a particular indicator should be explicit, as variation in numerator and denominator selection results in inconsistency of reporting and creates an opportunity for confusion in both the professional community and the recipient of care (85). The definition of success in IVF has to be simple and clear. Using a combination of parameters for reporting success (i.e. number of oocytes, number of ongoing implantations and number of deliveries) (86) seems exaggerated and unnecessary. Also, in the context of reporting research outcomes, choosing a different outcome parameter per trial and for different purposes (87) seems illogical.

One of the current most acceptable approaches for the numerator in defining success in IVF is the ongoing pregnancy rate. Other outcomes that have been suggested include the (term) (singleton) live birth (84). Recently *new* outcome parameters have been proposed. For example the singleton live birth rate per cycle (SLBRPCS) and multiple live birth per cycle started (MLBRPCS) (88) that reward efficacy (many healthy singleton babies) and penalizes

unsafety (multiple embryo transfer). and the cumulated singleton delivery rate (CUSIDERA) and cumulated twin delivery rate (CUTWIDERA) (89) which represents the combination of efficacy and safety. In 2004 the BESST (Birth Emphasizing a Successful Singleton at Term) endpoint was proposed: Singleton, term gestation, live birth rate of a baby per cycle (84). In addressing what constitutes the most relevant standard of success in assisted reproduction it was argued that pregnancy without consideration of obstetric and neonatal outcomes is no longer the objective. Practitioners acknowledge the significant contribution of multiple pregnancies to the risks and complications of assisted reproductive technology. However, despite universal agreement on the need for a reduction of this iatrogenic complication (90) trends in multiple pregnancies and deliveries have not declined (91,92). As high-risk pregnancies, twin gestations should be considered complications of assisted reproductive technology treatment and not counted as successes (93,94). If the object is a healthy baby, the specification of 'term gestation' is also justified. Term gestation is well defined, internationally agreed and able to be retrieved in all countries. However the outcome healthy singleton birth will appeal to obstetricians but is unlikely to find favour with patients. Couples on the threshold of IVF treatment may find it difficult to see beyond the short-term gains of a pregnancy, and focus on the longer term benefits of a healthy singleton child.

Whether twin pregnancies should be excluded when calculating success rates in IVF remains a point of debate (95). The definition of a twin birth as 'a complication' with the only acceptable outcome of infertility treatment being a single live birth is considered to be unnecessary and unsympathetic to couples who require ART in order to achieve pregnancy (95). A singleton birth policy for ART will multiply costs and discomfort for couples who require IVF, desire two children and have no physical impediment to successful completion of a twin pregnancy (95). Twins due to IVF account for only 1.4% of total premature births in the US. Furthermore, infants from multiple births have a greater chance of survival than singleton infants, of the same birth weight, gestational age, and ethnic origin (96).

Others questioned including 'term' in the definition of success in IVF because the aetiology of preterm birth among singletons is largely unknown and probably multifactorial (97,98). Numerous studies suggest that singleton infants born after IVF treatment are at increased risk for low birth weight, preterm delivery and fetal growth restriction in comparison with naturally conceived infants (99,100,101). However questions remain about whether these risks stem from the IVF treatment or from the underlying infertility of the couples using these treatments.

In addition to the numerator of the definition of success in IVF the denominator is also of great importance. One of the current most acceptable approaches for defining success in IVF is success per started cycle also taking cancelled cycles into account. The exclusion of cycles from which oocyte retrieval is not attempted is inappropriate. Oocyte retrieval is a significant component of assisted reproductive technology, accounting for much of stress, financial burden and almost all of the surgical risk (102). Moreover the cost of follicular stimulation is not insignificant, nor is the emotional burden of a cycle that is terminated prior to oocyte retrieval. Others are convinced that the cumulative delivery rate per stimulated cycle after all embryo transfers, fresh and frozen have been performed should be calculated (103,104,86). This strategy highlights the importance of cryopreservation programmes when implementing elective single embryo transfer (eSET) strategies.

In practice however the one piece of information that a woman or a couple really want to know is the likelihood of having a healthy baby at the end of a course of treatment (subsequent treatment cycles) or after a certain time period (105).

1.5 Study objectives

One of the main problems in IVF are multiple pregnancies. Awareness is growing that the ever–increasing contribution of assisted reproductive technology to multiple births in the developed world is no longer acceptable. Reducing multiple births in IVF is possible by performing single embryo transfer. The most important strategy to introduce single embryo transfer on a large scale will be to improve success in IVF while reducing the number of embryos transferred. In general success in IVF is presented per cycle. This has led to complex, stressful stimulation protocols resulting in high drop out rates. Adopting a new primary endpoint (term live birth per time period) will result in clinicians and scientists being encouraged to develop and apply patient-friendly stimulation protocols with less stress and discomfort, and fewer side effects and chance of complications such as the ovarian hyperstimulation syndrome. Milder stimulation strategies enables subjects, due to shorter duration and better patient tolerance, to have more cycles in the same time period. More cycles means additional pregnancy chances, which can compensate for the reduction in live birth per cycle due to milder treatment strategies. The present thesis addresses novel approaches for defining and achieving success in IVF and their consequences.

Firstly, the optimal way of defining success in IVF and the possible consequences adopting such a definition is discussed. Secondly, a meta-analysis comparing the outcome of IVF in PCOS and non-PCOS women is presented. Ovulation induction (with anti-estrogens or

gonadotropins) has the undesired side effect of inducing a high percentage of multiple pregnancies. IVF with single embryo transfer could be a feasible option to reduce single embryo transfer. The aim of this study was therefore to assess whether results of IVF in PCOS and non-PCOS women are comparable and whether studies investigating single embryo transfer may also apply to PCOS women. Thirdly, two randomised studies were performed evaluating the effects on the cumulative term live birth rates of reducing the number of replaced embryos. In a first trial a two versus three embryo strategy was compared in women over 38 years of age. The main objective of this study was to show that reduction of twin pregnancies can be obtained without a reduction in the overall term live birth rate per treatment. In a second trial, which was conducted in women under 38 years of age it was the objective to study whether mild ovarian stimulation and single embryo transfer would 1) prevent multiple birth rates while maintaining similar overall term live births per given time period, 2) reduces psychological and physical complaints, 3) improves efficiency (cost-effectiveness) of IVF treatment combined to standard ovarian stimulation and dual embryo transfer.

2. The next step to improving outcomes of IVF: Consider the whole treatment

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2.1 Introduction

A debate article in human reproduction proposed that 'the singleton, term gestation, live birth rate per cycle initiated should be considered the best endpoint for IVF' (84). It was suggested that this outcome definition reflects precisely what a subfertile couple wishes to know when they embark an IVF treatment. In our view, IVF outcomes should be defined in broader terms which reflect the interests both of the couple and those providing health care. A couple embarking on IVF are presently focused on the traditional numerators and denominators of outcome as shown in Table 1. The goal of their treatment is the chance of having a healthy baby after completing an *IVF treatment* consisting of a series of IVF cycles and subsequent replacement of frozen embryos. This should be weighed against the associated discomfort, complications and costs which they will encounter along the way. The outcome of a single cycle is of interest, but only as part of the whole treatment. The information patients, providers and policy makers require is the chance of delivering a healthy baby per treatment started (106,105) or per defined treatment period. Should these criteria become the means by which IVF outcomes are measured, a number of beneficial consequences would ensue.

2.2 Focusing on the whole treatment: consequences for clinical practice.

2.2.1 Patient friendly stimulation protocols

Around 50% of those who initiate IVF will not conceive (107). This is partly due to the high drop out rates after an unsuccessful IVF cycle. European data reveal that up to 25% of patients who undergo a first IVF cycle refrain from further treatment (108), and are therefore deprived of additional chances of conceiving. This is not only due to costs, or poor prognosis (109) but also due to the stress and side effects of the treatment itself (32). By expressing results in terms of the delivery of a healthy baby per treatment started (or in a given time period), clinicians and scientists will be encouraged to develop and apply patient friendly stimulation protocols with less stress, discomfort, side effects and chances for complications such as the ovarian hyperstimulation syndrome.

The introduction of GnRH antagonists into clinical practice has enabled shorter treatment protocols to be applied since, in contrast to GnRH agonists, treatment can be limited to the days in the mid-to-late follicular phase truly at risk of a premature LH rise (58). Moreover,

since this approach enables the endogenous inter-cycle FSH rise to be utilized rather than suppressed, it has opened the way to the development of mild stimulation protocols in which exogenous FSH administration is limited to the mid-late follicular phase (110,111,112,113).

Mild stimulation protocols may reduce drop outs from IVF and therefore increase the overall number of cycles per patient resulting in increased overall birth rates per started treatment. Shorter, patient friendly stimulation protocols may increase efficiency, enabling more cycles to be carried out in a given period than is possible with conventional stimulation protocols. Increasing exposure to chances of pregnancy while reducing exposure to the complications of conventional ovarian stimulation also offers a formula for reducing costs.

2.2.2 Single Embryo Transfer

In a debate series in Human Reproduction, Land and Evers suggest adopting an outcome measure - the corrected singleton live birth rate per cycle started - that rewards efficacy (many healthy singleton babies) and penalizes unsafety (multiple pregnancies) (88). We would agree that the ideal numerator for determining IVF outcome is a term singleton baby. However, Dickey et al proposed that multiple outcome measures are necessary when evaluating IVF success and that twin as well as singleton births should be counted as IVF successes (95). While healthy term twins may be perceived as a good outcome, twins in general are at higher risk of neonatal morbidity and mortality (14,114), and the current consensus is that multiple pregnancies should be prevented. One approach to the problem of reporting IVF results may be the implementation of a scoring system where singletons ‘count higher’ than twins (score 1 versus 0.5) but both are recognised as preferable to no pregnancy and higher order multiple pregnancies (score 0). In this way twin pregnancies contribute to the pregnancy rate per treatment but are also relatively penalized (72).

2.3 Healthy Baby

In this, and other articles in the current debate series, the phrase ‘healthy baby’ is frequently referred to. Intuitively such an outcome is desirable not only for prospective parents but also for health care providers. The meaning of ‘healthy’ in this context remains to be defined. A recent study has added to concern that even singleton babies born after conventional IVF may be at increased risk of prematurity and the associated health risks (115). By inserting the word ‘term’ into the numerator of singleton baby, additional encouragement would arise to develop IVF treatments in which the risk of prematurity was further limited.

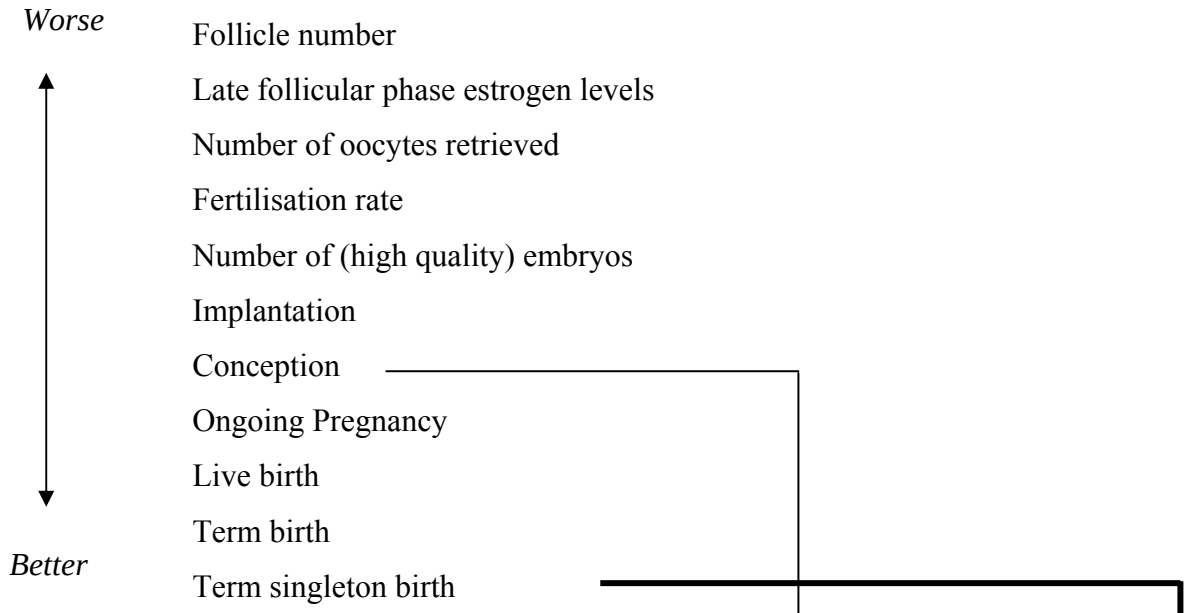
2.4 The integrated picture

Combining mild stimulation protocols with single embryo transfer is consistent with the emphasis on reducing complications for mother and child. This maybe at the price of a minor drop in pregnancy rate per cycle (46,37), but the same overall pregnancy rate per total IVF treatment may be achieved in the same amount of time, for similar costs with less patient stress and discomfort and most importantly with the virtual elimination of multiple pregnancies. It has recently been shown that counselling over the risks of multiple pregnancy may be insufficient to convince couples to opt for elective single embryo transfer (116). In contrast, if they can be reassured that their chance of achieving the goal of treatment will not be compromised, patients are receptive to the idea of transferring one rather than more embryos. Were IVF success rates to be expressed in terms of delivery of a term single baby per IVF treatment or in a certain time period, then such reassurance may be readily given, and single embryo transfer on a large scale more rapidly introduced.

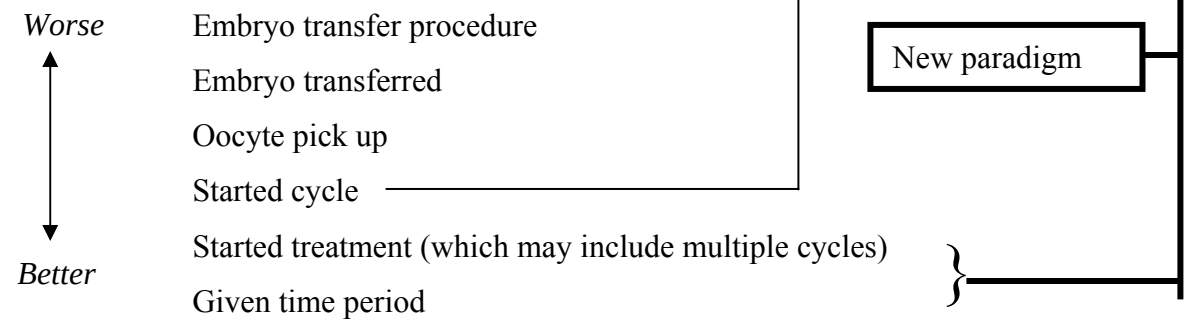
We postulate that the combination of mild stimulation and single embryo transfer would reduce the overall costs of treatment, both to couples and society, partly by reducing the indirect costs related to pregnancy complications. This could be achieved despite an increased number of cycles compared to conventional IVF hyperstimulation and dual embryo transfer (117,35,37). We consider that the optimal numerator and denominator for defining outcome from IVF are the term, singleton birth rate per started IVF treatment (or per given period). Widespread adoption of this definition would be an important step towards achieving these goals.

Table 1. *Assessment of IVF treatment outcome: towards the optimal numerator and denominator.*

Numerators



Denominators



3. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome

Heijnen, E.M., Eijkemans, M.J., Hughes, E.G., Laven, J.S., Macklon N.S., Fauser B.C.
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3.1 Introduction

Anovulation is a common cause of infertility. About 70% of infertile women presenting with oligomenorrhoea or amenorrhoea exhibit normal follicle stimulating hormone (FSH) and oestradiol concentrations (World Health Organization [WHO], Type 2 anovulation) (77,78). Normogonadotropic anovulatory infertility can be identified in 18-25% of the couples presenting with infertility (79). Polycystic ovary syndrome (PCOS) represents the most common diagnosis within this patient group (80).

Pharmacological ovulation induction constitutes the first line treatment of choice in these women, aiming at mono-ovulation. Conventional strategies include the anti-oestrogen clomiphene citrate as first line (118) and exogenous gonadotropins as a second line intervention (119). Although overall cumulative singleton live birth rates of 71% have been described after conventional ovulation induction, the multiple pregnancy rate (especially with exogenous gonadotropins) is considerable (10%) (120). The development of multiple dominant follicles resulting in multiple pregnancies cannot always be prevented. Therefore the widespread use of gonadotropin ovulation induction may be questioned (121,6). Prospective cohort follow-up studies have identified patient characteristics upon initial screening capable of predicting clinical outcome like mono-ovulation and pregnancy (122,123). Moreover, different strategies generating mono-ovulatory cycles have recently been emphasized, including *weight* reduction and life style changes, insulin sensitizers (124), aromatase inhibitors (125) and laparoscopic electrocautery of ovaries (126).

In addition, assisted reproductive technologies (ART) like intra-uterine insemination (IUI) or in vitro fertilization (IVF) are increasingly applied (6) although well designed studies documenting efficacy and safety in PCOS are lacking in this patient group. Certainly, with improved outcome and the more frequent use of single embryo transfer, eliminating chances for multiple pregnancies, IVF has become a serious alternative to ovulation induction. In addition, favourable IVF outcomes have been reported applying in vitro oocyte maturation in PCOS (127). Despite this trend, uncertainty remains with regard to risk of ovarian hyperstimulation syndrome (OHSS), cycle cancellation rate, oocyte quality and fertilization

rates in PCOS women undergoing IVF. Furthermore it remains unclear whether pregnancy rates differ between PCO and non PCOS women. Most published data are derived from uncontrolled, observational studies with small study populations. The aim of this meta-analysis is to compare IVF outcome in women with and without PCOS, using the best available data.

3.2 Materials and Methods

3.2.1 Criteria for considering studies for this review

Studies in which PCOS patients undergoing IVF were compared with a matched control group were considered for this review. The characteristics of the control group are given in Table I. No IVF/intra cytoplasmatic sperm injection (ICSI) cycles may be performed in both groups. PCOS diagnosed in line with the Rotterdam consensus criteria was required (2 out of 3 of the following criteria: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries) (80). Patients within a study had to be treated with the same ovarian stimulation protocol. Information regarding patient and cycle characteristics like age and number of oocytes retrieved and pregnancy outcome was also required.

3.2.2 Search strategy for the identification of studies

A search strategy was carried out based on the following MESH headings: "Polycystic Ovary Syndrome"[MAJR]) AND ("Fertilization in Vitro"[MAJR] OR "Reproductive Medicine"[MAJR] OR "Reproductive Techniques, Assisted"[MAJR). In addition a handsearch of Human Reproduction 1991-2004 and Fertility Sterility 1988-2004 was conducted. In addition the pharmaceutical companies Ferring, Organon and Serono were invited to provide data from unpublished or ongoing studies relating to this topic. Finally, the bibliographies of identified studies were hand-searched.

3.2.3 Identification

The MESH headings strategy yielded 290 publications. No additional publications were identified after the hand-search of Human Reproduction and Fertility Sterility and no additional data was obtained from the pharmaceutical companies. One hundred and twenty nine publications were excluded because it was clear from the title that they did not fulfil the selection criteria. Five of the 129 excluded publications were read in full (EH) to check the validity of this selection procedure. From the remaining 161 articles, 101 were excluded on

the basis of the abstract (EH). Seven of the remaining 60 publications were considered by two independent readers (EH,NM) to fulfil the selection criteria for inclusion. Two more publications were included after the respective first author had provided additional necessary information. All the bibliographies of the included publications were checked and no additional articles were identified.

3.2.4 Methods of the review

No prospective randomised controlled trials were identified addressing our research question. We therefore searched for studies which compared IVF outcomes in PCOS patients with matched controls. The following information was extracted from potentially relevant studies: study characteristics, specified as matched control (retrospective/prospective), cohort study (retrospective/prospective) and cross-over, patient population characteristics, identifying study groups and outcome measures. From the 9 relevant studies ultimately selected for further analysis the following data were extracted (Table I): definition of PCOS, previous treatment before IVF, constitution of the control group, treatment protocol and number of patients in the study and control group. The primary endpoints were number of oocytes retrieved, number of oocytes fertilized, number of patients with OHSS and number of clinical pregnancies. Secondary endpoints are summarized in Table II.

3.2.5 Statistical analysis

Data from the studies in Table II were pooled if at least two studies reported a similar outcome characteristic. For each study, the difference in IVF related outcome parameters between PCOS and control groups, were computed from the reported data. When the outcome of interest was of a continuous nature (e.g. number of ampoules FSH) the difference in mean value between the two groups was calculated, together with standard error. These differences were pooled across studies, resulting in a Weighted Mean Difference (WMD). For binary outcome parameters (e.g. cancellation), the odds ratios per study were calculated and pooled after logarithmic transformation. Pooling was performed using the inverse of the variance as weight. Heterogeneity between studies was tested and random effects estimates were calculated using the likelihood method described by Hardy and Thompson, when at least 3 studies were available. It may occur that this calculation does not yield results, when the variation between studies is less than the random expected variation. In those cases there is definitely no heterogeneity. The 95% confidence intervals are presented for the WMD and

pooled odds ratio respectively, using both the direct weighted method and the random effects (heterogeneity corrected) method. The random effects method is the preferred because it remains valid when true heterogeneity between studies is present. Statistical pooling was preformed for the following outcome parameters: number of cycles, oocyte retrieval and embryo transfer, number of ampoules gonadotropins used, duration of stimulation, number of oocytes, number of oocytes fertilized and number of clinical pregnancies.

3.3 Results

Nine relevant studies were identified (128,81,129,82,130,131,132,83,133), reporting data on a total of 458 PCOS patients (793 cycles) and 694 matched controls (1116 cycles). Information about the studies including definition of PCOS and previous treatment is provided in Table I. The sample size varied across the trials (19-392 patients; 19-518 cycles). There was no difference in age between PCOS patients and controls (31.9 years versus 31.8 years), weighted mean difference (WMD) -0.1 years (95% CI -0.6;0.3). No significant statistical heterogeneity was detected between studies. The random effects estimate for age between PCOS and non PCOS women was -0.2 (95% CI -1.1;0.5). Information about weight or body mass index was only provided in 2 studies and therefore could not be pooled.

3.3.1 Cancellation Rate

PCOS patients demonstrated a significantly increased chance of cycle cancellation (12.8% versus 4.1%), odds ratio (OR) 0.5 (95% CI 0.2;1.0) (Figure 1). However, no significant difference was observed in the likelihood of embryo transfer per oocyte retrieval between the groups, OR 0.7 (95% CI 0.4;1.3). Heterogeneity between studies and random effects estimate could not be calculated for both outcomes.

3.3.2 Gonadotropins used

No significant difference was observed in the amount of gonadotropins used in PCOS patients compared with controls, WMD -1.8 ampoules (95% CI -4.2;0.5) (Figure 2a). No significant heterogeneity was detected between studies. The random effects estimate between PCOS and non PCOS women was -1.2 (95% CI -6.3;4.6).

3.3.3 Duration of Stimulation

The duration of stimulation was significantly longer in the PCOS group. The WMD was 1.2 days (95% CI 0.9;1.5) (Figure 2b). No significant statistical heterogeneity was detected

between studies. The random effects estimate between PCOS and non PCOS women was 0.9 (95%CI -0.6;2.1).

3.3.4 Number of Oocytes Obtained and Number of Oocytes Fertilized

Significantly more oocytes per oocyte retrieval were obtained in PCOS patients compared with controls, WMD 2.9 oocytes (95% CI 2.2;3.6) (Figure 3a). However, significant heterogeneity was detected between studies ($p = 0.005$). The random effects estimate between PCOS and non PCOS women was 3.4 (95% CI 1.7;5.1). In this case the WMD is definitely a too small estimate of the true variability of the number of oocytes per oocyte retrieval.

The number of oocytes fertilized did not significantly differ between PCOS patients and controls, WMD 0.1 oocytes (95% CI -1.4;1.6) (Figure 3b). Heterogeneity between studies and random effects estimate could not be calculated.

3.3.5 Number of Clinical Pregnancies

No significant difference was observed for the clinical pregnancy rate per started cycle (37.4% versus 32.3%), OR 1.0 (95% CI 0.8;1.3) (Figure 4a), the number of live births per started cycle, OR 1.0 (95% CI 0.7;1.5) (Figure 4b), the clinical pregnancy rate per oocyte retrieval, OR 1.0 (95% CI 0.7;1.7), the clinical pregnancy rate per embryo transfer, OR 1.1 (95% CI 0.8;1.3) (Figure 5) and the number of miscarriages, OR 0.9 (95% CI 0.5;1.5) (Figure 6). No significant heterogeneity in clinical pregnancy per started cycle, number of live birth per started cycle, clinical pregnancy per oocyte retrieval, clinical pregnancy per embryo transfer and number of miscarriages was detected between studies. The random effects estimate between PCOS and non PCOS women were respectively 1.1 (95% CI 0.7;1.7), 0.9 (95% CI 0.6;1.5), 1.0 (95% CI 0.5;2.8), 1.1 (95% CI 0.8;1.8), 1.0 (95% CI 0.5;1.8) for the 5 comparisons.

3.3.6 OHSS after Oocyte Pick Up

In the majority of studies, the incidence of OHSS was not clearly reported. Data regarding this risk were therefore difficult to pool. In one study there was a trend toward more cases of ovarian hyperstimulation syndrome within the PCOS group. The development of ascites requiring hospital admission occurred in 2 of the 19 (11%) of the PCOS cycles. Another study reported a 16.6% incidence of mild to moderate OHSS and a 3.9% incidence of severe OHSS requiring hospitalization in patients with PCOS. No information regarding the non-PCOS

patients was provided in either study. One study reported 3 cases of OHSS in the PCOS group and 1 case of OHSS in the non PCOS women.

3.3.7 Implantation Rate and Multiple Pregnancy Rate

Data regarding implantation rate were available but without standard error and therefore could not be pooled. Data regarding multiple pregnancy rate were reported in only 2 publications, and could also not be pooled.

3.4 Discussion

Meta-analysis in general has several drawbacks, such as dependence on the quality of the reporting of primary analysis findings and dependence on sufficient numbers of eligible studies to justify statistical analysis. This meta analysis has an additional disadvantage because of the use of matched control studies. Nevertheless the findings of this meta analysis contributes to systematizing the knowledge about outcomes of conventional IVF in women with PCOS.

The current meta-analysis demonstrates that despite the fact that more oocytes per cycle were obtained along with lower fertilization rates, PCOS and non-PCOS patients achieve similar pregnancy rates and live birth rates per started IVF cycle (Figure 7).

The results showed a significant reduction in oocyte retrievals per started cycle in the PCOS group. Only two publications provided information regarding the reason for cancellation before retrieval. One study reported insufficient ovarian response to be significantly more frequent in PCOS women compared with non PCOS controls (131). These authors suggested that patient selection after preceding ovulation induction may explain the over representation of poor responders in this group. The same study described a non-significant difference in the incidence of OHSS in the PCOS group compared with the control group. In contrast, another study found significantly more cycles cancelled in the PCOS-group because of imminent severe OHSS (6% versus 1%) (130). This is consistent with previous studies of OHSS incidence and cycle cancellation in women with PCOS (134,135). Specific characteristics of PCOS considered to explain the higher incidence of OHSS include the presence of polycystic ovaries (136,137,138), an LH:FSH ratio > 2 (139) and hyperandrogenism (140). Furthermore an increased expression of vascular endothelial growth factor (VEGF) mRNA within the hypertrophic stroma of polycystic ovaries has been associated with increased risk of OHSS (141).

No significant difference was observed in the number of ampoules used for ovarian stimulation between the groups. However the duration of ovarian stimulation was significantly extended in the PCOS group compared with the non PCOS group. There was some inconsistency between the studies regarding these outcome parameters. This reflects the different stimulation protocols used because of the ongoing development of medication over the period in which the studies were published. The stimulation protocols and use of GnRH agonist co-treatment differed between studies, but they were applied consistently to PCOS and control groups within individual studies. The stimulation protocols used in the studied are showed in Table I.

An increased number of oocytes were retrieved following ovarian stimulation in the PCOS group compared with controls, but the fertilization rate was higher in the control group resulting in an equal total number of oocytes fertilized in both groups. A number of published studies have addressed possible reasons for this observation. One study concluded that the number of healthy non-atretic follicles is probably not increased in PCOS women because a normal inhibin B level, produced by pre-antral and small antral follicles, was found in PCOS patients (142). Another study compared the oocyte quality before intracytoplasmic sperm injection after the removal of the cumulus cells in PCOS and non-PCOS patients (143). No significant difference in rate of metaphase II oocytes, rate of germinal vesicles oocytes and fertilization rate was showed between the two groups. This finding points to involvement of cytoplasmatic factors instead of involvement of the nuclear maturity of oocytes. A further study (132) investigated the chromosomal normality of unfertilized oocytes from patients with PCOS and patients with tubal infertility. Although no significant differences in oocyte aneuploidy rates were found between the two groups, a reduced fertilization rate was observed. The authors concluded that the reduced fertilization rate is not attributable to chromosomal aberrations or immaturity of oocytes recruited from patients with PCOS.

LH concentrations in PCOS patients are higher compared with controls (144). It has been suggested that elevated LH levels in PCOS are associated with an increased rate of miscarriage (145) although this has been disputed more recently by others (123,146). It has been proposed that using a GnRH agonist to suppress LH can reduce this risk (147). In our meta-analysis, one study compared stimulation protocols with or without GnRH agonist co-treatment (82). This study showed an improved cumulative conception rate, cumulative live birth rate and miscarriage rate in women treated with a GnRH-agonist in combination with gonadotropins compared with gonadotropins alone in women with PCOS.

In conclusion IVF seems an appropriate treatment option for PCOS patients. Many of the common beliefs concerning significantly reduced chances for success and increased complication rates in PCOS patients undergoing IVF could not be confirmed in the current meta analysis. Our study shows that a woman with PCOS has a similar chance for pregnancy or live birth per started IVF cycle is to that of non-PCOS women. Reducing the number of embryos transferred will probably reduce the risk of multiple pregnancy compared with ovulation induction. However, IVF remains a complex treatment with significant costs and risks. In particular the risk of OHSS should be taken seriously. More research is necessary to define the optimal place of IVF and ovulation induction therapies for anovulatory infertile PCOS patients and to investigate the specific role of strategies like life style changes, insulin sensitizers, aromatase inhibitors and laparascopic electrocautery of ovaries in the treatment strategy. Outcomes from IVF and single ET remains to be established for PCOS.

Table 1. Characteristics of studies regarding PCOS and a matched controlled group who were included in the study

Article	Definition PCOS	Previous Treatment	Control-group	Treatment Protocol	Study Population
Dor et al, Hum Rep, 1990	Anovulation/ Oligoanovulation AND Physical characteristics (obesity, hirsutism) AND LH/FSHratio>3 AND polycystic ovarian appearance on ultrasound	Failed to conceive after at least 6 ovulatory treatment cycles clomiphene citrate (CC) AND 4 treatment cycles HMG	Pure tubal factor patients Retrospective	CC + Human Menopausal Gonadotropin (HMG) OR HMG	16 PCOS (26 cycles) 37control (37 cycles)
Urman et al Fert Steril, 1992	Anovulation/ Oligoanovulation AND Hyperandrogenism (total T>2,43nmol/l)	CC resistant OR Failed to conceive after 6 treatment cycles CC AND 6-7 treatment cycles HMG	Pure tubal factor patients Retrospective Age matched	HMG OR GnRH agonist + HMG	9 PCOS (19 ET-cycles) 40 control (40 ET-cycles)
Homburg et al Fertil Steril, 1993	Anovulation/ Oligoanovulation AND/OR Hirsutism AND polycystic ovarian appearance on ultrasound	Failed to conceive after CC AND 6 ovulatory treatment cycles of gonadotropins	Pure tubal factor patients Retrospective Age matched	follicle stimulating hormone (FSH) + HMG GnRH agonist + FSH + HMG	68 PCOS (208 cycles) 68 controls (143 cycles)
Kodama et al Hum Rep, 1995	Anovulation/ Oligoanovulation AND Hormone disorders (elevated LH/FSH ratio>1,5) and/or elevated conc of ovarian androgens in serum (T>50ng/ml, and/or androstenedione>2ng/ml) AND polycystic ovarian appearance on ultrasound	Failed to conceive after at least 2 years of ovulation induction therapy with CC AND Ovulation induction therapy with gonadotropins. .	Not male factor patients Retrospective Age range matched	GnRH agonist + FSH + HMG	26 PCOS (78 cycles) 202 Control (423 cycles)
Hardy et al Hum Rep, 1995	Anovulation/ Oligoanovulation AND Clinical and/or biochemical evidence of hyperandrogenism AND polycystic ovarian appearance on ultrasound	Less than three previous IVF cycles	Prospective Pure tubal factor patients	GnRH agonist + HMG	84 PCOS (104 cycles) 84 control (116 cycles)
Sengoku et al Hum Rep, 1997	Anovulation/ Oligoanovulation AND LF :FSH ratio > 1.5 AND polycystic ovarian appearance on ultrasound	Failed to conceive after at least 3 treatment cycles with gonadtrophins	Pure tubal factor patients Retrospective Age matched	GnRH agonist + HMG	26 PCOS (49 cycles) 26 control (46 cycles)
Doldi et al Hum Rep, 1999	Anovulation/ Oligoanovulation AND	Failed to conceive after 4 ovulatory treatment	Pure tubal factor patients Retrospective	GnRH agonist + FSH	195 PCOS (271 cycles) 197 controls (247 cycles)

	Ferriman Gallwey score>7 for hirsutism AND Hyperandrogenaemia AND Elevating concentrations of LH or LH/FSH ratio>2 AND polycystic ovarian appearance on ultrasound	cycles with gonadotropins.			
Mulders et al RBMonline, 2003	Anovulation/ Oligoanovulation AND normal serumFSH and E2 concentrations AND Free Androgen Index>4 AND polycystic ovarian appearance on ultrasound	Clomiphene resistant OR Failed to conceive after 6 ovulatory treatment cycles with CC AND 6 treatment cycles with gonadotropins	Pure tubal factor patients Retrospective Age matched	GnRH agonist + FSH	10 PCOS (10 cycles) 9 controls (9 cycles)
Urman et al RBMonline, 2004	Anovulation/ Oligoanovulation AND Clinical and/or biochemical evidence of hyperandrogenism	Failed to conceive after CC AND 4-6 treatment cycles with gonadotropins	Retrospective Age matched Duration of infertility matched	GnRH agonist + FSH	24 PCOS (28 cycles) 31 control (55 cycles)

Table 2. Available information in selected studies

	Dor 1990	Urman 1992	Homburg 1993	Kodama 1995	Hardy 1995	Sengoku 1997	Doldi 1999	Mulders 2003	Urman 2004
no of patients	X	X	X	X	X	X	X	X	X
no of cycles	X	X	X	X	X	X	X	X	X
no of oocyte retrievals	X	X		X				X	
no of embryo transfers (ET)	X	X	X	X	X		X	X	X
age	X	X	X	X	X	X	X	X	X
BMI							X	X	
duration infertility		X		X		X			X
no of ampoules		X			X		X	X	X
duration stimulation					X			X	X
oestradiol on day HCG									
cancellations cycles (poor)				X				X	
cancellations cycles (hyper)				X				X	
OHSS severe		X		X					
no of oocytes	X	X	X	X	X	X	X	X	X
percentage fertilization	X	X	X	X	X	X	X	X	
no of oocytes fertilized	X			X	X	X		X	
no of embryos per ET		X	X		X	X	X	X	X
no of clinical pregnancies	X	X	X	X	X	X	X	X	X
no of livebirths	X	X					X		
no of miscarriages	X	X	X	X			X	X	X
no of multiple pregn rates			X					X	
implantation rate					X			X	X

Figure 1. *Odds ratio for cancellation rate comparing PCOS patients and matched control*

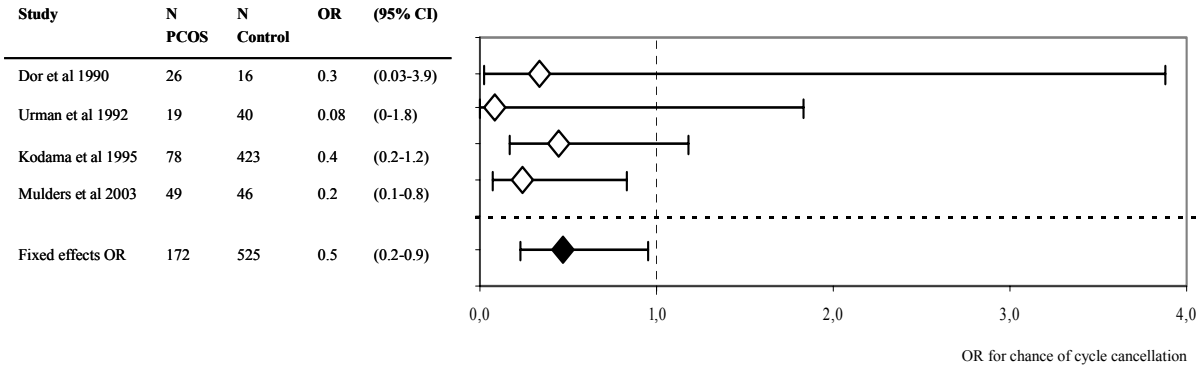
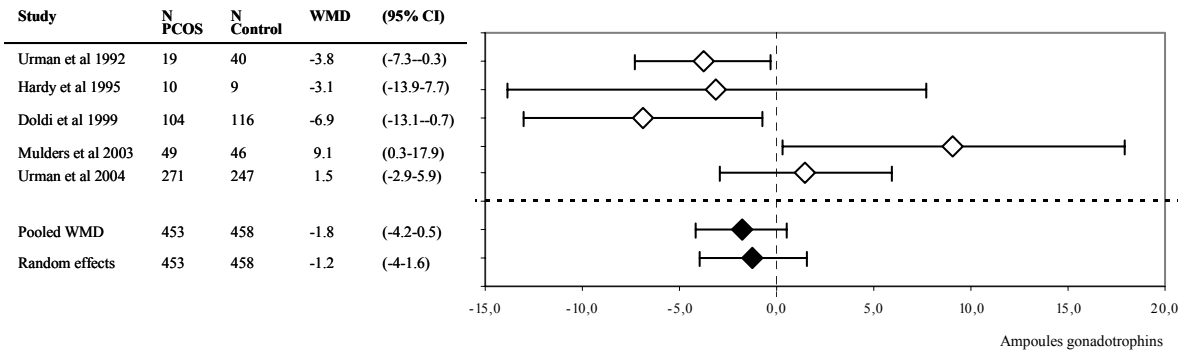


Figure 2. *Difference in amount of gonadotropins used (a) and duration of stimulation (b) for ovarian stimulation for IVF comparing PCOS patients and matched controls*

a.)



b.)

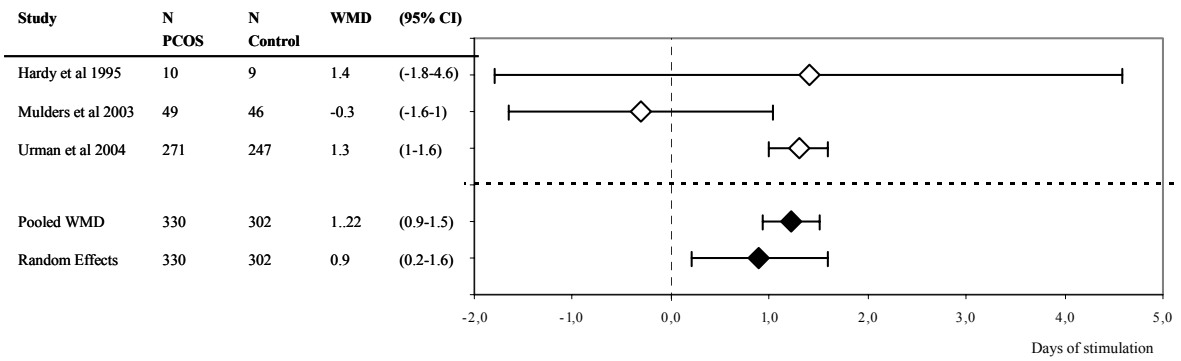
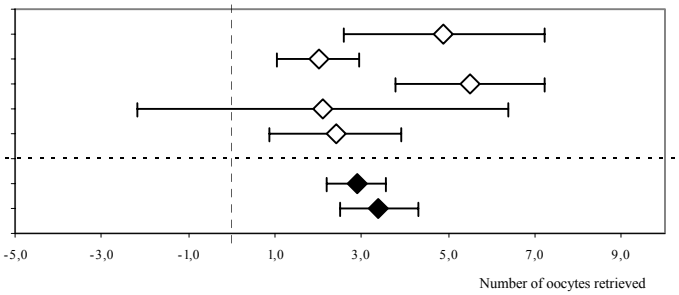


Figure 3. Difference in number of oocytes retrieved (a) and fertilised (b) during IVF comparing PCOS patients with matched controls

a.)

Study	N PCOS	N Control	WMD	(95% CI)
Dor et al 1992	26	37	4.9	(2.6-7.2)
Urman et al 1992	19	40	2	(1.1-2.9)
Kodama et al 1995	78	423	5.5	(3.8-7.2)
Mulders et al 2003	49	46	2.1	(-2.2-6.4)
Urman et al 2004	271	247	2.4	(0.9-3.9)
Pooled WMD	443	577	2.9	(2.2-3.6)
Random Effects	443	577	3.4	(2.5-4.3)



b.)

Study	N PCOS	N Control	WMD	(95% CI)
Dor et al 1992	26	37	0.6	(-2.2-3.4)
Kodama et al 1995	78	423	0.3	(-3.5-4.1)
Hardy et al 1995	10	9	-0.1	(-3.2-3)
Sengoku et al	28	55	-0.5	(-4--3)
Mulders et al 2003	49	46	-0.1	(-3.8-3.6)
Pooled WMD	163	515	0.08	(-1.4-1.6)

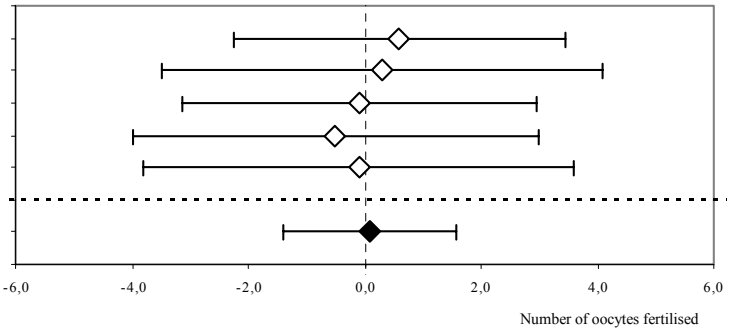
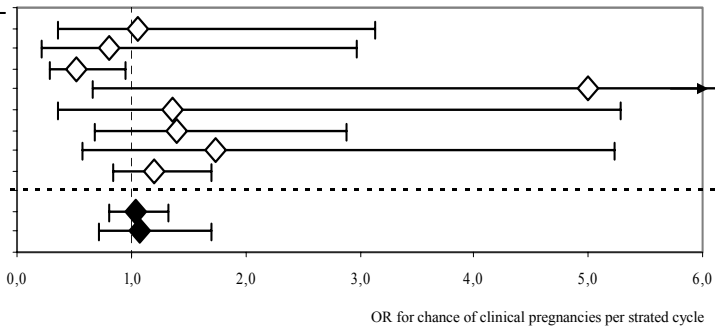


Figure 4. Odds ratio for number of clinical pregnancies (a) and live births (b) per started cycle comparing PCOS patients and matched controls undergoing IVF

a.)

Study	N PCOS	N Control	OR	(95% CI)
Dor et al 1990	26	16	1.1	(0.4-3.1)
Urman et al 1992	19	40	0.8	(0.2-3)
Kodama et al 1995	78	423	0.5	(0.3-1)
Hardy et al 1995	10	9	5	(0.7-38.2)
Sengoku et al 1997	28	55	1.4	(0.4-5.3)
Doldi et al 1999	104	116	1.4	(0.7-2.9)
Mulders et al 2003	49	46	1.7	(0.6-5.2)
Urman et al 2004	271	247	1.2	(0.9-1.7)
Fixed effects OR	585	952	1	(0.8-1.3)
Random effects	585	952	1.1	(0.7-1.7)



b.)

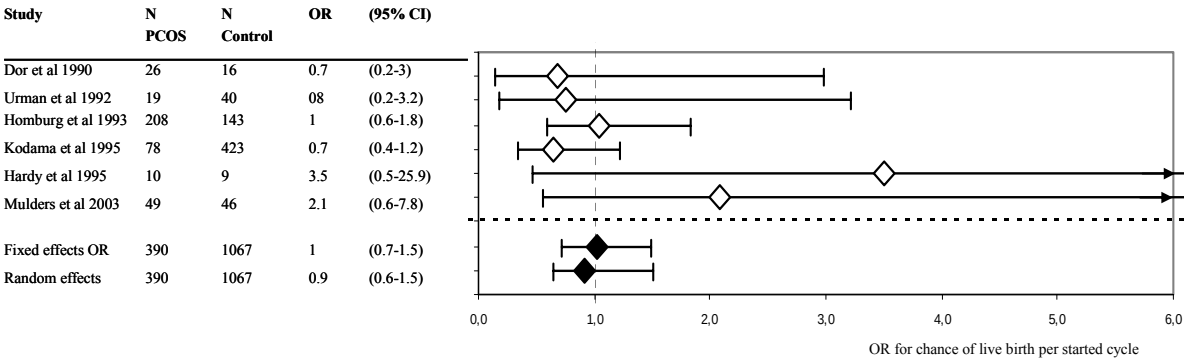


Figure 5. *Odds ratio for number of clinical pregnancies per embryo transfer comparing PCOS patients and matched controls undergoing IVF*

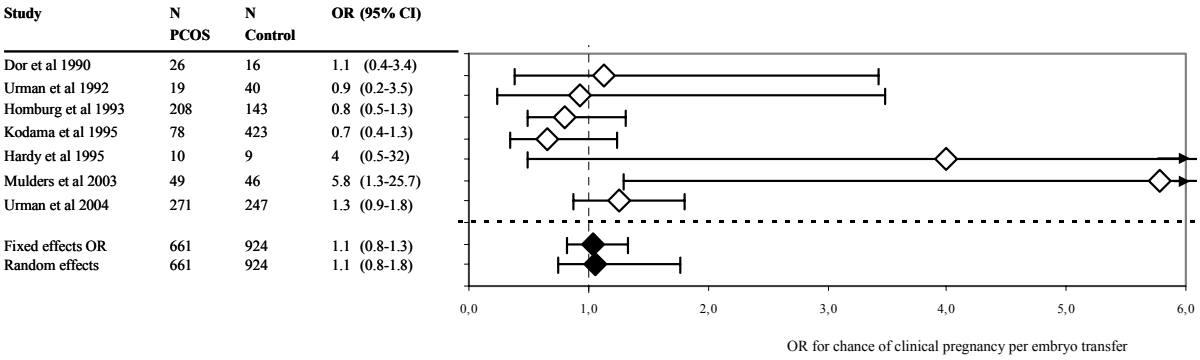


Figure 6. *Odds ratio for number of miscarriages per biochemical pregnancy comparing PCOS patients and controls undergoing IVF*

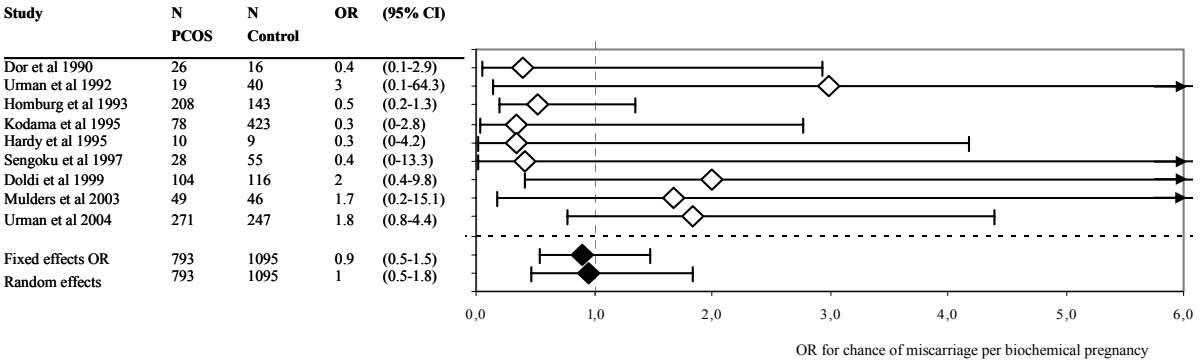
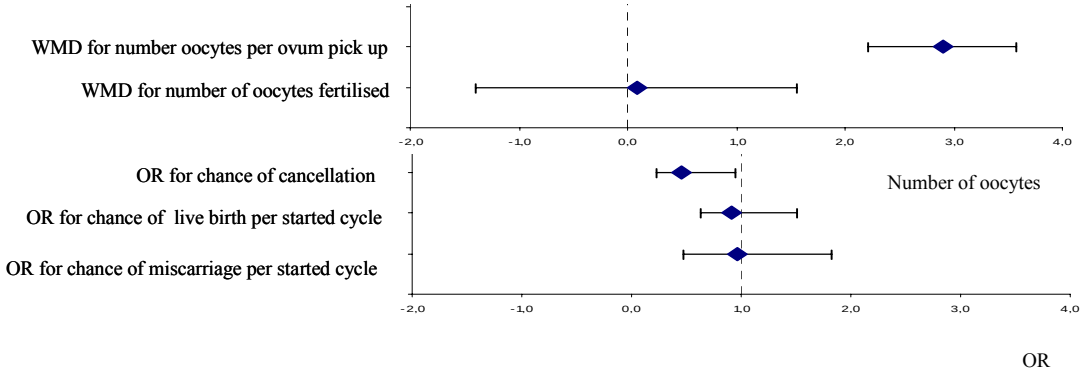


Figure 7. *Main findings of clinical outcomes of IVF in PCOS compared with matched controls*


WMD for number oocytes per ovum pick up
WMD for number of oocytes fertilised
-2,0
-1,0
0,0
1,0
2,0
3,0
4,0
OR for chance of cancellation
OR for chance of live birth per started cycle
OR for chance of miscarriage per started cycle
Number of oocytes
-2,0
-1,0
0,0
1,0
2,0
3,0
4,0
OR

4. Prevention of multiple pregnancies after IVF in women 38 and older: a randomised study

Heijnen, E.M., Klinkert E.R., Schmoutziger A.P., Eijkemans M.J., Te Velde E.R., Broekmans F.J.

Reproductive Biomedicine Online, Article In Press

4.1 Introduction

Multiple pregnancy rates after in vitro fertilization (IVF) treatment are substantial. In the Netherlands approximately 25% of the ongoing pregnancies after IVF is a multiple pregnancy and this is in line with rates observed in other European countries (148). Almost half of the children after IVF is part of a multiplet.

Multiple pregnancies are accompanied by a higher mortality and morbidity rate due to premature birth and low birth weight. Prematurity occurs in 5-10% of singleton pregnancies and in 40-60% of twin pregnancies after IVF (14). The same appears to be true for the risk of low birthweight which occurs in 5-10% and 50-71% respectively (149,150,151,152,153,154,55). Perinatal mortality is 5 times higher in twin pregnancies and the chance of neurological morbidity is 8 times higher compared with singleton pregnancies (14). All this implies that in twin pregnancies the chance of having one or two children that either have suffered perinatal death or have become severely neurological damaged may approach 8% versus 0.5% in singletons. Twin pregnancies also imply a higher risk for the mother such as preterm labour, gestation induced hypertension, diabetes and vaginal blood loss (153). Costs of an IVF treatment do not only contain the medical treatment costs but also the costs of obstetrical and neonatal care. Such costs are considerably higher in twin pregnancies (155,55). Clinicians and patients have become increasingly aware that multiple pregnancies should not be viewed as an undisputable success and should be avoided if possible.

Retrospective research has suggested that by transfer of 2 embryos instead of 3 in women under 35 years of age the pregnancy rate is not significantly different whereas the multiple pregnancy rate is significantly reduced in the group where 2 embryos were transferred (156). This finding has led to a major decrease in the rate of transfer of 3 embryos, at least in most European centers.

Recent studies showed that in patients younger than 38 years, in whom at least 3 good quality embryos were available, single embryo transfer (SET) compared to dual embryo transfer yields reduced ongoing pregnancy rates (36,40,41,157,43,44). However, this reduced

rate has to be set against the advantage of the elimination of multiple pregnancies (43,44). The reduced success rates may be compensated by performing an additional treatment cycle or by applying a high-quality frozen-thawed embryo program (43,44).

No randomised controlled trials in this research field have been performed in women above 38 years. Because implantation will considerably decrease with age (158) pregnancy rates are decreased by a factor 2 and ongoing pregnancy rates are only one third of those in the younger age class (159). Therefore most clinicians agree that SET is not advisable in women of 38 years and older (51). Little is known on the feasibility of transferring 2 in stead of 3 embryos in women of this age in order to decrease the incidence of multiple gestations. The present study aims to answer the question whether dual instead of triple embryo transfer during IVF treatment in patients over 38 years will substantially reduce the number of multiple pregnancies while the chance of a term (>37 weeks gestational age) live birth per started treatment still remains acceptable. The outcome parameter term live birth per treatment instead of per cycle is used because the goal of an IVF treatment is having a healthy baby after completion of an IVF treatment consisting of a series of IVF cycles and subsequent replacement of frozen embryos.

4.2 Materials and Methods

4.2.1 Study design

A two center controlled randomised study was performed. Randomisation was carried out using sealed envelops opened by the study coordinator on the phone. Study approval was obtained by the local ethics committee of the University Medical Centre Utrecht and the Rijnstate Hospital Arnhem, the Netherlands.

4.2.2 Patients

Patients on the waiting list for IVF or IVF/ICSI were recruited for the study. Recruitment took place in 2 hospital centers for reproductive medicine in the period October 2001 through December 2003. Patients were eligible for inclusion in the study if they were 38 years and older and had an indication for an IVF or IVF/ICSI treatment either for the first time or after a previous IVF or IVF/ICSI childbirth. No other inclusion criteria were applied. Patients were informed about the study by word of mouth by a doctor and in writing by a patient information leaflet. Randomisation was performed during the IVF or IVF/ICSI intake after

checking for inclusion and exclusion criteria. Written informed consent was obtained from all patients.

4.2.3 Treatment groups

All participants were randomised into one of two embryo transfer strategy groups. The first group was intended to undergo a transfer of a maximum of 2 embryos in the first 3 cycles (dual embryo transfer strategy: DET-group). In order to compensate for a possible reduction in pregnancy rate in this group, patients were offered a fourth reimbursed cycle in which the choice for the transfer of 2 or 3 embryos was left to the couple. The second group was intended to have the transfer of a maximum of 3 embryos in the first 3 treatment cycles (three embryo transfer strategy: TET-group). Randomisation for the whole treatment period was performed before information about embryo quality was available because we wanted to investigate a general policy applicable in clinical practice based on age without pre-selection on embryo quality.

4.2.4 Ovarian Stimulation Protocol

All cycles were performed by a long agonist suppression protocol (leuprolide, Lucrin: Abbott B.V., Amstelveen, The Netherlands; 0,2 mg/day, sc, or triptorelin, Decapeptyl: Ferring B.V. Hoofddorp, The Netherlands; 0,1 mg/day, sc). After downregulation was established recombinant FSH (recFSH) (Gonal-F; Serono Benelux B.V., Amsterdam, The Netherlands, or Puregon; N.V. Organon, Oss, The Netherlands), in a sc dose of 150 IU daily was started (stimulation day 1). Dose adjustments during the first cycle or in subsequent cycles were performed on an individual basis. Human chorionic gonadotropin (hCG) (Profasi, 10.000 IU, sc; Serono Benelux B.V., Amsterdam, The Netherlands, or Pregnyl, 5000-10.000 IU, sc; N.V. Organon, Oss, The Netherlands, or Ovitrelle, 250 microg, sc; Serono Benelux B.V., Amsterdam, The Netherlands) was administered for final oocyte maturation when the largest follicle had reached a diameter of at least 18 mm and at least 1 additional follicle > 14 mm was observed. 36 hours later oocyt retrieval was performed and embryos were transferred 3 or 4 days after oocyte pick up. Luteal phase support was started on the day of oocyte pick up.

4.2.5 Methods of analysis

Little information is available on cumulative term live birth rates in subsequent cycles in this age group. Moreover we did not know whether patients were willing to remain in the randomised group if they did not get pregnant in the first one or two cycles. Therefore, we

decided to perform a pilot study first in which we aimed to include approximately 50 patients. Depending on the results a decision on the continuation of the trial was to be taken or suggestions for further research would be given. The two treatment groups were compared using the t-test and the χ^2-test. A $p<0.05$ was considered statistically significant. The mean number of cycles, oocyte pick ups en embryo transfers were compared using a Mann Whitney U test.

The primary outcome measure was the cumulative term (>37 weeks gestational age) live birth rate. Additionally, we provided information about the live births. To calculate the primary endpoints we first performed an intention to treat analysis (ITT-analysis) and constructed a Kaplan Meier survival curve, in which non-pregnant patients who did not proceed to a subsequent cycle were censored. This method assumes that these patients would have had the same chance of getting pregnant as the patients who did continue treatment (non-informative censoring). However, it is well possible that the cumulative rate will be too optimistic if patients with poorer prognosis drop out selectively (160,161,127). Therefore, an adapted Kaplan Meier curve was calculated, in which we assumed that the patients who did not continue treatment had no chance of getting pregnant (162). The first curve represents an optimistic chance, the second curve a pessimistic chance and we assume that the true cumulative term live birth rate is somewhere in between. Second a per-protocol analysis (PPA) was performed to account for couples who switched from the DET strategy to the TET strategy being not pregnant after the first or second cycle. The cumulative term live birth rate for 4 cycles in the DET-group and 3 cycles in the TET-group was compared using the confidence interval of the difference between the 2 groups and a z-test.

Statistics Package for Social Sciences for Windows, version 11.5 (SPSS Inc., USA) was used for data analysis.

4.3 Results

Fourty five patients were included in the study. A total of 112 cycles were performed, 66 in the DET-group and 46 in the TET-group. The flowchart of the study according to CONSORT guidelines is shown in table 1.

The two groups were comparable regarding patient characteristics, cycle characteristics and treatment characteristics except for number of cycles, oocyte pick up and embryo transfers due to the treatment strategy (Table 2).

In the dual embryo transfer group, 23 first cycles, 20 second cycles, 15 third cycles en 8 fourth cycles were conducted. In the triple embryo transfer group, 22 first cycles, 15 second

cycles and 9 third cycles were carried out. In the DET-group 3 patients had 3 embryos transferred in the fourth cycle. The optimistic cumulative term live birth rate (assuming that drop outs have the same chances as patients who continued) in the DET-group after 4 cycles was 47.3% and in the TET-group after 3 cycles was 40.5%. The difference between the DET and TET-group was 6.8% in the favour of the DET-group (95% CI -25;38) (p=0.7). The pessimistic cumulative term live birth rate in the DET and TET-group did not differ statistically (Table 3). In the DET-group 4 patients (17.4%) switched to another embryo transfer policy whereas 0 patients switched in the TET-group. Two patients in the DET-group (8.6%) conceived spontaneously. When excluding this patients in the analysis (per-protocol analysis) the optimistic and pessimistic cumulative term live birth rate in the DET and TET-group did not differ statistically (Table 3). The cumulative singleton live birth after 4 cycles in the DET-strategy and 3 cycles in the TET-strategy was 47.3% versus 37.0%.

The percentage of patients with at least one top quality embryo (Day 3: ≥8 cells, <10% fragmentation; Day 4: Morula, complete compaction, <10% fragmentation) in the DET-group was 54% and in the TET-group 67%. This difference was not statistically significant (p=0.3).

Transferring the required number of embryos per strategy was not always possible. In the DET-group in 20% of the started cycles embryo transfer of 2 embryo's was not possible because there were less than 2 embryos available. In the TET-group in 28.2% of the started cycles embryo transfer of three embryos was not possible because there were less than 3 embryos available. Cryopreservation in the DET-group was possible in 6 cycles and in the TET-group in 1 cycle. Transfer of cryopreserved embryos did not lead to an ongoing pregnancy. The ongoing (>12 weeks) implantation rate was 7.5% (95% CI 3.5;13.8) in the DET-group and 11.6% (95% CI 6.3;19) in the TET-group. De difference between the 2 groups was not significant (p=0.3).

In the DET-group there were no multiple pregnancies 0% (95% CI 0;24). In the TET-group there were 3 twin pregnancies 30% (95% CI 7;65). The difference in twin rate was marginally significant (p=0.05).

The mean gestational age in the DET-group was 39.8 weeks (range 38.1 – 42.3 weeks) and in the TET-group 39.5 weeks (range 35.4 - 42.1 weeks) (p=0.8). The mean birth weight in the DET-group was 3729.8 grams (range 2020 - 5030 grams) and in the TET-group 3298,3 grams (range 2000 – 4240 grams) (p=0.3). One child in a singleton pregnancy from the DET-group suffered intra uterine death after 31.5 weeks of gestation.

Up till the date of November 1, 2005 in the DET-group all patients had continued treatment after 1 completed cycle, 1 patient did not continue treatment after 2 cycles and 5

patients did not continue treatment after 3 cycles. The total rate of couples not completing the treatment strategy for other reasons than getting pregnant was 26%. In the TET-group 1 patient did not continue treatment after 1 cycle and 4 patients after the second cycle. The total rate of couples not completing the treatment strategy was 23% in this group. There was no significant difference in patient or cycle characteristics between the drop outs and patients who finished the full treatment strategy.

4.4 Discussion

This study is the first randomised controlled trial comparing cumulative ongoing pregnancy rates after dual and triple embryo transfer in women of 38 years and older. It suggests that by applying dual instead of triple embryo transfer in subsequent cycles as standard strategy in patients of 38 years and older it is possible to reduce multiple pregnancy rates. Since the study was set up as a feasibility study, the numbers are too small to justify firm conclusions. The difference in the number of multiplets is obvious but the confidence intervals are wide and statistical significance on the edge.

In our experience it was quite difficult to recruit couples from those who were considered eligible, possibly due to the fact that couples in this age group anticipate an advantage of replacing a high number of embryos. To ensure that a difference in pregnancy rates is indeed smaller than 10% it would take 600 couples, based on the present findings. Such a study would imply a multi center set up in more than one country, an almost impossible endeavour.

Large but retrospective studies did not find differences in pregnancy rates per cycle performing DET compared to TET (52,53). Obviously such studies lacks the insight into the accumulation of pregnancies in subsequent cycles. Our findings show a trend in reduction of the per cycle chance of pregnancy when the number of embryos transferred is reduced. However, from the data shown it appears that application of a two embryo transfer strategy in women over 38 years will not change the final perspective of obtaining the desired healthy child. Furthermore the multiple pregnancy rate was significantly lower in the DET-group.

To accept the DET approach in daily practice it is important that, instead of looking at success in IVF treatment in terms of ongoing pregnancy rate per cycle, physicians and patients learn to look at success in terms of term live birth per whole IVF treatment or per treatment period (163). When using milder, more patient friendly, stimulation protocols the term live birth per whole IVF treatment or per treatment period could become higher because the drop

out rate may possibly be decreased and more IVF cycles can be conducted in the same period of time (164).

By taking live birth per whole IVF treatment as endpoint the discussion will arise whether a twin counts as 1 or 2 live births. A patient who delivers 2 babies will be less inclined towards starting a next IVF treatment for a second child. Especially in women 38 years and older having 2 babies from one serie of IVF attempts may be the only way to obtain a family with two children. To date it is not clear how to incorporate this item in the process of deciding on embryo transfer strategy, where health of the offspring is balanced against the desire for a completed family.

In the light of the ongoing discussion on single and dual embryo transfer in women younger than 38 years the issue of the use of DET or TET in women above 38 years is very much comparable. By replacing less embryos the live birth rate per cycle seems to drop but by conducting an extra treatment cycle the cumulative term live birth rate after more cycles will be equal in the DET and the TET-group. In our study, transfer of cryopreserved embryos did not result in additional pregnancies. In larger groups this could possible prove to be different, although it is reasonable to assume that cryopreservation and transfer of cryopreserved embryos is less frequent in women above 38 years (165).

The study of cumulative cycles in this trial delivered methodological problems in the course of the subsequent treatment cycles. First, there is the problem of drop outs. The overall drop out rates in the course of four and three treatment cycles were not different from those reported in the literature (160,32,166). Drop outs hamper the simple calculation of cumulative term live birth rates. To deal with this problem it was decided to calculate socalled optimistic and pessimistic scenarios (167,168).

A second problem within this study are the patients who switched from a DET to TET strategy in the course of the study period. For this reason we also conducted a per protocol analysis. Four patients in the DET-group switched from their allocated number of 2 embryos to transfer into 3 embryos and as such can be considered protocol violators. Therefore they were not included in the per-protocol analysis. Moreover, two patients in the DET-group became spontaneously pregnant between treatment cycles. These patients were also not included in the per-protocol analysis since the spontaneous pregnancies are not a direct result of the treatment given. Despite a considerable number of switchers and spontaneous pregnancies in the DET-group the per protocol analysis did not show a significant difference between both strategies. This finding proves that the almost identical cumulative term live

birth rates between both strategies in the intention to treat analysis were not caused by switchers or spontaneous pregnancies.

A third methodological issue that emerged in the course of the study was the number of embryos that became actually available for transfer. Older women are expected to have less follicles, less oocytes and therefore less embryos available for transfer (169,170). In our study women had a relatively high number of oocytes at oocyte pick up. Except for the inclusion criteria mentioned in the materials and methods no other inclusion criteria were used to include patients. All patients between 38 and 45 years with an indication for IVF had the possibility to embark the study protocol. In our study in both treatment strategies about 20% could not receive the allocated number of embryos because there were simply not enough embryos.

Introducing dual embryo transfer in women above 38 years may require big efforts from both the clinician and the couple. The couple should be made aware of the balance between their short term desire for offspring and their long term appreciation of raising healthy children. If structured, written information about risks and complications of multiple pregnancies and the consequences of the transfer of less embryos is provided, patients will probably become more inclined to the transfer of 2 embryos rather than 3. Introducing the dual embryo transfer as a standard policy, from which deviation is not allowed as a principle, patients may not easily put pressure on the physician to obtain consent for a 3 embryos transfer. However, if patients have to pay for the IVF cycle by themselves, choosing for dual embryo transfer when being well informed about the lower pregnancy rate will be a difficult choice. If a country has an adequate reimbursement system there is a main task for the politicians to create the legislation in such a manner that dual embryo transfer in women of 38 years and older is mandatory (171,48).

In summary, this study suggests that in women of 38 years and over a dual embryo transfer strategy after IVF may result in similar cumulative pregnancy rates compared with a triple embryo transfer strategy, while reducing multiple pregnancy rates. This seems to be at the expense of an increase in the number of cycles needed to obtain these results, an expense that seems nicely balanced against the great advantages of multiple pregnancy prevention.

Table 1. Flow chart according to CONSORT guidelines

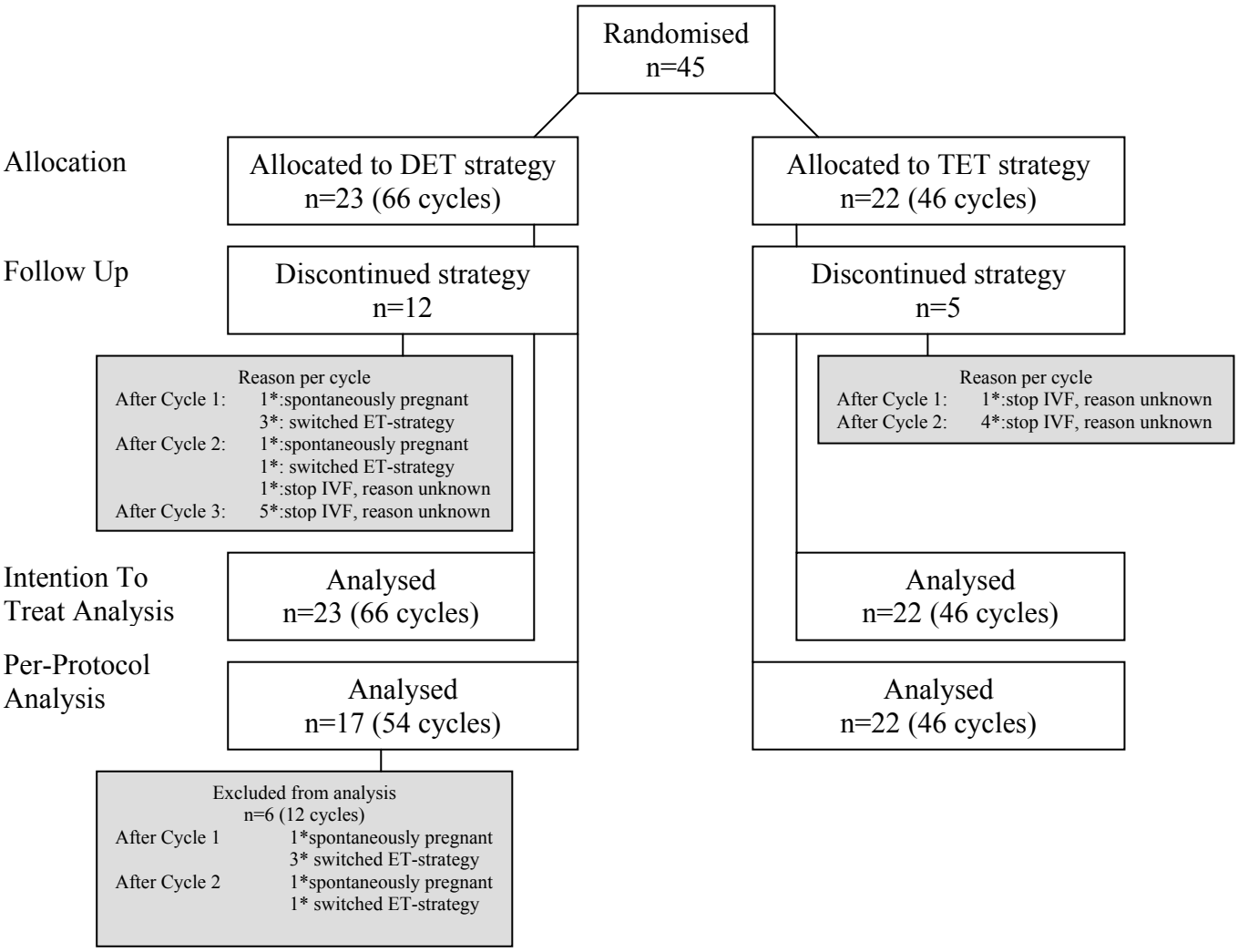


Table 2. Characteristics of patients, cycles and treatments in the DET-group and TET-group. All characteristics are based on the initial randomisation.

		DET-group	TET-group	p
Characteristics per patient		***23 patients***	***22 patients***	
Age (years)		40.8 (±1.7)	41.1 (±2.5)	NS
Dur inf (years) (range)		3.7 (±2.5)	3.2 (±2.4)	NS
Primary Infertility (%)		57	41	NS
Cause of inf (%)	***Cervical***	4.3	0	NS
	Anovulation	0	4.5	NS
	Tubal	21.7	22.7	NS
	Male	39.1	22.7	NS
	Unexpl	34.8	50	NS
No of Cycles (NC)		2.9 (±1.1)	2.1 (±0.9)	0.01*
NC with Oocyte Pick Up		2.7 (±1)	2 (±0.8)	0.01*
NC with Embryo Transfer		2.6 (±1)	1.9 (±0.8)	0.02*
Characteristics per cycle		***66 cycles***	***46 cycles***	
No cancelled cycles[a]		4 (6)	3 (6.1)	NS
No oocytes[b]		7 (3-12)	6 (2-14.6)	NS
No embryos[b]		3.5 (1-8)	4 (1-9.6)	NS
≥3 embryos available[a]		40 (60.6)	33 (71.8)	NS
No embryos transferred[b]		2.0 (1-3)	2.7 (1-3)	<0.001*
No cryopreserved embryos[b]		0.3 (0-5)	0.07 (0-3)	0.14
	Cycle no	**1 2 3 4**	**1 2 3**	
No of Started Cycles		23 20 15 8	23 16 9	NS
No Clin Pregn		7 7 2 2	6 3 2	NS
No Ong Pregn		3 4 2 2	6 2 2	NS

No Singlet Preg	3	4	2	2	4	2	1	NS
No Multi Preg	0	0	0	0	2	0	1	0.05**
No Live Birth	3	4	2	1	6	2	2	NS
No Term Live Birth	3	4	2	1	5	2	1	NS

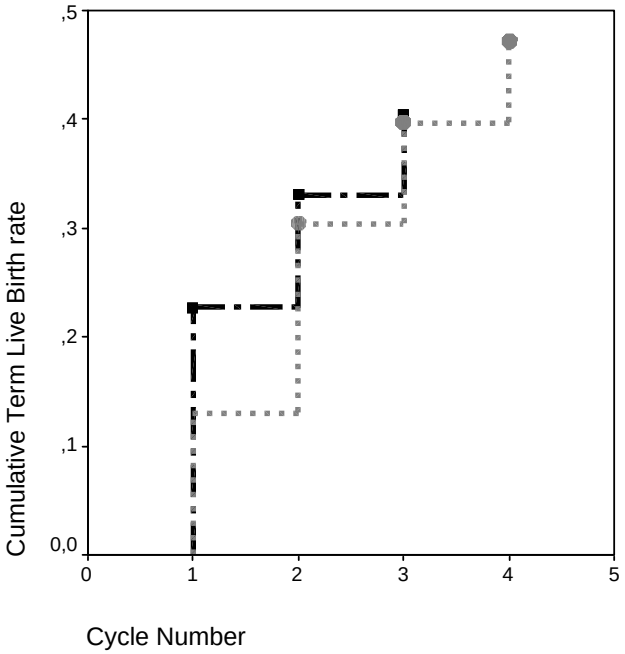
Values are mean (±standard deviation) or *[a]number (percentage), *[b]mean (range) per embryo transfer, *Mann Whitney U test, **Pearson χ^2 test

Table 3. Cumulative Optimistic (Opt) en Pessimistisc (Pess) Term Live Birth rate (%) in DET and TET group for intention to treat and per protocol analysis

	Intention to Treat Analysis				**Per Protocol Analysis**			
	Opt DET	**Opt TET**	**Pess DET**	**Pess TET**	**Opt DET**	**Opt TET**	**Pess DET**	**Pess TET**
1 cycle	**13**	**22.7**	13	22.7	8.7	22.7	8.7	22.7
2 cycles	**30.4**	**33**	30.4	31.8	29	33	26.1	31.8
3 cycles	**39.7[1b]**	**40.5[1ab]**	39.1[2b]	36.4[2ab]	41.9[3b]	40.5[3ab]	34.8[4b]	36.4[4ab]
4 cycles	**47.3[1a]**		43.5[2a]		41.9[3a]		34.8[4a]	

[1a] difference 6.8% (95% CI -25;38) p=0,7;[1b] difference -0.8% (95% CI -31;29) p=0.96 ;*[2a] difference 7.1% (95% CI -21;36) p=0.6;*[2b] difference 2.7% (95% CI -26;31) p=0.9;*[3ab] difference 1.4% (95% CI -31;34) p=0.9 ; *[4ab] difference -1.6 (95% CI -30;26) p=0.9

Figure 1. Cumulative Optimistic Term Live Birth rate (%) in DET and TET-group for intention to treat analysis.



5. Comparison of different treatment strategies in in vitro fertilisation with cumulative live birth over a given period of time as primary endpoint: Methodological considerations on a randomised controlled non-inferiority trial

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5.1 Introduction

The public health challenge for IVF today is to increase availability and acceptability and reduce adverse effects without compromising effectiveness. This paper will address the methodological issues in designing a trial to test a less complex protocol against a common version of the standard current protocol.

In vitro fertilisation (IVF) has been the treatment of choice in severe tubal infertility. For most other indications, IVF is applied as a last therapy after the failure of other treatment modalities. The high costs of the treatment, the burden of the ovarian stimulation for the patient and the complications (136), most notably the high chance of a multiple pregnancy and the associated costs, have prohibited the widespread use of IVF as a first line treatment option (117,111). However, the recent introduction of gonadotropin-releasing hormone (GnRH) antagonists has opened novel possibilities for milder stimulation protocols, which are better tolerated by the patient and less costly than the conventional stimulation regimens (26,112). Moreover, there is a growing awareness that the high rate of multiple pregnancies may be greatly reduced by a restricted, single embryo transfer policy (6,40,172,173,43,50). In theory, these developments hold a promise for the future by reducing complications for both mother and child.

Single compared to dual embryo transfer has reduced success rates per fresh embryo transfer cycle, which can only be overcome by establishing a high-quality frozen-thawed embryo program (43). The pregnancy rates per cycle following GnRH antagonist co-treatment have been shown to be slightly, but significantly, inferior to those of the classical GnRH agonist long protocol (26). Nevertheless, the mild stimulation approach might have advantages when evaluated over an entire (multiple cycle) treatment strategy, since the amount of time needed to complete a single IVF cycle is less and the costs of stimulation are reduced (26,112). More cycles could on average be performed in the same period of time for the same amount of money. Due to the better tolerability for patients, dropout rates may be reduced, so that the number of patients reaching pregnancy within a given period of time could very well be higher compared to the conventional ovarian stimulation approach, with

similar costs per pregnancy (163). Hence, a mild ovarian stimulation protocol with GnRH antagonist co-treatment could offer a means to compensate for reduced pregnancy chances when single embryo transfer is considered. Applying such an approach, pregnancy rates will be reduced when evaluated per cycle (46,37), but not for a given treatment period, which is more relevant to the patient. The importance of defining success of infertility therapies as live birth per treatment started instead of per cycle has been stressed recently (105). The time has come to seriously reconsider the definition of successful IVF (6), and design future studies accordingly.

We designed a randomised controlled trial to investigate whether IVF using mild ovarian stimulation combined with single embryo transfer is not inferior in clinical effectiveness, more patient friendly and more efficient in cost-effectiveness compared with conventional treatment. In this paper, the design of the study is presented and discussed in detail.

5.2 Methodological Considerations

The study is designed as a 2-arm randomised controlled non-inferiority effectiveness trial. The treatment strategies are mild ovarian stimulation with GnRH antagonist co-treatment along with the transfer of a single embryo versus 'standard' ovarian stimulation combined with pituitary down-regulation through the administration of a GnRH agonist long protocol, and transfer of two embryos. In brief, patients with a regular indication for IVF (with or without the addition of intra-cytoplasmic sperm injection (ICSI)), female age < 38 years, normal menstrual cycle (interval between periods 25-35 days) and without severe obesity or underweight (Body mass index 18-28 kg/m^2) were eligible for the study. Two academic medical centres (Rotterdam and Utrecht) participated in the study. Patient data are collected on standard patient-record forms. Patients will be followed-up for a maximum of 12 months treatment plus resulting pregnancy, until 6 weeks post-term. Analysis will be performed according to the intention-to-treat principle. The primary outcome measures are: (1) pregnancy within one year after randomisation leading to term live birth, (2) total costs per couple and child up to 6 weeks after expected delivery (3) overall patient discomfort within one year of randomisation. In the next sections, we will describe the background of the study and motivate the choices that were made in the design of the study.

5.2.1 Treatment protocols

The two treatment protocols were executed in a standardised fashion, as depicted in Figure 1. In the standard, GnRH agonist long protocol, two-embryo transfer (ET) arm, standard ovarian stimulation is performed. After approximately 2 weeks GnRH agonist subcutaneous (s.c.) daily, starting during the mid-luteal phase of the pre-treatment cycle (leuproline, 0.2 mg/day; or triptoreline, 0.1 mg/day, depending on the clinic), ovarian stimulation is started with a starting dose varying between patients from 112.5 to 150 IU/day recombinant FSH (recFSH) s.c.. The recFSH dose can be adjusted in subsequent cycles if needed. Human chorionic gonadotropin (hCG) 10,000 IU s.c. is administered for the induction of final oocyte maturation, when the largest follicle reaches at least 18 mm in diameter and at least 1 additional follicle > 15 mm is observed (112). Oocyte retrieval and fertilization are performed according to standard procedures, as described previously (174,175). A maximum of 2 (best quality) embryos is transferred (176). Luteal phase supplementation by progesterone, 600 mg/day, intravaginally is started at the evening of oocyte pick-up and continued until 12 days thereafter. In case good quality excess embryos are available they are cryopreserved and transferred in the subsequent unstimulated cycle, according to standard procedures (177). The maximum number of IVF cycles is 3.

In the mild, GnRH antagonist co-treatment, single ET arm, mild ovarian stimulation is performed by a fixed starting dose of 150 IU recFSH s.c. per day, initiated on cycle day 5. GnRH antagonist (ganirelix, 0,25 mg/dag; or cetrorelix, depending on the clinic) is administered s.c. if at least 1 follicle ≥ 14 mm is observed (112). The starting day or dose can be adjusted in subsequent cycles. Similar criteria apply for hCG, for oocyte retrieval and fertilization procedures as in the standard group. Only the best quality embryo is transferred. Standard luteal phase support, and criteria to cryopreserve embryos will be applied as in the standard arm. The maximum number of mild IVF cycles is 4.

5.2.2 Background ovarian stimulation

In the standard long-protocol ovarian stimulation, the pituitary-ovarian axis is suppressed through the administration of a GnRH agonist. Subsequently, “high dose” gonadotropins are needed over a long period of time to let the FSH levels rise above the threshold for ovarian stimulation, and the FSH ‘window’ is widened for an extended recruitment of follicles. A heterogeneous cohort of follicles is recruited in this way.

In mild ovarian stimulation, natural recruitment of follicles is achieved by the inter-cycle FSH rise (178) and exogenous FSH is administered only during the mid-follicular phase, allowing more than one follicle to gain dominance (112). This mode of stimulation interferes less with natural follicle selection and results in a lower number of aneuploid embryos, as shown recently (179).

5.3 Trial design

5.3.1 Effectiveness versus efficacy

The current trial is an effectiveness trial, aimed at answering the question: will the treatment strategy under consideration achieve the desired benefits in everyday routine practice. This type of trial is also referred to as a management trial (180) and should be distinguished from an efficacy or explanatory trial, which answers the question: can a treatment work under ideal circumstances (181,182). In an effectiveness trial, inclusion criteria and clinical protocols should resemble everyday reality. We used broad inclusion criteria and different pharmaceutical products, according to the daily routine in the two participating centres. The multi-centre design in itself leads to results that are more relevant to daily practice and less idealized than a highly controlled single centre trial.

5.3.2 2 versus 4 arms

By combining the choice between two ovarian stimulation strategies with the choice between single and dual ET, 4 different combinations are possible, at least in theory. The current study compares only two arms: mild ovarian stimulation and GnRH antagonist co-treatment combined with single ET versus standard stimulation and GnRH agonist co-treatment combined with dual ET. The reason for this choice is both pragmatic (the statistical power of a four arm trial would be much less, given the number of participants that could feasibly be recruited) as well as conceptual (the current comparison is between the standard 'gold standard' treatment strategy in Northern Europe at the time of design of the study (183) and a new, potentially more patient -and child- friendly integrated approach). The possibility to perform more cycles in the same period of time (because of better patient tolerance) renders mild stimulation a suitable combination with single embryo transfer. More cycles means additional pregnancy chances, which can compensate for the reduction in live birth rate per cycle due to the use of GnRH antagonist co-treatment along with the transfer of a single embryo. The acceptance of the proposed treatment strategies is illustrated by the timely accrual of patients into the study as depicted in Figure 2.

A maximum of three fresh IVF cycles was chosen in the standard arm, for practical reasons: it is the number of cycles traditionally covered by insurance in the Netherlands. In the new treatment strategy, one extra cycle was allowed to let patients realize the potential of more cycles in the same amount of time. The cumulative number of cycles completed by the first 200 patients included is depicted in Figure 3.

The other two alternatives have a priori disadvantages: mild stimulation with dual ET might give more pregnancies over time, but does not reduce the twin pregnancy rate. Standard stimulation with single ET does not diminish the physical and psychological burden of the standard stimulation regime. Lower pregnancy rates have been observed (46,37) following the transfer of fresh embryos only, and similar when cryo transfer is also considered (43). A cryo policy is also applied in the current study.

5.3.3 Non-inferiority versus equivalence: one-sided versus two-sided testing

The study is a non-inferiority trial. A non-inferiority trial is appropriate when a new intervention has fewer adverse effects and/or lower costs, and one might accept a little less than the benefit of the standard intervention to gain this advantage in adverse effects or costs. It is well established that the overall costs of pregnancy as well as the complications are greatly reduced by single ET, due to the elimination of twin pregnancies (117,35,184,56,55). If we are able to demonstrate that the mild stimulation/single ET strategy is not worse in clinical outcome compared with the standard strategy, the reduction in multiple pregnancies with their associated higher complications and costs will become decisive in favour of the new strategy. Even when the new strategy would be less effective, the reduction in costs may still make it the more efficient option. Therefore, the focus in the statistical comparison will be to establish that the mild stimulation, single ET strategy is not inferior, within a predefined margin, to the long protocol, dual ET strategy, i.e. a one sided hypothesis.

We calculated the required sample size for the study on a non-inferiority criterion derived from cost-effectiveness considerations. We used the total costs of one IVF treatment cycle of 1,500 Euro from Goverde et al (109), and data regarding costs of pregnancy, separately for singletons and for twins from Wolner-Hanssen et al (55), 5,300 and 46,000 Euro respectively, including costs of delivery, neonatal care and disability. Furthermore, we chose 45% as the total live birth rate in the standard IVF arm (with a maximum of 3 cycles), of whom 30% are twins, based on annual reports of Utrecht and Rotterdam IVF data, which is compatible with other published Dutch data (185,162). The expected costs per live birth would then be 26,000 Euro. We assumed that the mild stimulation, 1 ET strategy (with a

maximum of 4 cycles) could have a lower cumulative live birth rate but also lower costs, due to the absence of twin pregnancies. We tested a range of differences (from -5% to -15%) in live birth rate between the new and the standard strategy and calculated at each specified difference the costs per extra live birth of the standard strategy compared to the experimental strategy. This cost-effectiveness ratio varied from 90,000 Euro (at a difference of -5%) to 25,000 Euro (at -15% difference). At a difference of -12.5%, cost were 35,000 Euro. At this latter figure we (rather arbitrarily, and only for the calculation of sample size) considered the standard strategy no longer acceptable. Therefore, we used a difference in live birth rate between the experimental and the standard strategy of -12.5% as the critical threshold for non-inferiority.

The number of patients should be at least 200 per arm (400 in total) to assure with 80% power that the *lower bound of the 95% one-sided confidence interval* around the difference in live birth rate between the experimental and the standard group will not fall below –12.5%, in case there is no difference in reality. The use of a one-sided alpha is allowed in this case since we have a non-inferiority trial (186). Normally, one-sided confidence intervals are disdained because they prohibit testing a treatment-effect in the direction opposite to anticipation. Here, the opposite direction would be that the new strategy is really inferior. However, it would be of no concern that the new strategy were so inferior that the difference was *statistically* significant: as long as the difference remains -with 95% confidence- within the predefined non-inferiority margin, it is not *clinically* relevant.

5.3.4 Randomisation

Block-randomisation, stratified by clinic, was applied to achieve balance between the two groups within each centre. Randomisation was performed by sealed envelopes available at a central location in both centres. Envelopes were opened by the treating physician at the IVF-intake. As appropriate for an effectiveness trial, the analysis will be according to the intention-to-treat principle, meaning that all patients will be analysed in the group they were randomised to, whether they received the allocated treatment or not. This also applies to patients who cross over to the other treatment group. Again, this is in line with the spirit of an effectiveness trial, since in everyday practice patients may also display a preference for another treatment modality than the one they started with.

5.3.5 Numerator: cumulative live birth as end-point

We defined as primary outcome a pregnancy leading to a term live birth. Term live birth is defined as live birth after a normal gestational length of 37 to 42 weeks. The debate is ongoing whether twins should be regarded as a success (6) or as a complete medical failure. From the clinical perspective, a term twin birth without complications is definitely a success. However, the increased rate of complicated deliveries, preterm births, and low birth weight (all giving rise to increased chances for perinatal morbidity or mortality) associated with twin pregnancies, have led to the opinion that medical intervention in infertility should preferably aim at establishing a singleton pregnancy (6). Our choice of term live birth as primary outcome was made to give a fair advantage to healthy twin births, instead of counting all twins as failure. In this way the increased chance of complications of twins will be expressed in the higher rate of preterm deliveries and discounted proportionally in the outcome.

5.3.6 Denominator: per treatment period versus per cycle

For an effectiveness trial, the natural focus is not on the (technical) results per cycle, but rather on the overall result that a patient may expect over a given treatment period (105). Therefore we have chosen an analysis per treatment period, which will allow the treatment strategy that is best tolerated by the patients and requires the least amount of time per cycle, to realize more treatment cycles -thus more ‘chance exposure‘- than the other treatment strategy. Dropouts who do not wish to receive any more treatment will be assumed to have a zero chance of the outcome, i.e. a pessimistic assumption (162). In this way we establish a statistical penalty for dropout due to intolerability of the treatment. The time period of analysis will start from the moment of randomisation, to avoid post-randomisation selective dropout.

5.4 Health economics considerations

The economic evaluation of the study uses the societal perspective, which is central to health economics as it explicitly considers the question of how to get the most benefit from the scarce resources available to a society (187). It implies that not only medical costs, i.e. costs made within the health care sector, should be included, but also non-medical costs, when relevant. For both medical and non-medical costs, we consider direct costs, defined as directly related to the health care problem (infertility) and treatment (IVF) under consideration as well as indirect costs, which are made after the treatment period.

The costs of the two IVF strategies at hand can be distinguished into two stages:

(1) the costs of IVF treatment itself, starting with the first IVF cycle and ending with the outcome of the last IVF-cycle within a given time period (being pregnant, no pregnancy or drop out);
(2) the costs of antenatal, peripartal and post partum care in women who have become pregnant after IVF treatment.

Since the applied embryo transfer policy during treatment will affect costs during pregnancy, the cost analysis should include all costs from the start of the first IVF cycle up to and including the costs of post partum care. Post partum costs will be counted until 6 weeks post term, since the term period (40 weeks gestation) is the only time horizon that is uniformly applicable to all patients. Costs are measured as the product of health care resource use ('volumes') and cost per unit estimates ('prices').

The costs of IVF treatment are distinguished into direct medical costs in the hospital and outside the hospital, as well as non-medical direct costs. Direct medical costs in the hospital consist of scheduled and unscheduled outpatient visits, number of IVF cycles, personnel time per cycle, use of GnRH analogues and rec-FSH, costs of ultrasound and hormonal monitoring, the embryo transfer procedure and costs associated with complications. Outside hospital costs consist of GP visits, while indirect non-medical costs include travel and time costs and absence from work/sick leave due to treatment or complications. Cost volumes in the treatment stage are recorded with case record forms (CRFs), hospital-based management and budgetary information systems, patient questionnaires and literature. Prices of hospital-based care are estimated as 'true' economic costs (including fixed costs and overhead), as variable costs, and in terms of reimbursement fees. Out of hospital care is priced with reference values for the Netherlands (188). To describe the variability in costs between the two centers, resource use and critical cost parameters are documented for each participating center separately.

The costs of pregnancy and obstetric care can be distinguished into direct medical costs in the hospital (secondary obstetric care) and direct medical costs outside the hospital (e.g. primary obstetric care, GP care, etc.). The pregnant patient will receive questionnaires covering three months periods of their pregnancy, regarding the out of hospital costs. The last questionnaire covers the period around the calculated term date, until 6 weeks thereafter. This means that the neonatal costs are covered for a 6-week period post term. For preterm births, the postnatal period that we consider will therefore be extended resulting in higher costs, as is customary in studies on neonatal care (189).

The incidence of disabilities is markedly increased in multiple pregnancies, and the associated long-term costs might be included in a cost analysis (190). In our study we will add the costs related to long-term health consequences in a scenario analysis, i.e. we will repeat the calculations, with projected costs of life-long disability added to the cost analysis.

5.5 Psychological Considerations

Since many decades, outcome measures of medical interventions have not been restricted to rates on survival, mortality, morbidity, and – in reproductive medicine – pregnancies, but have involved other life aspects as well. Many of these are subsumed under the denominator of 'quality of life'. Quality of life measures encompass: (1) global measures of patient satisfaction, (2) multi-dimensional measures of health status (which often include social, psychological and physical dimensions), (3) disease-specific measures that chart problems associated with a specific illness, and finally (4) domain-specific measures that focus on a specific psychological outcome, such as anxiety or depression. Case reports have shown that IVF treatment is sometimes accompanied by intense moments of stress and emotional instability. Aside from being caused by physical stimuli, this emotional instability can also be attributed to the fact that patients swing between hope for a successful pregnancy and fear of failure. When choosing psychological outcomes to be included in an IVF effect study, it therefore seems essential to register negative emotions and moods, rather than assessing psychopathology.

Most psychological effect studies that have been carried out in a medical setting involved patients with a chronic disease. Often, retrospective questionnaires that cover a relatively long period of time are applied in these studies, since short term psychological changes are less relevant in the context of chronic illness. In case of episodic diseases or treatments (e.g. migraine and its medication), diary measures are used to monitor the day-to-day mood fluctuations that may accompany the different stages of the disease and the treatment. While the use of diary measures may reduce recollection-bias (van den Brink *et al.*, 2001), compliance to retrospective questionnaires may be better, as keeping a diary might be a burden to patients. In small studies, interviews are sometimes conducted to explore patients' reactions more thoroughly. Given the complexity of IVF treatment, a combination of retrospective questionnaires and diary measures would be optimal for recording both its long-term and short-term psychological effects.

Many previous studies examining the psychological consequences of IVF treatment have used depression and anxiety as their main outcome variables. These outcomes are

usually measured at a few specific moments during IVF treatment (often before or after a treatment cycle) with retrospective questionnaires, like the Spielberger's State and Trait Anxiety Inventory (STAI) and Beck's Depression Inventory (BDI). Other outcomes that are frequently measured with retrospective questionnaires in psychological IVF studies are marital adjustment and self-esteem. Aside from these general adjustment measures, some studies have used infertility-specific stress measures. The Fertility Problem Inventory (FPI) for example, measures five domains of stress that are specific to infertility: social concern, sexual concern, relationship concern, need for parenthood and rejection of childfree lifestyle. Infertility-specific stress measures are believed to be more sensitive to patient responses to infertility and its treatment than general stress measures. The use of standardized diaries to measure psychological variables is not widespread in the IVF field, with the exception of the Daily Record Keeping Chart (191). This questionnaire has been developed to assess daily emotional, physical and social reactions to infertility treatment.

In the present study a combination of retrospective and diary measures is used to ascertain both the long-term and the short-term effects of IVF treatment. During the first IVF treatment cycle both negative and positive affect are assessed daily with the use of the Daily Record Keeping Chart, which has shown good criterion-related and convergent validity and good internal consistency (192). Additionally, subjects are asked to fill in three retrospective questionnaires several times during the first treatment cycle: After randomisation (baseline), on the first day of ovarian stimulation (to assess the effects of pituitary down-regulation) and after embryo transfer. This last moment is considered to be the most stressful stage of IVF treatment by many patients (193). The retrospective questionnaires are also used to measure possible psychological effects during subsequent IVF cycles. To gain insight in possible side effects related to IVF treatment, self-reported physical discomfort is measured with the somatic subscale of the Hopkins Symptom Checklist (194). The Dutch version of the Hopkins Symptom Checklist has shown adequate to good test-retest reliability, internal consistency and validity (195). Additionally, subjective sleep quality is measured with the Subjective Sleep Quality Scale, a Dutch questionnaire (196), which consists of ten items on various aspects of sleep. This scale has shown good reliability and homogeneity. Finally, stress is assessed with the Hospital Anxiety and Depression Scale (197), which have been developed as a screening tool to detect anxiety and depression in medical patients. The Dutch version of the HADS (198) has shown good test-retest reliability, homogeneity and internal consistency in previous studies.

5.6 Discussion

In the current paper we describe the design of a study attempting to answer the question whether the use of a mild ovarian stimulation protocol (using GnRH antagonist co-treatment) combined with single embryo transfer is not inferior to a standard stimulation protocol (using GnRH agonist co-treatment) with dual ET, while resulting in reduced patient discomfort and lower overall costs per pregnancy.

Success of IVF treatment has for long been focussed towards technical aspects of the treatment: The number of follicles harvested, the fertilization or implantation rate. The only outcome of interest to the patient, and therefore the one that should be of interest to the doctor, is whether the procedure will lead to the desired result, a healthy baby (106,84,105). All other outcome measures are no more than surrogate for this endpoint. Treatments should be evaluated against this outcome measure. A point of ongoing discussion is how to define "healthy". Certainly, pre-term and higher order multiple births are outcomes that should be avoided if possible, but increased perinatal morbidity is also reported following twin pregnancies (6). Should a distinction between twins versus higher order multiples be made or should only a singleton, term delivery be regarded as a success? The current study uses a term live birth as primary clinical outcome measure, which implies that adverse effects of multiple pregnancies will be reflected in a higher rate of pre-term births.

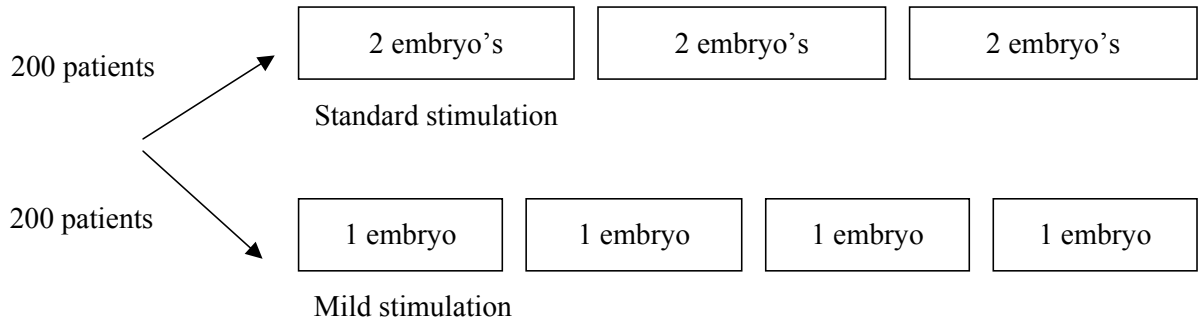
In the field of infertility treatment, the chances of success come in discrete, biologically defined, portions of time, i.e. the menstrual cycle of the woman. Because of the ease of analysis and the simplicity of the cycle concept, the focus in the literature on treatment results has been almost entirely on results per cycle, particularly in IVF. An improvement seems the reporting of cumulative pregnancy rates per patient over multiple cycles (105). However, like in other medical fields, the interest of the patient will be how long it will take until the desired outcome is reached. Obviously, duration of treatment is also related to costs. Cumulative rates over a number of cycles are not very informative if it remains unknown how long it will take to finish the treatment. Thus, the concept to assess success rates per given time interval should be considered. In our study we hypothesized that the mild stimulation method may lead to a shorter duration of a single treatment cycle and therefore the possibility to do more cycles in the same amount of time compared to the standard method.

However, success rates –regardless of how this is defined- still should not be the only outcome used when comparing treatment options. The costs associated with the treatments, the patient discomfort, side effects and complications (mainly ovarian hyperstimulation syndrome and multiple pregnancies as mentioned earlier) should also be part of the equation.

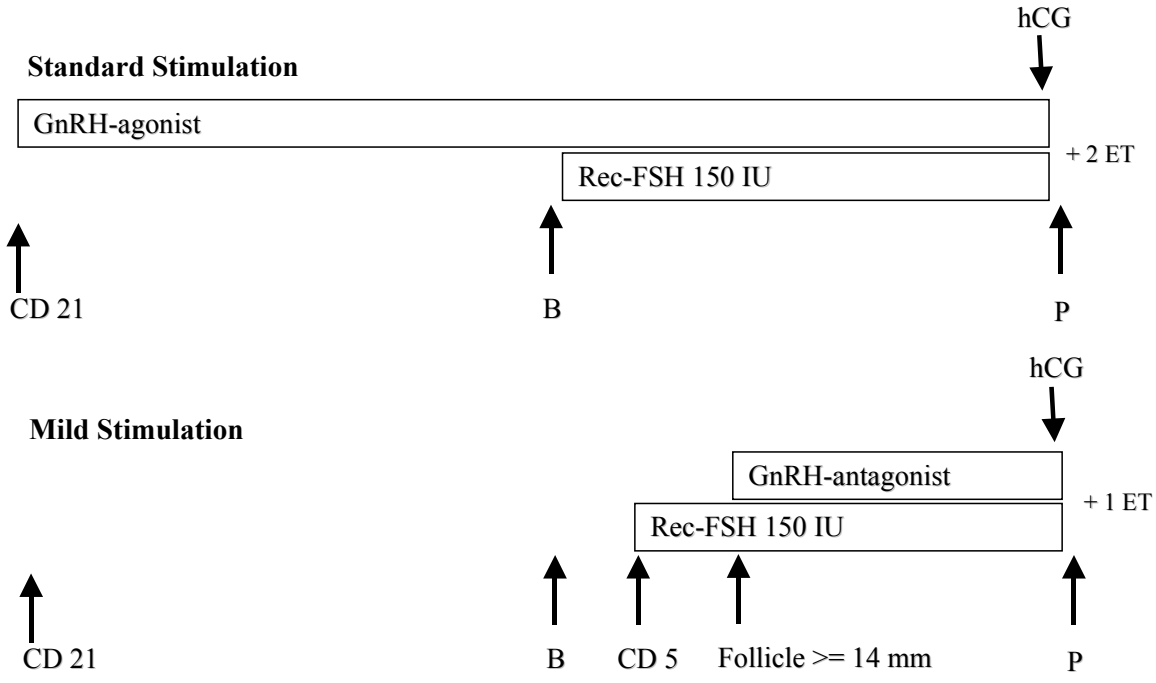
In the current study we measure all these aspect in order to give an integrated evaluation of the tested two treatment strategies. In case one treatment strategy is comparable to the other as far as success is concerned, but with a reduced complication rate, and better in the psychological and cost dimensions, it is clearly preferable. In other cases, the costs and patient stress and discomfort will be related to the success rate in a cost-effectiveness analysis. The preferability will then depend on how high the extra costs and psychological burden of the most successful treatment strategy are per extra pregnancy. The design of this study allows assessing all these aspects and obtaining a complete evaluation of two treatment strategies.

Figure 1. Schematic overview of the study design.

a) The standard stimulation, 2 ET and the mild stimulation, 1ET arms



b) Standard and mild stimulation protocol per cycle



CD 21: day 21 of the preceding cycle
B: day of bleeding
CD 5: day 5 of the cycle
P: day of follicle puncture for oocyte retrieval

Figure 2. *Accrual rate of the trial: Cumulative number of patients included in the study against calendar time.*

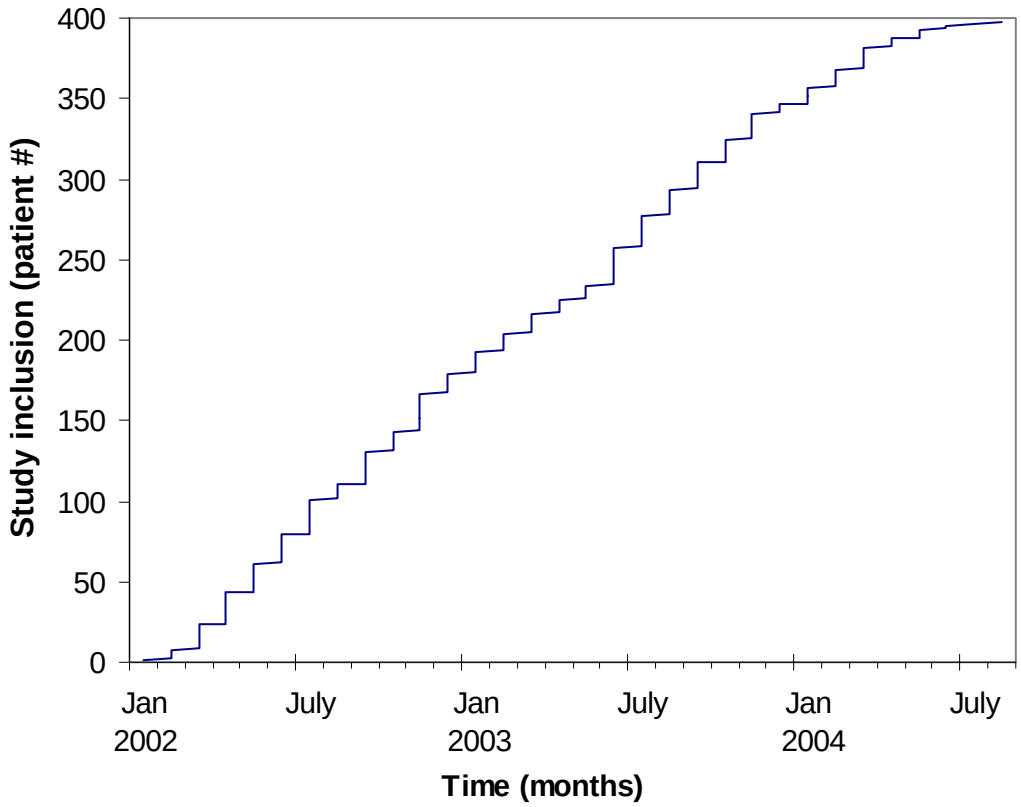
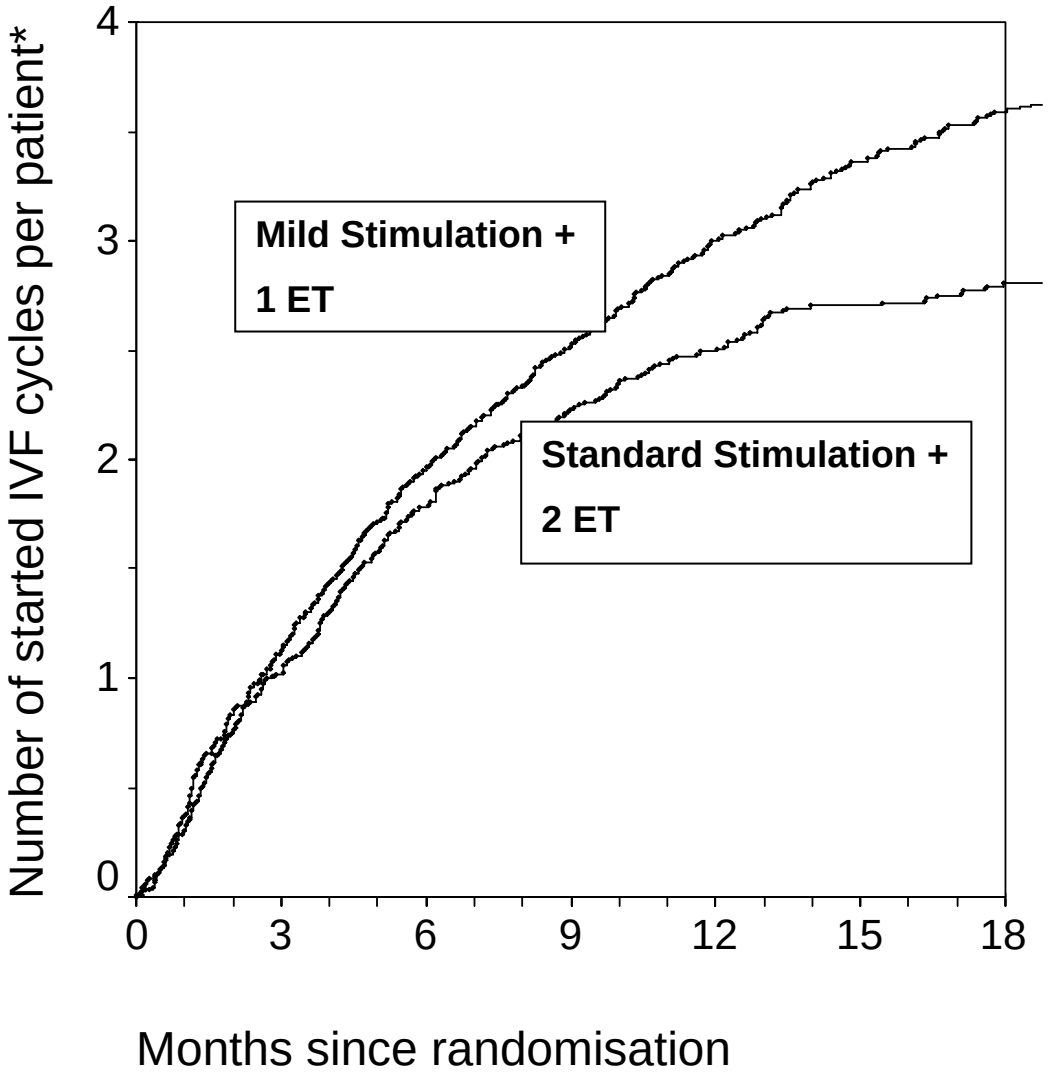


Figure 3. *Cumulative number of started IVF cycles per patient against time since randomisation, separately for the standard stimulation + 2 ET and mild stimulation + 1 ET group. Couples who became pregnant are censored: the curve represents the theoretical number of cycles in case no one would become pregnant.*



6. A mild strategy in IVF results in favourable outcomes in terms of term live birth, cost and patient discomfort.

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6.1 Introduction

In vitro fertilization (IVF) is a complex treatment for infertility involving costly ovarian stimulation regimens (64), substantial patient discomfort (111,32) and considerable chances of complications (138,6). Applied ovarian stimulations protocols aim to generate many oocytes in order to compensate for inefficiencies in the laboratory procedures and to generate multiple embryos for transfer into the uterus.

Standard stimulation protocols involve the co-treatment with GnRH agonists to desensitize the pituitary gland (199). In contrast to GnRH agonists, GnRH antagonist treatment can be limited to the days in the mid-to late follicular phase at risk for a premature LH rise (58) allowing for the endogenous inter-cycle FSH rise to be utilized rather than suppressed (178). Mild stimulation protocols in which exogenous FSH administration is limited to the mid to late- follicular phase of the menstrual cycle have been shown to represent a feasible novel approach in stimulating growth of multiple dominant follicles for IVF (111,112). A potential drawback of GnRH antagonist co-treatment may be a minor reduction in efficacy per cycle (26). However, mild stimulation protocols may reduce patient discomfort by diminishing symptoms associated with pituitary down regulation (111) leading to fewer drop-outs from IVF (200), and thereby creating additional pregnancy chances in subsequent IVF cycles (32).

Significantly increased infant mortality and morbidity associated with premature birth have led to (higher order) multiple pregnancies being considered as the most important complication associated with IVF treatment (117). The financial impact of multiple births on health care resources has been shown to be greater than the costs of IVF treatment itself (201,173). Multiple pregnancies arising from IVF can be avoided by the transfer of a single embryo (SET). The observed minor decrease in pregnancy rate per cycle following SET can be overcome by establishing a high-quality cryopreservation program for surplus embryos (providing additional pregnancy chances after transfer in subsequent cycles) (173,43) or by an additional IVF cycle (41). An increasing number of Northern European centers currently offer SET as standard practice in a young women (202,203). However, the widespread

implementation of SET into daily practice is hindered by the perceived need to maximize pregnancy chances per cycle (163).

Further development of IVF may be facilitated by challenging current concepts of "success" in assisted reproduction (105). Defining success in terms of chances for term live birth (or healthy child) per IVF treatment period (which may include multiple cycles) in relation to cost, patient discomfort and chances for complications as recently suggested by the Cochrane Menstrual Disorder and Subfertility group (204) would reduce the emphasis on maximizing single cycle outcome. Strategies involving shorter and milder ovarian stimulation protocols (including GnRH antagonist co treatment) and single embryo transfer may allow for more IVF cycles in the same period of time, resulting in similar term live birth rate per treatment period despite a minor reduction in birth rate per treatment cycle. Moreover, such a mild strategy may reduce patient discomfort by using a milder stimulation protocol while lowering costs by virtually eliminating multiple pregnancies. The present multi-centre effectiveness study was designed to test the hypothesis that a mild in vitro fertilization strategy can achieve the same term live birth rate within 1 year compared to standard treatment, while reducing patient discomfort, multiple pregnancies and cost.

6.2 Methods

6.2.1 Patients

Patients with an indication for IVF or IVF/ Intracytoplasmic Sperm Injection (ICSI) based on tubal, male or unexplained infertility were recruited in two Academic Medical Centers (Rotterdam and Utrecht) between February 2002 and March 2004 (205). Patients under < 38 years with a normal menstrual cycle (cycle length between period 25-35 days) and without severe obesity or underweight (body mass index 18-28 kg/m^2) were eligible for the study.

6.2.2 Study Design

This study was designed as a 2-arm randomised controlled, non-inferiority, effectiveness trial (205). The study was approved by the local ethics committee of both participating centers and all patients signed informed consent. Patients were randomly assigned to undergo either a mild ovarian stimulation with GnRH antagonist co-treatment combined with SET ("mild" treatment group) or a standard ovarian stimulation protocol including a GnRH agonist long-protocol combined with the transfer of 2 embryos ("standard" treatment group). In order to compensate for a possible reduction in pregnancy rate, patients in the mild treatment group were offered an extra reimbursed cycle on top of the three cycles normally reimbursed in the

Netherlands. It was estimated that within 1 year after commencing treatment, the majority of subjects undergoing standard treatment would complete 3 cycles whereas those undergoing the shorter, mild treatment would complete 4 cycles.

The randomisation sequence was computer generated with random blocks of size 4 and 6, stratified by center in order to maintain balance between the two groups within both centres. The allocated treatment assignments were subsequently put in numbered sealed envelopes available at a central location in both centres. Envelopes were opened by the treating physician at the IVF-intake after written informed consent was obtained.

In the mild treatment group ovarian stimulation was performed by a fixed starting dose of 150 IU recombinant FSH (recFSH) (Gonal-F®; Serono Benelux B.V., Amsterdam, The Netherlands, or Puregon®; N.V. Organon, Oss, The Netherlands) subcutaneous (s.c.) per day, initiated on cycle day 5. GnRH antagonist co-treatment 0.25 mg/day (Cetrorelix®; Serono Benelux B.V. or Ganirelix®; N.V. Organon) was administered if at least 1 follicle ≥ 14 mm diameter was observed by ultrasound, as previously described (112). The starting day or dose of recFSH could be adjusted in subsequent cycles. Induction of final oocyte maturation by human chorionic gonadotropin (hCG), oocyte retrieval, fertilization *in vitro* and luteal phase supplementation was performed according to standard procedures, as described previously[205]. Only the best quality embryo was transferred (176) on day 3 or 4 of culture. Supranumerary high quality embryos were cryopreserved and thawed for transfer in a subsequent unstimulated cycle, as previously described (177). One or 2 embryos were transferred after cryopreservation according to patient preference. Cryopreserved embryos were thawed for transfer before continuing to a subsequent IVF cycle.

In the standard treatment arm, a GnRH agonist (leuproreline 0.2 mg/day, Lucrin®; Abbott B.V., Amstelveen, The Netherlands; or triptoreline 0.1 mg/day, Decapeptyl®; Ferring B.V., Hoofddorp, The Netherlands) was started in the midluteal phase of the preceding cycle. After approximately 2 weeks of GnRH agonist administration, ovarian stimulation was initiated with a starting dose of 150 IU/day recFSH s.c.. The recFSH dose could be adjusted in subsequent cycles, if considered necessary. Similar criteria were applied for hCG administration, for oocyte retrieval and fertilization procedures as in the mild treatment group. A maximum of 2 (best quality) embryos were transferred after culturing for 3 to 4 days. Standard luteal phase support, and criteria for cryopreservation of embryos were applied.

The primary outcome parameters chosen for this study were: (1) pregnancy within one year of treatment after randomisation leading to term (≥ 37 weeks gestation) live birth, (2)

total costs per couple and child up to 6 weeks after expected delivery, and (3) patient discomfort and distress during IVF treatment.

6.2.3 Cost calculations

The costs of the two IVF strategies were distinguished into two stages: costs of IVF treatment itself ending with the outcome of the last IVF-cycle (being pregnant, no pregnancy or drop out), and the costs of antenatal, peri- and post partum care until 6 weeks after the expected delivery date in women who had conceived within the treatment period.

The volumes of health care use were multiplied by the corresponding unit prices. The costs of IVF treatment were calculated from direct medical costs associated with care and indirect non-medical costs (travel and time costs, absence from work). The costs of pregnancy and obstetric care were distinguished into direct medical costs in the hospital (secondary obstetric care), direct medical costs outside the hospital (e.g. primary obstetric care, GP care, etc.) and indirect non-medical cost (206). Cost volumes were recorded with case record forms (CRFs), hospital-based management and budgetary information systems, patient questionnaires and literature (205).

6.2.4 Evaluation of patient stress and discomfort

The Hospital Anxiety and Depression Scale (HADS) (range: 0-21) (197), the somatic subscale of Hopkins Symptom Checklist (HSCL-S) (range: 0-24) (194) and the Subjective Sleep Quality Scale (SSQS) (range: 10-0) (196), were used to assess patient stress (anxiety and depression), physical discomfort and sleep quality, respectively. These questionnaires have been described elsewhere (205). Women completed the questionnaires at baseline (just after randomisation), directly following the first embryo transfer and one week after the outcome of subsequent cycles (cancellation, pregnancy test).

To estimate overall patient discomfort during the first year after randomisation, the 'area under the cumulative score within 12 months' curves were calculated per patient for the 4 psychological dimensions. These areas were compared between the study groups with ANCOVA, after adjusting for baselines scores. As more cycles were to be expected in the mild compared to the standard treatment group within 1 year (i.e. 4 instead of 3), this implies higher cumulative discomfort scores, given similar scores per cycle.

6.2.5 Calculation of sample size

The total live birth rate after 3 cycles in the standard strategy was estimated at 45% with 30% twins. The expected costs per live birth were estimated at €26,000 using the total cost of one IVF treatment (€1,500) and cost of singleton and twin pregnancies (€5,300 versus €46,000) as described in the literature(109,55). It was expected that the mild strategy would result in a lower cumulative birth rate but also a lower twin pregnancy rate. A range of differences (from -5% to -15%) in live birth were tested and costs per extra live birth at each specified difference were calculated. At a difference of -12.5%, the cost per additional live birth in the standard strategy compared with the mild strategy was calculated to be 35,000 Euro. This was deemed to be excessive, and therefore -12,5% was used as the critical threshold for non-inferiority (205). Two hundred patients per arm were required to assure with 80% power that the lower bound of the 95% one-sided confidence interval around the difference in term live birth rate was within -12,5%.

6.2.6 Statistical analysis

Statistical analysis was carried out according to the intention-to-treat principle. In addition, an analysis was performed in which switchers (patients who prefer another stimulation protocol or embryo transfer policy) were excluded. The Kaplan-Meier method was employed where patient drop-outs were considered to have a zero chance of a term live birth (no censoring) (107). In this way we established a statistical penalty for drop out due to unacceptable burden of the treatment. Patients who achieved an ongoing pregnancy *not* leading to term live birth were censored at the time that pregnancy occurred. The cumulative term singleton live birth was calculated using the same method. Couples who did not start a subsequent cycle within 6 months received a questionnaire in order to obtain all information about pregnancies occurring within 1 year after randomisation.

6.3 Results

Four hundred and four patients were included in the study and a total of 769 cycles were performed within 1 year (444 in the mild group and 325 in the standard group). The flow-chart of the study according to CONSORT guidelines is shown in Figure 1.

The mean age in the total study population was 32.8 ± 3.1 (S.D.) years, the duration of infertility was 3.6 ± 2 years and the BMI was 23.1 ± 2.6 kg/m^2. The percentage of patients with primary infertility was 73.3%. The cause of infertility was 54.7% male factor, 16.6% tubal factor and 22.3% unexplained or other reason. Both treatment groups were comparable with respect to these patient characteristics (data not shown).

In the mild strategy group, 191 first, 138 second, 75 third, 30 fourth and 6 fifth IVF cycles were carried out within 1 year. In the standard group 188 first, 99 second, 39 third and 6 fourth IVF cycles were conducted. The mean number of started cycles, oocyte retrievals and embryo transfers in 1 year were respectively 2.3 ± 1.2, 1.8 ± 1.1 and 1.5 ± 1.0 in the mild group and 1.7 ± 1.0, 1.6 ± 0.9 and 1.4 ± 0.9 in the standard group (P-value respectively < 0.001; 0.008 and 0.5, t-test). The mean duration of injections was 8.5 ± 2.7 in the mild group and 25.3 ± 6.8 in the standard group ($p<0.001$),

Out of 96 ongoing pregnancies in the mild treatment group within 1 year, 11 were spontaneous, 78 arose from fresh embryo transfer, 6 were from cryopreserved embryos and 1 occurred after 'escape' intra-uterine insemination due to low ovarian response to stimulation. The number of total term live births resulting from 1 year of mild treatment was 86. Out of 103 of ongoing pregnancies in the standard treatment group, 5 were spontaneous, 93 after fresh embryo transfer and 5 were from cryopreserved embryos. The number of total term live births resulting from 1 year of treatment was 86.

The 1-year cumulative rate of pregnancy leading to term live birth was 43.4% in the mild group and 44.7% in the standard group (Figure 2). The difference between the mild and standard group was 1.3% in favour of the standard group, with a lower limit of the one-sided 95% confidence interval equal to –9.8%. The percentage of multiple pregnancies per randomised couple in 1 year of IVF treatment was 0.5% (95% CI 0.0;2.7) in the mild strategy and 13.1% (95% CI 8.7;18.6) in the standard strategy (P < 0.001, Chi-square test). Table 1 shows the characteristics of children born from pregnancies within 12 months after starting IVF. The miscarriage rate was 15.0% in the mild group and 17.1% in the standard group. The 1-year cumulative rate of pregnancy leading to singleton term live birth after 1 year was 43.4% in the mild group and 35.7% in the standard group (Figure 2).

In the mild treatment group 12 patients (5.8%) switched to another stimulation protocol or embryo transfer strategy, whereas 15 patients (7.5%) switched in the standard group. When excluding these patients in the analysis, the 1-year cumulative rate of pregnancy leading to term live birth rate was 43.2% in the mild group and 44.6% in the standard group.

The mild stimulation strategy resulted in lower average total costs per IVF treatment within 12 months and pregnancy up to 6 weeks after expected date of delivery (per couple and child) (€ 8,333 versus €10,745; P = 0.006, t-test*)* (Table 2). The IVF treatment costs within this period were similar for both strategies (€ 3,459 versus € 3,304). The costs of the obstetric and postnatal period were higher for the standard strategy (€ 2,547 versus € 4,899), due to more outpatient visits and hospital admissions, higher delivery costs, and greater absence

from work, mainly caused by multiple pregnancies. The non-medical costs were also higher for the standard strategy (€ 2,327 versus € 2,542).

Figure 3 shows the distribution of the raw scores for 4 psychological parameters in cycles performed during the first year after randomisation for both the mild and the standard group. The areas under the cumulative score curves over cycles performed within 12 months were equal among the two treatment strategies for scores on the HADS-A ($p = 0.9$), the HADS-D ($p = 0.8$), the HSCL-S ($p = 0.5$) and the SSQS ($p = 0.3$).

6.4 Discussion

To our knowledge, the current study is the first randomised controlled trial comparing cumulative term live births, total costs per couple and patient stress after different treatment strategies during a given period of time rather than per treatment cycle. This study demonstrates that in women less than 38 years of age, a mild strategy in IVF involving GnRH antagonist co-treatment together with single embryo transfer results in similar 1-year cumulative pregnancy rates leading to term live birth compared with a standard strategy. Moreover, overall patient discomfort within 1 year is similar despite a minor increase in average number of IVF cycles. Multiple pregnancy rates are greatly reduced and overall costs per term live birth are lower in the mild strategy group.

Previous studies focusing on outcomes in single cycles (40,157,43) have shown that SET in women less than 36 years is highly effective in reducing multiple pregnancies, but at the expense of a lower pregnancy rate per cycle. Although a reduced pregnancy chance per cycle was also observed for the mild strategy in the present study, similar cumulative 1-year pregnancy rates leading to term live birth of approximately 45% were shown to occur. In order to achieve this goal, the lower pregnancy rate per cycle is compensated by a slight increase in the average number of cycles. Because the duration of a mild stimulation cycle is shorter, more cycles can be performed in the same period of time. Therefore the percentage of couples finishing treatment within 1 year does not differ between the two groups (66.8% in mild group versus 71.9% in standard group ($p=0.3$)). When only singleton live birth was taken as a measure for treatment success, as proposed by some investigators (84), the 1 year cumulative term singleton rate was higher in the mild treatment group compared with the standard treatment group.

When calculating the chance of term live birth per 12 months per couple, we counted twin live births as being equivalent to 1 live birth. However, it may be argued that a term-born twin should count as 2 live births. Term-born twins may be perceived as a positive outcome,

reducing the need for subsequent IVF treatments. However, in addition to the increased perinatal morbidity, mortality and long term health consequences associated with twin pregnancies, parents of multiple pregnancies have shown to be at greater risk of depression and anxiety (207,208). Furthermore, when weighing the benefits of one compared with two embryos, account should also be taken of the live births which may occur following the subsequent transfer of surplus embryos (209).

Another methodological issue relevant to the present study is the means of calculating the cumulative pregnancy rates leading to term live birth. In this study, the Kaplan Meier method to calculate the 1-year cumulative pregnancy rates differs from the standard method often used in calculating cumulative success rates in infertility (107). Generally it is assumed that drop outs have a similar chance for pregnancy than patients continuing treatment (censoring). Because all information concerning pregnancies occurring in 1 year was available, an intention to treat analysis including all pregnancies could be performed to calculate the true cumulative term live birth rate without making assumptions with regard to the pregnancy chance among the drop outs (no censoring). Therefore, this cumulative term live birth rate is lower than usually found in the literature. Censoring does not punish for high drop out rates during treatment (for example due to patient discomfort) and is therefore not appropriate when outcome parameters are employed which take treatment-related stress into account.

Although more cycles were performed in the mild treatment group within one year, overall patient discomfort was similar among the two strategies during that year. In calculating the cumulative discomfort score over time, the assessments of discomfort at the end of each IVF cycle were used. The stress level may have varied during and between treatment cycles. Nevertheless, patient discomfort associated with the mild strategy appeared to be stable over time whereas the level of discomfort related to standard treatment increased during subsequent treatment cycles. Questionnaires were returned by just 50% in both treatment groups. Although this may reflect the complexity and frequency of the measurements, the response rate was within normally reported ranges for this type of psychological assessment (210). The degree of non-response might have resulted in an underestimate of symptoms in both groups, since questionnaires were perhaps less likely to be completed by women under greater stress due to their perceived additional burden.

The potential health economic benefits arising from SET have thus far been the subject of few studies (35,54,55). A recently published randomised trial demonstrated a SET strategy to be associated with lower total costs per cycle compared to cycles were 2 embryos were

transferred due to a considerable reduction of multiple pregnancies (201). Despite the higher average number of cycles performed with the mild strategy (and consequently higher monitoring and indirect costs) the overall costs per pregnancy within 1 year leading to term live birth were lower compared to the standard treatment strategy. This was mainly due to the reduction in multiple pregnancies. The postnatal period of cost assessment was limited to 6 weeks after the expected date of delivery. This probably resulted in a conservative estimate of the additional costs arising from premature deliveries, since prematurity often has in long term health consequences (211).

The findings of the current study highlight the medical, health economic and psychological benefits of mild strategies in women less than 38 years of age in IVF treatment. However, if these results are to be widely implemented, IVF outcomes should be redefined in broader terms, better reflecting the interests of the couple, the child and providers of health care. In other medical fields, such oncology, it is normal practice to present success of a treatment strategy as survival rate per given time period and also include side effects (212,213). The aim when embarking on IVF treatment is the delivery of a healthy baby (or babies) within a certain time period (consisting of a series of IVF cycles and subsequent replacement of frozen embryos). This needs to be weighed against the associated discomfort, chances for complications and costs. Adopting the endpoint 'term-delivery per time period' would encourage the adoption of patient friendly stimulation protocols and single embryo transfer. In conclusion, the findings of this study may contribute to the more widespread use of mild ovarian stimulation and SET in clinical practice. Additional measures required to aid widespread adoption of this approach will include better education of both patients and health care providers regarding the chance and definition of success, the risks associated with multiple pregnancies (48) and ideally, the institution of reimbursement systems which encourage, rather penalize SET (214,215).

Table 1. *Pregnancy outcome following IVF treatment (for a maximum of 1 year) comparing a mild versus standard strategy involving a total of 404 couples and 769 cycles.*

	Mild Strategy*	**Standard Strategy**	
	Singleton	Singleton	Multiple
Live Birth (total) (n)	91	76	26
Live born children (n)	91	76	51**
Late preterm live birth (n) (≥ 32 - 37 weeks gestation)	2	6	6
Early preterm live birth (n) (< 32 weeks gestation)	3	1	3
Birth weight (g)	3,339 ± 757	3,349 ± 757	2,340 ± 726

*One triplet occurred in the mild treatment group (Gestational age < 32 weeks, birth weight: 1340 gram).

**One twin pregnancy resulted in one intra-uterine death and one live birth

The difference in distribution of gestational age of the live births between the standard and mild treatment group is significant (p-value = 0.04).

Table 2. *Total costs (€) of IVF treatment over 12 months including costs of pregnancies up to 6 weeks after delivery (per couple).*

	Mild		**Standard**		**Significance***
	(Mean	± SD)	(Mean	± SD)	P
IVF Treatment					
Technical Procedures	1,083	± 734	991	± 584	*0.16*
Medication	1,626	± 1088	1,737	± 1069	*0.3*
Monitoring	750	± 561	576	± 693	*0.006*
Indirect costs	1,948	± 2280	1,740	± 1845	*0.3*
Pregnancy and neonatal period					
Medical costs	2,547	± 4,553	4,899	± 10,746	*0.01*
Indirect costs	379	± 1,177	802	± 2,270	*0.03*
Total costs	**8,333**	**± 5,418**	**10,745**	**± 11,225**	***0.006***

* independent groups t-test

Figure 1. *Flow chart according to the CONSORT guidelines showing the number of cycles analysed in the 12 months intention to treat analysis and the number of drop outs during the entire treatment.*

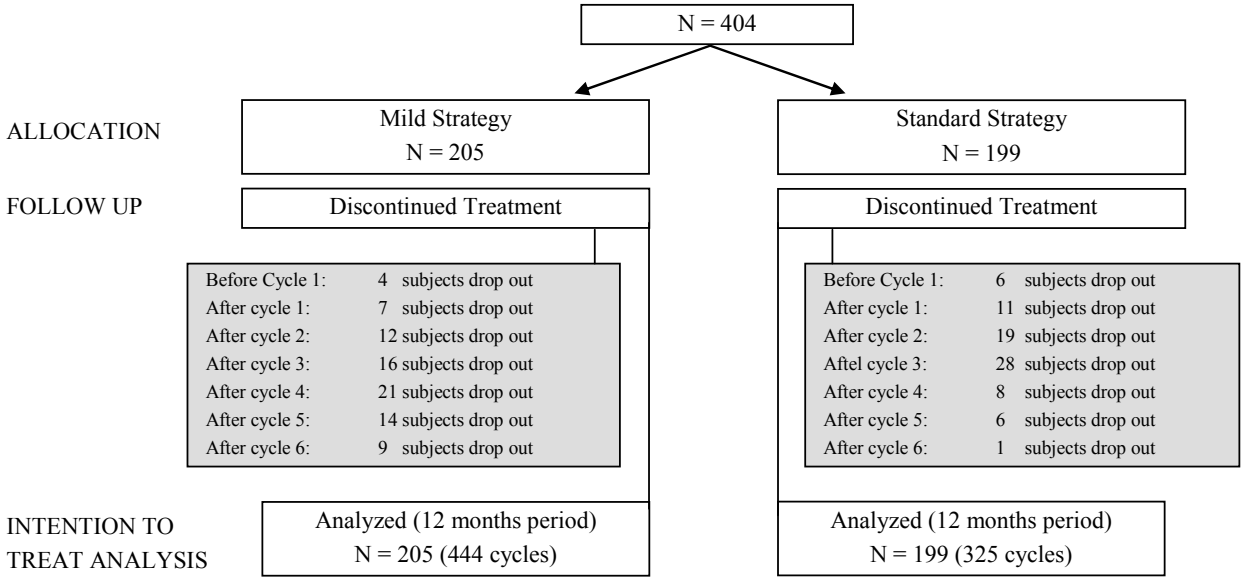


Figure 2. *Realistic cumulative term live birth rate within 12 months after starting IVF in 404 couples, comparing a mild ovarian stimulation plus single embryo transfer strategy (triangles) with a standard ovarian stimulation plus dual embryo transfer strategy (diamonds). The singleton live birth rate after 12 months is also presented in the graph.*

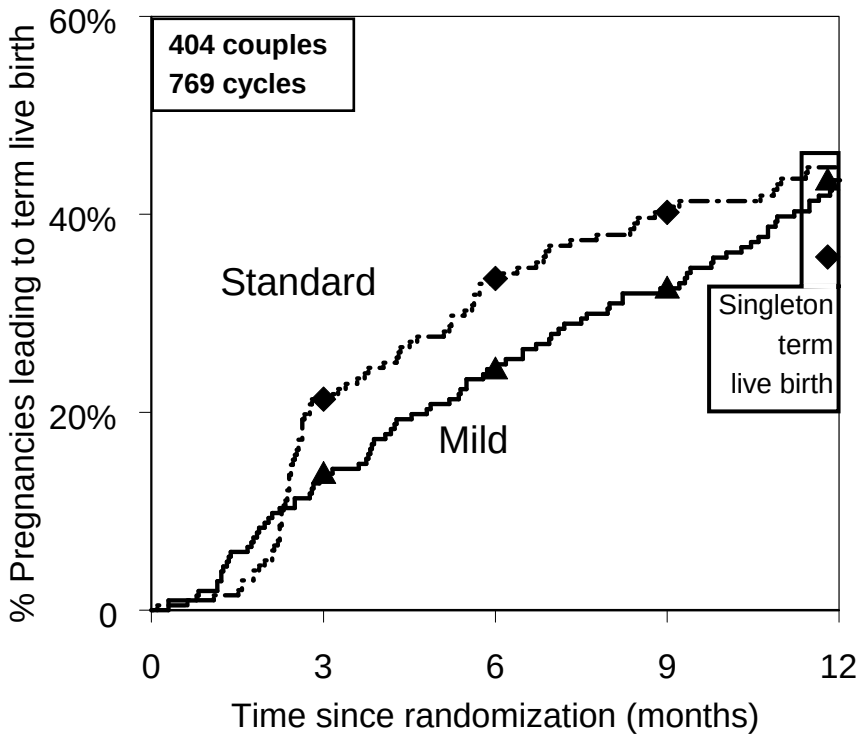
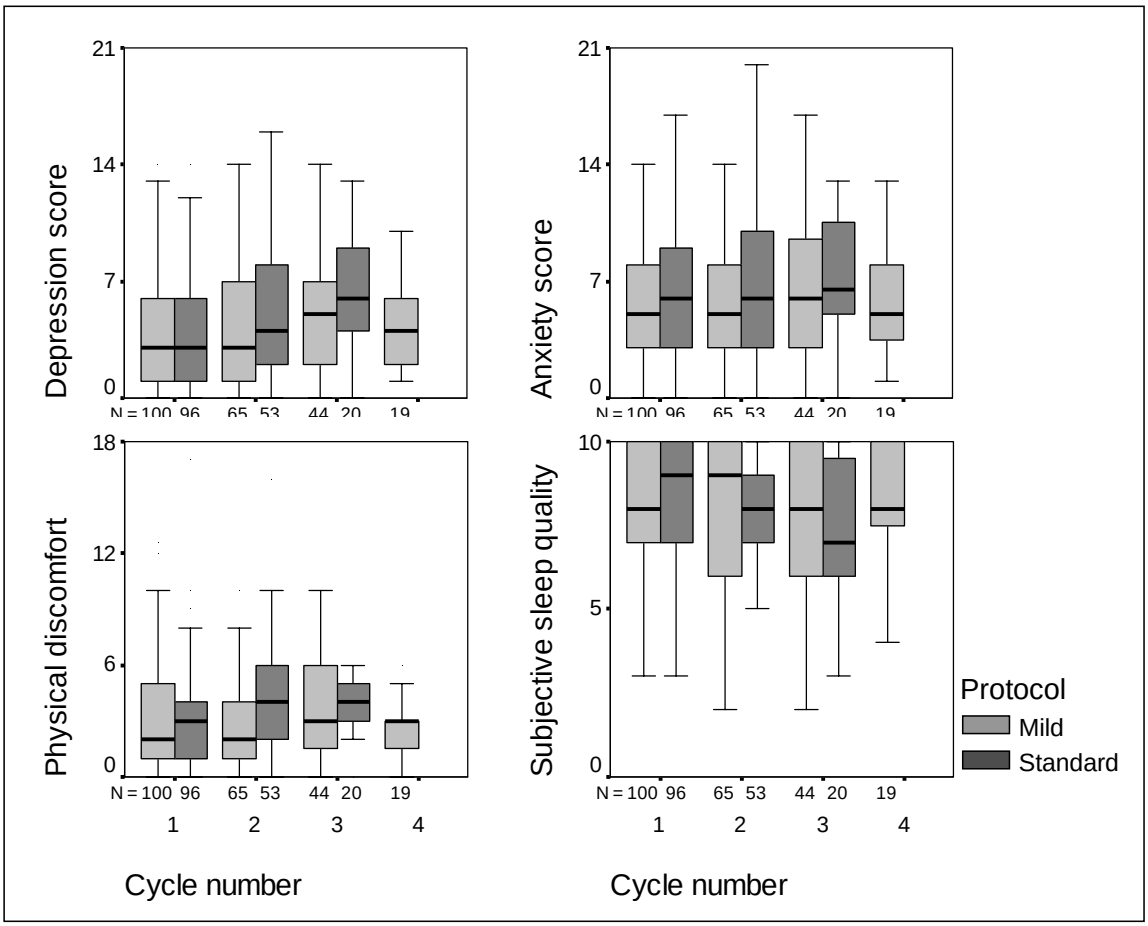


Figure 3. *Adjusted means of the scores on the 4 psychological dimensions: Anxiety, Depression, Physical discomfort (higher score means more anxiety, depression and physical discomfort) and Subjective sleep quality (higher score means better sleep quality) of cycles performed for both the mild and the standard treatment group within 12 months.*



7. Health economics of two IVF strategies: Mild ovarian stimulation in combination with single embryo transfer versus standard ovarian stimulation with dual embryo transfer

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Submitted

7.1 Introduction

The increasing success of IVF in the 1990s lead not only to an increased pregnancy rate, but also to an increase in the incidence of multiple births(6). Several cost studies have demonstrated the impact of multiple births on health care resources (16,37,201). The standard IVF regimen with the transfer of two embryos has a inherent high probability of multiple pregnancies, resulting in high costs due to intensive antenatal surveillance, increased chances for complications of both mother and child, hospital admissions, and perinatal and post partum care (37,56,55). The financial burden of multiple births on health care resources has been calculated to be greater than the costs of IVF treatment itself (216). There is a growing awareness that the high rate of multiple pregnancies can be greatly reduced by a single embryo transfer (SET) policy (217,43,6). However, single embryo transfer results in a lower live birth rate per cycle(218,43). There is a clear need for the further evaluation of efficacy and economic consequences of SET.

The introduction of gonadotropin-releasing hormone (GnRH) antagonists into clinical practice has enabled the development of novel milder ovarian stimulation protocols (111). Mild stimulation might be advantageous when evaluated over an entire (multiple cycle) treatment period, since the amount of time needed to complete a single IVF cycle is reduced, the costs of stimulation are lower (26,112) and the patient drop out rate may decrease. Mild treatment strategies with SET may result in more IVF cycles in the same period of time and therefore result in a similar term live birth rate per treatment period compared with standard stimulation protocols with the transfer of 2 embryos (41). Such a mild treatment strategy may also reduce costs by eliminating multiple pregnancies. As reported previously, a mild treatment strategy in IVF (mild ovarian stimulation with GnRH antagonist co-treatment and SET) results in similar cumulative term live birth rates within one year compared with a standard treatment strategy (“long” ovarian stimulation protocol, including GnRH agonist co-treatment and transfer of 2 embryos) in women less than 38 years of age, while greatly reducing multiple pregnancy rates (163).

Recently published randomised trials comparing the costs of single and dual embryo transfer (201,219), differed from our study in that costs were calculated per cycle and both groups were stimulated with the standard long protocol. Other cost studies comparing single and dual embryo transfer were not randomised controlled trials, but were based on theoretical extrapolations or decision-analytic calculations and were mainly based on one IVF cycle (35,54,55). These studies suggested lower costs for SET. The aim of this paper is to provide detailed information concerning the economic consequences of two different treatment strategies including ovarian stimulation protocols and embryo transfer policies during consecutive treatment cycles.

7.2 Methods

7.2.1 Study design

The study protocol was approved by the ethics review board of both participating University Medical Centers (Utrecht and Rotterdam). Patients with an indication for IVF or Intracytoplasmic Sperm Injection (ICSI) treatment in two academic medical centres were recruited in the period February 2002 through March 2004 (205). Patients with a regular indication for IVF or IVF/ICSI (tubal, male, unexplained), female age < 38 years, normal menstrual cycle (cycle length between period 25-35 days) and without severe obesity or underweight (body mass index 18-28 kg/m^2) were eligible for the study. Patients were randomly assigned to undergo either mild stimulation with GnRH antagonist co-treatment combined with single embryo transfer (mild strategy) or a 'standard' ovarian stimulation protocol where pituitary down regulation was established using a GnRH agonist long-protocol combined with dual embryo transfer (standard strategy). In order to compensate for a possible reduction in pregnancy rate per cycle, patients in the mild treatment group were offered an extra reimbursed treatment cycle on top of the three cycles reimbursed at that time in the Netherlands. It was considered that 12 months after commencing treatment, 3 cycles of standard IVF would be feasible for most couples, while 4 mild strategy cycles would be possible in the same period of time, due to the shorter duration and lower psychological burden. The study design has been described in great detail previously (205).

The primary endpoint for this study was defined as total costs of IVF treatment per couple within 12 months after randomisation, including costs of resulting pregnancy and postnatal costs of the mother and the infant(s) up to six weeks after the expected day of delivery. Since cumulative ongoing pregnancy rates within one year resulting in term live births were almost similar for both treatment groups (44.7% in the standard treatment group

versus 43.4% in the mild treatment group) (220), the economic evaluation in the current analysis is primarily designed as a cost-minimization analysis (CMA).

7.2.3 Cost calculations

The costs of the two IVF strategies were assessed in two stages. Firstly, the cost of IVF treatment itself, starting with the first IVF cycle and ending with the outcome of the last IVF-cycle within one year (pregnant, no pregnancy or drop out). Secondly, the cost of antenatal, peripartum and post partum care were analysed in women who became pregnant after IVF treatment.

Medical costs were calculated by multiplying the volumes of health care use with the corresponding unit prices. The costs of IVF treatment were distinguished into medical costs in the hospital (intramural), extramural medical costs, and non-medical costs. Medical costs in the hospital consist of scheduled and unscheduled outpatient visits, number of IVF cycles, personnel time per cycle, use of GnRH analogues and recombinant FSH, costs of ultrasound and hormonal monitoring, the embryo transfer procedure and costs associated with complications. Extramural medical costs consist of general practitioner (GP) consultations, and social worker. Non-medical costs are associated with travel and absence from work/sick leave due to the treatment or associated complications. Cost volumes in the treatment stage were recorded with case record forms (CRFs), hospital-based management and budgetary information systems, patient questionnaires and literature (Figure 1).

The costs of pregnancy and obstetric care were distinguished into medical costs in the hospital (secondary obstetric care) and medical costs outside the hospital (e.g. primary obstetric care, GP care, etc.). Pregnant patients received several questionnaires regarding health care use each covering three month periods of their pregnancy. The final questionnaire covered the period around the calculated term date, until 6 weeks thereafter. This means that the neonatal costs are covered for a 6-week period post-term. For pre-term births, the postnatal period is therefore longer and costs higher than for term births (189). In order to receive medical information regarding birth, questionnaires were sent to the responsible obstetrician.

For the most important cost items, unit prices were determined by following the micro-costing method (221), which is based on a detailed inventory and measurement of all resources used. During the determination of unit prices 2 embryos were transferred in the majority of cycles. Therefore all unit prices are determined for the transfer of 2 embryos. The calculation of the unit price of the IVF treatment consisted of detailed measurement of

investments in manpower, equipment, materials, housing and overhead. The salary schemes of hospitals and other health care suppliers were used to estimate costs per hour for each caregiver. Taxes, social securities and vacations were included, as well as the costs of the time that could not be assigned to other patients. The costs of equipment included those of depreciation, interest and maintenance. Costs for inpatient days in hospital were calculated from real, basic costs per day using detailed information from the financial department of the hospital. For the unit price per inpatient day in hospital, a distinction was made between general and university hospitals. These estimates included overhead and indirect costs. Other charges assaulted with inpatient and outpatient care were derived from previous publications[188], in order to make our results more comparable with other research and to make these unit costs independent from the specific hospital prices. For these items we used charges as a proxy of real costs. In the Netherlands a 'fee for service' system is used for the remuneration of medical interventions and diagnostic procedures. In order to calculate the costs for medication, we used pharmacotherapeutic charges. Costs caused by loss of economic productivity due to absence from work were also taken into account, using charges (188). Appendix A gives an overview of the cost categories and data used in the cost calculations.

7.2.4 Statistical analysis

Analysis was carried out according to the intention-to-treat principle. For an effectiveness trial, the focus should not be the cost per cycle but rather the overall cost that a patient may expect over a given treatment period (including cryo cycles) (105). Therefore we elected to base the analysis on a one year treatment period, which would allow the treatment strategy that is best tolerated by the patients and requires the least amount of time per cycle, to realize more chance of success than the other strategy. We used the Kaplan-Meier method, in which it is assumed that dropouts who do not wish to receive any more treatment have a zero chance of the outcome, i.e. a realistic assumption (no censoring) (107). The time period of analysis started from the moment of randomisation, to avoid post randomisation selective dropout.

Missing cost items arising due to non-response to the questionnaires were imputed, and stratified by randomisation arms to avoid the loss of data. For this purpose, the AregImpute method in S-plus (MathSoft. Inc., Seattle, WA, version 2000 was used). A comparison of the costs between both treatment strategies was performed with the independent groups t-test.

7.3 Results

7.3.1 Patient characteristics and clinical outcomes

404 patients were included in the study (Table 1). The mean number of started cycles within 1 year was 2.3 in the mild and 1.7 in the standard treatment group ($p < 0.001$, t-test). The 1-year cumulative pregnancy rate leading to term live birth rate was 43.4% in the mild group versus 44.7% in the standard group. The percentage of multiple pregnancies per ongoing pregnancy in 1 year of IVF treatment was 1.1% in the mild strategy and 29% in the standard strategy ($p < 0.001$, Chi-square test). The incidence of ovarian hyperstimulation syndrome requiring outpatient visits or hospital admission was 1.3% in the mild treatment group and 3.6% in the standard treatment group ($p = 0.04$, Chi-square test). For an extensive description of the characteristics and clinical outcomes see our earlier publication (220).

7.3.2 Costs per cycle

The response rate of the economic evaluation questionnaires during treatment was 81% for all IVF cycles and did not differ significantly between the 2 treatment strategies. Almost 75% of the pregnant women responded to at least two of the three economic evaluation questionnaires during pregnancy and the neonatal period. The mean direct medical costs per IVF cycle were lower for the mild strategy (€ 1,569 versus € 1,987; p=0.001), mainly due to lower costs for medication and technical procedures (Table 2). Per cycle, women in the mild treatment strategy had on average fewer days of sick leave during pregnancy as compared with the standard treatment strategy (23 versus 30; p=0.029).

For the mild strategy, the duration between cycles was shorter (88 ± 49 days versus 109 ± 38 days; $p < 0.001$). The cumulative treatment costs of the standard treatment strategy were higher in the first four months. However, over the complete 12 month period, treatment costs of the mild treatment strategy were comparable with those of the standard strategy (Figure 2).

IVF treatment, pregnancy, and the neonatal period revealed lower total costs for the mild strategy (€ 8,333 versus € 10,745; p=0.006), represented in Table 3. The costs of intramural care during IVF treatment was significantly higher for the mild strategy (€750 versus €576; p=0.006), which is due to the higher mean total number of cycles within one year. The medical costs during pregnancy for the mild strategy were half the costs of the standard strategy (€530 versus €1,061; p=0.03), due to the requirement for more medical care (outpatient visits, hospital admissions). Furthermore, the costs of the obstetric and postnatal period per ongoing pregnancy were significantly higher for the standard strategy, due to more

hospital admissions and more prolonged duration in hospital for mother and child. The cost per ongoing pregnancy leading to term live birth was €19,156 in the mild strategy and €24,038 in the standard strategy.

Figure 3 illustrates the extent to which the higher costs for the standard strategy can be attributed to multiple pregnancies. Within 12 months after randomisation there were 16 pregnancies leading to preterm live birth (< 37 weeks) in the standard treatment group, versus 6 in the mild treatment group (p=0.02) as illustrated by Figure 3. Early pre-term life birth (< 32 weeks gestation) resulted in relatively low costs, primarily due to a relatively low neonatal survival rate. Late pre-term life birth (32-37 weeks gestation) did result in relatively high total IVF treatment costs.

7.4 Discussion

We have previously published the clinical data of this study, which showed that in women younger than 38 years, a mild strategy in IVF may result in similar ongoing pregnancy rates leading to cumulative term live births within 1 year compared with a standard strategy, while greatly reducing multiple pregnancy rates (220). In the current study we measured the consequences of both IVF treatment strategies in terms of costs in order to give an integrated evaluation of the health economics of the two treatment strategies. The overall costs during 12 months of treatment were lower for the mild strategy compared with the standard strategy, despite a higher average number of IVF cycles for the mild strategy. This is mainly due to the benefit of the reduction of multiple pregnancies and thereby reduction of pre-term life birth in the mild strategy.

The real advantage of the mild strategy is the avoidance of the very high long-term costs resulting from the increased morbidity of twins after birth (35,222,223). In the current study, the neonatal costs were covered until 6 weeks after expected date of delivery. The long-term medical prognosis for the children born in this study period cannot be predicted but the future costs for these children (in some cases severely ill) are likely to be very large (211). The incidence of disabilities is markedly increased in multiple pregnancies, and the associated long-term costs would certainly have impact on cost analysis because indirect long term costs will out way perinatal costs (222,211). This strengthen our conclusion that the mild treatment strategy with SET is much more cost-effective. Standard used effectiveness outcomes in economic evaluation studies, such as quality adjusted life-years were not employed, because their use in certain pregnancy situations can be difficult to interpret and sometimes misleading (224).

The findings of an earlier randomised controlled trial were consistent with the results of the present study, showing lower total costs with the SET strategy as compared with the dual embryo transfer (201,219). Moreover, the SET strategy also resulted in a marked reduction in the costs of paediatric health care, due to a considerable reduction of multiple pregnancies[(201)]. Another randomised trial concluded that one cycle SET was less expensive, but also less effective compared to one cycle dual embryo transfer. It depends on the society's willingness to pay for one extra IVF cycle, whether a single cycle dual embryo transfer is preferred from a cost-effectiveness point of view (219). Other studies comparing costs of SET and dual embryo transfer were not randomised controlled trials, but all used theoretical extrapolations or decision-analytic calculations (35,54,55). De Sutter and colleagues suggested that the cost per child born was the same for single as for dual embryo transfer (35). This was explained by the fact that higher pre- and neonatal cost due to multiple pregnancies arising after dual embryo transfer balanced by higher cost for more SET cycles needed to obtain the same number of children (56). However, when costs are calculated per term live birth instead of child born (and a twin was calculated as one instead of two) costs for dual embryo transfer would be more expensive than for SET, which can be explained by the four fold higher cost of pregnancy of a twin instead of a singleton that they used in their calculations. When calculating the chance of term live birth per 12 months per couple, we counted twin live births as being equivalent to 1 live birth. It may be argued that a term-born twin should count as 2 live births. A term born twin may be perceived as a positive outcome, reducing the need for subsequent IVF treatments. However, in addition to the increased perinatal morbidity, mortality and long term health consequences associated with twin pregnancies, parents of multiple pregnancies have shown to be at greater risk of depression and anxiety (207,208). Furthermore, when weighing the benefits of the transfer of 1 or 2 embryos, account should also be taken of the live births which may occur following the subsequent transfer of surplus embryos (209), of which more will remain when just one fresh embryo is transferred

In general, performing more mild IVF treatment strategies will increase the number of cycles needed to obtain the same number of live births when compared with the standard treatment strategy. Despite this higher average number of cycles for the mild strategy, and thereby high treatment costs, we found in our study that overall costs per term live birth were cheaper compared to the standard treatment strategy, mainly due to the health economic benefits of the reduction of multiple pregnancies in the mild stimulation approach. The impact of multiple gestations and their associated complications on costs is dramatic.

The debate is ongoing whether twins should be regarded as a success (6). From a clinical perspective, a term twin birth without complications may be reported as success. However, the increased rate of complicated deliveries, pre-term births, and low birth weight (all giving rise to increased chances for perinatal morbidity or mortality and long term health consequences) and negative psychosocial implications for parents or children (18) associated with twin pregnancies, have led to the opinion that medical intervention in infertility should preferably aim at establishing a singleton pregnancy (163,84). This study might contribute to the introduction of single embryo transfer on a large scale. The clinician and health care providers should be aware that an extra treatment cycle may be considered a low medical price for the prevention of the lifelong compromised quality of life. The couple should be made aware of the balance between their short-term desire for offspring and the long-term appreciation of healthy children. If structured, written information about risks and complications of multiple pregnancies and the consequences of the transfer of fewer embryos is provided, patients may become more inclined to the transfer of 1 embryo rather than 2 (57,116). An adequate reimbursement system is an important point to make single embryo transfer work (48). Society will carry a large part of the costs for the complications associated with multiple pregnancy and birth. Governments therefore might have regulatory interest in how IVF is performed. By funding IVF, they will accrue costs in the short term, but might also be able to establish guidelines for the number of embryos transferred. The possible need for higher number of treatment cycles, to achieve pregnancy after one-embryo transfer, might increase treatment costs. However, in the long run, governments will profit by saving the costs of complications associated with multiple pregnancies.

Table 1. Characteristics of 404 patients randomised to the mild strategy or the standard strategy of IVF

	Mild	**Standard**	**P**
Randomised (n)	205	199	
Mean number of cycles within 1 year (n)	2.3	1.7	P < 0.001
Pregnancy within 1 year leading to term live birth (n)	86	86	NS
Cumulative term live birth rate within 1 year (%)	43.4	44.7	NS
Multiple pregnancies per randomised couple (%)	0.5	13.1	P < 0.001

Source: Heijnen, 2006 (Heijnen et al., 2006)

Table 2. Intramural medical costs (€) per cycle for the standard and mild IVF treatment

	Mild (Mean ± SD)	Standard (Mean ± SD)	Significance[1] P
Medication			
GnRH analogue[2]	155 ± 71	235 ± 70	< 0,001
FSH	585 ± 236	816 ± 337	< 0,001
Technical procedures			
Oocyte retieval and laboratory	323 ± 210	352 ± 184	0,038
Embryo transfer	151 ± 112	222 ± 110	< 0,001
Embryo cryo transfer	17 ± 68	14 ± 60	NS
Intramural care			
Ultrasound	151 ± 69	157 ± 94	NS
Hospital admission	26 ± 167	72 ± 471	0,059
Control visits	42 ± 51	43 ± 59	NS
laboratory	108 ± 123	65 ± 82	< 0,001
Total costs per cyclus	1,559 ± 608	1,977 ± 803	0,001

[1]independent groups t-test

[2]GnRH antagonist for mild treatment and GnRH agonist for standard treatment

Table 3. Total costs (€) of IVF treatment in 404 patients within 12 months including costs of resulting pregnancy up to 6 weeks after delivery (per couple)

	Mild (Mean ± SD)	**Standard** (Mean ± SD)	**Significance[1]** P
IVF treatment			
Technical procedures	1,083 ± 734	991 ± 584	NS
Intramural care	750 ± 561	576 ± 693	0,006
Medication	1,626 ± 1,088	1,737 ± 1,069	NS
Indirect costs[2]	1,948 ± 2,280	1,740 ± 1,845	NS
Pregnancy and delivery			
Medical costs during pregnancy	530 ± 984	1,061 ± 2,076	0.03
Delivery	449 ± 931	504 ± 854	NS
Neonatal period			
Hospital admission mother	542 ± 375	1,088 ± 1,164	<0.001
Hospital admission child	342 ± 374	1,653 ± 1,337	<0.001
Maternity care	684 ± 498	593 ± 348	NS.
Indirect costs[2] (pregnancy+neonatal)	379 ± 1,177	802 ± 2,270	0,03
Total costs	**8,333 ± 5,418**	**10,745 ± 11,225**	**0,006**

[1] independent groups t-test

[2] indirect costs involve transportation costs and absence from work/sick leave

Figure1. Flow chart of the economical evaluation measure points

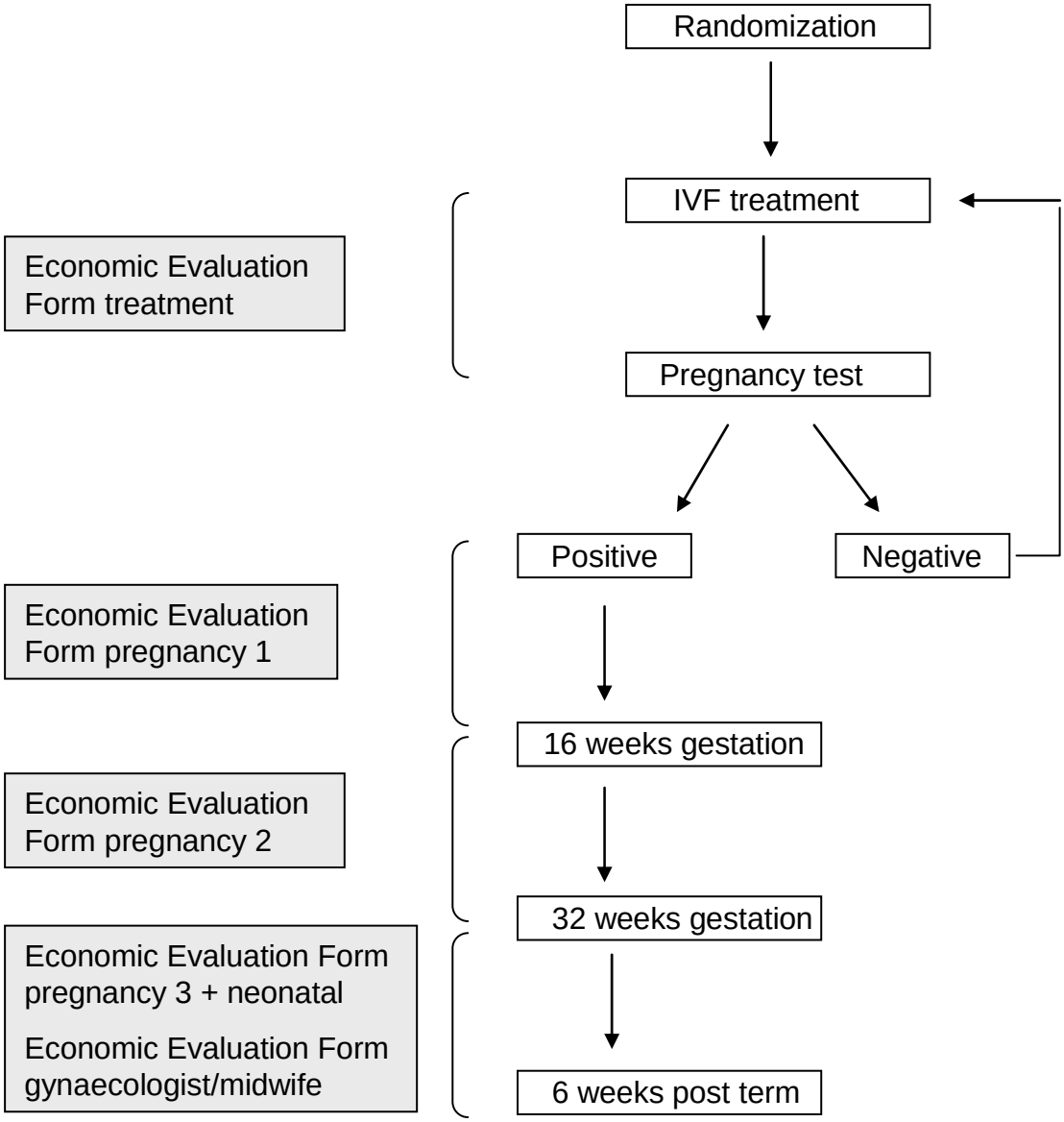
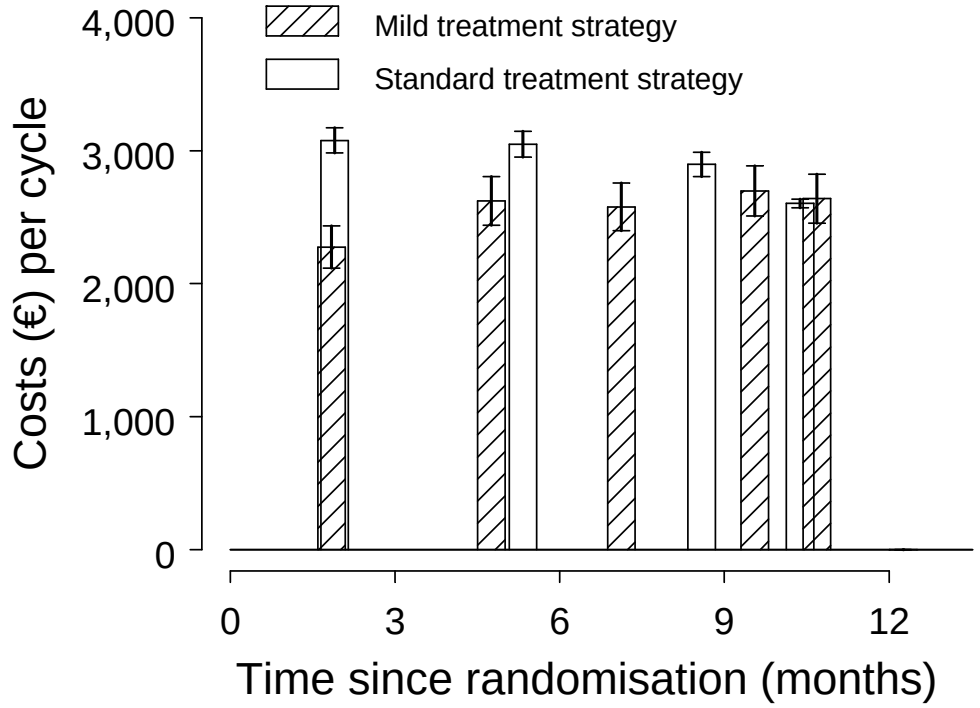


Figure 2. *Mean treatment costs per cycle (bars) and cumulative treatment costs (lines) within 12 months after starting IVF in 404 couples, comparing the mild approach (hatched) with the standard approach (white). The median time since randomisation of each cycle is indicated by the placing of the bars.*



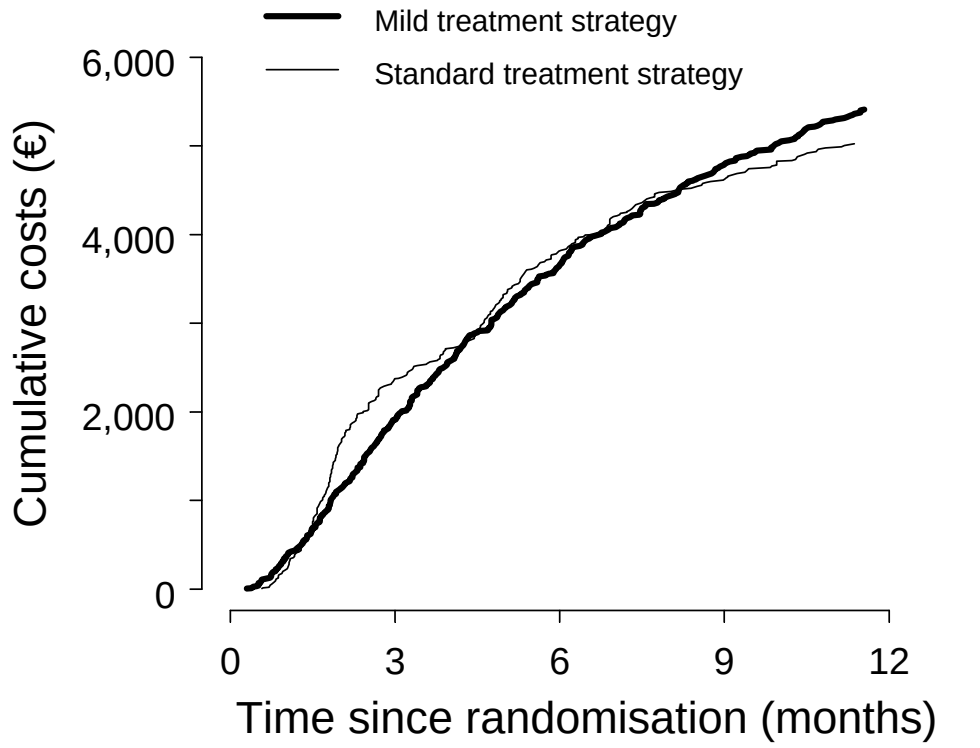


Figure 3. *Total costs (€) of IVF treatment up to 6 weeks after calculated term, comparing singleton (open bullet) with multiple (black bullet) pregnancies by gestation duration.*

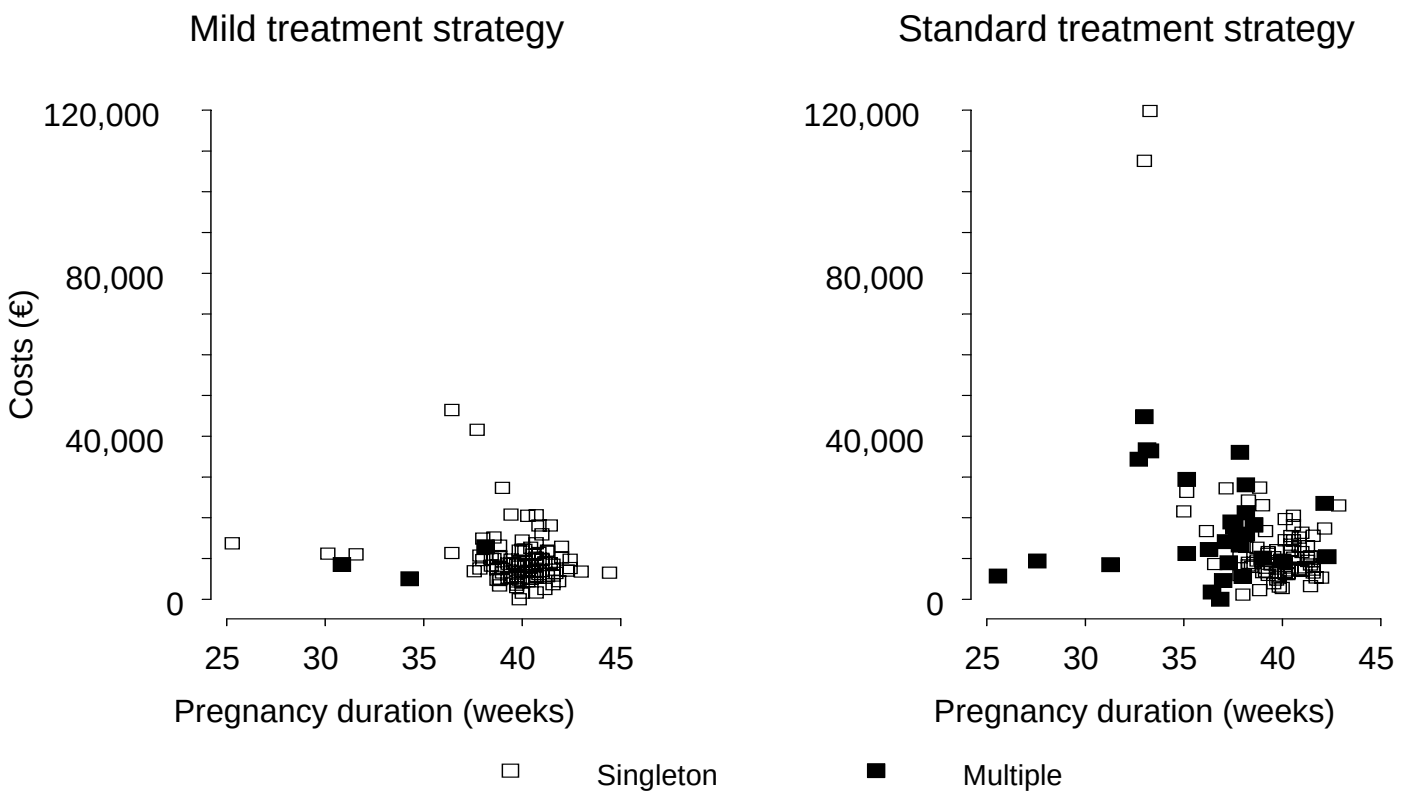


Table Appendix. Cost categories and data used in cost calculations

Cost category	**Parameter**	**Data collection volume of care**			**Cost estimate**
		CRF (physician)	***Questionnaires patient***	***Questionnaire obst/gyn***	***(unit price)***
Technical procedures					
Punction	Strategy	*			Real costs
Laboratory	Strategy	*			Real costs
Embryo transfer	Strategy	*			Real cost
Intramural care					
Hospital (academic)	Days	*	*	*	Real costs
Hospital (general)	Days	*	*	*	Real costs
NICU/MCU	Days	*	*	*	Real costs
Physician (academic)	Visits	*	*	*	Charges
Physician (general)	Visits	*	*	*	Charges
Echoscopy	Number	*		*	Charges
Prenetal research				*	Charges
Other therapy	Number			*	Charges
Delivery	Category		*	*	Literature
Medication					
GnRH	Strategy	*			Cost price
FSH	Days	*			Cost price
HCG/Progesteron	Days	*			Cost price
Extramural care					
Obstetrician	Visits		*	*	Charges

General practitioner (inpatient)	Number	*	Fees
General practitioner (home visit)	Number	*	Fees
Social worker	Number	*	Charges
Maternity nurse	Days	*	Charges
Non-medical costs			
Travel costs	Distance	*	Guidline
Absence from work	Days	*	Guideline

8. General Discussion

The aim of this thesis is to discuss the optimal way to define success in IVF and to show how the implementation of new outcome parameters can contribute to the development of alternative approaches of success in IVF in different patient groups. Firstly a meta-analysis was conducted to compare outcomes of IVF in women presenting with polycystic ovary syndrome (PCOS), characterized by 2 out of 3 of the following criteria: Oligo and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and poycystic ovaries. Furthermore two randomised controlled trials were performed. One feasibility trial comparing a dual embryo transfer policy and a triple embryo transfer policy in women of 38 years and older. Secondly, a randomised effectiveness trial was performed evaluating the cumulative term live birth rate of two different treatment strategies; the mild stimulation/ gonadotropin releasing hormone (GnRH) antagonist co-treatment protocol combined with single embryo transfer or a standard stimulation/GnRH agonist long-protocol in combination with the transfer of two embryos. This trial only involves 2 arms instead of the four possible combinations for conceptual and practical reasons. Conceptual, because the mild stimulation, (due to shorter duration and better patient tolerance), was expected to enable subjects to have more cycles in the same time period. More cycles means additional pregnancy chances, which can compensate for a possibly reduction in live birth per cycle due to the use of GnRH antagonist co-treatment along with the transfer of a single embryo transfer. In addition it makes sense to combine a mild approach, generating a reduced number of multiple follicles with the transfer of a reduced number of embryos. Practical because, given the number of participants that could feasibly be recruited over a given period of time, the statistical power of a four arm trial would significantly reduced.

The proposed optimal outcome parameter in this thesis is the cumulative term live birth rate per time period or per treatment period. This should be weighed against the associated discomfort, complications and costs. The first randomised trial presented in this thesis showed that in women of 38 years and older the transfer of 2 embryos after IVF may result in similar cumulative term live birth rates compared with the transfer of three embryos provided that a higher number of treatment cycles is accepted. The principle finding presented in this thesis is that the application of a mild strategy in women under 38 does not reduce the chance of achieving the goal of a term live birth within 1 year. Recent studies have shown that even in patients younger than 38 years where at least 3 good quality embryos are available, single embryo transfer yields reduced ongoing pregnancy rates compared to the transfer of two embryos (43). However, these studies provide no insight into outcome over a series of

cycles. Our findings also imply that the mild strategy will reduce the per cycle chance of pregnancy. However, cumulative term live birth rates of approximately 45% are still possible if the necessity of an additional treatment cycle is accepted. This is shown by the absence of a significant difference between the cumulative term live birth within 1 year comparing both strategies. As such, the couple will face no reduction in the overall potential to have a child, provided they undergo more 'mild' cycles in the same period of time. This will probably also count for PCOS women because the meta-analysis presented in this thesis has shown that IVF outcome is comparable between PCOS and non-PCOS women. However more research is necessary to develop patient friendly mild stimulation protocols for PCOS women. In general, PCOS women are excluded in studies investigating new milder stimulation protocols.

If the mild approach is to be adapted into daily practice, it is important that, instead of considering success from IVF treatment in terms of ongoing pregnancy rate per cycle both physicians and patients regard success in terms of a treatment period while also taking the risks, complications and patient discomfort into account (163). The debate as to whether twins should be regarded as a successful outcome continues (6). From a clinical perspective, a term twin birth without complications may be considered as a success. However, the increased rate of complicated deliveries, preterm births, and low birth weight (99,225) (which gives rise to increased perinatal and longterm morbidity) have led to the opinion that medical intervention in infertility should aim primarily at establishing a singleton pregnancy (6). The perinatal morbidity and mortality directly related to multiple births overwhelm any argument in favour of more rapid family building by means of multiple births. In addition, the incidence of stress fatigue and depression is increased in patients from twins (21). Yet, both patients (21,86,226) and infertility doctors (90) remain insufficiently aware of medical complications and parent stress associated with multiple births.

As mentioned before similar cumulative 1-year pregnancy rates leading to term live birth were shown to occur in both groups. In this study the Kaplan Meier method was applied in a different way than usually applied in calculating cumulative success rates in infertility (107). Generally it is assumed that drop outs have a similar chance for pregnancy as patients continuing treatment (censoring). Because all information concerning pregnancies occurring in 1 year was available, an intention to treat analysis including all pregnancies could be performed to calculate the real life cumulative term live birth rate without making assumptions with regard to the chance of pregnancies of the drop outs (no censoring). Therefore, this cumulative term live birth rate is lower than usually found in the literature. Censoring does not take into account the effects of high drop out rates during treatment (for

example due to patient discomfort) and is therefore not appropriate when outcome parameters are employed which take patient discomfort into account.

Term live birth rates should not be the only outcome used when comparing both IVF treatment options. The costs and psychological burden associated with the treatments should also be part of the equation. In section 7 of this thesis we measured the economic consequences of both IVF treatment strategies in order to provide an integrated evaluation of the effects and costs. In this study, the total costs were related to the success rate in a cost-effectiveness analysis. We concluded that the costs per ongoing pregnancy within 1 year resulting in term live birth are less for the mild strategy compared to the standard strategy, indicating that mild ovarian stimulation with single embryo transfer represents a reasonably approach not only medically and ethically, but also from an economical point of view.

In the study presented in section 7 of this thesis, we clearly demonstrate that costs of pregnancy, delivery and neonatal care differ between both strategies and that the overall costs are influenced heavily by the higher costs due to multiple pregnancies. Despite the slightly higher average number of cycles for the mild strategy, and thereby high treatment costs, we found in our study that overall costs per ongoing pregnancy were cheaper compared to the standard treatment strategy, mainly due to the health economic benefits of the reduction of multiple pregnancies in the mild stimulation approach.

Patient discomfort should also be considered when comparing IVF treatment strategies. By developing treatment strategies with less psychological complaints the drop out rate during treatment may decrease and as a consequence the term live birth rate per treatment (period) may increase. Pituitary down-regulation with GnRH agonist is associated with elevated levels of physical discomfort (29). In the week before the start of ovarian stimulation, women who were undergoing pituitary down-regulation reported more often symptoms like headache, abdominal pain and sore muscles than the control group (210). During subsequent treatment stages, however, no differences were found in physical discomfort between the two study groups. This suggests that “milder” ovarian stimulation might not result in reduced patient discomfort. However, since average treatment duration is shorter when using mild stimulation, patients suffer from physical complaints for a shorter period of time. In additional, overall discomfort within a year is comparable in both groups despite the fact that the average number of IVF cycles is increased in the mild strategy group.

The way to define success in IVF proposed in this thesis and the described study can contribute to the introduction of single embryo transfer on a large scale. Evidence is also

provided that triple embryo transfer in women of 38 years and older will not increase success rates per treatment and as such opens the possibility of restricting the number of replaced embryos to 2 even in this supposed low prognosis group as the individual potential for pregnancy will not become forfaited. Introducing single embryo transfer in women under 38 years may require big efforts from both the clinician and the couple. The couple and the clinician have to be aware that (less than) an extra treatment cycle within 1 year seems a reasonable price for the prevention of chances for the lifelong consequences of (severely) damaged children related to multiple birth (173). The couple should be made aware of the balance between their short-term desire for offspring and their long term appreciation of raising healthy children. In addition, the interest of the child itself and his/her quality of life and financial burden for society related to live long handicaps should be taken into consideration. If structured, written and oral information about risks and complications of multiple pregnancies is provided reassuring overall similar chances for offspring per started treatment, patients will probably become more inclined to the transfer of 1 embryo rather than 2. The development of patient friendly stimulation protocols can contribute to the introduction of single embryo transfer at large. Introducing single embryo transfer as a standard policy, from which deviation is not allowed as a principle, patients may not easily put pressure on the physician to obtain consent for a 2 embryos transfer. In Sweden and Belgium the law obliges single embryo transfer in women younger than 36 years (173,48). This has resulted in the transfer of 1 embryo in the majority of patients and in a decrease in multiple pregnancies. However, if patients have to pay for IVF themselves, choosing for single embryo transfer after being informed about the associated lower pregnancy rate may be difficult. If a country has an adequate reimbursement system there is an important task for the politicians and health insurance providers to modify the legislation in such a manner that single embryo transfer in women of 37 years and younger is stimulated (48). Part of this requires that the reimbursement system *per cycle* has to be replaced for a system of payment per overall treatment or per healthy child born.

Society will carry a large part of the costs for the complications associated with multiple pregnancy and birth. Governments therefore might have regulatory interest in how IVF is performed. By covering IVF by health insurance, they will accrue costs in the short term, but might also be able to establish guidelines for the number of embryos transferred. The possible need for a higher number of treatment cycles to achieve pregnancy after single embryo transfer will increase treatment costs. However, in the long run, governments may profit from reimbursing IVF treatments, which are restricted to one-embryo transfer, by

saving the costs of complications associated with multiple pregnancies. In addition, much more attention should be focussed towards additional pregnancies from cryopreserved surplus embryos (since the transfer of a single embryo will result in more embryos for cryostorage) and patient selection for single ET based on multi-variate models rather than chronological age per se.

Summary

Chapter 1:

Over the past 20 years, attention has been mainly focussed on how to improve pregnancy rates in IVF while the appropriate balance between success, risks and costs has been inadequately addressed. The most important complication of IVF is multiple pregnancy. Preterm delivery and low birth weight is the major cause of mortality and morbidity in multiple pregnancy. Another serious complication in IVF is the ovarian hyperstimulation syndrome. The incidence of multiple pregnancies can be decreased by the transfer of one embryo in women younger than 38 and two embryos in women of 38 years and older and by identifying those treatment cycles at particular risk of leading to multiple pregnancy. The ovarian hyperstimulation syndrome and other complications of IVF can be prevented by applying milder stimulation protocols. To compare different treatment strategies (stimulation protocol and embryo transfer policy) it is important to use a simple and clear consistent definition of success in IVF.

Chapter 2:

Changing the way in which successful in vitro fertilisation (IVF) treatment is defined offers a tool to improve efficacy while reducing costs and complications of treatment. Crucial to this paradigm shift is the move away from considering outcomes in terms of the single IVF cycle, and towards the started IVF treatment as a whole. We propose the most informative endpoint of success in IVF to be the term singleton birth rate per started IVF treatment (or per given time period) in the overall context of patient discomfort, complications and costs. These endpoints are not only important for patients but also for clinicians, health economists and policy makers. Such an approach would encourage the development of patient friendly and cheaper stimulation protocols with less stress, discomfort and side effects. The combination of mild ovarian stimulation with single embryo transfer may provide the same overall pregnancy rate per total IVF treatment, achieved in the same amount of time for similar direct costs, but with reduced patient stress and discomfort, and the near complete elimination of multiple pregnancies. This would offer major health and indirect cost benefits. If IVF success rates were to be expressed in terms of delivery of a term single baby per IVF treatment (or in a given treatment period), the introduction of single embryo transfer on a large scale would be facilitated.

Chapter 3:

The meta-analysis described in this section was conducted to compare outcomes of standard in vitro fertilization (IVF) in women presenting with polycystic ovary syndrome (PCOS) and non-PCOS patients. Studies in which PCOS patients undergoing IVF were compared with a matched –no male factor- control group were considered for this review. A definition consistent with the Rotterdam consensus criteria of PCOS was required and all patients within a given study had to be treated with the same ovarian stimulation protocol. Information regarding patient characteristics and pregnancy outcome was also required. Nine out of a total of 290 identified studies reporting data on 458 PCOS patients (793 cycles) and 694 matched controls (1116 cycles) fulfilled these inclusion criteria. PCOS patients demonstrated a significantly reduced chance of oocyte retrieval per started cycle, (odds ratio (OR) 0.5 (95% CI 0.2;1.0)). However, no difference was observed in chance of embryo transfer per oocyte retrieval between the groups (OR 0.7 (95% CI 0.4;1.3)). Significantly more oocytes per retrieval were obtained in PCOS patients compared with controls (random effects estimate 3.4 (95% CI 1.7;5.1). The number of oocytes fertilized did not differ significantly between PCOS patients and controls, weighted mean difference (WMD) 0.1 oocytes (95% CI -1.4;1.6). No significant difference was observed in the clinical pregnancy rates per started cycle, OR 1.0 (95% CI 0.8;1.3). The incidence of ovarian hyperstimulation syndrome (OHSS) after oocyte retrieval was rarely reported. This meta-analysis demonstrates an increased cancellation rate, but more oocytes retrieved per retrieval and a lower fertilization rate in PCOS undergoing IVF. Overall PCOS and control patients achieved similar pregnancy and live birth rates per cycle.

Chapter 4:

The aim of this chapter is to answer the question whether dual instead of triple embryo transfer in subsequent cycles in patients over 38 years will substantially reduce the number of multiple pregnancies while the chance of a term live birth remains at an acceptable level. A randomised controlled two-centre trial was performed. 45 patients, 38 years or older were randomised. Dual embryo transfer over a maximum of 4 cycles (DET-group) or triple embryo transfer over a maximum of 3 cycles (TET-group) was performed. The cumulative term live birth rate was 47.3% after 4 cycles in the DET-group and 40.5% after 3 cycles in the TET-group. The difference between the DET and the TET-group is 6.8% in favour of the DET-group (95% CI -25;38) (p=0.7). The multiple pregnancy rates in the DET and TET-group were 0% (95% CI 0;24) and 30% (95% CI 7;65), respectively (p=0.05). In the DET patients the mean number of treatment cycles was 2.9 compared to 2.1 in the TET-group (p=0.01). In

women of 38 years and older a dual embryo transfer strategy after IVF may result in similar cumulative term live birth rates compared with a triple embryo transfer strategy provided that a higher number of treatment cycles is accepted.

Chapter 5:

This chapter discusses the design of a clinical study to evaluate the effectiveness, health economics and patient discomfort of two treatment algorithms in in-vitro fertilisation (IVF), involving differences in both ovarian hyperstimulation and embryo transfer policies. A randomised controlled clinical trial was performed in two large centres. The tested treatment strategies are: A) mild ovarian hyperstimulation (including GnRH antagonist co-treatment) together with the transfer of a single embryo, versus a standard hyperstimulation regimen (with GnRH agonist long protocol co-treatment), and the transfer of two embryos. The primary study endpoints were; (1) pregnancy within one year after randomisation leading to term live birth; (2) total costs per couple and child up to 6 weeks after expected delivery, and (3) overall patient discomfort within one year of randomisation. Power considerations for this study were an overall cumulative pregnancy rate of 45% with the standard treatment strategy and non-inferiority of the new treatment strategy was defined as a no more than 12.5% lower live birth rate compared to the standard treatment strategy. For a power of 80% and alpha = 0.05, a total number of 400 subjects was required. Analysis will be performed according to the intention-to-treat principle. The trial is an ongoing two-centre trial in The Netherlands. As anticipated, from February 2002 until March 2004, 410 patients have been enrolled in the study. Further follow-up (12 months for treatment, and 9 months for pregnancy) is required for live birth as endpoint. Inclusion of study participants has been very good and is completed. Final data analysis can be performed at the end of 2005.

Chapter 6:

The aim of this chapter was to establish whether a mild in-vitro fertilization treatment strategy can achieve the same term live birth rate within 1 year compared to standard treatment, while reducing patient discomfort, multiple pregnancies and cost. A randomised controlled two-arm, two-centre effectiveness trial was performed. Four hundred and four patients were assigned to undergo either a mild stimulation/gonadotropin releasing hormone (GnRH) antagonist co-treatment protocol combined with single embryo transfer or a standard stimulation/GnRH agonist long-protocol in combination with the transfer of two embryos. The primary study endpoints were; (1) pregnancy within one year after randomisation leading to term live birth;

(2) total costs per couple and child up to 6 weeks after expected delivery, and (3) overall patient discomfort within one year of randomisation. The cumulative pregnancy rate resulting in term live birth after 1 year was 43.4% in the mild treatment group and 44.7% in the standard treatment group. The respective multiple pregnancy rate per couple was 0.5% versus 13.1% (P<0.001) and total costs were € 8,333 versus € 10,745 (P=0.006). The areas under the cumulative score curves for anxiety, depression, physical discomfort and sleep quality within one year were equal between the two treatment groups. Mild ovarian stimulation together with single embryo transfer in IVF can result in similar cumulative term live birth rates and patient discomfort over 1 year of treatment compared to standard stimulation with two embryo transfer, while significantly reducing multiple pregnancy rates, and overall costs.

Chapter 7:

This chapter compared the economic costs of a mild treatment strategy and single embryo transfer to the standard treatment strategy with dual embryo transfer. 404 patients were randomly assigned to; (I) mild ovarian stimulation/gonadotropin-releasing hormone (GnRH) antagonist co-treatment and single embryo transfer, or (II) standard ovarian stimulation/GnRH agonist co-treatment and dual embryo transfer. The primary outcome parameter was total costs of IVF treatment within 12 months after randomisation including costs of resulting pregnancy and postnatal costs of the mother and the infant(s) up to six weeks after term. The mild strategy was associated with lower hospital costs per IVF cycle (€1,569 versus €1,987; p=0.001) and, despite a significantly increased number of IVF cycles (1.7 versus 2.3; p<0.001), in lower average total costs during the first year (€8,333 versus €10,745; p=0.006). This was mainly due to higher costs of the obstetric and postnatal period for the standard strategy. The higher delivery costs and longer hospital admission of mother and child were mainly caused by multiple pregnancies. The cost per ongoing pregnancy leading to term live birth was €19,156 in the mild strategy and €24,038 in the standard strategy. Despite an increased mean number of IVF cycles within one year, from an economical perspective, the mild treatment strategy is more advantageous, assuming equal effectiveness. This advantage will further increase in the long-term, due to health economic benefits arising from physical and mental handicaps later in life.

Chapter 8:

This chapter discusses the conclusions which could be drawn from the work presented in the current thesis.

Samenvatting

Hoofdstuk 1:

Gedurende de laatste 20 jaar is binnen de IVF de aandacht voornamelijk uitgegaan naar de verbetering van zwangerschapsresultaten. Hierdoor is er te weinig aandacht besteed aan de juiste balans tussen succes, risico's en kosten. De belangrijkste complicatie van een IVF behandeling is een meerlingzwangerschap. Een partus premature en een laag geboortegewicht zijn de belangrijkste oorzaken van mortaliteit en morbiditeit in meerlingzwangerschappen. Een andere belangrijke complicatie in IVF is het ovariële hyperstimulatiesyndroom. De incidentie van meerlingzwangerschappen kan verminderd worden door het terugplaatsten van 1 embryo in vrouwen jonger dan 38 jaar en van 2 embryo's in vrouwen van 38 jaar en ouder en door het identificeren van de cycli met een hoog risico op meerlingzwangerschappen. Het ovariële hyperstimulatie syndroom kan voorkomen worden door het gebruiken van mildere stimulatie protocollen. Voor een goede vergelijking van verschillende behandelingsstrategieën (stimulatieprotocollen en embryo-terugplaats-beleid) is het belangrijk een duidelijke consistente definitie van succes in IVF te gebruiken.

Hoofdstuk 2:

Het veranderen van de manier waarop succes in IVF gedefinieerd wordt kan leiden tot een verhoging van de effectiviteit terwijl de kosten en complicatie van een behandeling afnemen. Succes per IVF cyclus zou vervangen moeten worden door succes per gestarte IVF behandeling (meerdere cycli). De a term geboren eenling per gestarte IVF-behandeling (of per tijdsperiode) rekening houdend met patiëntvriendelijkheid, complicaties en kosten is in onze ogen het meest informatieve eindpunt. Dit eindpunt is niet alleen van belang voor patiënten maar ook voor artsen, gezondheidseconomen en beleidsmakers. Een dergelijke benadering zal uitnodigen tot de ontwikkeling van patiëntvriendelijke en goedkope stimulatieprotocollen met minder stress en bijwerkingen. De combinatie van milde stimulatie protocollen met het terugplaatsten van 1 embryo kan dezelfde zwangerschapskans per gehele IVF-behandeling als gevolg hebben, in dezelfde tijdsperiode met gelijke kosten, maar met minder stress en andere ongemakken voor de patiënt en met het voorkomen van meerlingzwangerschappen. Het op grote schaal invoeren van het terugplaatsen van 1 embryo zou geholpen worden met het definiëren van succes in IVF als de kans op de geboorte van een a term geboren eenling per gehele IVF behandeling.

Hoofdstuk 3:

Ter vergelijking van de uitkomsten van een conventionele IVF behandeling in PCOS vrouwen en non-PCOS-vrouwen is een meta-analyse uitgevoerd. Voor deze meta-analyse werden studies beoordeeld waarin PCOS-patiënten die IVF ondergingen vergeleken werden met een vergelijkende controle groep (geen mannelijke factor). De definitie voor PCOS die in de studie gebruikt werd moest vergelijkbaar zijn met de Rotterdam consensus criteria voor IVF. Alle patiënten binnen een studie moesten met eenzelfde stimulatieprotocol behandeld worden. En de publicatie moest informatie bevatten over patiëntenkarakteristieken en zwangerschaps uitkomst. Negen van de 290 geïdentificeerde studies rapporteerden data over 458 PCOS-patiënten (793 cycli) en 694 gematchte controle subjecten (1116 cycles) voldeden aan bovenstaande inclusiecriteria. PCOS-patiënten lieten een significant verminderde kans op een oocyten punctie per gestarte cyclus zien, (odds ratio (OR) 0.5 (95% CI 0.2;1.0)). Desondanks werd er geen verschil gezien tussen de groepen in de kans op een embryo terugplaatsing per oocyten punctie (OR 0.7 (95% CI 0.4;1.3)). Significant meer oocyten per punctie werden verkregen in PCOS patiënten in vergelijking met de controle groep (random effect schatting 3.4 (95% CI 1.7;5.1). Het aantal bevruchte oocyten verschilde niet significant tussen de PCOS patiënten en de controle groep, weighted mean difference (WMD) 0.1 oocyten (95% CI -1.4;1.6). Er werd geen significant verschil gezien in de kans op een klinische zwangerschap per gestarte cyclus, (OR 1.0 (95% CI 0.8;1.3)). De incidentie van het ovariële hyperstimulatiesyndroom na de oocyten punctie werd in de meeste publicaties niet gerapporteerd, Deze meta-analyse liet in PCOS-vrouwen die een IVF behandeling ondergingen een verhoogde kans op het cancellen van de cyclus zien, meer oocyten per punctie en een lagere kans op bevruchting van de oocyten. PCOS-vrouwen hadden eenzelfde kans op een zwangerschap en een levend geborene per cyclus als de controle groep.

Hoofdstuk 4:

Kan het terugplaatsen van 2 embryo's in plaats van 3 in vrouwen van 38 jaar en ouder het aantal meerlingzwangerschappen na IVF verminderen terwijl de kans op een a term levend geborene acceptabel blijft? Om deze vraag te beantwoorden werd een gerandomiseerd gecontroleerd onderzoek in twee centra uitgevoerd. 45 patiënten, 38 jaar of ouder werden gerandomiseerd. Het terugplaatsen van twee embryo's gedurende een maximum van 4 cycli (DET-groep) werd vergeleken met het terugplaatsen van drie embryo's gedurende een maximum van 3 cycli (TET-groep). De cumulatieve kans op een a term levend geborene was 47.3% na 4 cycli in de DET-groep en 40.5% na 3 cycli in de TET-groep. Het verschil tussen

de DET en de TET-groep is 6.8% in het voordeel van de DET-groep (95% CI -25;38) (p=0.7). De kans op een meerlingzwangerschap in the DET en TET-groep was respectievelijk 0% (95% CI 0;24) en 30% (95% CI 7;65) (p=0.05). Bij de DET patiënten was het gemiddelde aantal IVF-cycli 2.9 vergeleken met 2.1 in de TET-groep (p=0.01). In vrouwen van 38 jaar en ouder resulteert het terugplaatsen van twee embryo's in een gelijke cumulatieve kans op een a term levend geborene. Dit in vergelijking met een strategie waarin 3 embryo's worden teruggeplaatst. Hiervoor zijn iets meer behandelings cycli nodig.

Hoofdtuk 5:

Dit hoofdstuk beschrijft het design van een klinische studie ter evaluatie van de effectiviteit, kosten en patiëntvriendelijkheid van twee behandelingsalgoritmen in IVF, bestaande uit verschillen in zowel het stimulatie protocol als het terugplaats beleid. Een gerandomiseerd gecontroleerd onderzoek werd uitgevoerd in twee grote centra. De twee strategieën zijn: A) milde ovariële hyperstimulatie (met GnRH antagonist) samen met het terugplaatsen van een embryo versus een conventioneel ovarieel hyperstimulatieprotocol (met een GnRH agonist lang protocol), en het terugplaatsen van 2 embryo's. De primaire studie eindpunten zijn; (1) zwangerschap binnen een jaar na randomisatie resulterend in een a term levend geborene. (2) de totale kosten per paar en kind tot 6 weken na de uitgerekende datum en (3) het totale patiëntenongemak binnen een jaar na randomisatie. De power berekening van deze studie ging uit van een overall cumulatieve zwangerschapskans van 45% met de conventionele behandeling strategie en non-inferiority van de milde behandelings strategie en was gedefinieerd als niet meer dan 12.5% verschil in de ondergrens van de kans op een levend geborene in vergelijking met de conventionele behandelings strategie. Voor een power van 80% en een alpha = 0.05, moeten er 400 subjects geïncludeerd worden. De analyse is uitgevoerd volgens het intention-to-treat principe. Volgens plan zijn er van februari 2002 tot maart 2004, 410 patiënten geïncludeerd in de studie. Verdere follow-up (12 maanden behandeling en 9 maanden zwangerschap was nodig omdat live birth het eindpunt is. De finale analyse heeft eind 2005 plaatsgevonden.

Hoofdstuk 6:

In dit hoofdstuk worden de resultaten van de onderzoeksopzet uit hoofdstuk 5 besproken. Het doel van dit hoofdstuk is om vast te stellen of een milde IVF strategie een zelfde kans op een a term levend geborene tot gevolg heeft binnen een jaar in vergelijking met de standaard strategie. Terwijl de kans op een meerlingzwangerschappen en de kosten afneemt en de

patiënt vriendelijkheid van de behandeling toeneemt. Er werd een gerandomiseerd, gecontroleerd, twee-armig effectiviteits onderzoek uitgevoerd met twee armen. 404 Patiënten werden gerandomiseerd voor een milde ovariële hyperstimulatie (met GnRH antagonist) in combinatie met het terugplaatsen van 1 embryo versus een conventioneel ovariële hyperstimulatie protocol (met een GnRH agonist lang protocol), en het terugplaatsen van 2 embryo's. The primaire studie-eindpunten waren; (1) zwangerschap binnen een jaar na randomisatie resulterend in een a term levend geborene. (2) de totale kosten per paar en kind tot 6 weken na de uitgerekende datum en (3) het totale patiënten ongemak binnen een jaar na randomisatie. The cumulative kans op een zwangerschap leidend tot een a term levend geborene binnen een jaar was 43.4% in the milde groep en 44.7% in the standaard groep. De kans op een meerlingzwangerschap per paar was respectievelijk 0.5% versus 13.1% (P<0.001) and de totale kosten zijn € 8,333 versus € 10,745 (P=0.006). Binnen een jaar was er was geen verschil in de oppervlaktes onder de curve voor angst, depressie, lichamelijke klachten en kwaliteit van slaap binnen een jaar. Milde ovariële stimulatie in combinatie met het terugplaatsen van 1 embryo resulteert in een gelijke cumulatieve kans op een a term levend geborene en een gelijke hoeveelheid patiënten ongemak na 1 jaar in vergelijking met de standaard stimulatie in combinatie met het terugplaatsen van 2 embryo's. Het aantal meerlingzwangerschappen en de totale kosten zijn minder bij de milde strategie.

Hoofdstuk 7:

Dit hoofdstuk vergelijkt de kosten van de milde strategie en het terugplaatsen van 1 embryo met de kosten van de standaard strategie en het terugplaatsen van 2 embryo's. 404 Patiënten werden gerandomiseerd voor een milde ovariële hyperstimulatie (met GnRH antagonist) in combinatie met het terugplaatsen van een embryo versus een conventioneel ovariële hyperstimulatie protocol (met een GnRH agonist lang protocol), en het terugplaatsen van 2 embryo's. De primaire uitkomstmaat was de totale kosten van een IVF behandeling binnen 12 maanden na randomisatie. De kosten van een eventuele zwangerschap ontstaan gedurende deze 12 maanden en de postnatale kosten van moeder en kind tot 6 weken na de uitgerekende datum werden ook meegnomen in de berekening. De milde strategie was geassocieerd met lagere ziekenhuiskosten per IVF cyclus (€1,569 versus €1,987; p=0.001) en, ondanks een significante toename in het aantal IVF-cycli (1.7 versus 2.3; p<0.001), in lagere gemiddelde totale kosten gedurende het eerste jaar (€8,333 versus €10,745; p=0.006).

Dit was voornamelijk het gevolg van hogere obstetrische en postnatale kosten voor de standaard strategie. De hogere kosten van de bevalling en de langere opnameduur van moeder

en kind werden voornamelijk veroorzaakt door de meerlingzwangerschappen. De kosten per doorgaande zwangerschap resulterend in een a term levend geborene waren €19,156 in de milde strategie en €24,038 in de standaard strategie. De milde behandelingsstrategie is, ondanks een toename van het aantal cycli binnen een jaar, vanuit een economisch perspectief voordeliger. Dit voordeel zal op de lange termijn verder toenemen omdat door een afname in tweelingzwangerschappen ook het aantal lichamelijke en geestelijke handicaps in het latere leven zal afnemen.

Hoofdstuk 8:

Dit hoofdstuk bespreekt de conclusies die getrokken kunnen worden uit dit proefschrift.

Publications

Publications presented in the present thesis

Heijnen EM, Macklon NS, Fauser BC. What is the most relevant standard of success in assisted reproduction? The next step to improving outcomes of IVF: consider the whole treatment. Hum Reprod. 2004 Sep;19(9):1936-8

Heijnen EM, Eijkemans MJ, Hughes EG, Laven JS, Macklon NS, Fauser BC. A meta-analysis of outcomes of standard IVF in women with polycystic ovary syndrome. Hum Reprod Update. 2006 Jan-Feb;12(1):13-21

Heijnen EM, Klinkert ER, Schmoutziger AP, Eijkemans MJ, Te Velde ER, Broekmans FJ. Prevention of multiple pregnancies after IVF in women 38 and older: a randomised study. Accepted RBM Online

Eijkemans MJ, **Heijnen EM,** de Klerk C, Habbema JD, Fauser BC. Comparison of different treatment strategies in IVF with cumulative live birth over a given period of time as the primary end-point: methodological considerations on a randomised controlled non-inferiority trial. Hum Reprod. 2006 Feb;21(2):344-51

Heijnen EM, Eijkemans MJ, De Klerk C, Polinder S, Beckers NG, Klinkert ER, Broekmans FJ, Passchier J, Te Velde ER, Macklon NS, Fauser BC. A mild strategy in IVF results in favourable outcomes in terms of term live birth, cost and patient discomfort. Accepted Lancet

Polinder S, **Heijnen EM,** Macklon NS, Habbema JD, Fauser BJ, Eijkemans MJ. Health economics of two IVF strategies: Mild ovarian stimulation in combination with single embryo transfer versus standard ovarian stimulation with dual embryo transfer. Submitted

Publication from the author related to the present thesis

de Klerk C, **Heijnen EM**, Macklon NS, Duivenvoorden HJ, Fauser BC, Passchier J, Hunfeld JA. The psychological impact of mild ovarian stimulation combined with single embryo transfer compared with conventional IVF. Hum Reprod. 2006 Mar;21(3):721-7

Verberg MF, Macklon NS, **Heijnen EM**, Fauser BC. ART – Iatrogenic Multiple Pregnancy?

Submitted

Abstracts and presentations from the author related to the present thesis

Heijnen EM, Fauser BC Meer minder, minder meer? Presented at Organon IVF weekend November 21, 2004, Kurhaus Scheveningen

Heijnen EM, Eijkemans MJ, de Klerk C, Beckers NG, Klinkert ER, Broekmans FJ, Passchier J, Te Velde ER, Habbema JD, Macklon NS, Fauser BC. Milde stimulatie en terugplaatsing van een embryo voor IVF: Kan IVF simpeler en veiliger? Presented at the voorjaarsvergadering van de Vereniging voor Fertiliteitsstudies (VFS). April 15, 2005, Antwerp, Belgium.

Heijnen EM, Eijkemans MJ, de Klerk C, Beckers NG, Klinkert ER, Broekmans FJ, Passchier J, Te Velde ER, Habbema JD, Macklon NS, Fauser BC. Mild Stimulation and Single Embryo Transfer: Towards safer and simpler IVF. Presented at the 21th annual meeting of the European Society of Human Reproduction and Embryology (ESHRE). June 19 – June 22, 2005. Copenhagen, Denmark.

Heijnen EM, Fauser BC. How to define successful IVF? Presented at the 23th Meeting of the Portuguese Society for Reproductive Medicine. November 4-5, 2005, Coimbra, Portugal

Heijnen EM, Eijkemans MJ, de Klerk C, Beckers NG, Klinkert ER, Broekmans FJ, Passchier J, Te Velde ER, Habbema JD, Macklon NS, Fauser BC. Alternative approaches in IVF. Presented at the NVOG Gynaecongres. November 10-11, 2005, Papendal, The Netherlands.

Heijnen EM, Fauser BC. Is single embryo transfer the answer to multiple pregnancy? Presented at Born in ART a Revolution of Parenthood February 17-18, 2006, Milan, Italy

Heijnen EM, Eijkemans MJ, De Klerk C, Polinder S, Beckers NG, Klinkert ER, Broekmans FJ, Passchier J, Te Velde ER, Macklon NS, Fauser BC. A randomised trial comparing a standard versus a mild treatment strategy, considering term live birth, cost and patient discomfort during one year of treatment. Presented at the 22th annual meeting of the European Society of Human Reproduction and Embryology (ESHRE). June 18 – June 22, 2006, Prague Czech Republic.

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