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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.

Complications associated with IVF

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.

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.

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.

Alternative Approaches in IVF

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

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 Rate 1 ET	Delivery Rate 1 ET	Twin Pregn (%)	Pregnancy Rate 2 ET	Delivery Rate 2 ET	Twin 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**

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).

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. Obvi-

ously 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.

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).

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.

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).

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.