Single embryo transfer and IVF/ICSI outcome: a balanced appraisal

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This review considers the value of single embryo transfer (SET) to prevent multiple pregnancies (MP) after IVF/ICSI. The incidence of MP (twins and higher order pregnancies) after IVF/ICSI is much higher (~30%) than after natural conception (~1%). Approximately half of all the neonates are multiples. The obstetric, neonatal and long-term consequences for the health of these children are enormous and costs incurred extremely high. Judicious SET is the only method to decrease this epidemic of iatrogenic multiple gestations. Clinical trials have shown that programmes with >50% of SET maintain high overall ongoing pregnancy rates (~30% per started cycle) while reducing the MP rate to <10%. Experience with SET remains largely European although the need to reduce MP is accepted worldwide. An important issue is how to select patients suitable for SET and embryos with a high putative implantation potential. The typical patient suitable for SET is young (aged <36 years) and in her first or second IVF/ICSI trial. Embryo selection is performed using one or a combination of embryo characteristics. Available evidence suggests that, for the overall population, day 3 and day 5 selection yield similar results but better than zygote selection results. Prospective studies correlating embryo characteristics with documented implantation potential, utilizing databases of individual embryos, are needed. The application of SET should be supported by other measures: reimbursement of IVF/ICSI (earned back by reducing costs), optimized cryopreservation to augment cumulative pregnancy rates per oocyte harvest and a standardized format for reporting results. To make SET the standard of care in the appropriate target group, there is a need for more clinical studies, for intensive counselling of patients, and for an increased sense of responsibility in patients, health care providers and health insurers.

Key words: IVF/ICSI/multiple pregnancy reduction/single embryo transfer

Introduction

European and American registries of medically assisted reproduction indicate high multiple pregnancy (MP) rates after IVF/ICSI (Keith and Oleszczuk, 1999; Schieve et al., 1999, 2002; European IVF-monitoring programme, 2001, 2002, 2004; Kiely and Kiely, 2001), ~25% for twins and ~3–5% for high order multiple pregnancies (HOMP). Approximately half of all IVF/ICSI children belong to a set of multiples. Non-IVF treatments, comprising ovulation induction and ovulation enhancement with or without intrauterine insemination, are responsible for another sizeable proportion of MP. An excellent review of this multidimensional problem was produced by an expert group consisting of both European and American experts, who made the cautious recommendation that triplets should be avoided and twins minimized (Bertarelli Group, 2003).

It has always been silently accepted that a high proportion of iatrogenic twins and HOMP was the price to be paid for a reasonable success rate of a treatment that is physically and emotionally demanding and, in most cases, expensive. Many twins are delivered healthy and advances in neonatal medicine have decreased mortality and morbidity of premature babies from MP, but the problem remains huge. Statistics convince less than personal experience and tragedy. IVF pregnancies are frequently not followed by the reproductive health providers, blurring the perception of IVF as a treatment with risks and complications.

The idea that we must adapt our perception and definition of a ‘success’ is gaining ground. A positive pregnancy test is not a success; a health baby is. Many twins or HOMP are not successes. Two healthy babies at the same time are a success as well, but obstetricians know that it is difficult to predict which MP will end well and which will not. Predictive factors of complications in MP can rarely be used as a basis to transfer one versus more embryos. The problem is not any one particular twin ending in the birth of two perfect children, but the epidemic size of the complications. These comprise a statistical increase...
in (severe) pathologies, as well as an increase in average costs for multiple pregnancies, deliveries and neonatal care (Hildebaugh et al., 1997; Wolner-Hanssen and Rydstroem, 1998; De Sutter et al., 2002; Ericson et al., 2002; Garceau et al., 2002; Ellison and Hall, 2003); severe parenting stress experienced by parents of multiples (Ostfeld et al., 2000; Glazebrook et al., 2004); and the lifelong support needed for mildly or severely disabled children.

MP cause several well-documented pathologies, extensively reviewed elsewhere (Tan et al., 1992; Tallo et al., 1995; Dho et al., 1997, 1999; Pons et al., 1998a,b; Senat et al., 1998; T. Bergh et al., 1999; Koudstaal et al., 2000a,b; Wennerholm and Bergh, 2000, 2004; Rydstroem and Herailb, 2001; Klemetti et al., 2002; Lynch et al., 2002; Strömberg et al., 2002; Wang et al., 2002; Helmerhorst et al., 2004; Wennerholm, 2004). They comprise a maternal risk for obstetric complications, hypertension, pre-eclampsia, preterm labour, anaemia and an increased Caesarean section rate; fetal or neonatal risk for increased mortality, lower gestational age, lower birthweight, respiratory distress syndrome, necrotizing enterocolitis, sepsis, intracranial haemorrhage, congenital malformations, the twin–twin transfusion syndrome, cerebral palsy and long-term neurological complications; and family complications of a psychological, social and financial nature.

Multifetal pregnancy reduction has been the safety valve to escape the complications of HOMP. This secondary prevention is not the best possible solution. Although the clinical outcome of reduced HOMP usually reduced to twins is similar to that of naturally conceived twins (Maymon et al., 1995; Antsaklis et al., 1999; Dood and Crowther, 2004), it offers no solution for the quantitatively much larger problem of twins, which are not as a rule reduced to singletons. There is psychological reluctance in future parents to accept and integrate this procedure in their personal lives. Most patients prefer primary prevention (C. Bergh et al., 1999). Prenatal diagnosis of genetic defects is also more difficult (Brambati and Tului, 1995).

Transferring just one embryo even of ideal morphology in an ideal recipient was a taboo, because it was feared that the pregnancy rate (PR) would drop below that of our nearest neighbour or below the layman’s and the media’s expectations (Coetsier and Dhont, 1998; Faber, 1997). Even if society is willing to pay for the extra costs incurred, money can never restore health irreparably lost, and, even if it could, society should ask whether this is really needed, provided chances to conceive after IVF/ICSI are not substantially decreased without running those risks. There is a strong philosophical argument for placing responsibility for our children’s good health at their start of life with all those involved in IVF/ICSI (patients, health care providers, health insurers, politicians) (Pennings, 2000; ESHRE Task Force on Ethics and Law, 2003).

Does the available evidence support judicious SET as a valuable tool to minimize MP?

### Clinical data on single embryo transfer (SET)

To date, relatively few publications have been dedicated to clinical reports of SET.

### Published randomized trials

Only four truly prospective randomized trials have been published: three European studies, of which two utilized day 3 SET (Gerris et al., 1999; Martikainen et al., 2001) and one >90% day 2 SET (Thurin et al., 2004), and one American study using single blastocyst transfers (Gardner et al., 2004) (Table I). It is, however, not appropriate to make a reliable meta-analysis of these trials. All four have a randomized design but they compare different things. In our own study, patients were randomized

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of cycles</th>
<th>Single embryo transfer</th>
<th>Double embryo transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pregnancy rate (%)</td>
<td>Twins (%)</td>
</tr>
<tr>
<td>Randomized trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gerris et al. (1999)</td>
<td>53</td>
<td>10/26 (38.5)</td>
<td>1/10</td>
</tr>
<tr>
<td>Martikainen et al. (2001)</td>
<td>144</td>
<td>24/74 (32.4)</td>
<td>1/24</td>
</tr>
<tr>
<td>ardner et al. (2004)</td>
<td>48</td>
<td>14/23 (60.9)</td>
<td>0/14</td>
</tr>
<tr>
<td>Thurin et al. (2004)</td>
<td>661</td>
<td>91/330 (27.6)</td>
<td>1/91</td>
</tr>
<tr>
<td>+ cryo</td>
<td>131/330 (39.7)</td>
<td>1/131</td>
<td>216/453 (47.6)</td>
</tr>
<tr>
<td>Total</td>
<td>906</td>
<td>139/453 (30.7)</td>
<td>3/139 (2.16)</td>
</tr>
<tr>
<td>Cohort studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gerris et al. (2002)</td>
<td>1152</td>
<td>105/299 (35.1)</td>
<td>1/124</td>
</tr>
<tr>
<td>De Sutter et al. (2003)</td>
<td>2898</td>
<td>163/579 (28.2)</td>
<td>1/163</td>
</tr>
<tr>
<td>Tiitinen et al. (2003)</td>
<td>1494</td>
<td>162/470 (34.5)</td>
<td>2/162</td>
</tr>
<tr>
<td>Catt et al. (2003)</td>
<td>385</td>
<td>49/111 (44.1)</td>
<td>1/49</td>
</tr>
<tr>
<td>Gerris et al. (2004)</td>
<td>367</td>
<td>83/206 (40.3)</td>
<td>0</td>
</tr>
<tr>
<td>Martikainen et al. (2004)</td>
<td>1111</td>
<td>107/308 (34.7)</td>
<td>1/107</td>
</tr>
<tr>
<td>Total</td>
<td>7407</td>
<td>669/1973 (33.9)</td>
<td>6/591 (1.0)</td>
</tr>
</tbody>
</table>

Data for the Martikainen (2001) study and the Thurin (2004) study show both the fresh and the cryo-augmented pregnancy rates. HOMP = high order multiple pregnancies; NA = not applicable.
between receiving one versus two top quality embryos, strictly defined as an embryo with <20% fragmentation, four or five blastomeres on day 2 and ≥7 blastomeres on day 3 after fertilization and no multinucleation in any of the blastomeres. Such embryos were shown to have an ongoing implantation potential of ~40% (Van Royen et al., 1999). The aim of this study was to find out what would be the difference in PR when one or two embryos of documented similar high implantation potential are transferred in a homogeneous group of ideal patients (<34 years of age, first IVF/ICSI cycle). In the four-centre Martikainen study, including patients in different treatment ranks and using traditional criteria of embryo selection, the aim was to show that single and double embryo transfer (DET) yield similar results. The conclusion of the study, that a 32.4% pregnancy rate after SET is not significantly different from a 47.1% pregnancy rate after DET, was statistically valid, but does not convince. Thurin et al.’s (2004) study is an 11-centre Scandinavian study in women <36 years of age in their first or second treatment cycles who were randomized to receive either one excellent fresh embryo and one frozen–thawed embryo (one-plus-one) in case no pregnancy occurred versus two fresh embryos. Embryos were selected on the basis of fragmentation, number of blastomeres and multinucleation. It showed that a strategy of one-plus-one transfer (39.7%) did not result in a substantial reduction in ongoing pregnancy rate when compared with double embryo transfer (43.5%). However, the fresh PR was 91/330 = 27.6% after SET versus 142/330 = 43% after DET (OR = 1.56; 95% CI = 1.26–1.93). Gardner et al.’s (2004) study compared the transfer of one with two day 5 embryos in a selected good prognosis group.

Taken together, the mean fresh pregnancy rate after SET in the four studies was 30.7% with 2.16% twins and 47.6% after DET with 33.8% twins.

Published cohort studies

A summary of results from a total of 7407 cycles published in six cohort studies comparing SET with DET is also shown in Table I. The mean pregnancy rate after SET was 33.9% with 1.0% twins versus 35.0% after DET with 32.6% multiple pregnancies. In most of these studies, SET was elective SET, i.e. SET was performed on the condition that an excellent quality embryo was available from a cohort of several embryos. Taken together, these data suggest that elective SET (transfer of a high competence embryo) yields the same pregnancy rate as indiscriminate DET. This is because high success rates after the transfer of two high competence embryos in DET is balanced down by the low success rate after the transfer of two poor quality embryos. This is where the importance of optimal embryo selection and of a validated definition of a high competence embryo is all-important.

A balanced appraisal of published results

These data illustrate two points of paramount importance with respect to SET. First, cryopreservation is a very important tool in reducing twins after IVF/ICSI. Second, transferring the ‘two best’ embryos always yields more pregnancies than transferring ‘the’ best embryo. There is no point in saying that SET equals DET. This is clearly shown when comparing the results after SET versus DET between the randomized and the cohort studies. In the former, there is a clear difference between both (DET, 216/453 versus SET, 139/453; OR = 1.55; 99% CI = 1.24–1.94).

SET is closely tied up with optimal embryo selection and it should only be applied if an embryo with putative high competence is available. We have defined it in our way (Van Royen et al., 1999), others in another way (see ‘SET and embryo selection’ below). It can be applied to a smaller or to a larger proportion of the patient population. The essential point should not be missed: optimized embryo selection, however and for whomsoever it is performed, is a tool that can be used in two opposite directions. It can be used to perform SET in a substantial proportion of patients, maintaining an overall PR in the vicinity of the natural conception rate for a normally fertile couple (~30%) but lowering the twinning rate substantially. Or it can be used to perform optimized DET in that same patient population, increasing the overall PR to >30% but ‘accepting’ an elevated twinning rate in the programme. It is understandable that to some centres, primarily looking at the PR, SET appears to be a step backward, whereas for others, looking primarily at safety, it is a step forward. The decision is a matter of judgement and trade-off.

Generally, randomization is a valuable method for clinical research aims, resulting in a similar distribution of confounding variables over the study arms. It is an excellent tool to demonstrate efficacy of concept for a particular treatment, in a patient population fulfilling specified inclusion criteria. However, in a clinical IVF/ICSI programme, which cannot be a never-ending randomized trial, one needs clear criteria to transfer one or two embryos in real-life situations, utilizing a validated selection method, yielding results that have equal clinical relevance because they represent the efficiency of the treatment.

The difference between SET and DET in randomized trials could be anticipated; only the extent of the difference is correctly made clearer (~50% more pregnancies after DET). In the real-life cohort studies, no difference between SET and DET exists, because SET means the elective transfer of a highly selected embryo of predictable implantation potential, whereas DET means a mix of transfers of two embryos with excellent, intermediate or poor implantation potential. These trials are useful to establish what is the trade-off point at which a mean physiological PR can be reached with an acceptably low (<10%) twinning rate. In addition, the significant difference found in the randomized studies disappears when one cryopreservation cycle is added to the fresh SET cycle.

In summary, the only reasonable interpretation of the combined data of these ten studies (see Table I) can be no other than favouring the use of SET of the appropriate embryo in the appropriate patient to achieve an acceptable twinning rate.

The European experience with elective SET

A summary of policies for the prevention of MP in Europe is given in the experts’ meeting report of the Bertarelli Group (2003).

In Belgium, before the reimbursement, just a few centres were applying SET in an increasing proportion of cycles from 1997 onwards. These represented only ~20% of all cycles in
Belgium, hence the effect on the national statistics up to the year 2002 was just a trend towards more SET and towards fewer twins with triplets becoming exceptional.

From July 1, 2003 onwards, a reimbursement system for six IVF/ICSI cycles in a lifetime has been set up, based on the clinical experience of Belgian groups. Beneficiaries are patients who fall under the Belgian health insurance provisions up to the age of <43 years of age. For each oocyte recovery, the hospital receives €1187 on a central account, which is booked by the centre for reproductive medicine. This amount covers the collection of both oocytes and sperm, including microepididymal sperm aspiration (MESA)/testicular sperm extraction (TESE), laboratory costs of fresh and frozen–thawed transfers resulting from each oocyte harvest and related costs for registration and follow-up. The medical costs (consultations, sonographies, oocyte recovery, embryo transfer) and drugs are covered by the standard system of third-party reimbursement through the health insurance companies. The crux is that (mainly neonatal) savings from the reduction in twins and the disappearance of triplets make up for the money needed to cover six cycles, thus providing access to treatment to all who need it and at the same time ensure quality outcome. There is also compulsory online registration of all cycles.

Pivotal in the whole exercise is the judicious application of SET. Depending on the woman’s age and the rank of the trial, the maximum number of embryos to transfer is regulated. All women aged <36 years in their first cycle receive one embryo, independent of its morphological assessment. The concept of a ‘top quality embryo’, as proposed by some authors (Gerris et al., 1999, 2003; Van Royen et al., 1999), has not been withheld, because embryo selection method and expertise cannot be simply transferred from one centre to another. In so doing, each centre must attempt to identify ‘the best’ embryo. In the same age group, in second treatment cycles, one embryo is transferred unless of insufficient quality, again allowing centres to use their own selection criteria. In older women or in subsequent cycles, the number of embryos to transfer never exceeds two except in women aged >39 years, where there is no imposed maximum.

All eyes are now on two outcome figures: the percentage of twins in the whole Belgian IVF/ICSI population (which used to be ∼25%) and the mean ongoing PR per treatment cycle. Authorities will also keep a stern eye on other variables; the total number of cycles performed and the total expenditure. Some increase in these figures is anticipated since some patients previously refrained from treatment for purely financial reasons. No official figures are as yet available to illustrate this ‘IVF/ICSI boom’, although in some centres, but not all, some increase is reported.

In our own centre, as in others, twinning rates have dropped to ∼7% in 2003 without significant decrease in overall PR and a complete disappearance of triplets (Figure 1; adapted from Gerris et al. 2004), apart from the rare but not exceptional dizygotic triplets. The number of children born as part of a set of twins fell from 80 in 1998 to <20 in 2003 (Figure 2).

In Finland, SET has been applied widely for several years. On a national level, the incidence of IVF/ICSI twins has significantly decreased and even the total national birth registry shows a decrease in the proportion of twins (Vilska and Tiitinen, 2004). SET has been combined very successfully with cryopreservation (Tiitinen et al., 2001) and was shown to be very successful in oocyte donation (Söderström-Anttila et al., 2003).

A Scandinavian prospective randomized trial in five Swedish, two Norwegian and four Danish centres shows that SET is now largely accepted in these countries. In Sweden, the practice seems to be in concordance with the regulation of the National Board on Health and Welfare stating that in principle only one embryo should be replaced apart from exceptional circumstances, which seem to be loosely defined. In this study, 661 women of <36 years of age in their first or second IVF/ICSI cycle were randomized to either two embryos (DET) or one embryo (SET); cycles with one embryo that did not lead to an ongoing pregnancy were followed, if possible, by one transfer of one frozen–thawed embryo (FET). The cumulative pregnancy rate after SET + FET was 39.5%, which is not substantially lower than the 43.5% after DET (Thurin et al., 2004).

In another report, the effects of the recent legislation on embryo transfer policy on results and pregnancy outcome in a Swedish unit were analysed (Olofsson et al., 2004) and it was confirmed that SET can be readily introduced on a large scale according to the policy in Sweden without compromising results and outcome.

In Germany, the Embryo Protection Act rules that no more than three oocytes can be cultured further than...
the two-pronuclear (2PN) stage and that no embryos can be frozen. This compels German embryologists to select the embryos for transfer at the 2PN stage, which is not only likely to be suboptimal, but also hinders the application of SET, because the average implantation rate of embryos selected at the 2PN stage seems to be lower than for early cleavage embryos or blastocysts. This law makes it hard to apply SET. Switzerland and Austria have a similar ruling.

In Italy, the situation at present is even more restrictive, since no more than three oocytes can be fertilized and all the embryos that result have to be replaced, leading to a completely anti-SET situation. Triplets reappear in centres that had almost completely eliminated them. Ethical concerns about respect for human life and protection of the family and offspring have the deplorable effect of burdening women with legislation that does not reflect biomedical reality (Robertson, 2004).

Dutch IVF centres seem convinced of the value of SET, as testified by an increasing number of Dutch publications addressing clinical or health-economic aspects of SET (Lukassen et al., 2004; Van Montfoort et al., 2004). However, the recent decision of the Dutch government to withdraw refunding of the first treatment cycle (previously three cycles were reimbursed) may hamper the application of SET, though hopefully a reasoned appeal may overturn this decision. Several studies are under way which address the issue of the optimal approach when putting reimbursement and transfer policy together in the balance.

In the UK, there is a legal restriction of the number of embryos to transfer to two, unless there are exceptional circumstances. The Human Fertilization and Embryology Authority has stated that it would like to move to SET if this ‘does not disadvantage anyone’ and Britain’s leading IVF pioneer has spoken in favour of it (Edwards, 2003).

Southern European countries (France, Spain, Portugal, Greece) have until now not produced clear evidence of a substantial proportion of SET cycles and do not have legislation setting a limit to the number of embryos transferred.

Indications and exceptions: SET for whom?

The essential prerequisites to introduce elective SET are simple. There must be a high baseline ongoing PR of the programme in the group of good prognosis patients (e.g. first and second cycles in women <38 years of age) in combination with a compellingly high MPR, and there must be an efficacious cryopreservation programme.

We should make a distinction between compulsory, medical and elective SET.

### Compulsory SET

Obviously, if only one embryo is available for transfer, SET is compulsory (cSET). Since in the majority of these cases the only available embryos are of poor quality, mean implantation rates in published series of cSET are low (Table II) (Giorgetti et al., 1995; Vilska et al., 1999; Gerris et al., 2001, 2002; De Sutter et al., 2003a; Tiitinen et al., 2003). These poor results are due to the fact that the majority of these cycles occur in poor prognosis patients (poor responders, older women, intrinsic fertilization defects).

### Medical SET

There are women in whom a multiple pregnancy represents an a priori increased risk compared to the overall population. Congenital anomalies of the uterus, bad obstetric history, previous loss of a twin, previous severe prematurity in a singleton, ishmic insufficiency, severe systemic disease (e.g. insulin-dependent diabetes) and the explicit wish of recipients to avoid a twin pregnancy, constitute absolute contraindications against DET.

### Elective SET

Elective is derived from the Latin ‘eligere’ which means ‘to choose and give preference’. By definition, elective SET means that there is choice from among two or more embryos suitable for transfer, with the purpose of transferring only one embryo. Therefore, embryo criteria for eligibility must be defined and validated, so that selection is optimized. This should be done using one or a set of criteria in a prospective setting where the outcome of each individual embryo is documented, ideally using only SET, which creates the possibility of one-to-one observations.

The theoretical possibility of transferring only one embryo in all cycles can for the present be discarded. Hence the challenge is to define the subgroup of patients who should receive one embryo. A number of older retrospective studies examined which clinical factors correlate with the chance for pregnancy or MP (Staessen et al., 1992; Svendsen et al., 1996; Templeton and Morris, 1996; Bassil et al., 1997; Commenges-Ducos et al., 1998; Minaretzis et al., 1998; Roseboom et al., 1995; Hsu et al., 1999; Shapiro et al., 2000, 2001). These were based on the transfer of two or more embryos. Most of the factors that were found to correlate (age being the most important one), are in fact themselves correlated with intrinsic embryo implantation potential (e.g. number of oocytes, number of normally fertilized 2PN zygotes, number of ‘good looking’ embryos, low dose of FSH needed, good ovarian response), emphasizing

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**Table II. Published results of compulsory single embryo transfers (SET)**

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of compulsory SET</th>
<th>No. of implantations</th>
<th>Implantation rate (%)</th>
<th>No. of live births</th>
<th>Live birth rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giorgetti et al. (1995)</td>
<td>858</td>
<td>88</td>
<td>10.3</td>
<td>62</td>
<td>7.2</td>
</tr>
<tr>
<td>Vilska et al. (1999)</td>
<td>94</td>
<td>19</td>
<td>20.2</td>
<td>15</td>
<td>16.0</td>
</tr>
<tr>
<td>Gerris et al. (2002)</td>
<td>86</td>
<td>26</td>
<td>30.2</td>
<td>19</td>
<td>22.1</td>
</tr>
<tr>
<td>Tiitinen et al. (2003)</td>
<td>205</td>
<td>39</td>
<td>19.0</td>
<td>31</td>
<td>15.1</td>
</tr>
<tr>
<td>De Sutter et al. (2003)</td>
<td>211</td>
<td>21</td>
<td>10.0</td>
<td>19</td>
<td>9.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1454</strong></td>
<td><strong>193</strong></td>
<td><strong>13.3</strong></td>
<td><strong>146</strong></td>
<td><strong>10.1</strong></td>
</tr>
</tbody>
</table>
the dominant impact of the embryo factor as compared to the patient factor.

Others have approached the problem using theoretical mathematical prediction models (Martin and Welch, 1998; Trimarchi, 2001; Hunault et al., 2002) or made recommendations towards DET (Staessen et al., 1992, 1993; Vauthier-Brouzes et al., 1994; Tasdemir et al., 1995; Roest et al., 1997; Templeton and Morris, 1998; Milki et al., 1999; Dean et al., 2000; Ozturk et al., 2001).

One study tried to identify patients most suitable for SET on the basis of a multivariate analysis of >2000 IVF/ICSI cycles with DET. These were found to be women aged <35–37 years in their first or second treatment cycles, with at least two embryos and without tubal pathology as an indication for IVF (Strandell et al., 2000).

There have also been some overt pleas for SET (Coetsier and Dhont, 1998; Gerris and Van Royen, 2000; Dhont, 2001).

A specific group that in our opinion should be actively counselled towards SET are women who obtained a non-ongoing pregnancy in a first IVF/ICSI cycle. For psychological reasons these patients often ask for two embryos in a second cycle, and it may be difficult to convince them to accept SET although there is evidence that these are very good prognosis patients (Croucher et al., 1998; Bates and Ginsburg, 2002).

Are there acceptable exceptions?

Even with SET as standard policy for IVF/ICSI, there will always remain a subgroup of patients in whom the transfer of more than one embryo is acceptable as an exception, based on sound medical considerations.

Cost for the patient is not a medical exception, but in many countries the high cost of IVF/ICSI for the patient causes an understandable objection to SET, although of course the health-economic considerations remain the same. This problem can only progressively be overcome by making the treatment more accessible to those who need it.

A real exception is female age. In some countries SET is performed up to age 38 or even 40 years. Above that age the transfer of more than one embryo is performed more liberally. Further clinical research will have to elucidate which are the optimal transfer algorithms in this age group. The potential role of aneuploidy screening may also be situated here, but to date, methodologically sound proof to that effect does not exist.

In patients with non-obstructive azoospermia, the recovery by TESE of sperm for ICSI at times is so low that only one or two treatment trials can be performed. Should more than one embryo be obtained in such unfavourable circumstances, the transfer of more than one embryo may be warranted. Other circumstances where only very small numbers of sperm are available, e.g. freezing before chemotherapy or ‘end of stock’ of a particular sperm donor, can be also considered exceptions.

Patients undergoing preimplantation diagnosis because of genetic disease frequently have just one or two unaffected embryos available to them after a long and technically complicated and expensive treatment, perhaps creating a relative contraindication to limit the number of embryos to one, although this should be weighed against the odds of a MP during the counselling.

Another example is oocyte donation. Depending on the availability of oocytes, which in some countries is much lower than the demand, or due to practical arrangements, where oocytes from one donor are shared by more than one acceptor, a dilemma may arise between the transfer of more than one embryo versus cryopreservation. However, a Finnish group has reported excellent results with SET in an oocyte donation programme (Sörderström-Anttila, 2003).

SET and embryo selection

Opinions vary as to whether SET should or should not be accompanied by some strategy of optimized selection of the embryo to transfer. If embryo selection is pushed too far and embryos considered as having a high implantation potential are too strictly defined or if the proposed technique is too complicated or expensive, the number of SET cycles will remain low and the impact on the twinning rate limited. If on the other hand, only one embryo is transferred without using strict selection criteria, the PR might drop below what is acceptable.

Documented ongoing implantation is the gold standard for a particular embryo’s competence. Published data show it to be a continuous biological variable, varying between 0% for the ‘worst’ and ~60% for the ‘best’ embryos. Labelling an embryo as a ‘top quality embryo’ or a ‘high implantation potential embryo’ or a ‘putative high competence embryo’ remains a clinically useful (when communicating with patients) but intrinsically oversimplified representation of this graduated implantation potential. This is illustrated in Table III (adapted from Van Royen et al., 2001) showing the observed implantation rates of embryos with known outcome (one-to-one observations). Is has been previously shown that embryos with MNB have very low implantation rates of ~5% (Pickering et al., 1995; Kligman et al., 1996; Jackson et al., 1998; Palmstierna et al., 1998; Pelinck et al., 1998; Van Royen et al., 2003).

Efforts have been made to correlate embryo implantation potential with characteristics of follicles (Van Blerkom et al., 1997; Van Blerkom, 1998), the oocyte (Van Blerkom et al., 1995, 2000; Van Blerkom, 1997; Palmstierna et al., 1998; Antczak and Van Blerkom, 1999; Ebner et al., 1999; Tesarik and Greco, 1999; Tesarik et al., 2000; Wilding et al., 2001), the fertilized zygote (Shoukri et al., 1997; Sakkas et al., 1998, 2001; Ludwig et al., 2000a,b; Bos-Mikich et al., 2001; Lundin et al., 2001; Montag and van der Ven, 2001; Salumets et al., 2001; Windt et al., 2004), the early cleaving embryo, either on day 2 (Giorgetti et al., 1995; Ziebe et al., 1997; Scott and Smith, 1998; Tesarik et al., 2000; Ebner et al., 2001; Martikainen et al., 2001; Hardarson et al., 2001; Laverge et al., 2001; Van der Auwera et al., 2002; Kovačić et al., 2002; Salumets et al., 2003; Tiitinen et al., 2003; Martikainen et al., 2004) or day 3 (Van Royen et al., 1999; Gerras et al., 1999, 2004; Coskun et al., 2000; Desai et al., 2000; Huisman et al., 2000; Scott et al., 2000; Wittemer et al., 2000; Lavergne et al., 2001; De Placido et al., 2002; Karaki et al., 2002; Rienzi et al., 2002; Utsunomiya et al., 2002, 2004; De Sutter et al., 2003a; Söderström-Anttila et al., 2003; Gerris, 2004; Kolibianakis et al., 2004; Thurin et al., 2004; Van Montfoort et al., 2004), the morula (Tao et al., 2002) and the blastocyst (Gardner et al., 1998, 2000, 2004; Rijnders and Jansen, 1998; Coskun et al., 2000; Huisman et al., 2000; Milki et al., 2000; Shapiro et al., 2000; Van der Auwera et al., 2002; Zollner et al., 2002; Rienzi et al., 2002; Abdelmassih et al., 2003).
Morphological characteristics that have been studied comprise the following: morphology of the oocyte; ATP content and mitochondrial distribution in oocytes; pronuclear body breakdown; number and symmetry of distribution of nucleolar bodies in zygote pronuclei; early (25–27 h after fertilization) or late first cleavage for day 2 embryos; number and symmetry of blastomeres, fragmentation and presence or absence of multinucleation in early cleaving embryos; number of blastomeres undergoing compaction and the morphology of the compaction process in day 4 morulas and blastocyst morphology in day 5 or day 6 embryos. Dynamic characteristics such as pyruvate and glucose metabolism (Gardner et al., 2001) or amino acid turnover (Houghton et al., 2002) have also been studied but not in an immediate clinical context.

Morphology alone cannot disclose whether a particular embryo will implant or not because we are looking at a statistical correlation, not at an individual measurement, and because morphology alone cannot disclose all the information contained in the embryo.

Table IV summarizes data from a number of studies of different sizes and aims, from which in each case the maximum PR, the maximum implantation rate (IR) per embryo and the mean number of embryos transferred are shown. In some of these studies, however, selection for transfer was not based on the criterion studied but on traditional day 2 and/or day 3 observations. Some studies are clinical, either randomized comparisons or cohort studies, which clearly stated the embryo selection technique. Others are retrospective analyses correlating PR and IR with particular recorded embryo characteristics either used or not used as a selection method. Some studies concern the overall IVF/ICSI population, others only very good prognosis patients. Therefore, comparison is flawed and conclusions hard to draw.

In studies applying elective SET (shown in bold), the PR (IR) varies between 27 and 61%. Excluding the two outliers (Gardner et al., 2004; Van Montfoort et al., 2004), variation lies between 28 and 50%, averaging ~35%. If anything can be concluded, it is that there is more than one valid selection method and that the average maximum IR is ~35% per ‘best’ embryo. It cannot be inferred from this table which is the optimal selection method for SET. To date, the best indicator to compare the performance of a particular selection procedure is to look at the maximum ongoing IR in studies that transferred one selected embryo. Even in these studies, patient selection bias precludes formal comparison. It is always possible to find a subset of patients (high responders, very young women) where the implantation rate is unusually high. This does not make the proposed procedure the ideal one for the overall population.

Studies focusing on early first cleavage agree that this is a good indicator to distinguish between embryos with high and low implantation potential, but, excluding the one study that stated actual selection took place on the basis of day 2 morphology (Salumets et al., 2003), the average reported IR varies between 14 and 28%. This is lower than the IR reported in studies where routine selection was based on day 2/3 characteristics, varying between 27 and 48%, averaging ~35%. Another variant of IR (60%) is reported in one study utilizing day 5 embryo transfers in highly selected patients (Gardner et al., 2004). When used as a routine selection stage, IR for day 5 embryos also average ~35%. Culturing several high quality day 3 embryos, if available, to blastocysts may add selective power, increasing the chance of transferring the best embryo available, but this subgroup of patients is small and the hypothesis has not been proven.

Table III. Implanted fraction of embryos without observed multinucleation, assessed by the number of blastomeres on day 2 (44–46 h) and day 3 (66–69 h) and the percentage of fragmentation (1: <10%; 2: <20%)a

<table>
<thead>
<tr>
<th>Fragmentation</th>
<th>Cells on day 2</th>
<th>Cells on day 3</th>
<th>100% implantation</th>
<th>0% + 100% implantation</th>
<th>Implantation fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>8</td>
<td>187</td>
<td>429</td>
<td>43.6</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>9</td>
<td>12</td>
<td>30</td>
<td>40.0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>9</td>
<td>8</td>
<td>20</td>
<td>40.0</td>
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<td>13</td>
<td>30.8</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>8</td>
<td>6</td>
<td>20</td>
<td>30.0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>10</td>
<td>3</td>
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<td>27.3</td>
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<tr>
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<td>38</td>
<td>26.3</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>19</td>
<td>26.3</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>8</td>
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<td>19</td>
<td>83</td>
<td>22.9</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>7</td>
<td>10</td>
<td>45</td>
<td>22.2</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
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<td>3</td>
<td>5</td>
<td>2</td>
<td>10</td>
<td>20.0</td>
</tr>
</tbody>
</table>

a≥10 embryos in each group; only embryos with an observed implantation rate of >20% shown.
Adapted from Van Royen et al. (2001).
Table IV. Maximum reported pregnancy rates, implantation rates rate per embryo transferred and mean number of embryos transferred after IVF/ICSI, arranged by day of appearance of the characteristic studied (not necessarily used for selection)

<table>
<thead>
<tr>
<th>Study</th>
<th>Day after fertilization</th>
<th>Maximum pregnancy rate (%) reported</th>
<th>Embryos/embryo transfer</th>
<th>Maximum implantation rate (%) reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ebner et al. (1999)</td>
<td>Day 0</td>
<td>NES</td>
<td>NES</td>
<td>20</td>
</tr>
<tr>
<td>Shoukir et al. (1997)</td>
<td>Day 1</td>
<td>33.3</td>
<td>2.67</td>
<td>23.6</td>
</tr>
<tr>
<td>Sakkas et al. (1998)</td>
<td>Day 1</td>
<td>25.9</td>
<td>2.8</td>
<td>14</td>
</tr>
<tr>
<td>Scott and Smith, 1998</td>
<td>Day 1 + zyg</td>
<td>65</td>
<td>3.7</td>
<td>28</td>
</tr>
<tr>
<td>Scott and Smith, 1998</td>
<td>Day 1 + zyg</td>
<td>65</td>
<td>3.7</td>
<td>28</td>
</tr>
<tr>
<td>Ludwig et al. (2000a,b)</td>
<td>Day 1</td>
<td>22</td>
<td>3</td>
<td>NES</td>
</tr>
<tr>
<td>Sakkas et al. (2001)</td>
<td>Day 1</td>
<td>45.0</td>
<td>2.2</td>
<td>23.8</td>
</tr>
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<td>Montag et al. (2001)</td>
<td>Day 1</td>
<td>37.9</td>
<td>2.3</td>
<td>20.5</td>
</tr>
<tr>
<td>Salumets et al. (2001)</td>
<td>Day 1</td>
<td>29.9</td>
<td>1</td>
<td>29.9</td>
</tr>
<tr>
<td>Lundin et al. (2001)</td>
<td>Day 1</td>
<td>40.5</td>
<td>1.92</td>
<td>28</td>
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<tr>
<td>Bos-Mikich et al. (2001)</td>
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<td>55</td>
<td>3.6</td>
<td>18</td>
</tr>
<tr>
<td>Salumets et al. (2003b)</td>
<td>Day 1</td>
<td>50</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Windt et al. (2004)</td>
<td>Day 1</td>
<td>33.3</td>
<td>3.2</td>
<td>NES</td>
</tr>
<tr>
<td>Gioretti et al. (1995)</td>
<td>Day 2</td>
<td>15.6</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Ziebe et al. (1997)</td>
<td>Day 2</td>
<td>49</td>
<td>2</td>
<td>26</td>
</tr>
<tr>
<td>Vilska et al. (1999b)</td>
<td>Day 1</td>
<td>35.8</td>
<td>1</td>
<td>35.8</td>
</tr>
<tr>
<td>Martikainen et al. (2001c)</td>
<td>Day 1</td>
<td>32.4</td>
<td>1</td>
<td>32.4</td>
</tr>
<tr>
<td>Hardason et al. (2001)</td>
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<td>52.9</td>
<td>1.98</td>
<td>36</td>
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<tr>
<td>Laverge et al. (2001)</td>
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<td>44.4</td>
<td>2.48</td>
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<td>35.1</td>
<td>1.9</td>
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<tr>
<td>Kovací et al. (2002)</td>
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<td>1.5</td>
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<tr>
<td>Tüitinen et al. (2003b)</td>
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<tr>
<td>Gardner et al. (1998)</td>
<td>Day 3</td>
<td>66</td>
<td>3.7</td>
<td>30.1</td>
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<tr>
<td>Van Ruyen et al. (1999)</td>
<td>Day 3</td>
<td>63</td>
<td>2</td>
<td>49</td>
</tr>
<tr>
<td>Gerris et al. (1999c)</td>
<td>Day 3</td>
<td>42.3</td>
<td>1</td>
<td>42.3</td>
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<tr>
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<td>74.1</td>
<td>2</td>
<td>48.1</td>
</tr>
<tr>
<td>Desai et al. (2000)</td>
<td>Day 3</td>
<td>41.9</td>
<td>3.4</td>
<td>18</td>
</tr>
<tr>
<td>Huisman et al. (2000)</td>
<td>Day 3</td>
<td>26.4</td>
<td>1.9</td>
<td>18</td>
</tr>
<tr>
<td>Coskun et al. (2000)</td>
<td>Day 3</td>
<td>39</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>Wittemer et al. (2000)</td>
<td>Day 3 + zyg</td>
<td>39.3</td>
<td>1.8</td>
<td>26</td>
</tr>
<tr>
<td>Scott et al. (2000)</td>
<td>Day 3 + zyg</td>
<td>57</td>
<td>3.2</td>
<td>31</td>
</tr>
<tr>
<td>Laverge et al. (2001)</td>
<td>Day 3</td>
<td>44.1</td>
<td>2.49</td>
<td>23.8</td>
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<tr>
<td>De Plácido et al. (2002)</td>
<td>Day 2/3 + zyg</td>
<td>75</td>
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<tr>
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<td>24.4</td>
<td>3.5</td>
<td>12.7</td>
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<tr>
<td>Rienzí et al. (2002)</td>
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<td>2</td>
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</tr>
<tr>
<td>Utsunomiya et al. (2002)</td>
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<td>2.9</td>
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</tr>
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<td>35.1</td>
<td>1</td>
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</tr>
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<td>De Sutter et al. (2003c)</td>
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<td>28.1</td>
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<td>Kolbianakis et al. (2004)</td>
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</tr>
<tr>
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<td>27</td>
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<td>Thurin et al. (2004c)</td>
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<td>1 + 1</td>
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</tr>
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<td>Tao et al. (2002)</td>
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<td>Day 5</td>
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<td>2.2</td>
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</tr>
<tr>
<td>Rijnders and Jansen, 1998</td>
<td>Day 5</td>
<td>53</td>
<td>2.25</td>
<td>30</td>
</tr>
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<td>1.9</td>
<td>26</td>
</tr>
<tr>
<td>Coskun et al. (2000)</td>
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<td>Shapiro et al. (2000)</td>
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</tr>
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<td>Milks et al. (2000)</td>
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<td>Gardner et al. (2000)</td>
<td>Day 5</td>
<td>87</td>
<td>2</td>
<td>70</td>
</tr>
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<td>Abdelmassih et al. (2001)</td>
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<td>29</td>
<td>2</td>
<td>29</td>
</tr>
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<td>Van der Auwera et al. (2002)</td>
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<td>Zollner et al. (2002)</td>
<td>Day 5 + zyg</td>
<td>19.6</td>
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<td>Day 5</td>
<td>21.2</td>
<td>1.4</td>
<td>34.4</td>
</tr>
</tbody>
</table>
No single static observation gives all the information contained in an embryo’s morphology. It is more logical to consider that a combination of different observations, preferably reflecting different aspects of implantation potential, should be used; e.g. it is likely that blastomere asymmetry and multinucleation both reflect aneuploidy (Hardarson et al., 2001). From the point of view of the prevention of MP, including twins, the discussion regarding the optimal stage to transfer remains largely academic. It seems more important to begin implementing SET rather than wait for any final proof as to which selection technique is ‘better’, because the question will always be: better for whom? Better for the average patient or for particular subgroups of patients? Although some centres proclaim excellent results after single blastocyst transfer (Gardner et al., 2004), others found the merit of blastocyst versus cleavage stage embryo transfer not to exist for the overall population (Coskun et al., 2000; Rienzi et al., 2002; Kolibianakis and Devroey, 2002; Bungum et al., 2003; Blake et al., 2004; Kolibianakis et al., 2004; Utsunomiya et al., 2004). Certainly, the fact that an embryo reaches the blastocyst stage per se does not mean that it will implant (Evsikov and Verlinsky, 1998; Magli et al., 1998, 2000; Sandalinas et al., 2001) and blastocyst culture cannot be considered a prerequisite for SET.

In summary, the introduction of SET compels embryologists to optimize their embryo selection procedure. It appears that much can be improved with respect to cleavage stage selection, which previously was often suboptimal; this was concealed by replacing a compensatory high number of embryos, thus leading to routine blastocyst culture with an unproven advantage.

Whether routine preimplantation aneuploidy screening is superior to (a combination of) morphology characteristics also remains unproven. Limited available evidence suggests that PGD serves mainly to prevent miscarriage, and after PGD, the ~35% ‘limit’ seems to appear again (Obasaju et al., 2001).

It should be underlined that to obtain a high ongoing PR, factors other than embryo competence play a role in the IVF/ICSI treatment sequence, e.g. ovarian stimulation, optimized laboratory conditions at oocyte retrieval and embryo transfer, transfer technique and endometrial receptivity. As we are moving towards more and more SET, these factors are of great importance.

All these considerations relate to fresh embryo transfers. Although routine day 2/3 assessment may at present appear to be at least as good as routine day 5 assessment, it remains possible that freezing may give better results at the blastocyst stage than at other stages of development (Veeck, 2003). Should further prospective trials prove this to be the case, the academic discussion concerning day 3/day 5 may conclude in favour of both.

### The role of cryopreservation

One benefit of SET is an increase in the number of embryos available for cryopreservation (De Neubourg et al., 2002). Optimized cryopreservation of embryos after SET is part of the strategy to decrease multiple pregnancies (Jones et al., 1995, 1997a, b; Gerris et al., 2003). Formerly a solution to an ethical problem—what to do with supernumerary embryos?—cryopreservation is now a goal in itself. It increases the cumulative PR per oocyte harvest and even allows patients who ‘desire’ a twin pregnancy to have their ‘delayed’ twin.

To date, there have not been many publications on the true cryo-augmentation effect after a fresh SET cycle.

In a group of 127 Finnish patients who had a fresh SET, 49 became pregnant (38.6%) and 34 delivered (26.8%); those without ongoing pregnancy had a total of 129 FET resulting in another 32 pregnancies and 32 deliveries. This increased the PR per patient to 62.4% and the delivery rate to 52.8% (Tiitinen et al., 2001, 2003). One other Finnish study showed a PR of 15% after the transfer of one and of 16% after two frozen-thawed embryos (Martikainen et al., 2001). In an Italian study the fresh clinical PR after DET was 56% for day 3 embryos and 58% for day 5 embryos. After one cryopreservation cycle the cumulative clinical PR rose to 85% for day 3 and to 66% for day 5 embryos (Rienzi et al., 2002). In a Swedish study, the transfer of one frozen-thawed embryo after a failed fresh cycle increased the cumulative PR (39.5%) to the same as after the transfer of two embryos (43.5%) (Thurin et al., 2004). In a Dutch study (Van Montfoort et al., 2004), the cumulative ongoing PR rose from 24 to 34% in SET patients and from 34 to 38% after DET. An Australian group performed a fresh transfer of either a single blastocyst or two blastocysts (PR of 44 and 59% respectively) followed by a frozen-thawed cycle of maximum two embryos, raising the PR per patient to 74% in the SET group and 70% in the DET group respectively (Catt et al., 2003). The twinning rates were 2 versus 44% for the fresh SET versus the fresh DET and 5 versus 28% after cryo-augmentation. A small recent Japanese study of 66 patients (Uchiyama et al., 2004) obtained a fresh PR of 44.9% in 66 fresh SET cycles and a cryo-augmented PR of 72.4% after 29 patients underwent a subsequent transfer of one frozen-thawed blastocyst. In this study, embryos had been cryopreserved by vitrification. Two American groups (Damario et al., 2000; Veeck, 2003) obtained

### Table IV. Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Day after fertilization</th>
<th>Maximum pregnancy rate (%) reported</th>
<th>Embryos/embryo transfer</th>
<th>Maximum implantation rate (%) reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frattarelli et al. (2003)</td>
<td>Day 5</td>
<td>69.2</td>
<td>2.0</td>
<td>43.5</td>
</tr>
<tr>
<td>Gardner et al. (2004*)</td>
<td>Day 5</td>
<td>60.7</td>
<td>1</td>
<td>60.7</td>
</tr>
<tr>
<td>Kolibianakis et al. (2004)</td>
<td>Day 5</td>
<td>39.5</td>
<td>1.8</td>
<td>26.6</td>
</tr>
<tr>
<td>Utsunomiya et al. (2004)</td>
<td>Day 6</td>
<td>25.8</td>
<td>1.2</td>
<td>24.1</td>
</tr>
</tbody>
</table>

*NES = not explicitly stated; zyg = zygote.*

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**Further Reading**

1. **Frattarelli** et al., 2002; Kolibianakis and Devroey, 2002; Bungum
2. **Studies utilizing single embryo transfer in bold.**
3. **NES = not explicitly stated; zyg = zygote.**
equally encouraging results after the transfer of thawed embryos, frozen at the pronuclear and the blastocyst stage respectively, but these frozen–thawed cycles did not take place after fresh SET cycles.

Further development of cryotechnology and increasing application of frozen–thawed cycles is becoming an integral part of an IVF/ICSI programme applying SET.

Elective SET as a clinical research model

Obstetric and neonatal outcome of IVF/ICSI pregnancies

SET of a top quality embryo could serve as a model for the prediction of early pregnancy outcome (De Neubourg et al., 2004a) and to study the obstetric and neonatal outcome of singletons and twins born after IVF/ICSI in a high proportion SET programme. It has been shown (Oehsenkuhn et al., 2003; Helmerhorst et al., 2004) that the outcome of singletons but not of twins after IVF/ICSI in a standard DET programme is worse than that of naturally conceived singleton or twins. Other authors found a worse outcome specifically for dizygotic twins after IVF/ICSI (Lambalk and van Hooff, 2001). The study of a sufficiently large cohort of singletons after SET may show whether we are looking at a patient effect, a treatment effect or an embryo effect.

It allows to answer the question whether reducing the number of embryos to transfer to and also reduces the incidence of the ovarian hyperstimulation syndrome. Preliminary data suggests this not to be the case (De Neubourg et al., 2004b).

Congenital anomalies after IVF/ICSI

Observation of children born after SET may confirm the largely settled debate on the incidence of congenital anomalies in IVF and/or ICSI children (Ericsson and Kjällén, 2001; Sutcliffe et al., 2001; Anthony et al., 2002; Bonduelle et al., 2002; Hansen et al., 2002; Bonduelle, 2004a,b).

Monozygotic twinning

Monozygotic twinning occurs more frequently (~1.5–2.0%) after IVF/ICSI than after natural conception (0.42%). Some authors have suggested that this could be due to blastocyst culture (Peramo et al., 1999; Sheiner et al., 2001; Tarlatzis et al., 2002; Wright et al., 2004), to zona pellucida (ZP) manipulation as when performing ICSI, subzonal insemination or partial zona drilling of the ZP or mechanical assisted hatching (Hershlag et al., 1999; Abuskeika et al., 2000; Schieve et al., 2000; Sills et al., 2000) or to an effect of age (Abuskeika et al., 2000). Others have found it to be independent of micromanipulation (Schachter et al., 2001) or of prolonged culture (Cassuto et al., 2003). The common hypothesis is alterations in the micro-architecture of the ZP (Alikani et al., 1994). One case–control study of 22 monozygotic twins suggests a positive effect of assisted hatching (Schwe et al., 2000; OR = 3.2, 95% CI = 1.2–8.0 for MP and OR = 3.8; 95% CI = 1.8–9.8 for singletons) and another case–control study of 207 monozygotic twins suggests a positive effect of blastocyst culture (Wright et al., 2004; OR = 3.91; 95% CI = 2.96–5.17 for MP and OR = 3.92; 95% CI = 3.92–5.17). One problem is that many HOMP are reduced, which makes it difficult to collect reliable data. SET may help in the correct diagnosis and registration of monozygotic twins, since their exact incidence after multiple embryo transfer remains difficult.

Impact of SET on registries and of registries on SET

SET has an important potential with respect to assisted reproduction registries and the relationship can be mutually positive. SET enforcing the quality of registries and registries serving to improve the quality of IVF/ICSI through monitoring an expected increase in SET. The effect of SET should be observed in national, regional and world assisted reproduction registries. Notwithstanding much interest and oral good-will, the much desired decrease in twinning rates has not yet been observed in national or European registries (European IVF-monitoring programme, 2001, 2002, 2004). This is in part due to the fact the data are released with a delay of some years. The only country where a decrease in the incidence of twins has already been demonstrated both in the IVF/ICSI registry and in the national total birth registry is Finland (Vilksa and Tiitinen, 2004).

Health-economic considerations

Although the main reason to apply SET is the health of the children at their start of life, financial considerations are also important. The increased utilization of hospital care in assisted reproduction children is the consequence of MP (Ericson et al., 2002). The estimated cost for an IVF singleton after IVF was calculated to be three times that of a twin (Wolmer-Hanssen and Rydstroem, 1998); an American group found a twin twice and a triplet 15 times as expensive as a singleton (Hidebaugh et al., 1997). Others have used a health-economic model to establish that SET and DET are financially equivalent per live-born child. SET needs more cycles and yields fewer children per cycle but DET yields higher obstetric and mainly neonatal costs per child (De Sutter et al., 2002, 2003b) and these effects balance out each other. They conclude that SET is to be preferred because most of the extra cost and care starts only after birth. This means cost and care for special education, increased pediatric morbidity, neurolinguistic and developmental retardation etc.

A Dutch retrospective cost analysis showed that an IVF twin pregnancy costs on average €10,000 more than an IVF singleton pregnancy (Lukassen et al., 2004). In a real-life prospective comparison between elective SET versus DET in women <38 years of age in their first IVF/ICSI cycle, elective SET of one high competence embryo was as efficient as DET (40% PR on both groups), but the cost per child was only approximately half after elective SET (Gerris et al., 2004). In all studies, as in the models, the major cost driver was the higher neonatal cost for prematurely born children.

Cost-effectiveness is also strongly dependent on the age of the female partner (Mol et al., 2000). An international survey of IVF costs revealed huge differences both in accessibility and cost per cycle between countries (Collins, 2002). In the USA the existence of (partial) insurance coverage had a depressive effect on the number of embryos transferred (Jain et al., 2002; Reynolds et al., 2003), although the mean number of embryos transferred in centres in states with complete coverage was still high. Reimbursement is not the whole story, although it incites a sense of responsibility in patients, serves as a lever to overrule
short-sighted measures for quick success and puts the quality of the child at the centre of the debate.

In Belgium, it has been calculated that the money saved by avoiding half of the MP suffices to finance all IVF/ICSI in a year. This is the basis for the Belgian reimbursement system. Everyone is supposed to win: the children (quality of health), the patients (accessibility to treatment), the government (less neonatal care costs) and the IVF centres (more accessibility means more work). The Achille’s heel of the system is the number of cycles: if this rises too much because of the higher accessibility, total costs for the governmental health insurance system could rise. In the larger scheme of things, in Europe, where the negative demographic trends provoke political talk about importing labour to maintain the ageing population which is dependent on social security provisions and pensions, creating desired healthy children will help to create healthy tax-payers.

In a wider health-economic perspective, it is also important to agree on when to begin with IVF and in whom it makes sense to refund it. In a systematic review of 2547 papers it was found that initiating treatment with intrauterine insemination (UII) is more cost-effective than IVF (Garceau et al., 2002). This has to be interpreted with care, because there is an overlap between patients suitable for UII and patients suitable for IVF; many patients have very poor chances for success with UII; and multiples after IUI must also be taken into account. The bottom line is that in order to keep the total number of IVF/ICSI cycles under control, UII will have to be given an important place in an integrated and evidence-based approach of high quality reproductive medicine.

**What is the best standard of success?**

Acknowledgement of the risks and complications of MP and the introduction of SET as a partial solution has sparked a lively debate on what is the best standard to measure or to express the quality ( = efficiency and safety) of individual IVF programmes.

An Australian team (Healy, 2004; Min et al., 2004) launched the concept of ‘BESST’ (birth emphasizing a successful singleton at term) as the best standard of success of assisted reproduction programmes. A lively discussion in Human Reproduction on this topic shows reluctance by some opinion leaders to accept rules to avoid twins (Alper, 2004; Davies et al., 2004; Dickey et al., 2004) or to consider BESST the best criterion (Griesinger et al., 2004). Some hold that no single outcome measure is satisfactory to evaluate the success of an assisted reproduction programme and that the essential aim of infertility treatment should be a healthy low order (singleton or twin) birth (Dickey et al., 2004). This stands in sharp contrast to the ethical standards issued by the ESHRE Task Force on Ethics and Law (2003) which makes four recommendations: SET, public funding for assisted reproduction, MP reported as complications not successes, and a uniform method of presenting results. Others hold in-between opinions, suggesting the application of SET and the use of fully descriptive reports where all variables can be separately read.

The BESST score was proposed with the sensible aim to correctly compare data between centres by recognizing the importance of the delivery of a single healthy baby as an appropriate endpoint. However, this score is sensitive to changes in IR which are not reflected in the BESST score: increasing overall success rates due to increased IR yields higher MPR but the BESST score will remain unaffected. Therefore this score does not appropriately reflect the IR and the proportion of MP in a particular IVF/ICSI programme. We calculated the evolution of the BESST score (per started cycle) in our programme since the introduction of SET and found that an increase in the score of 19.1% in 1998 to 22.2% in 2002 did not accurately reflect the decrease in twin pregnancies from 33.6 to 11.7% over that period.

Three measures of quality of a programme are advocated by a Danish group (Pinborg et al., 2004), one reflecting the pre-in vitro phase (number of oocytes per aspiration), one the in vitro phase (number of ongoing implantations per embryo transferred) and one the post-in vitro phase (number of deliveries per embryo transferred).

It cannot be the aim of each centre to reinvent SET. SET is not a ‘high tech’ discovery; it is just an example of common sense and reason in medicine. It is more of a philosophical idea than a technical innovation. This is most clearly expressed by Land and Evers (2004) who consider the best measure for a high quality assisted reproduction programme is indeed the percentage of SET performed in the centre.

It is heartening to see that on the other side of the Atlantic serious concern regarding the health and economic impact of MP has also been voiced (Jones and Schnorr, 2001; Adamson and Baker, 2004; Schieve and Reynolds, 2004), although some authors do not share the idea that twins should be considered a ‘complication’ instead of a success (Dickey et al., 2004) or stress the idea that SET is applicable to a small proportion of patients only (Alper, 2004). Nevertheless, SART intends to revise its guidelines. For most women aged < 35 years, SART will recommend the transfer of no more than two embryos and will suggest that SET be considered for those ‘with the most favourable prognosis’. Some American authors have taken the initiative to start a cautious application of elective single blastocyst transfer (Gardner et al., 2004; Milki et al., 2004).

**The patient’s perspective: information and counselling**

One of the most important challenges for providing safe IVF/ICSI treatment resides in the proper counselling of patients. Patients need proper and complete information (Buckett and Tan, 2004; D’Alton, 2004) about the fact that prevention is possible without serious decline in the chances for pregnancy, especially if combined with cryopreservation and if results are expressed (and patients or health insurers charged) per oocyte harvest and not per cycle or per transfer.

There are differences among patients, embryologists and clinicians in their perceptions of the desirability of MP (Hartshorne and Lilford, 2002). These have different origins and are determined by a mix of objective elements (statistics of chances for success versus risks for complications) and subjective factors (hope versus bad obstetric experience and the ‘happy twin’ illusion in the patient; wanting to perform versus a sense of responsibility in the professionals). Decisions imply a trade-off between the informed patients’ autonomy and the physicians’ clinical judgement, increasingly including medico-legal considerations.

This takes time, patience, commitment and personal conviction from those who treat and counsel. It also takes insight into
how patients think, or feel, about their chances and about risks they do not always understand (Ryan and Van Voorhis, 2004).

A British study investigated whether patients’ willingness to accept a hypothetical policy of SET changed with the method of providing information. The information that their chance for a pregnancy would not decrease due to SET and that fresh and frozen transfers would imply a fixed charge led to acceptance in a similar proportion of respondents who received a standard information pack (82%), an additional information leaflet (83%) or a personal discussion session (87%) (Murray et al., 2004).

A Danish group analysed attitudes of IVF/ICSI-twin mothers towards twins and embryo transfers, and found that only a quarter of these mothers agreed to SET (Pinborg et al., 2003). They also found that the delivery of a child with very low birthweight and hence morbidity was predictive of high acceptance of SET. It is noteworthy that not much was known concerning the ~20% of women who did not respond to the questionnaire and who may have been the ones with a bad obstetric outcome. They conclude that SET requires extensive counselling. It also illustrates that mothers of twins, even with some degree of disability, always love their children and would go through the same efforts and risks to have them. As long as they are under the erroneous conviction that their twin was the result of a choice between having twins or no children, they will not easily agree with SET.

The importance of complete information and of personal experience is shown by two observations. First, SET is more easily accepted, in fact often welcomed or even requested, by patients who have already obtained an IVF/ICSI pregnancy. Second, when starting to apply SET, it was not easy to convince patients to participate in a randomized trial (Gerris et al., 1999), which therefore had to be limited to very young patients in their first treatment cycle and who had at least two top quality embryos, whereas at present, it would be difficult to find patients to agree to DET. Other authors have had the same experience.

Conclusion and future perspectives

Elective SET, combined with subsequent transfer with frozen–thawed embryos, allows a high PR to be maintained with a dramatic decrease in MP. This allows a similar proportion of infertile patients to become pregnant. The medical complications, human suffering and expense that can thus be avoided should constitute a sufficiently strong argument to demand a reimbursement of IVF/ICSI costs, linked to a strict embryo transfer policy to avoid MP as well as a strict policy of acceptance of treatment to avoid over-consumption.

It has been argued that in the near future, SET should be the default policy for good prognosis IVF/ICSI patients. If at least one high competence embryo is available, only one should be transferred. Its definition should preferably be based on a large and ever-increasing number of one-to-one observations (Table III), so that selection is refined by experience. Since criteria cannot be extrapolated from one laboratory to another, the best option seems to be to allow centres build their own expertise. The pioneering groups in SET have a proportion of SET cycles > 60%; hence, the exception is non-SET. This is probably the way forward to a global implementation and should be welcomed. On the other hand, there will always remain a subgroup of patients in whom the transfer of more than one embryo remains acceptable.

An alternative to elective SET is the use of natural cycle IVF/ICSI (Pelinvck et al., 2002). Although this approach has its attractions (easy, cheap, repeatable, no ovarian hyperstimulation syndrome) its efficacy per cycle is low. The strength of elective SET resides in the fact that ovarian stimulation allows selection of the putative most competent embryo. Nevertheless, not much is known about implantation potential of embryos from natural cycles as compared with stimulated cycles (Zbie et al., 2004) and the possibility of natural cycle IVF/ICSI should perhaps be included as an option in some patients. Ovarian stimulation schemes might be adapted towards a lower dose approach, without compromising the possibility of choice. The search for ‘the’ best embryo to transfer should remain within reasonable limits, dictated by the intrinsic genetic limitations of human embryos.

The impact of SET will depend on the size of the group in whom it is applied. If criteria are very strict, the impact will be small, but the overall pregnancy rate is likely not to decrease. If the inclusion criteria are very liberal, the twinning rate will drop markedly, but the overall pregnancy rate might decrease somewhat, although the decrease appears to be much less than what was feared and it can be compensated by improved cryo-augmentation. To find the optimal trade-off between ongoing PR and twinning rate is the foremost clinical challenge for each IVF centre.

Clinical judgements regarding SET differ between centres and countries. The idea of SET represents more of a philosophy than a technique. Both aspects should inform wise actions.

References

Single embryo transfer and IVF/ICSI outcome


Kolibianakis EM, Zikopoulos K, Verpoot W, Joris H, Van Steirteghem AC and Devroye P (2004) Should we advise patients undergoing IVF to start a cycle leading to a day 3 or a day 5 transfer? Hum Reprod, 19,2545–2549.


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