Impact of patients’ choice for single embryo transfer of a top quality embryo versus double embryo transfer in the first IVF/ICSI cycle

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BACKGROUND: The aim of this study was to evaluate the impact of transferring a single top quality embryo in the first IVF/ICSI cycle of patients <38 years old who chose to have one or two embryos transferred. METHODS: A total of 262 patients participated in the study, and 243 transfers were performed: 156 (64%) patients chose the transfer of a single top quality embryo, if available, and two non-top quality embryos if not available; 87 (36%) patients chose to have a double embryo transfer regardless of embryo quality. RESULTS: In the first group an ongoing pregnancy rate of 40% (63/156) with a twin pregnancy rate of 2% (1/63) was achieved. In the second group the ongoing pregnancy rate was 44% (38/87) with 26% (10/38) twin pregnancies. In the patient group with only one embryo transferred, irrespective of the patient’s choice, the ongoing pregnancy rate was 43% (54/127) with no twin pregnancies. For the study population as a whole, the ongoing pregnancy rate was 42% (101/243) with 11% (11/101) twins. CONCLUSION: We conclude that the introduction of single embryo transfer in the first IVF/ICSI cycle is highly acceptable in women <38 years old.

Key words: embryo selection/single embryo transfer/twin pregnancy prevention

Introduction

The increasing success of IVF/ICSI in the 1990s has not only lead to an increased pregnancy rate, but also to a high percentage of multiple, mostly twin, pregnancies. Multiple pregnancy rates of ≥30% have been reported. The obstetrical risks and neonatal outcome, and the psychosocial and economical implications of multiple pregnancies for the children and their parents are important (Bergh et al., 1999; Schieve et al., 2002). Several steps towards reduction of multiple pregnancies have already been undertaken by many groups (Staessen et al., 1993; Templeton and Morris, 1998; Gerris et al., 1999; Vilska et al., 1999). Many elements must be considered in the discussion of how to reduce twin (and multiple) pregnancies in the IVF/ICSI population. Obviously, transfer of a single embryo is one answer. However, a previous study (Giorgetti et al., 1995) has shown that the pregnancy rate is directly related to embryo quality and may drop dramatically after compulsory single embryo transfer (SET). Therefore, the introduction of SET as a linear measure needs to be critically evaluated. Key factors in the discussion of SET are patient selection and embryo quality.

With regard to embryo quality, different groups have addressed the issue from different angles, but the endpoint is the same: the selection of embryos with the highest implantation potential. Some groups adhere to blastocyst culture and transfer (Gardner et al., 2000). Others prefer to critically evaluate pronuclear (Scott and Smith, 1998; Ludwig et al., 2000) and cleavage stage embryos on day 2 or 3 (Jackson et al., 1998; Pelinck et al., 1998) after fertilization.

We have previously shown (Van Royen et al., 1999) that embryos with a high implantation potential can be detected if the following criteria are met: embryos with four or five blastomers on day 2 and seven or more cells on day 3, with no more than 20% fragmentation and with the absence of multinucleated blastomers during the whole observation period.

These criteria were tested in a prospective randomized trial (Gerris et al., 1999) and led to an ongoing pregnancy rate of 38.5% after transfer of one such embryo; these embryos were therefore described as top quality embryos. The most salient conclusion of this study was that using the above described criteria, it was possible to introduce SET into daily clinical practice without a significant drop in the ongoing pregnancy rate. This is an important argument for the patient, especially if the couple considers a twin pregnancy to be a success in itself.

Subsequent to this study, we decided to introduce SET into clinical practice in a prospective cohort study. From our own data, we had calculated that after double embryo transfer (DET) 80% of twin pregnancies occurred in the two first IVF/ICSI cycles and that the implantation potential substantially
decreased from 38 years of age onwards. Patients prone to multiple pregnancies have been previously described (Staessen et al., 1993) as women <37 years old in their first three IVF/ICSI cycles with a minimum of six embryos of good quality. Coetsier and Dhont defined good prognosis for pregnancy patients as women <36 years old with more than three embryos with good embryo score available for transfer in their first three cycles (Coetsier and Dhont, 1998). Strandell et al. showed that the age of the patient and the number of embryos transferred are independent factors to predict multiple birth (Strandell et al., 2000).

Patients were thoroughly counselled about embryo transfer in their first IVF/ICSI cycle with regard to pregnancy rate and chance of a multiple pregnancy.

The data about the patients’ choice, patients’ characteristics, embryo quality, pregnancy chances and multiple pregnancy rates are described.

Materials and methods

Patients

From January 1, 2000 until December 12, 2001, 262 patients agreed to participate in the study after signing an informed consent. A total of 243 patients (93%) had embryo transfer. In 126 cycles (52%) there was male factor infertility, in 57 cycles (23%) female infertility, in 39 cycles (16%) mixed pathology and in 21 cycles (9%) idiopathic infertility. The mean age of the patients was 31.0 years (SD = 3.53).

Among the 19 cycles with no embryo transfer, two patients had no oocytes at the time of oocyte retrieval, in one patient no sperm could be obtained, 10 cycles showed fertilization failure and in six cycles no embryos suitable for transfer were obtained. The fertilization method was IVF in 140 (58%) cycles and ICSI in 103 (42%) cycles.

Ovarian stimulation protocol

Patients were treated with the long GnRH agonist desensitization protocol, starting in the mid-luteal phase with 6×100 µg buserelin (Suprefact; Hoechst, Germany) intranasally for 3 weeks. Gonadotrophin stimulation (Metrodin HP or Gonal-F; Serono, Geneva, Switzerland) was initiated if basal vaginal sonography showed a thin endometrium and no ovarian cysts. Stimulation was initiated with 150 IU of Metrodin HP or Gonal-F s.c. except in patients with known poor response, where 225 IU was used. The criterion for hCG administration was at least three mature follicles with a diameter of 18 mm. A total of 10 000 IU hCG (Profasi; Serono) was given i.m. exactly 37 h before oocyte retrieval.

IVF/ICSI procedure

Motile sperm were isolated from fresh semen in a two-step protocol: gradient centrifugation followed by migration–sedimentation. The micro-epididymal sperm aspiration (MESA) and testicular sperm extraction (TESE) procedures are described elsewhere (Silber, 1997; Tournaye, 1997). In all cases of MESA/TESE, frozen–thawed aspirates or biopsies were used.

Oocyte retrieval was performed vaginally under ultrasound guidance. Cumulus–oocyte complexes were isolated from the follicular aspirates and washed in Medi-Cult medium. Each was placed individually in a 25 µl microdrop of Ménézo B2 medium (C.C.D., Paris, France) under mineral oil (Sigma, St Louis, MA, USA) and incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. For standard IVF, 3–5 h after retrieval every oocyte was inseminated with ~20 000 motile sperm and incubated overnight. The ICSI standard procedure was performed.

Embryo quality assessment

Approximately 16–19 h after insemination/injection, normal fertilization was checked. All oocytes containing two clearly visible pronuclei were placed together in one fresh 10 µl microdrop of Ménézo B2 medium (maximum 10 oocytes/drop) and cultured for another 24 h. The next day (40–43 h after insemination/injection) the embryos were separated and each transferred to a 10 µl drop of Medi-Cult M3 medium for a further culture of 24 h. Every embryo was scored for the total number of cells, and the presence of anuclear fragments and multinucleated blastomeres. From the moment day 2 embryo criteria were recorded, embryos were cultured separately.

On day 3 (64–67 h after insemination/injection) embryo quality was evaluated again. Selection for embryo replacement was made according to the top quality embryo selection criteria, defined as follows: four or five blastomeres on day 2; seven or more blastomeres on day 3; and <20% fragmentation and total absence of multinucleated blastomeres at any stage of early cleavage. A final selection of embryos for transfer was based on implantation fractions (Van Royen et al., 2001). Embryos with >50% fragmentation were considered unsuitable for transfer. Supernumerary embryos were frozen. The criteria for cryopreservation of embryos were those which at the time of the transfer had six or more cells and <20% fragmentation with an absence of multinucleated blastomeres. Embryos with four cells on day 2 and seven or more cells on day 3 with up to 30% fragmentation were also cryopreserved.

Counselling about embryo transfer

In this study, patients <38 years old in their first IVF/ICSI cycle or after a previous delivery were thoroughly counselled about the embryo transfer as follows. We informed them of the chances of obtaining a pregnancy after transfer of a single embryo of top quality and how this would not decrease the chance of obtaining a pregnancy as compared with the results for the whole IVF/ICSI patient group. Because of our concern to keep the pregnancy rates stable at the time, we advised couples who chose SET of a top quality embryo but who did not produce such an embryo to have two embryos transferred. The final choice of whether to have one or two embryos transferred was left to the couple.

Embryo transfer technique

All transfers were performed on an outpatient basis using a Wallace embryo transfer catheter (Sims Portex Ltd, Hythe, Kent, UK) consisting of an inner and an outer catheter. The outer catheter was introduced first using a guidewire. Care was taken to limit the introduction of the outer catheter to a maximum of 4 cm into the cervix, in order to minimize potential microtrauma of the uterine cavity. The loaded inner catheter was then passed through the outer catheter until a total distance of 6 cm between the external os and the tip of the inner catheter was reached. The embryo(s) were gently expelled into the cavity in a volume of <30 µl Medi-Cult M3 medium. After removal, the catheter was checked under the microscope to ascertain that the embryo(s) were deposited in the uterine cavity. Strict care was taken that all clinicians followed the instructions for embryo transfer in a similar way.

Luteal phase

In all cycles, luteal phase was supported with 3×200 mg of micronized natural progesterone (Utrogestan; Laboratoires Piette International, Belgium) administered vaginally.

An ongoing pregnancy was defined as a conception cycle with at
least one fetal sac with a positive heartbeat reaching beyond 12 weeks amenorrhoea. Consequently, for the calculation of the ongoing implantation rate, biochemical conceptions, clinical miscarriages or extrauterine pregnancies were excluded from the calculation.

For statistical analysis, confidence interval analysis and $\chi^2$-test were used.

**Results**

From the 262 patients who agreed to participate in the study, 243 (92%) had embryo transfer. A total of 156 (64%) patients elected the transfer of one embryo of top quality if available and 87 (36%) patients elected to have two embryos transferred irrespective of the quality. The mean (±SD) age of patients was 31.3 ± 3.3 and 30.4 ± 3.9 years in the in the SET and DET groups respectively. Male factor infertility was 50% in the SET group and 54% in the DET group, female factor was 24% in the SET group and 23% in the DET group, combined factor infertility was 19% in the SET group and 10% in the DET group, and idiopathic infertility was 7% in the SET group and 13% in the DET group. These differences were not significant.

A total of 120 patients who chose to have SET received SET with 105 top quality SETs and 15 non-top quality SETs. Of these 15, 13 were compulsory SETs; there was only one embryo suitable for transfer. The other two patients decided to have SET regardless of the embryo quality. Thirty-six patients received two non-top quality embryos. The clinical outcome for the patients whose choice was SET is listed in Table I. There was an ongoing pregnancy rate of 43% after transfer of one top quality embryo and 40% after transfer of one non-top quality embryo. In the patient group receiving two non-top quality embryos, an ongoing pregnancy rate of 33% was obtained with one twin pregnancy (8%).

Of the 87 patients choosing DET, 80 actually received two embryos with seven patients receiving SET because only one embryo was suitable for transfer. Table II gives an exact overview of the embryos and their quality as well as the outcome. Patients choosing and receiving two embryos had an ongoing pregnancy rate of 44% and a 29% chance of obtaining a twin pregnancy. Although the chances of pregnancy were higher when two top quality embryos were available, the twinning rate also increased. The ongoing pregnancy rates after DET were 73, 35 and 26% after transfer of two top quality embryos, one top and one non-top quality embryo, and two non-top quality embryos respectively.

If we consider only data on SET (Table III), regardless of the choice of the patient, 43% (54/127) of the patients had an ongoing singleton pregnancy. In the particular case of embryos of non-top quality, a 40% (8/20) ongoing pregnancy rate was achieved.

With the strategy of counselling patients about the excellent chances of pregnancy with SET and about the increased chances of an adverse outcome with twin pregnancies, and allowing the couple to choose, an overall ongoing pregnancy rate of 42% was achieved. The twin pregnancy rate was 11% (Table IV).

The ongoing implantation rate of all top quality embryos (both from SET and DET) transferred was 72/159 (45.3%), and 29/154 (18.8%) for non-top quality embryos transferred (odds ratio = 2.4; 95% confidence interval: 1.7–3.5). Of the 243 patients, 156 (64%) had at least one top quality embryo.

In the group of patients choosing SET, 248 embryos in 156 cycles were cryopreserved and in the group choosing DET, 44 embryos in 87 cycles were cryopreserved. This means 1.59 embryos per cycle for SET versus 0.51 embryos per cycle in the DET group ($P < 0.0001$). The impact on the cumulative pregnancy rate per cycle could not yet be calculated at this point in the study.

**Discussion**

Although the obvious strategy to prevent multiple pregnancies is to apply SET, it was only after our prospective randomized trial (Gerris et al., 1999) showed that patients receiving SET had a 38.5% chance of obtaining a pregnancy that we felt at ease to do so in all first IVF/ICSI cycles. Although in this study the DET group had a significantly higher pregnancy rate (74.1%), the fact that the pregnancy rate for the SET group was similar to the pregnancy rate for the whole programme convinced us to proceed with SET.

Vilska et al. introduced the SET strategy in the Finnish population and compared elective SET (medical reasons, patients choice, risk of ovarian hyperstimulation syndrome) with SET because there was only one available embryo (compulsory SET) and reported pregnancy rates of 29.7% per cycle in the first group and 20.2% in the latter group (Vilska et al. 1999). During the same time period, DETs led to a pregnancy rate of 29.4%, but with a twin pregnancy rate of 23.9%. If the results of frozen–thawed embryos of the elective SETs were taken into account, a cumulative pregnancy rate of 47.3% was obtained per oocyte retrieval. On the basis of these data, SET was highly recommended, especially in women <35 years old and particularly if high-grade embryos were available for transfer.

Martikainen et al. reported a multicentred randomized study that compared SET versus DET in a first and second IVF/ICSI cycle in a patient group that had at least four good quality embryos available for transfer. The pregnancy rate was 32% in the SET group and 47% in the DET group (not significantly different, $P = 0.01$), but with 5 versus 39% twin pregnancies (significantly different) (Martikainen et al., 2001).
Table II. Clinical outcome of patients whose choice was double embryo transfer (DET)

<table>
<thead>
<tr>
<th>Transfer</th>
<th>DET</th>
<th>DET 1 top</th>
<th>DET 2 top</th>
<th>Total DET</th>
<th>SET</th>
<th>SET 1 top</th>
<th>SET 2 top</th>
<th>Total SET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>26</td>
<td>23</td>
<td>31</td>
<td>80</td>
<td>2</td>
<td>5</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Singleton pregnancies</td>
<td>13</td>
<td>5</td>
<td>7</td>
<td>25</td>
<td>1</td>
<td>2</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Twin pregnancies</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Ongoing pregnancies</td>
<td>19</td>
<td>8</td>
<td>8</td>
<td>35</td>
<td>1</td>
<td>2</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Percentage of twins</td>
<td>32</td>
<td>38</td>
<td>13</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Ongoing pregnancy rate (%)</td>
<td>73</td>
<td>35</td>
<td>26</td>
<td>44</td>
<td>50</td>
<td>40</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Ongoing implantation rate (%)</td>
<td>48</td>
<td>24</td>
<td>15</td>
<td>28</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

SET = single embryo transfer.

Table III. Clinical outcome after all single embryo transfers (SET) in the first IVF/ICSI cycle, irrespective of patients’ choice

<table>
<thead>
<tr>
<th>Transfer</th>
<th>SET</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>107</td>
<td>127</td>
</tr>
<tr>
<td>Ongoing singleton pregnancies</td>
<td>46</td>
<td>54</td>
</tr>
<tr>
<td>Ongoing pregnancy rate (%)</td>
<td>43</td>
<td>43</td>
</tr>
</tbody>
</table>

Table IV. Clinical outcome of the first IVF/ICSI cycle irrespective of the patients’ choice

<table>
<thead>
<tr>
<th>Transfer</th>
<th>SET</th>
<th>DET</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>127</td>
<td>116</td>
<td>243</td>
</tr>
<tr>
<td>Singleton pregnancies</td>
<td>54</td>
<td>36</td>
<td>90</td>
</tr>
<tr>
<td>Twin pregnancies</td>
<td>0</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Ongoing pregnancies</td>
<td>54</td>
<td>47</td>
<td>101</td>
</tr>
<tr>
<td>Percentage of twins</td>
<td>0</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>Ongoing pregnancy rate (%)</td>
<td>43</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>Ongoing implantation rate (%)</td>
<td>43</td>
<td>25</td>
<td>23</td>
</tr>
</tbody>
</table>

SET = single embryo transfer; DET = double embryo transfer.

The present study showed an ongoing pregnancy rate of 42% in the first IVF/ICSI cycle with a twin pregnancy rate of 11% regardless of the choice of the patient. On the other hand, if patients chose SET of a top quality embryo, or two embryos if there was no top quality embryo available, then the pregnancy rate was 43% with only 2% twin pregnancies.

If two non-top embryos were transferred—whether it be in the SET group with no top quality embryo available or in the DET group with no top quality embryos available—the twin pregnancy rate was 10%.

The conclusion of this study is that SET can easily be introduced into a twin-prone situation, i.e. patients <38 years old in their first IVF/ICSI cycle, yielding a high pregnancy rate and a low risk of twin pregnancy. Nowadays, many IVF centres as well as patients are convinced that twin pregnancies should and can be avoided in the first IVF/ICSI cycle. Nearly as important as avoiding twin pregnancies is the pregnancy rate. It is only because a high pregnancy rate can be achieved with SET that it became acceptable to clinicians who could then convince their patients.

Embryo selection plays a pivotal role in the success of SET. Embryos labelled as top quality show an ongoing implantation rate of 45.3 versus 18.8% for non-top quality embryos. The non-top quality embryos are also considered as having the second best implantation potential. These data correspond to the implantation potential of top quality embryos as calculated by Van Royen et al. (Van Royen et al., 2001). Presently, no data on the implantation potential of single blastocyst transfers are available. Studies of double blastocyst transfer show a pregnancy rate of 39% (Coskun et al., 2000) with 38% of twin pregnancies. Gardner et al. have described pregnancy rates of up to 87% with a twinning rate of 61% after transfer of two very high quality blastocysts and concluded that the way is set for SET (Gardner et al., 2000).

A reduction in the number of embryos transferred leaves a higher number of embryos for cryopreservation. If we take into account the pregnancies that will occur from the embryos that were cryopreserved in the SET cycle and add these to the pregnancy rate of SET (fresh cycle), then a cumulative conception rate may be calculated that probably will further increase the pregnancy rate of SET. This has been shown by Tiitinen et al. who obtained a cumulative delivery rate for elective SET and frozen–thawed embryo transfer (one or two embryos transferred) of 52.8% with a 7.6% twin rate (Tiitinen et al., 2001).

The challenge now lies in the implementation of SET into the general practice of IVF/ICSI and into the rest of the IVF/ICSI programme. As twin- and multiple-prone patients have been identified and described by ourselves and others (Staessen et al., 1993; Coetsier and Dhont, 1998), extension of SET to the second, third and maybe fourth cycle is under evaluation. Any further progress in embryo selection will only make it more easy to do so.

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References


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