Day 2 elective single embryo transfer in clinical practice: better outcome in ICSI cycles

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BACKGROUND: Single embryo transfer (SET) after IVF/ICSI has been shown to result in an acceptable pregnancy rate in selected subjects. In our unit, SET is routinely carried out among women under the age of 36 in the first or second treatment cycle when a top-quality embryo is available. In order to define further the selection criteria for SET, we have analysed the outcome of elective SET (eSET), including the cumulative pregnancy rate after frozen embryo transfers, performed in the years 2000–2002 in the Oulu Fertility Center. METHODS: During the study period, a total of 1271 transfers were performed, and in 468 cycles SET (39% of all transfers) was carried out. Of the SET cycles, in 308 cases a top-quality embryo was transferred on day 2 and extra embryos were frozen. Of these eSET cycles, ICSI was carried out in 87 cycles (28%). RESULTS: The overall clinical pregnancy rate per transfer was 34.7% in the eSET cycles. In the eSET ICSI cycles, the clinical pregnancy rate was significantly higher than in the corresponding IVF cycles (50.6 versus 28.5%, P<0.001). The cumulative pregnancy rate per patient after fresh and frozen embryo transfers was also significantly higher after ICSI (71.2 versus 53.4%, P<0.01). CONCLUSIONS: A high cumulative pregnancy rate per oocyte retrieval can be achieved after eSET in daily clinical practice. The implantation rate of fresh top-quality embryos in the ICSI cycles was significantly higher than in the IVF cycles, possibly due to more successful selection of the embryo for embryo transfer on day 2 after ICSI. In addition, our data suggest that embryo quality is a more important determinant of outcome than the age of the woman.

Key words: assisted reproductive technology/ICSI/IVF/multiple pregnancy/single embryo transfer

Introduction

The increasing number of multiple pregnancies, mostly as a result of assisted reproductive technology (ART), is a matter of concern in many countries. There is now much evidence that an acceptable pregnancy rate (30–40%) can be achieved by single embryo transfer (SET) in selected groups of subjects (Gerris et al., 1999; Vilska et al., 1999; Martikainen et al., 2001). In our prospective randomized multicentre study, the clinical pregnancy rate (PR) was 32.4% after SET and 47.1% after double embryo transfer (DET), and the cumulative PR was 47.3% after SET and 58.6% after DET (Martikainen et al., 2001).

Current data suggest that it will be necessary in the future to carry out more SETs in order to reduce the number of multiple pregnancies. After we finished our prospective randomized trial, the SET policy has gradually been implemented in our daily clinical practice. At present, SET is performed in >50% of cases. As a result of this change, the multiple PR has decreased to <10% in our unit. To convince clinicians and patients of the benefits of this policy, the results of the randomized trials, carried out with relatively small populations, need to be confirmed in daily clinical practice. It is essential to obtain more data on the selection criteria with regard to women undergoing SET. The purpose of this study was to evaluate the outcome of elective SET (eSET) cycles in two age groups and to compare IVF and ICSI cycles in order to be able to better select subjects for SET.

Materials and methods

During the study period, 2000–2002, a total of 1271 day 2 transfers were carried out in the Infertility Clinics of Oulu University and the Family Federation of Finland. Of these cycles, 803 DETs and 468 SETs were performed. In 308 cycles, a top-quality embryo was available for transfer, and extra embryos were frozen.

Embryo transfer was carried on day 2 after oocyte collection. The criteria for a top-quality embryo were: normal fertilization (2PN), 4–5 blastomeres on the day 2, <20% fragmentation and no multinuclear blastomeres. The criteria for freezable embryos were <20% fragmentation and no multinuclear blastomeres.
Table I. Clinical characteristics of the women in the eSET group

<table>
<thead>
<tr>
<th>Variable</th>
<th>IVF (n = 221)</th>
<th>ICSI (n = 87)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.7 ± 4.3</td>
<td>31.2 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Aetiology of infertility (%)</td>
<td>6.9 ± 2.5</td>
<td>6.8 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Male factor</td>
<td>52 (23.5%)</td>
<td>50 (56.6%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Low fertilize</td>
<td>48 (21.7%)</td>
<td>32 (36.8%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Tubal</td>
<td>36 (16%)</td>
<td>32 (36.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>45 (20%)</td>
<td>20 (23.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Mixed</td>
<td>6 (3%)</td>
<td>3 (3.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>Other female</td>
<td>29 (13%)</td>
<td>21 (24.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Unexplained</td>
<td>67 (30%)</td>
<td>30 (34.4%)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>3.9 ± 2.6</td>
<td>4.1 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Oocytes</td>
<td>14.0 ± 6.9</td>
<td>15.4 ± 6.8</td>
<td>NS</td>
</tr>
<tr>
<td>Cleaved embryos</td>
<td>9.2 ± 4.8</td>
<td>8.2 ± 4.6</td>
<td>NS</td>
</tr>
<tr>
<td>Frozen embryos</td>
<td>6.3 ± 4.2</td>
<td>5.7 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>Top embryos</td>
<td>2.5 ± 2.0</td>
<td>2.3 ± 0.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD. NS = not significant.

The characteristics of the study subjects and the treatment cycles are shown in Table I.

**Ovarian stimulation**

Ovarian stimulation was performed after pituitary downregulation with buserelin (Supercure®, Hoechst AG, Frankfurt, Germany) or nafarelin (Synarel®, Syntex Nordica AB, Södertälje, Sweden) for at least 10 days, followed by daily injections of recombinant FSH (recFSH; Gonal-F®, Laboratoires Serono; or Puregon®, Organon, Oss, The Netherlands). Oocyte pick-up was performed 34–36 h after the injection of 5000–10 000 IU of HCG (Profasi®, Ares-Serono; or Pregnyl®, Organon).

Oocytes/embryos were cultured in Universal IVF medium (Medicult A/S, Copenhagen, Denmark), Scandinavian IVF Science IVF-medium (Scandinavian IVF Science, Gothenburg, Sweden) or Sydney IVF Fertilization/Cleavage medium (Cook IVF, Queensland, Australia). One or two embryos were transferred into the uterine cavity 46–50 h after oocyte retrieval. Remaining good-quality embryos were frozen using a slow freezing protocol with 1,2-propanediol as the cryoprotectant.

Natural progesterone (200 mg × 3, Lugesterone, Leiras, Finland) was given transvaginally for luteal support for 14 days. Clinical pregnancies were confirmed by transvaginal ultrasonography.

**Statistical analysis**

The data were collected from a database created by Babe-3.0 computer software (X-treme-Solutions, Oulu, Finland).

Variables in the study groups were compared by using χ² tests and two-tailed t-tests with P < 0.05 as the limit of significance.

**Results**

During the study period, 803 DETs were performed. The overall clinical PR was 31.8% without any significant difference between IVF (165/507; 32.5%) and ICSI cycles (90/296; 30.4%). A total of 468 SETs were performed. In 160 cycles, only one embryo was available for transfer and the clinical PR in this group of compulsory SETs was 12.9%. In the remaining 308 cycles, a top-quality embryo was transferred and extra embryos were frozen. In these eSET cycles, the overall clinical PR was 34.7%. The outcome of fresh embryo transfer is shown in Table II. The clinical PR per transfer was 28.5% in the IVF group (63 pregnancies from 221 transfers) and 50.6% in the ICSI group (44 pregnancies from 87 transfers). There was one pair of monozygotic twins after eSET in the ICSI group.

In the IVF group, there were 250 frozen embryo (FET) cycles carried out among 144 women, and in the ICSI group, 61 transfers among 42 women. The outcome of these transfers is shown in Table III. The clinical PR per transfer was 23.6% in the IVF group (59 pregnancies from 250 transfers) and 34.4% in the ICSI group (21 pregnancies from 61 transfers). In the FET cycles, the majority of patients received two embryos. There were four twin deliveries in the IVF group (10.3%) and two in the ICSI group (13.3%). There were 17 SET cycles in the IVF group and three SET cycles in the ICSI group.

Up to now, there have been 122 clinical pregnancies in 118 patients in the IVF group and 65 clinical pregnancies in 62 patients in the ICSI group, giving cumulative PRs per patient of 53.4% in the IVF group and 71.2% in the ICSI group (P < 0.01). In the ICSI group, in which there are no ongoing pregnancies, a live birth rate per patient of 58.6% was achieved.

**Discussion**

The overall clinical PR after eSET was 34.7%, a figure that is in accordance with that in our randomized study (Martikainen et al., 2001) and a recent report from another Finnish clinic (Tiitinen et al., 2003). Putting together the data, it is obvious that an acceptable PR can be achieved after eSET in normal daily clinical practice.

In previous literature concerning SET, IVF and ICSI cycles have not been analysed separately. Surprisingly, we found, for the first time, that the clinical PR was significantly higher in the ICSI cycles. When we re-analysed the data in our previous randomized study (Martikainen et al., 2001), a similar difference between IVF and ICSI cycles (29 versus 44%) was also found in that population. Hence, this difference appears to be consistent in our IVF/ICSI programme.

The implantation rate of the top-quality embryos obtained after ICSI was significantly higher than that of the top-quality embryos obtained after IVF (52 versus 28%, P < 0.01), while the implantation rate of the non-top-quality embryos was not different between ICSI and IVF cycles (13 versus 14%). In addition, with regard to the outcome of the DETs, there was no
significant difference in the implantation rate between ICSI and IVF cycles during the study period.

There appears to be variation in the success rate after ICSI between clinics. In our unit, the results after ICSI have been slightly better than after IVF since ICSI was started. In our unit, and in all other clinics in Finland, the criteria for ICSI have been consistently strict, and ICSI is performed in ~30% of cases. This is in contrast to many other countries, where ICSI is carried out in 50–100% of cases (Nygren and Nyboe Andersen, 2002). In the literature, there is no evidence of the advantage of more widespread use of ICSI. In a randomized study, in which IVF and ICSI were compared in cases of non-male-factor infertility, no significant difference in outcome was observed when two embryos were transferred (Bhattacharya et al., 2001).

The reason for the difference observed in the PR between IVF and ICSI cycles is unclear. One explanation could be the different etiology of infertility in these two groups. In the ICSI treatment group, most of the cases (80%) had male factor as a reason for treatment, while in the IVF group the etiology was more heterogeneous. Subgroup analysis within the IVF group revealed a similar outcome regardless of the reason for treatment. Within the ICSI group, the PR was also similar in both the male factor and the low fertilization group. Hence, the data suggest that the higher implantation rate is not solely explained by the different groups of subjects. However, it must be stressed that this study is retrospective, and all possible biases therefore cannot be excluded. To elucidate this issue further, a randomized study, in which ICSI and IVF are being compared in cases of non-male factor infertility, has been started in our unit.

One possible explanation for the higher implantation rate in the ICSI cycles could be the superiority of the ICSI technique. The mean total numbers of cleaved, frozen and top-quality embryos obtained were similar after IVF and ICSI (Table I). Hence, the ICSI technique seems not to have any particular beneficial effect on embryo morphology, but, for some reason, day 2 selection of the best embryo for eSET is apparently more successful after ICSI.

We have observed continuous improvement in the freezing results in our unit concomitantly with the increased use of SET. In this study population, the proportion of FET pregnancies was 47.5% in the IVF group and 32.3% in the ICSI group, indicating the efficiency of the cryopreservation programme. In the FET cycles, in which the SET policy was not followed, the PR per embryo transfer was higher in the ICSI group, with the difference almost reaching statistical significance (23.6 versus 34.4%, \( P = 0.08 \)). This result suggests better frozen embryo quality after ICSI. In these cycles, the twin rate was 10.3% in the IVF group and 13.3% in the ICSI group. These figures are slightly higher than that after fresh embryo transfer in our overall population but lower than usually observed after DET. Hence, in the future, more emphasis should be given to the embryo transfer policy in FET cycles (Tiitinen et al., 2001), and more SETs should be performed in these cases, too.

In this study, the cumulative PR per patient was 53.4% in the IVF group and 71.2% in the ICSI group. These figures are even higher than in our previous randomized study (Martikainen et al., 2001). In the ICSI group, a live birth rate per patient of 57.5% was recorded.

In this study, most of the women were younger than 36 years, and there were 39 women >36 years of age. In this older group of women, there were eight clinical pregnancies in 11 women (PR 73%) after ICSI and nine pregnancies in 28 women after IVF (PR 32%). This result supports the view that embryo morphology is a more important determinant of outcome than the age of the woman, and that eSET might also be warranted in older women if a top-quality embryo is available.

At the moment, the proportion of SET cycles is >50% in our unit and the twin rate is ~7% in the ongoing pregnancies. Despite this, the overall clinical PR has been >30%. Hence, the more clinical experience of SET is gained, the higher the proportion of women eligible for the procedure. In our unit, practically all couples accept the SET policy after being informed of the results obtained. In Finland (with 17 units), the proportion of SET cycles increased to 30% up to the year 2001 but, despite this, the multiple PR is still ~20%. Therefore, it is likely that in order to reach a multiple PR of 10%, more SETs should be performed.

This study indicates that the SET policy can be implemented in clinical practice, resulting in a very high cumulative PR in this good prognosis group of subjects. The results obtained in our clinic and in other clinics in Finland (Tiitinen et al., 2003) encourage the shift to SET policy, associated with efficient use of cryopreservation, to bring about the optimal outcome and increase the safety of ART treatment.

References


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