The role of the number of replaced embryos on intracytoplasmic sperm injection outcome in women over the age of 40


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In order to examine if the transfer of more than three embryos has any beneficial effect on the outcome of intracytoplasmic sperm injection (ICSI) cycles in women aged >40 years, a retrospective analysis was made of all the ICSI cycles which were performed in this age group from 1 October 1991 to 31 December 1995. A total of 525 cycles was performed in 321 patients. In 413 cycles, at least one normally fertilized embryo was available for transfer. In 271 cycles, one to three embryos were replaced while in the remaining 142 cycles at least four embryos were replaced. There was no difference in implantation rate (number of gestational sacs/number of embryos transferred), after the transfer of one to three embryos (5.2%), compared with the transfer of at least four embryos (5.1%). The pregnancy rate/embryo transfer and the clinical pregnancy rate/embryo transfer were, however, higher when at least four embryos were replaced than was the case with one to three embryos (27.5 versus 11.8%, \( P < 0.0001 \) and 20.42 versus 9.96%, \( P < 0.005 \), respectively). There were no statistically significant differences in the delivery rates, multiple pregnancy rates or spontaneous abortion rates. The pregnancy rate and the clinical pregnancy rate after ICSI in women >40 years of age are related to the number of embryos replaced.

Key words: female age/implantation/intracytoplasmic sperm injection (ICSI)/number of embryos replaced/pregnancy rate

Introduction

The progressive decline in female fecundity that accompanies advancing age is a commonly recognized physiological phenomenon. Previous studies have documented an age-dependent decrease in female fecundity beginning at age 30, with a more obvious decline in the late 30s and virtual loss of reproductive potential by the mid-to late-40s (Stovall et al., 1991; Navot et al., 1994). This age-related reproductive failure is manifested in both lower pregnancy rates (PR) and higher rates of early pregnancy loss with advancing maternal age (Stein, 1985; Romeu et al., 1987).

Success of in-vitro fertilization (IVF)–embryo transfer and ICSI has also been reported to be adversely affected by increasing age (Piette et al., 1990; SART-ASRM, 1995; Devroey et al., 1996; Templeton et al., 1996). The decline in fertility may be accounted for either by a reduction in implantation rates or by a rise in spontaneous abortion rates in women over the age of 40 (Stein, 1985; Abdalla et al., 1993).

Considerable debate persists about the factors responsible for this age related decline in women's fecundity. Several hypotheses have been suggested to explain the inefficiency of assisted reproductive technology (ART) in women of advanced age. Most of these hypotheses attribute reduced pregnancy rates to factors associated with either endometrial (Meldrum, 1993), or oocyte ageing (Navot et al., 1994; Sauer et al., 1994). According to the first theory, factors such as defective hormonal milieu, poor uterine blood flow and inadequate endometrial proliferation cause reduced endometrial receptivity and hence implantation failure. According to the alternative explanation, factors associated with reduced embryo quality, ageing of oocytes and chromosomal imbalance are responsible. The primary explanation, however, is reduced implanting ability of the embryo due to poor oocyte quality (Yaron et al., 1993; Alsalili et al., 1995; Hull et al., 1996).

Another constant observation in many studies is that the incidence of multiple pregnancies is inversely related to female age (Balen et al., 1993; Navot et al., 1994; Oehninger et al., 1995).

A possible policy to improve the success rate of ART in women over the age of 40 may therefore involve the transfer of more than three embryos, when available, in such women. There is uniformity of opinion in the medical community that the number of embryos transferred during ART procedures should be restricted in order to minimize the risk of multiple pregnancy. However, legislative restrictions do not take into account the age-related decrease in female fecundity. Voluntary restrictions in some centres consider this age related decrease but in others do not (Staessen et al., 1992; Balen et al., 1993; Staessen et al., 1993). A possible alternative could be the use of oocytes from young donors, a procedure with well documented success (Meldrum, 1993; Sauer et al., 1994).

The aim of this study was to examine the outcome of intracytoplasmic sperm injection (ICSI) cycles in women over the age of 40 according to the number of embryos replaced and also to evaluate whether the transfer of more than three embryos, when available, has any beneficial effect on the outcome of ICSI in this age group.

Materials and methods

A total of 525 cycles of ICSI, which were performed in 321 women older than 40 years, have been analysed retrospectively. These cycles
Oocytes were retrieved, in 11 cycles (9.8%) no spermatozoa were available for intracytoplasmic injection, in 46 cycles (41.1%) none of the injected oocytes fertilized normally (2PN) and in 46 cycles (41.1%) available for intracytoplasmic injection, in 46 cycles (41.1%) no mature oocytes were transferred. In 24 of these 112 cycles (21.4%) no mature oocytes were available for transfer, in group 2 (n = 271) one to three embryos were replaced, and in group 3 (n = 142), four or more embryos were transferred.

All patients received the same regimen of ovarian stimulation which has been extensively reported elsewhere (Smitz et al., 1987). This was the ‘long’ protocol with the combined use of a gonadotrophin-releasing hormone agonist (GnRHα, buserelin; Suprefact; Hoechst, Frankfurt, Germany) and human menopausal gonadotrophins (HMG) (Humegon; Organon, Oss, The Netherlands; Profasi; Serono, Geneva, Switzerland). The GnRHα was usually commenced on day 1 of the menses at a dose of 100 μg six times daily. The agonist was not administered during the night. When serum oestradiol was <40 ng/l, progesterone <0.3 ng/l and no follicles of diameter >6 mm were found by vaginal ultrasound scan, stimulation with HMG was started. The initial dose was at least 150 IU daily i.m. for 4 consecutive days and if by the fifth day serum oestradiol had not increased by 40% of the preceding value, the dose was increased by at least 75 IU. Monitoring was performed by serial vaginal ultrasound scans and serum oestradiol assays. When at least three follicles of diameter ≥17 mm were recorded by ultrasound scan, then 10 000 IU of human chorionic gonadotrophin (HCG) (Pregnyl; Organon, Profasi; Serono) was injected i.m. Oocyte retrieval was carried out 36 h later under vaginal ultrasound guidance.

Oocyte handling and ICSI procedures have been extensively described elsewhere (Van Steirteghem et al., 1993, 1995). Fertilization was confirmed by the presence of two pronuclei ~16 h after ICSI and further embryonic cleavage was noted (Nagy et al., 1994). The embryos were scored according to the equality of size of the blastomeres and the number of anucleate fragments (Deschacht et al., 1988; Staessen et al., 1989). Cleaved embryos with <50% of their volume filled with anucleate fragments were eligible for transfer (Van Steirteghem et al., 1993). Embryos were transferred ~48 h after oocyte recovery. The luteal phase was supplemented with progesterone administered vaginally or with HCG administered i.m.

Pregnancy was confirmed by detecting increasing serum HCG concentrations on two occasions at least 12 days after embryo transfer. Patients were considered to be clinically pregnant if there was a positive pregnancy test together with ultrasonographic visualization of at least a gestational sac at 7 weeks. The implantation rate was defined as the ratio of the number of sacs/number of embryos transferred.

Prenatal diagnosis was proposed by chorionic villous sampling at 9–10 weeks of gestation or by amniocentesis at 16 weeks of gestation. Genetic counselling was given subsequent to prenatal diagnosis and for the planning of a prospective follow-up study of the children born after ICSI. The referring gynaecologist and the patients were asked to provide detailed information of the evolution of the pregnancy and the outcome of the delivery (Bonduelle et al., 1995; Wisanto et al., 1995).

Statistical analysis was performed using the χ² test of association to examine differences in proportions. A value of P < 0.05 was considered statistically significant.

### Results

An embryo transfer was performed in 413 out of 525 cycles of ICSI (78.7%). In 112 cycles no embryo was available for transfer. In 24 of these 112 cycles (21.4%) no mature oocytes were transferred.

### Table I. Characteristics of the cycles in relation to the number of embryos replaced

<table>
<thead>
<tr>
<th>No. of cycles</th>
<th>0 embryos</th>
<th>1–3 embryos</th>
<th>≥4 embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ETs</td>
<td>112</td>
<td>271</td>
<td>142</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>42.1 ± 2</td>
<td>41.9 ± 2</td>
<td>41.8 ± 2</td>
</tr>
<tr>
<td>Age (median)</td>
<td>42</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Age (range)</td>
<td>40–49</td>
<td>40–48</td>
<td>40–48</td>
</tr>
<tr>
<td>No. of embryos replaced (mean ± SD)</td>
<td>0</td>
<td>2.1 ± 1</td>
<td>4.7 ± 1</td>
</tr>
</tbody>
</table>

### Table II. Outcome of embryo transfers

<table>
<thead>
<tr>
<th>No. of ETs</th>
<th>0 embryos</th>
<th>1–3 embryos</th>
<th>≥4 embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancies (PR³)</td>
<td>32 (11.8)</td>
<td>39 (27.5)</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Clinical pregnancies (Cl.PR³)</td>
<td>37 (10.0)</td>
<td>29 (20.4)</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>5.20</td>
<td>5.10</td>
<td>NS</td>
</tr>
<tr>
<td>Deliveries (delivery rate³)</td>
<td>19 (7.0)</td>
<td>18 (12.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>3 (11.1)</td>
<td>5 (17.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Spontaneous abortions</td>
<td>7 (25.9)</td>
<td>10 (34.5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Figures in parentheses represent percentages.

³Per embryo transfer.

ET = embryo transfer; PR = pregnancy rate; NS = not significant.

### Discussion

The influence of the number of embryos transferred on pregnancy outcome is shown in Table II. The pregnancy rate was significantly higher in those women in whom more than four embryos were replaced, i.e. 27.5 versus 11.8% when one to three embryos were transferred (P < 0.0001). The clinical pregnancy rate was also significantly higher in the same group of women, i.e. 20.4% when at least four embryos were replaced versus 10.0% when one to three embryos were replaced (P < 0.005).

In contrast, there were no statistically significant differences in implantation rate, multiple pregnancy rate or spontaneous abortion rate, although these rates were higher when at least four embryos were replaced than when one to three embryos were replaced. The delivery rate, although 1.8 times higher in group 3 than in group 2 (12.7 versus 7%), was not statistically significantly different (P = 0.083). All the eight multiple pregnancies were twins. There were no multiple pregnancies of higher order than twins. Among these multiple pregnancies, selective reduction was performed from twin to singleton in two cases, one in each group. One woman with a twin pregnancy after the transfer of four embryos miscarried at the 13th week of gestation. The remaining twin pregnancies led to the delivery of five sets of twins and two singletons (after...
the two selective reductions). In two women, one from group 2 and one from group 3, an induced abortion was performed because of the prenatal diagnosis of chromosomal abnormalities (both trisomy 21). There were no ectopic or heterotopic pregnancies in this study population.

Discussion

The decline in fecundity which women experience as they progress through their reproductive life has been extensively studied and is well established (Stovall et al., 1991; Navot et al., 1994). This phenomenon has also been observed in cases of ART, and especially after IVF–embryo transfer and ICSI (Piette et al., 1990; SART-ASRM 1995; Devroey et al., 1996; Templeton et al., 1996).

The lower pregnancy rates in older women undergoing ART treatment may be attributed to oocyte senescence or to an age-related decline in endometrial receptivity (Meldrum, 1993; Yaron et al., 1993; Isaili et al., 1995).

In order to compensate for reduced fecundity in women >40 years, the number of embryos transferred may be increased. This strategy is greatly limited by the reduced number of embryos available for these patients and from the well-known relationship between the incidence of multiple pregnancies and the number of embryos replaced.

The data from our study highlight clearly that the pregnancy rate per embryo transfer was significantly increased in women ≥40 years of age, when at least four embryos are transferred (27.5 versus 11.8%, P < 0.0001). The clinical pregnancy rate per embryo transfer was also statistically significantly increased in the same group (20.42 versus 9.96%, P < 0.005). The delivery rate was 1.8 times higher when at least four embryos were replaced, but this did not attain statistical significance, probably because of the limited number of deliveries.

The implantation rate was similar in both groups (5.2 versus 5.1%), and this rate was within the range found by the same centre in a previous case-controlled study and similar to the findings by Hull et al., who recently reported an implantation rate of 6.1% in IVF–embryo transfer cases in women >40 years of age (Devroey et al., 1996; Hull et al., 1996). There is convincing evidence that the main reason for the decreased implantation rate with advancing maternal age is related to poor oocyte quality rather than to uterine receptivity (Navot et al., 1994; Oehninger et al., 1995). The results from oocyte donation programmes clearly indicate that donated oocytes from young donors implant satisfactorily in cases where the patients’ own oocytes fail (Yaron et al., 1993; Sauer et al., 1994). Fertilization and embryo cleavage rates are not affected adversely by age and the numbers of embryos available reflect the number of oocytes obtained (Hull et al., 1996). Age-related oocyte factors commonly lead to aneuploidy, including mosaicism in the cleaving embryo, but without interfering with fertilization, including the formation of a two-pronucleate zygote. Expression of the embryonic genome would occur only after the 4–8-cell stage and might account for the later wastage by failure of implantation and clinical spontaneous abortion despite apparently normal fertilization (Angell, 1994; Munne et al., 1995).

It is obvious that increasing the number of embryos transferred also increases the chances of achieving a pregnancy, but there is a parallel risk of increasing the incidence of high-order multiple pregnancies. This leads to a high rate of spontaneous abortions and other obstetric and neonatal complications and finally, to increased maternal morbidity and perinatal morbidity and mortality. Associated with this phenomenon is the fact that in the last decade the majority of assisted reproduction centres world-wide have restricted the number of embryos replaced to three or fewer, some voluntarily and others as a result of legislative restrictions (Balen et al., 1993; Staessen et al., 1993).

Our results indicate that in women ≥40 years of age, there is no significant difference in multiple pregnancy rates or in spontaneous abortion rates, when four or more embryos were replaced. Probably in a larger series, with more multiple pregnancies, there would be a difference. In addition, there were no high-order multiple pregnancies (e.g. triplets). In two cases of twins, one in each group, a selective reduction was performed at the patients’ request. This procedure is a possible approach, especially to high-order multiple pregnancies, but it may create other medical, ethical or psycho-social problems for the patient (Bollen et al., 1993).

With the social trend to delay childbearing because of careers, the treatment of the older infertile female has become a major challenge to today’s fertility specialist. When deciding on how many embryos to transfer, a compromise should be reached between optimizing chances of conception and an acceptable multiple pregnancy rate.

In conclusion, bearing in mind the limitations of a retrospective study, our data demonstrate that the probability of pregnancy after ICSI in women ≥40 years of age is related to the number of embryos replaced. Because the implantation rate was the same in the two groups studied, a good strategy to improve the pregnancy rate might be to increase the number of embryos replaced. Given that the multiple pregnancy rate and the spontaneous abortion rate were not significantly higher when four or more embryos were replaced than when three or fewer embryos were replaced, it seems advisable to replace a minimum of four embryos, where available, in this group of patients, in order to improve their chances of success and with little risk of multiple pregnancy.

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