Female age predicts embryonic implantation after ICSI: a case-controlled study


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From 1 October 1991 until 31 December 1993, 1270 cycles for intracytoplasmic sperm injection were performed. Of these, 71 (5.6%) were carried out in women ≥40 years of age. The semen characteristics in couples ≥40 years of age were similar. The mean male age for the older group of women was 47.1 years (range 34-67) versus 35 years (range 25-71) for the younger group of women (P < 0.001). The mean female age was 41.9 years (range 40-47) and 31.8 years (range 23-39). The numbers of cumulus-oocyte complexes and metaphase-II oocytes were significantly lower in women ≥40 years of age (P < 0.001). The mean numbers of replaced embryos were respectively 2.3 (133/59) in women ≥40 years of age and 2.5 (160/63) in women <40 years of age. The delivery rate per retrieval and per transfer was significantly lower in women ≥40 years of age (P < 0.05). The delivery rates per retrieval and per transfer were respectively 7% (5/71) and 8.5% (5/59) in the older group of women versus 22.5% (16/71) and 25.4% (16/63) in the younger group. Female age is the predictive factor for embryonic implantation.

Key words: delivery rate/intracytoplasmic sperm injection/male infertility/women ≥40 years of age/women <40 years of age

Introduction

In recent years intracytoplasmic sperm injection (ICSI) has developed successfully with regard to male-factor infertility (Van Steirteghem et al., 1993a,b). It is a well known phenomenon that ongoing pregnancy rates after assisted reproductive technology decrease significantly with advancing age (Fivnat, 1990, 1993). In women ≥40 years the delivery rate is almost negligible (Schwartz, 1983; Greenhall and Vessey, 1990; Lansac, 1995). The aim of this study was to analyse the pregnancy outcome after ICSI in male factor infertility in women ≥40 years of age. A case-controlled study is reported comparing the outcome of 71 ICSI procedures in women ≥40 years of age with women <40 years old.

Materials and methods

Out of 1270 women stimulated for oocyte retrieval and ICSI, 71 (5.6%) were ≥40 years of age. For each patient ≥40 years of age a subsequent cycle of a patient <40 years undergoing ICSI was included as a reference.

In the group of women ≥40 years of age, the mean age was 41.9 years (range 40-47) and their partners' age was 47.1 years (range 34-67). In the reference group of women <40 years of age the mean age was 31.8 years (range 23-39) and for their partners' 35 years (range 25-71) (P < 0.001). All couples suffered from pure male factor infertility. The mean duration of infertility was 11.7 years (range 2-12) in women ≥40 years of age and 8.6 years (range 4-12) in women <40 years of age. The mean basal follicle stimulating hormone (FSH) values were 6.5 IU/l (range 1.5-13.7) in women <40 years of age and 9.7 IU/l (range 4.7-16.3) in women ≥40 years of age (P < 0.05). For semen concentration and motility WHO guidelines, and for morphology Kruger criteria were applied (Kruger et al., 1986; WHO, 1992). Table I describes volume, concentration, motility and morphology of the male partners in the two female age groups (≥40 years and <40 years of age). No significant differences were observed.

Ovarian stimulation regimens have been extensively reported elsewhere. A luteinizing hormone releasing hormone (LH-RH) analogue, Buserelin (Suprefact®: Hoechst, Frankfurt, Germany), was commenced on day 1 of the menses at a dose of 100 µg per puff six times daily. The agonist was not administered during the night. When serum 17-β oestradiol was ≥40 ng/l and progesterone ≤0.3 µg/l, and when no follicles of >6 mm were found on ultrasound, stimulation with human menopausal gonadotrophin (HMG; Humegon®, Organon, Oss, The Netherlands; Pergonal: Serono, Geneva, Switzerland) was started. Gonadotrophin stimulation was started with 150 IU for 4 consecutive days and if at the fifth day serum oestradiol was not increased by 40% of its preceding value the dose was increased by 75 IU. Where at least three follicles of ≤17 mm diameter were recorded at vaginal ultrasound 10 000 IU human chorionic gonadotrophins (HCG; Pregnyl®, Organon, Oss, The Netherlands; Profasi®: Serono, Geneva, Switzerland) was injected (Smits et al., 1987). Oocyte retrieval was carried out 36 h later. The luteal phase was supplemented with 600 mg micronized progesterone vaginally administered three times daily (Utrogestan®, Pitee, Brussels, Belgium) (Smits et al., 1993).

Oocyte handling and ICSI procedures have been extensively described elsewhere (Van Steirteghem et al., 1993a,b). Two-pronuclear (2PN) fertilization was confirmed by the presence of two pronuclei approximately 16 h after microinjection and further embryonic cleavage was noted as described previously (Nagy et al., 1994). Further embryonic development was assessed under an inverted microscope (×200 or ×400 magnification) 40 h after sperm injection. The embryos were scored accordingly. Type A or excellent embryos were defined as embryos in which all blastomeres were of equal size or, if of unequal size, were without anucleate fragments. Type B or good embryos had blastomeres of equal size or, if of unequal size, had a maximum of 20% of the volume of the embryo filled with anucleate fragments. In type C or fair embryos, anucleate fragments were present in 20-50% of the volume of the embryo (Staessen et al., 1994).
A clinical pregnancy was determined by visualization of a gestational sac by vaginal ultrasound examination at 7 weeks. An ongoing pregnancy was recorded if fetal heart activity was positive at >12 weeks. Chorionic villi sampling at 7 weeks, amniocentesis at 16 weeks and a follow-up of children born was proposed (Bonduelle et al., 1994).

**Statistical analysis**

The statistical tests were carried out two-tailed at the 5% level of significance. The comparison of continuous variables in patients <40 years of age and patients ≥40 years of age was performed using the t-test for independent measurements. The \( \chi^2 \) test was applied for the comparison of the discrete variables.

**Results**

As indicated in Table I, no significant differences were found in relation to semen volume, concentration, motility and morphology in the two female age groups, i.e. ≥40 years and <40 years of age.

As demonstrated in Table II, the mean numbers of cumulus-oocyte complexes and metaphase-II oocytes were significantly lower in women ≥40 years of age (\( P < 0.001 \)). The 2PN fertilization rate of intact oocytes after injection was similar in women ≥40 years of age and <40 years of age. In women ≥40 years of age 184 embryos were transferable, 133 were transferred and 42 were cryopreserved for later use. In women <40 years of age 309 embryos were transferable, 160 were transferred and 106 were cryopreserved.

As indicated in Table III, for women ≥40 years of age, in 83.1% of the cases (59/71) and for women <40 years of age in 88.7% of the cases (63/71) an embryo transfer was performed.

The percentage of excellent embryos per egg retrieval was significantly decreased (\( P = 0.017 \)) in women ≥40 years of age, on average, i.e. 0.17/retrieval versus 0.61/retrieval in women <40 years of age (Table IV).

On average, 2.3 embryos (133/59) were replaced in women ≥40 years of age and 2.5 embryos were replaced in women <40 years of age.

The ongoing implantation rate (>12 weeks of gestation) was significantly lower in women ≥40 years of age, i.e. 4.5% (6/133) versus 14.3% (23/160) in women <40 years of age (\( P = 0.003 \)).

The delivery rate per ovum retrieval and per transfer was significantly lower in women ≥40 years of age (\( P < 0.01 \)). In women ≥40 years of age, out of 71 retrievals, five patients delivered (7%) and 16 delivered in women <40 years of age (22.5%) (Table III).

**Discussion**

It is well known that fecundity decreases progressively with advancing age (Schwartz, 1983; Greenhall and Vessey, 1990; Lansac, 1995). This phenomenon is also observed after assisted reproductive technology, and especially after in-vitro fertilization (TVF) (Fivnat, 1990, 1993).

As expected, in women ≥40 years of age the mean serum FSH concentrations were significantly increased as compared to the younger age group. It is important to notice that no pregnancies were observed if the basal FSH level was over 7.9 IU/L. This observation indicates that serum FSH concentration is a most important predictive factor.

The data of our case-controlled study highlight clearly that the delivery rates per cycle and per transfer are significantly decreased in women ≥40 years of age. In this study, couples suffering from male factor infertility were chosen because infertility was not caused by the female partner.

We were interested to know, with reference to ICSI, whether the embryonic implantation capability would be lower in women ≥40 years of age. Our data clearly demonstrate that in such women the ongoing implantation rate for an embryo obtained after ICSI is only 4.5%, which is significantly less than in women <40 years of age (14.3%). These data are in
An increased follicle disappearance was found at 38 years of age and subsequently significantly fewer metaphase-II oocytes were available for injection. This observation is in agreement with the mathematical model suggesting that follicle dynamics are age dependent. It is true that significantly fewer cumulus-oocyte complexes were retrieved in women ≥40 years of age and subsequently significantly fewer metaphase-II oocytes were available for injection. This observation is in agreement with the mathematical model suggesting that follicle dynamics are age dependent.

### Table III. Pregnancy and delivery rate per egg retrieval and per transfer after intracytoplasmic sperm injection (ICSI) in women ≥40 years of age and <40 years of age

<table>
<thead>
<tr>
<th>Female age</th>
<th>≥40</th>
<th>&lt;40</th>
<th>χ² test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrievals (n)</td>
<td>71</td>
<td>71</td>
<td>0.003</td>
</tr>
<tr>
<td>No. of transfers (%)</td>
<td>59 (83.1 %)</td>
<td>63 (88.7 %)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of embryos replaced (mean)</td>
<td>2.3</td>
<td>2.5</td>
<td>0.000</td>
</tr>
<tr>
<td>Ongoing implantation rate (&gt;12 weeks) (%)</td>
<td>6/133 (4.5)</td>
<td>23/160 (14.3)</td>
<td>P = 0.003</td>
</tr>
<tr>
<td>Clinical pregnancies (n)</td>
<td>8/71 (11.3)</td>
<td>24/71 (33.8)</td>
<td>P = 0.003</td>
</tr>
<tr>
<td>per cycle (%)</td>
<td>8/59 (13.5)</td>
<td>24/63 (38.0)</td>
<td>P = 0.004</td>
</tr>
<tr>
<td>Ongoing pregnancies (n) (&gt;12 weeks)</td>
<td>5/71 (7.0)</td>
<td>19/71 (26.8)</td>
<td>P = 0.018</td>
</tr>
<tr>
<td>per retrieval (%)</td>
<td>5/59 (8.5)</td>
<td>19/63 (30.2)</td>
<td>P = 0.025</td>
</tr>
<tr>
<td>Deliveries</td>
<td>5/71 (7.0)</td>
<td>16/71 (22.5)</td>
<td>P = 0.018</td>
</tr>
<tr>
<td>per retrieval (%)</td>
<td>5/59 (8.5)</td>
<td>16/63 (25.4)</td>
<td>P = 0.025</td>
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</table>

NS = not significant.

<table>
<thead>
<tr>
<th>Embryo quality</th>
<th>Female age</th>
<th>≥40 years</th>
<th>&lt;40 years</th>
<th>Significance of difference (χ² test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>0.17</td>
<td>0.61</td>
<td>0.004</td>
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<tr>
<td>%/cycle*</td>
<td>3.6</td>
<td>10.2</td>
<td>0.017</td>
<td></td>
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<tr>
<td>Good</td>
<td>1.97</td>
<td>2.94</td>
<td>0.015</td>
<td></td>
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<tr>
<td>%/cycle</td>
<td>59.8</td>
<td>45.8</td>
<td>0.017</td>
<td></td>
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<tr>
<td>Fair</td>
<td>0.44</td>
<td>0.79</td>
<td>0.018</td>
<td></td>
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<tr>
<td>%/cycle</td>
<td>19.0</td>
<td>17.7</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

NS = not significant.

*For each cycle, the frequency of excellent, good or fair quality embryos was expressed as a percentage of the total number of embryos obtained in that cycle. In the Table, the percentages of embryos over all cycles are given.

A similar policy on an increased number of oocytes to be replaced in women ≥40 years of age in GIFT cycles has already been proposed (Craft et al., 1988).

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### References


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