Intracytoplasmic sperm injection, results in women older than 39, according to age and the number of embryos replaced in selective or non-selective transfers

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The aim of this study was to assess the results of intracytoplasmic sperm injection (ICSI) in a large cohort of women older than 39 according to age and to embryo transfer policy. In all, 736 ICSI cycles were analysed retrospectively. In 576 (78.3%) cycles an embryo transfer was carried out. Embryo transfer was defined as non-selective when all the available embryos were transferred, and as selective when fewer than the available number of embryos were replaced. A statistically significant gradual decrease in the number of embryos available for transfer, the number of good or excellent quality embryos available for transfer, the pregnancy rates, the clinical pregnancy rates, the implantation rates and the viable pregnancy rates was found with advancing age. No viable pregnancies ensued in women from 45 years old onwards. There was a statistically significant gradual increase in the pregnancy rates, the clinical pregnancy rates, the implantation rates and the viable pregnancy rates from non-selective to selective transfers. The results were similar in women with five or more embryos available, irrespective of the embryo transfer policy. It seems, therefore, that the ovarian reserve and the chances for a successful pregnancy decrease gradually with advancing age, and it is pointless to treat women from 45 years old onwards. A subgroup of patients with better ovarian response and more embryos available for transfer have higher chances of conception. Conception and implantation rates depend mainly on the quality of the transferred embryos. However, the implantation capacity of the embryos is generally lower irrespective of their good morphology.

Key words: embryo quality/embryo transfer/female age/implantation/intracytoplasmic sperm injection

Introduction

Fertility, defined as the ability to achieve a pregnancy, declines gradually over a woman’s reproductive lifespan. Although this decline seems to begin from the age of 30 years, it is more obvious between 35 and 40 and increases dramatically thereafter. This phenomenon has been demonstrated in couples attempting natural conception and occurs despite the continuation of apparently normal ovulatory cycles (Menken et al., 1986). Data from therapeutic donor insemination programmes have also documented a decline in fertility with maternal age (CECOS, 1982). Moreover, this age-dependent decrease in female fertility has been confirmed by the lower success rates with advancing age in women undergoing various assisted reproduction techniques (Tan et al., 1992; Devroey et al. 1996; Hull et al. 1996; Marcus and Brinsden 1996; Templeton et al., 1996). In addition to the impaired ability to become pregnant, the risk of miscarriage is also increased in older women, further decreasing the chances of a successful delivery (Abdalla et al., 1993).

The decline in female reproductive potential correlates with ovarian factors (Navot et al., 1991; Scott and Hoffmann, 1995; Wallach, 1995; Marcus and Brinsden, 1996; Templeton et al., 1996), although a slight contribution from reduced uterine receptivity cannot be totally excluded (Marcus and Brinsden, 1996; Templeton et al., 1996). The ovarian reserve decreases with advancing age, as indicated by the constant and unrelenting reduction in the numbers of oocytes (Faddy and Gosden, 1996). Furthermore, there is a concomitant decrease in the quality of the oocytes, as indicated also by the increased incidence of oocyte aneuploidy (Munne et al., 1995).

However, an important aspect of the diminished ovarian reserve and of the associated decline in reproductive potential is that the timing of its onset is highly variable, since it is a biological and not a chronological phenomenon (Scott and Hofmann, 1995; Wallach, 1995; Marcus and Brinsden, 1996). Ovarian response to stimulation seems to be a reliable indicator of the ovarian reserve (Scott and Hofmann, 1995; Marcus and Brinsden, 1996). Thus, women >40 years old with adequate follicular development may represent a subgroup of patients with higher chances of conception (Scott et al., 1995; Roest et al., 1996; Tarlatzis et al., 1996; Vandervorst et al., 1997), especially when combined with a more aggressive embryo transfer policy (Widra et al., 1996; Adonakis et al., 1997). The aim of this study was to assess the results of intracytoplasmic sperm injection (ICSI) in a large cohort of women >39 years old. It is an extension of our previous study (Adonakis et al., 1997) in an increased number of cycles, examining also the effect of age and the numbers of embryos replaced in selective or non-selective transfers.

Materials and methods

A total of 736 ICSI cycles, which were performed in 443 women >39 years old from 1 October 1991 until 31 December 1996, has been retrospectively analysed. The indications for ICSI were: (i) total...
absence or <5% of normal fertilization after standard IVF and (ii) <500 000 progressively motile spermatozoa in the whole ejaculate. Ovarian stimulation was induced by human menopausal gonadotrophin (HMG; Humegon®, Organon, Oss, The Netherlands or Per-
gonal®, Serono, Geneva, Switzerland) in association mainly with the
gonadotrophin-releasing hormone analogue (GnRHa) buserelin (Suprefact®, Hoechst, Frankfurt, Germany). The ovarian stimulation
protocols have been described in detail elsewhere (Smits et al., 1988;
Staessen et al., 1989). At an appropriate time in the stimulation,
10 000 IU of human chorionic gonadotrophin (HCG; Pregnyl®;
Staessen et al., available for transfer with the patient’s age was examined using
Statistics and the outcome of the delivery (Wisanto to provide detailed information on the evolution of the pregnancy
Genetic counselling was given in view of the prenatal diagnosis and
over 12 weeks of gestation.

Ultrasound examination together with a positive HCG test. The
presence of at least one gestational sac at 7 weeks of gestation on
12 days after embryo transfer. Clinical pregnancy was defined as the
serum HCG concentrations on two occasions, 4 days apart, at least
administered i.m. Pregnancy was confirmed by detecting increasing

50% of the volume of the embryo (Staessen C, or fair quality, embryos, anucleate fragments are present in 20–
embryo filled with anucleate fragments. In the third category, type
C, or fair quality, embryos, anucleate fragments are present in 20–
50% of the volume of the embryo (Staessen et al., 1992).

Embryos were transferred 48 h after oocyte retrieval. The embryo
transfer was defined as non-selective when all the available embryos
were transferred and as selective when the number of embryos
transferred was fewer than the number of embryos available for
transfer. This was a team proposal and a combined decision with the
patient. The following groups of embryo transfers were studied: non-
selective transfer of one embryo, non-selective transfer of two
embryos, non-selective and selective transfers of three embryos, non-
selective and selective transfers of four embryos, and transfer of five
or more embryos. In the latter group, both non-selective and selective
transfers of five or six embryos were included, since in only 10 out
of 98 cycles embryos available were seven or more. Moreover, in
women with five or more embryos available, two different groups of
embryo transfers were studied: selective transfer of three or four
embryos and transfer of five or six embryos.

The luteal phase was supplemented with progesterone (Utrogestan®,
Piette, Brussels, Belgium) administered vaginally or with HCG
administered i.m. Pregnancy was confirmed by detecting increasing
serum HCG concentrations on two occasions, 4 days apart, at least
12 days after embryo transfer. Clinical pregnancy was defined as the
presence of at least one gestational sac at 7 weeks of gestation on
ultrasound examination together with a positive HCG test. The
implantation rate was defined as the ratio of the number of sacs and the
number of embryos transferred (Hall et al., 1996). Viable
pregnancy was defined as the presence of at least one living fetus
over 12 weeks of gestation.

Prenatal diagnosis was proposed by chorionic villus sampling at
9–10 weeks of gestation or by amniocentesis at 16 weeks of gestation.
Genetic counselling was given in view of the 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 on the evolution of the pregnancy and the outcome of the delivery (Wisanto et al., 1996; Bonduelle et al., 1996).

Statistics
The correlation between the number and quality of the embryos
available for transfer with the patient’s age was examined using
polynomial regression analysis. The correlation between the number
and quality of the available embryos was examined using linear
regression analysis. The differences between the pregnancy and the
implantation rates were compared using the χ2 test or the χ2 test for
overall trend.

Results
In 576 (78.3%) out of the 736 ICSI cycles analysed here, an
embryo transfer was carried out. In 160 cycles there was no
embryo available for transfer. In 33 cycles no mature oocyte
was retrieved, in 17 cycles no spermatozoa were available for
ICSI, in 75 cycles none of the injected oocytes fertilized
normally and in the remaining 35 cycles none of the fertilized
oocytes cleaved. In all, 108 pregnancies ensued. In 83 of these,
at least one gestational sac was detected (clinical pregnancies),
while 53 pregnancies continued after 12 weeks of gestation
(viable pregnancies). Forty-one women delivered, two had a
second trimester abortion, while the final outcome is not known
at the time of writing for 10 pregnancies.

The pregnancy rates, the clinical pregnancy rates, the
implantation rates and the viable pregnancy rates per started
cycle and per cycle with embryo transfer according to age are shown in Table I. A statistically significant gradual decrease
(P < 0.0001) in the percentage of cycles with embryo transfer
was observed with advancing age. Moreover, there was a
statistically significant gradual decrease in the pregnancy rates
(P = 0.002), the clinical pregnancy rates (P < 0.05), the
implantation rates (P = 0.01) and the viable pregnancy rates
(P < 0.05) with advancing age. No ongoing pregnancy has
been observed in women from 45 years old onwards. The proportion
of preclinical and clinical abortions was also high
(Table I).

Regression analysis of the mean numbers of the available embryos
(y = −0.0233x2 − 0.0543x + 3.6707, r2 = 0.938),
the mean numbers plus the standard deviation of the available embryos
(y = −0.0714x2 + 0.235x + 6.5539, r2 = 0.954),
and the mean numbers minus the standard deviation of the available embryos (y = 0.0248x2 − 0.3436x + 0.7875, r2 = 0.476) according to age are shown in Figure 1. A significant
negative correlation (P < 0.001) between the number of
available embryos and the age of the patient was found.

A regression analysis of the mean numbers of the embryos available
(y = −0.0233x2 − 0.0543x + 3.6707, r2 = 0.938),
the mean numbers of type A (excellent quality) embryos available
(y = −0.0127x2 − 0.0774x + 2.7359, r2 = 0.844)
and the mean numbers of type A plus B (excellent or good quality) embryos available (y = 0.0001x2 − 0.0291x + 0.3056,

r2=0.813) according to age is given in Figure 2. A gradual
decrease in the number of good or excellent quality embryos
was found with advancing age.

The distribution of the cycles according to the number of
embryos available is shown in Figure 3. A gradual decrease
in the number of cycles as the number of embryos increased
was observed. A linear regression analysis of mean numbers
of type A (excellent quality) embryos available (y = 0.0812x
− 0.1516, r2 = 0.524), and mean numbers of type A plus B
(excellent or good quality) embryos available (y = 0.81x
Figure 1. Regression analysis of (a) the mean numbers of the available embryos \( y = -0.0233x^2 + 0.0543x + 3.6707, r^2 = 0.938 \), (b) the mean numbers plus the standard deviation of the available embryos \( y = -0.0714x^2 + 0.235x + 6.5539, r^2 = 0.954 \) and (c) the mean numbers minus the standard deviation of the available embryos \( y = 0.0248x^2 - 0.3436x - 0.7875, r^2 = 0.476 \) according to age.

Figure 2. Regression analysis of (a) the mean numbers of embryos available \( y = -0.0233x^2 - 0.0543x + 3.6707, r^2 = 0.938 \), (b) the mean numbers of type A and B (excellent or good quality) embryos available \( y = 0.0001x^2 - 0.0291x + 0.3056, r^2 = 0.813 \) and (c) the mean numbers of type A (excellent quality) embryos available \( y = -0.0127x^2 - 0.0774x + 2.7359, r^2 = 0.844 \) according to age.

Figure 3. Distribution of the cycles according to the number of embryos available for transfer.

Figure 4. Linear regression analysis of the mean numbers of (a) type A (excellent quality) embryos available \( y = 0.0812x - 0.1516, r^2 = 0.524 \) and (b) the mean numbers of type A and B (excellent or good quality) embryos available \( y = 0.81x - 1.1164, r^2 = 0.968 \) according to the total number of embryos available for transfer.

In all, 16 multiple pregnancies were achieved: 15 twins and one triplet (non-elective transfer of four embryos). Six (40%) out of 15 continued as singleton viable pregnancies (vanishing twins) and one ended as a miscarriage. No difference was found between the different types of embryo transfer in the clinical and the viable multiple pregnancy rates, expressed either per transfer (Table II) or per clinical and per viable pregnancy.

The results according to the groups of embryos transferred in women with five or more embryos available are shown in Table III. The pregnancy rates, the clinical pregnancy rates, the implantation rates, the viable pregnancy rates and the multiple pregnancy rates were similar between women with selective embryo transfer of three or four embryos and women with embryo transfer of five or six embryos, although a trend towards better results was observed in women with selective embryo transfer of three or four embryos.
Table I. ICSI results according to the age of the patients

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>222</td>
</tr>
<tr>
<td>41</td>
<td>166</td>
</tr>
<tr>
<td>42</td>
<td>110</td>
</tr>
<tr>
<td>43</td>
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<td>44</td>
<td>62</td>
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<tr>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>46</td>
<td>56</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>(χ² for trend)</td>
</tr>
</tbody>
</table>

| Cycle started (n) | 191 |
| Cycle started per started cycle | 86 |
| Pregnancy (n) | 46 |
| Pregnancy per cycle (%) | 20.7 |
| Pregnancy per transfer (%) | 24.1 |
| Preclinical abortions (n) | 11 |
| Preclinical abortions per cycle (%) | 15.8 |
| Preclinical abortions per transfer (%) | 18.3 |
| Clinical pregnancies (n) | 35 |
| Clinical pregnancies per cycle (%) | 15.1 |
| Clinical pregnancies per transfer (%) | 19.1 |
| Implantation rate (%) | 6.7 |

| Transfer (n) | 166 |
| Transfer per started cycle | 78.9 |
| Transfer pregnancy (n) | 46 |
| Transfer pregnancy per cycle (%) | 33 |
| Transfer pregnancy per transfer (%) | 25.2 |
| Transfer preclinical abortions (n) | 10 |
| Transfer preclinical abortions per cycle (%) | 1.1 |
| Transfer preclinical abortions per transfer (%) | 1.3 |
| Transfer clinical pregnancies (n) | 9 |
| Transfer clinical pregnancies per cycle (%) | 1.2 |
| Transfer clinical pregnancies per transfer (%) | 1.3 |
| Transfer implantation rate (%) | 1.9 |

Table II. ICSI results in women >40 years old according to the number of embryos transferred

<table>
<thead>
<tr>
<th>1 Non-select.</th>
<th>2 Non-select.</th>
<th>3 Non-select.</th>
<th>4 Non-select.</th>
<th>&gt;5 Select. &amp; non-select.</th>
<th>3 Select.</th>
<th>4 Select.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles (n)</td>
<td>105</td>
<td>101</td>
<td>94</td>
<td>73</td>
<td>98</td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td>Pregnancies (n)</td>
<td>2</td>
<td>10</td>
<td>14</td>
<td>20</td>
<td>26</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>Pregnancies per transfer (%)</td>
<td>1.9</td>
<td>10.0</td>
<td>14.9</td>
<td>27.4</td>
<td>26.5</td>
<td>28.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Preclinical abortions (n)</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Preclinical abortions per transfer (%)</td>
<td>1.9</td>
<td>7.0</td>
<td>10.6</td>
<td>21.9</td>
<td>21.4</td>
<td>24.0</td>
<td>27.3</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>1.9</td>
<td>3.5</td>
<td>3.5</td>
<td>6.4</td>
<td>5.0</td>
<td>10.7</td>
<td>7.7</td>
</tr>
<tr>
<td>Abortions (n)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Ectopics (n)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Viable pregnancies (n)</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Viable pregnancies per transfer (%)</td>
<td>0.9</td>
<td>5.0</td>
<td>6.4</td>
<td>13.7</td>
<td>12.3</td>
<td>20.0</td>
<td>16.4</td>
</tr>
<tr>
<td>Clinical multiple pregnancies (n)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Clinical multiple pregnancies per transfer (%)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>4.1</td>
<td>7.1</td>
<td>8</td>
<td>3.6</td>
</tr>
<tr>
<td>Viable multiple pregnancies (n)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Viable multiple pregnancies per transfer (%)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2.7</td>
<td>3.1</td>
<td>4</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Discussion

The growing trend in delayed childbearing in Western countries for a variety of socio-economic reasons has increased the demand for therapy from infertile women >40 years old (Bopp et al., 1995; Marcus and Brinsden 1996; van Kooij et al., 1996). More insight will help physicians to counsel their infertile couples appropriately, especially when an advanced and expensive treatment such as ICSI is needed. The present study examines the results of ICSI in a cohort of women >39 years old, the largest to our knowledge from one single centre. It is limited to ICSI results and cannot be extrapolated to IVF, since more chromosomally abnormal embryos may be present in the ICSI population due to possible male abnormality. In IVF, with possibly more chromosomally normal embryos, more ongoing pregnancies may be expected. The findings of this study confirm the generally accepted decrease in pregnancy rates with advancing age in women treated with various forms of assisted reproductive techniques (Tan et al., 1992; Abdelmassih et al., 1996; Devroey et al., 1996; Dor et al., 1996; Hull et al., 1996; Templeton et al., 1996; Widra et al., 1996). Despite the application of an advanced therapy, the chance of a successful pregnancy in this group appears limited due to the intrinsic reduced fertility coupled with a higher risk of fetal loss (Hull et al., 1996; Widra et al., 1996). Moreover, it seems that treatment of patients from 45 years old onwards is pointless, as also observed by Widra et al. (1996).

In our study population, a statistically significant gradual decrease in the mean number of the available embryos per started cycle was observed with advancing age. This finding reflects the diminished ovarian reserve with advancing age.
since fertilization rates after ICSI do not depend on sperm quality and are similar in women >40 years and in younger patients (Devroey et al., 1996; Hull et al., 1996). A parallel gradual decrease in the available embryos of good or excellent quality was also found. However, the number of the embryos available for transfer may differ from patient to patient, as is shown in Figures 1 and 3. Some women of even older age may have a better response to stimulation and thus more embryos available for transfer. These patients seem to represent a subgroup with better chances of conception (Roest et al., 1996; Tarlatzis et al., 1996; Vandervoort et al., 1997). Moreover, a more aggressive embryo transfer policy has been proposed in order further to increase pregnancy rates, since implantation rates are lower in older women and thus the risk of multiple pregnancies limited (Azem et al., 1995; Widra et al., 1996; Adonakis et al., 1997). It has therefore been proposed to transfer up to six embryos, rather than four, in women >40 years old (Widra et al., 1996; Adonakis et al., 1997). Cryopreservation of embryos in women >40 years could be an alternative. However, we were not able to obtain acceptable pregnancy rates with frozen–thawed embryos in women ≥40 years of age.

The routine application of pre-implantation genetic diagnosis (PGD) in older women and the subsequent elective transfer of euploid embryos have been recently proposed as an alternative in order to improve the pregnancy rates, to reduce the abortion rates, to avoid high-order multiple pregnancies and to prevent the birth of children with common trisomies (Verlinsky and Kuliev, 1996). Two approaches have become available for PGD of aneuploidies on the basis of fluorescent in-situ hybridization (FISH), i.e. the cleavage-stage biopsy and FISH analysis of one or two blastomeres or the FISH analysis of polar bodies removed from the oocytes (Verlinsky and Kuliev, 1996). However, there is still debate concerning the safety, the accuracy and the necessity of this approach (Reubinoff and Shushan, 1996) in the absence of controlled randomized studies. Moreover, PGD is available only in a limited number of centres all over the world.

The variation in the numbers of embryos available for transfer, as well as the different embryo transfer policies, are reflected in the different groups of embryo transfers. A statistically significant gradual increase was found in pregnancy rates, clinical pregnancy rates, implantation rates and viable pregnancy rates when the number of the embryos available for transfer increased in cases of non-selective transfer as well as with the selective transfer of fewer than the available number of embryos in women with adequate ovarian response. It therefore seems that the achievement of pregnancy depends mainly on the quality of the transferred embryos, since success rates were higher in the selective embryo transfers. The increased pregnancy rates with the transfer of more embryos in the non-selective transfers may simply be due to the parallel increase in the number of good or excellent quality embryos replaced.

However, even in the selective transfers, the implantation rates never reached high values and these values were far from the implantation rates achieved in younger women (Staessen et al., 1992). This is probably due to a decrease in the implantation capacity of the embryos irrespective of their excellent or good morphology. Age-related oocyte factors commonly lead to aneuploidy in the cleaving embryo (including mosaicism), without interfering with fertilization and early stages of embryo development. Expression of the embryonic genome would occur only after the four-cell to eight-cell stage and might impair implantation and normal evolution of the pregnancy despite apparently normal fertilization and embryo morphology (Munné et al., 1995; Adonakis et al., 1997).

The lower implantation capacity of the embryos in women >40 years old is also expressed in the lower than expected multiple-pregnancy rates irrespective of the group of embryo transfer (Azem et al., 1995). Moreover, most of the multiple pregnancies involved were twins, as also observed by other investigators (Azem et al., 1995). On the other hand, there is an increased possibility of a higher-order multiple pregnancy (Walters, 1996). However, this seems to be associated with the transfer of three or more embryos (even non-selective) and not only to the transfer of five or more embryos, since the triplet pregnancy was achieved with a non-selective transfer of four embryos. This is important when embryo culture conditions are improved or duration of embryo culture is increased. In this condition a more conservative embryo transfer policy could be adopted.

Approximately half of the twin pregnancies continued as viable singletons. This may be an advantage of the more aggressive embryo transfer policy, since at least a viable ongoing pregnancy ensued from the replacement of higher numbers of the embryos available.

The effectiveness of a more aggressive embryo transfer policy in women >39 years old with five or more embryos available was evaluated by comparing two different groups of embryo transfer. The similar results obtained either by a selective transfer of three or four embryos or by the transfer of five or six embryos are probably due to the selection of the

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**Table III. ICSI results in women 40 years old and over with five or more embryos available according to the number of embryos transferred**

<table>
<thead>
<tr>
<th>Number of Embryos Transferred</th>
<th>&lt;4 embryos selective</th>
<th>≥5 embryos selective &amp; non-selective</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles (n)</td>
<td>93</td>
<td>98</td>
<td>191</td>
<td>NA</td>
</tr>
<tr>
<td>Pregnancies (n)</td>
<td>31</td>
<td>26</td>
<td>57</td>
<td>NA</td>
</tr>
<tr>
<td>per transfer (%)</td>
<td>33.3</td>
<td>26.5</td>
<td>29.8</td>
<td>NS (χ²)</td>
</tr>
<tr>
<td>Precleinal abortions (n)</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancies (n)</td>
<td>24</td>
<td>21</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>per transfer (%)</td>
<td>25.8</td>
<td>21.4</td>
<td>23.6</td>
<td>NS (χ²)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>8.4</td>
<td>5.0</td>
<td>6.3</td>
<td>NS (χ²)</td>
</tr>
<tr>
<td>Abortions (n)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Viable pregnancies (n)</td>
<td>18</td>
<td>12</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>per transfer (%)</td>
<td>19.4</td>
<td>12.3</td>
<td>15.7</td>
<td>NS (χ²)</td>
</tr>
<tr>
<td>Clinical multiple pregnancies</td>
<td>4</td>
<td>7</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>per transfer (%)</td>
<td>4.3</td>
<td>7.1</td>
<td>5.8</td>
<td>NS (χ²)</td>
</tr>
<tr>
<td>Viable multiple pregnancies</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>per transfer (%)</td>
<td>3.2</td>
<td>3.1</td>
<td>3.1</td>
<td>NS (χ²)</td>
</tr>
</tbody>
</table>

*Selects transfer of three or four embryos, *non-selective (mainly) or selective transfer of five or six embryos, NS: not significant.
best embryos in cases of selective transfer. This also confirms that the achievement of pregnancy depends on embryo quality. Thus, in order to benefit from an aggressive embryo transfer policy, five or more good quality embryos need to be transferred. As shown in Figure 5, this can be achieved when more than seven embryos are available for transfer, but this is possible in a limited number of patients because usually fewer oocytes are available.

In conclusion, it seems that the ovarian reserve and the chances of a successful pregnancy decrease gradually with advancing age in women from 40 years old onwards. Moreover, it is pointless to treat women from 45 years old onwards. A subgroup of patients with adequate ovarian response and more embryos available for transfer have greater chances of conception. Conception and implantation rates depend mainly on the quality of the transferred embryos. However, the implantation capacity of the embryos is generally lower irrespective of their good morphology. Thus, until the full elucidation of the validity of routine PGD, a more aggressive embryo transfer policy in women >40 years old may improve pregnancy rates, since the risk of high-order pregnancies seems to be limited. The effectiveness of this policy seems also to be related to the quality of the embryos available for transfer.

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