Oocyte donation programme: results obtained with intracytoplasmic sperm injection in cases of severe male factor infertility or previous failed fertilization

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Introduction
Oocyte donation was originally performed only in patients suffering from premature ovarian failure (Lujten et al., 1984) and surgical castration. Today this procedure is commonly used in patients who have undergone several unsuccessful attempts at in-vitro fertilization (IVF)-embryo transfer (Remohi et al., 1993), in menopausal women over 40 years old (Sauer et al., 1990) and, recently, in women past their natural menopause, i.e. women over 50 years (Borini et al., 1990; Borini et al., 1993). In most cases, the male partners are also older and, even if age does not seem to influence fertility in the male (Borini et al., 1994), semen abnormalities may present more often than in the general population.

The practice of intracytoplasmic sperm injection (ICSI) has become widespread and high success rates have been reported throughout the world in cases of severe male factor infertility (Van Steirteghem et al., 1993). It is not clear that the injection technique was absolutely necessary in the cases reported here, even though the criteria for ICSI were present, i.e. previous failed fertilization and severe male factor infertility (Van Steirteghem et al., 1993).

The aim of this preliminary report is to show results obtained in couples who underwent embryo transfer from donated oocytes microinjected using the ICSI technique with their own spermatozoa.

Materials and methods
Our programme employs anonymous non-paid donors. Oocytes were donated by women aged «35 years undergoing assisted reproductive treatment for infertility problems. Between May 1995 and July 1995, 15 patients aged between 37 and 51 years underwent a total of 15 donation cycles. Each recipient received oocytes donated by only one donor. Each donor donated oocytes to one or more recipients.

Donors
Oocyte donors were 11 patients undergoing assisted reproductive treatment cycles who wished to donate their excess oocytes anonymously. Their mean age was 30.5 years (range 21–35). Women and their partners signed an informed consent form. Details about the screening are described elsewhere (Flamigni and Borini, 1995).

Recipients
A total of 15 women aged between 37 and 51 years were treated; 12 had undergone premature ovarian failure and three were at natural menopause (i.e. >50 years old).

All patients underwent a preliminary blood, cardiographic and pulmonary work-up. Hysteroscopy was used to confirm the presence of an adequate uterine cavity.
The male partner submitted a semen sample for sperm analysis. Both partners signed an informed consent form.

In all, 10 patients needed the ICSI procedure for severe male factor and five for previous failed fertilization. Table I reports semen parameters of each male partner.

During the transfer cycle, women first received micronized 17β-oestradiol (Estrace; Mead Johnson Laboratories, Evansville, IN, USA). The dose was increased from 2 to 6 mg over a period varying from 10 to 18 days depending on the patient. Natural micronized progesterone (600 mg, Ultragestan: Piette, Brussels, Belgium) was started on the day of oocyte retrieval via the vaginal route. Transfers were performed on the third day of progesterone administration into the uterus. In the case of pregnancy, progesterone therapy was continued for 65 days after transfer. Before the transfer cycle, endometrial development was assessed in a ‘mock’ cycle as described elsewhere (Flamigni et al., 1993).

### Oocyte handling

Oocytes were denuded from cumulus and corona radiata by incubation for <1 min in HEPES-buffered Earle’s medium containing 80 IU/ml hyaluronidase (type VIII, Sigma Chemical Co., St Louis, MO, USA). The surrounding cumulus and corona cells were then removed by aspiration in and out of a hand-drawn glass pipette of ~200 μm diameter. The oocytes were then observed under an inverted microscope at ×100 in order to assess the maturation stage by observing the presence of the first polar body. The eggs were incubated for 3–4 h and observed again to assess whether more oocytes had extruded the first polar body. The microinjection procedure was performed on all metaphase II oocytes.

### Semen preparation and injection procedure

After evaluation of count, motility and morphology, sperm selection was carried out using the mini-Percoll technique (Ord et al. 1990). We performed the microinjection procedure using microinjection and holding pipettes made by Cook (Cook Australia, Queensland, Australia). We followed the injection technique described by Van Steirteghem et al. (1993). Briefly, a single spermatozoon was aspirated tail-first into the tip of the microinjection pipette. The oocyte was held by the holding pipette with the polar body at 12 or 6 o’clock and the microinjection pipette was introduced across the zona pellucida into the ooplasm with 1–2 pl of medium. The injection pipette was withdrawn carefully and the oocyte was released from the holding pipette.

### Results

A total of 62 oocytes were donated (mean ± SD 4.1 ± 1; range 3–6). The 46 metaphase II oocytes were injected (mean ± SD 3 ± 1.1; range 1–6). Thirty-one oocytes fertilized (mean ± SD 2 ± 0.8; range 1–4), and 29 became embryos after 24 h further in-vitro culture (mean ± SD: 1.9 ± 1; range: 0–4). A total of 25 embryos were transferred (mean ± SD 2 ± 0.5; range 1–3). Table I details semen characteristics, ICSI procedure results and pregnancy outcome.

In two cases there were no embryos to transfer. Transfer was performed in 13 women. A maximum of three embryos were replaced in the uterus in the attempt to avoid multiple pregnancies as far as possible. A total of five clinical pregnancies was obtained, one a twin pregnancy. The pregnancy rates per cycle started and per transfer were 33.3 and 38.4% respectively. There were no ectopic pregnancies. The patient with the twin pregnancy miscarried at 12 weeks of gestation. The other four pregnancies are ongoing. All pregnant patients underwent amniocentesis, resulting in four normal cytogenetic samples. There were no statistical differences in terms of fertilization and pregnancy rates between couples receiving ICSI for previous failed fertilization and those for severe male factor infertility (fertilization rate 73.3 versus 64.5% and pregnancy rate 40 versus 30% respectively).

### Discussion

Our preliminary results show that infertile couples in whom the female partner requires donated oocytes and the male partner suffers from severe male factor infertility can achieve pregnancy by undergoing oocyte donation combined with the ICSI technique.

Oocyte donation is commonly used for many clinical situations: premature ovarian failure, natural menopause (>50 years).

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Table I Semen characteristics, ICSI outcome and pregnancy outcome

<table>
<thead>
<tr>
<th>Couple no.</th>
<th>Sperm count (X10⁶/ml)</th>
<th>Motility (%)</th>
<th>Normal forms (%)</th>
<th>No. of oocytes injected</th>
<th>No. of embryos transferred</th>
<th>Pregnancy outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.1</td>
<td>40</td>
<td>18</td>
<td>6</td>
<td>3</td>
<td>Ongoing</td>
</tr>
<tr>
<td>2</td>
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<td>25</td>
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<td>3</td>
<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>3.3</td>
<td>30</td>
<td>22</td>
<td>3</td>
<td>2</td>
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</tr>
<tr>
<td>4</td>
<td>1.1</td>
<td>5</td>
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</tr>
<tr>
<td>5</td>
<td>7.6</td>
<td>20</td>
<td>16</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.3</td>
<td>40</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7.7</td>
<td>40</td>
<td>10</td>
<td>2</td>
<td>1</td>
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</tr>
<tr>
<td>8</td>
<td>35.0</td>
<td>20</td>
<td>14</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>78.0</td>
<td>30</td>
<td>10</td>
<td>1</td>
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<tr>
<td>10</td>
<td>2.6</td>
<td>5</td>
<td>16</td>
<td>3</td>
<td>2</td>
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</tr>
<tr>
<td>11</td>
<td>18.0</td>
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<td>3</td>
<td>2</td>
<td>Ongoing</td>
</tr>
<tr>
<td>12</td>
<td>13.5</td>
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<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>22.0</td>
<td>20</td>
<td>10</td>
<td>4</td>
<td>2</td>
<td>Abortion, 12 weeks</td>
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<td>17.5</td>
<td>60</td>
<td>10</td>
<td>3</td>
<td>2</td>
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<tr>
<td>15</td>
<td>33.0</td>
<td>60</td>
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<td>3</td>
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</tr>
</tbody>
</table>

*Patients 1–10: Severe male factor; 11–15: previous failed fertilization.

b<5% rapid progressive motility.

*Twin pregnancy.
old), surgical castration, as well as repeatedly unsuccessful IVF attempts. It more frequently happens that a woman who needs egg donation has a male partner with severe semen anomalies, a situation that gives rise to two different problems. First, in many countries (e.g. Italy), paid donors are forbidden, with the result that infertile patients undergoing assisted reproductive treatment cycles are themselves the only source of eggs. Often, only a few oocytes are donated, a situation that contrasts with the second problem, i.e. severe male factor infertility, which requires a large number of oocytes to be available in order to have any chance of fertilization. In cases of severe male factor infertility in the past, IVF was unsuccessful in our centre. In many centres, a failed oocyte donation attempt often means the infertile couple is placed on a long waiting list for another donation. The only possibility for such couples would normally be to try embryo donation, a well accepted practice prior to development of the ICSI procedure. Now 95% of men, after counselling, decide to use their own spermatozoa for the ICSI technique.

ICSI is recognized world-wide as a treatment for severe male factor infertility and for couples who have undergone repeated failed fertilization cycles. The high fertilization rate that can be obtained with the ICSI procedure means attempts may be made to fertilize even several eggs. ICSI and oocyte donation were adopted in our centre for couples who otherwise needed embryo donation or egg and semen donation (pre-implantation adoption).

Five couples in this study had undergone previous attempts using standard or modified IVF techniques (Ord et al., 1990; 1993; Borini et al., 1992) with negative results. The remaining 10 couples received ICSI because of semen characteristics. Of course it is not clear that ICSI was absolutely necessary in these cases, but it is extremely difficult to suggest to a couple that standard IVF should be used after a failed fertilization cycle or when semen characteristics are extremely poor.

Since pregnancy and implantation rates are similar to those we usually obtain in our oocyte donation programme, even if 15 cases are not enough to draw any firm conclusions, it seems that the ICSI procedure does not affect the outcome of such cycles. This is further evidence that ICSI is a successful technique.

Even if there is nothing physiologically remarkable or unexpected, this study is to our knowledge the first report on the ICSI procedure being adopted in conjunction with oocyte donation, and hence the first description of pregnancies obtained using this method.

Since indications for oocyte donation have been extended to many clinical situations other than premature ovarian failure, resort to the ICSI procedure will be useful and inevitable in many cases.

Considering today’s high fertilization and pregnancy rates, if in the future all doubts of possible fetal anomalies are removed, ICSI might be the procedure of choice in cases of oocyte donation when only a few eggs are available.

In conclusion, ICSI can be performed with good results to help couples where the male partner has severe semen anomalies and the female partner requires oocyte donation.

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**References**


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