Rescue ICSI of unfertilized oocytes after IVF

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BACKGROUND: Failed fertilization after IVF occurs in 10–20% of cycles. Conflicting results of rescue fertilization by ICSI have been reported. We therefore compared the success rate in terms of fertilization and pregnancy of cycles in which rescue ICSI was performed with those from a matched control group of primarily ICSI cycles.

METHODS: Unfertilized oocytes from IVF cycles with total fertilization failure where at least four metaphase II oocytes were available were treated by ICSI (group I; \( n = 120 \)). A matched control group was established with patients undergoing ICSI during the same period (group II; \( n = 280 \)).

RESULTS: Both fertilization rate and the proportion of embryos with four blastomeres on day 2 after ICSI were significantly higher in the control group \((P < 0.05)\). Embryo quality, however, was comparable in both groups. The pregnancy rate in the control group was 25.3% whereas in group I with rescue ICSI, no pregnancy was obtained.

CONCLUSIONS: Although unfertilized oocytes after IVF can be fertilized by ICSI, the developmental potential of the ensuing embryos is very poor. Therefore, rescue ICSI after total failure of fertilization is not recommended.

Key words: ICSI/IVF/pregnancy/reinsemination/total fertilization failure

Introduction

Total failure of fertilization (TFF) after IVF, in cases where the sperm seems to be normal, is one of the most frustrating experiences for the infertile couple as well as for the infertility team. Depending on the criteria for using either IVF or ICSI, this event occurs in 10–25% of IVF cycles (Chen et al., 1995). Various reasons for failed fertilization have been forwarded, but in most cases these are speculative. Sperm defects (Kruger et al., 1988; Liu and Baker, 2000), disturbances in sperm–oocyte interaction and oocyte abnormality have all been proposed as possible causes of failed fertilization after IVF (Bedford and Kim, 1993; Van Bleekom et al., 1994). 

From the beginning of IVF, various procedures to salvage the lost cycle have been attempted. Reimplantation of the unfertilized oocytes 24 h after oocyte retrieval has been tried. (Boldt et al., 1987; Calderon et al., 1993). After the advent of micromanipulation techniques, partial zona dissection (PZD), subzonal insemination (SUZI) and finally ICSI were introduced to rescue oocytes after unsuccessful IVF (Malter et al., 1989; Nagy et al., 1993; Van Steirteghem et al., 1993; Wiker et al., 1993; Imoedemhe and Sigue, 1994; Lundin et al., 1996; Morton et al., 1997; Bussen et al., 1997; Loong et al., 1997; Yuzpe et al., 2000). Although pregnancies have been reported after rescue of failed fertilization, most reports were relatively inconclusive. A large prospective study on the success of rescue ICSI in terms of pregnancy is still lacking.

The aim of our study was to compare the results of cycles with TFF in which rescue ICSI was performed with a matched control group of ICSI cycles, both in terms of fertilization and pregnancy outcome and to answer the question whether rescue ICSI is worth trying.

Materials and methods

Patients

Complete lack of fertilization occurred in 148 out of 1412 (10.5%) IVF cycles between September 1996 and June 2000. A rescue ICSI procedure was performed in all TFF cycles \((n = 120)\) where at least four metaphase II (MII) oocytes were available (group I).

All couples in the IVF group met standard criteria for admission to IVF, including normal semen motility and density as assessed by published criteria (World Health Organization, 1992). The matched control group (group II) was selected retrospectively and consisted of 280 couples undergoing an ICSI procedure during the same period. Seventy patients from each year of the study period were matched to group I patients according to age, type of stimulation and number of oocytes retrieved.

ICSI was performed when \(< 50,000\) sperm with type ‘a’ progressive motility (World Health Organization 1992) and good morphology were present in the whole ejaculate. The overall proportion of ICSI versus IVF cycles was \(\sim 62\%\) in our IVF centre at that time.

Stimulation protocol and oocyte retrieval

Normogonadotrophic patients with normal-sized ovaries on ultrasound were treated either with a GnRH agonist and hMG in a short protocol (group I, 71; group II, 160) or clomiphene citrate followed by hMG (group I, 14; group II, 35). In the flare-up protocol, buserelin
(Suprefact®, Hoechst) was used in a dose of 0.4 ml per 24 h s.c. starting from day 1–2 of the cycle and hMG (Humegon®, Organon or Pergonal®, Serono) or FSH (Metrodin®, Serono) was added on day 3 of the cycle, starting with a dose of 150 IU clomiphene citrate (Clomid®; Ciba-Geigy) was given at a dose of 100 mg per day from day 3 to day 7 of the cycle and the stimulation was continued with hMG, two ampoules per day from day 8 onwards.

Patients with polycystic ovaries (confirmed by ultrasonography; group I, 35; group II, 85) were treated with a long protocol, using either a short-acting GnRH agonist (Suprefact) or a long-acting GnRH agonist (Decapeptyl®, Beaufourt–Ipsen or Decapeptyl depot®, Ferring). The GnRH agonist was administered on day 21 of the previous menstrual cycle. After desensitization of the pituitary gland was confirmed, FSH and/or hMG were given in individually adapted doses, as a rule starting with 150 IU per day (Metrodin® or Pergonal®; Ares-Serono; or Humegon®, Organon). Ovulation was triggered with hCG (Pregnyl®; Organon Inc., Canada), 10 000 IU i.m., when at least three follicles had a diameter of 19 mm and serum estradiol concentration was >1000 pg/ml. Oocytes were retrieved 35 h after hCG administration by transvaginal ultrasound-guided follicular puncture under sedation with i.v. pethidine and diazepam.

**Sperm preparation**

Semen was collected by masturbation after 3–5 days of sexual abstinence. The ejaculates were allowed to liquefy (at 37°C) and examined within 0.5 h after collection. The evaluation according to published criteria (World Health Organization, 1992) was carried out under a light microscope. After examination sperm samples were cryopreserved for potential later use. For ICSI, either fresh or cryopreserved sperm were used. In three cases, motile sperm retrieved from the IVF incubation plate were used for rescue ICSI.

The semen preparation was performed as follows. An appropriate volume of fresh semen or cryopreserved sample, after 15 min of thawing in room temperature, was diluted with 5 ml of Earle's medium. After centrifugation (10 min at 800 g, room temperature) the supernatant was removed and replaced with another 5 ml of Earle's medium. After a second centrifugation, the supernatant was once again removed and the pellet was overlaid with 1 ml Earle's medium and incubated (37°C, 5% CO₂ in air) to perform separation by swim-up. For ICSI both supernatant and pellet, if needed, were used.

**Assessment of fertilization and embryonic development**

In IVF cases, cumulus–corona radiata–oocyte complexes were inseminated in 4-well plates (Falcon) with 50 000–150 000 motile sperm (Ménezo BII medium and placed in the CO₂ incubator (Heraeus, Germany) at a temperature of 37°C in 5% CO₂ in air. Fertilization was checked 18–20 h after insemination. Absence of pronuclei and a second polar body in the perivitelline space were proof that fertilization had not occurred. This was further confirmed after granulosa cells and sperm bound to the zona pellucida were removed enzymatically (hyaluronidase) and mechanically.

Unfertilized metaphase II oocytes were microinjected as described previously (Van Steirteghem et al., 1993), immediately after failed fertilization was observed and oocytes were checked for survival and fertilization 24 h later (Nagy et al., 1994). Fertilization was considered to be normal when two individual or fragmented polar bodies and two pronuclei were present. The quality of embryos was evaluated 48 h after ICSI. Embryos were graded as good (<20% anucleate fragments) fair (20–50% anucleate fragments) and poor (>50% anucleate fragments). Embryos with <50% fragmentation were eligible for transfer.

A maximum of three embryos was transferred into the uterus on day 3 after ICSI by using a Wallace (UK), Labotect (Labotect GmbH, Germany) or Frydman (OSI, France) catheter. On the day of hCG injection, a sham transfer with the Frydman catheter was performed to probe the cervical canal. The Wallace and Frydman catheters were used randomly for non-complicated transfers, the Labotec catheters were used for difficult transfers. The distribution of these three types of catheters was equal in group I and group II.

Supernumerary embryos were cultured with Vero cells and cryopreserved if they reached the blastocyst stage. The luteal phase was supported with intravaginally administered micronized progesterone, 600 mg daily (Uterogestan®, Piette, Belgium).

**Pregnancy**

Pregnancy was confirmed when rising serum β-hCG concentrations were found on at least two successive occasions. Clinical pregnancy was defined by the presence of a gestational sac and fetal cardiac activity by ultrasound at ~7 weeks of pregnancy.

**Statistical analysis**

Statistical evaluation was performed using Student's t-test, χ²-test, Fisher's exact test or the Mann–Whitney test as appropriate. Differences were considered significant at P < 0.05.

**Results**

There were no statistically significant differences in duration of infertility, past or present illnesses and age between the two groups. The mean age was 32.9 ± 5.0 (SD) years for female patients and 34.8 ± 5.9 (SD) years for male patients. The number of cycles and mean number of metaphase I (MI), MI, germinal vesicle (GV) oocytes retrieved and estradiol levels in the two groups are given in Table I. No significant differences in oocyte recovery were observed between the groups, except for the number of GV oocytes, which was significantly higher in the control group (group II). The mean serum estradiol level expressed per follicle was not significantly different between groups. The fertilization rate, expressed as the proportion of 2PN oocytes per successfully injected oocytes and as the proportion of 4-cell embryos on day 2 after ICSI, was significantly higher in group II. Embryo quality, however, assessed on day 2, was comparable in both groups (Table II).

The percentage of cycles with transfer and the mean number of embryos transferred were significantly higher in the control group (Table III). Seventy-one pregnancies were obtained in the control group (pregnancy rate of 25.3%, clinical pregnancy

| Table I. Serum estradiol level, number of oocytes and their quality in group I and group II |
|---------------------------------|--------|--------|
| Cycles                          | Group I | Group II |
| Total no. of oocytes            | 120     | 280     |
| Mean (SD) E₂/follicle (pg/ml)   | 212.1 (90.1) | 228.6 (109.7) |
| Mean (SD) no. of MII oocytes    | 6.1 (2.5) | 5.8 (2.1) |
| Mean (SD) no. of GV oocytes     | 0.18 (2.2) | 0.7 (1.6) |
| Total no. of MII oocytes        | 5.8 (1.1) |
| Refractile bodies (%)           | 18      | 6       |
| Perivitelline debris (%)        | 8       | 3       |

*P < 0.05.

E₂ = estradiol; MII = metaphase II; MI = metaphase I; GV = germinal vesicle.
There are, however, no ascribed to abnormalities of the sperm (Liu et al., 1989). Presumed to be present (Mahadevan and Trounson, 1984; Jeulin et al., 1986; Liu and Baker, 2000). Poor or absent binding of sperm to the zona pellucida, for instance, is readily ascribed to abnormalities of the sperm (Liu et al., 1989a,b). There are, however, no firm data to substantiate this hypothesis.

When the sperm is normal according to the classical criteria, an as yet undisclosed sperm defect is due to some sperm defect. When the sperm is normal according to the criteria for performing either IVF or ICSI. The low rate we observed is due to the fact that IVF was only performed when sperm quality were established. But even if apparent sperm, failure of fertilization after in-vitro insemination occasionally occurs. Although fertilization, albeit at a lower rate compared with the control group, was obtained after ICSI of unfertilized oocytes, no pregnancies were established.

Because fertilization at IVF is considered the ultimate proof of the fertilizing capacity of the sperm, there is an intuitive tendency to conversely assume that failure of fertilization is due to some sperm defect. When the sperm is normal according to the classical criteria, an as yet undiscovered sperm defect is presumed to be present (Mahadevan and Trounson, 1984; Jeulin et al., 1986; Liu and Baker, 2000). Poor or absent binding of sperm to the zona pellucida, for instance, is readily ascribed to abnormalities of the sperm (Liu et al., 1989a,b). There are, however, no firm data to substantiate this hypothesis.

Pregnancies either spontaneously or in subsequent IVF cycles do occur in patients with previous unexplained failure of fertilization, which suggests that occult sperm deficiencies, if they exist, are transient. There are, however, several other possible reasons for oocytes not being fertilized after IVF when the sperm is apparently normal. Bedford et al. demonstrated that inability of apparently competent sperm to penetrate the zona is often associated with ooplasmic anomalies such as refractile bodies, extra groups of chromosomes, chromatin rings or masses as well as one or more pronuclei with one or no polar bodies (Bedford et al., 1993). Also further downstream, a number of defects in the fertilization process such as sperm fusion with oolemma, nuclear decondensation or formation of the male pronucleus and failed oocyte activation have been reported (Ducibella et al., 1995; Flaherty et al., 1995; Palermo et al., 1997). Besides a disordered sperm–oocyte interaction, there are a number of developmental defects of the oocyte which can impede fertilization and/or the reproductive outcome. Spontaneous cytoplasmatic activation resulting in premature cortical granule loss and zona pellucida hardening (Ducibella et al., 1995) or cytoplasmic degeneration, manifested by refractile bodies, can affect fertilization (Serhal et al., 1997). Arrest or asynchrony of cytoplasmatic maturation can impact on the reproductive outcome of fertilized oocytes (Kubiak, 1989).

Faced with the frustrating experience of all oocytes remaining unfertilized the day after in-vitro insemination, all kind of attempts have been made to rescue the cycle by reinsemination or micromanipulation of the oocytes. Lundin et al. were the first to report pregnancies by microinjection of unfertilized IVF oocytes (Lundin et al., 1996). Other centres also reported successes with pregnancy rates ranging from 6.9 to 20.7% (Bussen et al., 1997; Morton et al., 1997; Yuzpe et al., 2000). However, in some cases, mixed transfers of IVF-ICSI embryos were performed (Table IV). Not all reports, however, are positive and a number of authors have reported negative results similar to ours and advise against the practice of rescue ICSI (Chen et al., 1995; Park et al., 2000).

Fertilization of oocytes by rescue ICSI indicates that the intrinsic fertilizing capacity of the sperm is intact in most cases. The very poor outcome in terms of pregnancy indicates that some intrinsic or developmental defect of the oocyte is

### Table II. Outcome of ICSI of 1 day old oocytes (group I) and fresh oocytes (group II)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of 2PN/no. of injected oocytes</td>
<td>30.4</td>
<td>56.1</td>
</tr>
<tr>
<td>48 2PN/cycle (SD)</td>
<td>1.84 (1.83)*</td>
<td>3.3 (2.67)*</td>
</tr>
<tr>
<td>Mean no. of embryos per cycle</td>
<td>1.78 (1.62)*</td>
<td>3.69 (2.71)*</td>
</tr>
<tr>
<td>% of two blastomeres embryos (no.)</td>
<td>32.2 (69)*</td>
<td>15.5 (160)*</td>
</tr>
<tr>
<td>% of four blastomeres embryos (no.)</td>
<td>52.3 (112)*</td>
<td>71.9 (742)*</td>
</tr>
<tr>
<td>Fragmentation of embryos at second day [% (no.)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20%</td>
<td>50 (56)</td>
<td>46 (344)</td>
</tr>
<tr>
<td>20–50%</td>
<td>29 (33)</td>
<td>29 (215)</td>
</tr>
<tr>
<td>&gt;50%</td>
<td>21 (23)</td>
<td>25 (183)</td>
</tr>
<tr>
<td>No. of co-cultured embryos/no. of blastocysts obtained (%)</td>
<td>48/0 (0)</td>
<td>407/92 (22.6)</td>
</tr>
</tbody>
</table>

*P < 0.05.

PN = pronuclei.

### Table III. Number of embryos per transfer and pregnancy outcome after ICSI of 1 day old oocytes (group I) and fresh oocytes (group II)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean no. of embryos transferred (SD)</td>
<td>1.66 (0.68)*</td>
<td>2.0 (0.5)*</td>
</tr>
<tr>
<td>No. of transfers/cycles (%)</td>
<td>100/120* (83.3)</td>
<td>264/280* (94.3)</td>
</tr>
<tr>
<td>Pregnancy (β-hCG) (%)</td>
<td>0</td>
<td>58/280 (20.7)</td>
</tr>
<tr>
<td>Pregnancy* per started cycle (%)</td>
<td>0</td>
<td>58/264 (21.9)</td>
</tr>
</tbody>
</table>

*P < 0.05.

**Confirmed by ultrasound.

rate of 21.9% per transfer or 20.7% per started cycle). In group I with rescue ICSI, no pregnancy was obtained. About half of these patients (n = 54) were subsequently treated by ICSI. The pregnancy rate in the first ICSI cycles of these patients was 14.8%.

### Discussion

The rate of total failure of fertilization after IVF in our programme (10.5%) was at the lower limit of reported rates that vary from 10 to 25%. The variation in reported rates of failed fertilization is dependent on the criteria for performing either IVF or ICSI. The low rate we observed is due to the fact that IVF was only performed when sperm quality was within the normal range of fertile sperm. But even with apparently normal sperm, failure of fertilization after in-vitro insemination occasionally occurs. Although fertilization, albeit at a lower rate compared with the control group, was obtained after ICSI of unfertilized oocytes, no pregnancies were established.

Regarding the number of defects in the fertilization process such as sperm–oocyte interaction, there are a number of developmental defects of the oocyte which can impede fertilization and/or the reproductive outcome. Spontaneous cytoplasmatic activation resulting in premature cortical granule loss and zona pellucida hardening (Ducibella et al., 1995) or cytoplasmic degeneration, manifested by refractile bodies, can affect fertilization (Serhal et al., 1997). Arrest or asynchrony of cytoplasmatic maturation can impact on the reproductive outcome of fertilized oocytes (Kubiak, 1989).

Faced with the frustrating experience of all oocytes remaining unfertilized the day after in-vitro insemination, all kind of attempts have been made to rescue the cycle by reinsemination or micromanipulation of the oocytes. Lundin et al. were the first to report pregnancies by microinjection of unfertilized IVF oocytes (Lundin et al., 1996). Other centres also reported successes with pregnancy rates ranging from 6.9 to 20.7% (Bussen et al., 1997; Morton et al., 1997; Yuzpe et al., 2000). However, in some cases, mixed transfers of IVF-ICSI embryos were performed (Table IV). Not all reports, however, are positive and a number of authors have reported negative results similar to ours and advise against the practice of rescue ICSI (Chen et al., 1995; Park et al., 2000).

Fertilization of oocytes by rescue ICSI indicates that the intrinsic fertilizing capacity of the sperm is intact in most cases. The very poor outcome in terms of pregnancy indicates that some intrinsic or developmental defect of the oocyte is...
likely to be present in cases of failed fertilization after IVF with normal sperm. This hypothesis is supported by the success of oocyte donation in cases of previously and repeatedly failed fertilization (Borini et al., 1996). Ageing of the oocyte due to delayed fertilization presumably is the most important reason for the poor outcome. A low blastulation rate has been observed in cases of delayed fertilization (Gullet et al., 1998). This was confirmed by our study, although no blastocysts were obtained after prolonged co-culture with Vero cells. The time interval between oocyte retrieval and fertilization is critical for an optimal embryo development. While incubation of oocytes for a period of 3 h before ICSI improves the fertilization rate and the embryo quality (Rienzi et al., 1998) an incubation period of 8 h seems to be the limit beyond which the outcome of ICSI will be affected (Jacobs et al., 2001). Prolonged culture of mouse oocytes can lead to zona hardening, increased parthenogenic activation and impaired embryonic development (Fukuda et al., 1992). Nagy et al. demonstrated that the fertilization rate decreased and that the incidence of abnormal fertilization increased with oocyte ageing (Nagy et al., 1995). Reinsemination of oocytes might also be associated with a risk of genetic defects in the offspring. Fertilization in vivo of aged mouse oocytes has a negative effect on the male and female pronuclear chromosomes (Boerjan and Saris, 1991). More recently, a study on unfertilized oocytes after IVF (Wang et al., 2001) found that the spindle of most aged oocytes was partially or completely disassembled when observed using a polscope. Chromosomal analysis of failed fertilized oocytes after IVF showed that about one-third of the oocytes had an abnormal karyotype, and that in about one-third of the unfertilized oocytes sperm penetration occurred, but premature chromosome condensation was present (Kunathikom et al., 2001).

The normal pregnancy rate obtained in the patients with previously failed fertilization who were subsequently treated by ICSI proves that in the majority of cases, failure of fertilization is not due to an intrinsic and permanent defect of either sperm or oocytes.

In conclusion, rescue ICSI, although resulting in fertilization, gives very poor results in terms of pregnancy. Prolonged incubation of oocytes presumably affects the developmental capacity of the embryo. Based on our experience, rescue ICSI of unfertilized oocytes after IVF does not seem to be worthwhile.

### Table IV. Comparison of studies on rescue ICSI of unfertilized oocytes after IVF

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample size (cycles)</th>
<th>Mixed transfers</th>
<th>Case-control study</th>
<th>Consecutive study</th>
<th>Pregnancy rate per transfer % (per cycle, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yuzpe et al. (2000)</td>
<td>32 TFF/PFF</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>20.7 (18.7)</td>
</tr>
<tr>
<td>Bussen et al. (1997)</td>
<td>1</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>One live-born child</td>
</tr>
<tr>
<td>Morton et al. (1997)</td>
<td>54 TFF</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>16.6 (14.8)</td>
</tr>
<tr>
<td>Lundin et al. (1996)</td>
<td>57 TFF/PFF</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>6.9 (3.5)</td>
</tr>
<tr>
<td>Present study</td>
<td>120 TFF</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>0</td>
</tr>
</tbody>
</table>

TFF = total fertilization failure; PFF = partial fertilization failure.

### References


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