Comparison between intracytoplasmic sperm injection and in-vitro fertilization (IVF) with high insemination concentration after total fertilization failure in a previous IVF attempt

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Introduction

Intracytoplasmic sperm injection (ICSI) has progressively replaced all other microinjection procedures for overcoming intractable male-factor infertility and has emerged in a relatively short time as a routine procedure for many in-vitro fertilization (IVF) programmes. Since the introduction of ICSI in our centre, we advocate the use of ICSI only when previous fertilization failures with IVF had occurred or the numbers of harvestable motile spermatozoa are less than $1 \times 10^6$ per ejaculate. We sympathize with the policy of Tucker et al. (1993) and Baker et al. (1993) that ‘the main aim should always be to use the simplest and least expensive procedures, with the greatest long-term chance of healthy children’. Edirisinghe et al. (1997) have reported that the incidence of chromosomal abnormalities in unfertilized oocytes after IVF and ICSI was similar. However, the increased rate of sex chromosomal abnormalities in ICSI pregnancies (Liebaers et al., 1995; Meschede and Horst, 1997) as well as the recent reclassification of the birth defects reported in infants born after ICSI by Kurinczuk and Bower (1997) has expanded the concerns about the safety of ICSI and potential risks for the offspring. In our view, this enhanced the necessity to apply ICSI only when fertilization of oocytes with IVF is seriously compromised.

Previous studies have made comparisons between IVF and ICSI and exhibited discrepant results. Due to the use of different insemination concentrations, very divergent fertilization rates were demonstrated after IVF. But although fertilization rates observed with ICSI were significantly higher (Payne et al., 1994; Hall et al., 1995; Calderon et al., 1996a,b), Hall and co-workers (1995) reported no significant difference in implantation and pregnancy rate between ICSI and IVF with high insemination concentrations. In this prospective randomized study with sibling metaphase II oocytes, we aimed to investigate whether couples with total fertilization failure in a previous IVF attempt should be offered an additional IVF attempt with elevated insemination concentration or should be treated with ICSI.

Materials and methods

Patients

During a 6 month period, couples entered this prospective randomized study with sibling metaphase II oocytes to compare the fertilization rates of ICSI and conventional IVF with high insemination concentrations. In order to get couples motivated to participate in this study, they were assured that at least three mature oocytes would be injected. The protocol was reviewed and approved by the Dutch national ethical committee (KEMO). The couples selected were informed about the unknown aspects of this treatment and were asked for written informed consent.

Twenty eight couples meeting the criteria of suffering from long-standing infertility and who had experienced total fertilization failure in at least one previous conventional IVF attempt in our IVF centre were selected for this study. The majority of these patients were
suffering from male factor infertility, although repeated failed fertilization was also observed in four cases of tubal pathology or unexplained infertility. In cases of male factor infertility, at least 1 × 10⁶ progressively motile spermatozoa were present in the whole ejaculate.

Five couples dropped out, since less than five metaphase II oocytes were obtained in the treatment cycle, so ultimately 23 couples were included in this study. Eight couples had suffered from complete fertilization failure in one previous IVF attempt and 11 couples had total failure of fertilization in two previous IVF attempts. The other four couples had obtained only one fertilized oocyte in one of the previous IVF cycles. However, the overall fertilization rate in their previous cycles never exceeded 5%. In total, these 23 couples underwent 40 previous IVF cycles in which 404 cumulus–oocyte complexes were inseminated. In four cycles only one fertilized oocyte was obtained and replaced, but no pregnancy was obtained. The mean (±SD) female partner age was 33.6 ± 3.6 years (range 27–40 years) and the mean duration of infertility was 6.6 ± 2.9 years (range 3–14 years).

In previous semen analyses of the 23 couples, oligoasthenoteratozoospermia, according to WHO criteria (WHO, 1992), was observed in 10 cases. The mean total motile sperm count was 5.6 ± 4.3 × 10⁶ (range 1.0–13.6 × 10⁶) and the mean percentage of normal forms was 18.0 ± 9.9% (range 3–29%). A double sperm defect was detected in nine cases. The mean total motile sperm count and percentage of normal forms for this group was 30.6 ± 25.6 × 10⁶ (range 4.3–79.0 × 10⁶) and 27.1 ± 13.5% (range 13–50%) respectively. In four cases, a single sperm defect was observed with a mean total motile sperm count of 32.6 ± 4.9 × 10⁶ (range 26.7–38.5 × 10⁶) and a mean percentage of normal forms of 21.3 ± 19.1% (range 5–44%). In two of the latter cases, patients were diagnosed as unexplained infertility, as teratozoospermia was caused by a marginal sperm defect.

Follicular stimulation
In all patients, controlled ovarian stimulation was carried out by the administration of gonadotrophin-releasing hormone analogue (GnRHa) (Lucrin®; Abbott, Amstelveen, The Netherlands) in a long protocol, followed by human menopausal gonadotrophins (HMG) (Pergonal®; Serono Benelux BV, The Hague, The Netherlands) and pure follicle-stimulating hormone (FSH) (Metrodin®; Serono) from cycle day 3. Ovulation was induced by the administration of human chorionic gonadotrophin (HCG; 10 000 IU i.m.) (Profas®; Serono). Previously, the protocol has been described in detail (Van Kooij et al., 1996).

Semien preparation
Except for one cycle, IVF and ICSI were performed using freshly ejaculated sperm. After liquefaction, semen concentration and motility were assessed microscopically. If less than 5 × 10⁶ motile spermatozoa were observed in the total ejaculate, a second semen sample was required. The semen samples were diluted 1:1 with human tubal fluid (HTF) medium, supplemented with 10% of a pasteurized human plasma-protein solution (Red Cross Central Blood Transfusion Laboratory, Amsterdam, The Netherlands). After centrifugation over a discontinuous gradient of two layers of Percoll, sperm pellets were washed twice by resuspension and centrifugation. If possible, spermatozoa were allowed to swim up. Sperm concentration and motility of washed sperm suspensions or swim-up fraction were assessed by means of a Makler chamber.

Collection and preparation of oocytes
Oocytes were recovered by transvaginal ultrasound-guided follicle aspiration, 34–36 h after HCG administration. Immediately before micromanipulation, cumulus and corona cells were removed enzymatically by incubating the oocytes in HEPES-buffered HTF medium containing 80 IU/ml hyaluronidase (Type VIII, Sigma, St Louis, MO, USA) for up to 3 min. Enzymatic removal was enhanced mechanically by aspirating the oocytes in and out of hand-drawn and fire-polished Pasteur pipettes. The denuded oocytes were examined to assess integrity and maturity. Only those oocytes that had reached metaphase II (MII) and extruded the first polar body were used in this study and treated either with ICSI or conventional IVF.

After randomly choosing three oocytes for ICSI, the remaining sibling MII oocytes were divided into two groups. ICSI oocytes were injected subsequently after denudation, whereas IVF oocytes were inseminated with 1 × 10⁶ to 1 × 10⁷/ml motile spermatozoa; i.e. if available an at least threefold (range ×3–12.5) increased sperm concentration in comparison to the previous IVF attempts. In six cases, sperm quality was too poor to increase the insemination concentration. The IVF oocytes were incubated in droplets of 50–100 µl IVF medium covered with lightweight parafin oil. Insemination and ICSI procedures were performed 40–41 h post HCG administration.

Intracytoplasmic sperm injection
Both holding and injection micropipettes were ready-made (Humagen Fertility Diagnostics, USA), and the ICSI procedure was carried out at ×300 magnification using Hoffman modulation optics.

Immediately before injection, sperm suspension was added to a 10 µl droplet of 10% polyvinylpyrrolidone (PVP; Medicut, Copenhagen, Denmark). Injection of oocytes was performed in microdroplets of HEPES-buffered HTF medium. All microdroplets were covered with lightweight parafin oil.

A single motile spermatozoon with apparently normal morphology was immobilized by touching its tail with the injection pipette and then aspirated tail first into the injection pipette. After securing the oocyte with the polar body at the 6 or 12 o’clock position onto the holding pipette, the micropipette was pushed through the zona pellucida and the oolemma into the ooplasm at the 3 o’clock position. When penetration of the oolemma was verified by aspirating some cytoplasm, the spermatozoon was slowly ejected. The injection pipette was withdrawn gently and the oocyte was released from the holding pipette. The ICSI procedure was repeated until all MII oocytes were injected.

Assessment of fertilization and cleavage
About 16–18 h after insemination and microinjection, the oocytes were examined for the presence of pronuclei and polar bodies. Fertilization was considered normal when two clearly distinct pronuclei were present. If a single pronucleus was observed, a second evaluation was carried out 4–6 h later.

Cleavage of the fertilized oocytes was assessed after a further 24 h and 48 h culture period. Depending on the age of the female partner, up to three normally cleaved embryos (<38 years, up to two embryos; ≥38 years, up to three embryos) were replaced. Supernumerary embryos of good morphological quality were cryopreserved.

Pregnancy testing
Biochemical pregnancies were defined as a positive result of a urine β-HCG pregnancy test, 18 days after follicle aspiration. Clinical pregnancies were confirmed by ultrasound at 7–8 weeks of gestation and pregnancies were considered to be ongoing when there was at least one fetus with vital heartbeat after 12 weeks of gestation.

Statistical analysis
After a random selection of 23 oocytes in both groups, a McNemar’s test was applied as a paired comparison of the fertilization rates obtained with IVF and ICSI.
Table I. Results with conventional in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) of sibling oocytes in 23 cycles

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<th>Conventional IVF</th>
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<td>MII oocytes</td>
<td>85</td>
<td>143</td>
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<td>Oocytes with:</td>
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<td>2 pronuclei</td>
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<td>3 pronuclei</td>
<td>0</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>1 pronucleus</td>
<td>0</td>
<td>9&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>no fertilization</td>
<td>85</td>
<td>13&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Cleaved embryos</td>
<td>82</td>
<td>91.1</td>
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<tr>
<td>Replacements</td>
<td>23</td>
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<tr>
<td>Replaced embryos</td>
<td>48</td>
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<tr>
<td>Implantation rate</td>
<td>11</td>
<td>22.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Pregnancies</td>
<td>8</td>
<td>34.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Ongoing pregnancies</td>
<td>6</td>
<td>26.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Live birth rate</td>
<td>9</td>
<td>18.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup>Per embryo replaced.  
<sup>b</sup>Per embryo replacement.  
<sup>c</sup>Significantly different from IVF result (P < 0.001).

**Results**

Twenty three couples were included in this study where at least five mature MII oocytes were obtained in the treatment cycle. In 22 cycles, freshly ejaculated sperm samples were used, and in one cycle ICSI and IVF was performed with frozen–thawed ejaculated spermatozoa of the male partner. In nine cases spermatozoa were allowed to swim up, since enough motile spermatozoa were present in the ejaculate. In seven cases a second ejaculate was needed in order to obtain sufficient motile spermatozoa to inseminate with high sperm concentrations.

In total, 269 cumulus-oocyte complexes were retrieved by ultrasound guided oocyte retrieval; a mean of 11.7 complexes per cycle. After enzymatic and mechanical removal of the surrounding cumulus and corona cells, 255 (94.8%) oocytes revealed an intact zona pellucida and clear cytoplasm. Nuclear maturity of these 255 intact oocytes was assessed just prior to the ICSI procedure; 228 (89.4%) had reached the metaphase II stage and extruded the first polar body, whereas 17 (6.6%) were still in the germinal vesicle stage and 10 (3.9%) were metaphase I oocytes, which had undergone germinal vesicle breakdown but not yet extruded the first polar body. Before sibling MII oocytes were randomly divided into the ICSI and IVF group. Of the 228 MII oocytes, 143 were treated with ICSI and 85 MII oocytes were treated with conventional IVF and inseminated (Table I).

Despite the use of high insemination concentrations of up to 1×10^7 motile spermatozoa per ml, no fertilization at all was observed in the 85 MII oocytes treated with IVF. Even after a second evaluation 20–24 h after insemination, in none of the inseminated MII oocytes was a pronucleus observed. A significantly higher (P < 0.001) fertilization rate was observed after ICSI. Of the 143 injected oocytes, 90 (62.9%) were normally fertilized and contained two pronuclei, whereas 13 oocytes (9.1%) showed no sign of fertilization at all. Nine (6.3%) oocytes showed only one pronuclei and three pronuclei were recorded in 10 (7.0%) oocytes. Twenty-one (14.7%) oocytes were degenerated, most likely due to damage during the injection procedure. No differences were observed between the fertilization rates of the group with a single, double and triple sperm defect. Of the fertilized oocytes, 82 (91.1%) cleaved of which 72 (80%) developed into transferable embryos, i.e. embryos with <50% fragmentation. In all 23 cycles at least one embryo, obtained by ICSI, could be replaced. One single embryo was transferred in one cycle (4.3%), two embryos in 19 cycles (82.6%) and three embryos in three cycles (13.0%). Eight pregnancies (34.8%) were achieved, but a gestational sac without a vital heartbeat was diagnosed in two cases. The remaining six (26.1%) pregnancies resulted in the delivery of nine healthy children, yielding an implantation rate and live birth rate per embryo replaced of 22.9 and 18.8% respectively.

**Discussion**

In our IVF programme, conventional IVF is only applied when at least 1×10^6 progressively motile spermatozoa are present in the total ejaculate of at least one semen analysis. Routinely oocytes are inseminated with 2×10^5 motile spermatozoa per ml. The insemination concentration is elevated if the semen characteristics showed 10% or less normal morphology in one or more previous semen analyses, according to the WHO criteria (WHO, 1992). In addition, the insemination concentration is increased when on the day of the oocyte retrieval, a motility of less than 10% or a marginal sperm count is observed. Also in cases where patients experienced total failure of fertilization or a disappointing fertilization rate in a previous IVF treatment cycle, the insemination concentration is increased five- to tenfold. Whenever fertilization failed in two previous attempts, despite the use of elevated insemination concentrations, couples were advised to refrain from further IVF treatment. However, since several studies have demonstrated the benefit of using high insemination concentrations or microdrops in cases of previously failed IVF attempts (Baker et al., 1993; Fishel et al., 1993; Tucker et al., 1993), and with the introduction of ICSI at our centre, it is questionable whether these couples should be offered an additional IVF treatment with increased insemination concentration or whether they should be treated with ICSI.

In the present study, 23 couples were included who had experienced total failure of fertilization in at least one previous IVF attempt in which elevated insemination concentrations had already been applied. Although the precise number of MII oocytes in all those previous IVF cycles is unknown, the overall fertilization rate would not have exceeded 1%, whereas for the four individual couples who had one fertilized oocyte in one of their previous cycles, the fertilization rate never exceeded 5%. Since for all 23 couples the fertilization of oocytes with conventional IVF was compromised, in the present study the insemination concentration was further increased and microdrops were used. Although insemination concentrations of up to 1×10^7 motile spermatozoa per ml were used, no fertilization was observed in the 85 conventionally inseminated oocytes. The fertilization rate achieved with ICSI was signific-
antly higher; 62.9% of the injected oocytes were normally fertilized. In order to include only intact MII oocytes in this comparing study, oocytes were enzymatically denuded to assess integrity and nuclear status. It can be suggested that the complete failure of fertilization in the IVF group could be caused by a possible negative effect of enzymatic denudation of the oocytes before insemination. However, previous studies showed no significant difference in fertilization rate between cumulus-intact and cumulus-denuded oocytes (Mahadevan and Trounson, 1985; Magier et al., 1990). Lavy et al. (1988) even reported a significantly higher fertilization rate of hyaluronidase denuded oocytes in patients with total fertilization failure in previous IVF cycles.

Several reports are published in which ICSI and conventional IVF were compared with sibling oocytes (Payne et al., 1994; Calderón et al., 1995; Hall et al., 1995; Aboulghar et al., 1996a,b). Aboulghar et al. (1996a,b) made the comparison between ICSI and IVF within three groups of patients who were beginning their first IVF treatment cycle. In the group with tubal factor infertility with normal semen, they observed a significant higher fertilization rate per retrieved oocyte with IVF, but the pregnancy and abortion rate between ICSI and IVF showed no statistically significant difference. In a second study, they found no significant difference in the fertilization rate in the group of patients with unexplained infertility, whereas in the group of patients with borderline semen, they found a significant difference between the fertilization rate of ICSI (59%) and IVF (27.1%) oocytes. The incidence of total fertilization failure in those cycles was 22.7% in the group with unexplained infertility and 45.8% in the group with borderline semen. If the patients who had no fertilization were excluded, there was no significant difference in the fertilization rate between ICSI and conventional IVF. This suggests that with the insemination concentration of only 1×10^6 spermatozoa per ml, a kind of ‘all or nothing’ chance of fertilization exists in cases of male factor infertility. Probably, fewer patients would have experienced total fertilization failure if higher insemination concentrations had been used as in the present study. This is also the case in the study of Payne et al. (1994), who also treated patients with poor sperm morphology (<20% normal) with both ICSI and conventional IVF. In that study sibling MII oocytes were inseminated with 5×10^4 motile spermatozoa per ml. The overall fertilization rate of ICSI and IVF oocytes differed significantly, 76% versus 15%, and the incidence of total fertilization failure in the IVF group was 61%. It remains to be established whether fewer patients would have experienced no fertilization if the insemination concentration had been doubled.

Calderón et al. (1995) reported a similar prospective and randomized study with sibling oocytes. The 34 couples included in their study suffered from male factor infertility. Seven couples experienced total failure of fertilization in a previous IVF treatment cycle. The overall fertilization rate obtained with ICSI (49.5%) was significantly higher than the fertilization rate obtained with IVF (19.5%). Only two of the seven couples (28.5%) with no fertilization in a previous attempt achieved fertilization of one oocyte with conventional IVF (7.4%). However, the total number of patients with total failure of fertilization in those 34 cycles, as well as the insemination concentrations used in the study, are not described.

Baker et al. (1993) stated that their fertilization rates for patients with <20% normal morphology ‘approximately doubled’ by utilizing insemination concentration from 2×10^5 to 1×10^6 motile spermatozoa per ml. Hall et al. (1995) used insemination concentration of 1×10^6 motile spermatozoa per ml in their study to evaluate a sibling oocyte comparison between IVF and ICSI for patients with severe teratozoospermia. They reported a fertilization rate of 59% with IVF and 67% with ICSI. No significant difference in implantation and pregnancy rate was demonstrated with either technique.

Summarizing the data from the studies quoted above reveals that once fertilization is obtained with conventional IVF using elevated insemination concentrations, embryo quality, implantation rate and pregnancy rate do not appear to be compromised. Therefore we adhered to strict inclusion criteria at the introduction of ICSI in our centre and now advocate the use of ICSI only when IVF treatments with elevated insemination concentrations have failed or when the numbers of motile spermatozoa are <1×10^6 per ejaculate and fertilization is severely compromised. In the present study, we assessed the validity of the decision whether IVF with increased insemination concentration or ICSI should be performed after total fertilization failure in a previous IVF treatment cycle. The couples selected had an insemination concentration of at least 0.3×10^6 motile spermatozoa per ml in their latest IVF attempt. Despite the further increased insemination concentration, no fertilization was observed, whereas with ICSI excellent fertilization and pregnancy rates were achieved. Although the technical difficulties, costs and the concerns for safety and long-term effects of ICSI cannot be ignored, currently ICSI is the most efficacious form of assisted fertilization. So for couples with failed fertilization in a previous IVF attempt with elevated insemination concentrations, ICSI should be the treatment of choice.

References
ICSI versus IVF with high insemination concentrations


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