Should ICSI be the treatment of choice for all cases of in-vitro conception?

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The objective of this study was to examine different clinical scenarios of in-vitro conception, viz. fertilization with conventional IVF, IVF with high insemination concentration (HIC) and intracytoplasmic sperm injection (ICSI), and assess on a sibling oocyte comparison the hypothesis that ICSI should be performed in all cases requiring in-vitro conception. ICSI with husband’s spermatozoa had a higher incidence of fertilization as compared with IVF or IVF with HIC with donor spermatozoa (if previous failure of fertilization had occurred) for unexplained infertility. Similarly, ICSI with husband’s spermatozoa had as high an incidence of fertilization as IVF with donor spermatozoa for patients with severe oligozoospermia, asthenozoospermia and/or teratozoospermia, even when the spermatozoa were not selected for their morphology. Two studies were performed to assess ICSI in potential oocyte-related failure of IVF, viz. when fertilization occurred in >50% of oocytes for one group of patients, and in <50% of oocytes in a second group. In both of these studies a significant proportion of the oocytes that failed to fertilize with conventional IVF eventually fertilized after ICSI. The overall conclusion was that ICSI as a first option offers a higher incidence of fertilization, maximizes the number of embryos and minimizes the risk of complete failure of fertilization for all cases requiring in-vitro conception. However, among other concerns, current knowledge of ICSI as an outcome procedure does not provide the confidence to use this process in all cases of IVF for the time being.

Key words: assisted conception/ICSI/infertility/IVF/micro-injection

Introduction

Intracytoplasmic sperm injection (ICSI) with its high fertilization and pregnancy rates has gradually replaced conventional IVF and other types of micromanipulation as first-line therapy in couples with severe male factor infertility. Since the report of the first human pregnancies achieved by the injection of the single spermatozoon into a human oocyte (Palermo et al., 1992), this technique has been applied extensively world-wide. ICSI ensures high fertilization and pregnancy rates regardless of sperm concentration, motility or morphology, even when epididymal or testicular spermatozoa are used (Devroey et al., 1994; Nagy et al., 1995a,b; Silber, 1995), with the resultant extension of this technique to patients for whom conventional IVF may be an option, including infertile partners with unexplained infertility (Aboulghar et al., 1996a).

Considering the high success rate of ICSI, it is reasonable to consider this technique for all cases requiring in-vitro conception, with a limitation for some cases of female infertility but specifically taking into consideration the age of the woman (Oehninger et al., 1995), and notwithstanding cost and the need for qualified laboratory personnel and facilities (Yang et al., 1996). In contrast, some authorities advocate the use of ICSI only when previous fertilization failure with IVF has occurred, or the number and/or quality of available spermatozoa is not appropriate for IVF; others have expressed the view that the main aim should always be to use the simplest and least expensive procedure, with the greatest long-term chance of healthy children (Baker et al., 1993; Tucker et al., 1993). Previous studies comparing IVF and ICSI have given inconsistent results. Due to the use of different insemination concentrations, divergent rates of fertilization were demonstrated after IVF. Although the rates of fertilization observed with ICSI were significantly higher (Payne et al., 1994; Calderon et al., 1995; Aboulghar et al., 1996a,b), it was reported in one study (Hall et al., 1995) that there was no significant difference in implantation and pregnancy rates between ICSI and IVF with high insemination concentrations.

Here we present results of our studies on sibling metaphase II (MII) oocytes. During these prospective randomized studies our aim was to investigate the suggestion that ICSI should be offered as a treatment of choice for all cases of in-vitro conception.

Materials and methods

Patients

In a multicentre study during a 5-year period between 1994 and 1998, 221 patients entered this prospective randomized study with sibling MII oocytes to compare the incidences of fertilization between ICSI and conventional IVF (inseminating with 0.1×10⁶ motile spermatozoa) or IVF with high insemination concentration (HIC) (inseminating with 0.5×10⁶ motile spermatozoa) in five different
clinical situations (see below). Patients were fully briefed and consented to the procedures undertaken.

**Group 1: Idiopathic failed conventional IVF (spermatozoa with normal count, motility and morphology); insemination with husband’s spermatozoa**

Thirty-seven patients with unexplained failed conventional IVF in a previous treatment cycle were included in this group. Sibling MII oocytes from these patients were divided randomly into two groups and inseminated with husband’s spermatozoa: (i) 206 oocytes were inseminated with husband’s spermatozoa using HIC; and (ii) 212 oocytes were inseminated with husband’s spermatozoa using ICSI.

**Group 2: Idiopathic failed conventional IVF with HIC (spermatozoa with normal count, motility and morphology); insemination with donor spermatozoa**

Eighteen patients with idiopathically failed HIC in a previous treatment cycle were included in this group. Sibling MII oocytes from these patients were divided into two groups randomly and were inseminated with donor spermatozoa: (i) 80 oocytes were inseminated with donor spermatozoa using IVF; and (ii) 81 oocytes were inseminated with donor spermatozoa using ICSI.

**Group 3: Patients unsuitable for conventional IVF with husband’s spermatozoa (with one or more abnormalities in count, motility or morphology) but put forward for IVF with donor spermatozoa or ICSI with husband’s spermatozoa**

Thirty-six patients were unsuitable for conventional IVF with husband’s spermatozoa due to one or more abnormalities in count, motility or morphology. Patients were divided into three groups depending upon the type of abnormality. Sibling MII oocytes obtained from each group of patients were further divided into two subgroups randomly and were inseminated either with donor spermatozoa by using conventional IVF or by ICSI with spermatozoa obtained from the husband.

**A: Unsuitable for IVF due to low count**

Nineteen patients were unsuitable for IVF with husband’s spermatozoa due to low count. The subgroups were as follows: (i) 81 oocytes inseminated by using conventional IVF with donor spermatozoa; and (ii) 76 oocytes micro-injected with spermatozoa obtained from the husband.

**B: Unsuitable for IVF due to low motility and count**

Nine patients were unsuitable for IVF with husband’s spermatozoa due to low motility and count. The subgroups were as follows: (i) 54 oocytes were inseminated by using conventional IVF with donor spermatozoa; and (ii) 62 oocytes were micro-injected with spermatozoa obtained from the husband.

**C: Unsuitable for IVF due to abnormal morphology (100% teratozoospermia)**

Eight patients were unsuitable for IVF with husband’s spermatozoa due to 100% teratozoospermia. The subgroups were as follows: (i) 45 oocytes were inseminated by using conventional IVF with donor spermatozoa; and (ii) 56 oocytes were micro-injected with spermatozoa obtained from the husband.

**Group 4: Successful conventional IVF (no abnormal semen parameters were noted and >50% oocytes fertilized); re-insemination of unfertilized oocytes using ICSI with husband’s spermatozoa**

In 72 patients more than 50% of oocytes were successfully fertilized with conventional IVF. In this group of patients 131 unfertilized oocytes were re-inseminated by ICSI with spermatozoa obtained from the husband.

**Group 5: ‘Unsuccessful’ conventional IVF (no abnormal semen parameters were noted and <50% oocytes fertilized); unfertilized oocytes were re-inseminated by using IVF with HIC or ICSI with husband’s spermatozoa**

In 58 patients >50% oocytes were unfertilized (i.e. <50% fertilized) after conventional IVF. In this group of patients, 108 unfertilized sibling oocytes were re-inseminated by IVF with HIC and 96 with ICSI with spermatozoa obtained from the husband.

**Semen preparation**

IVF, IVF with HIC and ICSI were performed using freshly ejaculated spermatozoa. After liquefaction, sperm concentration and motility were assessed microscopically. After centrifugation for 10 min at 500 g followed by passage over a discontinuous gradient of two layers of Percoll (Sigma, Poole, UK), sperm pellets were washed twice by resuspension and centrifugation for 5 min at 250 g (Fishel et al., 1995a). A Makler chamber was used to assess sperm concentration and motility of washed sperm suspensions. It should be noted that since 1997 Percoll has been replaced with Sil-Select (Feretipro, Beernem, Belgium) because Percoll was withdrawn from use in human assisted reproduction techniques. Duration and speed of centrifugation was the same as with Percoll.

**Collection of oocytes and their preparation**

Oocytes were recovered by transvaginal ultrasound-guided follicle aspiration, 34–36 h after human chorionic gonadotrophin (HCG) administration. After randomly choosing the oocytes for ICSI, cumulus and corona cells were removed enzymatically immediately before micromanipulation by incubating the oocytes in HEPES-buffered ICSI medium [complete minimal essential medium (MEM) supplemented with 4.5 mg/ml human serum albumin (HSA; Sigma) containing 80 IU/ml hyaluronidase (type III; Sigma)] for up to 2 min. Enzymatic removal was enhanced mechanically by aspirating the oocytes in and out of hand-drawn fine pipettes. The demuded oocytes were examined to assess integrity and maturity. Only MII oocytes with extruded first polar body were micro-injected in this study.

**Conventional IVF and IVF with HIC**

After randomly choosing the oocytes for ICSI, the remaining sibling MII oocytes were inseminated either by conventional IVF or IVF with HIC in various clinical situations as described above. For conventional IVF and IVF with HIC, oocytes were inseminated with 0.1×10⁶ or 0.5×10⁶ (0.2×10⁶–1.0×10⁶) motile spermatozoa/ml respectively of insemination medium (IVF medium; Medicult, Copenhagen, Denmark) (Fishel et al., 1995b).

**Intracytoplasmic sperm injection**

Details of the ICSI procedure have been described previously (Fishel et al., 1999a). Briefly, immediately before injection, spermatozoa were added to a 10 µl droplet of 10% polyvinylpyrrolidone (PVP; Medicult). Injection of oocytes was performed in microdroplets of HEPES-buffered ICSI medium (Medicult) covered with lightweight paraffin oil (Sigma).

A single spermatozoon with apparently normal morphology was immobilized by cutting across its tail with the injection pipette and then aspirating it, tail first, into the injection pipette. After securing the oocyte onto the holding pipette, with the polar body at the 6 or 12 o’clock position, the injection pipette 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 released from the holding pipette.
Table I. Comparison between incidence of fertilization achieved by IVF with high insemination concentration (HIC) and by ICSI using husband’s spermatozoa on the sibling oocytes retrieved from patients \((n = 37)\) with failed conventional IVF cycle (group 1)

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<th>HICa</th>
<th>ICSIa</th>
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<tr>
<td>No. of oocytes</td>
<td>206</td>
<td>212</td>
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<tr>
<td>No. of oocytes fertilized</td>
<td>69 (33%)</td>
<td>128 (60%)</td>
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*Significantly different, \(P < 0.001\).

Assessment of fertilization

At about 16–18 h after insemination and micro-injection, the oocytes were examined for the presence of pronuclei and polar bodies. Fertilization was considered normal when two clearly distinct pronuclei were present.

Embryo quality

Embryos were assessed on the basis of membrane integrity and regularity of blastomeres, on the presence of cytoplasmic fragmentation, and on the rate of cleavage: as ‘normal’, slow or arrested embryos (Fishel et al., 1985).

Statistical analysis

A \(\chi^2\)-test was applied as a paired comparison of the incidences of fertilization obtained with IVF, IVF with HIC and ICSI.

Results

Group 1

In 37 patients with idiopathic failed conventional IVF, 206 sibling MII oocytes were inseminated using HIC with husband’s spermatozoa. Out of these oocytes, 69 fertilized normally (33%). On the other hand, out of 212 sibling MII oocytes which were micro-injected with husband’s spermatozoa, 128 fertilized normally (60%) \((P < 0.001)\) (Table I).

Group 2

In 18 patients with idiopathic failed conventional IVF with HIC, 80 sibling MII oocytes were inseminated by conventional IVF using donor spermatozoa. Out of these oocytes, 48 fertilized normally (60%). On the other hand, out of 212 sibling MII oocytes, which were micro-injected with donor spermatozoa, 128 fertilized normally (60%) \((P < 0.001)\) (Table I).

Group 3

Group 3A

Nineteen patients were unsuitable for conventional IVF due to low sperm count. In this group, 81 sibling MII oocytes were inseminated with IVF using donor spermatozoa. Out of these oocytes, 46 fertilized normally (57%). On the other hand, out of 76 sibling MII oocytes, which were micro-injected with husband’s spermatozoa, 49 fertilized (64%) (not significant) (Table III).

Group 3B

Nine patients were unsuitable for conventional IVF due to low sperm count and motility. In this group, 54 sibling MII oocytes were inseminated with IVF using donor spermatozoa. Out of these, 38 oocytes fertilized normally (70%). On the other hand, out of 62 sibling MII oocytes which were micro-injected with husband’s spermatozoa, 47 fertilized (75%) (not significant) (Table III).

Group 3C

Group 3A: Low count \((n = 19)\)

No. of oocytes fertilized 122 (68%) 144 (74%)

Group 3B: Low count \(\leq\) motility \((n = 9)\)

No. of oocytes fertilized 46 (57%) 49 (64%)

Group 3C: 100% teratozoospermia \((n = 8)\)

No. of oocytes fertilized 38 (70%) 47 (75%)

*Not significant.

Table II. Comparison between incidence of fertilization achieved by conventional IVF with high insemination concentration (HIC) and by ICSI using donor spermatozoa on the sibling oocytes retrieved from patients \((n = 18)\) with failed IVF with HIC cycle

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<th>IVFa</th>
<th>ICSIa</th>
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<tr>
<td>No. of oocytes</td>
<td>80</td>
<td>81</td>
</tr>
<tr>
<td>No. of oocytes fertilized</td>
<td>48 (60%)</td>
<td>64 (79%)</td>
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</table>

*Significantly different, \(P < 0.015\).

Table III. Comparison between incidence of fertilization achieved by conventional IVF with donor spermatozoa and by ICSI with husband’s spermatozoa in patients who were unsuitable for IVF with husband’s spermatozoa due to compromised semen parameters

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<th>IVFa</th>
<th>ICSIa</th>
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| Group 3: Overall data \((n = 36)\)
| No. of oocytes | 180  | 194   |
| No. of oocytes fertilized | 122 (68%) | 144 (74%) |

Group 3A: Low count \((n = 19)\)

No. of oocytes fertilized 81 76

Group 3B: Low count \(\leq\) motility \((n = 9)\)

No. of oocytes fertilized 46 (57%) 49 (64%)

Group 3C: 100% teratozoospermia \((n = 8)\)

No. of oocytes fertilized 38 (70%) 47 (75%)

*Not significant.
**Embryo quality**

No significant difference in embryo quality was noted between the embryos within groups, or between groups of patients when monitored to day 2 or day 3 in culture after fertilization. These observations were the same as we had noted previously (Hall et al., 1995), and therefore we adhered strictly to the protocol described (Hall et al., 1995).

**Discussion**

In patients with unexplained failure of a previous conventional IVF cycle (normal count, motility and morphology of spermatozoa), ICSI with husband’s spermatozoa showed a higher incidence of fertilization as compared with IVF with HIC using husband’s spermatozoa (P < 0.001) (Table I). Similarly, in patients with a previous idiopathic failed HIC cycle, an improved incidence of fertilization was seen after ICSI with donor spermatozoa as compared with conventional IVF with donor spermatozoa (P < 0.015) (Table II). These results showed that ICSI resulted in a statistically superior overall number of fertilized oocytes as compared with conventional IVF or IVF with HIC in the absence of any abnormal semen parameter. However, ICSI with husband’s spermatozoa did not significantly improve the incidence of fertilization as compared with conventional IVF with donor spermatozoa of sibling oocytes in the presence of at least one abnormal semen parameter (Table III). As embryos of mixed origin (i.e. IVF and ICSI from the same patient) were often transferred, and as pregnancy analysis would be between women with uncontrolled differences (e.g. age), this aspect of data outcome was not assessed.

Groups 4 and 5 involved comparison of unfertilized oocytes from patients with >50% or <50% fertilization rates. Although there are some reports that aged oocytes can be fertilized and result in healthy, live offspring, it is well known that this is an inefficient and unreliable procedure. Indeed, the Human Fertilisation and Embryology Authority (HFEA) of the UK has banned this approach. However, the object of this study was to quantify the number of oocytes that could have been fertilized in addition to those fertilized by IVF if, in these particular cases, ICSI had been performed. It is assumed that the oocytes had not subsequently gained the potential for fertilization since the original IVF insemination; and this is borne out by the general experience that such oocytes perform badly after re-insemination by IVF. Similar results were observed from Group 5. Therefore, from this study we conclude that such groups of patients would have benefited by having an increased number of oocytes fertilized had ICSI been performed initially.

IVF was initially developed for female infertility due to tubal disease. Using this technique, fewer spermatozoa are required to obtain oocyte fertilization than with natural intercourse or intrauterine insemination. This feature of IVF has made it an attractive option even in male factor patients in whom surgical or pharmacological therapy has failed or is inapplicable. However, conventional IVF is not very successful in the presence of compromised semen parameters. In these cases, HIC has been shown to be beneficial (Baker et al., 1993; Fishel et al., 1993; Tucker et al., 1993). Currently, several male factor abnormalities including varying degrees of oligozoospermia, asthenozoospermia, oligoasthenozoospermia and teratozoospermia are best treated by ICSI.

Several studies have compared the results of IVF in male factor and non-male factor patients along with the various micromanipulation techniques in these patient groups. These studies have shown that fertilization and pregnancy rates in patients undergoing IVF for male factor are lower than for patients undergoing IVF for different aetiology. A large comparative series from Centre for Reproductive Medicine in Brussels, Belgium, revealed an incidence of fertilization of 68% among 960 cycles in 480 couples undergoing IVF for female tubal factor and of 23% among 226 cycles in 175 strictly male factor couples (Tournaye et al., 1992). The Society for Assisted Reproductive Technology (SART) data also support these findings; male factor pregnancy rates for all centres in the USA were 18%, while pregnancy rates for couples with no male factor were 24% (AFS/SART, 1994).

Unlike IVF, ICSI in general is not dependent on qualitative factors of spermatozoa (apart from vitality) (Palermo et al., 1993; Fishel et al., 1994). In a later study (Mansour et al., 1995), no significant difference was found in the incidence of fertilization between patients with <5% normal forms using strict criteria (an incidence of fertilization of 59.0% per oocyte) and patients with >5% normal forms (an incidence of fertilization of 57.3% per oocyte). ICSI is also independent of sperm motility per se, whereas absence of or an extremely low proportion of rapid progressive motility in fresh semen indicates a high risk of complete fertilization failure with conventional IVF (Verheyen et al., 1999).

Although teratozoospermia has been associated with poor fertilization using conventional IVF, whilst fertilization with ICSI is not affected routinely, most of the published comparative studies failed to assess sibling oocytes to account for uncontrollable differences between groups. Conventional IVF with ICSI in sibling oocytes in men with poor sperm morphology was evaluated (Payne et al., 1994) and defined as <20% normal forms, based on World Health Organization criteria (WHO, 1992). However, in that study poor semen morphology was not always the only abnormal semen parameter and this accounted for the study’s extremely poor overall incidence of fertilization per oocyte of 15% with conventional IVF compared with 76% per oocyte when ICSI was used.

Similar findings were observed when conventional IVF was replaced with IVF with HIC. When sibling oocytes were used from patients with previously failed conventional IVF, either on a single or two previous occasions in which there was no binding to the zona pellucida, 37% of oocytes fertilized with HIC versus 58% with ICSI in patients with <5% normal forms. The number of oocytes fertilized in patients with >5% normal forms was equivalent whether IVF with HIC or ICSI was used (Fishel et al., 1995b). Similarly, in another study with sibling MII oocytes retrieved from patients with total fertilization failure in a previous IVF attempt, ICSI appeared to be far superior to an additional IVF attempt with elevated HIC, as of the 143 injected (ICSI) oocytes, 62.9% fertilized normally, whereas none of the 85 IVF inseminated sibling.
oocytes was fertilized (Kastrop et al., 1999). These studies show the advantages of ICSI in patients with compromised semen parameters.

Several studies have reported an increase in the adverse perinatal outcome of pregnancies obtained after ICSI-embryo transfers (Rizk et al., 1991; Alsalili et al., 1995). However, different studies have shown no additional risk after ICSI (Bonduelle et al., 1995; Govaerts et al., 1996). Although congenital malformations and sex chromosome abnormalities seem to be slightly higher after ICSI, a statistically significant difference has not been identified (Liebaers et al., 1995). In a recent retrospective study, pregnancy outcome of 145 ICSI pregnancies was matched with a similar number of IVF pregnancies. Results showed no difference in the rates of preclinical (15%) and clinical abortions (11% versus 15%). Four ectopic pregnancies were observed in the IVF group and none in the ICSI group. In the ICSI group, two therapeutic alternatives before proceeding with ICSI, at least for the preclinical (15%) and clinical abortions (11% versus 15%). choice, higher costs, increased time and the current issues different studies have shown no additional risk after ICSI expensive and more cost-effective technique can be used to

References
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