Intracytoplasmic sperm injection does not overcome an oocyte defect in previous fertilization failure with conventional in-vitro fertilization and normal spermatozoa

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A cohort comprising a total of 447 oocyte aspirations due to male factors (n = 258) or to previous fertilization failure by IVF in the presence of normal sperm parameters (n = 189) was studied. We found a significantly reduced implantation and pregnancy rate per transfer in the group with previously failed IVF attempts compared to the male factor group (P < 0.001). No differences were found in age, number of oocytes retrieved, number of embryos transferred or quality of embryos scored at the time of transfer. However, the fertilization and cleavage rates were found to be reduced in the group with previous failed IVF cycles. It is therefore concluded that previous fertilization failure despite normal sperm parameters in an IVF cycle may not be alleviated by the intracytoplasmic sperm injection (ICSI) procedure. These patients might suffer from oocyte defects as well.

Key words: failed fertilization/inertility therapy/in-vitro fertilization methods/microinjection/sperm—ovum interaction

Introduction

Since intracytoplasmic sperm injection (ICSI) has been introduced on a larger scale (AliKani, 1993; Palermo et al., 1993; Trounson and Wood, 1993; Devroey et al., 1994; Matthews, 1994; Payne, 1994; Silber, 1994; Tsirgotos et al., 1994; Van Steirteghem et al., 1994a,b) this technique is increasingly used for severe oligoasthenozoospermia, in which the total count of motile spermatozoa is inadequate for in-vitro fertilization (IVF), and in cases with repeated failure of fertilization (Palermo et al., 1993, Redgment et al., 1994; Tsirgotos et al., 1994; Van Steirteghem et al., 1994a). Several publications report acceptable results following ICSI regardless of the indications and causes mentioned. The only detrimental impact on the outcome reported for ICSI has been age and extreme teratozoospermia (Redgment et al., 1994). Most publications have pooled results of ICSI following male factors with ICSI following previous fertilization failure in IVF with apparently normal semen samples. The failure of fertilization may be explained by lack of penetration of the zona pellucida, an oocyte activation failure or a defect of the oocyte. However, an estimate of oocyte-dependent defects in fertilization, cleavage and implantation has not been addressed in previous publications. It could be argued that, although it is possible by ICSI to induce pronuclei formation and cleavage, impaired implantation and cleavage might occur due to structural (i.e. cytoskeleton) or genetic (i.e. aneuploidy) failures.

In the present study we present a cohort of patients receiving ICSI either due to previous fertilization failure in a conventional IVF attempt with normal sperm parameters or due to severe oligoasthenozoospermia.

Materials and methods

Patients

A total of 447 consecutive IVF treatment cycles allocated to ICSI due to either severe male factor or failed previous fertilization by IVF despite normal sperm parameters performed at our unit between May 1994 and December 1995 were analysed.

The patients were allocated to the group named 'ICSI due to a male factor' or the group named 'ICSI due to failed previous fertilization' based on the following criteria:

Male factor (n = 258) The total number of progressive motile spermatozoa was <500 000 sperm per ejaculate.

Previous IVF failure (n = 189) The patient had one previous IVF cycle with a fertilization rate of <20% of the oocytes retrieved, and the sperm parameters according to WHO were normal including the morphology of fixed spermatozoa viewed by light microscopy.

All patients were counselled and gave their informed consent before the procedure. The mean female age was 32.5 years (23–44). There was no difference in the distribution of ages between the two groups.

Follicular stimulation

Follicular stimulation was carried out by the combination of gonadotrophin-releasing hormone agonist (GnRHa) buserelin acetate (Synarel, Syntex, Denmark), human menopausal gonadotrophin (HMG) (Pergonal; Serono) and human chorionic gonadotrophin (HCG) (Pregnyl; Organon, Lutterbuen, Denmark). Buserelin acetate was administered by nasal spray three times a day from day 21 in the previous cycle. 16 days later HMG 225 IU was given daily Ovarian response was monitored by transvaginal ultrasound scan and HCG, 10 000 IU, was administered when the leading follicles were >18 mm in diameter Transvaginal oocyte aspiration was performed under i.v. sedation 37 h after HCG injection. Luteal phase supplementation was administered with Progestan (Organon) 1 X3 pessaries a day.

Embryology

An ejaculate was produced by masturbation on the day of oocyte aspiration. Sperm assessment was performed according to the World....
Evaluation of study parameters

For estimates of population parameters mean and standard deviation are used. For comparison of these parameters Student's t-test was used. For comparison of differences between ICSI conducted for male factors or for previous fertilization failures, either Fisher's exact test or \( \chi^2 \) test were used as appropriate. A \( P \) value <0.05 was accepted as significant.

Results

A total of 220 patients with 258 oocyte aspirations was allocated consecutively to ICSI due to male factor; 38 patients had two attempts and none had three attempts.

A total of 162 patients with 189 oocyte aspirations was allocated consecutively to ICSI due to previous failure in fertilization. Of these, 27 patients had two attempts and none had three attempts. The number of transfers and the number of pregnancies as indicated by the presence of ultrasonically detected gestational sacs are displayed in Table I. This table includes patients with more than one ICSI treatment. The pregnancy rate of the previous failed IVF group was significantly lower than that of the male factor group. The number of oocytes collected, number of fertilized embryos and cleavage rate of fertilized embryos is shown in Table II.

We found a significantly lower fertilization and cleavage rate in the previous failed fertilization group compared to male factor group (Table I).

The mean number of oocytes retrieved per aspiration in the male factor group was 9.77 and in the previous IVF failure factor group (Table I).

No differences were found in the embryo score (Van den Abbeel et al., 1988; Staessen et al., 1990) or number of oocytes transferred between the two groups.

Discussion

This study comprises 447 IVF treatment cycles using intracytoplasmic sperm injection due to one of two indications; severe sperm factor or previously failed fertilization in conventional IVF with apparently normal sperm samples. A significant difference was found in the fertilization rate and cleavage rate in these two groups. Both the biochemical assays for pregnancy and later ultrasound scans revealing intrauterine living gestations were markedly lower in women who had had a previously failed IVF. This has not been thoroughly explored in previous studies, as the formation of gestational sacs has not been discussed in direct relation to the two indications for ICSI.

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### Table I. Intracytoplasmic sperm injection (ICSI) performed due to previous IVF failure or known male factor

<table>
<thead>
<tr>
<th></th>
<th>No. patients</th>
<th>Aspiration</th>
<th>Transfer</th>
<th>Pregnancy</th>
<th>Pregnancy/asp. (%)</th>
<th>Pregnancy/transf (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male factor</td>
<td>220</td>
<td>258</td>
<td>248</td>
<td>111</td>
<td>43</td>
<td>45</td>
</tr>
<tr>
<td>IVF failure</td>
<td>162</td>
<td>189</td>
<td>180</td>
<td>26</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

*Ongoing pregnancies with at least one fetal heart detectable by ultrasound. The mean number of embryos transferred in each group was 2.45

### Table II. Intracytoplasmic sperm injection (ICSI) performed due to previous IVF failure or known male factor

<table>
<thead>
<tr>
<th></th>
<th>No oocytes aspirated</th>
<th>No. at MI</th>
<th>No PN</th>
<th>No cleaved</th>
<th>Cleaved/MI (%)</th>
<th>PN/MI (%)</th>
<th>Cleaved/PN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male factor</td>
<td>2521</td>
<td>1905</td>
<td>1709</td>
<td>1599</td>
<td>84*</td>
<td>90*</td>
<td>94*</td>
</tr>
<tr>
<td>IVF failure</td>
<td>1349</td>
<td>1070</td>
<td>882</td>
<td>779</td>
<td>73*</td>
<td>82*</td>
<td>88*</td>
</tr>
</tbody>
</table>

* \( P < 0.05 \) for comparison of male factor with IVF failure.

MI: metaphase II eggs; PN: eggs with 2 pronuclei.
Nagy *et al.* (1993) showed no differences between the two groups concerning fertilization and pronuclei formation, but did not examine the possible difference in pregnancy rate. Their observations are not in accordance with the present findings, where we did detect a difference in the fertilization rate between the two groups. These cannot be explained by the possible impact of age as proposed by others (Redgment *et al.*, 1994), as our two groups exhibit similar age distributions.

It is very unlikely that the difference is due to an occasional selection error between the two groups.

In several previous studies (Redgment *et al.*, 1994; Tsingots et al., 1994; Van Steirteghem *et al.*, 1994a) the authors have merged the two categories investigated in the present paper, and have not defined the group with failed previous IVF cycles as couples having normal semen parameters.

Previous studies have shown that fertilization failure in couples with infertility due to a tubal factor and normal sperm parameters have a good prognosis for subsequent conventional IVF cycles giving a 97% fertilization rate in the second and third cycle (Liptz *et al.*, 1994). Calderón *et al.* (1995) obtained a 50% pregnancy rate in a group of seven couples with previous fertilization failure (six embryo transfers and three pregnancies) selected in almost the same way as our study. The reason for these discrepancies may be explained by the fact that the study by Liptz deals with tubal factor only, whereas we have included all types of previous fertilization failures and the group selected by Calderón is very low in number.

In the present study we have used the ICSI procedure in an attempt to overcome previously failed fertilization despite normal semen parameters and to compare the results with those of couples with severe semen problems also undergoing ICSI treatment. The reason for the very low fertilization and implantation rate in the first of these groups may be an oocyte factor or inborn error, which is not surmounted by intracytoplasmic sperm injection because further development is impaired, possibly because of several other failures in the oocyte (Sousa and Tesarik, 1994). Thus ICSI might not circumvent the problems for patients with unexplained failures in fertilization after routine IVF, as only a pregnancy rate of 14% was achieved in this group compared to a pregnancy rate of 45% per transfer when ICSI was used for treatment of a male factor.

**References**


