Special applications of intracytoplasmic sperm injection: the influence of sperm count, motility, morphology, source and sperm antibody on the outcome of ICSI

Zsolt P.Nagy1, Greta Verheyen, Herman Tournaye and André C.Van Steirteghem

Centre for Reproductive Medicine, University Hospital, Dutch-speaking Brussels Free University (Vrije Universiteit Brussel), Laarbeeklaan 101, B-1090 Brussels, Belgium

1To whom correspondence should be addressed

The relationship between the three basic parameters of ejaculated spermatozoa, i.e. concentration, motility and morphology, and the results of intracytoplasmic sperm injection (ICSI) were investigated in 838 microinjection cycles. A further 123 ICSI treatment cycles in which testicular spermatozoa were used for microinjection were also evaluated. The influence of anti-sperm antibodies (ASA) on the outcome of ICSI was investigated by analysing 55 cycles where the proportion of ASA-bound spermatozoa was ≥80%. After microinjection, oocyte intactness, fertilization, embryo cleavage, transfer and pregnancy rates were recorded and compared. The results showed that neither the type nor the extent of sperm impairment had an important influence on the outcome of ICSI when ejaculated spermatozoa were used. Only two very rare conditions had a strongly negative influence on the result of ICSI, i.e. where immotile (presumably dead) spermatozoa or where round-headed spermatozoa were injected into the oocyte. Neither the proportion of ASA-bound spermatozoa, the type of dominantly present ASA, nor the location of ASA on the spermatozoa had an important influence on fertilization, embryo development or pregnancy rates after ICSI. In most of the cycles combined with testicular biopsy (79%), there were enough motile spermatozoa present in the wet preparation for injection of all the oocytes. Injection of motile testicular spermatozoa led to a higher normal fertilization rate than did injection of non-motile spermatozoa (65 versus 21%). It can be concluded that injection of motile (living) spermatozoa into oocytes is the most important factor in determining good results with ICSI and that other sperm parameters do not have a strong influence on the outcome of ICSI.

Key words: fertilization/ICSI/micro-injection/pregnancy/sperm count/sperm morphology/sperm motility

Introduction

The successful introduction of intracytoplasmic sperm injection (ICSI) into clinical practice (Palermo et al., 1992; Van Steirteghem et al., 1993) was a breakthrough, especially for patients with andrological infertility. Experience has shown that the results of ICSI are very good when freshly ejaculated spermatozoa are used, regardless of sperm parameters (except complete globozoospermia) or other factors, such as the presence of very high concentrations of anti-sperm antibodies (Nagy et al., 1995a,b). The only condition in which the fertilization rate, and consequently the pregnancy rate, is lower after ICSI is when completely immotile spermatozoa are used for microinjection. In order to correlate different sperm parameters with the outcome of ICSI, a large number of cycles must be analysed in which ejaculated spermatozoa are used for microinjection.
Since the recent introduction of the use of testicular spermatozoa for microinjection (Schoysman et al., 1993; Devroey et al., 1994), we have learned that ICSI results in such cases are excellent, although somewhat lower in terms of fertilization rate than after injection of freshly ejaculated spermatozoa (Nagy et al., 1995c). It has also been observed that in most testicular biopsies where spermatozoa can be detected, at least a few sperm cells usually display a sluggish, twitching type of motility. Even in patients with 100% dead spermatozoa (complete necrozoospermia) in their ejaculate, motile spermatozoa can sometimes be recovered from a testicular biopsy (Tournaye et al., 1996a). Although when selecting spermatozoa for microinjection we always prefer to use one that has shown some sign of motility, on the basis of our experience with ejaculated spermatozoa, it has not always been possible to find motile spermatozoa in testicular biopsies (or to find enough motile spermatozoa for the number of oocytes) and, consequently, in a few cycles ICSI has had to be performed with non-motile testicular spermatozoa. In the present article, we have reviewed the influence of different basic sperm parameters and the extent of their impairment on the outcome of ICSI when ejaculated spermatozoa were used for microinjection and compared this to ICSI results where motile or immotile testicular spermatozoa were used for microinjection.

Materials and methods

Patients and sperm parameters

A total of 838 cycles in which ejaculated semen was used for microinjection were analysed in the present study (previously published as Nagy et al., 1995a). Inclusion criteria for patients for ICSI with freshly ejaculated semen were either failed or very low fertilization rates in the previous standard in-vitro fertilization (IVF) cycle(s) or very poor sperm parameters (<500 000 progressively motile spermatozoa in the whole ejaculate; Van Steirteghem et al., 1993). The outcome of ICSI in cycles involving freshly ejaculated semen obtained by masturbation was analysed in terms of the three conventional sperm parameters: total sperm count, motility and morphology. Total sperm count was analysed in three arbitrarily graded groups: group 1, no spermatozoa found in the initial semen sample(s) after checking the area of grids of the Makler or Neubauer counting chamber (57 cycles); group 2, total sperm count >0–1 × 10⁶ (97 cycles); group 3, total sperm count >5 × 10⁶ (684 cycles). Sperm motility (a+b+c motility) was analysed in four arbitrarily graded groups including only those cycles where at least four spermatozoa were present on the whole surface of the counting chambers: group 1, no motile spermatozoa present either in the initial semen sample(s) or after sperm treatment procedure, and consequently only immotile spermatozoa were used for microinjection (12 cycles); group 2, no motile spermatozoa present in the initial semen sample(s), but sufficient were recovered after semen treatment to inject all or most of the oocytes with motile spermatozoa (54 cycles); group 3, total motility 0–5% (19 cycles); group 4, total motility >50% (337 cycles). Sperm morphology was analysed in three arbitrarily graded groups after excluding cycles with zero sperm count and zero motility and including only those cycles where at least 100 spermatozoa were analysed for morphology: group 1, 0% normal morphology (48 cycles); group 2, 1–3% normal morphology, (125 cycles); group 3, ≥14% normal morphology (203 cycles).

In addition, 11 cycles were analysed in which ejaculated semen was used for microinjection from seven different patients who all had globozoospermia (Liu et al., 1995).

Another 55 ICSI cycles were analysed in which semen was used from 37 different patients who had anti-sperm antibodies (ASA) bound to at least 80% of their spermatozoa. The results of these patients with male immunological infertility were compared to the results of ICSI performed in 1767 cycles where an immunological factor was excluded (previously published as Nagy et al., 1995b).

In 123 ICSI cycles, testicular sperm extraction was performed for the purpose of alleviating male-factor infertility due to obstructive or non-obstructive azoospermia, with successful recovery of enough spermatozoa to inject all available oocytes. Ovarian stimulation was carried out by a desens-
Special applications of ICSI

itizing protocol using the gonadotrophin-releasing-hormone agonist buserelin (Suprefact; Hoechst, Brussels, Belgium) in combination with human menopausal gonadotrophin (HMG; Humegon; Organon, Oss, The Netherlands or Pergonal; Serono, Brussels, Belgium) and human chorionic gonadotrophin (HCG; Pregnyl from Organon or Profasi from Serono). Intravaginally administered progesterone (Utrogestan; Piette, Brussels, Belgium) was used for luteal-phase supplementation. The details of this stimulation protocol have been described previously (Smitz et al., 1988, 1992).

Semen and oocyte preparation

Sperm density and motility of ejaculated spermatozoa were evaluated according to the recommendations of the World Health Organization (WHO, 1992). Morphology was determined using the strict Tygerberg criteria (Kruger et al., 1986) after Diff-Quick staining (Enginsu et al., 1991) of the semen samples. Sperm treatment of the freshly ejaculated semen was as follows: (i) washing in Earle’s medium and centrifugation at 1800 g for 5 min, (ii) two-layer Percoll (47.5-95%) centrifugation at 300 g for 20 min, (iii) final concentration step by centrifugation at 1800 g for 5 min (Van Steirteghem et al., 1995). The presence of immunoglobulin (Ig)G antibodies was assessed by the mixed agglutination reaction (MAR) test (Ortho Diagnostic Systems, Beerse, Belgium). Antibodies of the IgA class were detected by the immunobead test or the IgA-MAR test (FertiPro, Ghent, Belgium). The percentages of spermatozoa coated with antibodies and their localization were recorded for both types of immunoglobulins.

The cumulus and corona-radiata cells were removed by incubation for 30 s in HEPES-buffered Earle’s medium containing 80 IU hyaluronidase/ ml (type VIII, specific activity 320 IU/mg; Sigma Chemical Co, St. Louis, MO, USA) and by aspiration of the complexes in and out of a hand-drawn glass pipette. ICSI was carried out only on metaphase II oocytes (Van Steirteghem et al., 1995).

Recovery of testicular tissue and treatment of testicular spermatozoa were as follows: testicular biopsy was performed under local or general anaesthesia. A small testicular incision was made and a specimen was placed in a Petri dish (Falcon Plastics, Becton-Dickinson, Aalst, Belgium) containing ~5 ml of modified HEPES-buffered Earle’s medium supplemented with 0.4% human serum albumin (HSA; Belgian Red Cross, Brussels, Belgium). After shredding the tissue, microscopic examination of biopsies was performed immediately using an inverted microscope at ×200 or ×400 magnification (Diaphot, Nikon Corporation, Tokyo, Japan), and the presence or absence of spermatozoa in the wet preparation was communicated to the operating theatre. If spermatozoa were present in the biopsy, no further testicular biopsy was obtained. In some instances, repeated sampling was needed, also from the contralateral side, in order to find a single spermatozoon. In some cases of obstructive azoospermia, testicular tissue was obtained by fine needle aspiration. A butterfly needle was inserted deeply into the testis and strong aspiration was exerted using a 10 ml syringe. This aspiration force usually resulted in the recovery of a minute amount of testicular tissue including spermatozoa. The contents of the needle were emptied directly into an injection dish which was used for ICSI. Where a testicular biopsy was performed, one single testicular specimen per testis was examined histologically. Testicular spermatozoa were prepared by dicing and squeezing a piece of testicular tissue in HEPES-buffered Earle’s medium and by centrifuging this medium for 5 min at 300 g (Verheyen et al., 1995). The pellet was kept without any further treatment until the moment of injection (Nagy et al., 1995c). Occasionally, a two-layer Percoll gradient (47.5 and 95%) centrifugation treatment procedure was used where large numbers of spermatozoa were observed in the biopsy specimen.

ICSI procedure and assessment of fertilization and embryo cleavage and establishment of pregnancy

The details of microtool preparation and microinjection procedures have already been described (Van Steirteghem et al., 1995) but, most importantly, whenever possible a motile spermatozoon with normal appearance was always selected from the concentrated sperm pellet for microinjection.
When ICSI was performed with testicular spermatozoa, the injection dish contained several droplets (20–30 drops) of 5 μl of HEPES-buffered Earle’s medium covered with mineral oil (M-8410; Sigma). The central droplet contained 10% polyvinylpyrrolidone solution (PVP; Sigma P 5288), and the pelleted sperm suspension was distributed over most of the droplets in the injection dish so as to facilitate the search for spermatozoa. The ICSI procedure was carried out on the heated stage of an inverted microscope (Diaphot, Nikon Corporation, Tokyo, Japan) at ×400 magnification using the Hoffman modulation contrast system (Modulation Optics Inc, Greenvale, NY, USA) (Van Steirteghem et al., 1995). Whenever possible, an apparently normal, motile spermatozoon was aspirated tail first into the injection pipette, removed from the separate sperm droplet and transferred first to the PVP droplet so as to clean the spermatozoon of its attached cells and debris. Usually, the necessary number of spermatozoa were collected into the PVP droplet first and oocytes were placed into the dish afterwards so as to avoid unnecessary exposure of the oocytes to suboptimal conditions such as possible temperature and pH fluctuations. For the injection, the oocyte was fixed on the holding pipette in such a way that the polar body was situated at 6 o’clock while the injection pipette was pushed through the zona pellucida at the 3 o’clock position into the cytoplasm (Nagy et al., 1995d), where the spermatozoon was delivered together with the smallest possible amount of medium. After injection the oocytes were washed, placed in 25 μl microdrops of B2 medium in a Petri dish and incubated at 37°C in 5% CO₂, 5% O₂, 90% N₂.

At 16–18 h after microinjection, oocytes were observed for survival and fertilization (Nagy et al., 1994). The number and aspect of polar bodies and pronuclei were recorded. The criteria for normal fertilization were the presence of two individualized or fragmented polar bodies together with two clearly visible pronuclei. Embryo cleavage and quality were evaluated 40–44 h after ICSI. According to the relative proportion of anucleate fragments present in the zona pellucida, they were categorized as: (i) excellent, with no anucleate fragments; (ii) good, where <20% of the embryo was fragmented; (iii) fair, where the relative amount of fragmentation was between 20 and 50% and (iv) poor, where >50% of the embryo was fragmented. Embryos with <50% fragmentation were selected for transfer; supernumerary embryos with <20% fragmentation were cryopreserved (Van Steirteghem et al., 1994). To reduce the risk of multiple pregnancies, and to avoid triplet pregnancies, only two embryos were transferred in selected cases (Staessen et al., 1993).

Pregnancy was detected by measuring serum HCG concentrations on at least two separate occasions 10 days after embryo transfer. Clinical pregnancy was determined by ultrasound screening of the fetal sac at 7 weeks of pregnancy.

**Data set and statistical analysis**

The data were analysed using the SPSS statistical package on an Inwork personal computer; 5% was defined as the level of significance. Intactness, fertilization, embryo quality, embryo transfer and embryo freezing rates were compared globally by the Kruskal–Wallis test and also by the one-way analysis of variance (ANOVA) test. If significant differences were present, then paired comparisons were performed by means of the Mann–Whitney U test and by the Tukey–HSD test. These tests were performed on the percentage values of the variables within each cycle. This approach was used in order to take the dependence of observations within each cycle into account and was necessary for a valid application of the tests of comparison, which assume independence of the observations. Because the results of these two tests always corresponded and because of the imbalance in the size of the groups compared, the non-parametric tests were preferred in the analysis of the data. Pregnancy rates were compared by means of the χ² test.

**Results**

**Sperm count and outcome of ICSI**

In the Makler or the Neubauer counting chamber, no spermatozoa were seen initially in the samples in 57 out of the 838 cycles where ejaculated semen was used. However, in these 57 cycles where no spermatozoa were found in the initial semen...
Special applications of ICSI

Table I. Results of intracytoplasmic sperm injection (ICSI) in relation to the total sperm count when ejaculated spermatozoa were used for microinjection. Adapted from Nagy et al. (1995a)

<table>
<thead>
<tr>
<th>Total sperm count</th>
<th>Group 1 '0'</th>
<th>Group 2 &gt;0-1 x 10⁶</th>
<th>Group 3 &gt;5 x 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>57</td>
<td>97</td>
<td>684</td>
</tr>
<tr>
<td>No. of injected oocytes</td>
<td>586</td>
<td>1038</td>
<td>6981</td>
</tr>
<tr>
<td>(% intact oocytes, mean ± SD)</td>
<td>(88.2 ± 12.3)</td>
<td>(92.1 ± 9.7)</td>
<td>(88.7 ± 14.9)</td>
</tr>
<tr>
<td>2 PN development</td>
<td>58.3a ± 25.5</td>
<td>64.5 ± 24.3</td>
<td>70.7b ± 23.5</td>
</tr>
<tr>
<td>(% of intact oocytes, mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryos transferred or frozen</td>
<td>74.8 ± 31.8</td>
<td>69.3 ± 24.5</td>
<td>70.7 ± 25.9</td>
</tr>
<tr>
<td>(as % of 2 PN oocytes, mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of embryo transfers</td>
<td>49 (86.0)</td>
<td>93 (95.9)</td>
<td>633 (92.5)</td>
</tr>
<tr>
<td>(% of started cycles)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. embryos transferred per transfer</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>No. of pregnancies (positive HCG) (per % of transfers)*</td>
<td>22 (44.9)</td>
<td>34 (36.6)</td>
<td>267 (42.2)</td>
</tr>
</tbody>
</table>

*aSignificantly different from b (P < 0.001 by the Mann–Whitney U test).
*bDifference between the groups not significant by the x² test.
2 PN = two pronuclei; HCG = human chorionic gonadotrophin.

...samples, it was usually possible to recover enough spermatozoa to inject all or most of the oocytes (Table I; previously published as Nagy et al., 1995a).

In the three sperm-count groups, 586, 1038 and 6981 oocytes were injected respectively. Normal fertilization rates were between 58 and 71% (P < 0.001). Testing for paired comparisons between groups revealed that the observed significant difference was due to the divergent results between groups 1 and 3. The proportions of oocytes with multiple pronuclei (≥3 PN) and with parthenogenetic activation (1 PN) were similar for the three groups.

Proportions of embryos transferred or frozen ranged from 69 to 75%. After analysis in greater detail, no significant difference was found in the distribution of excellent, good, fair and poor-quality embryos among the three groups. Total and clinical pregnancy rates (range 31–45% and 22–30% respectively) did not differ significantly among the three groups.

Motility of spermatozoa and outcome of ICSI

When ejaculated semen was used, in 12 cycles (group 1) where no motile spermatozoa were seen initially in the samples or after preparation, the oocytes were injected with immotile spermatozoa, while in the other 54 cycles (group 2) it was possible to perform ICSI with motile spermatozoa on the majority of oocytes (Table II; previously published as Nagy et al., 1995a). In group 1, where the sperm number was high enough, the eosin Y vitality test revealed that ~10% of the immotile spermatozoa were alive. In the four groups, 175, 503, 252 and 3493 oocytes were injected respectively. The 2 PN fertilization rate was lowest in group 1 (11%) and highest in group 4 (74%). The fertilization rate in group 1 was significantly lower than the in groups 2, 3 and 4. As a consequence, the proportions of transferable or frozen embryos were also different among the groups: lowest in group 1 (51%) and ranging from 66 to 72% in the other three groups. No pregnancy ensued in group 1, while in groups 2–4 the total pregnancy rate ranged from 23 to 42% (Table II).

Table III presents data from 123 cycles in which enough testicular spermatozoa to inject all the available oocytes were found. In group 1, in 97 of the 123 cycles it was possible to inject all the oocytes with motile spermatozoa (79%). On the other hand, in eight cycles (7%; group 2) all the oocytes had to be injected with non-motile spermatozoa because no motile spermatozoa were found. Finally, there were 18 cycles (14%) in which a total of 56 (39%) oocytes were injected...
with motile spermatozoa and 87 (61%) oocytes with non-motile spermatozoa because of the lack of motile spermatozoa (Table IV). The normal (2 PN) fertilization rate was significantly higher ($P < 0.001$) in the group in which all the oocytes were injected with motile spermatozoa (group 1; 65% of the number of intact oocytes) than in group 2, where only non-motile spermatozoa were microinjected (21%). No difference was observed in the quality of embryos across the groups.

The separate analysis of the 18 cycles in which a combination of motile and non-motile spermatozoa were used for ICSI showed that a larger proportion of the oocytes were injected with non-motile than with motile spermatozoa. Injection of motile spermatozoa resulted in a higher normal (2 PN) fertilization rate (64%) than did injection of non-motile spermatozoa (36%) in these sibling oocytes, but this difference was insignificant. Embryo quality and the proportions of transferred or frozen embryos were similar, regardless of whether motile or non-motile spermatozoa were used for microinjection.

**Sperm morphology and outcome of ICSI**

A total of 683 ICSI cycles were analysed in relation to sperm morphology (previously published as Nagy et al., 1995a). In all, 488, 1214 and 2146 oocytes were injected in the three groups respectively (Table V). Differences observed in the normal...
Special applications of ICSI

Table IV. Results of intracytoplasmic sperm injection (ICSI) in patients using motile (group 1) and non-motile testicular spermatozoa (group 2) within the same treatment cycle

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (motile spermatozoa)</th>
<th>Group 2 (non-motile spermatozoa)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>No. of injected oocytes</td>
<td>56</td>
<td>87</td>
<td>143</td>
</tr>
<tr>
<td>(% of intact oocytes, mean ± SD)</td>
<td>(88.6 ± 15.4)</td>
<td>(96.1 ± 7.8)</td>
<td>(92.3 ± 12.5)</td>
</tr>
<tr>
<td>2 PN development</td>
<td>64.0 ± 36.1</td>
<td>35.8 ± 25.2</td>
<td>49.9 ± 33.5</td>
</tr>
<tr>
<td>Embryos transferred or frozen (as % of 2 PN oocytes, mean ± SD)</td>
<td>83.7 ± 27.8</td>
<td>65.8 ± 17.3</td>
<td>74.3 ± 22.7</td>
</tr>
</tbody>
</table>

Table V. Results of intracytoplasmic sperm injection (ICSI) in relation to sperm morphology using ejaculated spermatozoa. Adapted from Nagy et al. (1995a)

<table>
<thead>
<tr>
<th>Percentage normal morphology</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>48</td>
<td>125</td>
<td>203</td>
</tr>
<tr>
<td>No. of injected oocytes</td>
<td>488</td>
<td>1214</td>
<td>2146</td>
</tr>
<tr>
<td>(% of intact oocytes, mean ± SD)</td>
<td>(90.9 ± 14.9)</td>
<td>(89.2 ± 14.9)</td>
<td>(89.3 ± 14.0)</td>
</tr>
<tr>
<td>2 PN development</td>
<td>67.8 ± 30.8</td>
<td>70.0 ± 20.0</td>
<td>74.8 ± 21.3</td>
</tr>
<tr>
<td>Embryos transferred or frozen (as % of 2 PN oocyte, mean ± SD)</td>
<td>67.8 ± 28.1</td>
<td>70.5 ± 26.3</td>
<td>69.6 ± 25.6</td>
</tr>
<tr>
<td>No. of embryo transfers (% of started cycles)*</td>
<td>42 (87.5)</td>
<td>120 (96.0)</td>
<td>193 (95.1)</td>
</tr>
<tr>
<td>No. of embryos transferred per transfer (per % of transfers)*</td>
<td>2.6</td>
<td>2.4</td>
<td>2.6</td>
</tr>
<tr>
<td>No. of pregnancies (positive HCG)</td>
<td>19 (45.2)</td>
<td>51 (42.5)</td>
<td>79 (40.9)</td>
</tr>
</tbody>
</table>

*Difference between the groups not significant by the $\chi^2$ test.

Fertilization rates among the four groups were statistically insignificant ($P = 0.05$ by the Kruskal–Wallis test; lowest in group 1, 68%; highest in group 3, 75%). The proportions of oocytes with 1 PN and with $\geq 3$ PN did not differ significantly among the three groups (ranges 2.8–4.5% and 3.9–5.7% respectively). No significant difference was observed in the distribution of embryo quality among the three groups. Transfer procedures were performed in 94.1% of all the started cycles (range 88–96%, which was not significant). The overall total and ongoing pregnancy rates per transfer were 41 and 29% respectively (no statistically significant difference between the groups).

In a separate analysis of 11 cycles in which all the spermatozoa displayed round heads and missing acrosomes (100% globozoospermia), the results of ICSI were distinctly different from those in which other types of 100% abnormal spermatozoa were used for microinjection (Liu et al., 1995). A total of 85 oocytes were injected in these 11 cycles (from seven different patients), all of them with motile round-headed spermatozoa. In all, 75 oocytes survived the injection (88%) and 14 oocytes fertilized normally (19% 2 PN fertilization as calculated on the basis of the number of intact oocytes). Ten embryos were transferred in four cycles; two patients became pregnant and one patient had an ectopic pregnancy. One of the two pregnancies ended in a pre-clinical abortion and one twin pregnancy went to term.

**Antisperm antibodies and the outcome of ICSI**

The results of this analysis are presented in Table VI. Normal fertilization rates (calculated as a
Z.P. Nagy et al.

Table VI. Results of intracytoplasmic sperm injection (ICSI) in patients with 80 to <100% mixed agglutination reaction (MAR)-positive (group 1) and with 100% MAR-positive (group 2) tests in comparison to ICSI patients where immunological infertility was excluded (control). Adapted from Nagy et al. (1995b)

<table>
<thead>
<tr>
<th>MAR test results for each group</th>
<th>Group 1 80 to &lt;100%</th>
<th>Group 2 100%</th>
<th>Total ≥80%</th>
<th>Control negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>15</td>
<td>40</td>
<td>55</td>
<td>1767</td>
</tr>
<tr>
<td>No. of injected oocytes (%)</td>
<td>197</td>
<td>403</td>
<td>600</td>
<td>18157</td>
</tr>
<tr>
<td>(% of intact oocytes, mean ± SD)</td>
<td>(92.3 ± 10.2)</td>
<td>(91.6 ± 11.2)</td>
<td>(91.8 ± 10.9)</td>
<td>(89.3 ± 14.0)</td>
</tr>
<tr>
<td>2 PN development (%)</td>
<td>68.0 ± 22.1</td>
<td>78.7 ± 17.6</td>
<td>75.7 ± 19.3</td>
<td>69.2 ± 24.3</td>
</tr>
<tr>
<td>(% of intact oocytes, mean ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryos transferred or frozen (as % of 2 PN oocytes, mean ± SD)</td>
<td>71.3 ± 27.8</td>
<td>63.0 ± 26.4</td>
<td>65.2 ± 26.8</td>
<td>69.4 ± 27.3</td>
</tr>
<tr>
<td>No. of embryo transfers (%)</td>
<td>15 (100)</td>
<td>38 (95.0)</td>
<td>53 (96.4)</td>
<td>1600 (90.6)</td>
</tr>
<tr>
<td>(% of started cycles)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of pregnancies (positive HCG) (per % of transfers)*</td>
<td>4 (26.7)</td>
<td>12 (31.6)</td>
<td>16 (30.2)</td>
<td>626 (39.1)</td>
</tr>
<tr>
<td>Clinical pregnancies (per % of transfers)*</td>
<td>4 (26.7)</td>
<td>10 (26.4)</td>
<td>14 (26.4)</td>
<td>502 (31.4)</td>
</tr>
</tbody>
</table>

*Significant difference between groups (P < 0.01 by the Mann–Whitney U and Tukey-HSD tests).

percentage of the intact oocytes) were high in both group 1 and group 2 (68 and 79% respectively; P = 0.069 by one-way ANOVA and P = 0.083 by Mann–Whitney U test). No differences were observed in the distribution of embryo quality when group 1 was compared to group 2. In group 1 and group 2, 50 and 44% of the embryos were transferred and 21 and 19% of them were frozen respectively. Initial (positive HCG) and ongoing pregnancy rates were similar: 27 and 27% in group 1 and 32 and 26% in group 2 respectively (Table VI). The normal (2 PN) fertilization rate was significantly higher (P < 0.05 by the Kruskal–Wallis test) for the group of patients with ASA than for patients without ASA (Table VI), which was due to the difference observed between group 2 (100% MAR positive) and the ASA-negative patients. The proportion of embryos of poor quality was significantly lower in the control group (P < 0.05 by the Kruskal–Wallis test), which was due to the difference between group 2 and the control group. No significant difference was observed in the total (positive HCG) and clinical pregnancy rates when MAR-positive patients were compared to the MAR-negative patients.

Normal fertilization rates were similar when analysed in relation to the type of antibody predominantly present on the spermatozoa, but the highest rate was found when both IgG and IgA antibody levels were ≥80% (84% 2 PN rate), in comparison with the group with high IgG but low IgA level (72% 2 PN rate) or with the group with high IgA and low IgG level (75% 2 PN rate). Embryo developmental rates (73, 68 and 53% respectively) and pregnancy rates (15, 29 and 33% respectively) were also similar in these three groups and any differences did not reach statistical significance.

No significant differences were observed in the 2 PN fertilization rates (85, 70 and 79% respectively), embryo developmental rates (59, 66 and 63% respectively) or in the total pregnancy rates (33, 17 and 24% respectively) when cycles were analysed in relation to the localization of anti-sperm antibodies on the spermatozoa (predominantly on head, predominantly on neck and tail, or mixed distribution respectively).

Discussion

In our study, we analysed the possible correlation(s) between three basic sperm parameters (total sperm
Special applications of ICSI

count, motility, morphology) and the outcome of ICSI. It is easy to conclude that, when ejaculated spermatozoa are used, a high 2 PN fertilization rate is obtained as long as some spermatozoa are found in the ejaculate, since such rates in semen with an extremely low sperm count are only slightly (although significantly) lower than when the sperm count is much higher. The observed 12% difference in the normal fertilization rates between the 0 sperm count group and the >5×10^6 sperm count group is statistically significant because of the large number of cycles included, but clinically it is not really important and is of no consequence for the embryo development rates or the pregnancy rates (Nagy et al., 1995a).

Important differences were noted in the pronuclear status of injected oocytes when results were analysed according to the motility characteristics of the ejaculated spermatozoa observed in the initial semen sample. Only 51% normal fertilization was observed in the group where no motility was present (group 1 and group 2 together, 66 cycles), while in the groups with 1–5% motility and with >50% motility uniformly high fertilization rates were observed (68–74%). In group 1, all of the oocytes had to be injected with immotile spermatozoa in spite of efforts to find motile spermatozoa. In these 12 cycles with immotile spermatozoa, the observed 2 PN fertilization rate was extremely low (11%; five out of the 12 patients had no fertilization). It seems a logical hypothesis, where living spermatozoa cannot be selected on the basis of motility, that the proportion of living spermatozoa microinjected into the oocytes will be the same as the proportion in the semen sample as determined by the vitality test. In these cases (where vitality assessments of spermatozoa were possible), the proportion of vital spermatozoa was ~10%. This suggests that ~10% of oocytes were probably microinjected with living spermatozoa, which in fact corresponds to the normal fertilization rate obtained in this group, implying that sperm vitality is a prerequisite for successful ICSI (Nagy et al., 1995a). This hypothesis is supported by the results of Bongso et al. (1989) and Terriou et al. (1993), who used immotile but living spermatozoa for subzonal microinjection, as well as by Casper et al. (1996), who used a hypo-osmotic swelling test to select living but immotile spermatozoa and obtained higher normal fertilization rates. Development rates of the normally fertilized oocytes in all the groups were high except when completely immotile spermatozoa were used, where the rate appeared reduced (owing to the small numbers in this group, this did not reach statistical difference). Transfer and pregnancy rates were equally high for all groups where motile spermatozoa were used for microinjection. In the group where only immotile spermatozoa were available, however, only five out of the 12 patients had an embryo transfer, none of them resulting in pregnancy.

Because of the recent more frequent use of testicular spermatozoa, the correlation between the presence or absence of motility of testicular spermatozoa and the outcome of ICSI was investigated (Tournaye et al., 1996b). An important finding of this study was that, in the majority of cases where spermatozoa were detected in the testicular biopsy, it was in fact possible to find motile spermatozoa. The motility of such spermatozoa was usually of a very sluggish, twitching type, but in a few cases a progressively forward motility was observed. Our experience is that, in cases of normal spermatogenesis or hypospermatogenesis, motile spermatozoa were more readily collected than in cases of germ-cell aplasia or of maturation arrest.

The mean normal fertilization rate was high (60%) after ICSI with testicular spermatozoa, and was similar to, or even somewhat higher than, previously reported (Nagy et al., 1995c; Silber et al., 1995). However, it was still lower than when freshly ejaculated spermatozoa were injected (Nagy et al., 1995c). There was a highly significant increase in the normal fertilization rate when motile rather than non-motile testicular spermatozoa were injected. The 2 PN fertilization rate was ~40% higher in cycles where only motile testicular spermatozoa were injected than in cycles where only non-motile testicular spermatozoa were injected. This indicates that the motility of testicular spermatozoa is an important predictor for the outcome of ICSI. There was also a difference in the normal fertilization rate in the group in which both motile and non-motile testicular spermatozoa were used for injection, depending on the type of spermatozoa.
If we compare these results with those obtained with freshly ejaculated semen, it is observed that injection of non-motile testicular spermatozoa correlates with a lower fertilization rate, as does injection of immotile ejaculated spermatozoa, although fertilization rates with the former were not so dramatically reduced (ranges between 21 and 36%). A possible explanation for this observed difference in the fertilization rate is the vitality rate. It was observed previously that the average vitality rate of non-motile ejaculated spermatozoa was ~10%, which is about the same as the 11% fertilization rate, thus suggesting that only vital spermatozoa are able to fertilize oocytes (Nagy et al., 1995a). It might be necessary to subclassify the dead sperm population. Spermatozoa that are ‘freshly’ dead (disruption of membrane integrity by either the ICSI needle for immobilization before injection or by freezing–thawing) are probably able to fertilize, while ‘long’ dead spermatozoa, where degenerative processes are more advanced, can no longer fertilize. It may be useful to determine how long after the spermatozoon dies does it lose its fertilizing capacity. This information could have practical importance in determining how long before ICSI spermatozoa can be collected (which is of relevance to difficult cases) without jeopardizing the fertilization rate.

It is probable that the vitality rate of non-motile testicular spermatozoa is much higher than that of non-motile ejaculated spermatozoa, where immotility is not the result of ultrastructural abnormalities of the spermatozoa. Although a vitality measurement of non-motile testicular spermatozoa has not been regularly attempted until now because of the low numbers of spermatozoa present, it may be postulated by analogy with the previous study that it is ~60%, which is in line with an earlier study carried out by Verheyen et al. (1995). This estimated higher vitality rate for non-motile testicular spermatozoa corresponds to the theoretical expectation that the proportion of vital spermatozoa retrieved directly from the source of production should be higher than is retrieved after the much longer delay occasioned by passage through the epididymis. This also implies that when only non-motile spermatozoa are found in ejaculated semen (where at least two or three samples have been shown to have an extremely low vitality rate) it is worth considering performing a testicular biopsy in the hope of recovering vital spermatozoa with a much greater fertilization potential. When results of ICSI were analysed according to the morphological characteristics of the spermatozoa, no differences were noted between the three groups either in the fertilization rate, the embryo developmental rate, the transfer rate or the pregnancy rate (Nagy et al., 1995a). The only morphological impairment which resulted in a lower fertilization rate was the total globozoospermia diagnosed in seven patients (Liu et al., 1995). The equally high results after ICSI in all the groups differed from what was observed in standard IVF, where the fertilization rate fell if the proportion of spermatozoa with normal morphology (determined by the strict criteria) was <14%, and especially when it was <4% (Kruger et al., 1988). One possible explanation of the higher fertilization rate obtained with ICSI is that for microinjection the ‘most normal-looking’ spermatozoon is selected within the limits of the microscope used for micro-manipulation (×400 magnification). A possible additional explanation is that as a consequence of the ICSI technique the morphologically abnormal spermatozoon, which would otherwise fail to penetrate the investments of the oocyte, is deposited directly into the cytoplasm. Total pregnancy rates (positive HCG) were uniformly high in each group independent of the extent of the morphological impairment of spermatozoa. An important observation is that embryo quality after injection of abnormal spermatozoa (group 1) is the same as in the groups where apparently normal spermatozoa were injected and the initial pregnancy loss (where no fetal sac was detected following HCG rise) was also not different. This implies that morphological abnormality of spermatozoa may reflect not so much a genetic abnormality of the male gametes (Martin and Rademaker, 1988; Rosenbusch et al., 1992) as an inability of the spermatozoon to penetrate the egg.

The analysis of the data of 11 cycles of patients with 100% globozoospermia revealed that the fertilization rate was severely impaired in comparison to those for spermatozoa with other types of
sperm abnormalities (Liu et al., 1995). It seems that, of the different types of total teratozoospermia, globozoospermia is the only pathological condition of sperm morphology that correlates with markedly reduced results after ICSI, even though the karyo-types of these spermatozoa appear to be normal (Rybouchkin et al., 1996). On the other hand, it should not be forgotten that, before the introduction of ICSI, it was not possible to obtain normal fertilization with acrosome-free, round-headed spermatozoa even by SUZI. For this reason, ICSI is the only method at present that can offer any help to patients with this sperm anomaly, as has also been confirmed by others (Lundin et al., 1994). Furthermore, it should also be noted that the fertilization rate after ICSI using round-headed spermatozoa is not uniformly low, being very low for some patients and acceptably high for others. This difference in the fertilization rates between patients might be explained by differences in the ultrastructural abnormalities that are associated with the globozoospermia.

The results show that patients whose infertility is essentially the result of high levels of antibody-coated spermatozoa can be successfully treated by ICSI (Nagy et al., 1995b). The fertilization, embryonic development and pregnancy rates after ICSI were not affected by the level of ASA-binding to the spermatozoa, by the type of immunoglobulin, or by the localization of the ASA on the spermatozoa. However, a non-significant increase in the fertilization rate occurred where 100% of the spermatozoa were coated with ASA, when the concentrations of both IgA and IgG were ≥80% and when ASA were located (also) on the heads of the spermatozoa. It was also established that MAR-positive patients had higher normal fertilization rates but also higher proportions of poor-quality embryos than did MAR-negative ICSI patients, while pregnancy rates seemed not to be affected.

One conclusion of this study is that neither the type nor the extent of sperm impairment has an important influence on the outcome of ICSI. Even in the most extreme cases of male-factor infertility, where cryptozoospermia, total asthenozoospermia or total teratozoospermia (except globozoospermia) is diagnosed from the initial semen sample or where all spermatozoa are coated with sperm antibodies, high fertilization and pregnancy rates can ensue from ICSI. It has been shown that only one condition has a strongly negative influence on the outcome of ICSI, i.e. where an immotile (presumably dead) spermatozoon is injected into the oocyte. Thus, the only ultimate criterion for successful ICSI is the presence of at least one living spermatozoon per oocyte in the semen preparation used for microinjection (Nagy et al., 1995a).

We can also conclude from the present study that when testicular biopsies had to be performed because of obstructive or non-obstructive azoospermia in conjunction with assisted fertilization treatment and where spermatozoa were present, in most such cases it was possible to find motile testicular spermatozoa. We also established that the normal fertilization rate is evidently lower when non-motile testicular spermatozoa are injected than when motile testicular spermatozoa are injected. However, the injection of non-motile testicular spermatozoa leads to a higher fertilization rate than the injection of non-motile ejaculated spermatozoa, suggesting a possible extension of the indications for testicular biopsy.

Acknowledgements

The authors wish to thank the clinical, paramedical and laboratory staff of the Centre for Reproductive Medicine, and especially the colleagues of the microinjection and IVF laboratory. Furthermore, we are very grateful to Mr Frank Winter of the Language Education Centre of our University for correcting the manuscript. This work was supported by grants from the Belgian Fund for Medical Research.

References


