Correlation between motility of testicular spermatozoa, testicular histology and the outcome of intracytoplasmic sperm injection

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The objective of the present study was to analyse the influence of motility on the results of intracytoplasmic sperm injection (ICSI) when testicular spermatozoa are used for microinjection and to correlate this with testicular histology. A total of 197 ICSI treatment cycles (167 couples) was analysed retrospectively in which testicular spermatozoa were used, because of complete azoospermia, for microinjection. Fertilization, embryo cleavage, transfer and pregnancy rates were evaluated and compared in relation to motility of testicular spermatozoa. In 170 cycles, histological diagnoses were compared with findings on motility. Injection of motile testicular spermatozoa (in 159 cycles) provided a higher normal fertilization rate than did injection of non-motile spermatozoa (in 14 cycles; 65 versus 45% respectively). Normal spermatogenesis was diagnosed in a significantly higher proportion and incomplete maturation arrest in a significantly lower proportion in the group of patients in which only motile spermatozoa were used for microinjection (65 and 10%), as compared to the group where exclusively non-motile spermatozoa were used (36 and 36%). Fertilization rate after ICSI was relatively high when non-motile testicular spermatozoa were used for microinjection, but use of motile testicular spermatozoa was associated with a still higher fertilization rate (except when histology of the testicular biopsy showed normal spermatogenesis), and therefore selection of motile testicular spermatozoa is always preferable for ICSI. Normal spermatogenesis predicts a greater probability, and maturation arrest a lower probability of recovering motile testicular spermatozoa.

Key words: azoospermia/ICSI/male infertility/sperm motility/testicular spermatozoa

Introduction

The successful introduction of intracytoplasmic sperm injection (ICSI) into clinical practice (Palermo et al., 1992; Van Steirteghem et al., 1993) has made it possible to alleviate nearly all types of infertility in a more efficient, more consistent and more reliable way than the use of conventional in-vitro fertilization (IVF). The greatest benefit, however, has been to those couples suffering from male-factor infertility and to those experiencing repeated failure or poor fertilization in standard IVF for undetermined reasons. It has been recently established that the results of ICSI are very good when freshly ejaculated spermatozoa are used, regardless of sperm parameters or other factors, such as the presence of very high levels of antisperm antibodies (Nagy et al., 1995a,b). The only circumstance in which the fertilization rate and, consequently, the pregnancy rate fell dramatically after ICSI using ejaculated spermatozoa was when completely immotile spermatozoa were microinjected (Nagy et al., 1995a). 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 (at least in terms of fertilization) than after injection of freshly ejaculated spermatozoa (Nagy et al., 1995c). It was also observed that in most testicular biopsies where spermatozoa were present, at least a few sperm cells usually displayed 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., 1996b). Although, in selecting spermatozoa for microinjection we always preferred to use a spermatozoon that had shown some sign of motility, on the basis of our experience with ejaculated spermatozoa it was not always possible in the case of testicular biopsy to find motile spermatozoa (or to find enough motile spermatozoa for the number of oocytes) and consequently in a few cycles ICSI had to be performed with non-motile testicular spermatozoa. The purpose of the present study therefore was to investigate whether injection of motile or non-motile testicular spermatozoa has any influence on the fertilization, embryo development and pregnancy rates after ICSI. Furthermore, we also wished to study the possible correlation between the presence of motile spermatozoa in the testicular biopsy and the histological diagnosis.

Materials and methods

During a period of 17 months, 197 cycles were performed (involving 167 couples) in which patients underwent testicular biopsy for the purpose of alleviating male-factor infertility due to obstructive or non-obstructive azoospermia, with successful recovery of enough spermatozoa to perform microinjection of all the available oocytes. In 170 of these 197 cycles, a histological examination of the testicular tissue was performed either at the pathology department of our hospital (Dutch-speaking Brussels Free University) or, in a few cases,
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at the pathology department of the referring centre. Histological
diagnoses of the testicular biopsies were categorized into four groups:
(i) germ-cell aplasia or Sertoli-cell-only syndrome (including cases
with focal spermatogenesis); (ii) maturation arrest (including cases
where focal spermatogenesis without arrest is present); (iii) hyposper-
matogenesis (or germ-cell hypoplasia); (iv) normal spermatogenesis
(Levin et al., 1979). The testicular biopsy was performed under a
local or general anaesthesia. A small testicular incision was made
and the specimen was placed in a Petri dish (Falcon Plastics, Becton-
Dickinson, Aalst, Belgium), containing ~5 ml modified HEPES-
buffered Earle’s medium supplemented with 0.4% human serum
albumin (HSA; Belgium Red Cross, Brussels, Belgium). After the
tissue had been shielded, microscopic examination of biopsies was
performed during the operation 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 done. In some
instances, repeated sampling was needed, also from the contralateral
side, in order to observe a single spermatozoon. One testicular
specimen per testis was sent for histology. This specimen was
routinely fixed in Bouin’s solution and stained with haematoxylin
and eosin for visualization of histological details. Testicular spermato-
zoa were prepared by dicing and squeezing a piece of testicular tissue
and eosin for visualization of histological details. Testicular spermato-
zoa were observed in the biopsy specimen.

Ovarian stimulation was carried out by a desensitizing protocol
using the gonadotrophin-releasing-hormone agonist buserelin (Supre-
fact; Hoechst, Brussels, Belgium) in association with human meno-
pausal gonadotrophin (HMG; Humegon; Organon, Oss, The
Netherlands; or Pergonal; Serono, Brussels, Belgium) and human
chorionic gonadotrophin (HCG, Pregnyl; Organon; Profasi, Serono).
Intravaginally administered progesterone (Utrogestan; Pitee, Brussels,
Belgium) was used for luteal-phase supplementation. The details of
this stimulation protocol have already been described elsewhere
were retrieved by vaginal ultrasound-guided puncture 36 h after HCG
administration. Where necessary Earle’s medium was used to flush
out CCOC. The cells of the cumulus and corona radiata were removed
by incubation of the CCOC for 1–2 min in HEPES-buffered Earle’s
medium, containing 80 IU/ml hyaluronidase (Type VIII, specific
activity 320 IU/mg; Sigma Chemical Co., St Louis, MO) and by
aspiration of the cumulus complexes in and out of a hand-drawn
glass pipette (diameter ~200 µm). The denuded oocytes were rinsed
in HEPES-buffered Earle’s medium and in B2 medium (bioMérieux,
Montalieu Vercieu, France). Metaphase II (MII) oocytes, as shown
by the presence of the first polar body in the perivitelline space, were
microinjected.

The injection dish contained several droplets (20–30 drops) of 5 µl
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)
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 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 in the
dish afterwards so as to avoid unnecessary exposure of the oocytes
to less than optimum conditions, such as possible temperature
alterations. 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.
The injection pipette was pushed through the zona pellucida at the 3
o’clock position and 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 and stored in 25 µl
microdrops of B2 medium in a Petri dish in an incubator containing
5% CO2, 5% O2, 90% N2. Sixteen to 18 h after microinjection,
the oocytes were checked for survival and fertilization (Nagy et al.,
1994). 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 injection. According to the relative proportion of anucleate
fragments present in the zona pellucida, embryos were assigned to
one of four categories: (i) excellent, where no anucleate fragments
were present; (ii) good, where <20% of the embryo was fragmented;
(iii) fair, where the proportion of fragments was 20–50%; (iv) poor,
where >50% of the embryo was fragmented. Embryos with <50% fragmentation were eligible for transfer. Supernumerary embryos with
<20% fragmentation were cryopreserved (Van Steirteghem et al.,
1994). To avoid multiple pregnancies, especially triplet pregnancies,
only two embryos were transferred in some cases (Staesen et al.,
1993). Exceptionally, in some older patients more than three embryos
were replaced.

Pregnancy was confirmed when serum HCG concentrations rose
on at least two separate occasions 10 days after embryo transfer.
Clinical pregnancy was determined by detecting the fetal heart beat
by ultrasonography at ~8–9 weeks of pregnancy.

Statistical tests were performed at the 5% level of significance.
The calculations were carried out using the SPSS statistical package
on an Inwork personal computer. Intactness, fertilization, embryo
quality, embryo transfer and embryo freezing rates were compared
by the Mann–Whitney U-test for the cycles with only motile testicular
spermatozoa (group 1) or only non-motile (group 2) testicular
spermatozoa. Total pregnancy rates were compared by means of the
χ2 test between the two groups. For cycles in which some of the
oocytes had been injected with motile spermatozoa and the rest
injected with non-motile spermatozoa, the percentage of intact oocytes,
fertilization, embryo quality, embryo transfer and embryo freezing
rates were compared using the Wilcoxon test.

Results

A total of 197 cycles (involving 167 couples) were evaluated
in which enough spermatozoa had been found to inject all the
available oocytes. In 159 of the 197 cycles (group 1) it was
possible to inject all the oocytes with motile spermatozoa
(80.7%). Conversely, in 14 cycles (7.1%; group 2) all the
oocytes had to be injected with non-motile spermatozoa
because no motile spermatozoa were found (Table I). Finally,
there were 24 cycles (12.2%) in which a total of 135 (38%)
oocytes were injected with motile spermatozoa and 225 (62%)
oocytes were injected with non-motile spermatozoa because
of the lack of a sufficient number of motile spermatozoa.

There were no differences between group 1 and group 2 in
the survival rates of the injected oocytes. The normal two-
The separate analysis of the 24 cycles in which a combination of motile and non-motile spermatozoa was used for ICSI shows that a larger proportion of the oocytes were injected with non-motile spermatozoa (62.5%) and only a smaller proportion of the oocytes with motile spermatozoa (37.5%). Injection of motile spermatozoa resulted in a higher normal (2PN) fertilization rate (58%) than did injection of non-motile spermatozoa (43%) in these sibling oocytes, but this difference was not significant ($P = 0.12$) according to the Wilcoxon test, possibly as a result of the insufficient number of cases involved. The proportions of oocytes displaying one (3.4 and 5.0% respectively) and three or more pronuclei (3.3 and 3.6% respectively), assessments of embryo quality (88.3 and 81.4% transferable quality embryos respectively) and the proportions of transferred or frozen embryos were similar here, regardless of whether motile or non-motile spermatozoa were used for microinjection. Seventy-eight embryos were transferred in 23 cycles and eight women became pregnant (34.8%). We have received information on five out of the eight pregnancies; four of these were singletons and one was a biochemical pregnancy.

In 170 cycles out of the 197 cycles (86.3%) the histological aspect of the testicular tissue was analysed. Normal spermatogenesis was diagnosed most frequently, in 100 out of the 170 cases (59%; Table II). The second most frequently observed histological diagnosis was the Sertoli-cell-only pattern (20%). Maturation arrest was present in 15% of the cases and hypospermatogenesis accounted for 6% of all the cases (Table II). Germ-cell aplasia and hypospermatogenesis were observed with similar frequencies in the three groups. However, the incidence of the maturation-arrest pattern was significantly lower in the group in which all the oocytes had been injected with motile spermatozoa (10%; group 1) than in the other two groups, in which oocytes had been injected exclusively (group

| Table I. Results of ICSI in patients after microinjection of motile testicular spermatozoa only (group 1), after microinjection of non-motile testicular spermatozoa only (group 2) |
|-----------------------------------|-------------------|-------------------|-------------------|-------------------|
| No. of cycles                     | Group 1            | Group 2            | Total             | Group 1 vs Group 2 |
| No. of injected oocytes           | 159               | 14                | 173               | NS                |
| % of intact oocytes (mean ± SD)   | 87.8 ± 17.1        | 92.8 ± 6.0        | 88.6 ± 15.7       | NS                |
| Pronuclear (PN) development       |                   |                   |                   |                   |
| (% of intact oocytes, mean ± SD)  |                   |                   |                   |                   |
| 2PN b                            | 64.9 ± 25.1        | 45.8 ± 26.3       | 61.1 ± 26.2       | 0.006             |
| 1PN                              | 2.8 ± 5.9          | 4.6 ± 6.5         | 3.2 ± 6.4         | NS                |
| 3PN                              | 3.9 ± 9.1          | 2.6 ± 8.1         | 3.8 ± 8.6         | NS                |
| Embryo development of 2PN oocytes |                   |                   |                   |                   |
| (% of cleaved embryos, mean ± SD) |                   |                   |                   |                   |
| Excellent                        | 4.8 ± 12.3         | 5.3 ± 11.1        | 4.8 ± 12.5        | NS                |
| Good                             | 46.8 ± 31.3        | 55.8 ± 30.9       | 47.7 ± 30.7       | NS                |
| Fair                             | 20.6 ± 25.0        | 15.7 ± 23.4       | 21.0 ± 25.2       | NS                |
| Poor                             | 25.2 ± 26.0        | 23.2 ± 24.8       | 24.1 ± 25.2       | NS                |
| Embryos transferred              |                   |                   |                   |                   |
| (as % of 2PN oocytes, mean ± SD)  |                   |                   |                   |                   |
| Embryos frozen                   | 11.6 ± 20.0        | 8.5 ± 23.0        | 10.8 ± 19.6       | NS                |
| No. of embryo transfers          | 150 (94.3%)        | 13 (92.9%)        | 163 (94.4%)       |                   |
| No. of transferred embryos       | 433 (94.3%)        | 36 (92.9%)        | 469 (94.4%)       |                   |
| No. of pregnancies (positive HCG) | 40 (26.7%)         | 4 (30.8%)         | 44 (28.0%)        |                   |

*The last group ‘total’ also includes an additional 24 cycles where microinjection of motile and non-motile testicular spermatozoa was performed within the same treatment cycle. A Mann–Whitney U-test was used to compare the results between group 1 and group 2. *bIn group 1 the 2PN rate was significantly different from group 2. *cThere was no significant difference in pregnancy rates between group 1 and group 2 by the $\chi^2$-test. HCG = human chorionic gonadotrophin.
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Table II. Distribution of testicular histological diagnosis in patients with only motile testicular spermatozoa (group 1), with only non-motile testicular spermatozoa (group 2) and with motile and non-motile testicular spermatozoa (group 3) within the same treatment cycle

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (131)</th>
<th>Group 2 (10)</th>
<th>Group 3 (19)</th>
<th>Total</th>
<th>(P (\chi^2\text{-test}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>159</td>
<td>14</td>
<td>24</td>
<td>197</td>
<td></td>
</tr>
<tr>
<td>No. of cycles with known histology</td>
<td>138</td>
<td>11</td>
<td>21</td>
<td>170</td>
<td></td>
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<tr>
<td>Histological diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germ-cell aplasia(^a)</td>
<td>79.4%</td>
<td>5.9%</td>
<td>9.2%</td>
<td>34</td>
<td>NS</td>
</tr>
<tr>
<td>Maturation arrest(^b)</td>
<td>53.9%</td>
<td>15.4%</td>
<td>30.8%</td>
<td>26</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hypospermatogenesis(^c)</td>
<td>70.0%</td>
<td>10.0%</td>
<td>20.0%</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Normal spermatogenesis(^c)</td>
<td>90.0%</td>
<td>4.0%</td>
<td>6.0%</td>
<td>100</td>
<td>0.02</td>
</tr>
</tbody>
</table>

\(^a\)NS = the number of patients is not significantly different between the three groups.
\(^b\)In group 1 the number of patients with maturation arrest was significantly different from that in group 2 or in group 3.
\(^c\)In group 1 the number of patients with normal spermatogenesis was significantly different from that in group 2 or in group 3.

Table III. Normal fertilization rates (% 2PN, mean ± SD) in relation to the different testicular histologies in patients with only motile testicular spermatozoa (group 1), with only non-motile testicular spermatozoa (group 2) and with motile and non-motile testicular spermatozoa (group 3) within the same treatment cycle

<table>
<thead>
<tr>
<th>Type of azoospermia</th>
<th>Group 1 (131)</th>
<th>Group 2 (10)</th>
<th>Group 3 (19)</th>
<th>Motile</th>
<th>Non-motile</th>
<th>Motile/Non-motile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-obstructive</td>
<td>60.8(^b) ± 25.6</td>
<td>31.2(^b) ± 23.7</td>
<td>69.1(^c) ± 36.6</td>
<td>40.8(^c) ± 32.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(maturation arrest and germ-cell aplasia)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstructive (normal spermatogenesis)</td>
<td>67.7 ± 22.7</td>
<td>69.3 ± 26.6</td>
<td>56.5 ± 31.0</td>
<td>55.8 ± 38.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)No. of cycles.
\(^b\)In group 1 the 2-pronuclear (2PN) rate was significantly different from group 2.
\(^c\)In group 3 the 2PN rate was significantly different after injection of motile testicular spermatozoa to that after injection of non-motile testicular spermatozoa.

Discussion

The single most important factor associated with the successful outcome of ICSI with ejaculated spermatozoa has been the use of a motile spermatozoon for oocyte microinjection (Nagy et al., 1995a). Because of the more frequent recent use of testicular spermatozoa, it seemed important to examine the correlation between the presence or absence of motility of testicular spermatozoa and the outcome of ICSI. Furthermore, we here relate the presence of sperm motility to the histological findings, since an earlier report indicated that the presence of motility might vary according to testicular histology (Tournaye et al., 1996a).

An important finding of this study is that in the majority of the cases where there were spermatozoa present in the testicular biopsy it was possible to find motile spermatozoa. Although in traditional thinking testicular spermatozoa are considered incapable of movement, some earlier studies have showed that spermatozoa recovered from the testes do display movement to some extent (Jow et al., 1993; Nagy et al., 1995c) and this
has been further confirmed by the present study. Because in our previous study (Tournaye et al., 1996a) the numbers of spermatozoa in a biopsy seemed to correlate with the historical findings, it may be suggested that the observed correlation in the present study between testicular histology and the time required to collect motile spermatozoa is simply due to the difference in sperm numbers, rather than because proportions of motile spermatozoa are very different in different testicular histopathologies. Our experience is that in cases of normal spermatogenesis or hypospermatogenesis much less time was usually needed to collect enough motile spermatozoa than in cases of germ-cell aplasia or of maturation arrest. There were only very few cases with normal spermatogenesis or hypospermatogenesis in which, despite the presence of large numbers of spermatozoa, no spermatozoa showed the slightest sign of motility.

The oocyte survival rate was similar across all the groups, regardless of whether sperm motility was observed prior to microinjection or not. This corresponds to our expectations and correlates too with previous findings where oocyte survival was independent of sperm source or sperm parameters (Nagy et al., 1995a,c).

The mean normal fertilization rate was high (61%) after ICSI with testicular spermatozoa, and was similar to, or even somewhat higher than, that in our previous report (Nagy et al., 1995c; Silber et al., 1995). However, this is still lower than the results of microinjection with freshly ejaculated spermatozoa (Nagy et al., 1995c). There was a highly significant difference in the normal fertilization rate when motile spermatozoa were injected, rather than non-motile spermatozoa. The 2PN fertilization rate was ~20% higher in cycles where only motile spermatozoa were used (group 1) than in cycles where only non-motile spermatozoa were used (group 2). This result indicates that motility of testicular spermatozoa is an important predictor for fertilization after intracytoplasmic sperm injection. In the group where motile and non-motile spermatozoa were used for injection, many more oocytes were injected with non-motile spermatozoa (62%) than with motile spermatozoa (38%). The likely reason for fertilization rates not being significantly different in this group is that not enough cases were involved and the type of statistical test applied (non-parametric) was less sensitive. It has been previously established that total absence of sperm motility is the only important factor, with a dramatically negative influence on fertilization and on pregnancy rates after ICSI when freshly ejaculated spermatozoa are used (Nagy et al., 1995a). If we compare the results of the present study with the results with freshly ejaculated semen, it is evident that the use of non-motile testicular spermatozoa does not correlate with such a dramatically low fertilization rate as does the use of non-motile spermatozoa from fresh ejaculate. A likely explanation for this observed difference in the fertilization rate is the difference in vitality rate, which is usually very low in ejaculates from patients with 100% asthenozoospermia (Nagy et al., 1995a) but much higher in testicular biopsies (Verheyen et al., 1995). This also implies that in cases where only non-motile spermatozoa are found in ejaculated semen (analysing at least two or three samples with 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.

It is important to note that normal fertilization rates correlated with the presence or absence of motility also when this was analysed separately according to the type of azoospermia. Results showed that the diagnosis of normal spermatogenesis was associated with a high fertilization rate irrespective of whether motile or non-motile testicular spermatozoa were used for ICSI. This means in practice that in cases of normal spermatogenesis, where besides non-motile spermatozoa there are usually many motile spermatozoa also present, sperm collection from testicular tissue extract is in itself fast and easy and there is no special need to look for motile spermatozoa, or therefore to take more than one biopsy specimen from the testis. Conversely, in cases of maturation arrest and germ-cell aplasia where it is usually very difficult to find spermatozoa at all, the need to find and collect motile sperm cells will make the work even more cumbersome. In this respect, in-vitro culture of testicular spermatozoa might be a useful procedure that can help to improve the proportion of motile spermatozoa present in the removed testicular tissue, as reported recently (Edirisinghe et al., 1996; Liu et al., 1996,1997) and thus to improve fertilization rates in non-obstructive cases although the positive effect of in-vitro culture in these publications has been more clearly demonstrated in obstructive azoospermic cases.

Embryo quality 2 days after ICSI seems to be independent of whether motile or non-motile testicular spermatozoa were microinjected. This confirms the observation made in the earlier study after the injection of non-motile ejaculated spermatozoa and further confirms our earlier hypothesis that the type of spermatozoa and/or sperm parameters do not have an important influence on the quality of the embryo. In other words, once normal fertilization has occurred, the quality of the resulting embryos will generally be independent of sperm factors.

No statistically significant difference appeared in the three groups as regards total pregnancy rates. However, the total pregnancy rate was somewhat higher in the third group where motile and non-motile spermatozoa were used for injection within the same cycle, but this was probably the result of the larger number of embryos transferred (per patient) than in the other groups. Information on the outcome of pregnancies is incomplete mainly because many patients were referred from abroad to our Centre (especially in this patient population who require testicular sperm extraction–ICSI procedure) who were very difficult to contact after their return. However, it seems that the outcome of the pregnancies in this patient population was not different from the outcome of other ICSI patient population.

For the majority of the male patients it was possible to establish a histological diagnosis before or after a testicular biopsy was taken. The proportions of patients in two historical categories (germ-cell aplasia and hypoplasia) were similar across the three groups but in the two other categories (maturation arrest and normal spermatogenesis) there were significant differences in the numbers of patients between the
three groups. From these results it may be concluded that the histological diagnosis of maturation arrest predicts with high probability a lack of motile spermatozoa in the wet preparation of the testicular biopsy. On the other hand, the diagnosis of normal spermatogenesis is associated with a much higher probability of the presence of motile testicular spermatozoa.

To sum up, we can conclude from the present study that where testicular biopsies had to be performed because of obstructive or non-obstructive azoospermia in conjunction with assisted fertilization treatment and where spermatozoa were found, in the majority of 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 into the oocytes than when motile testicular spermatozoa are injected, as suggested in our earlier study on ejaculated spermatozoa (Nagy et al., 1995a). However, the injection of non-motile testicular spermatozoa provides an acceptable fertilization rate, suggesting a possible extension of the indications for testicular biopsy. Finally, it may also be concluded that in cases of maturation arrest there is a lower probability of finding motile spermatozoa in the wet preparation from a testicular biopsy and in cases of normal spermatogenesis there is a higher probability of this.

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, MA of the Language Education Centre of our University for correcting the manuscript. We thank Ms Marie-Paule Derde for the statistical advice.

References


Received on August 14, 1997; accepted on January 19, 1998