ICSI outcome in patients with transient azoospermia with initially motile or immotile sperm in the ejaculate

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BACKGROUND: In patients with transient azoospermia, few sperm may be found in the ejaculate. We investigated the outcome of ICSI in patients with transient azoospermia. METHODS: Records of patients with transient azoospermia referred during a 42 month period were reviewed. If only immotile sperm were found, the sample was incubated with 30% human serum albumin (HSA) before motility re-assessment. If still immotile, mechanical assessment of sperm viability was utilized. Study groups were: (A) motile sperm; (B) motility achieved by HSA; (C) no motility, but viability assessed by a mechanical technique; and (D) control group with sperm counts from 1 to 5 × 10^6/ml. There were 57 couples (cycles) in the study group and 43 couples (cycles) in the control group.

RESULTS: Age, days of stimulation and endometrial thickness were comparable among groups. In 29.8% of the cycles, only immotile sperm were found. Fertilization and cleavage rates were higher in groups A and D than in groups B and C. Clinical pregnancy rate/cycle and live birth rate/cycle were not different among groups. No congenital malformations were found in newborns. CONCLUSION: Fertilization and cleavage rates were lower in patients with initially immotile sperm compared with those with initially motile sperm and oligoasthenoteratozoospermia patients. Clinical pregnancy and viable pregnancy rates were not statistically different among groups, although when only immotile sperm were present both clinical pregnancy and live birth rate were lower in comparison with cycles with motile sperm.

Key words: azoospermia/ICSI/immotile sperm motile sperm

Introduction
Patients diagnosed as azoospermic may occasionally be found to have sperm in the ejaculate. Tournaye described such a situation as ‘virtual azoospermia’ (Tournaye et al., 1995).

Testicular epidydimal sperm extraction (TESE) followed by ICSI is often advised for azoospermic patients for treatment of the couple’s infertility. It is not surprising that Ron-El et al. (1997) found that more than a third of their patients diagnosed as azoospermic, if asked to deliver sperm on the morning of a scheduled TESE procedure, were successful in doing so.

ICSI cycles in patients with severe oligoasthenoteratozoospermia (OAT) or non-obstructive azoospermia (NOA) can be complicated by unanticipated cancellation or need for an additional TESE procedure when sperm are unavailable on the day of oocyte pick-up (OPU). To avoid such an event in patients with a severe male factor, a strategy of sperm ‘pooling’ and cryopreservation was suggested (Lahav-Baratz et al., 2002). Eventually, in 32 of their 45 ‘transient azoospermic’ patients, fresh sperm were available on the OPU day, yet, in 13 patients the cryopreserved sperm were used for ICSI. Notably, in seven of their patients who had a previous TESE procedure due to azoospermia, ejaculated sperm cryopreservation was possible.

Nagy et al. (1995) have demonstrated that in patients with severe OAT, when sperm were obtained only after centrifugation of the ejaculate and used for ICSI, a 24.5% clinical pregnancy (CP) rate could be accomplished, comparable with a 22% CP rate in patients with a sperm count of 1–5 × 10^6/ml.

In patients with severe OAT, the ejaculate is frequently found to contain only immotile sperm. Immotile sperm may not be viable and their use for ICSI may result in lack of fertilization. Unfortunately, there is no ideal test of sperm viability assessment except for observing their motility. The hypo-osmotic swelling test was used to determine the viability of immotile sperm, but controversy surrounds this test or variants thereof owing to the deleterious effect it may have on sperm viability (Casper et al., 1996; Tsai et al., 1997).
The fertilization rate using immotile sperm may be quite low. Thus, Nagy et al. (1995) have used ejaculated immotile sperm for ICSI in 12 couples and obtained a low fertilization rate of 10%, and no pregnancies were achieved. The criteria for selection of viable immotile sperm were not reported. Nijs et al. (1996) incubated initially immotile ejaculated sperm for several hours, obtained motility in some and achieved a significantly higher fertilization rate with these sperm compared with totally immotile sperm.

Recently, a mechanical touch technique was described for evaluation of viability of testicular retrieved immotile sperm which resulted in a fertilization rate of 73.4% and pregnancy/patient rate of 30% (Marques de Oliviera et al., 2004). The aim of our study was to investigate the outcome of ICSI treatment in couples in whom the husband presented with 'transient azoosperma' and assess the occurrence of congenital malformations in newborns delivered to these couples.

Subjects and methods

The files of patients who had been referred to our IVF unit from January of 2000 until June of 2003 and diagnosed as having NOA were reviewed. Eligibility for inclusion in the study was that in those patients, at least on one occasion, sperm were not found even after extensive sperm analysis of the ejaculate, yet were found at least once in a following extensive search. As for the female partner, inclusion criteria were absence of congenital or acquired uterine anomalies and age ≤ 38 years.

All male patients were documented for past medical history, hormonal profile and testicular ultrasonography. A karyotype study and a Y deletion assessment were always advised, but for different reasons only some of the patients had these tests. Between one and two ejaculates were cryopreserved prior to the treatment cycle as a back-up in case no sperm would be found on the day of oocyte retrieval. When the sperm were contaminated with many leukocytes, appropriate antibiotic therapy was administered to patients before entering the IVF cycle.

Only couples in whom fresh sperm were used for oocyte fertilization were considered in the study.

Sperm analysis was performed using the Makler chamber (Sefi Medical Instruments, Haifa, Israel). Sperm morphology was assessed according to the WHO criteria (World Health Organization, 1999). When no sperm were found, the ejaculate was washed twice in HEPES-HTF medium (Irvine Scientific, Santa Ana, CA) supplemented with 10% synthetic serum substitute (SSS) at 300 g for 10 min. The pellet was resuspended in 10–15 µl of HEPES-HTF + 10% SSS using an inverted microscope (× 200 magnification). If sperm were found, and even where only a few were motile, the pellet from the second wash was divided into several 5 µl droplets of HEPES-HTF + 10% SSS under oil (extensive sperm analysis; ESA) in ICSI plates. However, if no motile sperm were found in the pellet of the second centrifugation, HTF + 30% human serum albumin (HSA) was added and centrifuged in the same conditions. The pellet was resuspended in HTF + 10% SSS and divided into several 5 µl droplets under oil and incubated for 90 min at 37°C and 5% CO2. The motile sperm, when found, were taken to the polyvinylpyrrolidone (PVP) droplet for tail crushing.

If no motile sperm were found after treatment with 30% HSA, mechanical assessment of sperm viability was utilized during the ICSI procedure. The mechanical touch technique used in our centre is similar to a procedure described recently (Marques de Oliviera et al., 2004) and involves pressing against the upper third of the immotile spermatozoon tail and the ICSI dish with the micropipette and observing a movement of the distal two-thirds of the tail. If the tail is flexible, the spermatozoon is considered viable.

Accordingly, the entire study group was divided into three subgroups: cycles with initially motile sperm (group A), cycles in which sperm motility was achieved by 30% HSA (group B) and cycles in which viability was assessed by the mechanical touch technique (group C). Only the first treatment cycle was considered in both study and control groups.

All patients were asked to abstain from intercourse for 72 h and to bring fresh semen on the day of oocyte retrieval. When sperm were not present in the first ejaculate even after ESA, the patient was asked to provide an additional ejaculate which was again screened by ESA.

The control group (D) comprised couples in whom the male had a sperm count of between 1 and 5 x 10⁶ sperm/ml in repeated sperm counts (at least two) and who were treated during the same time period as the study group. There were 61 couples in the study group and 43 in the control group.

Controlled ovarian stimulation (COS) was performed with the long, downregulation protocol as previously described (Tal et al., 2002) in 82.7% of cycles of the study group and 81.4% of cycles of the control group. In the rest of the cycles, it was either the short downregulation COS that was utilized, namely starting the GnRH agonist (Decapryl, Ferring; Lapidot, Cesarea, Israel) on the second day of a menstrual period and adding the gonadotrophins on the third day, or a COS in which gonadotrophins are initiated on day 3 of a menstrual cycle and a GnRH antagonist (Cetrotide 0.25 mg, Serono, Herzliya, Israel) is added when the leading follicle reaches a mean diameter of 14 mm. The last two COS protocols were utilized in a comparable number of cycles in the two groups.

An ICSI procedure was carried out in all cycles using a Nikon microscope with Nomarski optics with three-dimensional manipulators (Narishige, Tokyo, Japan) as fully described (Ben-Yosef et al., 1999). Fertilization was confirmed 16–20 h after injection by the presence of two distinct pronuclei under the inverted microscope. Cleavage was assessed 24 h later and the embryos were classified according to their morphological appearance (Veeck, 1991). Up to three embryos were selected for transfer into the uterine cavity 48 h (44 cycles in the study group and 35 in the control group) or 72 h (nine cycles in the study group and seven in the control group) after oocyte retrieval. The only reason for a 72 h transfer was an intervening weekend. We do not see any differences in CP rates between a day 2 or 3 transfer (unpublished data, Tal J).

Luteal supplementation consisted of 600 mg vaginal progesterone capsules daily (Utrogestan, Besims International Laboratories, France). Spare embryos were cryopreserved. However, results of transfer cycles with frozen–thawed embryos are not given because during a few months of the study period we had technical difficulties with the cryopreservation equipment and data are incomplete.

None of our patients had had a TESE procedure in the past. Since we perform a testicular biopsy for histological evaluation during the TESE procedure, we do not have a diagnosis of the testicular pathology.

 Estradiol serum levels were determined with a commercial enzyme immunoassay kit.

Endometrial thickness was assessed on the HCG day by a sonographic machine with a 6.5 MHz transvaginal probe. The limits of the total endometrial width were defined by the operator at the outer bright borders of the myometrium–endometrium interface in the B-mode uterine image.
Table I. General data

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Study group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>43</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>No. of cycles</td>
<td>43</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Female age</td>
<td>27.8 ± 4.0</td>
<td>28.7 ± 4.7</td>
<td>NS</td>
</tr>
<tr>
<td>Male age</td>
<td>32.5 ± 4.5</td>
<td>32.0 ± 4.6</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>3.7 ± 3.2</td>
<td>3.1 ± 2.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS = not statistically different.

A CP was defined when a gestational sac with fetal heart activity appeared in an ultrasonographic study performed 10–14 days after a positive serum pregnancy test.

Newborns were examined by the neonatologists within 24 h after delivery and then followed-up by paediatricians.

Statistics

Statistical analysis of discrete variables was performed using the χ^2 double classification test and the Fisher exact test where appropriate. Continuous variables were analysed using Student’s two-sample t-test. Statistical differences were considered significant at P < 0.05.

Results

In four of the 61 patients in the entire study, fresh sperm were not available on the day of oocyte retrieval and cryopreserved sperm were used. These couples were excluded from the study. Therefore, 57 couples were left in the study group and 43 in the control group. All had sperm in the ejaculate on the day of oocyte retrieval. Altogether, there were 57 treatment cycles in the study group and 43 in the control group.

Table I shows that the mean age of the women and the men did not differ between the two groups. The mean duration of infertility before IVF/ICSI treatment is also comparable.

Table II depicts some data related to the male spouses. Significantly more patients in the study group than in the comparable control group had an elevated FSH level (P = 3.4). The sperm parameters given in the table are for the oocyte retrieval day of 57 and one out of 43, respectively (P = 3.4).

Results

In four of the 61 patients in the entire study, fresh sperm were not available on the day of oocyte retrieval and cryopreserved sperm were used. These couples were excluded from the study. Therefore, 57 couples were left in the study group and 43 in the control group. All had sperm in the ejaculate on the day of oocyte retrieval. Altogether, there were 57 treatment cycles in the study group and 43 in the control group.

Table I shows that the mean age of the women and the men did not differ between the two groups. The mean duration of infertility before IVF/ICSI treatment is also comparable.

Table II depicts some data related to the male spouses. Significantly more patients in the study group than in the control group had an elevated FSH level (>12 IU/l), 10 out of 57 and one out of 43, respectively (P < 0.01). The sperm parameters given in the table are for the oocyte retrieval day of all treatment cycles.

For the control group, the mean ± SD sperm count was 3.4 ± 3.9 x 10^6/ml, motility 38.2 ± 24.5% and abnormal morphological forms 93.1 ± 12.9% (World Health Organization, 1999). In the study group in all cycles, only 300–400 sperm/ml were found after ESA. Sperm motility was described as ‘sluggish’ in 43.8% and as ‘good’ in 26.4% of the cycles. In 29.8% of the cycles, only immotile sperm were found.

In about three-quarters of the cycles of the study group, 80–90% of sperm were morphologically abnormal, and in a quarter only abnormal forms were found.

The main results of the treatment outcome are summarized in Table III. No differences were found between the four groups in the days of gonadotrophin stimulation, number of ampoules/cycle, endometrial thickness and estradiol level on the day of HCG administration. More oocytes were retrieved in the control group than in any of the study subgroups, P < 0.01; however, the number of oocytes retrieved did not differ among groups A–C.

In four women of the study group (four cycles), no oocyte was fertilized. Two were not fertilized in group A, and one in each of group B and C. In the control group, fertilization occurred in all cycles, but in one woman the only retrieved oocyte was arrested at 2PN. Thus, there were 53 embryo transfer cycles in the entire study group and 42 in the control group (Table III).

The fertilization rate was significantly higher when sperm were initially motile (group A, 76.5%) than in groups B and C with initially immotile sperm, 56.9 and 55.1%, respectively. Likewise, the fertilization rate was higher in the control group, 78.2% compared with groups B and C but not different compared with group A. There was no difference between the fertilization rates of the two groups with the initially immotile sperm.

Fertilization resulted in five out of 137 3PN zygotes (3.6%) in group A and one out of 27 (3.7%) in group C, but none in the other groups. The rate of 1PN after ICSI was two out of 179 (1.1%) and occurred only in group A.

The cleavage rate was significantly higher in group D (97.3%) compared with groups B (89.2%) and C (85.2%). The cleavage rate did not differ between groups A and D and B and C.

The percentage of grade 1 and 2 embryos (higher quality) was between 44.8 and 48.2% in the four groups and not different statistically. Likewise, the proportion of lower grade embryos (grades 3 and 4) did not differ between the four groups.

Table II. Hormonal and sperm parameters in the male patients

<table>
<thead>
<tr>
<th>Patients/cycles</th>
<th>Control group</th>
<th>Study group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with FSH &gt;12 IU/ml</td>
<td>1 (0.2%)</td>
<td>10 (27.5%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sperm count^a on OPU^b day</td>
<td>3.4 ± 3.9 (x 10^6/ml)</td>
<td>300–400/ml</td>
<td></td>
</tr>
<tr>
<td>Cycles with only immotile sperm^a</td>
<td>17 (29.8%)</td>
<td>15 (26.4%)</td>
<td></td>
</tr>
<tr>
<td>Cycles with ‘sluggish’ motility^a</td>
<td>25 (43.8%)</td>
<td>15 (26.4%)</td>
<td></td>
</tr>
<tr>
<td>Cycles with ‘good’ motility^a</td>
<td>93.1 ± 12.9%</td>
<td>42 (73.6%)</td>
<td></td>
</tr>
<tr>
<td>Pathological forms</td>
<td>93.1 ± 12.9%</td>
<td>42 (73.6%)</td>
<td></td>
</tr>
<tr>
<td>Cycles with 80–90% pathological sperm^a</td>
<td>15 (26.4%)</td>
<td>15 (26.4%)</td>
<td></td>
</tr>
<tr>
<td>Cycles with 100% pathological sperm^a</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

^a After extensive sperm analysis in the study group.

^b OPU = oocyte pick-up day.
The CP rate/cycle was not different among the four groups: 25.0, 18.2, 16.7 and 32.6% for A, B, C and D, respectively. Similarly, the CP rate/embryo transfer was not different among the four groups.

The live birth rate/cycle did not differ between the study groups and control group: 20.0, 18.2, 16.7 and 25.6% for A, B, C and D, respectively. Similarly, the implantation rate was comparable among groups: 12.1, 11.5, 7.1 and 16.4% for A, B, C and D, respectively.

Eight live deliveries occurred in group A, five singletons and three twins (37.5%), one after embryo reduction from triplets. There were also one first trimester and one second trimester miscarriage.

In the control group, 11 deliveries occurred, seven singletons and four twins (36.4%). There were two first trimester miscarriages.

Four biochemical pregnancies occurred in group A (10.0%) compared with two out of 43 (4.6%) in group D, with no statistical difference.

All newborns were examined by neonatologists in the neonatal care unit and later by paediatricians. During the 9–42 months (median 21) follow-up of these infants, no congenital malformation or abnormality was found.

### Table III. Treatment outcome in the first fresh transfer cycle

<table>
<thead>
<tr>
<th></th>
<th>Control group (D)</th>
<th>Initially immotile sperm (mechanical touch) (C)</th>
<th>Initially immotile sperm (HSA 30%) (B)</th>
<th>Motile sperm (A)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment cycles</td>
<td>43</td>
<td>6</td>
<td>11</td>
<td>40</td>
<td>0.92</td>
</tr>
<tr>
<td>Cycles with embryo transfer</td>
<td>42</td>
<td>5</td>
<td>10</td>
<td>38</td>
<td>0.63</td>
</tr>
<tr>
<td>Days of gonadotrophin stimulation</td>
<td>10.5 ± 2.2</td>
<td>10.3 ± 2.2</td>
<td>10.7 ± 2.5</td>
<td>10.1 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Estradiol level on HCG day (pmol/l)</td>
<td>7295 ± 3971</td>
<td>5236 ± 2782</td>
<td>5932 ± 3478</td>
<td>5534 ± 2973</td>
<td>NS</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>10.2 ± 2.4</td>
<td>9.9 ± 2.6</td>
<td>10.1 ± 2.3</td>
<td>10.6 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Oocytes/cycle</td>
<td>11.2 ± 6.8a</td>
<td>9.1 ± 3.6a</td>
<td>7.4 ± 3.9a</td>
<td>8.9 ± 5.5a</td>
<td>NS</td>
</tr>
<tr>
<td>MI oocytes</td>
<td>377495 (76.2%)</td>
<td>4964 (76.6%)</td>
<td>6584 (74.4%)</td>
<td>179248 (72.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>295/377 (78.2%)b</td>
<td>27/49 (55.1%)b</td>
<td>37/65 (56.9%)b</td>
<td>137/179 (76.5%)b</td>
<td>NS</td>
</tr>
<tr>
<td>Cleavage rate</td>
<td>287/295 (97.3%)c</td>
<td>23/27 (85.2%)c</td>
<td>33/37 (89.2%)c</td>
<td>131/137 (95.6%)c</td>
<td>NS</td>
</tr>
<tr>
<td>1PN (oocytes)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (1.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>2PN (arrested)</td>
<td>8 (2.7%)</td>
<td>0</td>
<td>0</td>
<td>6 (4.4%)</td>
<td>NS</td>
</tr>
<tr>
<td>3PN (zygotes)</td>
<td>0</td>
<td>1 (3.7%)</td>
<td>0</td>
<td>5 (3.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Embryos transferred/cycle</td>
<td>2.8 ± 0.9</td>
<td>2.7 ± 0.7</td>
<td>2.7 ± 0.8</td>
<td>2.8 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Embryo gradea</td>
<td>1–2</td>
<td>1021 (47.6%)</td>
<td>1329 (44.8%)</td>
<td>58121 (47.9%)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>3–4</td>
<td>112255 (51.8%)</td>
<td>1629 (55.2%)</td>
<td>63121 (52.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Positive β HCG/cycle</td>
<td>1643 (37.2%)</td>
<td>1/6 (16.7%)</td>
<td>2/11 (18.2%)</td>
<td>14/40 (35.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy/cycle</td>
<td>144/35 (32.6%)</td>
<td>1/6 (16.7%)</td>
<td>2/11 (18.2%)</td>
<td>10/40 (25.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy/ETb</td>
<td>144/42 (33.3%)</td>
<td>1/5 (20.0%)</td>
<td>2/10 (20.0%)</td>
<td>10/38 (26.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Live birth/cycle</td>
<td>114/45 (25.6%)</td>
<td>1/6 (16.7%)</td>
<td>2/11 (18.2%)</td>
<td>8/40 (20.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>19116/164 (16.4%)</td>
<td>1/14 (7.1%)</td>
<td>3/26 (11.5%)</td>
<td>14/116 (12.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Live deliveries</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Singleton</td>
<td>7 (63.6%)</td>
<td>1</td>
<td>2</td>
<td>(62.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>Twins</td>
<td>4 (36.4%)</td>
<td>0</td>
<td>0</td>
<td>3 (37.5%)f</td>
<td>NS</td>
</tr>
<tr>
<td>Early miscarriage</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Late miscarriage</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Biochemical pregnancy/cycle</td>
<td>2/43 (4.6%)</td>
<td>0</td>
<td>0</td>
<td>4/40 (10%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

NS = not significant among all subgroups by the χ^2 double classification test, Fisher’s exact test or the Students t-test where appropriate.

aP < 0.01 by the Students t-test for D compared with all other subgroups.

bP < 0.04 for subgroups A and B, A and C; P < 0.008 for B and D; P < 0.01 for C and D by the χ^2 test.

P < 0.05 for B and D; P < 0.03 for C and D both by Fisher’s exact test.

Grade 5 embryos (no blastomeres, only fragmentations) not included.

ET = embryo transfer.

fOne case after embryo reduction from triplets.

### Discussion

We have demonstrated in this study that the results of ICSI treatment in couples in whom the male is diagnosed as azoospermic but demonstrates intermittent existence of sperm in the ejaculate are favourable if sperm are motile, but less so if sperm are initially immotile.

The four features of sperm found in these patients were (Table II): (i) very few sperm found only after centrifugation and extensive search in droplets (300–400 sperm/ml); (ii) in ~40% of the cases, motility was described as ‘sluggish’, and many times only wiggling of the sperm tail attested to sperm viability; (iii) ~30% of the sperm samples contained only immotile sperm; and (iv) abnormal sperm forms (WHO criteria) ranged between 80 and 100%.

Thus the entire study group was divided into three groups according to the way that sperm viability was demonstrated. Motility was the obvious sign for viability in group A. Group B comprised initially immotile sperm, and motility was observed only after treatment with HSA which is known for its antioxidant potential and stabilizing effects on the sperm membrane (Armstrong et al., 1998).

A mechanical touch technique had been utilized when 30% HSA treatment had failed to clarify if sperm were viable.
viability was assessed in the immotile sperm. We have not used the hypo-osmotic swelling test for assessment of sperm viability because of the reported low fertilization rates (Casper et al., 1996) and the observed non-viability of sperm after 30 min of incubation in hypo-osmotic solution (Tsai et al., 1997).

The fact that parameters related to the hormonal treatment protocols and endometrial thickness on the HCG day were comparable among our four groups (Table III) affords the opportunity to study the ICSI treatment results in our couples. Moreover, we considered only the first treatment cycles of our patients, thus avoiding the confounding effect of various numbers of repeated cycles per patient on the treatment outcome.

The greater number of oocytes retrieved in the control group compared with each of the study subgroups is probably incidental since the mean age of the women and the COS protocols in all groups were similar. However, a number of 7–9 oocytes that were retrieved in the different study groups was usually sufficient to achieve at least 3–4 embryos for transfer.

In our study, the fertilization rate was significantly higher in cycles with initially motile sperm, groups A and control, 76.5 and 78.2%, respectively, compared with cycles with initially immotile sperm in groups B and C, 56.9 and 55.1%, respectively. These findings are consistent with other studies comparing fertilization rates in motile and immotile sperm from the ejaculate of patients with extremely severe OAT or transient azoospermia.

Thus, in one study, a 10.9% fertilization rate in cycles in which only immotile sperm were used (even after treatment) was significantly lower than the 60.2% in cycles in which sperm motility was achieved after treatment (Nagy et al., 1995). Lahav-Baratz et al. (2002) found a 52.5% fertilization rate using motile ejaculate sperm in patients with transient azoospermia.

Contrary to our findings, using the ejaculate of patients scheduled for TESE, Ron-El et al. (1997) found a 39% fertilization rate with motile sperm, which was not different from a 26% rate with immotile sperm. They did not report how viability was assessed in the immotile sperm.

In this regard, the study of Nijs et al. (1996) is interesting as they could show that immotile sperm from the ejaculate of severe OAT patients had a lower (53%) fertilization rate than immotile sperm retrieved from the testes (65%). They speculated that the ejaculated immotile sperm were aged because of a longer transit through the epididymis and contained fragile DNA, thus hampering pronucleus formation.

Oocyte parthenogenetic development may result from an ICSI procedure with non-viable sperm. It is therefore interesting that in the study by Nagy et al. (1995), the 1PN rate (parthenogenesis) in the immotile sperm group was 13.2%, significantly higher than in any other ICSI procedure with motile sperm. That was well correlated with 10% vital sperm found in a vitality test in the immotile sperm group. A significantly higher 1PN rate (17%) using immotile sperm compared with 5% with motile sperm was also found by others (Ron-El et al., 1997).

In our study, the 1PN rate was 1.1% with initially motile sperm (group A) but not in any of the other groups. It seems, therefore, that oocyte injection with immotile, non-viable sperm may lead to parthenogenesis, whereas this injection procedure effect is circumvented by normal fertilization when viable sperm are selected for injection. In this regard, the fact that no 1PN oocytes were detected in our group C with initially immotile sperm, assessed by the mechanical touch test, could be another attestation of their viability.

The cleavage rate was significantly lower in cycles with initially immotile sperm (groups B and C) compared with the control group, 89.2, 95.2 and 97.3%, respectively (Table III). Importantly, in both groups with initially immotile sperm, embryos for transfer were available in 15 of 17 cycles in which fertilization had occurred. A lower developmental rate of oocytes fertilized with immotile sperm compared with those with motile sperm was also observed by others. Nijs et al. (1996) found a 95% cleavage to 4- or 8-cell embryos of oocytes fertilized with immotile sperm, leading to embryo transfer in all their patients. Our finding that the cleavage rate was lower in cycles with initially immotile sperm despite assessment of their viability by either HSA treatment or the touch technique may point to some sperm defect needed for support of even early embryonic development which up to 4–8 cells is dependent on oocyte and sperm gene expression, and not on the embryonic genome per se.

Moreover, the fact that the good quality embryo rate was between 45 and 48%, with no difference among the four groups, was probably due not only to the oocyte quality of the relatively young women in our study but also to the use of viable sperm. This is in agreement with the finding of others who obtained fewer good quality embryos when totally immotile spermatozoa were used for ICSI (without selection for viability) compared with embryos derived from motile spermatozoa (Nijs et al., 1996).

The percentage CP rate/cycle, viable pregnancy/cycle and implantation rate was lower in all study groups compared with the control group, but the difference did not achieve statistical significance among the four groups. Results were especially low in cycles in which sperm viability was assessed by mechanical touch. The fact is that despite the almost 48% combined grade 1 and 2 embryos achieved in this group, only one of six cycles produced a viable pregnancy. Whether this is due to some sperm defect that does not support advanced embryonic development is questionable. Since we transferred embryos on day 2 or 3 after oocyte retrieval, embryonic development to the blastocyst stage was not tested, a procedure that could clarify some questions related to the survival and quality of embryos achieved by fertilization with immotile sperm.

The high rate of multiple pregnancies was evidently due to the transfer of more than two embryos in a large number of cycles. A policy of transfer of two embryos has been adopted in our unit since January of 2003 and we are witnessing a decrease in the multiple pregnancy rate.
The biochemical pregnancy rate was 10% in group A compared with only 4.6% in the control group. Although this difference was not statistically significant, it is worth investigating this issue in the future because it may reveal another aspect of sperm quality affecting early embryonic development in these patients with transient azoospermia.

In our small group of newborns, no clinically detectable congenital malformations were found during a 9–42 month follow-up. Only a few women in both study and control groups had either amniocentesis or chorionic villous sampling for genetic diagnosis, probably because of their relatively young age and the history of infertility.

In summary, infertile males who demonstrate transient azoospermia and usually very low sperm quality have a considerable chance by modern assisted reproduction techniques for a viable pregnancy when their sperm are motile. However, the pregnancy rate is markedly reduced when only immotile sperm are present. In patients with immotile sperm, a TESE procedure might be a better option as testicular sperm may be less affected by pathological processes such as DNA damage than ejaculated sperm (Greco et al., 2004).

References