ICSI outcomes in obstructive azoospermia: influence of the origin of surgically retrieved spermatozoa and the cause of obstruction

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BACKGROUND: Spermatozoa can be retrieved from the testis and epididymis of men with obstructive azoospermia (OA) and used for ICSI. However, it is unknown whether the outcome of ICSI depends on the cause of obstruction or the origin of surgically retrieved spermatozoa. METHODS: A cohort of 171 men with OA and normal spermatogenesis were included in this retrospective study. They were divided into three groups according to the site and origin of obstruction: 83 men had congenital bilateral absence of vas deferens; 55 and 33 had acquired epididymal and deferent duct obstructions, respectively. The outcome of 368 ICSI cycles was determined and compared according to the origin of spermatozoa: epididymal (n = 253) or testicular (n = 115). RESULTS: Fertilization and clinical pregnancy rates did not differ between spermatozoa of different origin (58.9% versus 51.9% and 22.1% versus 24.3% with epididymal and testicular spermatozoa, respectively). However, the miscarriage rate was significantly higher for testicular spermatozoa (35.7% versus 12.5% P < 0.05, x^2 test). Findings were similar whatever the aetiology of the OA. CONCLUSION: This study suggests that the use of testicular spermatozoa, even those generated during normal spermatogenesis, alters embryonic development and that epididymal spermatozoa should be preferentially used, irrespective of the aetiology of OA.

Key words: ICSI/microsurgical epididymal sperm aspiration/obstructive azoospermia/testicular sperm extraction

Introduction

Ten percent of male infertility cases involve azoospermia (Hendry, 1994), which is classified as either obstructive or non obstructive. Obstructive azoospermia is characterized by an occlusion or an absence of the reproductive tract preventing sperm cells from entering the seminal plasma before ejaculation. Non obstructive azoospermia is caused by absent or severely reduced sperm production by the testis, such that the ejaculate does not contain sperm (Jarow et al., 1989).

When the technology was developed to obtain pregnancies by intracytoplasmic injection of spermatozoa surgically retrieved from the testis or the epididymis, new hope was given to azoospermic men that they would be able to use their own gametes to become fathers (Craft et al., 1993; Silber et al., 1994). Spermatozoa can be obtained by microsurgical epididymal sperm aspiration (MESA) in cases of azoospermia due to a congenital bilateral absence of the vas deferens, failed vasoepididymostomy and otherwise irreparable obstruction (Tournaye et al., 1994). Spermatozoa can also be collected straight from the testis if the epididymis is inaccessible or if no motile spermatozoa are present in the epididymis (Devroey et al., 1994; Nagy et al., 1995).

High fertilization and good pregnancy rates can be obtained for both obstructive and non-obstructive azoospermia (Dohle et al., 1998; Palermo et al., 1999), although the quality of spermatozoa collected from the epididymis may differ from that collected from the testis. High abortion rates were reported when using testicular spermatozoa (Ghazzawi et al., 1998; Pasqualotto et al., 2002), suggesting that the male gamete could affect the developmental competence of resulting embryos. However, in most studies, it was not possible to determine whether the miscarriage rate (MR) associated with the use of testicular spermatozoa was related to a defect of spermatogenesis or to sperm immaturity.

In this study we compared the outcomes of ICSI for men with obstructive azoospermia and normal spermatogenesis according to the use of epididymal or testicular spermatozoa and the cause of obstruction.
Materials and methods

Patients
This retrospective study included 171 men presenting obstructive azoospermia at one of the two infertility centers, at Cochin or Saint Vincent de Paul Hospitals, between January 1996 and December 2001. Most (91%) suffered from primary infertility of 1 to 18 years duration. The mean age of the men was 36.7 ± 6.4 years (range: 23.2–55.4 years) at the time of semen collection. Azoospermia was confirmed on at least two occasions. All men had normal testicular volume and normal serum FSH concentration. Karyotype analysis was performed systematically and was normal in all the men. Testicular biopsy revealed normal spermatogenesis in all cases for which it was performed (n = 56). Therefore, it can be presumed that spermatogenesis was normal in all cases. The mean age of the female partners was 32.8 ± 4.4 years (range: 20.5–46.0 years). The participants gave informed consent.

Azoospermic men were divided into three groups according to the cause of obstruction. Eighty-three men had a congenital bilateral absence of the vas deferens (CBAVD) diagnosed by a lack of vas deferens at clinical examination, associated with a low semen volume, a semen pH <7 and very low fructose, carnitine and α(1-4) glucosidase concentrations in the seminal plasma. Ultrasound investigation confirmed the diagnosis. Before ICSI was undertaken for these men, the patient and his partner were tested for cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations to allow appropriate genetic counselling. Fifty-five patients had an acquired epididymal obstruction due to a history of epididymitis, a tuberculous epididymal head and flat epididymal tail at clinical examination, associated with low concentrations of carnitine and α(1-4) glucosidase but normal fructose in the seminal plasma. In all cases, the epididymal obstruction was confirmed by ultrasound examination or surgical exploration. In 33 patients, the acquired vas deferens obstruction was related to a history of vasectomy (n = 3), or of lower abdominal, scrotal or inguinal surgery. In this last group, the clinical examination revealed normal epididymitis; the semen volume was normal with low concentrations of carnitine and α(1-4) glucosidase in the seminal plasma. The time between vasectomy and surgical sperm retrieval was 10, 12 and 15 years (n = 3). In all cases, the diagnosis was also confirmed by ultrasound examination and/or surgical exploration.

Surgical sperm retrieval
All testicular or epididymal sperm retrievals were performed under general anaesthesia. Epididymal spermatozoa were aspirated by MESA from the most proximal part of the caput of the epididymis (Tourneay et al., 1994). When MESA did not provide sufficient motile spermatozoa for ICSI or when the status of the epididymis did not allow sperm collection, testicular spermatozoa were extracted from an open biopsy (TESE; Silber et al., 1994). In other cases, an open testicular biopsy was performed because it was technically simpler and less time-consuming. Large numbers of testicular spermatozoa were always collected, reflecting a presumably ongoing spermatogenesis. One small piece of the testicular biopsy was fixed in Bouin’s solution for histopathological examination and the remainder was sent to the laboratory for sperm processing.

Sperm processing
Following MESA, epididymal fluid was diluted in FERTICULT™, HEPES (JCD, Lyon, France) and the spermatozoa were prepared immediately for ICSI or for cryopreservation by addition of an equal amount (v/v) of freezing medium (SPERM FREEZE™; JCD) to obtain a final concentration of 15% glycerol.

To perform ICSI, fresh epididymal spermatozoa were selected by centrifugation (20 min, 300g) through a two-step PURESPERM™ (JCD) density gradient (90–45%). The pellet was then washed (10 min, 600g) and diluted in Upgraded B2 INRA medium (CCD, Suresnes, France). A 1μl aliquot of the final suspension was placed in the injection dish. Cryopreserved epididymal samples were processed in the same way as fresh spermatozoa after thawing at room temperature.

Following TESE, testicular biopsies were placed into Nunc tubes containing 1 ml of FERTICULT-HEPES medium before being transferred to Petri dishes containing 2 ml of the same pre-incubated medium. They were then crushed with sterile needles to dilacerate the seminiferous tubules and kept at 37°C, under 5% CO₂ in air for at least 1 h to facilitate the dispersal of spermatozoa into the medium. Spermatozoa were then used immediately for ICSI or cryopreserved by addition of an equal amount of cryopreservation medium (SPERM FREEZE).

When used immediately for ICSI, the medium of the mixture was layered on a 1ml phase (45%; PURESPERM) for 20 min at 300g and then washed in B2 INRA medium (600g, 10 min). The pellet was divided into 5μl drops in the cover of a Petri dish. The final suspension was covered with mineral oil (Medicult, Limonest, France) and kept for 1 h at 37°C and under 5% CO₂ in air before examination for the presence of motile spermatozoa (Royere et al., 1996). Cryopreserved testicular samples were processed in the same way as fresh spermatozoa after thawing at room temperature the day of ICSI.

Ovarian stimulation and oocyte pick-up
All female patients were treated with either a long or a short GnRH analogue (GnRHa, Decapeptyl®, Beaufour Ipsen Pharma, Paris, France) protocol, followed by ovarian stimulation with recombinant FSH. Oocytes were retrieved by transvaginal ultrasound-guided puncture of the ovarian follicles 36 h after HCG administration.

ICSI, assessment of fertilization, embryo cleavage, quality of embryo at day 2 and clinical pregnancy
ICSI was carried out on the stage of an inverted microscope (Nikon) at ×400 magnification using the Hoffman Modulation Contrast system. It was performed on all morphologically intact MII oocytes (second metaphase) and always with immobilized motile spermatozoa (Palermo et al., 1996). Oocytes were examined 16–18 h after microinjection. Normal fertilization was defined as the presence of two distinct pronuclei (PN) and a second polar body. Embryo cleavage was assessed 24 h later and embryos were scored according to the number of blastomeres and to the percentage of enucleate fragments (A: no fragments; B: 1–20% fragments; C: 21–50% fragments; D: >50% fragments). Up to three embryos were transferred into the uterine cavity 48 or 72 h after the oocyte puncture. Supernumerary embryos with <20% fragmentation were frozen and cryopreserved (Mandelbaum et al., 1988).

Pregnancy was detected by rising serum HCG concentration in two consecutive assays at least 12 days after embryo transfer. Clinical pregnancy was defined by the presence of at least one gestational sac observed by ultrasound scanning after 7 weeks. Miscarriage was defined as all spontaneous interruption of clinical pregnancy, not related to extra uterine implantation or medical abortion.

Statistical analysis
Numerical variables were compared using analysis of variance (ANOVA). Categorical variables were compared using the χ² test. All statistical tests were assessed at the 5% level of significance.
ICSI outcomes according to the origin of spermatozoa from 171 obstructive azoospermic patients

Comparison of variables according to the outcome of pregnancy

### Table I. ICSI outcomes according to the origin of spermatozoa from 171 obstructive azoospermic patients

<table>
<thead>
<tr>
<th></th>
<th>Testicular spermatozoa</th>
<th>Epididymal spermatozoa</th>
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<tbody>
<tr>
<td>No. of oocyte retrievals</td>
<td>115</td>
<td>253</td>
</tr>
<tr>
<td>Age of women (years)</td>
<td>33.0 ± 4.5</td>
<td>33.0 ± 4.2</td>
</tr>
<tr>
<td>Age of men (years)</td>
<td>38.2 ± 7.5</td>
<td>36.0 ± 5.7</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>1164 (10.1 ± 6.2)</td>
<td>2623 (10.4 ± 6)</td>
</tr>
<tr>
<td>No. of metaphase II (MI)</td>
<td>874 (7.6 ± 4.8)</td>
<td>1889 (7.5 ± 4.4)</td>
</tr>
<tr>
<td>No. of 2 pronuclei (PN)</td>
<td>454 (51.9%)</td>
<td>1112 (58.9%)</td>
</tr>
<tr>
<td>No. of cleaved embryos</td>
<td>483 (55.2%)</td>
<td>1192 (63.1%)</td>
</tr>
<tr>
<td>No. of embryos with &lt; 20% fragmentation %b</td>
<td>208 (43%)</td>
<td>501 (42%)</td>
</tr>
</tbody>
</table>

aPercentage of the total number of MII oocytes injected; bPercentage of the total number of embryos.

### Table II. Comparison of variables according to the outcome of pregnancy

<table>
<thead>
<tr>
<th></th>
<th>Without miscarriage</th>
<th>With miscarriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of women (years)</td>
<td>31.6 (22–39)</td>
<td>33.3 (1.1)</td>
</tr>
<tr>
<td>Age of men (years)</td>
<td>36.0 (27–51)</td>
<td>38.2 (31–47)</td>
</tr>
<tr>
<td>Rank of ICSI attempt</td>
<td>1.8 (0.1)</td>
<td>2.1 (0.4)</td>
</tr>
<tr>
<td>ICSI with cryopreserved spermatozoa (%)a</td>
<td>73.0</td>
<td>46.0</td>
</tr>
</tbody>
</table>

aRatio between the number of attempts performed with cryopreserved spermatozoa and the number of attempts with fresh spermatozoa.

The differences observed in MR were not influenced by the long or short GnRHa protocol used before ovarian stimulation. In case of short protocol, the MR was 44.4% when using testicular spermatozoa and 20% when using epididymal spermatozoa (n = 9 and 15 pregnancies, respectively; P = 0.05; χ² test). In case of long protocol, the MR were 31.6% when using testicular spermatozoa (n = 19 pregnancies) and 9.7% when using epididymal spermatozoa (n = 41 pregnancies; P < 0.05; χ² test). There was no significant difference in the MR coming after ICSI with fresh and cryopreserved sperm (17.3% and 28.5%, respectively; P > 0.05).

Four children were born with malformations (two with pylori stenosis, one with periventricular leukomalacia and one with pulmonary artery valvular stenosis).

### Results

**ICSI outcome with testicular or epididymal spermatozoa**

A total of 115 ICSI cycles were performed with testicular spermatozoa (84 with frozen and 31 with fresh spermatozoa) and 253 ICSI cycles with epididymal spermatozoa (200 with frozen and 53 with fresh spermatozoa) (Table IA). A majority of patients (n = 115) had ICSI attempts with only epididymal spermatozoa and 40 with only testicular sperm. Spermatozoa from both origins were used for 16 patients on two different cycles. Mean female and male ages and mean number of oocytes retrieved were similar in the two groups. The fertilization rates, embryo cleavage rates and embryo quality did not differ according to the site of sperm retrieval, either testicular or epididymal (Table IA). No difference in the implantation rate or in the clinical pregnancy and delivery rates were observed. However the MR was significantly higher for spermatozoa retrieved from the testis (35.7%) than for that from the epididymis (12.5%, P < 0.05, Table IB, Fig. 1). When examining ICSI results of only the first rank of attempts, the MR after ICSI with testicular sperm was also higher compared with the miscarriage rate after ICSI with epididymal spermatozoa (40.0 versus 11.8%, P < 0.05; χ² test). Similar significantly differences were obtained for the other ranks.

Although mean male and female age, and mean rank of ICSI attempts were slightly higher for those pregnancies that ended with a miscarriage, the difference to pregnancies without miscarriage was not significant (Table II; ANOVA). A significantly higher MR was also observed after ICSI using testicular spermatozoa in the subgroup of women under 35 years old (Fig. 1; P < 0.05; χ² test). The trend was similar for women who were ≥35 years old, but the difference not statically significant because the small size of this last group. The differences observed in MR were not influenced by the long or short GnRHa protocol used before ovarian stimulation. In case of short protocol, the MR was 44.4% when using testicular spermatozoa and 20% when using epididymal spermatozoa (n = 9 and 15 pregnancies, respectively; P = 0.05; χ² test). In case of long protocol, the MR were 31.6% when using testicular spermatozoa (n = 19 pregnancies) and 9.7% when using epididymal spermatozoa (n = 41 pregnancies; P < 0.05; χ² test). There was no significant difference in the MR coming after ICSI with fresh and cryopreserved sperm (17.3% and 28.5%, respectively; P > 0.05).

#### Outcome of transfers with thawed embryos according to the site of spermatozoa retrieval

One hundred and ninety-one transfers of thawed embryos, which had been frozen during the same ICSI attempts, were
performed. The mean number of transferred embryos was similar in the two groups. The implantation and clinical pregnancy rates were significantly lower for oocytes injected with testicular spermatozoa (Table III). Moreover, the MR was also significantly higher for oocytes injected with testicular spermatozoa (Table III). Two and 19 deliveries went to term in the testicular and epididymal groups, respectively, resulting in the birth of three and 21 babies, respectively, with no obvious congenital malformations.

When cumulating pregnancies obtained after transfer of fresh or frozen-thawed embryos, the MR was 39.4% when using testicular spermatozoa compared with 13.9% when using epididymal spermatozoa.

**ICSI outcome according to the cause of azoospermia and the site of spermatozoa retrieval**

The ICSI outcome was evaluated according to the site of sperm retrieval for each aetiological group (Tables IVA and IVB). Mean female and male ages were similar in all groups (Tables IVA and IVB). In the CBAVD group, the 2 PN fertilization, cleaved embryo (Table IVA) and delivery rates were significantly higher for epididymal spermatozoa whereas the MR was significantly higher for spermatozoa retrieved from testis (Tables IVB). For cases of azoospermia related to acquired epididymal or deferent duct obstructions, no difference was observed in 2 PN fertilization or embryo cleavage rates, or in embryo quality, according to the sperm origin (Table IVA). However, the MR was again significantly higher with testicular spermatozoa than with epididymal spermatozoa (Table IVB).

The influence of the site of obstruction was also evaluated by analysing ICSI results with epididymal spermatozoa in each aetiological group. The percentage of good quality embryos and clinical pregnancy rate were significantly lower for ICSI with epididymal spermatozoa from cases of acquired epididymal obstruction than from that of the two other types of obstructive azoospermia (Tables IVA and IVB).

**Table III. Outcome of transfers of thawed embryos according to the source of spermatozoa**

<table>
<thead>
<tr>
<th></th>
<th>Testicular spermatozoa</th>
<th>Epididymal spermatozoa</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of women (years)</td>
<td>32.2 ± 4.7</td>
<td>32.4 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>Age of men (years)</td>
<td>37.4 ± 7.8</td>
<td>36.0 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>No. of embryo transfers</td>
<td>55</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>No. of thawed embryos</td>
<td>101 (1.8 ± 0.7)</td>
<td>256 (1.8 ± 0.7)</td>
<td></td>
</tr>
<tr>
<td>Implanted (%)</td>
<td>6 (5.9%)</td>
<td>27 (10.5%)</td>
<td>&lt;0.05f</td>
</tr>
<tr>
<td>No. of clinical pregnancies (%)</td>
<td>5 (8.4%)</td>
<td>23 (16.3%)</td>
<td>&lt;0.05f</td>
</tr>
<tr>
<td>No. of miscarriages (%)</td>
<td>3 (60%)</td>
<td>4 (17.4%)</td>
<td>&lt;0.05f</td>
</tr>
<tr>
<td>No. of deliveries (%)</td>
<td>2 (3.6%)</td>
<td>19 (13.9%)</td>
<td></td>
</tr>
<tr>
<td>No. of newborns</td>
<td>3</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

*Ratio between the number of gestational sacs and the number of transferred embryos; \(^a\) Ratio between the number of clinical pregnancies and the number of oocyte retrievals; \(^b\) Ratio between the number of spontaneous pregnancy losses before 20 weeks of gestation and the number of clinical pregnancies; \(^c\) Ratio between the number of deliveries and the number of oocyte retrievals; \(^d\) \(\chi^2\) test; \(^e\) \(\chi^2\) test for small samples.

**Discussion**

The use of surgical sperm retrieval and ICSI has greatly improved treatment of azoospermic men, but the consequences of using immature sperm retrieved directly from the testis with normal or defective function are not fully known. We studied ICSI outcomes according to the site of sperm retrieval (testis or epididymis) and the aetiology of the obstructive azoospermia by analysing 368 consecutive ICSI cycles involving 171 obstructive azoospermic men with normal spermatogenesis. This is the largest series since a previous published report of a series of 17–138 ICSI cycles in case of obstructive azoospermia (reviewed by Nicopoullos et al., 2004b).

A number of methods have been proposed to surgically recover spermatozoa for ICSI. These methods include MESA, PESA (percutaneous epididymal sperm aspiration; Shrivastav et al., 1994), TESE or TESA (testicular sperm aspiration; Gorgy et al., 1998). The aim of this study was not to compare these various methods. The standard MESA procedure is a reliable technique that allows the retrieval of the maximum number of spermatozoa, which may be cryopreserved for several ICSI if needed. In our experience, up to five ICSI attempts could be performed and 43 mature oocytes could be injected after only one MESA. It allows the surgeon to make a complete scrotal exploration and, whenever indicated,
a vasoepididymostomy may be performed concomitantly. Furthermore, if motile epididymal spermatozoa cannot be collected, testicular extraction of sperm can be carried out during the same surgical procedure. However, MESA is more invasive and time-consuming and may cause more discomfort for the patient.

In our study, 2 PN fertilization, cleavage, embryo quality, implantation, clinical pregnancy and delivery rates were similar for spermatozoa retrieved from the testis and the epididymis. However, we found that the use of testicular spermatozoa for ICSI was associated with a rate of pregnancy loss that was significantly higher than that for epididymal spermatozoa (35.7% versus 12.5%, P < 0.05). Similar results were found after transfer of both fresh and frozen-thawed embryos, whatever the aetiology of obstructive azoospermia. Given the length of the studied period and even though the ICSI practitioners were already trained when the study started, data were re-analysed omitting the first and the second year of the study. We found similar observations leading to similar conclusions since 38.4% and 13.9% of miscarriage rates were observed when using testicular (n = 101) and epididymal (n = 159) sperm, respectively, for ICSI (P < 0.05; χ² test). Thus, an hypothesized bias due to a learning period during the study had no influence on the results. Similarly, to avoid possible bias related to the rank of treatment cycle and the fact that a proportion of treatments were in the same men twice or more, we also analysed only the results of the first cycle of treatments. Comparison with published data is difficult since the series are most often small and are reporting mixed cases of obstructive and non-obstructive azoospermia. Furthermore, the origin of the spermatozoa is not always mentioned. In our study, the 12.5% MR observed in pregnancies obtained with epididymal spermatozoa was similar for spermatozoa retrieved from the testis and the epididymis. However, we found that the use of testicular spermatozoa for ICSI was associated with a rate of pregnancy loss that was significantly higher than that for epididymal spermatozoa (35.7% versus 12.5%, P < 0.05). The difference in MR could not be explained by the age of women since it was also observed when analysing the subgroup of women who were <35 years old at the time of ICSI. It could also not be explained by the treatment used for ovarian stimulation.

The proportion of miscarriage when using testicular or epididymal spermatozoa is very different in the current literature. Comparison with published data is difficult since the series are most often small and are reporting mixed cases of obstructive and non-obstructive azoospermia. Furthermore, the origin of the spermatozoa is not always mentioned. In our study, the 12.5% MR observed in pregnancies obtained with epididymal spermatozoa was similar for spermatozoa retrieved from the testis and the epididymis. However, we found that the use of testicular spermatozoa for ICSI was associated with a rate of pregnancy loss that was significantly higher than that for epididymal spermatozoa (35.7% versus 12.5%, P < 0.05). The difference in MR could not be explained by the age of women since it was also observed when analysing the subgroup of women who were <35 years old at the time of ICSI. It could also not be explained by the treatment used for ovarian stimulation.

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sperm from obstructive azoospermic sperm, did not find any difference in the MR according to the origin of spermatozoa. However, the series is small (28 treatment cycles and 12 pregnancies with epididymal sperm and 14 treatment cycles and six pregnancies with testicular sperm, respectively) and, consequently, it is difficult to draw any firm conclusion. The 35.7% MR observed in pregnancies obtained with testicular spermatozoa is also in the previously reported range which varies from 12.5% (Palermo et al., 1999) to 40% (Nicopoullos et al., 2004) whatever the nature of azoospermia. In a larger series of 180 clinical pregnancies obtained after ICSI with testicular spermatozoa for obstructive azoospermia, a 14% clinical MR was reported (Vernaeve et al., 2003b); however, this study made comparison with epididymal spermatozoa.

The outcome of ICSI using non-ejaculated sperm may be influenced by various factors related to the female partner, sperm quality and the source of surgically retrieved sperm. The female partner’s age or ovarian reserve defined as the number of oocytes available for ICSI may significantly contribute to the pregnancy outcome and delivery after ICSI (Silber et al., 1997; Friedler et al., 2002). Furthermore, high maternal age is an important risk factor for early pregnancy loss (Ziebe et al., 2001). In our study, there were no significant differences in maternal age, female infertility factor rates and mean number of retrieved or micro-injected oocytes for ICSI with testicular and epididymal sperm.

**Could the use of frozen-thawed sperm influence ICSI and pregnancy outcome?**

We did not find any differences in ICSI outcomes related to the use of fresh or frozen-thawed spermatozoa, whether epididymal or testicular. This result is in accordance with most published studies, which report that sperm cryopreservation does not affect fertilization, embryo cleavage, pregnancy or abortion rates compared with fresh spermatozoa for either epididymal (Nagy et al., 1995; Silber et al., 1997; Tournaye et al., 1999; Habermann et al., 2000; Cayan et al., 2001; Friedler et al., 2002; Nicopoullos et al., 2004a) or testicular spermatozoa (Fischer et al., 1996; Ben-Yosef et al., 1999; Palermo et al., 1999; Friedler et al., 2002; Nicopoullos et al., 2004a).

**Could the source of spermatozoa influence the ICSI outcome?**

Published results concerning fertilization, pregnancy and miscarriage rates after ICSI with testicular spermatozoa are discordant. Some studies have reported similar fertilization and pregnancy rates for ICSI with ejaculated and epididymal spermatozoa (Ghazzawi et al., 1998; Monzo et al., 2001; Windt et al., 2002; Friedler et al., 2002). Other studies report impaired fertilization rates with epididymal spermatozoa but similar pregnancy rates (Palermo et al., 1999; De Croo et al., 2000), and others similar fertilization rates but impaired pregnancy rates (Osmanagaoglu et al., 2003; Vernaeve et al., 2003a). Miscarriage rates after ICSI with testicular spermatozoa have been reported to be higher (Ghazzawi et al., 1998; Pasqualotto et al., 2002) or similar (Palermo et al., 1999; Monzo et al., 2001; Friedler et al., 2002; Nicopoullos et al., 2004a,b) to ICSI with ejaculated or epididymal sperm. It is difficult to interpret these results and their causes because, apart from two studies (Palermo et al., 1999; Nicopoullos et al., 2004a), there was no systematic distinction between obstructive and non-obstructive azoospermia. This prevents consideration of the respective influence of spermatogenesis defects and of spermatozoa immaturity in miscarriage. Indeed, spermatozoa produced by defective spermatogenesis may be associated with a higher chromosomal aneuploidy rate (Mateizel et al., 2002) or other genetic alterations and may be responsible for embryos being less able to develop to the blastocyst stage (Balaban et al., 2001) and to implant (Ubaldi et al., 1999).

Our results show that testicular spermatozoa from normal spermatogenesis allow similar fertilization, early embryo pre-implantation development and pregnancy rates as epididymal spermatozoa but that they are responsible for a significantly higher MR, as previously suggested by Pasqualotto et al. (2002). Failure of embryo development after ICSI could be related to spermatozoa DNA damage (Morris et al., 2002), but it has been previously shown that DNA fragmentation as measured by Tunel assay and Comet assay was higher in epididymal than in testicular spermatozoa of men with obstructive azoospermia (Steele et al., 1999; Ramos et al., 2002). Therefore, other sperm defects might be responsible for the higher MR when using testicular spermatozoa.

We also found that the cause of obstruction may influence ICSI outcome when using epididymal spermatozoa. In this study, the embryo quality and pregnancy rates were lower after ICSI with epididymal spermatozoa in cases of acquired epididymal obstruction compared with CABVD or acquired deferent duct obstruction groups. However, no difference was found in the MR between the three groups. The epididymis is generally damaged because of infection or inflammation in case of acquired epididymal obstruction. Our results and those of others (Yamamoto et al., 1996; Nicopoullos et al., 2004b; Pasqualotto et al., 2005) suggest that there may be worse than normal results for ICSI with epididymal spermatozoa when the cause of obstructive azoospermia is infective or inflammatory. This tendency for poor ICSI results with epididymal spermatozoa in cases of obstructive azoospermia of infective origin requires further study and raises several questions, including what is the best source of spermatozoa to use in these cases.

In summary, our results show that the origin of surgically retrieved spermatozoa has an effect on the success of assisted reproduction, even when spermatogenesis is presumably normal. The significantly higher MR after ICSI with testicular spermatozoa than with epididymal spermatozoa in cases of obstructive azoospermia suggests that the immaturity of testicular spermatozoa may affect embryo development. More studies are required to determine the mechanisms of paternal influence on embryo development and long-term follow-up studies are needed to prove the safety of testicular sperm retrieval in assisted reproductive techniques.

**Acknowledgements**

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References