Diagnostic epididymal and testicular sperm recovery and genetic aspects in azoospermic men

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Various procedures for sperm recovery in azoospermic men have been described, from open testicular biopsy to simple needle aspiration from the epididymis and the testis. Fifty-one obstructive and 86 non-obstructive azoospermic men were treated to compare the recovery of spermatozoa obtained by percutaneous aspiration from the epididymis (PESA) and aspiration/extraction from the testis (TESA, TESE) with histopathology. If TESA failed, the work up proceeded with TESE. All patients were karyotyped. Spermatozoa were recovered by PESA or TESA in all obstructive men (51/51 patients). In 22 out of 86 patients with non-obstructive azoospermia, testicular spermatozoa could be successfully recovered by TESA. In five additional patients TESE was successful in recovering spermatozoa where TESA had failed. In 43 patients, neither TESA nor TESE was successful. Sixteen patients chose not to proceed with TESE. Seven out of 86 patients had an abnormal karyotype in the non-obstructive group (8%), none in the obstructive group. In the non-obstructive patient group testicular histopathology showed hypospermatogenesis, incomplete maturation arrest and germ cell aplasia with focal spermatogenesis in cases where spermatozoa were recovered and complete germ cell aplasia, complete maturation arrest and fibrosis in cases where no spermatozoa were found. Spermatozoa were recovered by PESA or TESA from all patients with obstructive azoospermia and from ~40% of patients with non-obstructive azoospermia by TESA or TESE. Retrieval of viable spermatozoa in the infertility work-up was highly predictable for sperm recovery in subsequent ICSI cycles. TESA performed under local anaesthesia seems almost as effective as more invasive procedures in recovering testicular spermatozoa, both in obstructive and non-obstructive azoospermic men.

Key words: azoospermia/epididymal sperm aspiration/male infertility/testicular sperm aspiration/testicular sperm extraction

Introduction

With the introduction of intracytoplasmic sperm injection (ICSI), the treatment of male infertility was revolutionized. The first pregnancy was reported in 1992 (Palermo et al., 1992). Modern sperm recovery techniques have made it possible to help men with both obstructive and non-obstructive azoospermia to achieve genetic fatherhood. In patients with obstructive azoospermia, viable spermatozoa from either the epididymis (Tournaye et al., 1994) or the testis (Craft et al., 1993; Schoysman et al., 1993) can be used for the ICSI procedure. Spermatozoa from the testis can also be used after recovery from men with deficient spermatogenesis or so-called non-obstructive azoospermia (Devroey et al., 1995; Kahraman et al., 1996). The recovery techniques for epididymal spermatozoa include open surgery by microepididymal sperm aspiration (MESA) (Temple-Smith et al., 1985; Silber et al., 1994) and a less invasive method, percutaneous sperm aspiration (PESA) (Craft et al., 1994; Shrivastav et al., 1994). Testicular spermatozoa can be recovered by testicular biopsy (testicular sperm extraction, TESE) (Schoysman et al., 1993; Nagy et al., 1995), which is an invasive method, or a simplified less invasive method of percutaneous aspiration with a fine needle, testicular sperm aspiration (TESA) (Tsirigotis and Craft, 1995).

Several studies have shown the ICSI technique to be a safe method. Follow-up studies of children born after intracytoplasmic sperm injection with ejaculated, epididymal or testicular spermatozoa have given no indication of an increased risk of major malformation or low birthweight babies (when separated for singletons and multiples) compared to children born after in-vitro fertilization (IVF) and children born after natural conception (Bonduelle et al., 1998; Wennerholm, 1998). The incidence of chromosomal aberrations in children born after ICSI has been a matter of concern. Structural chromosomal aberrations are more frequent in subfertile men, which lead to an increased risk of an unbalanced chromosomal constitution in the offspring or a risk of transmitting the same translocation and hence the infertility to the offspring (Baschat et al., 1996). Furthermore, the incidence of Klinefelter’s syndrome, and hence the risk of producing an aneusomic offspring is also higher in subfertile men (Cozzi et al., 1995; Chevret et al., 1995).

A possible increased risk of all chromosomal abnormalities has been suggested (In’t Veld et al., 1995) and current data from the largest cytogenetic study published so far (Bonduelle et al., 1998) indicate a slightly increased risk of de-novo chromosomal aberration and transmitted chromosomal aberration in children conceived after ICSI as compared to the general population.

In our IVF unit, treatment of azoospermic men with ICSI using surgically retrieved spermatozoa was initiated in 1994. A noticeable number of sperm recovery failures was soon encountered. Unsuccessful sperm recovery procedures have...
Sperm recovery in azoospermia

important emotional and financial implications if the woman concomitantly undergoes ovarian stimulation and oocyte retrieval. There seem to be no strong predictors for successful sperm recovery. Testicular histopathology was found to be the best predictor for viable spermatozoa in a study from Brussels (Tournaye et al., 1997). We therefore decided to perform diagnostic PESA/TESA or TESE in all our azoospermic men, both of obstructive and non-obstructive origin, before acceptance for IVF/ICSI treatment. Epididymal spermatozoa from diagnostic PESA were frozen for further use in ICSI cycles. When diagnostic sperm recovery procedures failed the couple was informed about adoption and insemination with donor spermatozoa (AID).

The aim of the present study was to correlate the recovery of spermatozoa obtained by percutaneous aspiration from the epididymis (PESA) and aspiration/ extraction from the testis (TESA, TESE) to histopathology. The recovery of spermatozoa was then correlated to the success rate of obtaining viable spermatozoa in subsequent ICSI cycles. A further aim was to examine the frequency of abnormal karyotypes among patients with non-obstructive azoospermia. Serum concentrations of follicle stimulating hormone (FSH) and maximum testicular volume were also evaluated in relation to sperm recovery.

Materials and methods

This study was carried out between October 1995 and December 1997 at the Centre for Reproductive Medicine, Sahlgrenska University Hospital, Göteborg University, Sweden. The patient population consisted of 137 consecutive male patients with azoospermia. Before the sperm aspiration/ICSI procedure the patients were subjected to diagnostic epididymal and/or testicular sperm aspiration (PESA, TESA) performed under local anaesthesia. If sperm recovery was successful, the couple was accepted for an IVF/ICSI procedure. Epididymal spermatozoa were frozen and used in further IVF/ICSI cycles, if possible. If recovery was not successful the patients were offered multiple testicular biopsies (TESE), which were performed under general anaesthesia. Two to three biopsies were taken on each side. Parts of the testicular biopsies were sent for histopathological examination. If sperm recovery with TESE also failed, adoption or AID was recommended. Spermatids are not used clinically for ICSI in our IVF unit.

In addition to the diagnostic puncture, the work-up included general medical history, information and a physical examination where the testicular volume was measured with an orchidometer. Karyotyping was performed from lymphocytes in a peripheral blood sample and serum concentrations of follicle stimulating hormone (FSH) were determined. In couples where the male suffered from congenital bilateral absence of the vas deferens (CBAVD) both the man and the woman were screened for possible cystic fibrosis (CF) gene mutations. The screening included the most frequent mutations (ΔF-508, 394delTT) and variations (5T allele) for the population.

PESA procedure

0.5 mg alfentanil (Rapifen®; Jansen-Cilag, Beerse, Belgium) was given i.v. followed by infiltration of 7–8 ml lidocaine (Xylocaine®) around the spermatic cord. Under sterile conditions, a 19 or 21 gauge butterfly needle was passed through the scrotal skin. Suction was applied with a 20 ml syringe and the negative pressure was maintained by clamping the tubing in the same manner as in the PESA procedure. The needle was pushed five or six times in different directions with quick thrusting movements into the testicular tissue. The needle was then slowly removed from the testis and the scrotal skin while the back pressure was maintained by the clamped tubing. The assistant used two pairs of fine tweezers to pick up the small tubules recovered by the needle. The clamps were then removed and the needle was flushed with culture medium and its content was expelled into a sterile tube containing culture medium. The procedure was repeated four to six times, trying to cover the whole ventral surface of the testis. The testicular tissue was dissected, incubated in culture medium and examined 1, 4, 24 and 48 h after puncture in the laboratory.

TESE procedure

Under general anaesthesia, a longitudinal incision was made in the scrotal skin and carried through the peritoneal tunica vaginalis exploring the tunica albuginea and the epididymis. Two or three incisions were made through the tunica albuginea in different regions of each testicle. A 0.5–1.0 cm biopsy of extruding testicular tissue was excised and cut into two pieces. The first part was placed in a sterile tube containing culture medium (IVF-50) and the tissue was further prepared and examined in the laboratory 1, 4, 24 and 48 h after surgery. The other part was put into a tube containing formaldehyde and sent for histopathological examination. The examination of the testicular tissue was mainly performed by one pathologist with a special interest in this field. After each biopsy, the tunica albuginea was closed with 4–0 vicryl sutures. No more than three biopsies on each side were performed.

Results

A total of 137 patients with azoospermia underwent diagnostic sperm aspiration. Fifty-one patients were judged to have obstructive and 86 patients non-obstructive azoospermia. In the obstructive group, spermatozoa were recovered in all patients with PESA or TESA. The mean ± SD serum FSH concentration in this group was 3.8 ± 2.0 IU/l. The mean testicular volume was 19.8 ± 4.4 ml. All patients had a normal karyotype. A large majority of the men with cystic fibrosis and/or CBAVD were positive for CF gene mutations. All the female partners were negative.

In the non-obstructive group, testicular spermatozoa could be successfully recovered by TESA in 22 out of 86 patients. In five additional patients TESE was successful in recovering spermatozoa, where TESA had failed. In 43 patients neither TESA nor TESE was successful. Sixteen patients chose not
to proceed with the TESE procedure. The mean serum FSH concentration was significantly higher, 23.9 ± 13.6 versus 16.1 ± 12.6 IU/l (P = 0.02), in the group where sperm recovery failed compared to the group where testicular spermatozoa were successfully retrieved. Also, the testicular volume was significantly lower, 13.7 ± 3.9 ml versus 16.4 ± 4.0 ml (P = 0.007), in the failure group compared to the successful group. However, the overlap between the groups for both these variables was great (Table I).

Seven out of 86 (8.1%) patients had an abnormal chromosomal karyotype. Four of these men had Klinefelter’s syndrome (47,XXY), one of whom was a mosaic (47,XXY/45,X/46,XY). Two patients had translocations [45,XY;rob(13;14)(q10;q10) and 46,XY; (6;15)(q13;p13) respectively]. One patient had a more complicated karyotype (48,XY + 2mar/45,X/46,XY).

In the non-obstructive group where spermatozoa could be recovered the testicular histology showed different types of defective spermatogenesis. It was either general: described as hypospermatogenesis in one or both testes; or focal: described as maturation arrest or germ cell aplasia with focci of normal spermatogenesis in the testicle(s).

In the group where spermatozoa could not be recovered, histology showed complete germ cell aplasia (Sertoli cell-only syndrome), complete maturation arrest and fibrosis. The histopathology of the biopsies often differed between the two testicles and occasionally also between biopsies within the same testicle.

A total of 105 ICSI cycles has so far been performed in the obstructive and the non-obstructive groups where viable spermatozoa earlier had been detected in the diagnostic procedure. In all of these cycles the repeated sperm recovery was successful.

**Discussion**

Epididymal and testicular sperm recovery in combination with ICSI now offers azoospermic men the possibility of fathering their own genetic children. Since the first reports of births of children conceived from surgically retrieved epididymal (Temple-Smith et al., 1985; Silber et al., 1994) and testicular spermatozoa (Devroey et al., 1995; Tournaye et al., 1995), several papers on larger series have been published (Mansour et al., 1997; Tournaye et al., 1997). Initially, when using these new techniques, it was enthusiastically claimed that almost 100% of azoospermic men were possible to treat (Silber, 1995). Further studies have shown that this may be true for men with obstructive azoospermia but in men with non-obstructive disorders the chance of retrieving viable spermatozoa is considerably smaller. In recent publications (Chen et al., 1996; Friedler et al., 1997; Mansour et al., 1997; Tournaye et al., 1997), the sperm recovery rate in non-obstructive azoospermic men varied between 40 and 70%. In these studies, sperm recovery was mainly performed by TESE. Our results, when using mainly the less invasive methods, PESA and TESA, are in accordance with these reports, showing a 100% recovery rate in obstructive patients and a 39% recovery rate in non-obstructive patients. TESA performed under local anaesthesia in men with non-obstructive azoospermia was recently reported to be a quick, reliable and easy method of obtaining testicular tissue (Malldis et al., 1994; Craft et al., 1997).

In our material among men with non-obstructive azoospermia, TESE was successful in only five of 48 (10%) cycles where TESA performance earlier had failed, whereas other reports have emphasized that TESE is superior in most cases (Friedler et al., 1997). The aspiration procedure in those cases was described as testicular fine needle aspiration (TEFNA), where only one puncture was made. Our TESA procedure involved several punctures in different directions in three to six areas at the ventral surface of the testis. With this technique, tissue samples from the testis were almost always obtained. Compared to the TESE procedure, the amount of tissue recovered by TESA was smaller but the present technique was able to reach deeper into the parenchyma, aiming for the rete testis, and thereby covered a larger area of the testis compared to one to two biopsies. The present strategy with TESA would also be beneficial if spermatogenesis is focal in some of these non-obstructive cases. This has recently been a matter of debate (Silber et al., 1997).

Obviously, the present technique seems to have a high predictive value for sperm recovery in subsequent ICSI cycles. In all ICSI cycles performed so far, where viable spermatozoa earlier had been detected in diagnostic procedures, all repeated sperm recoveries were successful.

In addition to simplicity, convenience for the patients and considerably lower costs, TESA might cause less damage to the testis compared to TESE. Transient adverse effects such as inflammations and haematomas after TESE were reported by Schlegel et al. (1997). They also reported that permanent devascularization occurred in one out of 64 patients after TESE. No per- or post-operative complications were reported in our series of 137 patients and no serious adverse effects have hitherto been reported after TESA.

The mean serum FSH concentration was significantly lower in the non-obstructive group where spermatozoa could be successfully retrieved compared to the group where sperm recovery failed. However, a great overlap in the distribution of FSH concentrations was evident, irrespective of the presence or absence of testicular spermatozoa. The same was found for the testicular volume, with a wide distribution in mean testicular

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**Table I. Serum follicle stimulating hormone (FSH) and testicular volume (mean ± SD) in men with obstructive and non-obstructive azoospermia**

<table>
<thead>
<tr>
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<th>Obstructive azoospermia</th>
<th>Non-obstructive azoospermia: successful sperm recovery</th>
<th>Non-obstructive azoospermia: unsuccessful sperm recovery</th>
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<tbody>
<tr>
<td>No. of patients</td>
<td>51</td>
<td>27</td>
<td>43</td>
</tr>
<tr>
<td>FSH (IU/l), (range)</td>
<td>3.8 ± 2.0</td>
<td>16.1 ± 12.6a</td>
<td>23.9 ± 13.6</td>
</tr>
<tr>
<td>Testicular volume (ml)</td>
<td>19.8 ± 4.4</td>
<td>16.4 ± 4.0b</td>
<td>13.7 ± 3.9</td>
</tr>
<tr>
<td>(range)</td>
<td>14–30</td>
<td>10–25</td>
<td>8–25</td>
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*P < 0.05 versus non-obstructive azoospermia unsuccessful sperm recovery (Student’s t-test).

*P < 0.01 versus non-obstructive azoospermia unsuccessful sperm recovery (Student’s t-test).
volume and a large overlap between non-obstructive patients with and without spermatozoa. These data are in accordance with previous reports (Tournaye et al., 1995; Friedler et al., 1997), showing that neither serum FSH nor testicular volume was predictive for the chance of retrieving spermatozoa.

The prevalence of chromosomal aberrations in this study was seven out of 86 (8%). In the group of men with poor sperm production, the incidence of Klinefelter’s syndrome is ~3% (Tournaye et al., 1996; Lundin et al., 1998). Among our patients with non-mosaic 47,XXX, so far no spermatozoa have been found. However, a recent study reported five out of five normal preimplantation embryos after ICSI with spermatozoa from testicular recovery of men with a non-mosaic 47,XXY chromosome constitution (Staessen et al., 1996). In two case reports of two men with a 47,XXY/46,XY mosaicism, hyperploidy (24,XX or 24,XY) was found in 0.9 and 2.09% of the spermatozoa, respectively (Cozzi et al., 1994; Chevret et al., 1995). The proportion of 24,XX and 24,XY spermatozoa was doubled in the first case and increased 5-fold in the second case, compared to normal men, where the frequency of disomy for the sex chromosomes in spermatozoa is 0.42% (Martin et al., 1996). Although the proportion of spermatozoa, disomic for the sex chromosomes, may vary from case to case in Klinefelter mosaics, depending on the variation of the degree of mosaicism in the sperm-producing cells, the risk of producing a 47,XXX or 47,XXY offspring must be considered.

As the incidence of chromosomal abnormalities such as translocations may be up to ten times higher in men with poor sperm production compared to men with normal sperm production (Lidegaard et al., 1998), the risk of producing an offspring with an unbalanced chromosome content must also be considered.

All possible chromosomal imbalances in the offspring due to the chromosomal aberrations described in this paper may be detected by traditional prenatal diagnosis or by preimplantation genetic diagnosis. However, trisomy 47,XXX, 47,XXY or 47,XY does not in general lead to major malformation or mental retardation (Meschede and Horst, 1997). When these aberrations are detected in the offspring, the alternatives of continuing the pregnancy or therapeutic abortion should be thoroughly discussed.

The karyotyping did not include screening for chromosome Y microdeletions. Analysis of microdeletions is only available in a few laboratories in Scandinavia. The function of the various loci on the Y chromosome is not known but it seems well established that microdeletions on the Y chromosome play a central role in male infertility (Simoni et al., 1998). Most of the deletions seem to be de novo. The outcome of sperm recovery and fertilization rate after ICSI among non-obstructive azoospermic men with deletions compares favourably with non-obstructive azoospermic men without deletions (Mulhall et al., 1997). Hence, our couples with severe male infertility are offered genetic counselling where they are informed about the possible risk of vertical transmission of deletions to the male offspring.

In summary, this study showed that spermatozoa may be recovered by PESA or TESA from all patients with obstructive azoospermia. Testicular spermatozoa may be recovered from ~40% of patients with non-obstructive azoospermia. TESA performed under local anaesthesia is effective, simple and cost-effective. Including a sperm aspiration in the infertility work-up in azoospermic patients seems to be an excellent way to predict successful sperm recovery in subsequent ICSI treatments. In addition, in the obstructive group, epididymal or testicular spermatozoa can be frozen for later ICSI treatments with high fertilization and pregnancy rates (Nagy et al., 1995), while in the non-obstructive group the freezing–thawing procedure of testicular spermatozoa is more unpredictable (Gil Salom et al., 1996; Verheyen et al., 1997).

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G. Westlander et al.


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