Sperm retrieval and fertilization in repeated percutaneous epididymal sperm aspiration

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Percutaneous epididymal sperm aspiration (PESA) for retrieval of spermatozoa for intracytoplasmic sperm injection (ICSI) is a new simplified technique in the treatment of men with obstructive azoospermia. There has been a fear that the PESA procedure, being blind, could cause damage to the epididymal duct system and make it impossible to retrieve spermatozoa if a repeated procedure is required. We report here on repeated PESA procedures from the same unilateral epididymis. Twenty-seven men with obstructive azoospermia were investigated retrospectively regarding sufficiency of the number of motile spermatozoa for ICSI, fertilization rate (FR) and possibility of collecting spermatozoa for cryopreservation in repeated PESA procedures. Sufficient motile spermatozoa for ICSI were found in a similar proportion of men at the first two attempts: 91 and 89% respectively. Fertilization rate and the possibility of collecting spermatozoa for cryopreservation were also similar at the first two PESA procedures: 62 versus 67% and 33 versus 33% respectively. At the third procedure, motile spermatozoa for ICSI were retrieved in 86% (6/7), FR was 47% and spermatozoa were cryopreserved in one case. Two men underwent a fourth PESA. In both cases, a sufficient number of motile spermatozoa for ICSI was found and FR was 62%. This study shows that in men with obstructive azoospermia, PESA can be repeated on the same unilateral epididymis up to three times, with good opportunity of retrieving sufficient motile spermatozoa for ICSI.

Key words: epididymal spermatozoa/fertilization/ intracytoplasmic sperm injection/ obstructive azoospermia/ percutaneous epididymal sperm aspiration

Introduction

In cases of obstructive azoospermia, spermatozoa can be obtained surgically. Temple-Smith et al. (1985) reported the first pregnancy with the use of spermatozoa obtained by microsurgical epididymal sperm aspiration (MESA) and in-vitro fertilization (IVF). However, the number of aspirated epididymal spermatozoa, and especially the motility of the spermatozoa, are generally low after MESA compared to ejaculated spermatozoa. The fertilization rate using MESA in combination with conventional IVF has been low (Silber et al., 1994), but since the introduction of intracytoplasmic sperm injection (ICSI) (Palermo et al., 1992), the fertilization rates with use of epididymal spermatozoa after MESA have improved (Tournaye et al., 1994). Microsurgical epididymal sperm aspiration in combination with ICSI has now become a common procedure in fertility clinics in the treatment of patients with obstructive azoospermia. However, MESA is a relatively complicated procedure which carries certain disadvantages such as the need for operation facilities and an experienced microsurgeon. Furthermore, postoperative morbidity such as pain, haematoma formation and infection may occur and repeated surgery in the explored scrotal area may be more complex as a result of postoperative fibrosis (Tsirigotis and Craft, 1995). As an alternative to MESA, a new simplified method, percutaneous epididymal sperm aspiration (PESA) has been introduced (Craft and Shrivastav, 1994; Shrivastav et al., 1994) for retrieval of epididymal spermatozoa for ICSI in patients with obstructive azoospermia. Spermatozoa obtained by PESA may also be cryopreserved for future use, avoiding the need to repeat the procedure (Tsirigotis and Craft, 1995).

There has been a fear that the blindness of the PESA procedure and the fact that several epididymal tubules are penetrated may lead to diffuse injury to the epididymal tubules or to haematoma in the epididymal tunica, causing further obstruction (Cha et al., 1997).

The aim of this retrospective study was to analyse the outcome of repeated PESA procedures with reference to the possibility of retrieving a sufficient amount of spermatozoa for ICSI, the fertilization rate and the possibility of collecting spermatozoa for cryopreservation.

Materials and methods

Between March 1995 and November 1997, PESA was performed on 130 men in three IVF centres. So far, the PESA procedure has been repeated in 35 of these men. The men either underwent a first diagnostic trial followed by a treatment cycle, or a first treatment cycle was followed by a new treatment cycle. Eight men were excluded from further analysis, since the first procedure had been carried out on the opposite side. The remaining 27 men with a mean age of 37 years of age (range 26–49 years) formed the study group. All men had a history of azoospermia, the aetiology of which was classified as epididymal or vas obstruction (n = 11), congenital bilateral absence of the vas deferens (CBAVD) (n = 6), vasectomy (n = 4), or peripheral neuropathy (n = 2). One patient had a history of anejaculation after treatment for testicular cancer which included...
ICSI was performed essentially according to Hamberger protocols used for conventional IVF/intracytoplasmic sperm injection stimulation with follicle stimulating hormone (FSH), following years). The treatment cycles included down-regulation and ovarian aspiration (TESA) was performed by needle biopsies, using 19- or 21-gauge butterfly needles. As the total volume of epididymal fluid retrieved during PESA is usually very low, it was difficult to quantify the total number of spermatozoa accurately in all PESA procedures. The sperm count is therefore not presented. However, the percentage of motile spermatozoa at each PESA was estimated. The fertilization rate in each treatment cycle was calculated. Percutaneous epididymal sperm aspiration was performed under local anaesthesia. Prilocaine hydrochloride 7–10 ml (Citanest® 10 mg/ml, Astra, Södertälje, Sweden) or lidocaine (Xylocaine, 10 mg/ml, Astra) was infiltrated around the epididymis. Gentle, negative pressure was applied as epididymal fluid was aspirated. The aspirate was washed out of the needle with 1 ml of IVF medium (Gamete100®, Scandinavian IVF Science, Göteborg, Sweden). If the aspiration was unsuccessful, up to three further punctures were performed on the same epididymis. If no motile spermatozoa were obtained by TESA, testicular sperm aspiration (TESA) was performed by needle biopsies, using 19- or 21-gauge butterfly needles. The female partners had a mean age of 33 years (range 24–40 years). The treatment cycles included down-regulation and ovarian stimulation with follicle stimulating hormone (FSH), following protocols used for conventional IVF/intracytoplasmic sperm injection (ICSI). ICSI was performed essentially according to Hamberger et al. (1995). In the majority of cases, the media used for sperm and oocyte culture were from Scandinavian IVF Science AB. In treatment cycles, the PESA procedures were scheduled to take place on the day of ovum retrieval. The spermatozoa selected for ICSI were either picked out directly from the aspirated fluid or selected after preparation by swim-up or a gradient centrifugation (Percoll; Pharmacia, Uppsala, Sweden), or by a combined swim-up and Percoll preparation.

Results

The first PESA was carried out as part of a treatment cycle in 22 cases and as a pretreatment trial in five cases (Table I). All second, third and fourth PESA were part of a treatment cycle. A sufficient number of spermatozoa for fertilization with ICSI was found in 91% of the patients (20/22) at the first PESA (Table I). In two cases, no motile spermatozoa for ICSI were found, and testicular spermatozoa were used for successful fertilization. Spermatozoa were aspirated at all of the first PESA carried out as pretreatment trials. In three of these five cases, spermatozoa could be cryopreserved. In a total of nine cases, spermatozoa obtained at the first PESA could be cryopreserved. The fertilization rate in the first treatment was 62% (Table II). There was one case of fertilization failure.

Sufficient spermatozoa for ICSI were found in 89% of the cases (24/27) at the second PESA (Table I). In three cases where spermatozoa had been found at the first PESA, no spermatozoa were found at the second one and testicular spermatozoa were used for fertilization. In nine cases, spermatozoa obtained at the second PESA could be cryopreserved. The fertilization rate was 67% (Table II). Seven men underwent a third PESA, and in six of these cases (86%) spermatozoa sufficient for ICSI were retrieved. In one case no spermatozoa were found from the earlier puncture site, and spermatozoa were retrieved after testicular sperm aspiration. The fertilization rate at the third PESA procedure was 47%. In two cases, a fourth PESA was carried out. In both cases, spermatozoa for ICSI were found and the fertilization rate was 62%. The sperm motility in each procedure ranged from a median of 3–10% and there was no significant difference in motility between the four procedures.

Except for minor bruising and swelling, there were no clinically significant per- or postoperative complications in any of the men in this study.

Discussion

In many cases of untreatable obstructive azoospermia, spermatozoa can be obtained from the epididymis by MESA or PESA. Even in cases of erectile dysfunction and ejaculation

retroperitoneal lymph node dissection. In three of the patients, the aetiology of the azoospermia was unknown. In total, 63 repeated PESA procedures on the same unilateral epididymis were performed. All 27 men underwent two, seven men had a third, and two men had a fourth PESA procedure carried out. The median time intervals between the procedures were 18 (range 7–44 weeks), 24 (range 16–41 weeks) and 20 weeks respectively.

As the total volume of epididymal fluid retrieved during PESA is usually very low, it was difficult to quantify the total number of spermatozoa accurately in all PESA procedures. The sperm count is therefore not presented. However, the percentage of motile spermatozoa at each PESA was estimated. The fertilization rate in each treatment cycle was calculated. Percutaneous epididymal sperm aspiration was performed under local anaesthesia. Prilocaine hydrochloride 7–10 ml (Citanest® 10 mg/ml, Astra, Södertälje, Sweden) or lidocaine (Xylocaine, 10 mg/ml, Astra) was infiltrated around the epididymis. Gentle, negative pressure was applied as epididymal fluid was aspirated. The aspirate was washed out of the needle with 1 ml of IVF medium (Gamete100®, Scandinavian IVF Science, Göteborg, Sweden). If the aspiration was unsuccessful, up to three further punctures were performed on the same epididymis. If no motile spermatozoa were obtained by TESA, testicular sperm aspiration (TESA) was performed by needle biopsies, using 19- or 21-gauge butterfly needles.

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Table I. The outcome of percutaneous epididymal sperm aspiration (PESA) regarding sufficiency of motile spermatozoa for intracytoplasmic sperm injection (ICSI) and cryopreservation, and sperm retrieval

<table>
<thead>
<tr>
<th>PESA</th>
<th>n</th>
<th>Sufficient motile spermatozoa for ICSI (%)</th>
<th>Sufficient motile spermatozoa for cryopreservation (%)</th>
<th>No spermatozoa retrieved (%)</th>
<th>Converted to TESA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>27a</td>
<td>20/22 (91)</td>
<td>9/27 (33)</td>
<td>2/27 (7)</td>
<td>2/22 (9)</td>
</tr>
<tr>
<td>2nd</td>
<td>27</td>
<td>24/27 (89)</td>
<td>9/27 (33)</td>
<td>3/27 (11)</td>
<td>3/27 (11)</td>
</tr>
<tr>
<td>3rd</td>
<td>7</td>
<td>6/7 (86)</td>
<td>1/7 (14)</td>
<td>1/7 (14)</td>
<td>1/7 (14)</td>
</tr>
<tr>
<td>4th</td>
<td>2</td>
<td>2/2 (100)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*aIncludes five pretreatment trials.

TESA = testicular sperm aspiration.

Table II. The outcome of percutaneous sperm aspiration (PESA) regarding number of treatment cycles with epididymal spermatozoa, percentage of motile spermatozoa and number and percentage of fertilized eggs

<table>
<thead>
<tr>
<th>PESA</th>
<th>No. of treatment cycles with epididymal spermatozoa</th>
<th>Percentage of motile spermatozoa (median)</th>
<th>No. of fertilized eggs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>20</td>
<td>5</td>
<td>110/177 (62)</td>
</tr>
<tr>
<td>2nd</td>
<td>24</td>
<td>3</td>
<td>170/253 (67)</td>
</tr>
<tr>
<td>3rd</td>
<td>6</td>
<td>10</td>
<td>28/60 (47)</td>
</tr>
<tr>
<td>4th</td>
<td>2</td>
<td>5</td>
<td>13/21 (62)</td>
</tr>
</tbody>
</table>
problems, epididymal spermatozoa can be retrieved by surgical procedures, although this is presently controversial.

An important aspect of these procedures is the failure rate. In a series of 28 men with obstructive azoospermia undergoing MESA, Silber et al. (1995) reported failure to obtain spermatozoa in 12 cases (42.8%). All 12 patients with no epididymal spermatozoa had undergone multiple previous scrotal surgeries and were massively scarred. In a PESA series reported by Meniru et al. (1997), in men with CBAVD and failed reversal of vasectomy, the rate of failure to obtain spermatozoa was 8% (10 of 122 patients). The failure rate with PESA in our study was ~10%, and did not change with repeated procedures.

The fertilization rate by ICSI after MESA has been reported to be 45–67% (Silber et al., 1995; Watkins et al., 1997) and the fertilization rate after PESA 54–62% (Meniru et al., 1997; Wood et al., 1997). The overall fertilization rate in this study was 63% (322/511 oocytes) and did not appear to change with repeated procedures. The efficacy of MESA in cases of failed PESA is a moot question. More importantly, the centre in which PESA is practised may not have easy access at short notice to equipment and personnel for MESA. However, testicular sperm aspiration (TESA), (Bourne et al., 1995; Hovatta et al., 1995; Westlander et al., 1998) or open biopsy (Silber et al., 1995) is an effective backup procedure in such circumstances, and the need for testicular biopsy will be limited to few cases. In this form of azoospermia, fertilization rates with testicular spermatozoa have been reported to be in the range of 52–68% (Aboulghar et al., 1997; Rosenlund et al., 1997), while in cases of non-obstructive azoospermia, the fertilization rates with such spermatozoa have generally been lower (Tournaye et al., 1996). The reasons for preferring PESA over TESA are that normally more spermatozoa are obtained, the epididymal spermatozoa are easier to work with and it is more likely that there will be sufficient spermatozoa for freezing.

Epididymal spermatozoa can be cryopreserved for future use, thus avoiding the need for repeated MESA/PESA in subsequent treatment cycles. Even when the motility of the epididymal spermatozoa is low, cryopreservation may be possible. The fertilization and pregnancy rates are reported to be at least as high with the use of frozen–thawed epididymal spermatozoa as with fresh epididymal spermatozoa (Cha et al., 1997; Lundin et al., personal communication). Hence, to reduce the need for repeated PESA, efforts should be made to cryopreserve spermatozoa obtained by these procedures. By cryopreservation in several portions, one straw can be thawed prior to the next treatment cycle in order to check sperm survival.

PESA is a simple procedure and does not need microsurgical experience. The trauma to the epididymis is minimal and in our study there were no operative or postoperative complications. Although retrieval of testicular spermatozoa is an alternative, there appears to be a potential risk of temporary or permanent ischaemic testicular injury due to devascularization after open testicular biopsy (Schlegel and Su, 1997). The physiological consequences of percutaneous testicular biopsies require future investigation. This and previous studies have shown that with PESA the sperm recovery rate in obstructive azoospermia is high, especially in men who have not undergone surgery to the epididymis. As shown in this study, PESA can be repeated with similar results.

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References


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