Serial sonography and colour flow Doppler imaging following testicular and epididymal sperm extraction

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Percutaneous epididymal sperm aspiration (PESA), percutaneous testicular sperm aspiration (TESA) and testicular sperm extraction (TESE) are invasive procedures and their consequences on the testis have not been clearly defined. In order to relate the sonographic and colour Doppler flow changes to the clinical data, 14 patients with non-obstructive and six with obstructive azoospermia were examined by the same roentgenologist immediately before, at 5 days, 2 weeks, 2 and 6 months after the surgical procedure. Testicular volumes remained unchanged during the follow-up period in both the non-obstructive and obstructive groups. Of the non-obstructive group, focal testicular lesions were seen in 20 of the 26 testes (77%) 5 days after the procedure and in 54% by 6 months. Ten were hypoechogenic, of which six converted to echogenic foci, three remained hypoechogenic and one disappeared at 6 months. The other 10 were echogenic lesions, three of which were no longer visible at 6 months and the remainder were unchanged. In the obstructive azoospermic group, focal lesions were not found. Extratesticular abnormality consistent with haematoma was demonstrated in four non-obstructive cases, which disappeared at the 6 month examination, and in none of the obstructive azoospermic patients. Whether residual focal lesions in the testes have long-term effects remains to be evaluated. In the obstructive azoospermic group, the aspirations performed did not leave any sonographic abnormalities.

Key words: azoospermia/in-vitro fertilization/intracytoplasmic sperm injection/testicular biopsy/testicular sperm extraction

Introduction

Since the introduction of the technique of using testicular spermatozoa for intracytoplasmic sperm injection (ICSI) by Schoysman et al. (1993) and Craft et al. (1993), percutaneous epididymal sperm aspiration (PESA), sperm aspiration (TESA) and testicular sperm extraction (TESE) have been offered to patients with obstructive and non-obstructive azoospermia (Devroey et al., 1994, 1995; Abuzeid et al., 1995; Bourne et al., 1995; Silber et al., 1995; Kahraman et al., 1996; Tournaye et al., 1996; Friedler et al., 1997). The procedures, although promising and exciting, are invasive and their consequences on the testis have not been clearly defined. Since the majority of patients need more than a single testicular sperm retrieval to achieve pregnancy, the risk of complications from the repeated procedure is increased.

Vascular injuries and inflammatory changes can occur following testicular surgery. After entering the scrotum, the testicular artery runs along the posterior aspect of the testis and penetrates the tunica albuginea to form the capsular arteries. These arteries have centripetal branches that enter the testicular parenchyma between the septa, separating the seminiferous tubules (Horstman et al., 1991). These centripetal branches under the tunica albuginea are end-arteries, and any injury to these vessels during testicular surgery may devascularize an area in the testis.

The purpose of this prospective study was to relate serial sonographic and colour flow Doppler changes to the clinical data following PESA, TESA and TESE.

Materials and methods

Patients with azoospermia were evaluated by physical examination of their genitalia, extensive work-up of several ejaculates, testicular and transrectal sonography, assessment of their hormonal profile and cytogenetic consultation including karyotyping. Obvious cases of obstructive azoospermia were designated to PESA. Up to six aspirations from each epididymis or testis were performed. When no spermatozoa were detected in the aspirated fluid, the surgical procedure was extended to TESA.

In all other patients in whom non-obstructive azoospermia was suspected (based upon palpation of the vas deferens and imaging of the seminal vesicles by transrectal sonography and elevated serum follicle stimulating hormone, [FSH]), fresh ejaculate was evaluated on the ovum retrieval day by extended sperm preparation (Ron-El et al., 1997). If no spermatozoa were observed, the patient underwent TESE, which included up to three biopsies in different areas of each testis. The surgical procedure was stopped when the embryologist identified mature forms of spermatozoa in the wet preparation. The PESA, TESA and TESE procedures are described extensively in our previous study (Friedler et al., 1997). In brief, PESA was performed using 21-gauge butterfly needles attached to a 20 ml plastic syringe as the aspiration device. The testicular biopsy was performed via a small incision in the mid-portion of the testes, and a substantial piece of the extruding testicular tissue was removed by small scissors.

Following vigorous tissue shredding, human tissue fluid (HTF)–HEPES–albumin medium supplemented with 7.5% synthetic serum (Irvine Scientific, Santa Ana, CA, USA) was added to the suspension obtained and incubated in 6 ml Falcon tubes (Becton-Dickinson, Aalst, Belgium), for 2 h (5% CO2 in air) at 37°C. The overlying pellet was then collected and centrifuged (300 g for 10 min). The remaining pellet was isolated, processed and finally examined in multiple droplets under oil (Embryo tested; Sigma, St Louis, MO, USA; cat. no. 8042–47–5). If no spermatozoa were observed the
Sonographic and colour Doppler imaging were performed immediately before the surgical procedure, and then at 5 days, 2 weeks, 2 and 6 months after the PESA/TESA/TESE procedures. Each testis was measured in three dimensions and the volume calculated by use of the formula for an ellipsoid. Testicular texture was evaluated in terms of echogenicity of the parenchyma, presence and size of focal lesions, intratesticular haematoma and colour flow Doppler imaging, using an Acuson 128XP/10 computed sonography unit (Mountain View, CA, USA) with a 7 MHz transducer. The presence of extra-testicular haematoma or hydrocele was also documented, as well as changes in size and texture of the head of the epididymis. All sonographic examinations were performed by the same roentgenologist, who was uninvolved with the type of surgical procedure the patient had undergone and his clinical follow-up.

Results

Twenty-six azoospermic patients underwent surgical procedures for sperm retrieval and serial sonography. Six were lost to follow-up after the sonographic examination at 2 weeks. Twenty patients were followed to 6 months and comprised the study group. Of these, 14 patients suffered from non-obstructive azoospermia and six from obstructive azoospermia. Two undescended testes, both in non-obstructive azoospermic patients, were not included in the study.

All men with non-obstructive azoospermia underwent the TESE procedure. Surgery was performed bilaterally in 12 of these patients and in a single testis in each of the two patients with cryptorchid testis, giving a total of 26 testes. All six patients with obstructive azoospermia underwent PESA. Three of them were aspirated only on one side, since enough spermatozoa were retrieved for the treatment as well as for freezing for future attempts. In two, bilateral PESA was necessary to collect spermatozoa, and in the sixth case bilateral TESA was performed, since PESA yielded no spermatozoa.

Testicular volume of the operated testes before and in examinations following the procedure is given in Table I. Testicular volumes remained unchanged during the follow-up period in both the non-obstructive and obstructive groups. The mean testicular volume in the non-obstructive group was approximately half that of the obstructive group.

Four testes, all in non-obstructive azoospermic patients, were found to be diffusely hypoechoic or heterogeneous on the pre-procedure sonography and throughout the study period. Of the non-obstructive azoospermic group, focal testicular lesions were seen in 20 of the 26 testes (77%) or in 12 of the 14 patients, examined several days after the procedure. At 2 months post procedure, two of the 20 focal lesions had resolved and by 6 months only 14 (54%) of the 26 testes (11 of 14 patients) showed residual abnormal areas. Of the 20 testes seen initially to have focal lesions, 10 were hypoechoic (Figure 1). At 6 months, six of the hypoechoic lesions had converted to echogenic foci, three remained hypoechoic and one had disappeared. Of the 10 echogenic lesions seen at the first post-procedure study, three were no longer visible at 6 months and the remainder were unchanged at that time. All the echogenic lesions seen initially to have focal lesions, 10 were hypoechoic (Figure 2). In the obstructive azoospermic group, focal lesions were not found, including the patient who had undergone bilateral TESA.

Colour Doppler imaging was normal in 35 of the 38 testes (92%) examined, and there was no case of complete devascularization of the testis following the procedure. Areas of decreased blood flow were seen in three testes at 2 months.

<p>| Table I. Sonographic calculation of testicular volume (ml) in non-obstructive azoospermic patients (n = 14) and obstructive azoospermic patients (n = 6) |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>At 5 days</th>
<th>At 14 days</th>
<th>At 2 months</th>
<th>At 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-obstructive azoospermia (n = 26)</td>
<td>Right testis 6.7 ± 2.6</td>
<td>6.5 ± 3.1</td>
<td>8.4 ± 2.2</td>
<td>7.1 ± 1.8</td>
<td>6.4 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Left testis 6.5 ± 2.6</td>
<td>7.1 ± 2.0</td>
<td>7.1 ± 2.0</td>
<td>6.5 ± 1.8</td>
<td>6.8 ± 2.3</td>
</tr>
<tr>
<td>Obstructive azoospermia (n = 12)</td>
<td>Right testis 15.3 ± 5.8</td>
<td>14.8 ± 4.8</td>
<td>16.4 ± 4.5</td>
<td>16.4 ± 4.5</td>
<td>17.1 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Left testis 16.5 ± 5.1</td>
<td>16.5 ± 3.9</td>
<td>16.3 ± 4.5</td>
<td>16.3 ± 4.5</td>
<td>16.6 ± 3.5</td>
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*aNo. of testes.
and in two of the three at 6 months. The decreased blood flow was localized to a hypoechoic area in the testis, whilst the remainder of the testis had normal vascular supply.

Extratesticular abnormality consistent with haematoma was demonstrated in four non-obstructive azoospermic cases. In each case a unilateral echogenic collection was seen adjacent to the anterior surface of the testis (Figure 3). In all four cases, the haematoma was not visualized at the 6 month post-procedure study. No extratesticular finding was demonstrated in obstructive azoospermic patients.

On pre-procedure sonography, in five cases, all of whom were non-obstructive azoospermic patients, the head of the epididymis was found, bilaterally, to be small and ill-defined, or not definitely visualized. In another case, an obstructive azoospermic patient, the head of the epididymis appeared unilaterally to be under-developed. Very small cysts, up to 0.4 cm in diameter, were seen in the head of the epididymis in four patients. In all cases, however, the cysts were present prior to the procedure, or not related to the type of procedure performed. Following PESA procedure, there was no evidence of a haematoma in the epididymis and no significant change in its size.

**Discussion**

Since testicular aspirations and biopsies are being increasingly performed in cases with obstructive and non-obstructive azoospermia, there is a need to study the impact of the procedure on the remaining testicular tissue. Concerns about the early and late complications following the surgical intervention and the timing of repeated testicular biopsy have been raised. Del Vento et al. (1992) found in stallions decreased echogenicity at the sites of the testicular biopsies and leukocyte infiltration in histology of the same area, up to 1 month after the procedure. Hypoechoic lesions and diffusely increased heterogeneity of testicular tissue were characterized in patients as the sonographic appearance of intratesticular bleeding associated with trauma 15 years ago (Anderson et al., 1983). These data were confirmed 10 years later (Corrales et al., 1993).

Changes in testicular texture on ultrasound examination following biopsy have been described recently by Harrington et al. (1996) and Schlegel and Su (1997). The incidence of intratesticular haematoma or a new area of increased echogenicity has been reported by Harrington et al. (1996) as 29%, 1 month after an open testicular biopsy. These areas of increased echogenicity were interpreted as parenchymatous scars, and persisted 6 months postoperatively.

Schlegel and Su (1997), studying patients with non-obstructive azoospermia who had undergone TESE, performed ultrasound on 17 at 3 months, 14 at 6 months and five at both 3 and 6 months. They found at 3 months after TESE, sonographic abnormalities in the testis suggestive of haematoma or resolving inflammation in 82% of the patients. Out of 14 patients undergoing sonography at 6 months, nine (64%) had changes consistent with parenchymal calcifications or linear scars. We found, similarly, focal lesions in 11 of 14 (79%) patients both at 2 and 6 months. We feel, however, that sonographic changes should be analysed per testis and not per patient. Accordingly, we found at 2 months post-procedure changes in 17 of 26 (64%) tests and in 14 of 26 (54%) tests at 6 months, in our group of non-obstructive azoospermic patients.

We observed echogenic foci with posterior acoustic shadowing on the surface of nine testes in the first postoperative examination. Since one would not expect calcification to develop within days after the procedure, this finding may represent sutures or even air trapped in the biopsy site. Indeed, in two cases, the echogenic focus with the posterior acoustic shadowing seen on initial examination disappeared on subsequent studies.

Both percutaneous and open biopsy of the testis have the potential for inadvertent vascular injury and permanent damage to the testis. Out of 31 patients who underwent TESE procedures and were followed by sonographic and colour Doppler examinations, Schlegel and Su (1997) reported two patients who developed progressive unilateral testicular atrophy. Colour Doppler showed in one testis a large region without blood

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**Figure 2.** Transverse sonogram of testis with superficial echogenic lesion (electronic calipers) and posterior acoustic shadowing (arrows).

**Figure 3.** Longitudinal sonogram showing large extratesticular haematoma (H), adjacent to testis (T).
flow, and in the other globally decreased arterial flow. In our study, small localized areas of decreased blood flow were seen on colour Doppler at 6 months in two patients, but the remainder of the tests had normal vascular pattern and testicular volumes were unchanged throughout the study period. These localized areas of decreased flow appeared to be in foci of chronic changes suggestive of fibrosis or scarring.

Testicular atrophy or complete devascularization of the testis is a major complication, and may be related to the number of biopsies taken from the tests. We performed up to a maximum of three biopsies per testis, and this may account for the absence of testicular atrophy in our series. With this policy, spermatozoa were found in the testicular tissue in six of the 14 non-obstructive azoospermic patients.

Whether residual focal lesions in the testes following TESE have long-term effects remains to be evaluated, but it seems unlikely that these small areas would adversely affect testicular function. It is interesting to note that in the obstructive azoospermic group, the aspirations performed did not leave any sonographic abnormalities.

References


Sonogram of epididymis and testis after aspiration or biopsy


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