

## Physiological consequences of testicular sperm extraction\*

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**Testicular sperm extraction (TESE) may provide spermatozoa for attempts at fertility with assisted reproduction; however, the physiological effects of TESE on testicular function are not well understood. In order to evaluate the effects of TESE on the testis, 64 patients were evaluated after TESE for non-obstructive azoospermia with physical examinations, serial scrotal sonography, histological analyses and evaluation of the success of repeated sperm retrieval attempts. At 3 months after TESE, 82% of evaluated patients had ultrasonographic abnormalities in the testis suggesting resolving inflammation or haematoma at the biopsy site. By 6 months, these acute inflammatory changes typically resolved leaving linear scars or calcifications. Two patients had documented impaired testicular blood flow, with complete devascularization of the testis for one patient after TESE with multiple biopsies. Repeat TESE procedures were far more likely to retrieve spermatozoa if the second TESE attempt was performed >6 months after the initial TESE procedure (80%), relative to those performed within 6 months (25%). Transient adverse physiological effects are common in the testis for up to 6 months after TESE. In addition, permanent devascularization of the testis can occur following TESE procedures with multiple biopsies. The risk of this complication may be minimized by using an open biopsy technique with optical magnification to directly identify testicular vessels.**  
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### Introduction

For men with non-obstructive azoospermia (NOA), retrieval of spermatozoa from the testis and use of these spermatozoa for assisted reproduction in intracytoplasmic sperm injection (ICSI) may provide an opportunity for fertility despite quantitatively limited sperm production. It has been well documented

that testicular spermatozoa have the ability to fertilize oocytes and produce viable embryos for men with NOA (Devroey *et al.*, 1995; Kahraman *et al.*, 1996; Schlegel *et al.*, 1997). Jow *et al.* (1993) suggested that spermatozoa could be found in a single testis biopsy sample from 34% of men with NOA. Multiple biopsies can be used to retrieve spermatozoa in 48–86% of men with NOA, suggesting the presence of patchy regions of minimal sperm production (Devroey *et al.*, 1995; Kahraman *et al.*, 1996; Tournaye, 1996; Schlegel *et al.*, 1997). The technique of acquiring testicular spermatozoa using testicular sperm extraction (TESE) was described by Schoysman *et al.* (1993) as well as Craft *et al.* (1993) for obstructive azoospermia and subsequently by Silber *et al.* (1995) and Devroey *et al.* (1995). Since then, TESE has been increasingly performed as a therapeutic procedure in conjunction with ICSI for both obstructive and non-obstructive azoospermia. Studies to date have focused on the remarkable potential to retrieve viable spermatozoa from the testis using TESE despite the histological findings of maturation arrest, Sertoli-cell-only, or hypospermatogenesis on testis biopsy that are characteristic of NOA. However, little is known about the physiological consequences of TESE on testicular function.

Spermatogenesis is an intricate process of 74 days duration that is highly sensitive to toxic effects and even minor alterations in temperature. Therefore, inflammatory changes in the testis following testicular surgery could adversely affect the spermatogenic process.

Vascular injuries can also occur from testicular surgery. The testicular blood supply is derived primarily from branches of the internal spermatic artery, with collateral branches off the cremasteric and vasal arteries (Schlegel and Chang, 1991). Regardless of its source, the testicular blood supply penetrates the tunica albuginea covering the testicular parenchyma and travels extensively under the tunica albuginea but over the surface of seminiferous tubules before penetrating between the septa separating the seminiferous tubules. The sub-tunical arteries are end-arteries. Ligation or division of these end-arteries during testis biopsy may devascularize a region of the testis. Elegant human autopsy studies of testicular blood supply have shown that no single area of the tunica albuginea can be blindly opened without potential injury to a major vessel of the testis, including the risk of complete testicular devascularization (Jarow, 1991).

The effects of a single diagnostic testicular biopsy on spermatogenic function have been previously suggested. Harrington *et al.* (1996) reported that 29% of single open diagnostic testicular biopsies resulted in intratesticular haematoma formation, with development of an intraparenchymal hypoe-

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choic region on high resolution ultrasound. Haematoma formation was also seen after percutaneous biopsy.

The multiple biopsies or removal of a larger sample of testis required for TESE may result in more inflammation and a greater disruption of spermatogenesis. Therefore, the quantitatively low sperm production present in men with NOA may be transiently abolished by any decrease in sperm production.

In order to evaluate further the physiological effects of TESE on subsequent testicular function, we assessed patients with serial ultrasound evaluations and compared sequential histological results of biopsy specimens and results of attempted sperm retrieval for men who underwent multiple TESE procedures.

### Materials and methods

All patients in this study underwent TESE for non-obstructive azoospermia and were subsequently evaluated at the New York Hospital–Cornell Medical Center, USA. The diagnosis of NOA was typically made based on pre-TESE diagnostic biopsy. When available, the biopsy was also used to rule out testicular carcinoma-in-situ. Clinical grounds for establishing the diagnosis of NOA included azoospermia in the presence of small volume testes with elevated serum follicle stimulating hormone (FSH) and an apparently empty epididymis and vas deferens. TESE procedures were performed with either multiple biopsies as described by Devroey *et al.* (1995), or as a single large biopsy (Schlegel *et al.*, 1997). All TESE procedures were performed in conjunction with planned in-vitro fertilization (IVF) cycles with ICSI for the female partner.

Patients were evaluated at 1, 3 and 6 months following TESE procedures. Initial post-TESE evaluation included history of any persistent testicular pain, change in testicular size, or scrotal swelling as well as direct evaluation of testicular volume by physical examination with an orchidometer. Testicular sonograms were obtained at 3 and 6 months following TESE. Sonographic data were obtained using an Acuson 128XP/10 computed sonography machine (Mountainview, CA, USA) with both 5.0 and 7.0 MHz probes. Sonographic findings that were considered acute included diffuse heterogeneity of testis parenchyma or focal hypoechoic regions. These lesions have been previously demonstrated after blunt scrotal trauma to reflect intratesticular bleeding and haematoma formation respectively (Anderson *et al.*, 1983; Fournier *et al.*, 1985; Gorrales *et al.*, 1993). Findings that were considered chronic or permanent included calcification on the surface of the testis or a linear hyperechoic focus without an associated region of hypoechogenicity. Testicular blood supply was evaluated by colour Doppler flow analysis of the testicular parenchyma.

Patients who underwent multiple TESE attempts were evaluated for the success of sperm retrieval relative to the interval between TESE procedures. Interval changes in testicular histology on Bouin's fixed sections of testicular tissue obtained during successive TESE procedures were also noted.

### Results

In all, 64 patients were evaluated after a total of 83 TESE procedures; 19 of the patients had more than one TESE procedure performed. The mean age of the patients was 38 years (range 25–64). Two patients complained of progressive unilateral testicular atrophy on follow-up after TESE. At 1 month following the TESE procedure, all patients were found to have minimal induration of the tunica albuginea correspond-

ing to the site of biopsy as well as slight induration under the skin incision. No acute perioperative complications such as a clinically evident haematoma or wound infection were detected.

### Ultrasonographic findings

Out of 17 patients who underwent testicular sonographic evaluation at 3 months post-TESE procedure, 14 (82%) had acute findings described either as hypoechoic areas (Figure 1) or heterogeneity of testicular parenchymal echotexture. These findings were consistent with haematoma and inflammation at the TESE site(s) or intratesticular bleeding from the TESE procedure. Of the four patients with acute findings on sonography 3 months post-TESE who had subsequent ultrasound evaluation, resolution of these abnormalities was seen at 6 months post-operatively. Residual, chronic findings included parenchymal calcifications or a linear hyperechoic focus. These persistent findings were suggestive of resolution of inflammation and formation of a scar. A summary of these findings is presented in Figure 2.

Overall, 14 patients had testicular sonograms performed 6 or more months after their original TESE procedure. Nine of these patients had similar chronic findings, most commonly parenchymal calcifications or a linear hyperechoic scar. Calcifications on the surface of the tunica albuginea, associated with tunical incisions and sutures, were typically seen. Of the nine patients with chronic ultrasonographic findings, five had these chronic findings documented >1 year post-TESE. Three patients (3/14, 21%) had findings consistent with acute inflammation or haematoma present. The two remaining patients in this group had normal testicular sonograms.

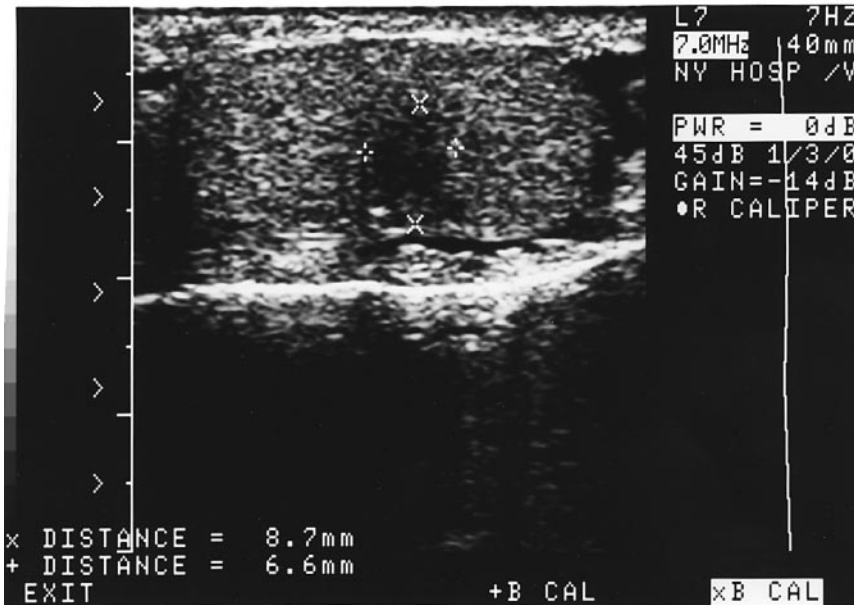
### Testicular blood flow analysis

The two patients who developed unilateral testicular atrophy after TESE (see above) were evaluated further with colour Doppler ultrasound. In the first patient, ultrasound of the atrophic testis revealed a large region without blood flow, confirming devascularization of the testis by the TESE procedure (Figure 3). This was the second TESE attempt for this patient; each attempt had involved multiple biopsies from the subsequently atrophied (right) testis. The second patient had arterial flow present in the atrophic testis which was globally decreased as compared to the contralateral testis. In addition the parenchyma was noted to have surface changes consistent with fibrosis and retraction.

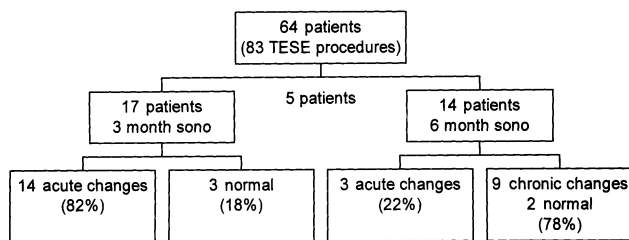
### Multiple TESE procedures

A total of 19 patients underwent multiple TESE attempts. All had viable spermatozoa retrieved for ICSI at the initial TESE procedure. Four of these patients had a second TESE procedure <6 months after the initial TESE. The interval between TESE procedures was 2, 3, 3 and 4 months for the four patients. In three patients no spermatozoa were retrieved. The fourth had spermatozoa retrieved but cytological evaluation demonstrated extensive inflammatory cells and debris, and spermatozoa with short tails. No normal fertilization or pregnancy was achieved using these spermatozoa.

For 15 patients who underwent multiple TESE attempts at retrieval of spermatozoa, the repeat procedure was made >6



**Figure 1.** Right testicular sonogram of a patient 3 months following testicular sperm extraction demonstrating an area of hypoechogenicity (outlined by cross-hairs).



**Figure 2.** Schematic overview of the results of sonographic evaluation of patients after testicular sperm extraction (TESE) procedures. Of the 31 patients who had sonographic follow-up examination (at 3 months,  $n = 17$ ; at 6 months,  $n = 14$ ), five were evaluated twice, at both 3 and 6 months.

months after the initial successful TESE procedure. In 12 of 15 (80%) attempts, testicular spermatozoa were retrieved during the second TESE procedure (Figure 4). These TESE-ICSI attempts resulted in successful deliveries for three patients, ongoing pregnancy for four patients, one ectopic pregnancy, one pregnancy with subsequent spontaneous abortion, one biochemical pregnancy, and spermatozoa which were not immediately used for ICSI for one couple; no pregnancy was achieved for one couple. Of three patients where no spermatozoa were retrieved, one patient had a repeat TESE attempt performed 7 months following his original procedure which again yielded no spermatozoa. Histological sections obtained at the second TESE attempt revealed diffuse sclerosis of seminiferous tubules with only rare spermatogonia and extensive peritubular fibrosis. It is possible that the sclerosis and fibrosis was a result of the previous TESE procedure and reaction, or that insufficient spermatogenic potential was present after the first TESE procedure to allow retrieval of spermatozoa on a second attempt.

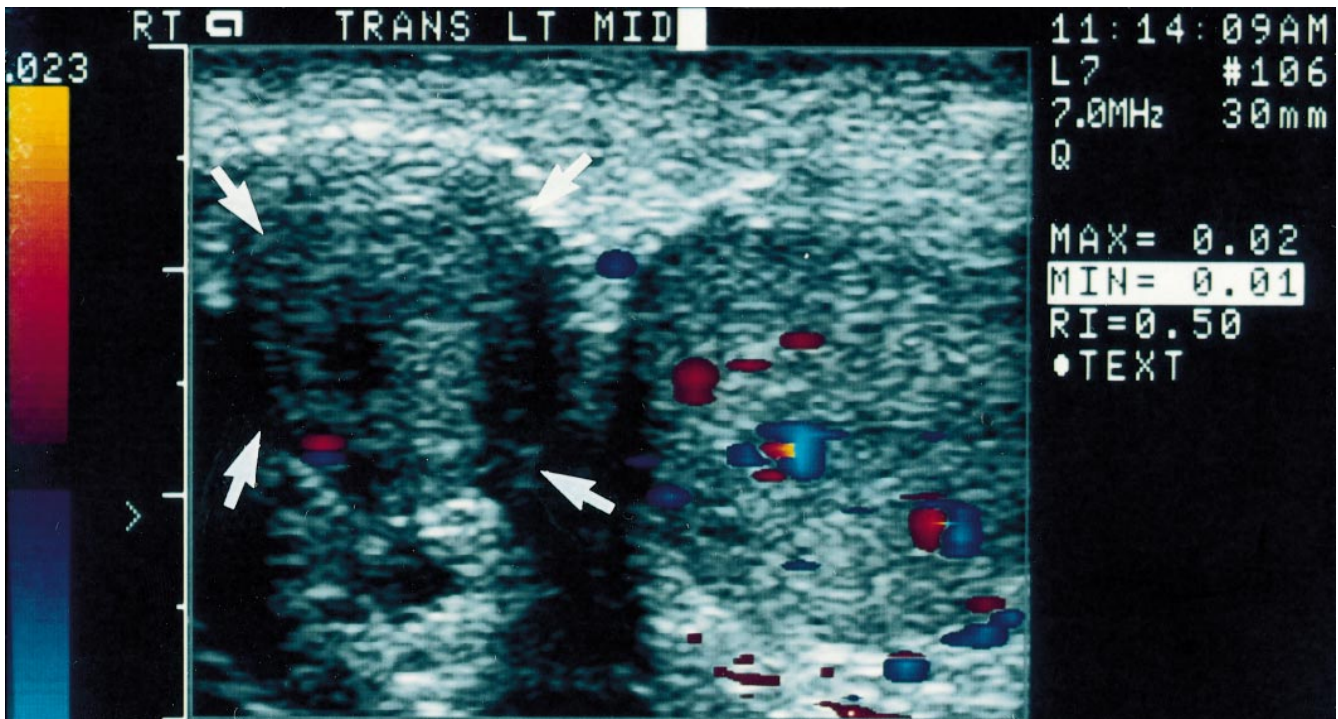
One patient had histological evaluation of the testis before and 3 months after a TESE procedure. The initial, baseline evaluation demonstrated late maturation arrest with numerous

spermatids. During the first TESE procedure, many spermatozoa were retrieved and injected. At 3 months after TESE, predominantly Sertoli-cell-only tubules were seen, with the most advanced spermatogenic cell being spermatogonia. The second TESE procedure did not retrieve spermatozoa. A third TESE procedure, delayed >6 months after the second TESE attempt, resulted in sperm retrieval for injection and a subsequent live delivery.

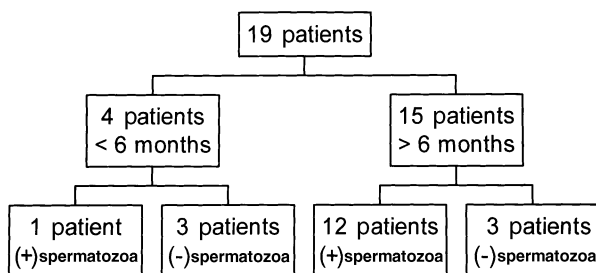
### Discussion

Retrieval of spermatozoa from men with NOA is now possible for some patients using the TESE procedure. This has provided a new treatment option for these men with testicular failure, who previously could only consider donor insemination of their wife or adoption as the potential routes to fatherhood. An understanding of the physiological effects of TESE on the testis and spermatogenesis may help to improve the delineation of the risks of this procedure as well as to plan repeat TESE procedures to optimize the chances of successful sperm retrieval.

Spermatogenesis can be affected by many conditions including varicoceles, cryptorchidism, genito-urinary infections as well as many environmental factors including heat, chemical exposures and drug use. Osegbe (1991) demonstrated that patients with unilateral epididymo-orchitis have focal areas of hemorrhage as well as infiltration with polymorphonuclear and mononuclear cells on testis biopsies taken during the acute phase of infection. Interestingly the contralateral, non-infected testicle on biopsy did not demonstrate the same inflammatory changes; however, a reduction in the population of germ cells as well as reduced spermatogenesis and spermiogenesis was seen. It was therefore postulated that unilateral inflammatory changes may adversely affect contralateral testicular function. Testicular surgery can also produce inflammation which may



**Figure 3.** Colour Doppler scrotal ultrasound in a man with symptomatic right testicular atrophy 3 months following a testicular sperm extraction procedure. A large devascularized region in the right testis (outlined by arrows) is observed.



**Figure 4.** The effect of interval between repeat testicular sperm extraction procedures is illustrated for men who have had an initial, successful sperm retrieval for non-obstructive azoospermia.

impair sperm development. In an animal model, Del Vento *et al.* (1992) found that transient sonographic changes (i.e. decreased echogenicity of testicular parenchyma at biopsy sites) and histological alterations (i.e. leukocyte infiltration at the site of biopsy) are detectable up to 1 month following testicular biopsy in stallions.

In this study we evaluated the physiological consequences of TESE on testicular function in men with non-obstructive azoospermia. We found that despite an outwardly normal scrotal examination, 82% (14 of 17) of patients had intratesticular abnormalities present on ultrasound suggestive of persistent haematoma and/or inflammation as long as 3 months following TESE. These findings were characterized by sonography as hypoechoic areas or increased heterogeneity of the testicular parenchyma. Hypoechoic regions and diffusely increased heterogeneity are characteristic of intratesticular bleeding associated with trauma (Anderson *et al.*, 1983; Fournier, *et al.*, 1985; Corrales *et al.*, 1993). The majority of these hypoechoic lesions were transient and appeared to resolve by 6 months after TESE,

leaving linear scars or calcifications visible on sonography. The persistence of what we have described as 'chronic findings' on ultrasound, as late as 1 year post-TESE procedure in five of the patients, suggests that these changes represent complete resolution of the inflammatory response associated with TESE rather than ongoing acute inflammation. Knowledge of these findings is important since the hypoechoic region on testis biopsy may be confused with the sonographic appearance of a testis tumour.

We chose to evaluate patients at 3 month intervals after TESE because of the nearly 3 month duration of spermatogenesis. Since active inflammation is frequently present within the testis at 3 months and has nearly always resolved at 6 months after TESE, we compared the success of sperm retrieval in TESE procedures performed <6 months with those performed >6 months after a prior successful TESE procedure. It was clearly shown that sperm retrieval was unlikely at an interval of <6 months after a prior TESE attempt, but highly likely (80%) >6 months after a prior successful TESE procedure. For the 15 couples who waited >6 months after the initial TESE procedure, ongoing pregnancy or live births were achieved for seven, one couple had a spontaneous abortion after clinical pregnancy, and one couple had an ectopic pregnancy.

In addition to the transient effects of a biopsy on spermatogenesis, permanent devascularization of the testis may occur after TESE procedures. Based on prior studies by Jarow (1991), we postulate that multiple incisions in the tunica albuginea used to retrieve spermatozoa may result in interruption of a sufficient proportion of testicular arteries to devascularize the testis. Therefore, avoiding multiple incisions in the tunica albuginea as well as minimizing repeated TESE attempts

is important to avoid the risk of permanent ischaemic testicular injury from TESE. It is also of concern that one patient who had spermatozoa retrieved on a prior TESE procedure was subsequently found to have extensive testicular fibrosis. It is possible that the TESE procedure caused intratesticular bleeding and the extensive intratesticular fibrosis subsequently detected. There is little evidence that multiple, blind testicular needle aspirations have any less of a risk of testicular injury than an open biopsy with identification of testicular vessels.

Spermatogenesis is an intricate and delicate process which is easily influenced by its environment. Although normal men produce 80–100×10<sup>6</sup> spermatozoa per day, men with non-obstructive azoospermia barely produce any spermatozoa. Any adverse effect on testicular function for a man with NOA will be likely to ablate the marginal level of sperm production present in such a man. The use of an open biopsy technique with optical magnification may maximize identification of sub-tunical vessels and minimize the risk of inadvertent vascular injury to the testis. We also recommend that at least 6 months elapse before a repeat TESE procedure is performed. The banking of frozen testicular tissue or spermatozoa retrieved during TESE procedures is advised to decrease the need for repeat TESE attempts and minimize injury to the testis. Gil-Salom *et al.* (1996) reported on 18 ICSI cases using frozen-thawed testicular spermatozoa, obtained primarily from men with obstructive azoospermia. The relative success of ICSI using freshly retrieved testicular spermatozoa versus frozen-thawed testicular spermatozoa for men with non-obstructive azoospermia in larger subsequent studies will elucidate the relative value of fresh versus frozen testicular spermatozoa.

Transient adverse physiological effects on the testis are common following TESE procedures. Organization and resolution of a haematoma and its associated inflammation frequently occur after TESE, and an associated detrimental effect on spermatogenesis may continue for several months. Although spermatozoa may occasionally be retrieved early after a prior TESE attempt, the frequent finding of intratesticular changes after TESE suggests that it would be prudent to avoid repeat TESE procedures on the same testis within 6 months, which would optimize the chance of sperm retrieval. Patients scheduled for TESE procedures should be counselled regarding the potential for testicular devascularization, especially if multiple biopsies are required.

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