Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision

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Testicular sperm extraction (TESE) is often an effective method for sperm retrieval from men with non-obstructive azoospermia. However, TESE has been a blind procedure that does not identify the focal sperm-producing areas of the testicle until after tissue has been excised from the patient. Experience with a new technique of microdissection of testicular tubules is presented here that identifies sperm-containing regions before their removal. Identification of spermatogenically active regions of the testicle is possible by direct examination of the individual seminiferous tubules. The underlying concept for this technique is simple: seminiferous tubules containing many developing germ cells, rather than Sertoli cells alone, are likely to be larger and more opaque than tubules without sperm production. In a sequential series of TESE cases for men with non-obstructive azoospermia, the ability to find spermatozoa increased from 45% (10/22) to 63% (17/27) after introduction of the microdissection technique. Microdissected samples yielded an average of 160,000 spermatozoa per sample in only 9.4 mg of tissue, whereas only 64,000 spermatozoa were found in standard biopsy samples that averaged 720 mg in weight (P < 0.05 for all comparisons). For men where microdissection was attempted, successful identification of enlarged tubules was possible in 56% (15/27) of cases. However, spermatozoa were retrieved with microdissection TESE for six men in whom sperm retrieval was unsuccessful with standard TESE approaches (35% of all men with spermatozoa retrieved). These findings suggest that microdissection TESE can improve sperm retrieval for men with non-obstructive azoospermia over that achieved with previously described biopsy techniques.

Introduction

Testicular sperm extraction (TESE) can provide spermatozoa from some men with non-obstructive azoospermia for intracytoplasmic sperm injection (ICSI). However, spermatogenesis is limited in men with non-obstructive azoospermia. Failure of testicular sperm extraction may occur in up to 57% of TESE attempts for men with non-obstructive azoospermia (Devroy et al., 1995; Kahraman et al., 1996; Friedler et al., 1997; Schlegel et al., 1997; Rosenlund et al., 1998). Failure to extract spermatozoa is more common if the semen is carefully examined immediately before attempted testicular sperm extraction. For men with non-obstructive azoospermia documented on multiple prior semen analyses, rare viable spermatozoa can be isolated from the ejaculate in up to 35% of cases using an extensive evaluation of the centrifuged semen specimen on the day of planned TESE (Ron-El et al., 1997). Since testicular sampling can be avoided for some men with non-obstructive azoospermia if a testis biopsy is deferred until the time of a programmed in-vitro fertilization (IVF) cycle, an approach of simultaneous TESE–ICSI can help prevent unnecessary surgery for sperm extraction. However, this treatment protocol may result in selection of even more difficult cases that remain for attempted TESE. Removing men with intermittent azoospermia or cryptozoospermia from treatment with TESE will make sperm retrieval even less likely to occur for the remaining selected patients who ultimately require sperm retrieval.

The approach of planned biopsy and cryopreservation before an IVF cycle can avoid the need for ovarian stimulation in cases where no spermatozoa are retrieved with biopsy. However, intentional biopsies for cryopreservation may be unnecessary if failure of ovarian stimulation is encountered in older women, or if spermatozoa from the ejaculate were adequate for ICSI without a biopsy, as noted above. In addition, extensive multi-biopsy approaches may be needed before spermatozoa are found in non-obstructive azoospermia (Tournaye et al., 1996; Ostad et al., 1998). Some investigators have applied fine needle aspiration for retrieval of spermatozoa from men with non-obstructive azoospermia. However, most investigators accept that open biopsies are required to achieve an optimal chance of finding the rare foci of testicular spermatozoa present within these poorly functioning gonads (Friedler et al., 1997; Rosenlund et al., 1998).

Several approaches have been described for isolation of the rare spermatozoa present in the testes of men with complete azoospermia and limited sperm production. Some authors have suggested a single large biopsy, others perform multiple limited biopsies, and other investigators have reported excising a majority of the volume of the testis in an effort to sample enough tissue to find spermatozoa (Oates et al., 1997; Silber et al., 1997; Ostad et al., 1998). Processing of specimens with erythrocyte-lysing buffer or enzymatic dispersion after biopsy removal have also been described (Crabbé et al., 1997; Nagy...
Figure 1. Optically magnified view of the initial incision in the tunica albuginea, as seen through an operating microscope. Note that the overall testicular length is only 1.5 cm, as is true for many men with non-obstructive azoospermia.

Figure 2. Intraoperative high-powered (×20–25) view of testicular parenchyma during attempted microdissection testicular sperm extraction. Arrows indicate enlarged tubules containing normal sperm production. Arrowheads indicate areas with sclerotic or Sertoli cell-only tubules. Scale bar = 350 µm.

Figure 3. An isolated region of normal-appearing tubules with spermatogenesis outlined by arrows. The adjacent testicular tissue contains primarily sclerotic tubules with no spermatogenesis. The surface of the tunica albuginea is labelled. This sperm extraction was carried out through a previously explored biopsy site, documented to contain active spermatogenesis. Microdissection allowed sperm retrieval without further extensive biopsies when no further frozen spermatozoa were available from the previous testicular sperm extraction procedure. Scale bar = 200 µm.

Figure 4. An isolated region of morphologically normal spermatogenic tubules is seen within an otherwise poorly functioning testis. The normal seminiferous tubules are outlined by the black and white arrows. The segment of the testis with normal tubules is located adjacent to the cut edge of the tunica albuginea. The tubules with spermatogenic tubules are extracted by traction on this region. Scale bar = 250 µm.

et al., 1997). Multiple testicular biopsies could result in the loss of significant amounts of testicular tissue, and can also interrupt the testicular blood supply that travels under the tunica albuginea, with risks of complete testicular devascularization and subsequent atrophy to the testis (Schlegel and Su, 1997). Scientific approaches to the isolation of small foci of sperm production within the testicle have not been defined.

In this study, a novel but conceptually simple approach to the identification and removal of the restricted areas of sperm production present in most men with testicular failure is described. Seminiferous tubules that contain only Sertoli cells without any germ cells are thinner than tubules containing a full complement of spermatogenic cells. The larger volume of intratubular germ cells within those tubules that have active spermatogenesis cause those tubules to be larger and more opaque, or whiter, than tubules without sperm production. The difference between the larger and smaller tubules is not visible without optical magnification. However, direct identification of sperm-producing tubules is possible with optical magnification at ×20–25 power. We have applied this concept of differential identification of tubules with excision of selected enlarged tubules in an attempt to improve sperm yield during TESE procedures for non-obstructive azoospermia. We present our results using this microdissection technique to enhance sperm retrieval during 27 attempted TESE procedures. Results from microdissection TESE were compared with results from the 22 consecutive prior sperm retrieval attempts using standard multiple biopsy TESE techniques.
Materials and methods

A sequential series of 49 men with non-obstructive azoospermia underwent attempted TESE in conjunction with a simultaneous programmed IVF–ICSI cycle. All men were confirmed to have non-obstructive azoospermia based on histological analysis of fixed testicular tissue specimens, stained with haematoxylin and eosin. Any treatable conditions were addressed before attempted sperm retrieval, including correction of hormonal abnormalities. No patients were excluded from treatment based on prior diagnostic biopsy evaluation. The presence of azoospermia was documented on multiple semen specimens, all processed with centrifugation at 1800 g and extensive, careful examination of the resuspended pellet. A repeat analysis was also performed on the morning of the planned sperm retrieval procedure. TESE procedures were cancelled if any viable spermatozoa were present in ejaculated or previously frozen testicular specimens. The initial 22 procedures were performed with a standard multi-biopsy approach and compared with results from a sequential series of 27 attempts at sperm retrieval in men with non-obstructive azoospermia using microdissection. The latter group of 27 men also had standard biopsies performed.

Standard biopsy

The technique for standard biopsies has been described previously in detail (Schlegel et al., 1997; Ostad et al., 1998). Briefly, a large (250–750 mg, depending on testicular volume) biopsy was obtained through a single incision after exploration in the testis that was larger or showed better histological evidence of spermatogenesis in diagnostic biopsies. Each biopsy sample was placed in simulated human tubal fluid (HTF) medium supplemented with 6% plasmanate (Schlegel et al., 1996), and dispersed by isolating individual seminiferous tubules with glass slides and mincing individual tubules. The resulting suspension from this tissue dispersion was further disrupted by passing the tissue suspension several times through a 24-gauge angiocatheter. Previous studies have demonstrated that this approach may improve sperm yield from testicular tissue obtained from men with non-obstructive azoospermia (Ostad et al., 1998). Each tissue sample was analysed by placing a microdroplet of dispersed tissue suspension in a disposable cell-counting chamber (Cell-Vu, Cat. No. DR600; Erie Scientific Co., Erie, PA, USA) and quantifying the presence and number of spermatozoa present. If no spermatozoa were present in the initial sample, then subsequent samples were taken from the same testis and subsequently from the contralateral testis until either (i) spermatozoa were identified, or (ii) further biopsy incisions were deemed likely to impair the testicular vasculature.

Microdissection TESE

The procedure for direct microscopic identification of functioning seminiferous tubules is referred to as microdissection TESE. As with the standard multi-biopsy approach, optical magnification (×6–8 power) was used to visualize blood vessels under the surface of the tunica vaginalis, allowing placement of biopsy incisions in avascular regions of the testis. However, instead of planning for multiple incisions in the tunica albuginea, an attempt was made to open widely the tunica albuginea near its midportion to optimize visualization of the testicular parenchyma without affecting the testicular blood supply (Figure 1). Direct examination of the testicular parenchyma was then carried out at ×20–25 magnification under the operating microscope. During this evaluation, an attempt was made to identify individual seminiferous tubules that were larger than other tubules in the testicular parenchyma. The typical appearance of seminiferous tubules in the testes of a man who had microdissection applied is shown in Figures 2 and 3. This examination was performed through as much of the testicular parenchyma as possible. Small (2–10 mg) samples were excised sharply from tubules that were larger and typically more opaque (whiter) (Figure 4). For each microdissected sample, the tissue around this area was then excised as a standard biopsy to evaluate the efficiency of sperm retrieval with microdissection compared with standard biopsy techniques. Each excised testicular tissue specimen was further cut into smaller pieces to allow spermatozoa to be released from the inside of the seminiferous tubules. The resulting suspension was examined as described above for standard biopsies. Additional incisions in the same or contralateral tests were made to find spermatozoa. The procedure was terminated when spermatozoa were retrieved or further biopsies were thought likely to impair the blood supply of the testis.

If no morphologically normal tubules were identified, then any tubules that differed from the remainder of the tissue in their size were excised as microdissected samples. If all tubules were seen to have an identical morphological appearance, then microdissection was not performed and standard biopsies were performed. All surgical procedures were consecutive and performed by one surgeon.

Controlled comparison of biopsy procedures

Excision of microdissected samples was compared with standard biopsies from the surrounding areas of testicular tissue based on the number of spermatozoa retrieved and the volume of testicular biopsy excised. The statistical significance of differences in number of spermatozoa and volume of tissue excised in these directly linked samples was analysed using paired t-tests. The proportion of men with spermatozoa retrieved with standard biopsy extraction or microdissection TESE followed by standard biopsies (as needed) was compared using chi-square analysis on computerized software (Excel, Microsoft Corp., Redmond, WA, USA).

Results

Differential identification of enlarged tubules within the testicular parenchyma was possible in 56% (15/27) of attempted microdissection TESE procedures. Application of the microdissection technique resulted in an improvement in sperm retrieval rates from 45% (10/22) per TESE attempt using only standard multi-biopsy techniques to 63% (17/27) with standard biopsy and microdissection attempted in sequential series of TESE procedures (P < 0.05). For the series of 27 patients who underwent a controlled comparison of standard large biopsies and microdissection, spermatozoa were found only with microdissection, but not in standard biopsies in 6/17 (35%) men with sperm found. For these men, spermatozoa would not have been available for ICSI if only standard biopsies had been performed. If these six patients had not had sperm retrieval with microdissection TESE, then the overall sperm retrieval rate would have been only 41% (11/27), similar to that observed in our historical control cases. Spermatozoa were found only with large random (control) biopsies, but not by microdissection in 1/17 (6%) cases. For another 10 men, spermatozoa were found with both microdissection and control biopsies, but retrieval was quantitatively improved with the optical magnification technique.

Average retrieval per sample was estimated at 160 000 ± 270 000 (mean ± SD) spermatozoa for microdissected samples and 64 000 ± 52 000 spermatozoa in adjacent standard biopsies, despite removal of 720 mg of tissue in standard biopsies compared with 9.4 mg in microdissected samples (P < 0.01.
and < 0.0001, respectively). No acute or chronic complications were noted after TESE for the 49 patients in this sequential series of TESE procedures.

Spermatozoa retrieved with microdissection resulted in fertilization of oocytes after 95/146 (65%) of attempted ICSI procedures, whereas only 51/98 (52%) of oocytes injected with spermatozoa in the control series of 22 attempted standard TESE procedures were fertilized (P = 0.01). The technique used for ICSI did not differ between the two sequential series of attempted TESE procedures.

Discussion

The discovery of the microdissection technique was an unintended result of observations made during TESE procedures. Based on our concerns about changes that occur within the testis after TESE, we began to use the operating microscope to examine the surface of the testis for subtunical vessels before making biopsy incisions (Schlegel and Su, 1997; Goldstein, 1998). After observing the testicular parenchyma with high-power magnification, it became obvious that individual seminiferous tubules could be examined and qualitative differences between those tubules were seen. The presence of larger tubules was apparently the result of different germ cell content within those larger tubules. These observations were then verified using quantitative analysis of the sperm content of tubules selected by microdissection, and these findings were compared with results obtained from adjacent tubules, selected at random by a standard biopsy sample.

Excision of testicular tissue can be limited with maximized yield of spermatozoa for men with non-obstructive azoospermia if a microdissection technique is used during TESE. Optical magnification allows identification of avascular regions of the testis to minimize risks of testicular injury. Despite removal of 70-fold smaller volumes of testicular tissue, sperm retrieval was better with small, microdissected tissue specimens when compared with standard biopsies. Microdissection requires the use of an operating microscope, and the technique initially requires time to examine the testicular parenchyma in detail. Moreover, experience with the microdissection approach is needed for the effective identification of larger tubules. However, the use of an operating microscope does not substantively change the procedure. All TESE procedures discussed in this manuscript were performed in an outpatient setting, and cases were conducted under local or general anaesthesia based only on patient preference.

Microdissection is easier to apply in men with smaller testes, for example those with classical Klinefelter’s syndrome. For these cases, a relatively small surface of testicular parenchyma is available for examination during microdissection. Microdissection is also easier to apply when the difference between tubular diameter is more prominent, as when a Sertoli cell-only pattern predominates throughout the testis. However, at least one case involving a man with predominantly maturation arrest was successfully treated with microdissection because the limited regions of sperm production were only identified under the microscope, and none of the standard, random biopsies contained spermatozoa. With microdissection, the ability to extract spermatozoa from more men with non-obstructive azoospermia means that a significant proportion of men who would have had unsuccessful IVF cycles, or had to revert to the use of donor spermatozoa, may have a new opportunity to achieve their goal of becoming the genetic fathers of children.

Microdissection TESE has several other potential advantages. The removal of standard or large biopsies burdens the embryology laboratory with the need for an extensive cell-by-cell search for spermatozoa through large volumes of testicular tissue in the laboratory. This incredibly labour-intensive search may possibly miss the rare and relatively small spermatozoa within a sea of seminiferous tubules and other cells. In addition, excision of multiple biopsy samples during TESE results in an impairment of testosterone production for up to one year after TESE (Manning et al., 1998.) Long-term follow-up of patients after microdissection TESE is underway to evaluate whether subtle changes in testicular function occur after this refined approach, in comparison with what has been seen previously after standard multi-biopsy TESE. To date, no apparent complications have occurred from the wide incision in the tunica albuginea, even when patients are followed with serial ultrasound examinations after TESE, as is routinely performed for our patients (Schlegel and Su, 1997.) The absence of complications may well be related to the fact that the incisions are made in an avascular region of the tunica albuginea, since the subtunical vessels can be identified and avoided. Therefore, the microdissection technique may prove safer than current multi-biopsy approaches, although the goal of the present study was only to demonstrate the efficacy and applicability of the technique.

Several groups have used fine needle aspiration for sperm retrieval in men with obstructive and non-obstructive azoospermia. This technique provides diagnostic information and reliable sperm yield in obstructive azoospermia with a minimally invasive technique (Craft et al., 1997). However, the number of spermatozoa retrieved is quantitatively limited (Sheynkin et al., 1998). Several controlled studies comparing fine needle aspiration with open testicular biopsy for men with non-obstructive azoospermia have demonstrated that open biopsy is more likely to retrieve spermatozoa than fine needle aspiration (Friedler et al., 1997; Rosenlund et al., 1998). In order to provide spermatozoa from the highest number of men with non-obstructive azoospermia, open biopsy should be performed either primarily or, if spermatozoa are not found, with fine needle aspiration. The results in this manuscript suggest that microdissection enhances sperm yield with open biopsies.

Microdissection may be the optimal initial approach for TESE in men with non-obstructive azoospermia. Standard biopsies can be applied for cases where enlarged spermatozoa-containing tubules are not identified during microdissection. The availability of microdissection appears to improve the frequency of sperm retrieval for men with non-obstructive azoospermia, despite the removal of dramatically less testicular tissue.

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References


