Symposium

Surgical sperm retrieval: Techniques and their indications

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ABSTRACT

Men with azoospermia can father a child through intra-cytoplasmic sperm injection if sperm can be retrieved from their epididymis or testis. Several percutaneous and open surgical procedures have been described to retrieve sperm. The various techniques and their merits are discussed in this review. In men with obstructive azoospermia, epididymal sperm can usually be retrieved by percutaneous epididymal sperm aspiration (PESA). If PESA fails then testicular sperm are obtained by needle aspiration biopsy (NAB). In men with non-obstructive azoospermia, there will be no sperm in the epididymis and testicular sperm retrieval is required. Percutaneous retrieval by NAB can be tried first. If that fails then testicular sperm extraction (TESE) from open microsurgical biopsies is performed using the single seminiferous tubule (SST) or the microdissection TESE techniques. The simplest, least invasive procedure should be tried first.

Key words: Azoospermia, epididymal, sperm retrieval, testicular

INTRODUCTION

In the past, the only options for treating azoospermia were reconstructive surgery (if there was obstruction) or donor insemination. Now, the advent of intra-cytoplasmic sperm injection (ICSI) has enabled many azoospermic men to become biological fathers using sperm obtained from their epididymis or testis. Pregnancy rates with ICSI are the same whether ejaculated, epididymal or testicular sperm are used.[1]

Epididymal sperm retrieval for *in vitro* fertilization (IVF) was first described for a case of secondary obstructive azoospermia.[2] The authors used a micro-pipette to puncture the exposed epididymal ductule and aspirate sperm. Subsequently, Silber popularized an open technique for men with vas aplasia, in which the epididymal ductule was dissected microsurgically, opened, aspirated, and then sutured close (MESA).[3] We modified this procedure by directly aspirating sperm from the exposed epididymis with a simple 26-G needle without any microsurgical dissection or suturing (OFNA).[4] Shrivastava *et al.*, described an alternative, simpler procedure in which the aspiration was performed percutaneously (PESA).[5]

Testicular sperm were first used for IVF and ICSI for men with obstructive azoospermia.[6-8] Subsequently, Devroey *et al*,.[9] showed that even in men with testicular failure sperm could be retrieved from the testis in many cases and ICSI successfully performed. Accordingly, men with testicular failure were subjected to multiple open biopsies in an attempt to locate focal areas of spermatogenesis. However, several studies showed that multiple conventional biopsies damaged the testes.[10,11] Hence, instead, Schlegal and Li[12] proposed selective micro-biopsies, using magnification to identify favorable seminiferous tubules (microdissection-testicular sperm extraction (TESE)), as a technique for taking extensive biopsies while minimizing damage. Subsequently, we described an alternative method of acquiring multiple micro-biopsies, while causing less trauma, by taking single seminiferous tubule biopsies through puncture holes in the tunica (SST technique).[4] Recently, the use of ultrasound has been described both for avoiding blood vessels during percutaneous procedures and for indentifying areas of increased vascularity that may be more likely to harbor sperm.[13,14]

This review will describe and compare the various methods of epididymal and testicular sperm retrieval.

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INDICATIONS FOR OPERATIVE SPERM RETRIEVAL

Retrieval of epididymal or testicular sperm for ICSI is indicated in the following cases:

- Obstructive azoospermia – when reconstruction has failed, or is not possible (e.g. vas aplasia or multiple blocks following tuberculosis), or if the couple chooses ICSI over surgery.
- Non-obstructive azoospermia - in those men who have areas of focal spermatogenesis.
- Failure to ejaculate during an ICSI procedure (if vibrator stimulation fails and electro-ejaculation is not available).
- Total astheno-/necrozoospermia - when all sperm are immotile they may be viable but immotile, or non-viable and hence immotile. In case of viable, non-*motile* sperm, these can be identified by the hypo-osmotic swelling test[15] and used for ICSI. However, in case of necrozoospermia (non-viable, immotile) it is necessary to use sperm aspirated from the testis since these are usually viable and may sometimes even be motile.[16]

SPERM RETRIEVAL TECHNIQUES

In men with obstructive azoospermia, sperm may be retrieved from the epididymis and/or the testis, while in men with non-obstructive azoospermia only testicular sperm retrieval procedures are useful.

The various procedures are listed in Table 1.

OPERATIVE TECHNIQUES

Microsurgical epididymal sperm aspiration[17,18]

Technique

The epididymis is exposed through a scrotal incision. Under an operating microscope the epididymal tunica is incised and an epididymal ductule is mobilized. The ductule is opened and the spermatic fluid that flows out is aspirated. Once enough sperm are recovered the ductule is closed with microsutures. If no sperm are found another ductule is dissected.

Advantages

Microsurgical visualization of the epididymis allows for precise, blood-free aspiration from multiple locations. A large number of motile sperm can be recovered and cryopreserved for future cycles of ICSI. Microsurgical handling of the ductule may preserve it for future repeat aspiration, if required.

Disadvantages

This is a time-consuming and demanding procedure that needs an operating microscope and a trained andrological microsurgeon. There is no evidence that closing the ductule microsurgically improves chances of future retrieval.

Open fine needle aspiration[19]

Technique

The epididymis is exposed and a ductule is directly punctured through the tunica, without any dissection, using a 26-G needle. Epididymal fluid is aspirated from the ductule. On withdrawing the needle, epididymal fluid continues to flow out of the punctured ductule and is aspirated from the epididymal surface. The ductular opening is not sutured.

Advantages

As in MESA, spermatic fluid can be aspirated under vision from different locations, thus obtaining the maximum number of sperm. Since no microsurgical dissection or suturing is involved, the procedure is very quick, does not need special equipment or training, and can be performed under local anesthesia in the operation theatre of the IVF unit.

Disadvantages

It is an open surgical procedure

Microsurgical epididymal sperm aspiration versus open fine needle aspiration

Since OFNA offers the advantages of MESA without its cumbersomeness it is the preferred technique when open epididymal aspiration is required.

Table 1: Sperm retrieval procedures

Procedure type	Open	Percutaneous
Epididymal	MESA; OFNA	PESA
Testicular	Conventional, MD-TESE, SST	TESA; NAB; Tru-cut

MESA = Microsurgical epididymal sperm aspiration; OFNA=Open fine needle aspiration; PESA=Percutaneous epididymal sperm aspiration; MD-TESE=Microdissection testicular sperm extraction; SST=Single seminiferous tubule biopsy; TESA=Testicular sperm aspiration; NAB=Needle aspiration biopsy

PERCUTANEOUS EPIDIDYMAL SPERM RETRIEVAL

Percutaneous epididymal sperm aspiration[20]

Technique

The scrotum is cleaned with antiseptic and then thoroughly washed with saline to eliminate any residual antiseptic. Under local anesthesia[21] the head of the epididymis is palpated and stabilized between thumb and forefinger. It is then punctured, directly through the scrotal skin, with a 26-G needle attached to a tuberculin syringe containing 0.1 ml of sperm-washing medium. An air bubble is kept between the medium and the rubber stopper of the plunger to prevent direct contact between the rubber and medium. The assistant pulls the plunger all the way to the top of the syringe thus creating a suction force. The needle is gently and slowly advanced through the epididymal ductule. The syringe is rotated 180 degrees and then withdrawn partially, staying within the epididymis. It is then advanced in a different direction, all the while maintaining suction. The

suction is partially released and the needle is withdrawn from the epididymis. Sometimes droplets of fluid can be seen entering the syringe, but on other occasions the epididymal aspirate may be so thin and scanty that there is no visible aspirate. The contents of the syringe are gently flushed into a dish and examined for the presence of sperm. If motile sperm are not seen the procedure is repeated at a slightly different location on the epididymal head (in obstructive azoospermia, better sperm are obtained from the proximal epididymis rather than the distal body or tail). Since this is a blind procedure sometimes several attempts are required before good quality sperm are found.

Some authors perform PESA using a larger butterfly needle[20] and a 20-ml syringe. The droplets of aspirated fluid can be easily seen in the tubing.

Advantages

The procedure is simple, quick, avoids open surgery and can be repeated.[20] We have had several cases in whom we have repeated the procedure two to four times and obtained adequate sperm each time.

Disadvantages

Since the epididymis is not visualized the location of puncture is guided by palpation alone and cannot be precisely controlled. As a result, occasionally the sperm-containing ductule may be missed. We have had occasional cases of vas aplasia where very little epididymis was present and was covered by fat: as a result PESA failed, but subsequent OFNA could retrieve motile sperm. Further, there is the possibility of puncturing a blood vessel and contaminating the sample with red blood cells.

Open versus closed epididymal sperm retrieval

Since PESA can obtain enough sperm for ICSI and for cryopreservation there is no reason to subject a patient to an open surgical procedure. If PESA fails then OFNA can be performed. Alternatively, percutaneous testicular sperm retrieval can be done to avoid an open procedure.

Testicular sperm aspiration[22]

Technique

This is like an aspiration cytology procedure. Under local or general anesthesia, a 22-G butterfly needle is jabbed around the testicular substance while applying suction with a 20-ml syringe. The aspirated fluid is checked for sperm. TESA is primarily useful in men with obstructive azoospermia,[23] but has also been used for men with non-obstructive azoospermia.[24] Color Doppler ultrasonography has been used to guide the aspiration so as to avoid blood vessels and reduce hematoma formation.[13]

Advantages

This is a simple, non-surgical procedure that can be performed without special training or equipment.

Disadvantages

Since this is a blind procedure there is the risk of puncturing a tunical vessel and causing a hematocele. Multiple passages of the needle through the testicular tissue damage a large number of seminiferous tubules and could cause intra-testicular hemorrhage. The total amount of cellular material is scanty and in our experience, and that of others,[25] TESA has often failed to recover sperm in men with non-obstructive azoospermia in whom sperm could be found when the testicular biopsy was examined.

Needle aspiration biopsy [19]

Technique

An 18-G scalp vein needle is introduced into the testis under local or general anesthesia. Once the needle enters the tunica, suction is applied using a 10-ml syringe. The needle is pushed in up to its hub, then pulled partly out (staying within the tunica) and then pushed in again. The needle may be rotated 180 degrees (to cut the tissue) and the out-and-in motion of the needle is repeated a few times till the aspirate is seen in the tubing of the needle. The tubing is then clamped to prevent tissue from being sucked into the syringe and the needle is withdrawn slowly out of the testis. As the needle emerges from the scrotal skin, a loop of the seminiferous tubule is pulled out. This is grasped with non-serrated microsurgical forceps and more tissue is pulled out of the testis. A varying amount of tubule can be extracted in this manner, depending on its quality. The syringe is disconnected, air aspirated, the syringe reconnected, the clamp removed, and the contents of the scalp vein needle are flushed into a dry dish. This results in recovery of a moderate amount of fluid (testicular aspirate, corresponding to TESA) and an additional amount of testicular tissue which is added to the tissue initially pulled out with the forceps. Both the fluid and the tissue are checked for sperm. Additional NAB biopsies are done if the initial tissue is scanty or if no sperm are found. This technique can be used both for diagnosis and therapeutic retrieval. There will be some loss of tubular architecture but sufficient detail is preserved for diagnostic histopathology. Marmar and Benoff have described a similar technique utilizing an 18-G angiocath. Further, they used an ultrasound probe to identify and avoid major blood vessels, thus preventing any hematoma formation in 78.3% of their patients.[26]

Advantages

This is a simple, quick, incisionless procedure that can obtain tissue equal to an open biopsy. Technically, it is similar to TESA, but its cellular yield is many times greater.

Disadvantages

Since it is a blind procedure with a large needle it carries the risk of producing a hematocele, of causing intra-testicular hemorrhage, or damaging the epididymis. Further, since the testis is not visualized, multiple biopsies cannot be plotted as accurately as when doing open biopsies.

Testicular sperm aspiration versus needle aspiration biopsy

Both TESA and NAB can retrieve enough sperm in cases of obstructive azoospermia; however, the NAB procedure provides both a TESA sample and a tissue biopsy and hence yields far more sperm than TESA. Hence, NAB is preferred over TESA, especially in cases where spermatogenesis is impaired. In a study of 34 men with non-obstrcutive azoospermia Friedler *et al.*,[27] could recover sperm in only 11% by needle aspiration as compared to 43% by TESE. Similarly, Ezeh *et al.*,[28] could retrieve sperm by an open biopsy in 17 of 22 men in whom aspiration had failed. In our experience, a NAB specimen has often shown sperm when the TESA sample from the same site had none.

Cutting needle biopsy

Technique

A testicular biopsy can also be obtained using a tissue-cutting biopsy needle[29] (e.g. Tru-cut needle or Biopty gun).

These dedicated biopsy needles are spring-activated. When the needle is placed against the testis and released, it enters the stroma, cuts a sliver of tissue and withdraws it into a sheath.

Advantages

This is a simple method that is routinely being used to biopsy a variety of tissues. It is particularly useful in men with testicular fibrosis in whom NAB may fail to retrieve sufficient tissue.

Disadvantages

Unlike the NAB technique in which a tubule is unraveled out of the testis, the biopsy needle cuts through a number of tubules thus causing more trauma. The amount of tissue recovered can be much less as compared to NAB. Also, these special needles represent an additional expense.

OPEN TESTICULAR SPERM RETRIEVAL

Conventional open biopsy

Technique

Through a scrotal incision the testis is exposed, the tunica is incised, and a piece of protruding testicular tissue is excised. The tunica is sutured and the incision is closed.

Advantages

It is an easy method that can be performed by any surgeon, and yields a good amount of tissue.

Disadvantages

It is an open surgical procedure. Further, during incision and closure of the tunica albuginea, and while excising the testicular tissue, the testicular vessels can be damaged. Testicular arteries are end-arteries, and hence multiple, conventional biopsies in men with testicular failure can demonstrably impair testicular function.[30,31]

Single seminiferous tubule – biopsy[4,19]

Technique

The scrotum is opened and the testis is exposed. An avascular area of the tunica is punctured with a 26-G needle. A prong of a micro-forceps is introduced into the puncture hole, dilating it till a loop of seminiferous tubule pops out. The seminiferous tubule is held with the micro-forceps, pulled out and examined under the operating microscope. If it seems favorable then more tubule is pulled out, excised, crushed and examined for sperm. If sperm are seen then additional tubule is pulled out from the same site. If no sperm are found, or if the tubule appears fibrous, then another area is punctured and another tubule is pulled out and examined. The procedure is repeated at multiple sites all over the testis till sperm are found or the entire testicular surface has been explored. There is no need to suture the tunica since each opening is very small. In very small testes, which are mainly fibrous, no tubule may pop out. In this situation an extended SST technique is used--the opening is widened a little so that both prongs of the micro-forceps can be pushed into the depth of the testicular tissue; the deeper tissues are grasped and the forceps is pulled out; fibrous tissue gets left behind and the better tubules are extracted.

Advantages

This technique allows extensive sampling of the testis without making any significant incision on the tunica. Only "healthy" tubules are biopsied. Since the tunica is not incised or sutured no blood vessel is damaged; hence, multiple biopsies can be taken without affecting the testis.

Disadvantages

It is an open surgical procedure. In the extended SST biopsy technique the deeper tubules are procured blindly.

Microdissection TESE - Microsurgical testicular sperm extraction[32]

Technique

The testis is exposed and a long incision is made in the tunica to expose the testicular parenchyma. The seminiferous tubules are gently separated and examined under an operating microscope. Fibrous tubules can be distinguished from "healthy fat" tubules that are more likely to contain sperm.[33]

Only the promising tubules are biopsied and examined for sperm. Dissection and biopsy are continued till adequate sperm are retrieved. The tunica is closed with a running prolene suture.

Advantages

Since only selective tubules are biopsied, less tissue needs to be removed resulting in less testicular damage. A large area of testicular tissue can be visually evaluated and biopsied, improving the chances of finding sperm in cases with

focal spermatogenesis, especially in men with Sertoli cell syndrome only and high follicle-stimulating hormone (FSH).[34] Ramasamy and Schlegel[35] reported retrieval of sperm by microdissection TESE in 56% of men in whom no sperm had been found in previous one to two biopsies, and in 23% of men with no sperm in previous three to four conventional biopsies.

Disadvantages

Though only a small amount of tissue is removed, the large tunical incision and the dissection of the testicular tissue can cause devascularization and fibrosis of the testis.

Single seminiferous tubule biopsy versus microdissection-TESE

Both techniques allow extensive sampling of the testicular tissue under vision. The SST technique samples the surface more efficiently, while the microdissection-TESE allows deeper sampling in one plane. The two techniques have not been compared. Since the SST technique is less traumatic (due to no incision on the tunica) we first use the SST technique and proceed to microdissection-TESE only if no sperm are found.

Open conventional biopsy versus microsurgical biopsy techniques

If a single biopsy shows sperm then the method of biopsy does not make a difference. However, in men with testicular failure, in whom multiple biopsies have to be done, microsurgical biopsy techniques - which minimize testicular damage while allowing extensive sampling - are preferred to the conventional open testicular biopsy. In a comparison of conventional multiple biopises versus the microdissection technique in 435 men with non-obstructive azoospermia, Ramasamy *et al.*[36] reported sperm retrieval rates of 32% and 57% respectively. Further, there were significantly fewer ultrasonically-detected acute and chronic testicular changes in the microsurgical group.

Percutaneous versus open testicular biopsy

The percutaneous NAB technique gives adequate tissue in most cases and is psychologically more acceptable to patients. The open methods (microdissection and SST) allow for extensive sampling and give the best chances of sperm recovery, especially when the testis is small and fibrotic. Hence, NAB should be tried first, followed by open biopsy if no sperm are found.

COMPLICATIONS OF SPERM RETRIEVAL PROCEDURES

The main potential complications of sperm retrieval procedures are hematoma,[10] infection, testicular parenchymal fibrosis and testicular atrophy. These can occur following both percutaneous and open procedures. After TESE, testosterone levels can drop by 20% or more and take over 12 months to recover.[36] Men with small compromised testes, who undergo multiple biopsies, are most at risk, and need to be monitored for the development of hypogonadism.[37]

ADDITIONAL CONSIDERATIONS DURING SPERM RETRIEVAL IN TESTICULAR FAILURE

Number of biopsies

How many biopsies should be taken before accepting that no sperm can be found? There is no consensus on this. However, published studies[38] and our experience suggest that in most cases where sperm are present some sperm will be found in the first four biopsies. Tournaye *et al.*,[39] reported the mean number of biopsies to retrieve sperm as 4.2 ± 4.5 in men with maturation arrest and as 2.8 ± 2.5 in men with Sertoli cell syndrome. A recent review of sperm retrieval techniques in non-obstrcutive azoospermia reported that most studies used one to four biopsies.[40] However, there will be the occasional outliers where sperm may be found after ten or more biopsies. Since micro-biopsies by the SST or microdissection-TESE technique are relatively non-traumatic we take six to 15 biopsies on each side (depending on the size of the testis) in our quest for sperm.

Predictors of finding sperm

If sperm were found in a previous biopsy then there is a high likelihood (but not a certainty) of finding sperm during subsequent TESE.[41-43]

If genetic studies show a Yq microdeletion in the AZFa or AZFb regions then sperm will not be found, while AZFc deletions are associated with a high chance of finding sperm.[44]

No other parameters can reliably predict whether sperm will be found on TESE. Sperm may be found in men with very small testes[45] and high FSH levels,[46] irrespective of the general histological pattern.

Cryopreserving scanty sperm in men with testicular failure

Since sperm will be found in only some men with testicular failure it would be ideal to first do a trial TESE and confirm the presence of sperm before subjecting the partner to the extensive hormonal stimulation required for IVF. However, this implies that it would be possible to cryopreserve the scanty sperm obtained in these men and retrieve them in adequate numbers after thawing, with no reduction in the fertilization and pregnancy rate as compared to fresh sperm. However, while it is generally accepted that sperm from the epididymis[47] and the testis[48] of men with obstruction (normal spermatogenesis) can be cryopreserved and used without affecting outcome, there is a significant decrease in motility and viability when testicular sperm are frozen and thawed,[49] and, hence, the recovery and use of sperm after cryopreservation in men with testicular failure who have

scanty sperm is still controversial. While several studies have shown equal results with fresh and frozen testicular sperm in men with non-obstructive azoospermia,[50,51] in the large study by Verheyen *et al.*[51] sperm could not be retrieved from the frozen sample in 20% of cases and in 30% of these failures sperm were not found during the second TESE procedure.

The fertilization and pregnancy rates are reported to be lower when testicular sperm from men with non-obstructive azoospermia are used as compared to testicular sperm obtained from men with obstruction and normal spermatogenesis.[52] However, a recent paper showed that when sperm are obtained from a non-obstructed testis are motile then the pregnancy rates are the same.[53] Hence, the recovery of motile sperm should be considered when evaluating the efficacy of testicular cryopreservation programs.

In practice, outcomes vary from laboratory to laboratory, and in India, some centers rely on a trial TESE with cryopreservation, whereas others prefer fresh TESE for men with testicular failure.

Role of special tissue processing methods

When very few sperm are present in the testicular tissue their detection may be facilitated if the tubules are properly lysed. Initially, this can be achieved mechanically by mincing the tissue and submitting it to multiple passes through a fine needle that is attached to a syringe.[12] Further, the tubules can be enzymatically dissected using collagenase.[54] Additionally, erythrocyte lysing buffer may be used to render the sample more clean.[55] Several studies have suggested that, particularly in men with testicular failure, incubating the testicular tissue for 24 to 72 h improves sperm motility[56] and may improve pregnancy rates.

Ultrasound-guided testicular sperm extraction

Recently, it has been suggested that the use of ultrasound to identify and biopsy areas having greater perfusion may yield better sperm.[14]

SUMMARY – CHOICE OF PROCEDURE

A recent Cochrane meta-analysis[57] of various techniques found "insufficient evidence to recommend any specific sperm retrieval technique". The authors' recommendation that the simplest and least invasive technique be used first matches our own experience and philosophy. Our approach is summarized below.

In obstructive azoospermia, PESA is the first choice. If PESA fails then OFNA or NAB is done.

In non-obstructive azoospermia, initially NAB is tried. If six to nine NAB biopsies from each side fail to procure adequate sperm, then multiple microsurgical biopsies by the SST method are taken bilaterally. If these do not reveal sperm then micro-TESE is tried. (If adequate tissue was obtained by NAB then we often do not proceed to open biopsies). A staged approach like this, starting with the least invasive technique, can help avoid open, more aggressive techniques in many cases.[58] There is no role for epididymal sperm retrieval in non-obstructive azoospermia.

When operative sperm retrieval is required for men with failure to ejaculate, a NAB biopsy will provide adequate number of sperm.

In men with total asthenozoospermia NAB is preferred to PESA since testicular sperm are more likely to be viable in these cases.

At centers where the sperm retrieval is performed by gynecologists, often TESA or NAB is used as the primary procedure in men with obstructive azoospermia, since the operator may not be comfortable doing a PESA. However, in cases of non-obstructive azoospermia it would still be worthwhile involving a urologist who could perform a microdissection-TESE or SST biopsy for the best chance of retrieval.

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