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Sperm Retrieval Techniques for the Management of Male Infertility

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ABSTRACT

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Infertility is an important public health problem faced by many couples in our country. The introduction of intracytoplasmic sperm injection (ICSI) for the treatment of male infertility and its success in azoospermic men demonstrating that the sperm retrieved from epididymis or testis were capable of normal fertilization and conception are the major achievements in the management of male infertility. This article reviews the various techniques for sperm retrieval which helps in the treatment of male infertility.

Keywords: Surgical Sperm Retrieval, Epididymal Techniques and Testicular Techniques, Complications of Sperm Retrieval Techniques and Contraindications

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INTRODUCTION

The social stigma and the mental trauma inflicted by infertility are so high that it is incorporated in the national reproductive and child health programme.[1] Male infertility accounts for 40-50% of infertility[2] and only 23% among these undergo treatment in our country.[3] Azoospermia is defined as the complete absence of spermatozoa in the ejaculate after centrifugation. Azoospermia is found in 1-3% male population and 10% of infertile males. Though associated with infertility azoospermic men retain sperm production at varying levels within the testis.[4] Sperm obtained by various retrieval techniques can be used in assisted reproductive techniques like in vitro fertilization (IVF) associated to ICSI.[5,6] Pregnancy rates with ICSI are the same whether ejaculated, epididymis or testicular sperm are used.[7]

Indications for Sperm Retrieval

Surgical sperm retrieval is indicated in a) obstructive azoospermia (OA) especially when reconstruction has failed or not possible due to vas deferens aplasia or multiple strictures due to tuberculosis or patient wishes, b) non-obstructive azoospermia (NOA) in patients with focal spermatogenesis, c) failed ejaculation during ICSI procedure or d) total astheno or necrozoospermia.[8] In case of immotile sperms, viable ones can be identified by hypo osmotic swelling test and used for ICSI.[9]

Evaluation of Patients Before Sperm Retrieval

The success rate and the type of sperm retrieval technique to be used depends on whether azoospermia is obstructive or non obstructive. Clinical history, physical examination, hormonal evaluation of FSH and testosterone levels are helpful in diagnosing around 90% of the type of azoospermia.[10,11]

Spermatogenesis is intact in OA but an obstruction is present somewhere between the epididymis and ejaculatory duct. Congenital causes of OA are cystic fibrosis, congenital bilateral absence of vas deferens (CBAVD) and Young's syndrome.[4-6,11] Acquired causes of OA are vasectomy, failure of vasectomy reversal, inguinal, pelvic or scrotal surgeries and trauma.[4,5]

Causes of NOA can be divided into a) Congenital-anorchia, cryptorchidism or testicular dysgenesis, genetic abnormalities like Y-chromosome deletions b) Acquired – trauma, testicular torsion, trauma, mumps orchitis, exogenous medications or toxins, irradiation, testicular tumours, varicocele and surgeries leading into testicular vascular compromise. Idiopathic NOA are also reported.[4]

Patients with OA have normal sized testis and hormone profile. The epididymis or seminal vesicles may be enlarged clinically. The pathognomonic features of OA are low volume ejaculate (<1.5 ml) which is

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acidic (pH<7.0) and azoospermic with absent or low fructose associated sometimes with non palpable vas deferens.[4,5] Cystic fibrosis transmembrane conductance regulator(CFTR) gene mutation is seen in about two-third of the patients with OA and CBAVD and counseling is advised before proceeding with sperm retrieval and ICSI because of high risk of cystic fibrosis transmission to the offspring.[4-6,12]

NOA is associated with small sized testis and elevated FSH level.[4,6] Patients with normal sized testis and semen volume and FSH level within normal range may have either OA or NOA. In such cases a testicular biopsy is helpful in proper diagnosis. The presence of normal spermatogenesis in testicular specimen indicates OA while maturation arrest, Sertoli cells only or decreased spermatogenesis usually indicates NOA.[4-6,12,13] Due to the heterogenous distribution of spermatogenesis in patients with NOA, the absence of spermatozoa in testicular biopsy does not completely excludes the chance of finding sperm elsewhere.[11,13,14]

In patients with idiopathic NOA, karyotyping and Y chromosome deletion testing are helpful.

Karyotyping abnormalities are seen in 10-15 % of NOA men where as 7-15% of men with NOA have Y chromosome microdeletion.[13,15] Y chromosome microdeletion restricted to AZFc region is associated with viable sperm within the testis where as AZFa and AZFb microdeletion is associated with complete absence of sperm.[16,17] Sperm aspirated from the testis is used in necrozoospermia since these are motile and viable.[10]

Sperm Retrieval Techniques

Sperm can be retrieved either from testis or epididymis in case of OA where as only testicular sperm retrieval is helpful in NOA cases. Open surgical and percutaneous methods are the two general sperm retrieval techniques.[8] The main goals of sperm retrieval are: (i) obtaining sufficient sperm for both immediate use and cryopreservation, (ii) retrieval of high quality sperm, and (iii) minimizing the reproductive tract damage to preserve testicular function for future sperm retrieval.[18]

Epididymal Sperm Retrieval Techniques

A. Microsurgical epididymal sperm aspiration (MESA): In this open technique described by Temple-Smith et al in 1985, scrotal incision exposes the epididymis after cutting the epididymal tunica under an operating microscope.[19] The epididymal ductule is dissected and the epididymal fluid is aspirated using needle. Multiple attempts of epididymal ductule dissection can be done if the sperms are not seen initially. The advantages of MESA are large number of sperms can be retrieved which can be cryopreserved for future ICSI, less chance of hematoma formation and with microsurgery; reconstruction of the ductule is possible. The disadvantages are the need for microsurgical instruments, operating microscope and expertise apart from this procedure being expensive and time consuming.[8,20,21]

B. Open fine needle aspiration (OFNA): In this open method, the exposed epididymal ductule is directly punctured through the tunica using a 26 gauge (G) needle to aspirate the fluid at different locations which increases the yield of quality sperm. Repeatability, non requirement of operating microscope, microsurgical expertise and yield of large number of sperms are the advantages of this procedure. The disadvantages are fibrosis and obstruction at aspiration site and post operative discomfort. Since OFNA helps to collect large number of sperms without much expertise and microsurgical instruments, it is the preferred open epididymal sperm retrieval technique.[8,20,22]

C. Percutaneous epididymal sperm aspiration (PESA): The percutaneous approach to retrieve sperm from epididymis was first described by Shrivastav et al in 1994.[(23)] In this technique a 26 G needle attached to a tuberculin syringe containing sperm wash medium is inserted into the epididymis through scrotum percutaneously. The negative pressure created by pulling the syringe plunger and in and out movement of the needle tip helps in epididymal fluid aspiration. The may be repeated at a different site preferably epididymal head if motile sperms are not obtained initially. Better quality sperms are obtained from the epididymal head rather than body and tail in case of OA. This procedure is cheap, simple, lesser expertise needed and easy to perform. Since percutaneous approach can obtain enough sperm for ICSI, PESA is preferred first followed by OFNA and MESA when epididymal sperm retrieval is considered. The disadvantages of PESA are retrieval of few sperms, risk of fibrosis and obstruction at puncture site and increased risk of hematoma or spermatocele formation.[8,20,23]

Testicular Sperm Retrieval Techniques

A. Testicular sperm aspiration (TESA): This technique is an alternative if PESA fails in cases of OA.[8,24] It can also be used for NOA cases.[25] In this procedure, an 18 or 22 G needle is inserted into the testis through scrotum percutaneously usually at the anterolateral and anteromedial aspect of the superior pole of the testis in an oblique angle to minimize

injury to the testicular artery branches running superficially beneath the tunica albuginea. The same technique is used as in PESA to aspirate fluid which is then flushed into a tube containing sperm wash medium.[20,26] Colour Doppler ultrasonography can be used to avoid blood vessels during puncture thereby reducing the incidence of hematoma and also helps in taking biopsy from areas with good perfusion to obtain better quality sperms.[27] Simplicity, cheap, easy to perform and non requirement microsurgical instruments are the advantages where as hematocele formation, intratesticular hemorrhage, few sperms for cryopreservation and low success rate in NOA cases are the drawbacks.[8,20]

B. Testicular fine needle aspiration (TEFNA): This technique was described by Turek et al in 1997, though it was used initially as a diagnostic method in case of NOA.[28] Later this procedure was used also for sperm retrieval in both OA and NOA cases. In this technique, a 23 G fine needle is attached to a 10 cc syringe and the fluid is aspirated using the same technique as in PESA or TESA. The aspirated fluid is then flushed into a tube containing sperm media. The advantages and disadvantages are the same as that for TESA with the additional benefit of lesser post procedural discomfort.[8,20,28]

C. Testicular sperm extraction (TESE): It was described by Devroey et al in 1995.[29] In this technique, testis is exposed by deepening the incision over the scrotum till albuginea is seen. A small incision over the albuginea with gentle pressure over the testis extrudes the testicular parenchyma, a 5×5 mm fragment of which is excised using sharp scissors and placed in sperm media. Multiple such specimens can be taken after which the albuginea and the scrotal wound is closed. No microsurgical expertise is needed and the procedure is repeatable. Retrieval of relatively few sperms, risk of impairment of testicular androgen production, testicular atrophy and post procedural discomfort are the drawbacks.[20,29-31]

D. Single Semeniferous Tubule (SST) biopsy: In this technique, the testis is exposed by opening the scrotum and an avascular area of testis is punctured using 26 G needle. A micro forceps is then introduced to dilate the puncture site using its prongs till a loop of semeniferous tubule is extruded out. It is examined under operating microscope and healthy thick semeniferous tubules are excised, crushed and examined for spermatozoa. The procedure is repeated at multiple sites. This technique helps in extensive sampling of the testis without many incisions over the albuginea. Relatively few sperms are retrieved in NOA cases.[8,22,32]

E. Microsurgical testicular sperm extraction (Micro dissection TESE): In this technique described by Schlegel in 1999, testicular sperm extraction is done with the help of an operating microscope.[33] The testis is exposed by incising the scrotum and a large single incision is made in an avascular area of the tunica albuginea to expose the testicular parenchyma. Healthy semeniferous tubules which are more likely to harbour viable sperm can be identified by its comparatively larger size and dissected under 16-25X magnification.[33,34] Deep testicular regions can be examined and testicular biopsies can be taken micro surgically if needed to extract the enlarged semeniferous tubules which are then placed in petridish containing sperm media. Retrieval of larger number of sperms with higher chance of cryopreservation, higher success rate in NOA cases and low risk of complications are the advantages where as requirement of technical expertise and microsurgical instruments, costlier and time consuming and risk of devascularisation and fibrosis of the testis are the disadvantages of this procedure.[8,20,33,34]

Complications of Sperm Retrieval Techniques

The complication rate varies depending on the sperm retrieval technique used. The main complications are hematoma, persistent pain, infections, testicular fibrosis and testicular atrophy which may occur with both percutaneous and open surgical methods.[8,20,30] Percutaneous approach is more commonly associated with hematoma formation compared to open approach. Conventional TESE is associated with increased risk of decrease in serum testosterone levels due to excessive tissue removal and testicular devascularisation compared to micro surgical TESE. The testosterone levels usually return to normal values within 12 months following the procedure.[35]

Considerations in Sperm Retrieval

There is no consensus on the minimum number of biopsies to be taken before stating that no sperm is found. Various studies suggest that sperm will be found in the first four biopsies if it is actually present.[8,36-38] With micro dissection TESE or SST biopsy techniques, 6 to 15 biopsies can be taken on each side without much complication.[8]

The detection of scanty sperms in the testicular tissue can be facilitated by properly lysing the tissue with collagenase after the tissue is minced and subjected

to multiple passes through fine needle attached to a syringe.[39,40] Erythrocyte lysing buffer can be used if the sample is mixed with blood.[41] It is suggested that incubating the testicular tissue for 24-72 hours improve sperm motility and improves pregnancy rates especially in men with testicular failure.[42]

Sperm retrieval may be performed on the same day of oocyte retrieval or one day before. Fresh sperm is preferable for ICSI because frozen and thawed sperm from NOA men have significantly impaired motility and viability.[43]

Success Rate and Outcome of Assisted Reproduction

The pregnancy rate achieved with retrieved sperm and ICSI is far better than that with IVF. The successful retrieval rate after percutaneous approach was 97.9% in OA men with about 86% success using PESA alone. TESA yielded about 90% success rate in cases of failed PESA. [44] Cryopreservation of sperm is essential in the treatment of male infertility with OA as the major problem, which helps in avoiding complications associated with repeated sperm retrieval procedures. Fertilization rates of 45-75% are seen with ICSI when epididymal or testicular spermatozoa from men with OA are used and pregnancy and live birth rates range from 26-57% and 18-55% respectively.[20,45-49] The fertilization and pregnancy rates are low in case of sperm retrieved from NOA cases compared to OA men. The sperm retrieval rate with TESA range from 10-30%.[20,38] Previous successful TESA or testicular histopathology showing hypospermatogenesis are good predictors of successful sperm retrieval in which retrieval rate range from 70-100%.[5,14] TESE have a retrieval rate of 49.5% and results in higher yield than fine needle aspiration (TEFNA) when multiple biopsies are used. Micro dissection TESE have retrieval rates ranging from 35% to 77%.[20,38] With the presence of motile sperms in retrieved samples from NOA men, the pregnancy rates are the same.

CONCLUSION

The success rate of sperm retrieval depends on the type of azoospermia and the retrieval technique used. Sperm retrieval rate is high in OA cases compared to NOA. Same technique yield more sperm when done on testis compared to epididymis in OA cases where as only testicular procedures are successful in NOA cases. Considering the complications and minimal difference in the sperm retrieval rate, less invasive percutaneous procedures are done first followed by open methods if sufficient sperm are obtained initially.[8] Which ever method is used collection of motile sperm is necessary to increase the efficacy of testicular cryopreservation programmes.

END NOTE

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Editor's Remarks: Infertility is increasing in the community especially among the well educated young population. These couples face social humiliation and are willing to take the trouble of treating the difficult problem especially with assisted reproduction, the results are variable. The article reviews the current techniques of sperm retrieval.

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