

EVALUATION OF EPIDIDYMAL AND TESTICULAR SPERM ASPIRATION IN AZOOSPERMIC INFERTILE MALES IN BASRAH

Murtadha MS Majeed Al-Musafer* & Safaa T Al-Maatoq@

*Professor of Urology, Basrah College of Medicine and Basrah General Hospital. @MB,ChB, Basrah General Hospital.

Abstract

This study aimed to evaluate the safety and efficacy of percutaneous epididymal and testicular sperm aspiration as a diagnostic technique to confirm sperm production and as a therapeutic technique to harvest sperms for use in the intracytoplasmic sperm injection and the indications for performing testicular biopsy in azoospermic infertile males.

Thirty married patients were included in this prospective study from February 2011 to December 2012 seen in Basrah General Hospital. Their age ranged from 20 to 40 years. All patients underwent full medical examination with laboratory tests which included seminal fluid analysis, serum leutinising hormone (LH), follicular stimulating hormone (FSH), testosterone, and prolactin in addition to color Doppler ultrasonography of the scrotum. Patients with history of undescended testes, varicocele, & testicular pathology were excluded from this study.

All patients showed normal physical examination with normal secondary sexual characters. The external genitalia were normal with normal sizes of their testes. The percutaneous epididymal and testicular sperm aspirations were positive in 12 out of 30 patients (40%). The rest had negative aspirations (60%). The testicular biopsy which performed in the patients with negative aspiration showed normal germinal epithelium with mature spermatozoa in only 5 patients out of 18 (28%) while the rest 13 patients had spermatogenic arrest (72%).

In conclusion, percutaneous epididymal and testicular sperm aspiration has been found helpful as a diagnostic technique for patients with non-reconstructable azoospermia. It is a minimally invasive sperm retrieval technique and appears to be an effective alternative to microsurgical epididymal sperm aspiration, which is more invasive and costly. It is less invasive than testicular biopsy and preferably performed as a first step procedure in an attempt to obtain sperms for both diagnostic and therapeutic purposes.

Introduction

Infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year¹. Conception is normally achieved within 12 months in 80 to 85% of couples who are not using contraceptive measures². Infertility affects both men and women. Male causes for infertility are found in 50% of these couples. In many couples, however, male and female factors are present. In case of a single factor, the fertile partner may compensate for the less fertile partner. Infertility then usually becomes manifest if both partners are

subfertile; this explains why in infertile couples there is often a coincidence of male and female factors. Reduced male fertility can be the result of congenital and acquired urogenital abnormalities, infections of the male accessory glands, increased scrotal temperature (varicocele), endocrine disturbances; genetic abnormalities and immunological factors. In 40–60% of cases there is abnormal seminal analysis and there is no relevant history or abnormality on physical examination and endocrine laboratory testing (idiopathic male infertility)¹.

primary spermatogenic failure is defined as impaired spermatogenesis originating from causes other than hypothalamic-pituitary diseases. The severe forms of primary spermatogenic failure have a clinical presentation as non-obstructive azoospermia. Typical findings from the physical examination of a patient with spermatogenic failure may be abnormal secondary sexual characteristics, gynaecomastia and low testicular volume and/or consistency. Follicle Stimulating Hormone (FSH) may be elevated (Hypergonadotrophic hypogonadism) or normal. Obstructive azoospermia means the absence of both spermatozoa and spermatogenic cells in semen and post-ejaculate urine due to bilateral obstruction of the seminal ducts¹.

Intratesticular obstruction has been reported in 15% of obstructive azoospermia³ and is usually caused by post-inflammatory obstruction of the rete-testis. Epididymal obstruction is the most common cause of obstructive azoospermia; affecting 30-67% of azoospermic men⁴.

Congenital forms of obstruction (disjunction between efferent ductules and corpus epididymis, agenesis/atresia of a short part of the epididymis) are rare. Young's syndrome, characterised by proximal epididymal obstruction and chronic sinopulmonary infections⁵, results from a mechanical blockage due to debris within the proximal epididymal lumen. Among the acquired forms, those secondary to acute (gonococcal) and subclinical (e.g. chlamydial) epididymitis are considered to be the most frequent⁶.

Azoospermia caused by surgery may occur after bilateral epididymal cyst removal. Vas deferens obstruction following vasectomy is the most frequent cause of acquired obstruction. About 2–6% of these men request vasectomy reversal. Of those undergoing vasovasostomy, 5–10% will also have an epididymal blockage due to tubule

rupture, making vasoepididymostomy mandatory⁷.

Congenital bilateral absence of the vas deferens (CBAVD) is found in 1:1600 men and in all men with cystic fibrosis. Men with CBAVD appear to have mutations of the cystic fibrosis gene in 85% of the cases. CBAVD can therefore be considered a genital form of cystic fibrosis⁸.

Ejaculatory duct obstruction is found in about 1–3% of obstructive azoospermia⁹. These obstructions can be classified as cystic or post-inflammatory. Cystic obstructions are usually congenital (Müllerian duct cyst or urogenital sinus/ejaculatory duct cysts) and are medially located in the prostate between the ejaculatory ducts. In urogenital sinus anomalies, one or both ejaculatory ducts empty into the cyst, while in Müllerian duct anomalies, ejaculatory ducts are laterally displaced and compressed by the cyst¹⁰. Post-inflammatory obstructions of the ejaculatory duct are usually secondary to urethroprostatitis¹¹.

Congenital or acquired complete obstructions of the ejaculatory ducts are commonly associated with low semen volume, decreased or absent seminal fructose and acid pH. The seminal vesicles are usually dilated (anterior-posterior diameter >15 mm) on transrectal ultrasound¹².

Typical clinical findings in men with obstructive azoospermia are a normal testicular volume, enlarged and hardened epididymis, nodules in the epididymis or vas deferens, absence or partial atresia of the vas deferens, signs of urethritis or prostatitis and Prostatic abnormalities on rectal examination. Obstructive lesions of the seminal tract should be suspected in azoospermic or severely oligozoospermic patients with normal-sized testes and normal endocrine parameters¹.

Percutaneous epididymal and testicular aspirations were first described in 1928 by Huhner as a diagnostic technique for cases

of infertility¹. Since then, the pathological findings of percutaneous epididymal and testicular aspirations have been shown to correlate with open testicular biopsies in 87 to 94% of cases. Using 20 to 23 gauge needles, this diagnostic procedures have been performed with few complications or adverse effects²⁻⁴.

Aim of the study

The aim of this study is to evaluate the *safety and *efficacy of percutaneous epididymal and testicular sperm aspiration as a diagnostic technique to confirm sperm production and as a therapeutic technique to harvest sperms for their subsequent use in the intracytoplasmic sperm injection (ICSI), and the indications for performing testicular biopsy.

* Regarding post-aspiration complications

* Sperm retrieval rate in comparison with the standard testicular biopsy

Patients & Methods

Thirty married patients with azoospermia were included in this comparative study from February 2011 to December 2012 in Basrah General Hospital. All patients underwent epididymal and testicular aspirations initially. Those with negative aspiration (no sperms in the aspirate) were subjected to the standard testicular biopsy procedure. Their age ranged from 20 to 40 years. All patients underwent full medical examination with laboratory tests which included two seminal fluid analysis, post ejaculate urine for sperms, serum Leutinising Hormone (LH), Follicle Stimulating Hormone (FSH), testosterone, and prolactin in addition to color doppler ultrasonography of the scrotum. Trans-rectal ultrasound (TRUS) not available in Basrah center. Patients have no past history of: Crypto-orchidism, Testicular pathology (torsion or trauma), Infection (tuberculosis or mumps orchitis), Chemotherapy/Radiotherapy, Surgery (orchidopexy, retroperi-toneal/pelvic, herniorrhaphy, prostatic surgery) Retrograde ejaculation.

All patients showed normal physical examination with normal secondary sexual characters and no gynecomastia. The external genitalia were normal with normal sizes of their testes, and normal endocrine parameters which include (serum LH, FSH, testosterone, and prolactin).

Method of epididymal sperm aspiration

After obtaining patient's consent, the aspirations were performed in a minor procedure room in Basrah general hospital and Al-manar fertility center with the patient under local anesthesia (spermatic cord block which was performed by injecting 5cc of 1% lidocaine into the spermatic cord at the pubic tubercle area bilaterally). Before aspiration, patients received a single intravenous dose of a broad-spectrum antibiotic (1gram of cefotaxime).

The epididymis was immobilized by the first assistant who stabilized the inferior two-thirds of the testicle. The scrotum overlying the epididymis was pulled taut. The superior pole of the testicle was presented to the operating surgeon who grasped the epididymis carefully between the thumb and index finger. A 23 gauge butterfly needle was directed into the caput of the epididymis and suction was applied by pulling back on the plunger of a 20cc syringe. The needle was delicately adjusted until there was return of opaque or off-white fluid in the butterfly tube. At this point the epididymis was gently squeezed in an effort to knead fluid into the tube (Figure 1). The needle was removed when the fluid return ceased, at the end of procedure a pressure on the site of aspiration for 5 minutes was applied.

Method of testicular sperm aspiration

Before aspiration, patients received a single intravenous dose of a broad-spectrum antibiotic (1gram of cefotaxime). After washing and draping

the genitalia in a sterile fashion, a cord block is given (Figure 2). Some men may still need oral or intravenous sedation; others can be done with only local anesthesia. The testis is held by the surgeon using the left hand, bringing the anterior surface of the testis tightly against the scrotal skin. A small amount of 1% lidocaine is infiltrated into the skin at the site of the aspirating needle puncture (23 gauge butterfly needle). Holding the aspirating needle device in the right hand, the anterior surface of the testis is punctured, and negative pressure is exerted on the syringe by pulling the pistol grip as far back as it will go. With steady negative pressure held, the needle is moved back and forth four to five times in different directions, never removing the

needle from the site of puncture. The pressure in the syringe is then gradually reduced by allowing the plunger to return to its original down position over a period of 30–60 seconds. This slow release prevents the aspirated tissue from being pushed from the needle back into the testis. Once there is no pressure in the syringe, the needle is withdrawn from the testis. Mild pressure on the puncture site is applied to minimize the risk of a hematoma.

Examination of the aspirate was performed by the cytopathologist for diagnostic and therapeutic purposes. A positive aspirate was defined by the presence of sperms in the aspirated specimen.

Figure 1: Percutaneous Epididymal Sperm Aspiration

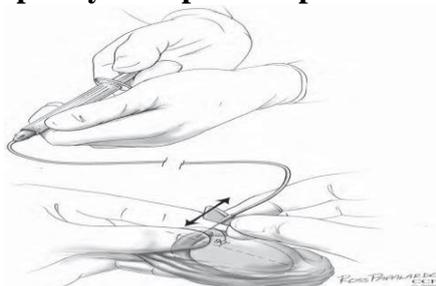
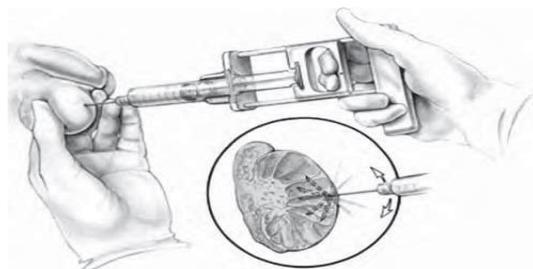


Figure 2: Testicular Sperm Aspiration



Open Testicular Biopsy

A standard testicular biopsy is still useful to determine the status of spermatogenesis. The testis to be biopsied is held positioning the epididymis posterior. The skin is pulled taut over the surface of the testis without pulling the testis up away from the patient, as this will cause discomfort. The 1.5-2 cm. incision is made and the tunica vaginalis is opened (Figure 3) exposing the anterior surface of the testis. A 4-0 absorbable suture is passed into the tunica albuginea in an area in which there does not appear to be any large blood vessels beneath the tunica (Figure 4). This suture is tied with a 4-5 in. tail that is tagged with a hemostat while the needle end kept intact to be used to close the incision after the biopsy is taken. The tag acts as a holding suture if the biopsy site slips from ones grasp and

needs to be pulled back into view. A 1-cm. horizontal incision is made in the tunica, and with gentle pressure exerted on the testis, a cluster of seminiferous tubules are extruded out of the incision and sharply excised with a small scissors (Figure 5). This tissue can be sent for standard histological evaluation, placing it in a preservative such as Bouin's solution to preserve its cellular architecture. Formalin is not used because it distorts the normal architecture. Tissue with sperm that is meant to be cryopreserved should be placed in an appropriate sperm nutrient medium to maintain sperm viability. Multiple small (2×4mm) pieces can be removed. The incision in the tunica albuginea is closed with a running interlocking suture (Figure 6). The tunica vaginalis, dartos muscle and skin are closed with absorbable 4-0 sutures.

Figure 3:

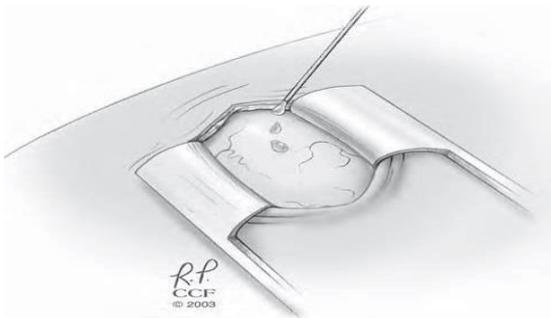


Figure 4:

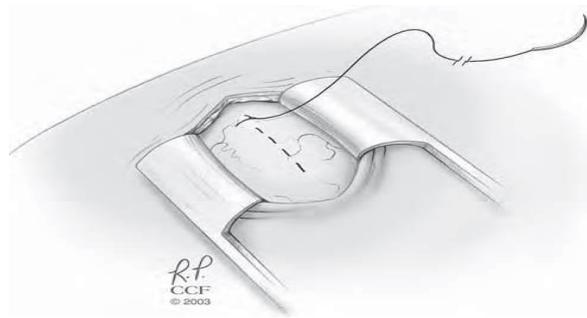
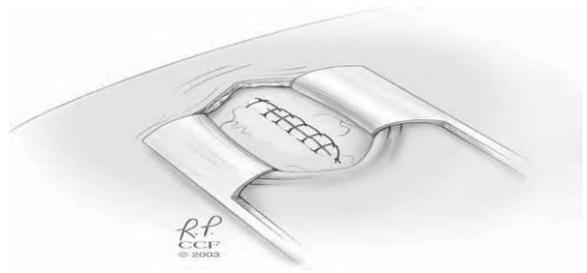


Figure 5:



Figure 6:



Results

All patients showed normal physical examination with normal secondary sexual characteristics. The external genitalia were normal with normal sizes of their testes and normal endocrine parameters which include serum LH, FSH, testosterone, and prolactin.

The percutaneous epididymal and testicular sperm aspiration was positive in

12 out of 30 patients (40%). The rest 18 patients had negative aspiration (60%) Table I & Figure I. The testicular biopsy which performed in patients with negative aspiration showed normal germinal epithelium, with mature spermatozoa in only 5 patients out of 18 (28%); while, the rest 13 patients had spermatogenic arrest (72%). Table II & Figure II.

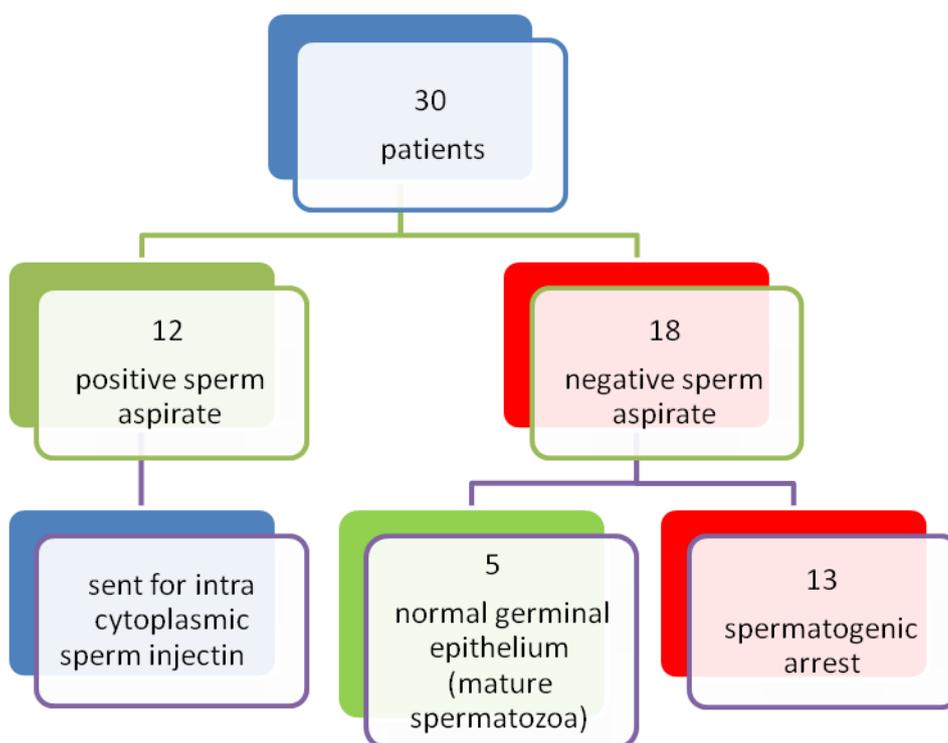


Diagram showing patients distribution according to aspiration

So the accuracy of percutaneous aspiration is (70.58%)* which is relatively high. Mild pain was a complain in all patients which was relieved by simple

analgesia. No significant complications had been reported after the percutaneous (epididymal and testicular) sperm aspirations.

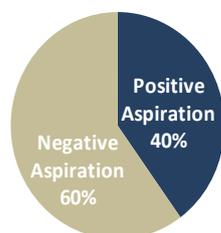
Table I: Results of Percutaneous (Epididymal and Testicular) Aspirations

Results	No. of patients	%	P- value
Positive aspirate	12	40%	<0.001
Negative aspirate	18	60%	
Total	30	100%	

By Z test, the P-value < 0.001 which is less than 0.05 so it is significant

*Accuracy rate = true positive cases of percutaneous aspirations / total true positive (of the percutaneous aspiration + testicular biopsy) X 100% = 12/(12+5) X 100 %

Figure (I): Results of Percutaneous epididymal and testicular sperm Aspiration

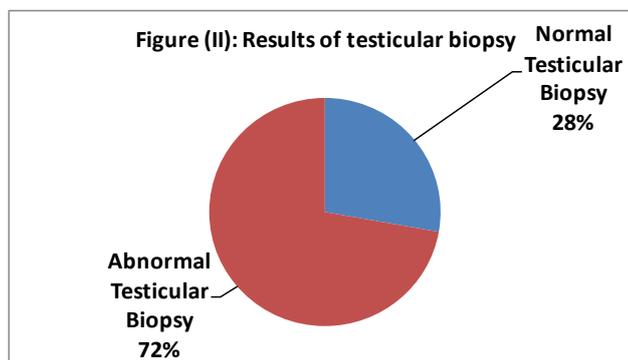


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Table II: Results of Testicular Biopsy

Results	No. of patients	%	p-value
Positive testicular biopsy	5	28%	=0.001
Negative testicular biopsy	13	72%	
Total	18	100%	

By Z test, the P-value = 0.001 which is less than 0.05 so it is significant



Discussion

With the increased use of gamete micromanipulation in the treatment of infertility, percutaneous aspiration techniques are now being evaluated for use as sperm harvest procedure for men with azoospermia¹³. Intracytoplasmic sperm injection has recently emerged as a technique that requires a limited number of viable sperms for successful fertilization^{14,15}. The first open sperm retrieval procedures such as microsurgical sperm aspiration were abandoned for less invasive techniques such as percutaneous epididymal sperm aspiration. Although initial fertilization rates were low, Craft et

al reported a fertilization rate of 49.3% and a pregnancy rate of 30% per transfer using sperm obtained with percutaneous sperm aspiration from the epididymis in conjunction with intracytoplasmic sperm injection^{16,17}.

Microsurgical sperm aspiration was first reported in 1980 as an effective approach for retrieving sperms from men with non-reconstructable obstructive azoospermia. It remains the most commonly used procedure for retrieving epididymal sperms in cases when the option of fathering genetic offspring was not previously possible. Yet microsurgical sperm aspiration is an operation that

typically requires general anesthesia, use of a microscope and direct exposure of the epididymis and testicle which carries the additional risks of infection and healing problems. On the other hand, percutaneous procedures (percutaneous epididymal or testicular sperm aspiration) were performed with the patient under local anesthesia which obviate the need for a scrotal incision¹⁸.

An important technical aspect of percutaneous procedures is the selection of needles. Craft et al used a 21 gauge butterfly needle for percutaneous aspiration of the epididymis¹⁸. For testicular aspiration Mallidis and Baker evaluated multiple needles, including hypodermic 19 to 25 gauge, lumbar puncture 22 to 23 gauge, CHIBA 20 to 23 gauge, Menghini 20 to 22 gauge and the Turner 20 and 22 gauge¹⁹. The 20 gauge Menghini and Turner biopsy needles with stylet penetrated easier with the least amount of tissue distortion. Gottschalk-Sabag et al, initially recommended using a 21 gauge needle because insufficient tissue was obtained with the 23 gauge needle²⁰. In more recent cases they used a 23 gauge needle but a larger syringe (20 cc) for suction²¹. In this study the 23 gauge butterfly needle resulted in adequate sperm quantity for intracytoplasmic sperm injection.

In addition, it is clear that 40% of the patients in this study who have positive percutaneous aspiration results did not need subsequent testicular biopsy with no risks of anesthesia nor complications of the procedure. Even so, the remaining 60% of patients with negative percutaneous aspiration results showed only 28% normal germinal epithelium with mature spermatozoa by testicular biopsy while the rest 72% had spermatogenic arrest.

Conclusion

Percutaneous sperm aspiration has been considered as a helpful diagnostic technique for patients with non-reconstructible azoospermia. It is a minimally invasive sperm retrieval technique and appears to be an effective alternative to microsurgical epididymal sperm aspiration (MESA), which is more invasive and costly procedure. It is also less invasive than testicular biopsy.

Recommendations

We recommend the use of percutaneous epididymal and testicular sperm aspiration as a first step procedure in an attempt to obtain sperms for both diagnostic and therapeutic purposes.

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