Fine Needle Aspiration Cytology of Testis in Male Infertility- A Review

*Alam MA,[1] Islam MS[2]

The technique of fine needle aspiration (FNA) may have a role as a reliable, quick and easy method of obtaining testicular cells. Recent advances in the management of male subfertility and in particular, the finding that spermatozoa recovered from epididymis and testis can result in embryo generation after intracytoplasmic sperm injection (ICSI), question the traditional role of open testicular biopsy for the assessment of spermatogenesis. The purpose of this article was to review various studies published on role of testicular fine needle aspiration cytology in male infertility and provide brief information on method of testicular fine needle aspiration, interpretation of testicular fine needle aspiration cytology for evaluation of spermatogenesis, its advantages, limitations and complications as compared to testicular biopsy.

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Introduction

Fine needle aspiration cytology (FNAC) of superficial as well as of deep seated lesions is now a well recognized diagnostic procedure for the diagnosis of neoplastic as well as non-neoplastic and inflammatory lesions. Recently it has gained popularity for its diagnostic and therapeutic role in male infertility .Since times immemorial the wife has always been blamed for infertility especially in third world countries. Failure to find sperms in post coital test, conducted by Max Huhner in 1913, raised the possibility that husband could be responsible for infertility. Approximately 20% cases of infertility are caused entirely by male factor with additional approximately 30% to 50% of infertile couples.[1.3] Azoospermia or absent sperm in semen occurs in approximately 5% to 10% of infertile men who are evaluated.[4] Azoospermia may be obstructive azoospermia (OA) or non obstructive azoospermia (NOA).The obstructive may have no significant effect on spermatogenesis and may be amenable to surgery where as before introduction of intracytoplasmic sperm injection (ICSI), the only available option for men with NOA was adoption or sperm donor. Assessment of spermatogenesis is an important component in the diagnostic algorithm of male infertility. Traditionally, the surgical testis biopsy has been the gold standard in this evaluation because it provides information in cases of both suspected obstruction and in failing on obstructed testes. Any technique to assess spermatogenesis must minimally invasive and must conserve as much testicular tissue as possible. It should also not only provide qualitative but quantitative information about spermatogenesis. In addition to answering the question whether sperm production is normal, it must also address whether sperm are present at all within the testis, as with advances in field of reproductive medicine, even a single sperm can now give men with NOA chance to enjoy biological fatherhood.[7] FNAC of the testis and scrotum is a simple, quick, minimally invasive and painless procedure. The sample can be obtained in outpatient deptt, can be more representative than biopsy as several separate punctures can be made, and there is no local scarring.

1. *Dr. Md.Ashraful Alam, Associate Professor, Department of Pathology, Rangpur Medical College, Rangpur
2. Dr. Md. Shahidul Islam, Assistant professor, Department of Urology. Rangpur Medical College, Rangpur
*For correspondence

The purpose of this article was to review studies published on role of testicular FNAC in male infertility and provide brief information on method of testicular fine needle aspiration, interpretation of testicular cytology and its possible complications.

Indications of Testicular Biopsy or FNA

1. **To differentially diagnose OA from - NOA in men with normal or borderline testicular size,** palpable ejaculatory ducts and normal follicular stimulating Hormone (FSH) serum labels. Testicular biopsy sets the histological diagnosis, i.e. whether azoospermia is caused by primary testicular failure, it is secondary to obstruction or there is a combination of these two conditions. Therapeutic management is then appropriately selected.
2. **In cases of OA with concurrent varicole.** Normal spermatogenesis in these men confirms obstruction, whereas hyper spermatogenesis or spermatogenesis arrest usually is interpreted as negative influence of varicole on the spermatogenesis, and therefore it is an indication for surgical correction.
3. **In cases of primary testicular failure with concurrent varicole.** Sertoli cell-Only Syndrome (SCOS) favors the diagnosis of primary testicular failure, whereas spermatogenesis arrest indicates that varicocele affects the spermatogenesis and therefore it should be corrected.
4. Other special indication for testicular FNA includes the detection of spermatozoa in azoospermic men prior to Assisted Reproduction Techniques(ART).Sperm detection in these cases is a good prognostic factor for sperm retrieval in subsequent open testicular biopsy(Testicular Sperm Extraction-TESE)If no sperm is retrieved during FNA, open biopsy is still indicated as a TESE-ICSI procedure (Inracytoplasmic Sperm Injection);counseling however, of the couple includes the option of sperm donor use. Common causes of azoospermia are idiopathic Non obstructive Azoospermia(INOA),cryptorchidism, Klinefelter syndrome,epididymal, vassal or ejaculatory duct pathology,and congenital bilateral absence of he vasa Deferentia(CBAVD).

FNA Technique

Testicular FNA is done under local anesthesia.[5,6] The scrotal skin is cleaned and spermatic cord block is achieved by 5 to 7 ml of two percent lidocaine. To quicken the distribution of anesthetic, spermatic cord is gently massaged after injection. After several minutes the testis is firmly palpated to ensure absence of pain. Then the testis is positioned with epididymis and vas deferens directed posteriorly, safe from injury. The scrotal skin is stretched taut over the testes by wrapping the scrotal skin behind the testes with a sponge. The testicular wrap serves not only as convenient handle to manipulate the testes but also fixes the scrotal skin over the testes for procedure.[13] Testes is aspirated at three different sites, upper, middle and lower part, using 21-23 G needle with 10 ml-20 ml syringe attached to it. Precise gentle in and out movement, varying from 5-8 mm are used. Testes can also be needled without local anesthesia, but only at one site and procedure should be completed in 10-15 seconds. The patient should rest for at least ten minutes after the procedure.[6] Both testes should be sampled when FNA is done for evaluation of spermatogenesis. Slides are prepared from the aspirated material and are fixed in alcohol and stained with Papanicolaou (Pap) stains or are air dried and stained with Giemsa stain.[2,5,9] Staining the smears with Giemsa or Pap is not superior to each other. Both staining methods should be used together in order to use

advantages of each method during the microscopic evaluation.[17] Giemsa stain may be superior to Papanicolaou stain in defining cell borders of spermatogenic cells but tail of spermatozoa is most readily visible with Pap stain.[14]

Reporting of Testicular FNAC for Evaluation of Spermatogenesis

Specimen Adequacy for FNA

If at least 200 cells could be counted on minimum one well spread slide, specimen is considered adequate.[2] Approximately 97% testicular FNA yield adequate specimen for evaluation of spermatogenesis.[7] 200-500 consecutive cells should be counted and percentage of different cells noted. Cytologic results are satisfactorily reproducible.[20]

Two cell populations are evident in cytology.[5, 9]

• Sertoli cells

• Cells in various stages of spermatogenesis

The spermatogenetic cells are divided into spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. Cytologic features of these cells are described below:

Sertoli cells: These cells have round vesicular nucleus with finely granular chromatin and large nucleolus. The nuclear outline is smooth. Cytoplasm is abundant, pale and vacuolated with poorly delineated border. These cells are fragile and so naked nuclei are common.

Spermatogonia: These are uninucleated mainly but may be binucleated or multinucleated. The nuclei are round or oval, slightly eccentric and dark or pale depending upon their chromatin density. The cytoplasm is homogenous and has well defined border. In air dried Geimsa stained smears the spermatogonia may resemble lymphomatoid blast.

Primary spermatocytes: These cells have large nucleus with thread like or coarse chromatin. Nuclear outline may be irregular. The cytoplasm if present is basophilic and it is more deeply stained at the periphery of the cell. Binucleated primary spermatocytes are common. Primary spermatocytes are either isolated or are present in groups with other spermatogenic cells or sertoli cells.

Secondary spermatocytes: These cells are rarely identified because of their shorter life span and immediate transformation to spermatids

Spermatids: They have much smaller nucleus than spermatocytes, corresponding to their haploid set of chromosomes. The nucleus is often centrally placed and finely granular. They may also be binucleated and have cytoplasmic vacuoles. They may be found in groups in normal testes, resembling sheets of epithelial cells.

Spermatozoa: They have oval nuclei with very dense chromatin. The tail is found on opposite side of acrosome.

Leydig cells: The aspiration of interstitial tissue is difficult and hence Leydig cells are usually not visualized in cytologic smears. If present, in smears they appear singly or in clusters. They are somewhat smaller than the sertoli cells and have central spherical or oval nucleus. Some are bi nucleated. They have abundant finely granular eosinophilic cytoplasm .

FNA interpretation

Depending upon the percentage of different cells counted the result is interpreted as one of the following [3,7,16]:

• Normal spermatogenesis

• Hypospermatogenesis

• Sertoli cell only (SCO)

• Maturation arrest.

• Atrophic pattern

Normal spermatogenesis: Smears show abundant cellularity with 10-20 spermatozoa per 400x field (HPF). Abundant primary spermatocytes and spermatids are present.

The ratio of spermatogenic to sertoli cell is at least 1.5:1 (60:40).

Hypospermatogenesis: Smears show relative decrease in all three germ cell types as compared to normal spermatogenesis. Less than 10 spermatozoa are visualized in each 400x field (HPF). Overall paucity of cell is seen, however all three kinds of germ cells are present including primary spermatocytes, spermatid and spermatozoa. Ratio of spermatogenic to sertoli cell is less than 1.5:1. Sertoli cells only/Germ cell aplasia: Smears show mainly sertoli cells.

Maturation arrest: Maturation arrest is divided into early maturation arrest and late maturation arrest. In early (premeiotic) maturation arrest smears have adequate cellularity and show numerous primary spermatocytes. No or only occasional spermatids or spermatozoa are visualized. In Late maturation arrest (Post meiotic arrest) normal number of primary spermatocytes and spermatids are present but spermatozoa are not seen or are only occasionally seen.

Atrophic pattern: Smears show mainly proteinaceous material and scant sertoli and leydig cell.

Various cell indices can be calculated with the help of differential cell count. Useful indices are spermatic index (Spermatozoa/ all spermatogenetic cell), sertoli cell index (Sertoli cell / all spermatogenic cell) and sperm – sertoli cell index (spermatozoa/sertoli cells). Progressively increasing value of sertoli cell index and progressively decreasing value of sperm – sertoli cell index is detected in normal spermatogenesis, maturation arrest, hypospermatogenesis and sertoli only cell syndrome respectively.[2]

Fine Needle Aspiration Mapping

FNA mapping is defined as aspiration of more than four FNA sites per testis.[13] This is performed most commonly as diagnostic test to detect presence and distribution of sperms in testis of men who have testicular atrophy or prior biopsy reveals abnormal or absent spermatogenesis. The idea behind FNA mapping is that there is geographic variation in presence of sperm in testes and these patches can be discovered by mapping even if biopsy shows absent sperm.

Testicular FNA in Assisted Reproduction

Testicular FNAC is also useful in assisted reproduction in two ways.[3,11] First FNA (MS) mapping can locate the area of spermatogenesis in failing testis and thus biopsy for retrieval can be directed to that particular site. Second, FNA itself can use for sperm retrieval instead of biopsy.

Advantages of FNAC

FNAC is less invasive and gives informative data on spermatogenesis of entire testes. Report can be issued quickly as compared to biopsy. Complications related to procedure are rare. It is simple, quick and inexpensive because surgical instruments are not required.[15] Local scarring doesn't occur.[9] It is well tolerated by patient. Infertile patients feel more secure with aspiration than with biopsy.[2] The material shows excellent preservation and various cell types can be identified.[6] FNAC guided TESE is useful alternative to blind biopsy.[12] Material obtained can be used for quantization of spermatogenesis by DNA Flow cytometry.[16]

Limitations of FNAC

FNAC is unable to provide architectural information of testes. It doesn't give information about thickness of tubular basement membrane, tubular diameter or status of interstitial tissue.[17] Testicular disorders leading to azoospermia such as

atrophy, fibrosis and Leydig cell hyperplasia can be diagnosed on basis of histology but are difficult to assess by FNA.[3] Some patients complain of prolonged pain but this can be relieved by scrotal support and analgesics.[2] Fairly experienced pathologist is needed to interpret the smears.[5] Neurogenic shock have been reported in patients who failed to rest after the procedure. Hematoma formation can be expected when thick needle (20G) is used.[6]

Conclusion

In spite of limitations, testicular FNAC represent an important diagnostic procedure in azoospermic men and a reliable prognostic parameter for successful sperm retrieval at TESE, particularly in NOA. Also sperm may be retrieved with fine needle aspiration alone for use in assisted reproduction. As assisted reproduction is gaining popularity worldwide, pathologists and urologists should develop skill and experience in performing the procedure and interpreting the reports in male infertility.

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