TESTICULAR FINE NEEDLE ASPIRATION IN MALE INFERTILITY: A REVIEW

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Summary

Fine needle aspiration cytology is a well accepted diagnostic tool in the evaluation of neoplastic as well as non-neoplastic lesions. Now it has gained popularity in the evaluation of infertility. The aim of this review article was to provide brief information on testicular fine needle aspiration cytology for evaluation of spermatogenesis as well as its procedure, advantages and limitations.

Key words: fine needle aspiration; cytology; testis; infertility; azoospermia

Introduction

Male factors are responsible for about half of all infertility cases[1]. Azoospermia is present in about 10-15% of men evaluated for infertility[2]. Azoospermia may be obstructive azoospermia or non-obstructive azoospermia (NOA). The obstructive cause 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 [3].

Assessment of spermatogenesis is an important component in the diagnosis of male infertility. Traditionally, the testicular biopsy has been the gold standard in this evaluation because it provides information in cases of both suspected obstruction and in failing unobstructed testes [4].

Testicular biopsy is well established and also the main investigative modalities in male infertility for evaluation of spermatogenesis[5]. It has been indicated to investigate seminiferous tubule function since the 19th century and was used clinically by Hotchkiss [6]. But the tissue sample in testicular biopsy is small and not representative of entire testis[5]. It is also invasive and traumatic especially when applied to both testes[7].

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Testicular fine needle aspiration (FNA) is an established technique for the evaluation of testicular and intrascrotal tumours, but it is only beginning to gain acceptance as a diagnostic and treatment tool for male infertility [8,9,10,11]. However its greatest value is in evaluation of spermatogenesis in azoospermic males, particularly in NOA, Where it can conserve tissue of already failing organ[3].

Fine needle aspiration of testes was first described by Max Hubner, however it was later in 1965 only that first fine needle aspiration (FNA) of human testes in men with fertility disorder was performed by scandinavian group pioneered by Obrant and Persson, still not fully describing the morphologic features of various stages of spermatogenesis. Later cytologic features of seminiferous epithelium was described by Schenek and Schill [7,12,13]. However testicular FNA did not gain popularity then because of limited awareness of the usefulness of the technique, lack of expertise in aspiration and interpretation of the cytological variations as well as paucity of information about architectural details on cytology remain limiting factors for more widespread adoption of this modality[1]. But later on many studies carried out showed that FNAC evaluated spermatogenesis of entire testes, was simple and less invasive, report could be issued quicker and there was good cytologic-histologic correlation [5,9,12,14,15].

Testicular FNA was also found therapeutic implication in assisted reproduction technique. Since the introduction of intracytoplasmic sperm injection (ICSI) in 1992, several studies of testicular sperm retrieval in azoospermic patients have been reported[16,17,18].

FNA technique

Usually FNA is done using the standard technique described by Zajicek[19]. Testicular FNA is done under local anaesthtic[5,7]. The scrotal skin is cleaned and spermatic cord block is achieved by 5 to 7ml of 2% Lidocaine. To quicker the distribution of anaesthetic, 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 procedures[20]. Testes is aspirated at three different sites, upper, middle and lower part, using 21-23G needle with 10ml-20ml syringe attached to it, precise gentle in and out movement varying form 5-8 mm are used. Testes can also be needled without local anaesthesia, but only at one site and procedure should be completed in 10-15 seconds. The patient rest for at least ten minutes after the procedure[7]. 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) stain or are air dried and stained with Geimsa stain. Staining the smears with Geimsa 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[21]. Geimsa stain may be superior to Papanicolaou stain in defining cell borders of spermatozoa[22].

Evaluation of spermatogenesis

• Specimen adequacy for FNA

If at least 200 cells could be counted on minimum one well spread slides, specimen is considered adequate. Approximately 97% testicular FNA yield adequate specimen for evaluation of spermatogenesis. 200-500 consecutive cells should be counted and percentage of different ells noted, cytologic results are satisfactory reproducible [3]. In cytology, sertoli cells, cells in various stages of spermatogenesis i.e spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa are noted [6, 23].

• Cytologic morphology of the cells [13,24].

Sertoli cells: Having round to oval nucleuses, with granular chromatin and prominent nucleolus. The cytoplasm is fragile, making the cells look naked.

Spermatogonia: These cells are uninucleated mainly but may be binucleated or multinucleated. The nuclei are round to 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 basoplilic 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: Are usually seen in groups. The nuclei of these cells are round to oval with fine granular clumped chromatin. No nucleolus is seen. The cytoplasm is scanty and vacuolated .

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

FNA interpretation

Based on various proportion of aspirated cells, the smear is interpreted as one of the following [13,14,25].

1. Normal spermatogenesis: Smears show spermatogonia, primary spermatocytes, spermatids, numerous spermatozoa and a proportional number of sertoli cells. The ratio of spermatogenic to sertoli cell is at least 1.5:1.

2. Hypospermatogenesis: This patttern is characterized by varying number of spermatozoa, spermatocytes, spermatids and sertoli cells. Ratio of spermatogenic to sertoli cell is less than 1.5:1.

3. Sertoli cell only/ Germ cell aplasia: Smears show mainly sertoli cells and no germ cells.

4. Atrophic pattern: Smears show mainly proteinaceous material and very scanty sertoli and leydig cell.

5. Maturation arrest: All types of germ cells except mature spermatozoa are present. It is divided into early and late maturation arrest. In early maturation arrest numerous primary spermatocytes are present but no or occasional spermatids are seen. In late maturation arrest, normal number of primary spermatocytes and spermatids are present but no spermatozoa is seen.

Cell indices: Various cell indices can be calculated with the help of differential cell count. Useful

indices are-
1. Spermatic index (ratio of mature spermatozoa to total spermatogenic cells).

2. Sertoli cell index (ratio of sertoli cell to all spermatogenic cells).

3. Sperm -sertoli cell index (ratio of spermatozoa to sertoli cell).

Progressively increasing value of sertoli cell index and progressively decreasing value of sperm sertoli cell index is detected in normal spermatogenisis, maturation arrest, hypospermatogenesis and sertoli cell only syndrome respectively[26].

Testicular FNA in assisted reproduction:

Testicular fine needle aspiration is also useful in assisted reproduction in two ways[3,20]. First FNA mapping can locate the area of spermatogenesis in failing testis and thus biopsy for sperm retrieval can be directed to that particular site. Second, FNA it self can be used for sperm retrieval instead of biopsy.

Advantages of FNA:
Like FNAC of other organs, testicular FNAC is also a simple,, quick and inexpensive outpatient (OPD) procedure. It 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 well tolerated by patient. Infertile patients feel more secure with aspiration than with biopsy. The material shows excellent preservation and various cell types can be identified. Good concordance has been observed between histology and cytology[3]. Material obtained can be used for quantitation of spermatogenesis by DNA flow cytometry[27] and other cytogenetic study[28].

Disadvantages or limitations of FNAC:
FNAC can not provide architectural information of testes, it does not give information about thickness of tubular basement membrane and status of interstitial tissue [6,28]. 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[14]. Some complain of prolonged pain, haematoma formation, neurogenic shock have been reported. Fairly experienced cytopathologist is needed to interpret the smears [1,29].

Conclusion

FNAC of testis is a simple, safe, inexpensive outpatient procedure. It yields adequate materials and in experienced hands, provides reliable diagnosis in patients with azoospermia.

Disclosure

All the authors declared no competing interestes.

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