TESTICULAR FINE NEEDLE ASPIRATION IN MALE INFERTILITY: A REVIEW

M Shahab Uddin Ahamad 1 Babul Osman Chowdhury 2 Mohammad Zobair 1

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 cases1. Azoospermia is present in about 10-15% of men evaluated for infertility2. 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 donor3.

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 testes4.

Testicular biopsy is well established and also the main investigative modalities in male infertility for evaluation of spermatogenesis4. It has been indicated to investigate seminiferous tubule function since the 19th century and was used clinically by Hotchkiss5. But the tissue sample in testicular biopsy is small and not representative of entire testis4. It is also invasive and traumatic especially when applied to both testes5.

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 infertility6,7,10,11. However its greatest value is in evaluation of spermatogenesis in azoospermic males, particularly in NOA, where it can conserve tissue of already failing organ1.

Fine needle aspiration of testes was first described by Max Huber, 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 Obroart and Persson, still not fully describing the morphologic features of various stages of spermatogenesis. Later cytologic features of seminiferous epithelium was described by Schleuek and Schill7,12,13. 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 modality1. 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 correlation5,7,12,14,15.

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 reported6,17,18.

FNA technique
Usually FNA is done using the standard technique described by Zajicek10. Testicular FNA is done under local anaesthetic5,7. 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\textsuperscript{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\textsuperscript{21}. 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\textsuperscript{21}. Geimsa stain may be
superior to Papanicolaou stain in defining cell
borders of spermatozoa\textsuperscript{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 cells noted,
cytologic results are satisfactory reproducible\textsuperscript{3}. In
cytology, sertoli cells, cells in various stages of
spermatogenesis i.e spermatogonia, primary
spermatocytes, secondary spermatocytes, spermatids
and spermatozoa are noted\textsuperscript{5,13}.

- Cytologic morphology of the cells\textsuperscript{11,24}.

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 uninnucleated 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 basopilic 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\textsuperscript{12,21,22}.

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 pattern 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
proteinsaceous 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
Mini Reviews

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. -seral 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 spermatogenesis, maturation arrest, hypospermatogenesis and sertoli cell only syndrome respectively. 

Testicular FNA in assisted reproduction:

Testicular fine needle aspiration is also useful in assisted reproduction in two ways. 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 itself 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. Material obtained can be used for quantification of spermatogenesis by DNA flow cytometry and other cytogenetic study.

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

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 interests.

References
11. Mahajan AD, Ali NI, Walwalkar SJ, Rege JD, Pathak HR. The role of fine needle aspiration
cytology of the testis in the diagnostic evaluation of infertility. BJU international 1999; 84: 485-888


