

Testicular touch imprint: reinforcing its role in male infertility

Sandhya V^{1,*}, Malur PR²

¹Consultant Pathologist, Apollo Hospital, Bangalore, ²Consultant Pathologist, KLES Hospital, Belgaum

***Corresponding Author:**

Email: sandhyavenkatachala@yahoo.co.in

Abstract

Introduction: Testicular biopsy plays an important role in the evaluation of male infertility. In the present decade of Mapping of testis for foci of spermatogenesis and testicular sperm extraction for the treatment of infertility, testicular touch imprint cytology has again become a vital adjunct to open biopsy. Only two large studies have correlated touch imprint cytology with open biopsy.

Aim: To correlate open testicular biopsy and Touch imprint in the evaluation of the cause of male infertility.

Materials and Method: 30 infertile men with a sperm count of less than 5 million/ml after sperm wash and swim-up method were subjected to open testicular biopsy. A touch imprint was taken before the specimen was fixed in Bouin's fluid. Hematoxylin and eosin staining was done. The biopsy and touch imprint were categorised into 5 groups based on Meinhard's classification.

Results: A 93.3% (28/30 cases) correlation was seen between open biopsy and touch imprint. Discordance was seen in 2 cases in which a definitive opinion was not possible in touch imprint cytology, while biopsy revealed tubular hyalinisation. Maturation arrest was the most common cause (76.67%) followed by Hypospermatogenesis, Sertoli cell syndrome and tubular hyalinisation, 6.7% each. A single case of obstructive azoospermia with normal spermatogenesis was seen.

Conclusion: High correlation between testicular touch imprint and open biopsy makes it a useful adjunct in not only the evaluation of infertility but also in its treatment by assisted reproduction techniques.

Keywords: Touch imprint, Infertility, Spermatogenesis, Azoospermia, Testicular mapping

Introduction

The male factor is responsible for infertility in 50% of the couples. Testicular biopsy has been the gold standard in the evaluation of male infertility after the initial semen analysis and endocrine work-up.⁽¹⁾ Touch imprint preparations have been infrequently studied, while literature review shows correlation studies between testicular FNAC (Fine needle aspiration cytology) and open biopsy.⁽²⁾ These studies show a good correlation of 89% between FNAC and biopsy. The present concept of mapping spermatozoa within the testicle requires examination of multiple locations. The presence of spermatozoa in touch imprint of the testicular biopsy identifies the ideal tissue obtained by mapping for testicular sperm extraction for assisted reproduction procedures like intracytoplasmic sperm injection.⁽³⁾ Thus the present study was undertaken to correlate touch imprint cytology with open testicular biopsy in evaluating the cause and also in the treatment by infertility.

Materials and Method

A total of 30 cases of infertile men whose sperm count was less than 5 million/ml after sperm wash and swim up were included in the study. They were subjected to open technique of testicular biopsy and in all the cases, touch imprint was also taken. The open biopsies were performed under spinal anesthesia. The testicular tissue was extruded and excised. The tissue was passed gently across 2 clean glass slides in circular fashion taking care not to exert pressure so as to distort the tissue. The slides were placed in 95% ethanol. The tissue was transferred in Bouin's fixative to the Pathology Department.

Bouin's fixative was used in place of formalin for better cell morphology. After processing and paraffin embedding, sections of 3-5 microns were cut. Hematoxylin and Eosin staining was done on both the sections and touch imprint.

The biopsies were categorized according to Meinhard's classification-Maturation arrest(MA), Hypospermatogenesis(HP), Sertoli cell only syndrome (SCOS) or germ cell aplasia, Tubular hyalinization (TH) and those with normal histology.⁽¹⁾ Touch Imprint smears were also categorized into 5 cytological groups as the biopsy.^(1,2) Presence of at least 200 cells was required to consider the imprint as adequate. Spermatogenic Cell identification was based on the criteria by Papic and Foresta.^(4,5) Sertoli cells are large with abundant fragile vacuolated cytoplasm, granular chromatin and large nucleolus. Intermediate cells with darkly stained nuclei and high nuclear cytoplasmic ratio were identified as spermatogonia, while cells with thread-like chromatin were spermatocytes. Spermatids were small sized cells with dark small nuclei and minimal cytoplasm. Spermatozoa had indistinct tail. The criteria for Hypospermatogenesis was Sertoli index (number of sertoli cells per 100 cells counted) above 50%, this index in a normal testis being less than or equal to 50%.⁽⁵⁾

Results

Of the 30 cases 15 were Azoospermic, 11 oligozoospermic, one oligoasthenospermic, 2 oligoteratospermic, and one oligoasthenopyospermic. Of the 30 cases, touch imprint was adequate for evaluation in 28 cases. Two cases revealed few fibroblast like cells

with RBCs and were considered to be inadequate for a definitive opinion. The cytological categories have been shown in Table 1.

One of the cases revealed spermatogenic cells in all stages of maturation with infrequent Sertoli cells (Fig. 1A) consistent with normal spermatogenesis. This was a case of obstructive Azoospermia. In hypospermatogenesis (Fig. 2A) germ cells in all stages of maturation were seen, but were diminished in number relative to Sertoli cells. Maturation arrest was at the level of spermatocyte with few spermatids in 20 of the 23 cases, while in the other three maturation arrest was at the level of spermatid (Fig. 3A). SCOS showed groups of Sertoli cells.

Biopsy was adequate in all the cases. At least 50 well preserved tubules were analysed in all the cases.

The findings were the same as shown in Table 1 except for the category of tubular hyalinization which constituted two of the thirty cases (6.7%). Hyalinised tubules with absence of Sertoli and germ cells were seen (Fig. 4B). The Two cases of Hypospermatogenesis showed marked thinning of germinal epithelium (Fig. 2B). Majority of the cases of maturation arrest were at the level of spermatocyte (Fig. 3B). The two cases of SCOS showed seminiferous tubules lined by Sertoli cells with Leydig cell hyperplasia in the interstitium (Fig. 4A).

Correlation was seen in 28 of the 30 cases (93.3%) between touch imprint and biopsy findings (Table 2). A definitive opinion in touch imprint was not possible in 2 cases (6.7%) in which biopsy showed tubular hyalinization.

Table 1: Shows distribution of cases in the 5 cytological groups by touch imprint

Touch imprint Diagnosis	No. of patients(n=30)*	Percentage
Normal spermatogenesis	1	3.3
Hypospermatogenesis	2	6.7
Maturation arrest	23	76.6
Sertoli cell only syndrome	2	6.7
Tubular hyalinisation	0	0

*Scant cellularity 2 (6.7%)

Table 2: Shows correlation between touch imprint and biopsy evaluation of spermatogenesis

Biopsy Touch imprint	Normal	Hypospermatogenesis	Maturation Arrest	Sertoli cell Only syn	Tubular Hyalinization
Normal	1	-	-	-	-
hypospermatogenesis	-	2	-	-	-
Maturation arrest	-	-	23	-	-
Sertolicell only syn	-	-	-	2	-
Scant cellularity	-	-	-	-	2
Total	1	2	23	2	2

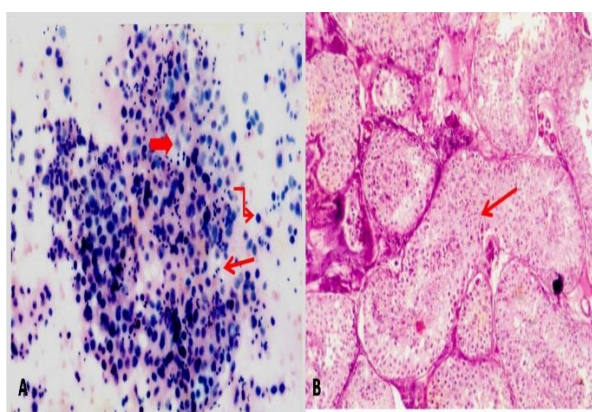


Fig. 1: A Imprint smear with spermatogenic cells in all stages, spermatozoa (thin red arrow) spermatocyte (yellow arrow) Sertoli cell (broad red arrow) (H&E 400x). **B** Normal biopsy with Spermatozoa (red arrow) (H&E 400x)

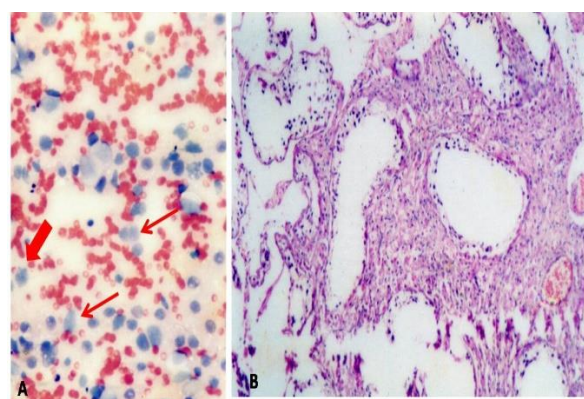


Fig. 2: A Shows more Sertoli cells (thin arrows) with other spermatogenic cells, spermatocyte (broad arrow) and spermatid in the centre (H&E, 400x). **B** Shows biopsy of the same case with thinned germinal epithelium (H&E, 100X)

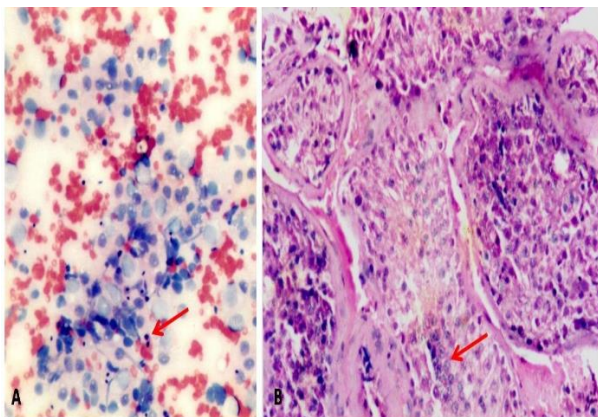


Fig. 3: A Maturation arrest at spermatid level (broad arrow) with spermatocytes (thin arrow) but no spermatozoa. (H&E,400X). **B** Maturation arrest at spermatocyte level, spermatocytes(arrow)H&E400x

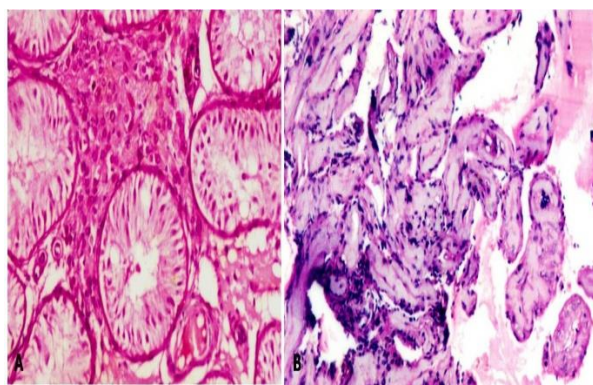


Fig. 4: A Shows tubules lined by only sertoli cells with leydig cells in the interstitium (H&E, 200X). **B** Shows hyalinised seminiferous tubules in testicular atrophy(H&E, 200X)

Discussion

Open testicular biopsy is vital not only in the diagnosis of male infertility but in the recent decade for Testicular sperm extraction (TESE).⁽⁶⁾ The pioneers in Cytological evaluation were Paptic and Foresta through their studies on testicular FNAC.^(4,5) Characterisation of different cell types and a cytological scoring system based on Sertoli index, Spermatic index and sperm-sertoli index have been described by Foresta.⁽⁵⁾ Testicular FNAC as a diagnostic tool in male infertility has been analysed by a good number of research works and has been correlated with biopsy in some of them.^(2,4,7,8,9,10) However only two studies have evaluated touch imprint cytology in male infertility.^(11,12) Hence the present study was done to correlate touch imprint cytology with open biopsy to determine the cause of male infertility.

In the present study we categorized the cases into 5 groups which corresponds to Meinhard's classification of biopsy.⁽¹⁾ However Kim ED and others have primarily classified touch imprint into 2 major groups- normal

spermatogenesis and maturation arrest.⁽¹¹⁾ Studies correlating testicular FNAC and biopsy have used Sertoli cell index, Spermatid index, sperm-Sertoli index to group the cases by cytology.^(2,7) This grouping has been done after assessing a minimum of 500 cells. FNAC yields a better cellularity compared to a touch imprint. Hence a differential count of spermatogenic cells can be performed and the indices be calculated. Our's being touch imprint, evaluation based on indices was not done, instead we used Meinhard's classification similar to other studies using touch imprint.^(11,12)

A correlation of 93.3% was seen in the present study between touch imprint and open biopsy. Discordance of 6.7% (2 cases) was seen. Both these were cases of tubular hyalinization with no germ cells or Sertoli cells on touch imprint. Identification of tubular hyalinization on touch imprint has been a difficult task as described by Person who opined that distinction between SCOS and TH is always not possible in cytology. However Verma and others have differentiated these two groups based on cellularity which is good in SCOS comprising of Sertoli cells and scant in TH.⁽¹⁴⁾ In our study also both the cases of TH showed scant cellularity, while SCOS showed groups of sertoli cells without germ cells and was identified in touch imprint.

A similar high correlation between touch imprint and biopsy has been reported by Yildiz-Aktas and others.⁽¹²⁾ In their studies, these authors found disagreement in one case where biopsy showed tubular hyalinization but a rare spermatozoa was seen on the imprint.

In the present study a 100% correlation between touch imprint and biopsy was seen in the cases of maturation arrest and normal spermatogenesis. However few studies have found discordance in maturation arrest at spermatid level as the touch imprint revealed spermatozoa which were not seen in biopsy. The authors have attributed this difference to the loss of tails of spermatozoa when thin sections are cut for histological preparation.⁽¹⁵⁾

Spermatogenesis varies geographically within a failing testis. FNA mapping can locate the area of spermatogenesis and thus biopsy for sperm retrieval (TESE-testicular sperm extraction) can be directed to that particular site.^(3,6) Tissue thus obtained can be subjected to touch imprint to confirm the presence of spermatozoa before it is processed to obtain sperm for IVF(In-vitro- fertilization). Thus touch imprint cytology has again taken a prime position both in the evaluation of the cause of infertility and to achieve fertilization in infertile men.

References

1. Meinhard E, McRai CU, Chisholm GD. Testicular biopsy in evaluation of male infertility. *BMJ* 1973;3:577-81.
2. Agarwal MS, Gupta A, Chaturvedi K, Lavania P. Assessment of the utility of testicular FNAC in infertile males with special reference to differential counts. *Indian J Urol* 2004;20:148-53.

3. Beliveau ME, Turek PJ. The value of testicular 'mapping' in men with non-obstructive azoospermia. *Asian Journal of Andrology* 2011;13: 225-30.
4. Pasic Z, Katona G, Skrabalo Z. The cytological identification and quantification of testicular cell subtypes, Reproducibility and relation to histologic findings in the diagnosis of male infertility. *Acta Cytol* 1988;32:697-706.
5. Foresta C, Varotto A, Scandellari C. Assessment of testicular cytology by FNA as a diagnostic parameter in the evaluation of the azoospermic subject. *Fertil Steril* 1992;57:858-65.
6. Jha R, Sayami G. Testicular fine needle Aspiration in evaluation of male infertility. *J Nepal Med Assoc* 2009;48:78-84.
7. Prasad U, Prasad U. Testicular cytology in azoospermia. *Int J Reprod Contracept Obstet Gynecol* 2015;4:647-52.
8. Bettell A, Ferlin A, Menegazzo M, Ferigo M, Tavolini IM, Bassi PF et al. Testicular fine needle aspiration as a diagnostic tool in non-obstructive azoospermia. *Asian J Androl* 2005;7:289-94.
9. Mehtora R, Chaurasia D. Fine needle aspiration cytology of the testis as the first line diagnostic modality in azoospermia: comparative study of cytology and histology. *Cytopathology* 2008;19:363-8.
10. Qublan HS, Al-Jader KM, Al-Kaisi, Al ghoweri AS, Abu-Khait SA. Fine needle aspiration cytology compared with open biopsy histology for the diagnosis of azoospermia. *J Obstet Gynecol* 2002;22:527-31.
11. Kim ED, Greer JA, Abrams J, Lipshultz LI. Testicular touch preparation cytology. *J Urol* 1996;156:1412-4.
12. Yildiz-Aktas IZ, Monaco SE, Khalbuss WE, Parwani AV, Jaffe TM, Pantanowitz L. Testicular touch preparation cytology in the evaluation of male infertility. *CytoJournal* 2011;8:24-34.
13. Persson PS, Ahren C, Obrant KO. Aspiration biopsy smear of testis in azoospermia. *Scand J Urol Nephrol* 1971;5:22-6.
14. Verma AK, Basu D, Jayaram G. Testicular cytology in azoospermia. *Diagn Cytopathol* 1993;9:37-42.
15. Meng MV, Cha I, Ljung BM, Turek PJ. Testicular Fine-Needle Aspiration in Infertile Men: Correlation of Cytologic Pattern with Biopsy Histology. *Am J Surg Pathol* 2001;25:71-9.