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Testicular fine needle aspiration cytology and biopsy correlation in male Infertility

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Abstract

Introduction: Testicular Fine needle aspiration cytology (FNAC) is Simple and minimally traumatic procedure with less number of complications at the same time more sites could be aspirated safely. As well as FNAC could give better morphological details of different stages of spermatogenesis.

Materials and Method: This study was done in 36 infertile men for a one year period to evaluate the cytological features of testicular FNAC and correlate with histological diagnosis, and to correlate various cell indices in different categories of cytological diagnosis.

Results: In the present study, in Cytology, the common diagnosis was found to be Maturation arrest with a corresponding Johnson's score of 3 to 7 was found to be the most frequent cause of male infertility. The correlation between the FNAC and biopsy was 91.9%. Sertoli cell index was found to be increased in hypospermatogenesis.

Conclusion: The overall accuracy of FNAC in this study was found to be 86% and well correlated with histological diagnosis. Hence testicular FNAC can be used for the diagnosis of male infertility.

Keywords: Testicular FNAC, Male Infertility, Sperm Count, Sertoli Cell Index, Johnson's Score.

Introduction

Although testicular biopsy has remained the gold standard in the diagnosis of male infertility for decades, testicular FNAC has picked up in recent years following Huhner, Obrant and persson, Papic et al, Schenck and Schill and Foresta et al who characterized different cell types in testicular cytological smears and demonstrated good correction of cytological diagnosis with histological diagnosis.[1-6] FNAC, a minimally invasive technique is one of the important investigations in diagnosis and management of infertility. FNAC could give better morphological details of different stages of spermatogenesis.

Materials and Method

This study was done on 36 cases, out of which 1 case was excluded as the sample did not contain testicular tissue. FNAC of testes followed by testicular biopsy was done under spermatic cord block. The cytological smears were stained with H&E and May Grunwald - Giemsa (MGG). The cytological features of testicular FNAC was then evaluated. Hundred consecutive spermatogenic and sertoli cells were counted in a well spread portion of the cytology smear and the Cell Indices were calculated. Cytological diagnosis was then correlated with sperm count, various indices and finally with histological diagnosis.

Cytological diagnosis was done based on various proportions of the different cell types.

The specimen was considered adequate if at least 200 cells could be counted in one well spread slide. Different cells in FNA smears were recognized by their nuclear and cytoplasmic characteristics. The smears were categorized into Six categories[7]:

Normal spermatogenesis: when the smears show spermatogonia, spermatocytes, spermatids, many spermatozoa and sertoli cells (around one third of the total spermatogenetic cells). (Fig. 1)

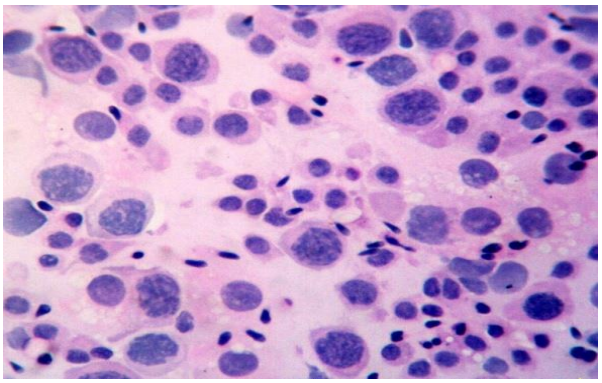

Fig. 1: Normal Spermatogenesis- (H&E 400X) smear shows Spermatogonia, primary spermatocyte, Spermatids and spermatozoa

Hypospermatogenesis: when all types of cells up to spermatozoa are present and the proportion of sertoli cells to spermatogenic cells is increased. (Fig. 2)

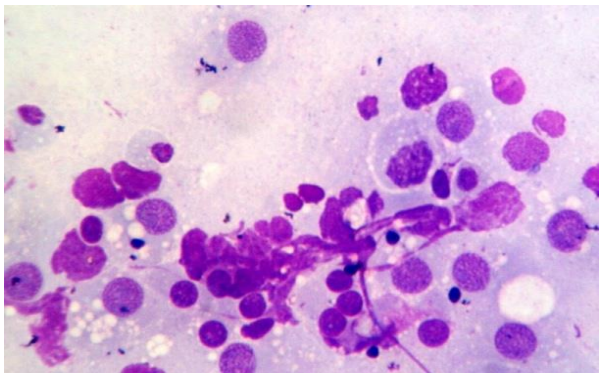

Fig. 2: Hypospermatogenesis- Smear shows Sertoli cells admixed with few spermatocyte and spermatids (MGG 100X)

Early Maturation Arrest: smears show more number of spermatogonia and primary spermatocytes along with absence of spermatids and spermatozoa. (Fig. 3&4)

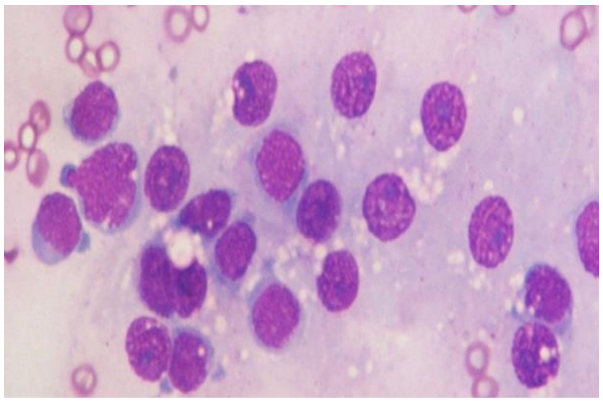

Fig. 3: Maturation Arrest –Spermatogonia level - (MGG 400X) Smear shows Sertoli cells and spermatogonia.

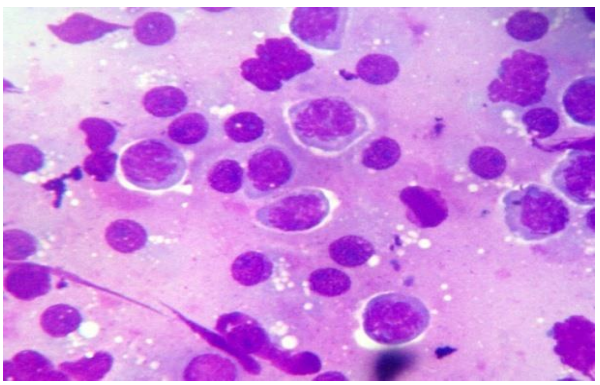

Fig. 4: Maturation Arrest– Spermatocyte level (MGG 400X) shows Sertoli cells admixed with primary spermatocyte and spermatogonia

Late Maturation Arrest: smears were characterized by the total absence of spermatozoa and increase in proportions of round and elongated spermatids along with spermatocytes and sertoli cells. (Fig. 5)

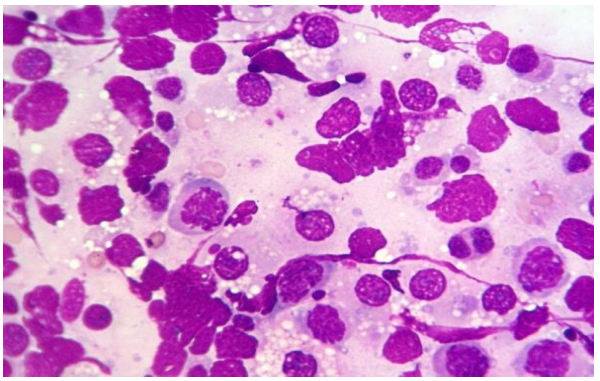

Fig. 5: Maturation Arrest – Spermatid level - Smear shows sertoli cells, primary spermatocyte, early and late spermatids.

Sertoli cell only syndrome (SCOS): Smears show only sertoli cells with prominent nucleoli and absence of spermatogenic cells. (Fig. 6)

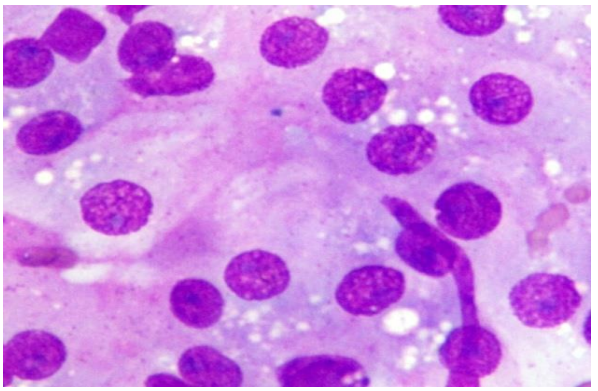

Fig. 6: Sertoli cell only syndrome- Smear shows singly scattered sertoli cells (MGG 1000X)

Testicular Atrophy: smears have scanty cellularity and few sertoli cells. (Fig. 7)

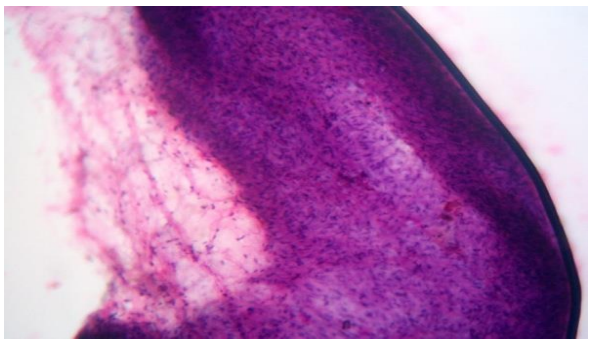

Fig. 7: Testicular atrophy- (H&E 100X) Smear shows only hyalinised stromal tissue

CELL INDICES were calculated. Spermatic index was calculated as the number of spermatozoa per 100 spermatogenic cells. Sertoli cell index (SEI) was calculated as the number of sertoli cells per 100 spermatogenic cells and Sperm- sertoli index (SPSEI) was expressed as the number of spermatozoa per 100

sertoli cells.[8] Then the mean value of these indices in each category of the cytological diagnosis was then taken and compared.

Testicular biopsies were evaluated by Johnsons scoring.[9] In the histopathology, five patterns were observed. They are Normal spermatogenesis (Fig. A), Hypospermatogenesis (Fig. B), Maturation Arrest (Fig. C,D,E), Germ Cell Aplasia (Fig. F) and Tubular hyalinization (Fig. G).[10,11]

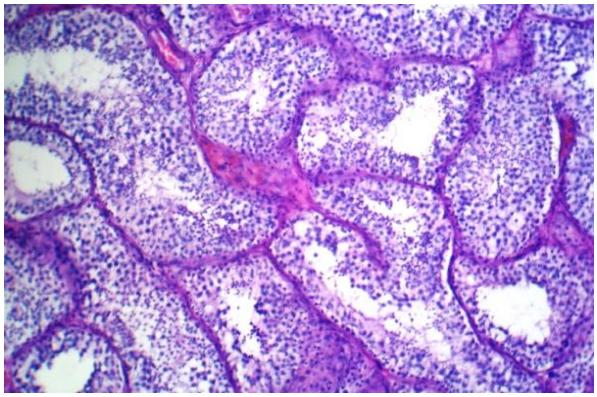

Fig. A: Normal Spermatogenesis (HPE -100X) Tubule showing all the stages of spermatogenesis

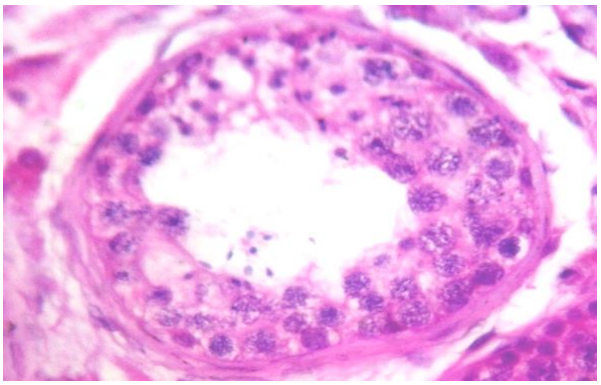

Fig. B: Hypospermatogenesis – Tubules showing reduction in number of germ cells

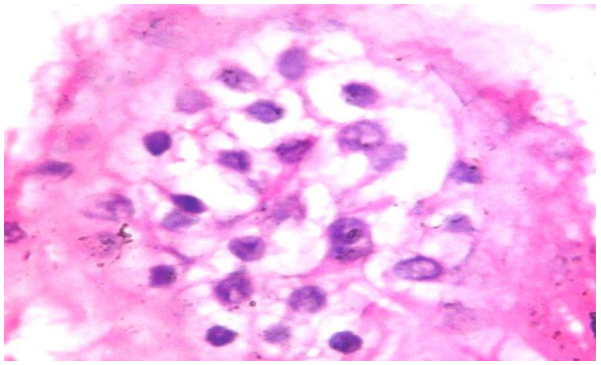

Fig. C: Maturation Arrest –Spermatogonia level- Tubule lined by spermatogonia and sertoli cells (400X)

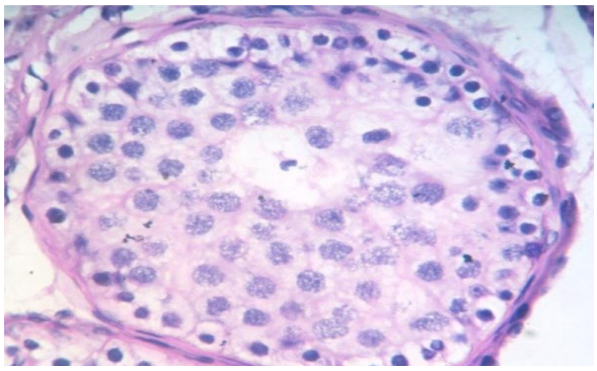

Fig. D: Maturation Arrest –Spermatocyte level (HPE- 400X)- Tubule shows arrest at the level of primary spermatocyte

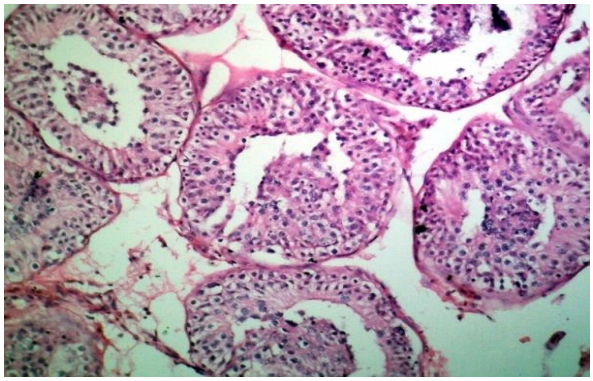

Fig. E: Maturation Arrest -Spermatid level (HPE-100X) Shows tubules with maturation arrest at the level of spermatids

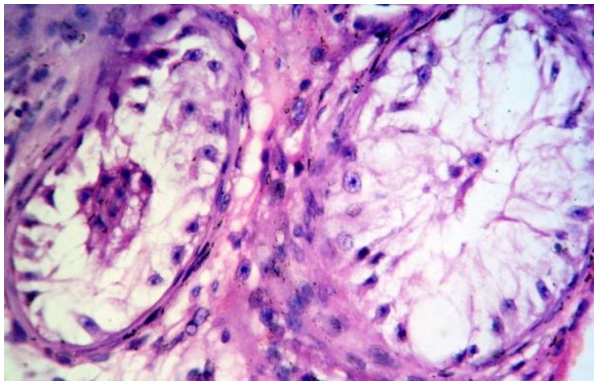

Fig. F: Germ Cell Aplasia (HPE 400X)- Shows tubules lined only by sertoli cells

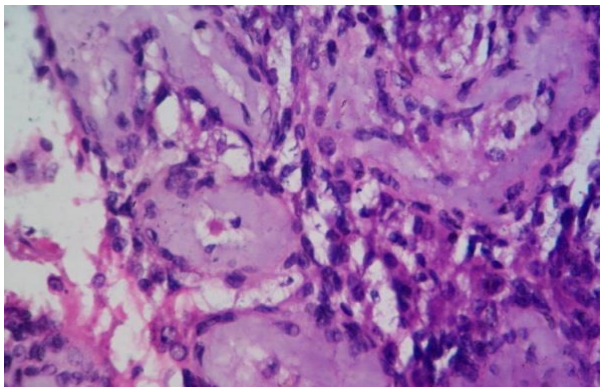

Fig. G: Tubular hyalinization – HPE (400X) - Shows hyalinised tubules with no germ cells or sertoli cell lining

Results

This study was done on 36 cases, out of which 1 case has been excluded as the sample did not contain testicular tissue. The results of 35 cases taken for study were tabulated. Almost all patients had Primary infertility except for one patient who had secondary infertility. The age group ranged from 20 to 40 years with a mean age of 30.4 yrs. The duration of infertility ranged from 2 years to 14 years with mean duration of 5.7 years. 27 patients were confirmed to be azoospermic after 3 semen analyses correlating with maturation arrest, Sertoli cell only syndrome (SCOS) and Testicular Atrophy in testicular biopsy. The remaining 8 patients were oligospermic correlating with hypospermatogenesis and normal spermatogenesis. The cytological diagnosis from 35 cases was depicted in Table1. The most frequent diagnosis on cytological report was Maturation arrest.

Table 1: Cytological diagnosis in 35 cases

FNAC diagnosis	No. of cases	% of total
Normal spermatogenesis	8	22.9
Hypospermatogenesis	8	22.9
Maturation arrest	12	34.2
Sertoli cell only syndrome(SCOS)	5	14.3
Testicular atrophy	2	5.7

Progressive decreasing values of Spermatic Index (SI) were seen in normal spermatogenesis, hypospermatogenesis and maturation arrest. Sertoli cell index **(**SEI) was found to be the most important index in distinguishing hypospermatogenesis from normal spermatogenesis since SEI was found to be elevated in hypospermatogenesis. Maturation arrest was distinguished from hypospermatogenesis by the SI which was severely decreased with absent spermatozoa. In Sertoli Cell Only Syndrome, SI and SPSEI were zero. Various indices in different categories are mentioned in Table 2.

Table 2: various cell indices in different categories of cytological diagnosis

Cytological diagnosis	No. of cases	Indices	
		Spermatic Index (S.I)	Sertoli Cell Index (SEI)
Normal spermatogenesis	8	54.25	43.75
Hypo spermatogenesis	8	6.2	123
Maturation arrest	12	0	99.3
Sertoli cell only syndrome	5	0	-
Testicular atrophy	2	0	-

Johnson Score correlation with HPE is 100% in case of Normal spermatogenesis, Tubular hyalinization and Germ cell aplasia. The predominant Johnson's score ranged between 3 and 7 indicating maturation arrest at spermatogonia, spermatocyte and spermatid levels. Correlations between Johnson's scoring and histology report are given in Table 3.

Table 3: Correlation between Johnson's scoring and histology report

Johnson's scoring	No. of cases	% of total	Histological report	No. of cases	% of correlation
9 &10	7	20%	Normal spermatogenesis	7	100%
8	6	17.1%	Hypo spermatogenesis	5	83.3%
7-3	13	37.2%	Maturation arrest	12	92.3%
2	7	20%	Germ cell Aplasia	7	100%
1	2	5.7%	Tubular Hyalinization	2	100%
Total	35	100%	Total	33	94.3%

There was complete agreement between the cytological findings and histological findings. Cytohistological correlation was shown in Table 4. The overall percentage of cytohistological correlation was 86%. Of 13 cases diagnosed as Maturation arrest in HPE, 10 cases were diagnosed the same in FNAC. Remaining 3 cases were

diagnosed as Hypospermatogenesis since the smears contained few mature spermatozoa. Of the 7 cases diagnosed as SCOS in HPE, 5 cases were diagnosed the same in FNAC. Remaining 2 of the cases were diagnosed as early maturation arrest, since some spermatogonia were seen in the FNAC.

Table 4: FNA-Histopathology Correlation

Patterns	HPE	FNAC	% correlation
Normal spermatogenesis	8	8	100%
Hypo spermatogenesis	5	5	100%
Maturation arrest	13	10	76.9%
Germ cell Aplasia (SCOS)	7	5	71.4%
Tubular Hyalinization	2	2	100%
Total	35	30	86%

Discussion

Male infertility is now a day a common problem. Although the technique of testicular FNAC was there for a long period it has become popular only in recent years. Though Testicular biopsy can differentiate an obstructive etiology of male infertility from an intrinsic testicular cause, it has its own complications like hematoma, fibrosis and scarring. On the other hand, FNAC is an easy and less invasive method associated with no or minimal complications. In cytological smears a report of presence or absence of sperm is adequate since in cases of hypospermatogenesis and maturation arrest, these patients may be helped by hormonal therapy. FNAC has minimum side effects whereas biopsy may result in fibrosis which hamper the process of sperm extraction for ICSI (Intra Cytoplasmic Sperm Injection).[12]

In our study out of 35 cases, 27 cases were found to be azoospermic and 8 cases found to be oligospermic. Out of these 27 azoospermic cases, 10 cases in FNAC were found to have mature sperms. This discordance was found to be due to obstructive causes except in two cases.

In the present study, maturation arrest with a Johnson's score of 3 to 7 was found to be the most frequent cause of male infertility. Correlation of various cell indices with cytological diagnosis showed an increase in Spermatic index (SI) and decrease in Sertoli index (SEI) in case of normal spermatogenesis, whereas progressive decrease in SI and increase in SEI were observed in hypospermatogenesis and maturation arrest. SEI was found to be more increased in hypospermatogenesis than in maturation arrest. In SCOS, SI and SEI was found to be zero. In considering all the results, the overall accuracy of FNAC in this was found to be 86%. FNAC was found to be 100% accurate in diagnosing normal spermatogenesis, hypospermatogenesis and tubular hyalinization. Overall sensitivity of this study was found to be 89.7% with specificity of 96.2%, positive predictive value of 89.2% and negative predictive value of 96.1%.

Testicular cytomorphological patterns given in various studies were compared with current study and shown in Table 5.[13-16] There was a wide variation among several studies and so the incidence varies in different studies. Our study showed a predominance of maturation arrest whereas some studies showed a predominance of hypospermatogenesis as the cause of male infertility. Two studies have shown germ cell aplasia as the predominant cause for infertility.

Table 5: Comparison of Incidence of Cytomorphological patterns with Other Studies

Author/yea r	Normal spermatogenesis	Hypo spermatogenesis	Maturation arrest	Sertoli cell only syndrome	Testicular Atrophy
Al-Jitawi et al, 1995[13]	10.2%	31.4%	-	30.2%	28.5%
Meng et al, 2001[14]	13.8%	17.2%	33.3%	35.6%	-
Qublan et al, 2002[15]	20.6%	26.5%	23.5%	29.4%	-
Singh et al, 1999[10]	15.63%	65.63%	3.3%	3.13%	12.5%
Verma et al 1992[16]	30.3%	42.3%	6.7%	2.6%	-
Current study	22.9%	22.9%	34.2%	14.3%	5.7%

Johnson Score correlation with HPE was 100% in case of Normal spermatogenesis, Tubular hyalinization and Germ cell aplasia. The predominant Johnson's score in our study ranged between 3 and 7 indicating maturation arrest at spermatogonia, spermatocyte and spermatid levels. The discordant cases indicate that if Johnson's score is done routinely during histopathology reporting, then the diagnostic accuracy could be improved. In Mona et al the predominant Johnson score was 2 indicating SCOS as shown in Table 6.[11]

Table 6: Comparison of Johnsons Score and HPE Report

Johnsons Score	Mona et al 2008[11]		Current study	
	Number of cases (50)	Percentage of total (%)	Number of cases (35)	Percentage of total (%)
9 & 10	12	24%	7	20%
8	4	8%	6	17.1%
7-3	14	28%	13	37.2%
2	17	34%	7	20%
1	3	6%	2	5.7%

Several references are available on correlation between FNAC and biopsy as given below in Table 7.[17-22] Most of the available references show an accuracy rate of >85% and our study shows accuracy (% agreement) of 86%. Correlation between histology and cytology in evaluating spermatogenesis exceeds 90% in most studies. Meng et al found discordant diagnosis between cytology and histology in 6% of cases.[14] In half of these, the discordance was due to additional information provided by FNAC. It is likely that the thin sections performed during the histological preparation "cut" tails of some spermatozoa, but these are well preserved when the whole FNA specimen is smeared on the glass.

Table 7: Literature on Correlation between FNAC and Biopsy

Studies (Year)	Number of Patients	Cytologic & Histologic Agreement (%)
Gottschalk- Sabbag et al (1993)[17]	47	87%
Mallidis et al (1994)[18]	46	94%
Mahajan et al (1999)[19]	60	97%
Rammou- Kinia et al (1999)[20]	30	87%
Odabas et al (1997)[21]	24	90%
Meng et al(2001)[14]	87	94%
Qublan et al (2002)[15]	34	96%
Srivastava et al (2004)[22]	46	95.6%
Current study	35	86%

In our study discordance was observed in 5 of 35 cases (14.3%). Out of this 5 cases, 3 cases reported as maturation arrest in biopsy were found to have mature spermatozoa (hypospermatogenesis) in cytological smears and the two other cases reported as Sertoli cell Only Syndrome in biopsy contained spermatogonia (maturation arrest).

Thus FNAC is less invasive and gives informative data on spermatogenesis of the entire testes. Report can be issued quickly as compared to biopsy. It is simple, quick and inexpensive because surgical instruments are not required. Local scarring doesn't occur. It is well tolerated by patient. The material shows excellent preservation and various cell types can be identified. There are also some limitations as 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.

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