The role of FNA cytology of the testis in management of male infertility (In Iraq)

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Abstract

Background and objectives: Fine needle aspiration cytology of the testis is now well recognized in diagnosis of testicular diseases. Recently it has also gained popularity for its diagnostic and therapeutic role in male infertility. The purpose of this article is to put light on role of testicular fine needle testicular 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, moreover to its coincidence with hormonal situation of the patient for diagnosis.

Methods: This is a prospective study for sample collection during the period from January 2009-Dec 2010. The samples studied included 152 patients underwent FNA of testis for evaluation of spermatogenesis , spermiogenesis & the presence of active mature sperms in addition to hormonal assay for FSH, LH, testosterone and prolactine correlating with clinical findings

Results: : The majority of cases of infertility were due to secondary maturation arrest (94 cases 61.84%) (P=0.03) followed by obstructive orchiopathy (38case 25%) & then the minority of cases were due to untreatable conditions including primary maturation arrest & total testicular atrophy

Conclusion: FNAC is the dependent method of Azospermic patient evaluation despite of the presence of controversial ideas depending on that the FNAC size is not a suitable window to be able to represent whole testis in evaluation of azospermia.

Keywords: Azospermia, fine needle cytology,reproduction, spermatogenesis

Introduction

Delivering epidemiological data has shown male factors infertility accounts 20% of prevalence rate and 1/3 is a product incorporate both male and female factor.Thus the predictive value for male factor domain has been estimated approximately 30% to 50% of infertile couples.[1] Azoospermia or absent sperm in semen occurs in approximately five to ten percent of infertile men who are evaluated.[2]

Azoospermia may be obstructive azoospermia or non obstructive (parenchymal disorder) azoospermia (NOA). There has been renewed optimism to tackle the obstructive cause following, intracytoplasmic sperm injection, in whom the only option for men with NOA. Assessment of spermatogenesis is an important component in the diagnostic algorithm of male infertility

Traditionally, the testicular biopsy has been the gold standard to boost this evaluation because it provides a para amount information about obstructive or non-obstructive testicular causes dictate of both suspected obstruction and in failing unobstructed testes. Any technique to assess spermatogenesis must be minimally invasive and must conserve as much testis tissue as possible.[3] It should also not only provide qualitative but quantitative information

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about spermatogenesis in addition to answering the question of whether sperm production is normal, it must also address whether any sperm are present at all within the testis. According to last advances in field of reproductive medicine, even a single sperm can now give reproductive men with NOA chance to enjoy biological fatherhood.[4] Testicular biopsy is well established as one of the main investigative modalities in male infertility for a long period of time in evaluation of spermatogenesis. But the tissue sample is small and not representative of entire testis.[5] It is also invasive and traumatic especially when applied to both testes.[6] Fine needle aspiration cytology (FNAC) of testes has proved to be useful in testicular tumors as well as in non neoplastic and inflammatory conditions of testes, less traumatic Needle aspiration of testes was first described by Max Huhner. First fine needle aspiration (FNA) of human testes in men with fertility disorder was performed by Scandinavian group which is pioneered by Obrant and Persson, still not fully describing the morphologic features at various stages of spermatogenesis. Later cytological features of seminiferous epithelium was described by Schenk and Schill[7]. However testicular FNA did not gain popularity because of lack of experience of pathologists in cytological analysis of various seminiferous tubular cells, because of fear of trauma to the testes, with hematoma formation by the procedure and because cytology was unable to give information about the tubular basement membrane and status of interstitial tissue.[6] But later on many studies carried out showed that FNAC evaluated spermatogenesis of entire testes was less invasive, report could be issued quicker and there was good cytological- histological correlation. [6,8] It was also concluded that information of tubular basement membrane and interstitial tissue was of no help in evaluation of spermatogenesis not only in diagnosis, but testicular FNA has 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. [9,10,11] These studies have encouraged the modifications in various sperm retrieval techniques Because biopsy procedures remove interstitial hormone-producing tissue non selectively with seminiferous tubules, substantial and possibly critical reductions in Leydig cell mass are possible and remain a concern for men who present with atrophic testes.[12] Also, testicular open biopsies have side-effects like hematomas, inflammation and especially permanent devascularization of the testis resulting in testicular atrophy.[13] So FNA of the testis for sperm retrieval have also started to gain popularity of FNA technique

Methods

The present study includes 152 male patients who presented with azospermia FNA of testis underwent to all patients ,for the majority of case were bilateral ,few of cases were unilateral FNA .Testicular FNA is done under local anesthesia . 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 rule out any tenderness. 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 accuracy & completness Using 21-23 G needle with 10-20 ml syringe attached to it. Testes is aspirated Precise gentle in and out movement varying from 5-8 mm are used.Testes can also be needled without local anesthesia after blocking of spermatic cord, but only at one site and procedure should be completed within 10-15 seconds. The patient should be rested for at least

ten minutes after the procedure.[14] 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 examination. [15] Geimsa 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.[16] Reporting testicular FNAC for 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[6] consecutive cells should be counted and percentage of different cells noted. Cytological results are satisfactorily Reproducible. two cell populations are evident in cytology, sertoli cells & cells in various stages of spermatogenesis.The spermatogenetic cells are divided into: .Spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. Cytological 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.[17]

Results

A total of 152 patients were included in this study. Ten men out of the total had single testis. The mean age of the patients studied was 42.5 years with a range from 20 years to 65 years the most predominant age group is belong to 30-39 years constitute 71 cases(46.7%) followed by age group(20-29)year constitute 40 cases (26.3%) & then the age group (40-49)year 32 cases(21%) Table(1) . The duration of infertility of the patients studied ranged from 1 year to 35 years with a mean duration of 17.5 years. Almost all patients were primarily infertile except for 5 patients who were having secondary infertility & in second marriage. Three of the patients had bilateral undescended testis, two patients unilateral . Five patients were operated to have uni or bilateral varicoceles. All of the patients were confirmed to be azoospermic after 3 semen analyses. A total of 152 fine needle aspiration cytology smears specimens obtained from infertile men were studied (3 men had unilateral left testis). The following were the diagnosis on cytological examination:The most frequent diag -nosis on cytological evaluation was normal sperma-togenesis with 38 cases (25%). They were cellular smears, which consisted of the total spectrum of spermatogenic cells admixed with Sertoli cells. The spermatogenesis cells showed transitional forms from spermatogonia to spermatozoa characterized by dimi-nution in nuclear size and condensation of chromatin Figure (3). The next common diagno-sis was hypo spermatogenesis with 32 cases (21%). The smears had less than normal amount of sperma-tozoa admixed with the other cells.The smears showed a high percentage of spermatogonia and primary spermatocytes along with absence of spermatids and spermatozoa. In present study there were 31 cases (20.4%) of early maturation arrest.Some smears were characterized by the total absence of spermatozoa and significant relative increase in proportions of round and elongated spermatids

along with spermatocytes and Sertoli cells. Late maturation arrest was seen in 31 cases (20.4%) Figure (5). The smears were cel-lular characterized by complete lack of germ cells and showed only Sertoli cells.In our study 12 cases (7.9%) showed Sertoli cell only syndrome Figure (6). Testicular Atrophy - In our study there were 8 cases (5.3%) of testicular atrophy. The smears had scanty cellularity mainly consisting of few Sertoli cells Figure (1). In evaluating the different patterns of diagnoses in azospermic patients it was found that in some of the azospermic patients, the diag-nosis was normal spermatogenesis (25%) suggestive of an obstruc-tive pathology. The accuracy in which these patients were diagnosed by FNAC was 100%.. This was closely followed by hypo spermatogenesis and maturation arrest. .The least frequent one diagno-sis was testicular atrophy Among the obstructive type & all types of secondary maturation arrests total (74) cases were got positive for active sperms, (40) cases belong to obstructive type got bilateral positive active sperms ,(20) cases got positive active sperm in left testis only & (16) cases got positive active sperm in right testis only among all of them (61) cases(40.13%) got fatherhood, figure 2 concordant results were obtained in testicu-lar cytology of 150 patients (98.7%). Dis-cordant results were seen in only 2 pa-tients. Of the 2 patients one had a testis which cytology proved to have normal sper-matogenesis while the contra lateral testis showed hypo spermatogenesis. The other patient had a cytology proven sertoli cell syndrome on one side while the other showed early maturation arrest. Of these 2 patients who had discordant findings the size of testes were quit variable. Regarding etiology of infertility in this study identified that in (128) case (84.2%) the causes were diagnosed among them (94) cases posi-tive history for sexually transmitted dis-eases (14) case with low testosterone less than 3ng/ML ,(10) case due to hyperprolac-tinemia more than 14ng/ML & (10) cases were iatrogenic, Table (2).

Table 1:distribution of the study population according to age group

Age groups	Number of cases	Percentages
20-29year	40	26.3%
30-39 year	71	46.7%
40-49 year	32	21%
50-59 year	6	4%
60 & above	3	2%
Total	152	100%

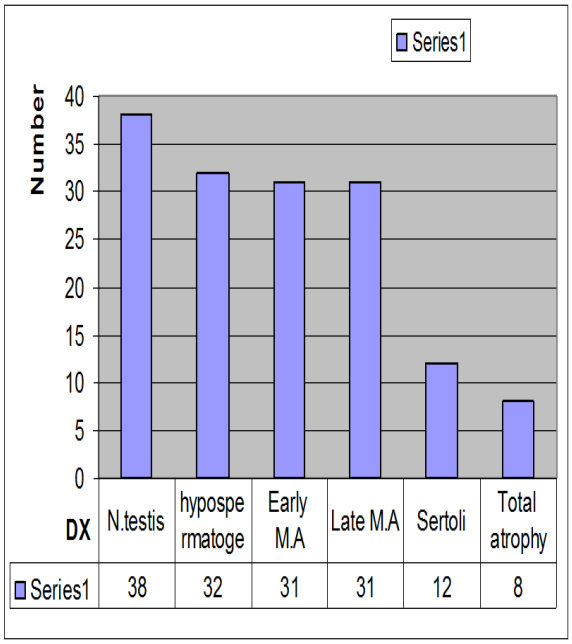

Figure 1: Cytological diagnosis & number of cases

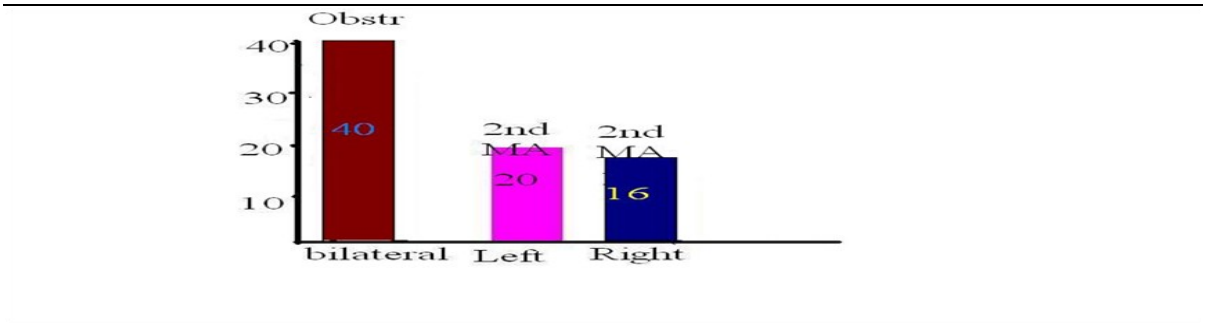

Figure2 : Side of positive sperms with number of cases

Table 2: Etiological classification & number of cases

Aetiology	Sexualy transmitted diseases	Testosterone	prolactine	Iatrogenic	Total
No of cases &%	94(61.84%)	14(9.2%)	10(6.57%)	10(6.57%)	128(84.2)

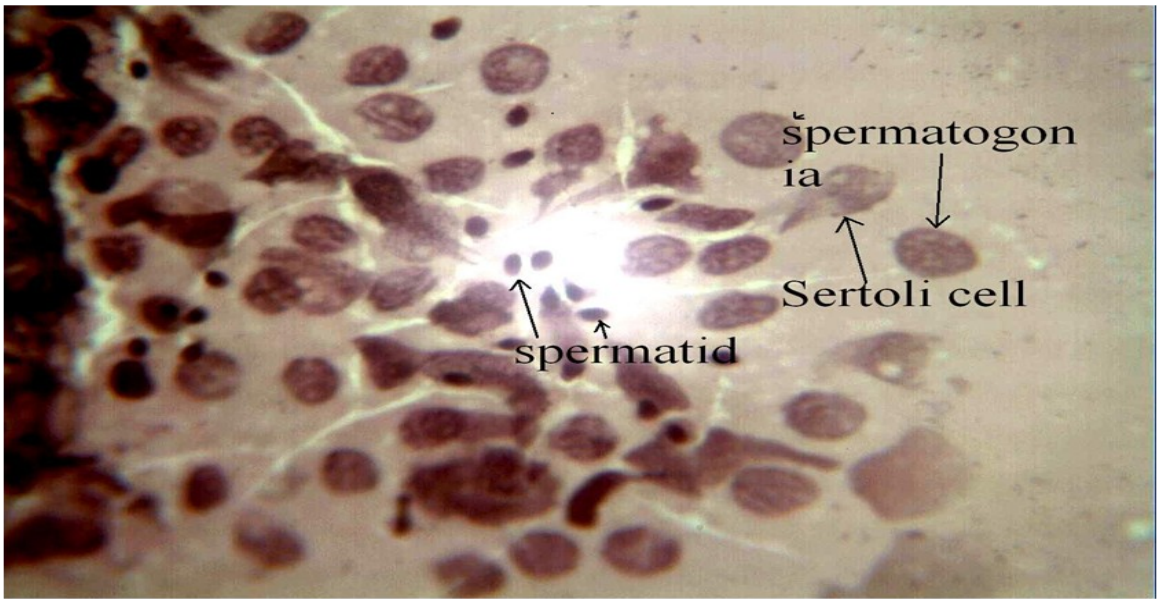

Figure3: Obstructive orchiopathy

Figure 4: Mature sperm

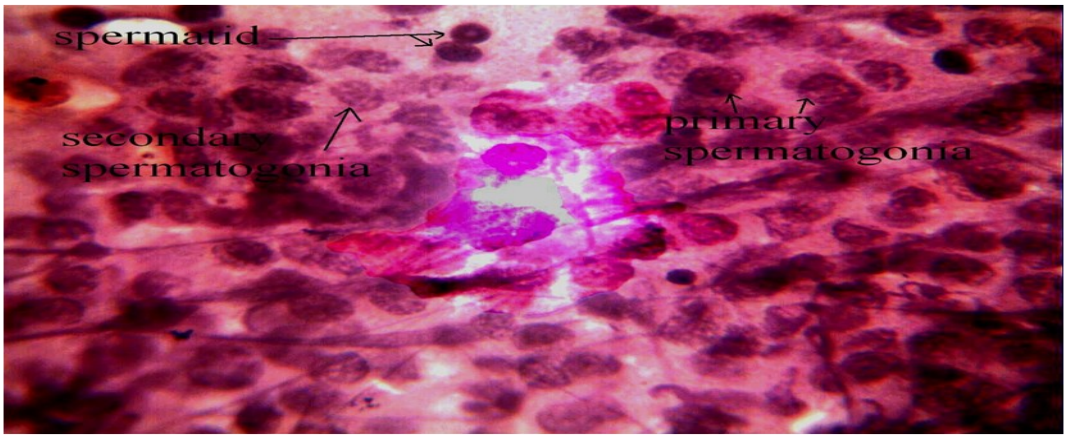

Figure 5: Secondary maturation arrest

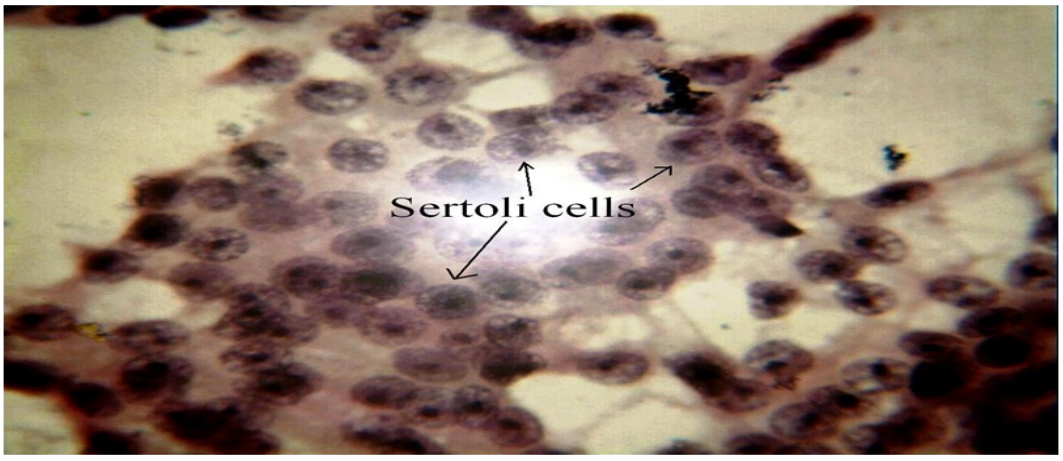

Figure 6: Sertoli cell only syndrome

Discussion

Huhner first used testicular puncture biopsies in the investigation of human infertility that exam-ined unstained samples for spermatozoa.[17] Later fine needle aspiration of the testis pioneered by Obrant and Persson (1971) was being proposed as a non-invasive technique.[18] Char-acterizing the cell types in cytological smears was straight-forward, with out much difficulty in recognizing germ cells and Sertoli cells. The material aspirated by FNAC is adequate and the various cell types can be identified by their distinctive morphology. Some authors have attempted to quantitatively analyze the population of germ cells. Ser-toli cells and spermatozoa in the cytological smear so as to reach a diagnosis.[19] In the 152 FNACs in this study the cytological patterns encountered showed that 25% had normal spermatogenesis & the majority of them were due to obstruction of vase differences by sexually transmitted diseases .which can be avoidable while the remaining were show-ing impaired spermatogenesis.. In our study there was good agreement between the cytological with positive active sperm and the chance of getting fatherhood 80.3% . An accuracy of positive sperms was 92 % was achieved in our study. In 4 cases, in our study, the material aspirated was scanty and therefore insufficient for a diagnosis. The correspond -ing histopathological diagnoses were a majority of 8 cases with testicular atrophy, 1 case of early maturation arrest and 1 case of normal spermatogenesis. Thus it might be pointed out that insufficient aspiration was mostly because of the relatively a cellular, fibrosis, atrophied testes. In diagnosing a correctable post testicular cause for in-fertility in those patients with azoospermia and cytology- proven normal spermatogenesis the accuracy of fine needle aspiration cytology compared to histopathology was 100%. Many similar studies have been conducted correlating the efficacy of cytological diagnosis with histological di-agnosis. Most of these studies show a similar degree of good correlation. [20,21]

Conclusion

The technique of testicular FNAC is simple, inex-pensive and minimally traumatic. More than 1 speci-men can be taken safely.Testicular FNAC gives an accuracy of 92% in the diagnosis of patients with male infertility.The material aspirated by FNAC is adequate and the various cell types can be identified by their distinc-tive morphology. This study proves that FNAC can evaluate accurately all classically defined histological types.FNAC obtained insufficient smears mainly in atro-phied testes.The accuracy of diagnosing normal spermatogenesis activity in obstructive azoospermia by FNAC was 100%.

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