Testicular Biopsy In Male Infertility (Study Of 80 Cases)

P U.R., G H.M., D K.J., S A.M.

Citation

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Abstract

Background: Infertility is one of the major problems in developed as well as many developing countries. About fifteen percent of all marriages face the problem of infertility. Aim: To find out the preventable and treatable cause of infertility which help to reduce its prevalence. Methods and Material: Semen analysis and testicular biopsy was carried out in 80 infertile male after detailed history and clinical examination. Results: History of smoking was encountered in 29 (34.12 %) cases, 56 (70 %) patients were clinically normal in terms of testis and scrotum. Varicocele (41.67 %) was the most common positive clinical finding responsible for infertility. Though the specific reason for sertoli cell only syndrome is not known, it was the most common abnormal histopathological finding (18.75 %), followed by maturation arrest, testicular atrophy and granulomatous inflammation of testis (11.25% each). On semen analysis, 71.25 % cases had azoospermia, 24.71 % cases had oligospermia and 2.35 % cases had normal sperm count. Oligospermia is usually associated with inflammatory etiology. Among oligospermics and normospermics, the sperm motility was more than 50 % in 14 patients (60.67 %). Among the azoospermic patients, 35.09 % showed normal spermatogenesis in their testicular biopsy. Although azoospermia is commonly associated with normal spermatogenesis, it is a hallmark of sertoli cell only syndrome (SCOS), testicular atrophy and maturation arrest. Conclusion: Testicular biopsy is invaluable in the azoospermics but its usefulness is limited in the oligospermics.

INTRODUCTION

Infertility is empirically defined as the inability of a couple to conceive after one year of coital activity without contraception.[1] Since the burden of a childless marriage is borne by the female, it was a gynecologist who attempted the first exploratory measures for the diagnosis of infertility in male.[2] The statistics of infertility show that about 15 to 20 percent of the marriages (every 7 th couple) face the problem of infertility in the developed countries.[1] In the developing countries the prevalence is variable, it is as high as 30% in Sub Saharan Africa to as low as 5 % in China.[3] Males alone are responsible for almost 30 % of all cases and in another 20 % both the partners have detectable abnormalities.[4] The ever increasing interest in the study of male infertility has stimulated the investigation of spermatozoal physiology, semen abnormality and the pituitary gonadal relationship and the microscopic study of the testicular tissue, making a bewildering array of diagnostic options available. The most basic and simple screening test for evaluation still remains the semen analysis and testicular biopsy. Semen analysis is relatively inexpensive and can provide valuable information if done correctly.[5,6] Ultimate aim of our study is to find the etiology for infertility and if treatable cause is find, to treat the cause and to improve the fertility of the patient. By all our means we try to reduce the burden of infertility in our society; by increasing the awareness in public.

MATERIAL AND METHOD

The present study was conducted in the department of pathology over a period of 2 years, starting from January 2005 to December 2006. This study is based upon the histopathological examination of the testicular biopsies. The biopsies were obtained from 80 infertile males with abnormal semen examination. The age of incidence was from 20 to 45 years. Cases for testicular biopsy were selected after taking detailed clinical history, thorough clinical examination and semen analysis. The detailed clinical history including age, the duration of active marriage life, duration of infertility, sexual relationship, frequency of coitus, premature ejaculation, psychological status and libido was noted. History regarding alcohol consumption, smoking and other substance abuse was also inquired into. Occupational exposure to radiation, heat and pesticides was asked. Past history of infection like mumps, measles, pneumonia, sexually transmitted diseases (STD), tuberculosis and any surgical procedure was also asked. Secondary sex characters and genital organs examination

was done. The systemic examination to exclude anemia, tuberculosis, diabetes mellitus was also carried out. Clinical diagnosis was confirmed by ultrasonography whenever necessary.

Semen analysis was done in all the patients and parameters like total sperm count, motility and presence or absence of abnormal forms were looked into. Before collection, the patients were advised for three days of abstinence. Semen samples were collected in the laboratory room in a clean, dry, biologically inert container. In case of oligospermic or azoospermic patients, three semen samples were collected on alternate day and thorough examination was carried out. Spermatozoa were counted using the hemocytometer chamber under high power in all four WBC square using semen diluting fluid consisting of sodium bicarbonate and formalin in distilled water[5,6,20] (1:20 dilution). The sperm counting was carried out same as WBC counting. Sperm count is one of the most useful tests for evaluation of fertility in men. As the name implies a semen specimen containing no spermatozoa on at least two examinations is said to be azoospermic. Before stamping azoospermic, the sample must be centrifuged and check the pellet for sperms. The count in oligospermia may range from very few spermatozoa to 20 million. To evaluate the motility, a small drop of liquefied semen was placed on a prewarmed slide, covered with a cover slip and was seen under high power. Smear must be prepared from pellet and stained with leishman stain to check the sperm morphology.[20] It is usually found that lower the count, higher the number of abnormal forms. Cases were categorized into three groups i.e. group I with azoospermia, group II with oligospermia (count <20 million / ml) and group III with normal count. According to motility they are divided in 4 grades.

Grade a (fast progressive) sperms are those which swim forward fast in a straight line-like guided missiles

Grade b (slow progressive) sperms swim forward but either in a curved or crooked line, slowly/slow linear/non linear

Grade c (non progressive) sperms move their tails, but do not move forward

Grade d (immotile) sperms do not move at all.

Sperms of grade c and d motility are considered of poor quality.

Testicular biopsy was done in all the azoospermics, oligospermics and normospermics with relevant clinical history. The testicular biopsy specimens were taken by urologist after proper cleaning of scrotum with an antiseptic solution, and the area around it is covered with sterile cloth. A local anesthetic was injected into the skin of the scrotum. Then a small incision was made through the skin, and a tiny piece of testicular tissue was removed using “Window’s microsurgical technique”.[10] Approximately 3 mm size specimen was excised using “No touch technique” and placed in Bouin’s fixative. A single stitch was used to close the incision in the testicle and another stitch is used to close the incision in the skin with an unabsorbable sutures. The scrotal area was then bandaged and patient was informed to wear an athletic support for several days after the procedure. The biopsy took approximately 15-20 minutes. Patient had been advised to refrain from sexual activity for 1 to 2 weeks after biopsy and avoid washing the area for several days.[10] After fixation the tissue was processed and paraffin embedded. Sections were cut and stained with haematoxylin and eosin stain. Special staining for elastic fibers, collagen and crystals of reinke were done wherever indicated. In the interpretation of testicular biopsy, attention was paid to the size, shape and population density of seminiferous tubules, the state of basement membrane, various stages of spermatogenesis and components of the interstitial tissue.[17] Some degree of subjectivity does occur in the interpretation of mild hypospermatogenesis and tubular hypoplasia, since there are no clear guidelines for the range of normality.[7] In the present study, testicular biopsy was reported individually by two different pathologists to reduce the subjectivity.

RESULTS

Eighty patients of male infertility between the periods from January 2005 to December 2006 were selected on the basis of semen examination. Patients with azoospermia, oligospermia and normospermia were selected for biopsy.

As table-1 shows, the age group of 25 – 35 years is the most common age group, 56 out of the selected 80 patients (app. 69.70 %) fall in this age group and after the age of 35 years, the number of cases significantly decreased. History of smoking was present in 29 (34.12 %) cases.

Table-2 shows that the majority of the patients, 56 out of 80 (70 %) were clinically normal. Clinically positive findings were found only in 24 patients. Out of these 24 patients, 10 (41.67 %) had varicocele. Thus varicocele was the most common positive clinical finding encountered, followed by hernia, cryptorchidism and small testes (16.67 % each). Out of all 80 cases, only 1 case of hernia and 2 cases of small

testes were bilateral.

During semen analysis 68 (80%) cases had normal semen volume. Out of the selected 80 patients of infertility, 57 (71.25%) were azoospermic, 21 (24.71%) were oligospermic and 2 (2.35%) were normospermic. Thus, azoospermia was the most common finding in semen analysis. Table IV shows that among the 23 patients (normospermic and oligospermic) studied for sperm motility, irrespective of the sperm count, 14 cases (60.87 %) had motility more than grade III and average motility was 50-70%.

In histopathological examination normal spermatogenesis was the most common finding encountered in 29 out of 80 cases, comprising approximately 36.25 %. Out of 9 reported cases of granulomatous inflammation, 7 had tuberculosis. As tuberculosis is one of the most common diseases in our country, it forms a major positive clinical finding and is usually associated with oligospermia and/or normospermia. Varicocele was the most common positive clinical finding and it was usually associated with normal spermatogenesis. Small testes and cryptorchidism were exclusively associated with sertoli cell only syndrome (SCOS) and atrophy of testes. Hydrocele was associated with the inflammatory lesions, both granulomatous orchitis and nonspecific epididymo-orchitis. When testes were present in hernial content, the histopathological examination showed various findings like normal spermatogenesis, sertoli cell only syndrome (SCOS) as well as atrophy of testes. Sertoli cell only syndrome (SCOS) was the most common positive histopathological finding comprising around 18.75 % of the cases. This was followed by maturation arrest, testicular atrophy and granulomatous inflammation of testes, having an incidence of 11.25 % each. As such, inflammation in general, including both granulomatous orchitis and nonspecific epididymo-orchitis (total 12 cases-15 %) were more common than maturation arrest and testicular atrophy. Maturation arrest as a lesion showed no clinical finding.

The table-3 shows that azoospermia was seen to be the exclusive finding in maturation arrest, sertoli cell only syndrome (SCOS), and testicular atrophy. Granulomatous as well as nonspecific inflammation was associated with oligospermia or normospermia. Azoospermia was the most common finding of semen analysis, comprising around 71.25 % of the cases. During histopathological examination, it appears that azoospermia was also associated with normal spermatogenesis (35.09 %).

Figure 1

Table 1: Age distribution table (n=80)

Age group (years)	No. of cases
20-25	20
26 – 30	36
31 – 35	20
36 – 40	03
>40	01
Total	**80**

Figure 2

Table 2: Clinical & Histopathological Correlation (n=80)

	Histopathological examination							
	Normal Spermatogenesis	Hypo spermatogenesis	Maturation arrest	SCOS	Atrophy	Granulomatous inf.	Non specific inf.	Total
Clinically Normal	21	3	9	10	3	8	2	**56 (70%)**
Hydrocele	-	-	-	-	-	1	1	**2**
Cryptorchidism	-	-	-	2	2	-	-	**4**
Hernia	1	-	-	1	2	-	-	**4**
Varicocele	7	3	-	-	-	-	-	**10**
Small Testes	-	-	-	2	2	-	-	**4**
Total	**29**	**6**	**9**	**15**	**9**	**9**	**3**	**80**
Incidence (%)	**36.25**	**7.50**	**11.25**	**18.75**	**11.25**	**11.25**	**3.75**	**100**

Figure 3

Table 3: Correlation of HPE & Semen Analysis (n=80)

	Semen analysis		
	Azoospermia (n=57)	Oligozoospermia (n=21)	Normospermia (n=2)
Normal spermatogenesis	20 (35.09 %)	9 (42.86%)	--
Hypospermatogenesis	4 (7.02%)	2 (9.52 %)	--
Maturation arrest	9 (15.79 %)	0	--
SCOS	15 (26.32 %)	0	--
Atrophy	9 (15.79 %)	0	--
Granulomatous infla.	0	7 (33.33 %)	2
Non Specific infla.	0	3 (14.29 %)	--
Total	57 (71.25 %)	21 (24.71 %)	2 (2.35 %)

Figure 4

Table 4: Sperm Motility (n=23)

MEAN MOTILITY	NO. OF CASES
50-70 %	14 (60.87 %)
<50 %	9 (39.13 %)

Figure 5

Table 5: Corelation Of Histopathological Examination

HISTOPATHOLOGICAL EXAMINATION	PRESENT STUDY (80 CASES)	PUROHIT et al[7] (50 CASES)	AHMAD et al[18] (80 CASES)
Normal Spermatogenesis	36.25 %	16 %	35 %
Hypospermatogenesis	7.5 %	26 %	10 %
Maturation Arrest	11.25 %	8 %	7.5 %
SCOS	18.75 %	26 %	7.5 %
Testicular Atrophy	11.25 %	18 %	35 %
Inflammatory Lesions	15 %	6 %	5 %

DISCUSSION

At the current level of advancement using the multidisciplinary approach involving the pathologist, gynecologist and the urologist, improvement in the quality of the ejaculate can be attained in a reasonably large percentage of men, provided that the diagnostic and prognostic measures available are used to their fullest extent. The semen analysis must be considered as the most important investigation in the evaluation of an infertile male.[5] Increase in the scrotal temperature due to tight wearing of the underwear and/or other causes also give variation in the sperm count.[9] Robinson[9] and associates cooled the scrotum of euspermic and oligospermic men with ice bag for 30 minutes daily for 2 weeks. A transient increase in the sperm concentration by about 3 folds was noted. Testicular biopsy is being re-evaluated as a step in the investigation of male infertility. With the advent of micromanipulation techniques in the field of fertility management, now even a single viable sperm can be used to fertilize an ovum. Testicular biopsy has gained therapeutic importance also.[10] As the name implies, testicular biopsy consists of an operative removal of a small bit of tissue from the testes, small enough to have no deleterious effects on the normal functioning of the gland, yet large enough to include a representative sample of the whole gland.

In the present study, the most common age group affected was 25 – 35 years comprising around 70 % of the cases. Similar finding (76 %) was achieved by Trupti et al[7] in 2004. In the present study, 25 % of the patients were below 25 years, whereas Trupti et al[7] found only 8 % of the patients in this age group. This is due to early age of marriage in India.

Proper history taking and clinical examination is also important as it is known that smoking decreases the sperm density as well as sperm motility, and to a lesser extent it cause an increase in the number of morphologically abnormal sperms.[11] History of smoking was noticed in 29 (34.12 %) patients. As smoking is increases in youngsters nowadays, it directly affect the fertility by increasing the abnormal density, motility and morphology in sperms.

Regarding the clinical examination of testes, 70.77 % (56 out of 80) of patients, who had normal sized testes, had abnormality in the semen examination. Trupti et al[7] found 35 out of 50 (70 %) were clinically normal with no symptoms, and had abnormalities in semen examination. Thus, the results are comparable. Though they were clinically normal they had azoospermia in semen examination and/or germ cell aplasia in histopathological examination. This occurs because we can clinically see only the size of testes or secondary sex characters. Although thorough semen examination was done, until and unless biopsy was done, we could not reach the proper conclusion.

The histological lesion associated with varicocele was hypospermatogenesis, which was similar to the changes reported by other workers.[12] Fertility is reversible after curing this condition.[12] Varicocele affects the function of testes in two ways. Firstly, chronic venous congestion leading to hypo spermatogenesis and atrophy of testes and secondly, the inhibitory effect of this raised scrotal temperature on sperms along with abnormal testicular hormonal environment due to regurgitation.[13]

Cryptorchidism was found in 5 % of the cases. The contralateral testes also had mild changes of atrophy in them, although clinically normal. In cases of cryptorchidism, it is important to perform orchidopexy before the age of puberty; otherwise an atrophic change start to appear in the contralateral testis also and also leads to a higher risk of bilateral malignancy.[12]

The present study shows 71.25 % of the cases (57 out of 80 patients) were azoospermic and 35.09 % of the azoospermia were associated with normal spermatogenesis. Similar finding were obtained by Trupti et al[7] and Ahmad et al,[18] while Kurien et al[19] found 50 % of the patients had normal spermatogenesis on fine needle aspiration cytology examination. These findings suggest that the obstructive etiology is one of the major causes responsible for male infertility and has a good prognosis. The obstruction may occur at the level of epididymis or vas deferens.[14] In case of

obstruction at the level of epididymis, the semen volume is normal, fructose is present, the PH is alkaline and no sperm precursor cells are seen on semen analysis.[14] On clinical examination they typically have normal sized firm testes, but epididymis is full and turgid.[14] In case of obstruction at the level of vas deference, the semen volume is low (0.5 ml or less), fructose is absent and the PH is acidic. The non obstructive azoospermia is commonly associated with maturation arrest, sertoli cell only syndrome (SCOS) and testicular atrophy. Similar findings were reported by Trupti et al.[7] Ahmad et al[18] found testicular atrophy (35 % of the cases) was the major cause of non obstructive azoospermia followed by maturation arrest and sertoli cell only syndrome (SCOS) (7.5 % each). Fertility in these non obstructive cases is not reversible.

The present study shows, 24.71 % of cases (21 out of 80 patients) were oligospermic, while Trupti et al[7] showed 46% (23 out of 50 patients) were oligospermic. Oligozoospermia was primarily associated with normal spermatogenesis and inflammatory lesions. Though semen analysis is an important screening test for evaluation, there are discrepancies in selecting cases for testicular biopsy on the basis of sperm count. We have taken sperm count of 20 million/ml as a cut off point for oligospermia and normospermia. Among all oligospermics and normospermics, mean motility was 50-70 % in 14 cases. Results are comparable to Trupti et.al.[7]

Azoospermia, is a common finding in both, normal spermatogenesis which is suggestive of obstruction and has a good prognosis and also in potentially bad prognostic lesions like sertoli cell only syndrome (SCOS) and maturation arrest. Azoospermia can be due to obstructive or non obstructive lesions. Some authors advocate biopsy to distinguish the type of azoospermia while others advocate quantitative methods. Biopsy is useful to evaluate spermatogenesis as well as for prognosis. On biopsy, normal histology suggests either partial or complete obstruction or possibility of antisperm antibodies.[7,12,18]

Regarding the findings of testicular biopsy for the evaluation of male infertility number of studies[7,11,16,18,19] are available. Trupti et al[7]observed hypospermatogenesis as a common lesion, while Ahmad et al[18] found testicular atrophy (35 %) as a major cause responsible for male infertility followed by sertoli cell only syndrome (SCOS) and maturation arrest (7.5 % each) Kurien et al[19] found maturation arrest and testicular atrophy as a common finding (12.7 % each) followed by sertoli cell only syndrome (SCOS) (3.6 %). We reported sertoli cell only syndrome (SCOS) as the most common lesion (18.75 %) followed by maturation arrest and testicular atrophy (11.25 % each). Whichever the histopathological finding, fertility is not reversible in these cases and so, prognosis is poor.

Out of 80 patients studied in this series, the best prognosis can be predicted for the patients having normal histology or obstructive lesions. The worst prognosis is predicted in sertoli cell only syndrome (SCOS), maturation arrest at primary spermatocytes level and testicular atrophy.

CONCLUSION

Smoking decreases the sperm density, sperm motility and cause morphologically abnormal sperms. History of smoking was encountered in 34.12 % cases.

Varicocele (41.67 %) was the most common positive clinical finding and it was associated with normal spermatogenesis.

Maturation arrest, sertoli cell only syndrome (SCOS), and testicular atrophy were exclusively associated with azoospermia.

Of all the cases studied, the diagnosis suggestive of obstruction was made in 36 % of the cases. Non obstructive pathology was found in 64 % of the cases.

In the 80 cases studied, the best prognosis was found in the lesion “suggestive of obstruction” and bad prognosis was found in sertoli cell only syndrome and maturation arrest.

Semen analysis and testicular biopsy provide valuable information about the etiology and the fertility potential of an individual.

Testicular biopsy is invaluable in the azoospermics but its usefulness is limited in the oligospermics.

Histological examination of the testis gives a correct assessment of the spermatogenesis and allows a rational choice for the future management by reconstructive surgery, hormonal therapy, artificial donor insemination or adoption.

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Author Information

PARIKH U.R., MD (Patho)
Assistant Professor in Pathology, Pathology Department, B.J.Medical College

GOSWAMI H.M., MD (Patho & Bact)
Professor of Pathology, Pathology Department, B.J.Medical College

DELIWALA K.J., MD (Gynec)
Assistant Professor in OB & GY, Pathology Department, B.J.Medical College

Shah A.M., MD (Patho)
Tutor in Pathology, Pathology Department, B.J.Medical College