TESTICULAR FINE NEEDLE ASPIRATION CYTOLOGY AND HISTOPATHOLOGY CORRELATION IN MALE INFERTILITY

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CERTIFICATE

This is to certify that this dissertation entitled **"TESTICULAR FINE NEEDLE ASPIRATION CYTOLOGY AND HISTOPATHOLOGY CORRELATION IN MALE INFERTILITY"** is a bonafide work done by **Dr. M. SRIDEVI** in partial fulfillment of the requirements of The TAMIL NADU DR.M.G.R. MEDICAL UNIVERSITY, Chennai for the award of M.D. Pathology Degree.

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DECLARATION

I declare that this dissertation entitled **"TESTICULAR FINE NEEDLE ASPIRATION CYTOLOGY AND HISTOPATHOLOGY CORRELATION IN MALE INFERTILITY"** done by me under the guidance and supervision of **Prof. Dr. P. Karkuzhali M.D.** It is submitted in partial fulfillment of the requirements for the award of the M.D., Pathology degree by The Tamilnadu **Dr. M.G.R. Medical University**, Chennai. This has not been submitted by me for the award of any degree or diploma from any other University.

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CONTENTS

INTRODUCTION

Male infertility is a common problem that can be devastating to a couple trying to conceive. The statistics of infertility shows that 15% of all marriages face in future the problems of infertility. The WHO has reported a core global prevalence of 5% infertility in the mid 70s. In approximately 30% of cases, significant abnormalities are found in the man alone, in another 20% of cases abnormalities are found in both the man and the woman. Thus in roughly 50% of infertile couples, the male factors is at least partially responsible for the failure to conceive. The diagnostic approach to a case of male infertility includes a detailed clinical history and a thorough physical examination supported by various laboratory investigations like semen analysis, hormonal evaluation, detection of anti-sperm antibodies, sperm function assays, ultrasound, vasography and invasive tests like testicular biopsy (or) FNAC (Fine Needle Aspiration Cytology). FNAC has become one of the important methods of investigations in cases of azoospermia and oligospermia. The technique of testicular FNAC is more than 30 to 50 years old, but has become popular only in recent years. Unlike FNA done in other sites, testicular FNA requires local anesthesia which could be the reason for the non-popularity of the technique. Therefore only few studies have been done so for.

Testicular biopsy can help us to differentiate a post-testicular, obstructive etiology of male infertility from an intrinsic testicular cause. Though biopsy provides some useful information regarding spermatogenesis and basement membrane status of

seminiferous tubules, it has its own complications like hematoma, fibrosis (very rarely), scarring and sampling only a small volume of tissue. FNAC on the other hand is reliable, quick, easy, less invasive and associated with no or minimal complications. **FNAC could give better morphological details of different stages of spermatogenesis.**

Studies have shown a good correlation between FNA and biopsy findings and abnormal findings in FNA can be followed up and evaluated further with a formal testicular biopsy. The concordance rate of FNA and histological diagnosis reached >85% in many studies with high specificity and sensitivity approaching >95%.

In the past, findings of ↑FSH (Follicular stimulating hormone) concentration often predetermined that infertility couples were recommended to use donor without further investigations. However, focal areas of spermatogenesis may exist in men previously considered to be devoid of spermatogenesis, such as in Sertoli Cell Only Syndrome (SCOS) & maturation arrest. In such cases it is now possible for testicular FNA 'mapping' for sperm recovery from different regions of the male reproductive system for Intracytoplasmic sperm Injection (ICSI) enabling fertilization. By performing FNA map prior to In Vitro Fertilization (IVF) cycle we can maximize testis parenchymal conservation in non-azoospermic men with varied pathology. When post-testicular obstructive cause is demonstrated, surgical correction may be indicated.

FNA cytology sometimes provides information not evident on histology, mainly

because of the heterogenecity of the pathologic process. The organ may contain more mature germ cell lineage in small foci far from the site of biopsy. The wider sampling area in the testis by FNA may result in a more accurate representation of pathology in the tissue.

AIMS AND OBJECTIVES OF THE STUDY

1. To evaluate cytological features of testicular FNAC for diagnosis of Male Infertility and to determine the diagnostic values and reliability of testicular FNA as a cytological sampling technique.

2. Considering histopathology as the 'gold standard' to study the correlation between cytological and histological diagnosis.

3. To correlate Johnson's Score of histopathology with that of histological diagnosis.

4. To evaluate the possibility of replacing biopsy of azoospermic testes by FNA for diagnostic and management purpose.

REVIEW OF LITERATURE

HISTORY:

Technique of testicular FNAC is more than 30-50 years old. The statistics of infertility shows that 15% of all marriages face in future the problems of infertility. Testicular FNAC was first attempted by Posner in 1905 and Huhner in 1928[1]. Later most of the work was done by Orbant in 1965[2] and Perssons in 1971[3] who characterized different cell types in cytological smears, and demonstrated good correlation of cytological diagnosis with histological categories. Mallidis and Baker in1994[4], AI-Jitawi et al in 1997[5] and Abdulla et al in 2000[6] had described on FNA biopsy technique for histological assessment in male infertility.

The role of testicular FNAC as a diagnostic parameter in the evaluation of azoospermic patients were studied by Foresta et al in 1992[7] and Shoshana et al in 1993[8]. Dajani et al in 1998[9] had studied the use of testicular FNAC by grading the cytological smears in 1000 infertile men. Later Rewat et al in 2007[10], AI-Rayes et al in 2000[11], Kurien et al in 2003[12], Bettella et al in 2005[13] and Srivastava et al in 2004[14] had also studied the role of testicular FNAC in male infertility. Bettella et al in 2005[13] studied the role of FNAC in Testicular sperm extraction (TESE) and Gupta et al in 2006[15] had described the role of testicular FNAC in patients with clinically obstructive azoospermia. Angelocarpiel et al in 2002 [16] had studied the use of FNAC in Cryptorchid males.

Craft et al in 1993[17], Schoysman et al in 1993[18], Tournaye et al in 1998[19] and Bourne et al in 1995[20] had described about the recovery of mature sperms from obstructive azoospermic patients for Intracytoplasmic Sperm Injection (ICSI). Later studies reported the successful recovery of mature sperm in patients with non-obstructive azoospermia (Devroy et al in 1995[21] and Friedler et al in 1997[22]).

Craft and Tsirigotis in 1995[23] had described that multiple needle biopsies could retrieve more spermatozoa than a single open biopsy. To determine more accurately which men with non-obstructive azoospermia (NOA) are candidates for ICSI, diagnostic and therapeutic testicular sperm extraction (TESE) with cryopreservation (Mulhall et al, 1997)[24] and real-time, multi-biopsy approaches were performed with either optical magnification (Schlegal et al, 1996)[25] or classic testis biopsy (Tournaye et al, 1996)[26].

Turek et al in 1999[27] had proved that by Testicular Sperm Extraction (TESE), In Vitro-Fertilization (IVF) Cycle cancellation can be reduced and can maximize testis parenchymal conservation in Non-Obstructive Azoospermia (NOA) men with atrophic testes. Turek et al in 2000[28] had studied the diagnostic findings from testes FNA mapping in obstructive azoospermia.

Infertility:

Infertility is defined as the inability to achieve pregnancy after one year of unprotected intercourse.

CAUSES OF MALE INFERTILITY

1. **Pre-testicular Causes** :

- Disorders of the hypothalamic or pituitary endocrine diseases (Thyroid or Adrenal disorders or Diabetes Mellitus)
- Metabolic disorders (Renal and Liver disease)
- Chronic Infection
- Drugs

2. **Testicular Causes:**

- Idiopathic Hypospermatogenesis or aspermatogenesis
- Developmental and genetic disorders (agonadism, cryptorchidism, SCOS and Klinefelter's Syndrome).
- Circulatory - varicocele or torsion.
- Inflammatory lesions – infections or immune causes
- Iatrogenic – chemical, radiation or surgical
- Environmental.

3. **Post-testicular causes (Genital)**

- Congenital Block: anomalies of excretory ducts or accessory glands.

- Acquired Block:

➢ Inflammatory lesions of the excretory ducts and accessory glands.

➢ Iatrogenic or post-traumatic lesions of the excretory ducts, accessory glands or ejaculation nerve plexus[29].

EVALUATION OF MALE INFERTILITY

1. Clinical History and Examination

2. Semen Analysis

3. Transrectal Ultrasonography

4. Vasogram

5. Testicular FNA

6. Testicular Biopsy

7. Others – Tests for antisperm antibodies, ova penetration, cervical mucus interaction, hormonal assays, karyotype analysis.[30]

CLINICAL HISTORY AND EXAMINATION

Infertility patients should be asked about their marital history, personal habits like

smoking/alcoholism/intake of drugs, diseases like DM/TB/HT/STD/Thyroid problems and also surgical history for maldescended testes/hernia repairs. They should be examined for external genital abnormalities, testicular size, testicular consistency and presence of varicocele. Then they should undergo routine examinations like HB%, blood sugar, urea, creatinine and urine routine examination followed by other investigations listed below:

INVESTIGATIONS

1. **SEMEN ANALYSIS:** Semen analysis should be the first step in the investigation. If any gross abnormalities detected, then the couple should be councelled for the need of assisted reproductive technology[31].

Collection of semen for analysis:

Collection is best done by masturbation failing which by coitus interruptus. The semen is collected in a clean wide mouthed dry glass jar. Sample so collected should be sent to the lab as early as possible so that the examination can be performed within 2 hours. Coitus should be avoided for at least 2 to 3 days prior to the test.

Normal Semen values (WHO 1999)[32]

Volume – 2 ml or more
PH – 7.2 to 7.8
Sperm concentration – 20 million/ml or more
Total Sperm Count ≥ 40 million per ejaculate
Motility – 50% or more progressive forward motility
Morphology – 30% or more normal forms
Vitality – 75% or more living
Leucocytes – <1 million/ml
Total Fructose – >13 mmol/ejaculate
Micro Agglutination Reaction (MAR) test – <10% sperms with adherent particles
Acid phosphate - 25,000 - 60,000 IU/ml
Zinc - 90 - 600mg/100ml

In the selected cases, acid phosphatase, zinc (prostatic fluid content) and fructose (seminal vesicle fluid content) are to be estimated. If the values are found lower than mentioned, it is not wise to interpret the analysis as abnormal. However, one should repeat the test at least twice at about two and half month's interval, before signing out

the report as abnormal. In the semen analysis, patients are said to have normal study when the above criteria's are fulfilled. If the sperm count is less than 20 million it means **Oligospermic** and **Azoospermic** if no sperms are found in the semen. **Aspermia** is a condition where there won't be any seminal ejaculate.

3. **TRUS (Trans rectal Uretheroscopy):** To visualize the seminal vesicles, prostate and ejaculatory ducts obstruction.

4. **VASOGRAM:** It is a radiographic study done to evaluate ejaculatory duct obstruction. It has been mostly replaced by TRUS.

5. **TESTICULAR FNAC:**

Diagnosis involves two steps:

1. Identification of the cell types present
2. The proportions of the cell population represented by each.

Two cell populations are evident in cytology.[33]

- Sertoli cells
- Cells of various stages of spermatogenesis

The spermatogenetic cells are divided into spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa.

Thus accurate recognition of the normal cell types in FNA of testis is the key to diagnosis. The MGG Staining method is used throughout. The following descriptions apply to MGG stained FNA smears.[34, 35]

Cytological features of these cells are described below:

Sertoli cells: They have round or oval nucleus with a rather smooth chromatin pattern. Large pale or blue nucleoli are usually present. The cytoplasm is abundant, pale slate blue and is usually foamy with poorly-defined borders. Although occurring singly, these cells usually form sheets or a loose matrix. When spermatogenesis is present the spermatogenic cells are interposed within the matrix. Bare nuclei presumably extruded from the matrix are common. Sertoli cells are invariably present even in the total absence of spermatogenesis. Their presence therefore gives the cytologist the confidence that the testis has been sampled correctly.

Spermatogenetic cells (in order of maturation):

Spermatogonia: They contain 16-20μm round or oval, slightly eccentrically placed nucleus with smooth finely condensed nuclear chromatin which is either pale staining (light) or dark staining. Nucleoli are not usually seen. The cytoplasm is homogenous and has well defined border. Spermatogonia are outnumbered by spermatocytes in normal spermatogenesis and may only occur in relatively small numbers. When, however, a maturation arrest is present they are often abundant.

Primary Spermatocytes: It's the largest germ cell. It contains a round nucleus, 14-20 μm, the size depending upon the state of maturation, with a heavy coarse chromatin pattern. The nuclear chromatin shows a 'chunky' appearance with a clear dark/light effect. The cytoplasm stains deeply hyper-basophilic and is moderate in amount. Nucleoli are not seen.

Secondary Spermatocytes: It contains a round nucleus, variable in size depending on maturation; from 8-16 μm. Binucleate forms are common. The chromatin pattern is coarse but to a lesser extent than that seen in the primary spermatocytes and exhibits a similar pattern. The cytoplasm is moderate in amount, basophilic but not hyperbasopholic as seen in the primary spermatocyte. As the cell matures towards spermatid the cytoplasm is often reasonably abundant. Nucleoli are not seen. These cells are rarely identified because of their shorter life span and immediate transformation to spermatids

Spermatids: A small cell, although the size is variable. The nucleus is less than 8 μm depending, as in the other spermatogenic cells, upon maturation stage. In the 'close to mature' stage, the nucleus of course resembles a sperm head. The nuclear chromatin is darkly staining and smooth. The cytoplasm is grey blue and often shows a ragged, uneven border. Sperm tails are commonly seen either in or protruding from the cytoplasm.

Mature Spermatozoa: They have oval nuclei with very dense chromatin. The tail

is found on opposite side of acrosome. This end point is proof that, spermatogenesis, the transformation of spermatid to spermatozoa, is functional. A maturation arrest at the spermatid level is not uncommon which would of course produce azoospermia and infertility and therefore a cytological conclusion that the testis is functioning normally must include observation of all stages of maturation.

Leydig (interstitial) cells: Leydig cells are relatively uncommon in testicular cytology when compared with the other cellular components. They are however usually present in small numbers if careful scrutiny is applied. The nucleus is 10-12 μm, round, darkly staining with a relatively smooth chromatin. The cytoplasm is abundant and stains basophilic. The cell borders are usually clearly defined in contrast to the poorly defined sertoli cell borders. The cytoplasm is also cleaner and smoother when compared with sertoli cells. Scattered green/blue granules are seen lying within the cytoplasm. These cells can occur singly but are usually seen in small sheets, similar in pattern to those cells seen in tissue sections lying between the tubules of the testis in clusters.

Mesothelial cells:

Mesothelial cells are an expected finding, picked up on the way in from the scrotal lining and occur in large monolayer sheets with moderately high N/C ratio. They have moderate amount of bluish cytoplasm & well demarcated cell borders, large nuclei & prominent nucleoli. Cytoplasmic vacuoles may be seen. It is important to identify the mesothelial cell and to differentiate it from sertoli cell in order to be certain that the

sample is indeed from the testis and not a failure.

Large numbers of degenerate autolysing cell forms are a feature of testicular cytology. This effect is presumed to be due to the rapid 'turnover' of the spermatogenic cells within the organ and consequent loss of many, not an artifact produced by the heavy handedness of the sampler.

Specimen adequacy for FNA:

If at least 200 cells could be counted on minimum in one well spread slide, the specimen is considered adequate. Approximately 97% testicular FNA yield adequate specimen for evaluation of spermatogenesis.

Based on various proportions of the different cell types, the smear is categorized into six groups[15]:

1. **Normal spermatogenesis** – This pattern is reported when the smears show spermatogonia, spermatocytes, spermatids, many spermatozoa and a proportional numbers of sertoli cells forming roughly one third of the total spermatogenetic cells.

2. **Hypospermatogenesis** – This pattern is described when all types up to spermatozoa are present and the proportion of sertoli cells to spermatogenic cells is increased.

3. **Early Maturation Arrest** – In this category smears will show a high percentage of spermatogonia and primary spermatocytes along with absence of spermatids and

spermatozoa.

4. **Late Maturation Arrest** – In these the 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.

5. **Testicular Atrophy** – The smears have scanty cellularity and few sertoli cells.

CYTOLOGICAL GRADING

Cytological smears were also graded according to Dajani et al[9] by counting 100 consecutive spermatogenic and sertoli cells in a well spread area with good cellularity in a random manner using light microscopy as follows:

Grade A: spermatozoa were detectable

A_1: Adequate production of fully developed spermatozoa

A_2: Low, scanty or rare fully developed spermatozoa.

Grade B: Germ cells seen but no spermatozoa present. Includes maturation arrest at the level of spermatid, spermatocyte or spermatogonia stages.

Grade C: Sertoli cell only pattern; no germ cells detected.

Grade D: Sclerosis or lack of both germinal and sertoli cells.

CELL INDICES:

Various indices were calculated according to Agarwal et al[37] as follows:

Spermatic index (S.I):

SI is the number of spermatozoa per 100 spermatogenic cells. Its normal value is 51.6 ± 12.4.

Sertoli cell index (SEI):

SEI is the number of sertoli cells per 100 spermatogenic cells. Its normal value is 42.4 ± 11.4.

Sperm- sertoli index (SPSEI): SPSEI is an expression of number of spermatozoa per 100 sertoli cells.

IN NORMAL SPERMATOGENESIS: SI and SPSEI will be higher than SEI.

IN HYPOSPERMATOGENESIS: SI and SPSEI will be lower than in normal spermatogenesis and SEI will be higher than in normal spermatogenesis and maturation arrest.

IN MATURATION ARREST: SI and SPSEI will be zero and SEI will be higher than in normal spermatogenesis.

IN SCOS: SI will be zero and SEI & SPSEI can not be calculated as there are no

spermatogenic cells.

TESTICULAR BIOPSY:

The specimens were studied histopathologically for evaluation of the following criteria[39, 40]:

- General architecture (150-250 μm diameters of tubules)
- Number of seminiferous tubules in specimen (usually 20-30 cross sections of tubules)
- Seminiferous tubules pattern
- Germ cell/ Sertoli cell ratio
- The Basement membrane
- Interstitial tissue
- Leydig cells
- Tubular Hyalinization
- Tunica albuginea
- Epididymis (if present in biopsy)

Methods to estimate the degree of spermatogenesis:

1. **Johnson Scoring**[41]

Score 10 -Complete Spermatogenesis and perfect tubules

Score 9– Many spermatozoa present but disorganized spermatogenesis

Score 8 – Only a few spermatozoa present

Score 7 - No spermatozoa but many spermatids present

Score 6 - No spermatozoa, only a few spermatids present

Score 5 - No spermatozoa or spermatids present, but many spermatocytes present

Score 4 –Only a few spermatocytes present

Score 3 -Only Spermatogonia present

Score 2 - No germ cells, only sertoli cells present

Score 1 - No germ cells or sertoli cells

The mean Johnson Score of normal testis is **9.39±0.24** and 60% or more of the tubules should score at10.

In oligospermia → 5.3±2.1

In pituitary hypogonadism → 3.95±1.4

In Klinefelter's Syndrome → 1.3±0.3

2. Silber-Rodiguer-Rigarr Method :

Counting round mature spermatids (small round, dark nuclei) per tubule cross section. At least 20 round spermatids/tubules – Ideally counting in 20 tubules. If it is 45, 40, 20 and 6-10 then the sperm count is approximately 85, 45, 10 and 3 million/ml. If sperm count is lower than expected it indicates partial obstruction[42].

3. **Estimating Germ Cells: Sertoli Cell Ratio (Sertoli Index):** Counting at least 30 tubule cross sections .This ratio is constant at about 13: 1 in young healthy men. Average of 12 sertoli cell per tubular cross sections is considered normal.

In the histopathology, five patterns are observed[40]. They are:

1. Normal spermatogenesis
2. Hypospermatogenesis
3. Maturation Arrest
4. Germ Cell Aplasia
5. Tubular hyalinization

1. **Normal spermatogenesis:** Usual testicular biopsy contains 3 to 5 tubules together

with portions of septa. Germ cells in all stages of spermatogenesis are seen within the seminiferous tubules in cases of normal spermatogenesis. Seminiferous tubules are so convoluted that they are mostly seen in cross sections. Maturation proceeds in an orderly overlapping helical pattern along the length of a tubule. So not all stages are seen within the tubules. Normal spermatogenesis is found in azoospermic patterns with excurrent duct obstruction.

2. **Hypospermatogenesis**: A reduction in the number of all germinal elements, including the late spermatids/spermatozoa is present in cases of hypospermatogenesis. Thus, histologically germinal epithelium may be hypoplastic and therefore thinner than normal. The organization of the germinal epithelium may be disrupted and immature germ cell may be found in the lumen in some instances. The interstitial (Leydig) cells are normal. It is associated with oligospermia in the range of 2 to 20 million sperms per ml and severe cases may show azoospermia. Associated changes may include tunica propria thickening, focal interstitial fibrosis and focal tubular sclerosis. In some cases the spermatogenic cells occupy only a small portion of the entire circumference of the tubules, whereas other tubules contain only sertoli cells. Many genetic, toxic, infectious and vascular disorders may produce the same morphologic changes.

3. **Maturation Arrest**: Histological examination reveals spermatogenesis proceeding normally through a specific stage at which point no further maturation of germ cells

is identified. The arrest may occur at the primary spermatocyte, secondary spermatocyte or early spermatid stage but most often occurs at the level of the primary spermatocyte. The tubules in these settings usually show identical morphologic changes in that all appear arrested at the same stage of spermatogenesis. Patients with complete maturation arrest at any stage shows no late spermatids/spermatozoa, whereas those with incomplete maturation arrest show arrest at different stages of spermatogenesis. In such cases, some tubules show spermatogenetic cells arrested at one stage (for e.g., spermatids) and adjacent tubules at another stage (for e.g., primary spermatocytes). Thus patients with complete arrest exhibit azoospermia, whereas patients with incomplete arrest may have varying degrees of oligospermia.

4. **Germinal Cell Aplasia:** It is also known as sertoli cell only syndrome (SCOS). It is the severe form of these disorders and is invariably accompanied by azoospermia. This finding is noted in 10 to 20% of all testicular biopsy specimens obtained from infertile men. Testicular histology reveals seminiferous tubules lined by sertoli cells with a complete absence of all germ cells. The oval nuclei of the sertoli cells are typically located in the mid-portion of their cytoplasm with their longitudinal diameter perpendicular to the basement membrane of the tubules. The diameter of the seminiferous tubule is reduced and the interstitium is usually minimally altered. Patients with this syndrome have small to normal sized testis associated with normal or elevated levels of FSH. Occasionally biopsies may have

predominantly sertoli cell only patterns, but careful evaluation reveals a few scattered germ cells, mostly spermatogonia. This represents germ cell hypoplasia of variable degrees. Rarely, testis biopsy may show a combination of areas with sertoli cell only patterns in combination with maturation arrest.

Testis biopsies in Klinefelter syndrome usually show absent spermatogenesis, tubular sclerosis and prominent Leydig cells often in the form of nodules. However some biopsies may also show some degree of spermatogenesis. Spermatozoa have been observed in the wet preparations of testis biopsies and a successful ICSI have been done.

5. **Tubular hyalinization**: It is also known as tubular sclerosis or "end-stage" testis disease as it is the end-product of many forms of tubular injury. All disturbances of spermatogenesis may ultimately evolve into this end-stage disease. Hyalinization of the tubules is associated with a loss of germinal epithelium, obliteration of the lumens and fibrosis of the interstitium. Sertoli cells may or may not be present. Leydig cells may be absent or decreased in number within the sclerotic interstitium. Foci of spermatogenesis may be seen in some of the preserved tubules, but even these tubules are probably obstructed by fibrous tissue and not connected to excretory ducts. Clinically, these testes are bilaterally atrophic and firm. This appearance may ultimately be found in undescended testes, remote chronic orchitis or ischemia, acquired gonodotropin deficiency, testes of karyotypic abnormalities, or without a known etiology.

Other patterns: In cases of hypogonodotropic hypogonadism, congenital or acquired lesions of the hypothalamus and pituitary the development and function of testes are affected by these disorders. It can be caused by tumours, inflammation or genetic and developmental syndromes. Various drugs, trauma, or irradiation of the hypothalamus may have the same consequences. All these conditions are characterized by low levels of serum gonadotropins. The testes are small and lined with immature sertoli cells that have round nuclei and inconspicuous nucleoli. Leydig cells are absent. In cases of isolated LH deficiency, normal spermatogenesis may be reduced. Treatment with GnRH given in a pulsatile manner may initiate and maintain spermatogenesis and cure infertility.

Excurrent Duct obstruction:-

Normal spermatogenesis is seen associated with dilated tubular lumens, disorganized germ cells, germ cells sloughing and thickened tunica propria.

The testicular biopsy is rarely pathognomonic of a single etiology. In addition, several patterns may be present in an individual biopsy specimen. Thus in most cases, a testicular biopsy does not result in the identification of a specific etiologic factor of a patient's infertility.

Fine Needle Aspiration versus biopsy:

FNA is quite capable of recovering sufficient material for cytological assessment

of infertility as compared with biopsy. Many have claimed that FNA is superior to biopsy. FNA cytology sometimes provides information not evident on histology mainly because of the heterogeneity of the testes, the organ may contain more mature germ cell lineage in small foci far from the site of biopsy. The wider sampling area in the testis by FNA may result in a more accurate representation of true histological geography and variation that exists within the organ. Despite a histological finding of no germ cells within the seminiferous tubules, clinical experience in men undergoing testicular sperm extraction and intracytoplasmic sperm injection has demonstrated that testis with this histology can harbor fertile sperm. This fact underlines 2 important practical points:

(1) The importance of careful examination of a testicular biopsy to find mixed histological patterns of infertility

(2) The benefit of wider geographic sampling, which can be achieved more practically by FNA than with multiple biopsies.

Many studies have revealed the concordance rate of FNA and histological diagnosis as >85% with high specificity approaching >95%.

MATERIAL AND METHODS

This study has been undertaken in 35 infertile men, aged 20-40 years, in the

Urology OT (Operation theatre), Madras Medical College, performed during the period of June 2007 – August 2009. All patients signed informed consent prior to aspiration.

Indications[43]:

ABSOLUTE

1. Azoospermia
2. Oligozoospermia
3. Teratozoospermia (Abnormal form)
4. Atypical cells in ejaculate

RELATIVE

1. Varicocele
2. Cryptorchidism
3. Chronic infection

Contraindication

1. Severe anaemia

2. Unco-operative patients

Patient's selection: The study was done in patients in whom three consecutive semen analyses showed oligospermia or azoospermia. In case of primary hypogonadism, FSH was found to be increased to three times the normal value and hence testicular

biopsy was not done in these cases. All semen analyses were done only after a period of abstinence of at least 4 days. A clinical examination was then conducted and relevant personal and clinical data were noted. Patients were then subjected to a FNA of the testes for cytological evaluation and open testicular biopsy was done for histopathological correlation.

Patient preparation:

Procedures and risks involved in the procedures were explained and informed consent was obtained. Routine investigations like Hb%, blood sugar, urea, creatinine and urine routine examination were done.

Semen Analysis:

All semen analyses were done only after a period of abstinence of at least 4 days.

The semen was examined for the following: colour, volume, ph, liquefaction time, sperm count, motility, morphology and leucocytes. Then the results are given as either normal (If sperm count > 20 million) or oligospermic (If sperm count <20 million) or azoospermic (If no sperms are seen in the semen).

FNA technique:

They were done in the Urology OT (Operation theatre); Madras Medical College with the patient in supine position, the scrotum was palpated to confirm the clinical

findings. Then spermatic cord block was done by injecting 5 ml of 1% xylocaine. Five minutes after injection of local anaesthesia, larger of the two testes was held with (L) hand and was positioned with epididymis in the posterior position. Testis was gently held between the thumb and the index finger. Using (R) hand, the testis was punctured with a 23 gauge needle attached to a 5 ml of disposable syringe. Needle was passed in an axis perpendicular to longitudinal axis of testes in the middle of the anterior surface of testis opposite to epididymis. With a to and fro motion of the needle, aspiration was done twice or thrice under negative pressure. Negative suction was released and tissue fragments were then expelled onto a clear glass slide, gently smeared. If too much pressure is applied it may lead to marked distortion and crushing artifacts. Open testicular biopsy was performed immediately following the procedure of FNAC of the testis[9]. Smears were air-dried and stained with May Grunwald - Giemsa (MGG) as well as fixed in 95% alcohol and stained with H&E.

Procedures:

1. May Grunwald - Giemsa (MGG) stain:

1. Smears are air dried, fixed in acetone free methanol for 30 minutes.
2. Stain in May Grunwald for 5 minutes
3. Stain in Giemsa for 15 minutes
4. Wash in buffer water (PH 6.8) for 5 minutes

5. Air dry the slides
6. Clear in xylene (optional) for 5 minutes
7. Mount the slides with DPX(Dibutyl Pthalate Xylene).

2. Hematoxylin and Eosin stain:

1. Smears are fixed in 85% Isopropyl alcohol for 20 minutes
2. Stain in Harris Hematoxylin for 5 minutes
3. Wash well in tap water
4. Differentiate in 0.5% acid alcohol for 3 to 5 seconds
5. Wash well in tab water for blueing for 10-20 minutes
6. Dip in 1% aqueous eosin for once
7. Wash in running tap water for 1 to 5 minutes
8. Slides are dried and mounted in DPX (Dibutyl Pthalate Xylene).

Testicular biopsy technique:

A 1 to 1.5 cm incision was made on the convexity of the centre of anterior portion of testes, through the portal of entry of needle, through the skin and tunica albuginea with size 11 scalpel blade. Gentle pressure on testis extruded a small amount (2 to 3 mm) of testicular parenchyma from the incision, which was then excised with a wet sharp pair of curved Iris scissors and immediately transferred to and fixed in

Bouin's fluid, specimen processed and stained with hematoxylin and eosin for HPE examination. After securing homeostasis, Tunica albuginea, Tunica Vaginalis and skin with subcutis were closed in layers with 3/0 vicryl sutures. A dry scrotal dressing was then applied[40].

Staining Procedure for HPE:

Hematoxylin and Eosin stain:

1. Dewax sections, hydrate through graded alcohols to water.
2. Stain in Harris Hematoxylin for 5 minutes
3. Wash well in tap water
4. Differentiate in 1% acid alcohol
5. Wash well in tab water for blueing for 10-15 minutes
6. Stain in 1% eosin for 1 to 2 minutes
7. Wash in running tap water for 1 to 5 minutes
8. Dehydrate in alcohols, clear in Xylol and mount in DPX.

Aftercare (follow -up):

The patients stayed in the recovery room for 30-90 minutes and were sent home on recovery from anaesthesia. No antibiotics or pressure scrotal dressings were applied. Patients were advised to keep the area dry and to report any bleeding, discharge or

swelling in the suture site. They were seen 2 weeks after the procedure, and enquiries were made about pain, swelling, infection and intercourse. Testicular volume was estimated with a Prader Orchidometer and the biopsy sites were examined.

The study populations of 35 infertile cases were studied on the basis of the following parameters:-

Age group (ranging from 20-40 years).

Duration of infertility (ranging from 2-14 years).

Sperm count and sperm count correlation with HPE and Cytological diagnosis. Based on sperm count, the cases were grouped under azoospermia if no sperms are seen in semen and Oligospermia if sperm count was <20 millions. In addition semen was also examined for colour, volume, ph, liquefaction time, sperm count, motility, morphology and leucocytes .

Cytological grading: Cytological smears were also graded according to Dajani et al[9] by counting 100 consecutive spermatogenic and sertoli cells in a well spread area with good cellularity in a random manner using light microscopy as follows:

Grade A: spermatozoa were detectable

A_1: Adequate production of fully developed spermatozoa

A_2: Low, scanty or rare fully developed spermatozoa.

Grade B: Germ cells seen but no spermatozoa present. It includes maturation arrest at spermatid, spermatocyte or spermatogonia stages.

Grade C: Sertoli cell only pattern; no germ cells detected.

Grade D: Sclerosis or lack of both germinal and sertoli cells.

Cytological diagnosis:

Based on various proportions of the different cell types, the smear is categorized into six groups[15]:

1. **Normal spermatogenesis** – This pattern is reported when the smears show spermatogonia, spermatocytes, spermatids, many spermatozoa and a proportional numbers of sertoli cells forming roughly one third of the total spermatogenetic cells.

2. **Hypospermatogenesis** – This pattern is described when all types up to spermatozoa are present and the proportion of sertoli cells to spermatogenic cells is increased.

3. **Early Maturation Arrest** – In this category smears will show a high percentage of spermatogonia and primary spermatocytes along with absence of spermatids and spermatozoa.

4. **Late Maturation Arrest** – In these the 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.

5. **Testicular Atrophy** – The smears have scanty cellularity and few sertoli cells.

CELL INDICES:

Various indices were calculated according to Agarwal et al[37].100 consecutive spermatogenic and sertoli cells were counted in a well spread portion of the smear and the percentage of spermatozoa per 100 spermatogenic cells (Spermatic index) and the number of sertoli cells per 100 spermatogenic cells (Sertoli cell index) were calculated based on Agarwal et al[37]. The mean value of these indices in each category of the cytological diagnosis is then taken and compared with different categories of cytological diagnosis.

Spermatic index (S.I):

SI is the number of spermatozoa per 100 spermatogenic cells.

Sertoli cell index (SEI):

SEI is the number of sertoli cells per 100 spermatogenic cells.

Sperm- sertoli index (SPSEI):

SPSEI is an expression of number of spermatozoa per 100 sertoli cells

In normal spermatogenesis: SI and SPSEI will be higher than SEI.

In hypospermatogenesis: SI and SPSEI will be lower than in normal spermatogenesis and SEI will be higher than in normal spermatogenesis and maturation

arrest.

In maturation arrest: SI and SPSEI will be zero and SEI will be higher than in normal spermatogenesis.

In SCOS: SI will be zero and SEI & SPSEI cannot be calculated as there are no sertoli cells.

HISTOLOGICAL DIAGNOSIS:

In the histopathology, the predominant pattern was taken into account and classified into the following five patterns [40]. They are:

i. **Normal spermatogenesis**: Germ cells in all stages of spermatogenesis are seen within the seminiferous tubules in cases of normal spermatogenesis

ii. **Hypospermatogenesis**: A reduction in the number of all germinal elements, including the late spermatids/spermatozoa is present in cases of hypospermatogenesis.

iii. **Maturation Arrest**: Histological examination reveals spermatogenesis proceeding normally through a specific stage at which point no further maturation of germ cells is identified. The arrest may occur at the primary spermatocyte, secondary spermatocyte or early spermatid stage.

iv. **Germ Cell Aplasia**: Testicular histology reveals seminiferous tubules lined by sertoli cells with a complete absence of all germ cells.

v. **Tubular hyalinization**: Hyalinization of the tubules is associated with a loss of germinal epithelium, obliteration of the lumens and fibrosis of the interstitium. Sertoli cells may or may not be present. Leydig cells may be absent or decreased in number within the sclerotic interstitium.

JOHNSON SCORING[41]:

100 consecutive seminiferous tubules were counted and the mean was taken and compared with histological report.

Score 10 - Complete Spermatogenesis and perfect tubules

Score 9 - Many spermatozoa present but disorganized spermatogenesis

Score 8 - Only a few spermatozoa present

Score 7 – No spermatozoa but many spermatids present

Score 6 - No spermatozoa, only a few spermatids present

Score 5 - No spermatozoa or spermatids present, but many spermatocytes present

Score 4 - Only a few spermatocytes present

Score 3 - Spermatogonia only

Score 2 - No germ cells, sertoli cells only

Score 1 - No germ cells or sertoli cells

The mean Johnson Score of normal testis is **9.39±0.24**

Statistical analysis:

The patients were statistically analyzed on the basis of the following formulas:

Sensitivity	a / (a + b) x100
Specificity	d / (c + d) x 100
Positive Predictive value	a / (a + c) x 100
Negative Predictive value	d / (b +d) x 100
a	True positive
b	False negative
c	False positive
d	True negative
a + b	Diseased
c + d	Not diseased

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. The age group ranged from 20 to 40 years (Table 1) & (Fig 1) with a mean age of 30.4 yrs.

Table 1: study population – Age distribution

Age	No. of cases(35)	% of total
20-30	16	45.7%
31-40	18	51.4%
41-50	1	2.9%
MEAN AGE		30.4 yrs

The duration of infertility ranged from 2 years to 14 years (Table 2) & (Fig 2) with mean duration of 5.7 years.

Table 2: study population- distribution of infertility duration

Duration (years)	No. of cases (35)	% of Total
1-5	21	60%
6-10	11	31.4%
11-15	3	8.6%
MEAN DURATION		5.74 years

Fig:1

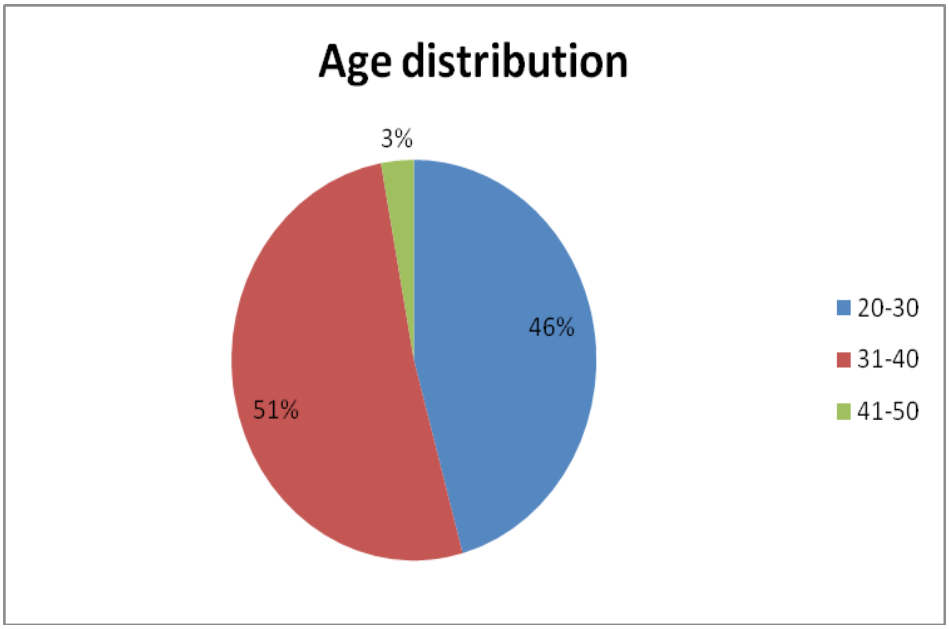
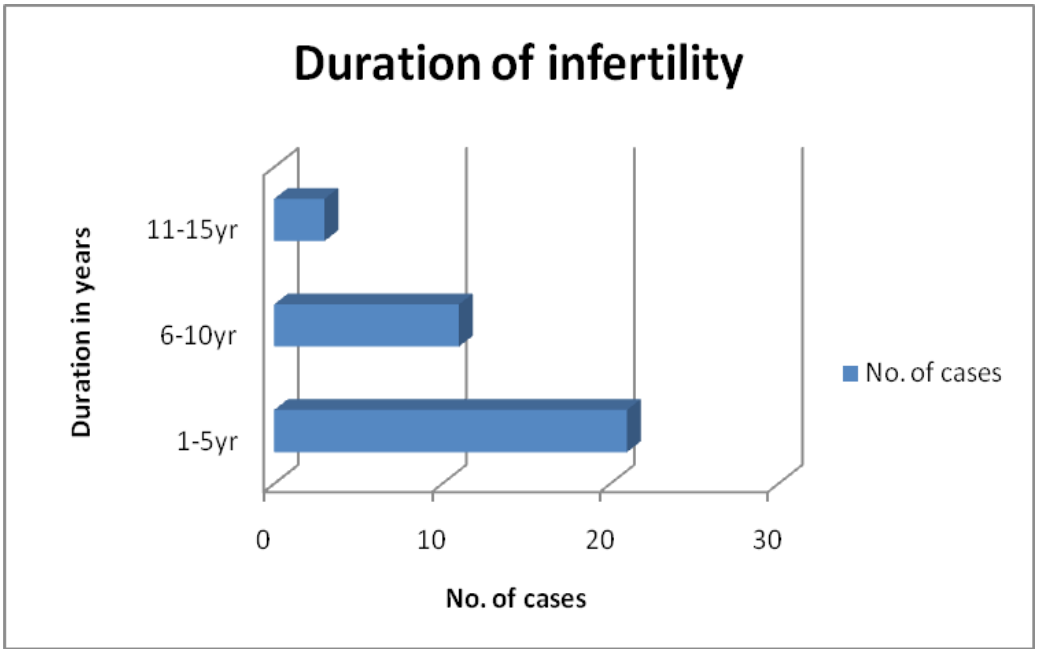


Fig 2:



Almost all patients had Primary infertility except for one patient who had secondary infertility. In semen analysis, the sperm count ranged from azoospermia to oligospermia (<20 million). Fig 3 shows the correlation of sperm count with HP and

cytology diagnosis.

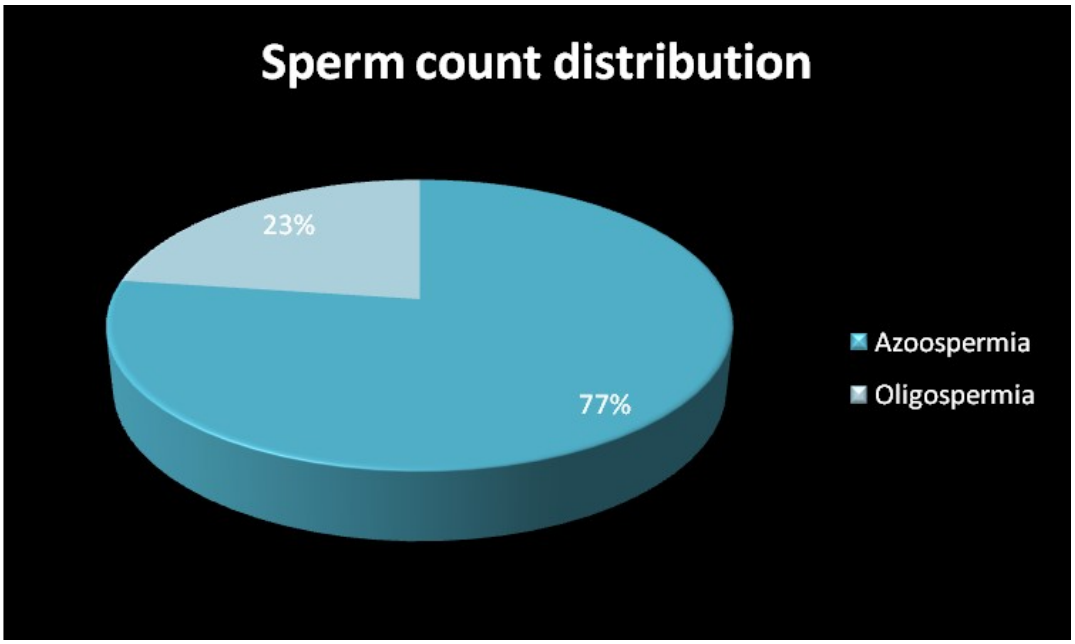
Azoospermia is correlated with maturation arrest, germ cell aplasia and tubular hyalinization. Oligospermia is correlated with hypospermatogenesis and normal spermatogenesis (table 3) & (fig.4):

Table 3: Sperm count correlation with HPE and Cytological diagnosis

Sperm count	No. of cases	% of Total	HP correlation		Cytology correlation	
			No. of cases	%	No .of cases	%
Azoospermia	27	77.14%	18	67%	17	63%
Oligospermia	8	22.86%	4	50%	6	75%
Total	35	100%	22	58.5%	23	69%

Out of 35 cases, 27 cases (77.14%) were azoospermic. Out of this 18 cases (67%) correlated with HP diagnosis and 17 cases (63%) correlated with cytological diagnosis. Discordance of 9 cases in HPE and 10 cases in cytology was found to be due to obstructive causes except in two cases. Out of 35 cases, 8 cases (22.86%) were oligospermic, out of which 4 cases (50%) correlated with HP diagnosis and 6 cases (75%) correlated with cytological diagnosis.

Fig:4



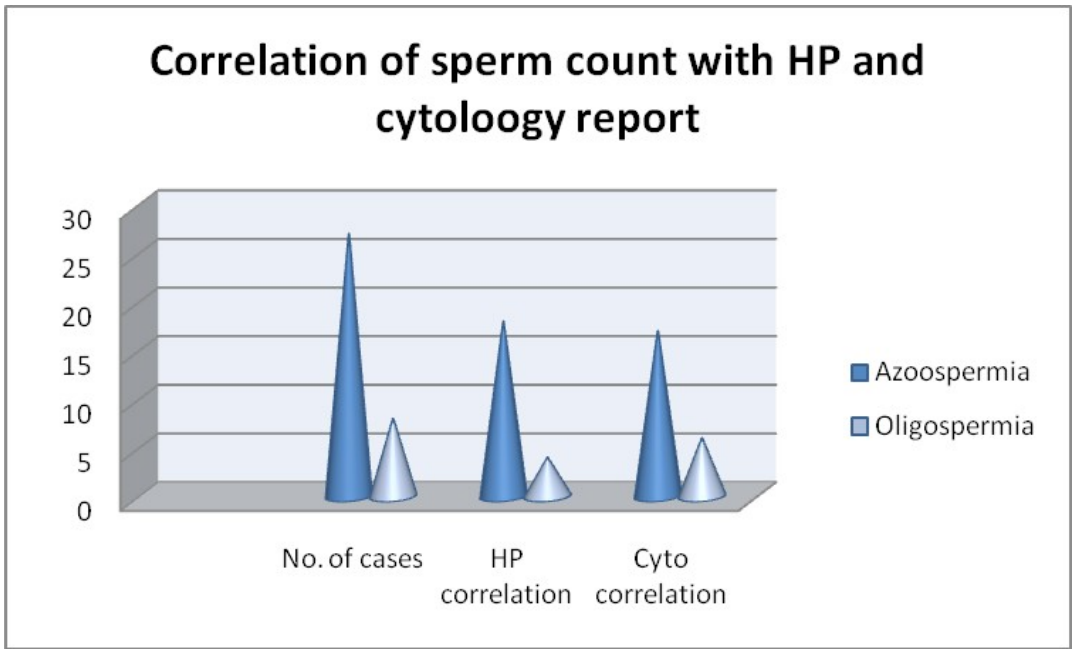


Fig:4

Cytological cell identification was done with the help of criteria given by Papic

et al[35] and Foresta et al[7]. Cytological smears were graded according to Dajani et al[9] and correlated with the cytological diagnosis (Table 4) & (Fig 5).

Table 4: Distribution of Cytological diagnosis and cytological grade

FNAC diagnosis	No. of cases (35)	% of total	Cytological grade
Normal spermatogenesis	8	22.9	A1
Hypospermatogenesis	8	22.9	A2
Maturation arrest	12	34.2	B
Sertoli cell only syndrome(SCOS)	5	14.3	C
Testicular atrophy	2	5.7	D

The most frequent diagnosis on cytological report was Maturation arrest (Fig. no. 9, 11, 13) with 8 cases. Next common diagnoses were Normal spermatogenesis (Fig. no.1-4) and Hypospermatogenesis (Fig. no.7) with 8 cases each. SCOS (Fig. no.16, 17) was seen in 5 cases and testicular atrophy (Fig. no. 20, 21) seen in 2 cases. Consequently the common cytological grades in the descending order of frequencies were B, A2 & A1, C & D.

Fig 5:

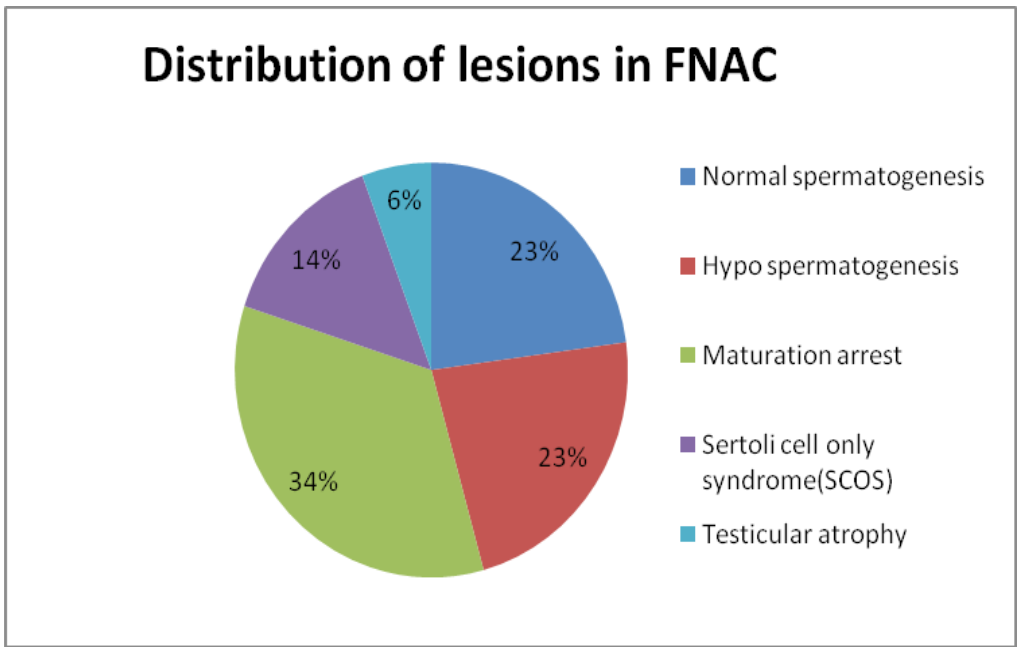
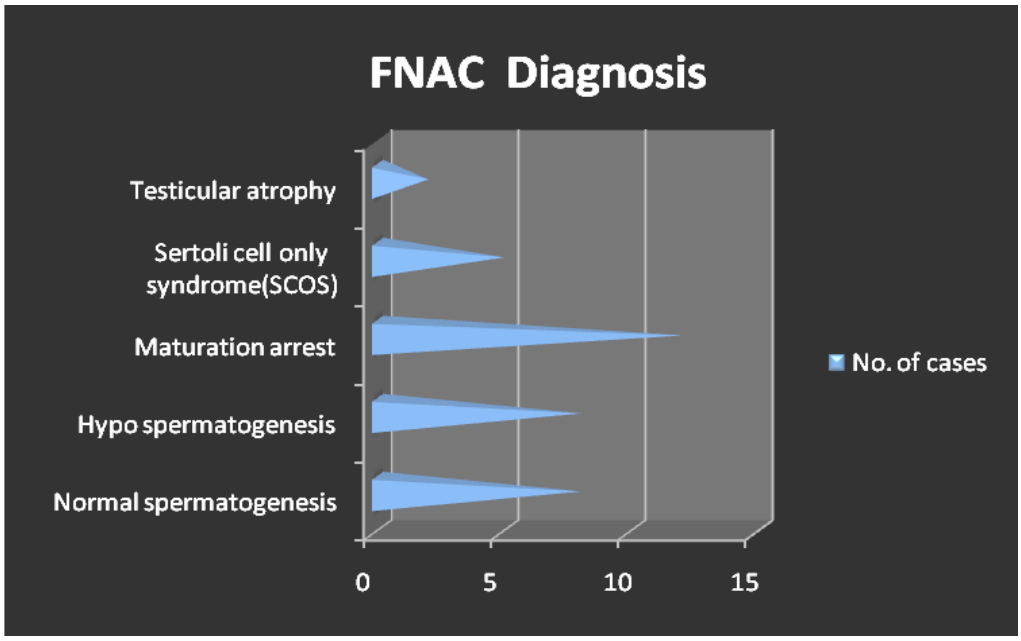


Fig 5:



100 consecutive spermatogenic and sertoli cells were counted in a well spread portion of the smear and the percentage of spermatozoa per 100 spermatic cells (Spermatic index) and the number of sertoli cells per 100 spermatogenic cells (Sertoli

cell index) were calculated based on Agarwal et al[37]. The mean value of these indices in each category of the cytological diagnosis is given in Table 5.

Table 5: 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	-

Progressive decreasing values of SI were seen in normal spermatogenesis, hypospermatogenesis and maturation arrest. 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 SCOS, SI and SPSEI were zero.

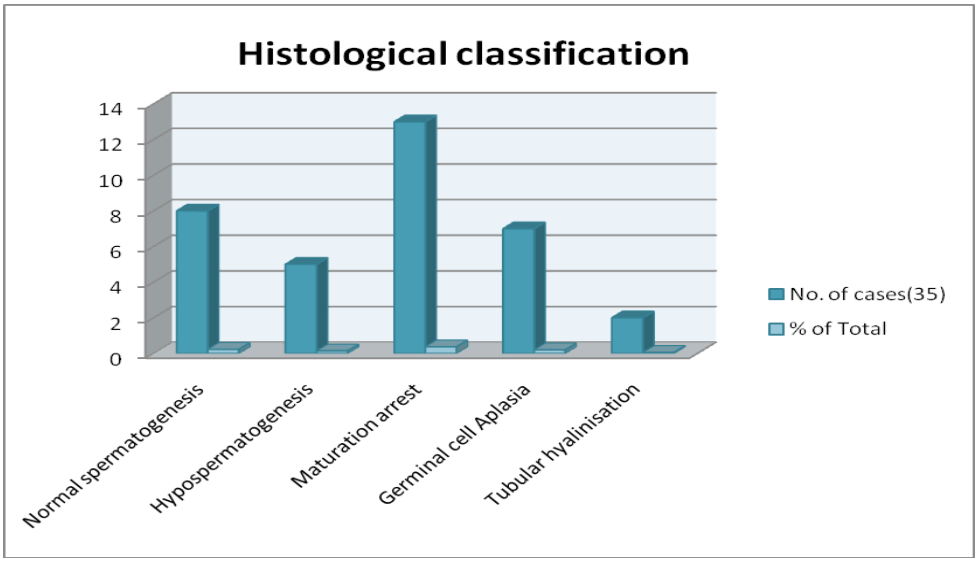
In histological classification, the predominant pattern was taken into account and presented in Table 5 & Fig 6

Table 5: Histological classification

Histological classification	No. of cases (35)	% of Total
Normal spermatogenesis	8	23%
Hypospermatogenesis	5	14.2%
Maturation arrest	13	37.1%
Germ cell Aplasia	7	20%
Tubular hyalinization	2	5.7%

Most frequent diagnosis was Maturation arrest (Fig. no.10, 12, 14, 15) with 13 cases (37.1%). Next common diagnosis was normal spermatogenesis (Fig. no.5, 6) with 8 cases (23%). Tubular hyalinization (Fig. no.22) was seen in 2 cases (5.7%). Germ cell aplasia (Fig. no.18,19) was seen in 7 cases and hypospermatogenesis (Fig. no.8) in 5 cases.

Fig: 6



In all the cases, Johnson's scoring was done. To do Johnson's scoring 100 consecutive seminiferous tubules were counted and the mean was taken and compared with histological report (Table 6 & Fig 7).

Table 6: Correlation between Johnson's scoring and histology diagnosis

Johnson's scoring	No. of cases	% of total	Histological diagnosis	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%

Most of the cases scored between 3 to 7 which means maturation arrest. Scores 9 & 10 correlated well with Normal spermatogenesis and score 2 and 1 with Germ cell Aplasia and Tubular Hyalinization. There were discordance in 2 cases, where score 7 was reported as Hypospermatogenesis and score 8 as Normal spermatogenesis.

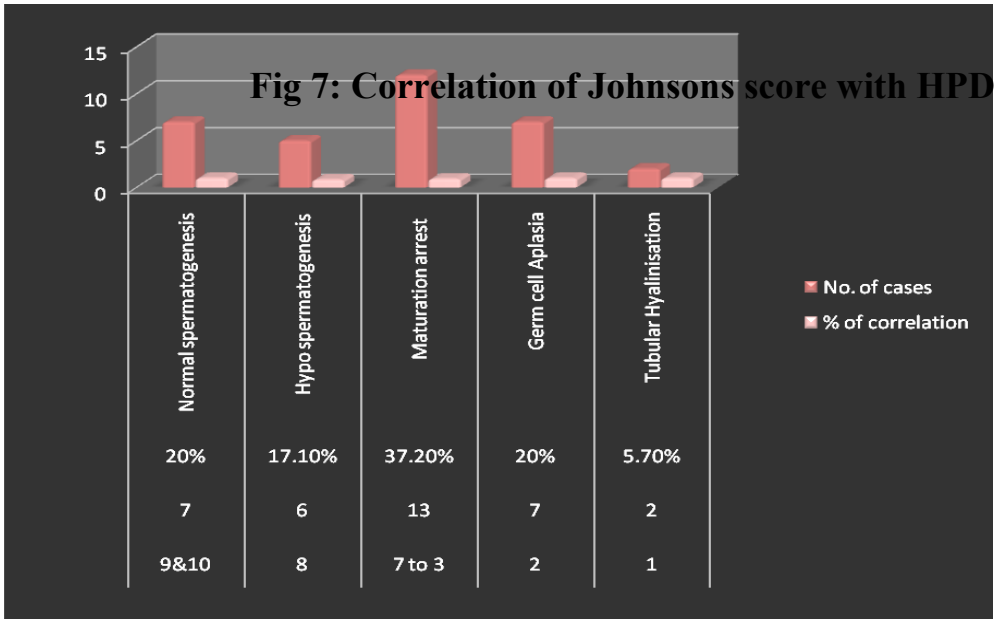


Fig 7: Correlation of Johnsons score with HPD

Considering histopathology as the gold standard for definitive diagnosis of any lesion, of the 35 cases of our study, 30 cases correlated well with FNAC. The overall percentage of correlation with respect to HPE was 86%. 8 cases diagnosed as Normal spermatogenesis by HPE were also diagnosed the same in FNAC with 100% correlation. Similarly 5 cases diagnosed as hypospermatogenesis and 2 cases diagnosed as tubular hyalinization were diagnosed the same in FNAC with 100% correlation. 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. The results of HPE and FNAC correlation are given in Table 7 & Fig 8.

Table 7: 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%

Fig 8:

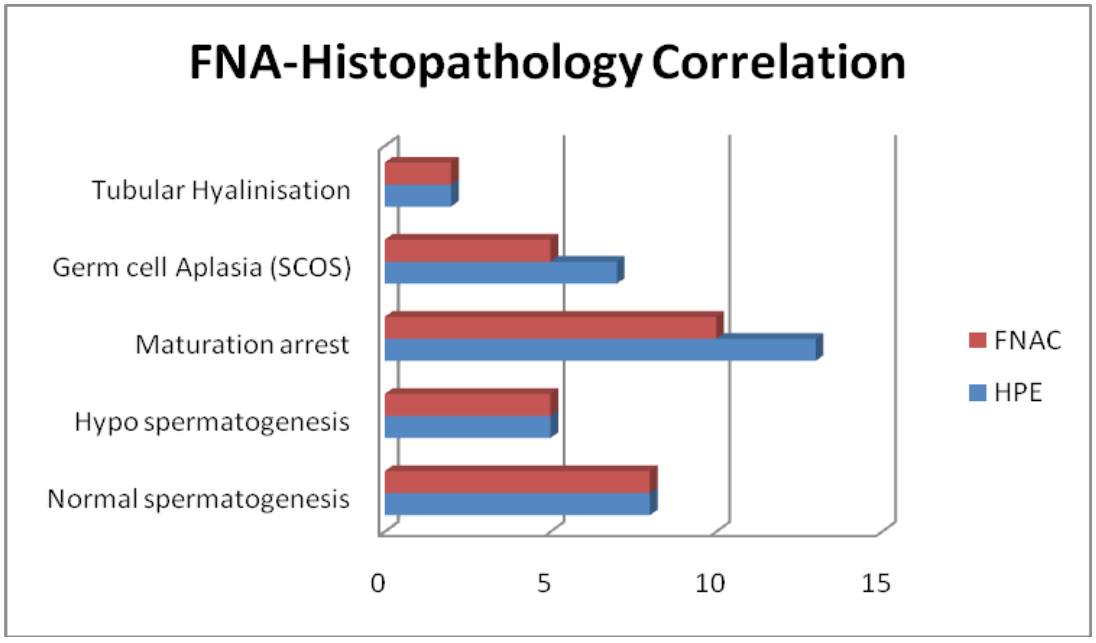
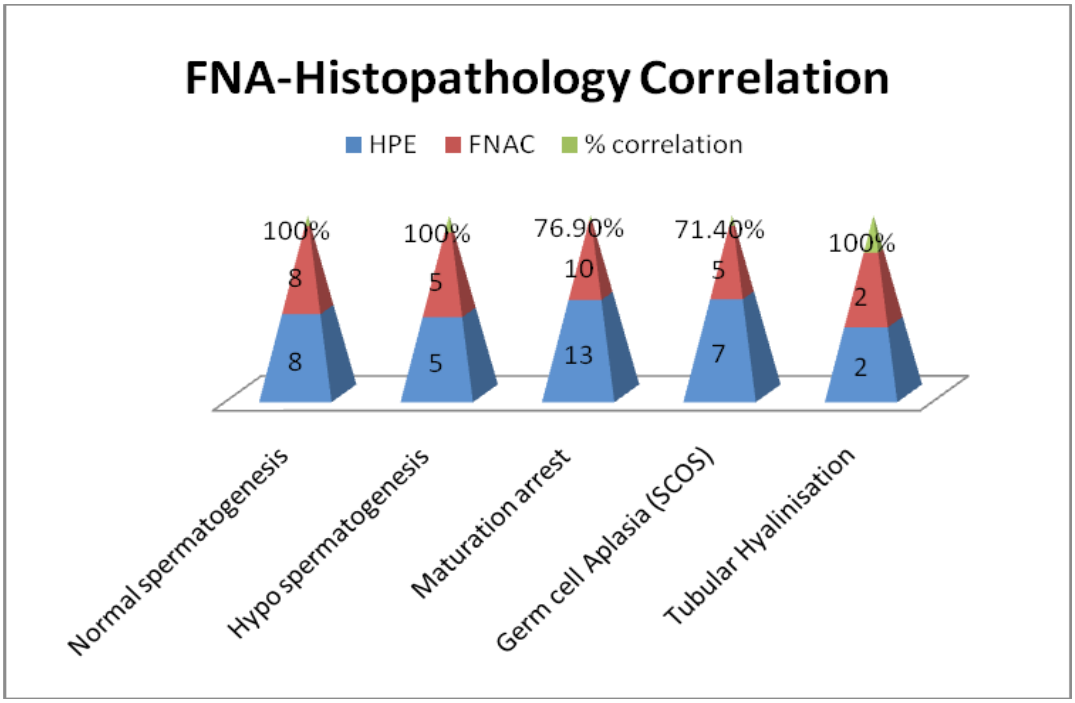


Fig 8:



Statistical Analysis:

FNA finding of normal spermatogenesis and tubular hyalinization showed sensitivity, specificity, positive predictive value, negative predictive value of 100%.

Table 8: Statistical Analysis

Patterns	**Sensitivity**	**Specificity**	**+ ve Predictive value (%)**	**-ve Predictive value (%)**
Normal spermatogenesis	100%	100%	100%	100%

Hypo spermatogenesis	100%	90%	62.5%	100%
Maturation arrest	77%	91%	83.3%	87%
Germ cell Aplasia	71.4%	100%	100%	93.3%
Tubular Hyalinization	100%	100%	100%	100%
Total	89.7%	96.2%	89.2%	96.1%

FNA finding of hypospermatogenesis showed a sensitivity of 100%, specificity of 90%, positive predictive value of 62.5%, and negative predictive value of 100%. FNA finding of maturation arrest showed a sensitivity of 77%, specificity of 91%, positive predictive value of 83.3% and negative predictive value of 87%.FNA finding of SCOS showed a sensitivity of 71.4%, specificity of 100%, positive predictive value of 100% and negative predictive value of 93.3%.

DISCUSSION

Open testicular biopsy has remained the cornerstone in the diagnosis of male infertility for decades. Testicular FNAC has picked up in recent years following Obrant and person[2], Papic et al[35], Schenck and Schill[34] and Foresta et al[7] who characterized different cell types in cytological smears and demonstrated good correction of cytological diagnosis with histological categories. FNAC is a minimally invasive technique which is one of the important investigations in diagnosis and management of infertility. In current era, microassisted fertilization techniques are of great help for infertile couples as nowadays the only requirement in these techniques is a viable sperm and ovum. Neither quality nor degree of motility is essential[6]. Therefore in cytological smears a report of presence or absence of sperm is also adequate. In cases of hypospermatogenesis and maturation arrest, these patients may be helped to some extent by hormonal therapy. FNAC serves the purpose with minimum side effects where as biopsy may result in fibrosis which hamper the process of sperm extraction for ICSI (Intra Cytoplasmic Sperm Injection).

This study has been done in 35 infertile males in Urology OT, MMC with mean age of 30.4 years and mean duration of infertility of 5.74 years to evaluate the cytological features of testicular FNAC for the diagnosis of male infertility, and to study the correlation between cytological and histological diagnosis. Out of these 35 cases, 27 cases were found to be azoospermic and 8 cases found to be oligospermic. Out of these

27 azoospermic cases, 9 cases in HPE and 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 cytological grade of B and 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%.

The results of this study of comparison of testicular FNAC and HPE in male infertility is in concordant with various studies conducted in the past and is compared with some of these studies here. The mean age of patients in various studies is given below (Table 1):

(TABLE 1): MEAN AGE WITH VARIOUS STUDIES

Studies	Mean Age(years)
Foresta et al (1992)[7]	27 yr
Mallidis & Baker et al (1994)[4]	34 yr
Dajani et al (1998)[9]	36 yr
Gupta et al (2006)[15]	27 yr
Current study	**30.4 yr**

The duration of infertility was found to be 2-10 yrs according to Mona et al and a mean duration of 2 yrs according to Foresta et al. In our study the mean duration is 5.7 yrs.

Bettella et al 2005[13] studied the role of FNAC in TESE (Testicular Sperm Extraction) and found mature spermatozoa by FNAC in 33 of 125 men (26.4%), in 24 of 42 patients with severe hypospermatogenesis (57.1%), 9 of 14 subjects with maturation arrest (64.3%) and none with SCOS. In our study mature spermatozoa were identified in 5 out of 5 patients with hypospermatogenesis, 3 out of 13 patients with maturation arrest and none with SCOS.

Agarwal et al 2004[37] had correlated the sperm count with HP report. Comparison our study with Agarwal et al is given below (Table 2).

TABLE 2: COMPARISON OF SPERM COUNT WITH HP REPORT

Sperm Count	Current Study		Agarwal et al ,2004[37]	
	No. of cases (35)	% correlation	No. of cases (50)	% correlation
Azoospermia	27	67%	27	81.5%
Oligozoospermia	8	50%	23	65.2%

Testicular cytomorphological patterns given in various studies are compared with current study (Table 3).

(TABLE 3): COMPARISON OF INCIDENCE OF CYTOLOGICAL TYPES WITH OTHER STUDIES

Author/year	Normal spermatogenesis	Hypo spermatogenesis	Maturation arrest	Sertoli cell only syndrome	Testicular Atrophy
Al-Jitawi et al, 1995[5]	10.2%	31.4%	-	30.2%	28.5%
Meng et al, 2001[45]	13.8%	17.2%	33.3%	35.6%	-
Qublan et al, 2002[46]	20.6%	26.5%	23.5%	29.4%	-
Singh et al, 1999[40]	15.63%	65.63%	3.3%	3.13%	12.5%
Plas et al, 1999[47]	14%	3%	36%	22%	7%
Seo et al, 2001[48]	-	41.6%	13.5%	44.9%	-
Rayes et al, 2000[11]	31%	13%	11%	39%	5%
Verma et al 1992[49]	30.3%	42.3%	6.7%	2.6%	-
Current study	**22.9%**	**22.9%**	**34.2%**	**14.3%**	**5.7%**

There is 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.

Dajani et al[9] had studied the use of testicular FNAC by grading the cytological smears in 1000 infertile men and found the common grade to be grade C which indicates SCOS. Our study showed that grade B is the commonest grade. Table 4 gives the comparison of our study with that of Dajani et al.

TABLE 4: COMPARISON OF CYTOLOGICAL GRADES

Cytological Grade	**A1**	**A2**	**B**	**C**	**D**
Dajani et al[9] (1998)	25.3%	17.1%	20.6%	34.6%	0.24%
Current study	**22.9%**	**22.9%**	**34.2%**	**14.3%**	**5.7%**

Mona et al[39] 2008 had studied the patterns of testicular histopathology in male in infertility and ranked the testicular biopsies according to the Johnsons scoring system .Table 5 gives the comparison of our study with Mona et al 2008.

TABLE 5: COMPARISON OF JOHNSONS SCORE AND HP REPORT

Johnsons Score	**Mona et al 2008[39]**		**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%

In Mona et al the predominant Johnson score was 2 indicating SCOS. In our study the predominant score ranged between 3 and 7 indicating maturation arrest at spermatogonia, spermatocyte and spermatid.

Several references are available on correlation between FNAC and biopsy as given below in table 6.

TABLE 6: LITERATURE ON CORRELATION BETWEEN FNA AND BIOPSY

STUDIES (Year)	**NUMBER OF PATIENTS**	**CYTOLOGIC & HISTOLOGIC AGREEMENT (%)**
Gottschalk- Sabbag et al (1993)[50]	47	87%
Mallidis et al (1994)[4]	46	94%
Mahajan et al (1999)[51]	60	97%
Rammou- Kinia et al (1999)[52]	30	87%
Craft et al (1997)[53]	19	84%

Odabas et al (1997)[54]	24	90%
Meng et al(2001)[44]	87	94%
Qublan et al (2002)[46]	34	96%
Srivastava et al (2004)[14]	46	95.6%
Current study	**35**	**86%**

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[44] found discordant diagnosis between cytology and histology in 6% of cases. 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.

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).

In our study the overall sensitivity is 90% and specificity is 96.2%. The following table 7 gives the comparison of sensitivity and specificity with that of various studies:

TABLE 7: COMPARISON OF SENSITIVITY AND SPECIFICITY

Study	Sensitivity	Specificity
Hussein et al 2005[55]	98%	100%
Betella et al ,2005[13]	44.6%	100%
Current study	**90%**	**96.2%**

In our study the sensitivity was 90% and specificity was 96.2%.

Thus FNAC is less invasive and gives informative data on spermatogenesis of the entire testes. Report can be issued quickly as compared to biopsy. Complications related to procedure are rare. It is simple, quick and inexpensive because surgical instruments are not required. Local scarring doesn't occur. 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. FNAC guided TESE is useful alternative to blind biopsy[44]. 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. Testicular disorders leading to azoospermia such as atrophy, fibrosis and Leydig cell hyperplasia can be diagnosed better on basis of histology but are difficult to assess by FNA. Some patients complain of prolonged pain but this can be relieved by scrotal support and analgesics. Fairly experienced pathologist is needed to interpret the smears. Neurogenic shock has been reported in patients who failed to rest after the procedure. Hematoma formation can be expected when thick needle (20G) is used[44].

SUMMARY & CONCLUSION

FNAC is a reliable, quick, easy and less invasive technique which is one of the important investigations in diagnosis and management of infertility and is associated with no or minimal complications. Testicular FNAC has picked up in recent years following Obrant and person[2], Papic et al[35], Schenck & Schill[34] and Foresta et al[7] who characterized different cell types in cytological smears and demonstrated good correction of cytological diagnosis with histological categories. In current era, microassisted fertilization techniques are of great help for infertile couples as nowadays the only requirement in these techniques is a viable sperm and ovum. Neither quality nor degree of motility is essential [6]. Therefore in cytological smears a report of presence or absence of sperm is also adequate.

This study has been done in 35 infertile males in Urology OT, MMC with mean age of 30.4 years and mean duration of infertility of 5.74 years to evaluate the cytological features of testicular FNAC for the diagnosis of male infertility, and to study the correlation between cytological and histological diagnosis. Maturation arrest is the common pattern seen in our study (37.1%) in Histology. Next common is the Normal spermatogenesis (23%).

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. The discordant cases indicate that if Johnson's score is done routinely during histopathology reporting, then the diagnostic accuracy could be improved.

In Cytology, the common diagnosis was Maturation arrest (34.2%) as in histology and the common cytological grade was B correlating with maturation arrest. Taking HPE as the gold standard for correct diagnosis, the correlation of FNA with HPE diagnosis is 86%. However, in testicular biopsy of male infertility, cytology could be considered as gold standard as histologically negative cases show sperm in cytology. So that sperm extraction for ICSI could be successfully done based on cytology report rather than histology report.

Our study showed an overall sensitivity of 90%, specificity of 96.2%, positive predictive value of 89.2% and negative predictive value of 96.1%. No major complications were encountered in relation to the procedure. The cytological technique can be considered as better than testicular biopsy for the following reasons:

A) Simple, expensive and minimally traumatic with less number of complications.

B) More sites could be aspirated safely. This is useful as the lesions are focal or mixed rather than as misinterpreted in histology.

C) Cytomorphological features of various cell types are identified correctly and can evaluate accurately all classically defined histological types.

D) Above all cytology yields sperms in histologically negative cases and the sperms could be extracted for successful ICSI.

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TESTICULAR FNAC & HISTOPATHOLOGY CORRELATION IN MALE INFERTILITY

Name: Age: Sex: IP no: Ref Unit:

History:

1. Smoker	
2. Alcoholic	
3. Drugs	
4. DM/ HT/ TB/ STD/ Thyroid problems	
5. Marital History	
6. Surgery: for maldescended testis/ Hernia Repair/ Varicocele	
7. Others	

General Examination:

Anaemia ☐ Jaundice ☐

Lymphadenopathy ☐ Built ☐ Cyanosis ☐

Local Examination:

Penis, Foreskin & External meatus :

Testicles: Size/ Consistency/ Volume :

Epididymes/ Vasa :

Scrotal Skin/ Varicocele :

Investigation:

4. Complete Haemogram :
5. Semen Analysis :

Volume :

Colour :

Liquifaction Time :

Viscosity :

Total Sperm count :

Motility :

Morphology :

Pus cells :

Others :

6. **Trans rectal Ultrasound (TRUS)/ Scrotal USG/ Vasogram**

7. **Testicular FNAC:**

8. **Testicular Biopsy:**

9. **Others:**

Diagnosis:

Complications:

KEY TO MASTER CHART

NS	Normal spermatogenesis
HYPO	Hypospermatogenesis
MA	Maturation arrest
SCOS	Sertoli cell only syndrome
GCA	Germ cell aplasia
TA	Testicular atrophy
TH	Tubular hyalinization
USG	Ultrasonogram
A	Azoospermia (absence of sperms in semen)
B	Oligospermia (sperm count less than 20 million)