Material and Methods
MATERIAL AND METHODS

The present study was conducted in the department of Medicine M.L.B. Medical College, Jhansi. Patient were selected from the patients coming to the out patient room of the Department of Medicine of this college.

Male partners of 51 couples complaining of infertility were selected for this study. The infertility was defined as "unprotected barren union for 1 year during which the husband and wife should have stayed together."

Investigation of the Male partner began with history, the patients History was noted down in the following format.

Inclusion Criteria: The following couples coming to the OPD of the medicine department were included in this study.

1. Those who failed to achieve pregnancy after one year of unprotected and regular intercourse.
2. All couples in the age group between 15 to 60.

HISTORY

(1) Name:
Occupation: Address:
Religion:
Age and date of birth:
Wife's name:
Wife's age:
Socioeconomic status:
How long have you been married?
2). **Complete history**: With special reference to -

- Age at the time of marriage
- Use of any contraceptive method.
- H/O infertility in parents.
- Duration of regular and unprotected sex,
- H/O- DM, hypertension, hernia surgery, diet, smoking, alcohol, trauma, testicular swelling, UTI, hemorrhoids, varicose veins radiation, undue stress, fever, urogenital infections, drug abuse, exposure to heat or chemicals.
- Sexual history- Libido, frequency of sex, quality of sex, overall sex life.

3). **Complete physical examination**:

(a) **General Exam**: Appearances, built, nutrition, BP, height, weight, secondary sexual character, lymph nodes.

(b) **Systemic Exam**: Examination of CVS, CNS, Respiratory system and Abdominal examination.

(c) **Genital Exam**:
   
   - Penis - Shape, foreskin, infection, ulcer discharge.
   - Testis - Shape, size, position, consistency, any associated lump.

(d) **General body habitus** - Gynaecomastia, obesity, eunuchoid appearance etc.

**INVESTIGATIONS**:

**General Investigation**

- Hb, TLC, DLC, ESR
- Blood Sugar
- Blood Urea, S. creatinine
- Urine (Routine & M/E).
- VDRL and TORCH
- USG Abdomen.

**PATIENT WORK UP PLAN**
**Special Investigation approach**

A. Sexual or ejaculatory → Abnormal → Sexual dysfunction

Dysfunction

Normal

Semen Analysis → Normal → Test for immunological factors → Normal

Find etiological factors

- No → Idiopathic Azoospermia
- Yes → Etiological diagnosis

Endocrine Evaluation

B. Semen Analysis: Normal pH: 7.5 - 7.8

Normal Volume: 2-5 ml.

Azoospermia → Idiopathic Azoospermia

Yes

No

Concentration $10^6$/ml → Oligozoospermia

≥ 20

Motility (% Motile) → Asthenozoospermia

≥ 40

Morphology % normal forms → Teratozoospermia

≥ 50

Vitality % live → Necrozoospermia

≥ 60

Seminal Fluid → Isolated Seminal fluid abnormality

Abnormal

Normal

Look for criteria of male accessory gland infection

**If Semen Analysis Abnormal**
1) Endocrine Evaluation
   Normal FSH - Obstructive causes
   Increased FSH Testicular cause.
2) Testicular biopsy: Normal - Obstructive cause.
3) Vasography - Abnormal - Obstructive cause.
4) Trans scrotal u/s - to find out site and etiology of obstructive cause.

**Indication of Testicular biopsy**

Normal FSH with -
   1) In azoospermic patients.
   2) Selected cases of severe oligozoospermia
   3) Testicular granulomatous disease.

**Cervical Mucus penetration Test**

To access the ability of spermatozoa to penetrate the cervical mucus barrier to know it fertilizing potential or human spermatozoa in vivo & in vitro

**Hypo-osmotic swelling test**

Swelling of tail of normal human spermatozoa when exposed to hypo-osmotic solution of fructose and sodium citrate differentiate it from nonfunctioning or nonviable sperm which fails to swell on exposure to such solution

**Scrotal Ultrasonography**

It is a reliable method of detection of varicocele/hydrocele. Thus, with above mentioned history/clinical examination, and Investigations, etiology of Infertility can be searched and can be worked accordingly.
**Normality criteria for semen sample:**

- Spermatozoa > 20 million/ml

- Motility > 40% with linear forward progression

- Morphology > 50% Normal (ideal) forms

- Viability > 60% live

- Agglutination: No

- Seminal Fluid:
  - Normal appearance
  - Normal Viscosity
  - Less than $10^6$ WBC/ml.

- Aspermia: No fluid

- Oligospermia < 1.5 ml semen

- Polyspermia > 6.0 ml semen

- Azoospermia: No spermatozoa in fluid

- Oligozoospermia < 20 million sperms/ml.

- Polyzoospermia > 250 million sperms/ml.

- Normozoospermia: 20-250 million sperms/ml

- Asthenozoospermia: Motility < 40% 2 hrs after ejaculation or qualitatively insufficient, without good forward propulsion.

- Necrozoospermia: Absence of motility

- Teratozoospermia: Too high number of abnormal sperm heads.
INSTRUCTIONS TO THE PATIENT

The semen should be brought for examination at least twice, first after period of abstinence of 3 to 5 days to have a standard for comparison; the second time after a period of abstinence that is normal for the patient. If a couple has intercourse everyday, it may well be that a specimen produced after 4 days of abstinence is quite normal, but with daily intercourse the sperm count, as well as the volume may be too low. The investigator could miss his important factor if he sticks rigorously to an examination after a period of abstinence of 4 days.

There are several ways for the patient to produce his semen for investigation except that an orgasm is always necessary. Fluid expressed from the penis by rectal massage, or obtained by puncture of the testes or epididymis, is not representative for the ejaculate produced by an orgasm. Also unacceptable is the examination of the drop of fluid that can be expressed from the penis after intercourse. Examination of the reflux semen after normal coitus used to be the method of choice for roman catholic patients when the religious doctrine was more severe than in later years. This method has the disadvantage that the collection often is incomplete, that the biochemical constitution may be greatly changed and that leucocytes and epithelial cells from the vagina may interfere with the Judgement. Much better is the method of coitus interruptus, although this has the disadvantages that the very first part of ejaculate may be lost, if the patient is not quick enough. Total recollection is guaranteed by using a condom, but this is to be depreciated because many condoms or the powder therein will interfere with the motility of the spermatozoa (pseudonecrozoospermia). Ignorance of this possibility has been the cause of the many misapprehensions in the past. If massage is not acceptable on
religious considerations, the cervical spoon may be used (Doyle 1948 and Schellen, 1958) although in this way one can not be sure that the complete ejaculate is recollected.

The best method to obtain the semen for investigation is the production of an orgasm by means of massage of the penis, either by the patient or by his wife. For this procedure it is better to dispense with the 'loaded' word masturbation. There is no difference between the seminal patterns if the semen is produced by massage or by interrupted coitus, as has been proved by various seminologist (Freund, 1962).

The semen has to be collected in glass Jar or a plastic Jar, supplied by the andrologist. This Jar must have been tested and found harmless to the motility of the spermatozoa. The receptacle should be kept at body temperature for some time before usage, to prevent cold shock. The semen has to be transported at out of door temperature. Only if this is near freezing point should it be kept under the clothes during transport. The specimen has to be delivered within two hours after ejaculation, preferably within one half hour.

COLLECTION

Two semen samples were collected from each patient. Sample was collected after minimum of 48 hours and not longer than 7 days of sexual abstinence and the two samples were collected not less than 7 days and not more than 3 months apart. If semen showed any abnormality, an attempt was made to collect a third sample but this time without abstinence, examination in usual ejaculatory frequency become subfertile or infertile. This is because it has been shown that when frequency of ejaculation is high the sperm counts and morphology is lower that what is after abstinence. So a person who is fertile on
examination after abstinence might in practice be at borderline levels of fertility. Semen was collected in a wide mouthed glass jar with cork by masturbation at the premises of Hospital only.

After each collection patient was asked to provide following information. This was important. This information is important for the person analyzing the semen and helps in interpretation of the results.

1. Time of collection

2. Time of analysis

3. Was any semen lost at this collection? Yes/No

4. Was the semen thick

5. When did you last ejaculate

6. With this masturbation did you feel
   (a) Produced more semen than at intercourse
   (b) Produced less semen than at intercourse
   (c) Produced the same quantity as intercourse

7. In the past 2-3 months did you have
   (a) had any major illness
   (b) had unusual alcohol consumption
   (c) taken drugs (specify)
   (d) Recent trauma to testes
   (e) had periods of constant stress
8. Could your semen be infected (hepatitis B, HIV, sexually transmitted disease) - specify.

**Analysis** The specimen was analyzed within 3 hrs. of collection. The volume was measured to the nearest volume in ml. Before further analysis the semen was vigorously shaken in the container. This was because semen contains fluids from various organs with differing viscosity, cellularity in various portions. Also with time the motile cells tend to settle as a function of time.

**A. THE SEMINAL PLASMA**

1. Volume

The volume of an ejaculate depends mainly on the contribution of the seminal vesicles. Normally this lies between 1.5 and 5.0 ml. A smaller part is derived from the prostate, 0.2- 1.0 ml and from the glands of Cowper and Littré, a few droplets only.

About 0.2 ml, containing the spermatozoa, is derived from the vasa deferentia and the epididymides. The average volume of a normal ejaculate after a period of abstinence of about three days, lies between 2 and 4 ml. The volume is fairly constant in most patients; the upper limit is reached after a abstinence of about four days. A volume of less than 1.5 ml or more than 6-8 ml is considered as abnormal.

**Relation to fertility**

**Low volume** : Although usually a volume of less then 1.5ml is considered as abnormal, this does not necessarily mean that the fertility is decreased. The importance of a low volume by itself and as a single factor is often exaggerated. It has often been presumed that a low volume prevents the formation of a vaginal pool, or that a small amount of semen is incapable of buffering the acid
vaginal secretion. In this respect, other factors also play a part: the position of the cervix to the vaginal pool, the acidity of the vagina and the occurrence or absence of orgasm in the wife, amongst others. However, a small volume may point to an abnormal function of the seminal vesicles and in that case there will indeed be a decrease fertilizing capacity because there are more factors involved.

**High volume**: It is often said that too high a volume (polyspermia) may have an adverse effect on the fertilizing capacity, because the sperm density will easily become too low. This is contrary to the situation in cattle, where the fecundity is sequestered by diluting the semen. Be this as it may, one should realize that the greatest number of spermatozoons is to be found in the first 1 or 1.5 ml of the ejaculate. This first fraction is reaches against the external orifice of the uterine cervix, there by reaching the mucus plug. What happens with the rest of the ejaculate is, most probably, of far less importance. The vagina of a woman who has never given birth contain more than 1.5ml semen. One can easily make the experiment by injecting into the posterior fornix a fluid of approximately the viscosity of semen. The higher the viscosity, the more will be retained, but rarely more than 2 ml. Consequently, all of the ejaculate above this limit will be spilled as the so-called reflux. It does not seem to be of much importance whether this reflux measures 1 or 4 or even 8 ml. as long as the first part of the ejaculate reached the cervical mucus first

\[ P^\text{H} \]

The initial $P^\text{H}$ depends mainly on the relation between the alkaline secretion of the seminal vesicles and the acid secretion of the prostate. After ejaculation the $P^\text{H}$ tends to decrease due to the formation of lactic acid from
glucose and fructose especially if the motility is good. The normal $P^H$ ranges from 7.4 to 8.5 if measured within one hour after ejaculation.

Relation To Fertility

An abnormally low pH, although in itself is no reason for a decrease in fertilizing capacity, is an important finding because of the usually accompanying abnormalities (low volume, low content of sugar).

VISCOSITY

The viscosity of the semen is the degree of stickiness that remains after liquefaction.

High viscosity: High viscosity may cause a decrease in the fertilizing capacity because the spermatozoa may not be able to pass from the semen into the cervical mucus.

THE SPERMATOZOA

SPERM COUNT

Formerly it was presumed that a density of less than 60 millions per ml were too low but now put at 20 millions per ml. This was first stated by Mcleod and Gold (1951) who made a follow up study on two groups of one thousand males. The first group had recently impregnated their wives, the second group is thought aid for a childless marriage. They found in both groups that with a density above the 20 million level a further increase in sperm count did not increase the case of conception.

In most males, fertile or infertile, the sperm count varies within rather narrow limits (Mcleod and Gold, 1951). There are exception however, and in some patients the fluctuations may even be up to five or tenfold.
Pathology

Low density

Oligozoospermia may be an artifact. If the first part of an ejaculate is lost during collection, the density in the collected second part will be low. Also an incomplete orgasm caused by psychological inhibition or a cramped state of the smooth muscle tissue may have the same result. Careful interrogation and re-instruction of the patient will reveal this possible factor. Furthermore, too frequent ejaculations and too short a period of continence will result in oligozoospermia in most men.

Rarely a low density is the result of too high a volume. Then, oligozoospermia without other abnormalities in the semen may be caused by too low a number of normally functioning tubules. It may be that the testes are too small or that part of the tubules are fibrotic, caused by some severe illness or inanition in the past. In still other cases, a situation develops where the germinal tissue disappears from the tubules, only the cells of Sertoli remaining. If fully developed this syndrome of depopulation of, Sertoli cell only syndrome causes azoospermia. In the incomplete form of partial aspermatogenesis, there will be oligozoospermia.

Hypospermatogenesis that is a diminished activity of the spermatogenic tissue, usually leads to oligosthenozoospermia. If there is disorganization in the tubules oligo-asthenoteratozoospermia will be the result.

Polyzoospermia

A density of more than 250 millions per ml is considered as abnormal. Doepfmer (1962) made an analysis of cases with a high sperm count and he distinguished different situations. If a high density is caused by a relatively low
volume in the presence of a normal total count, this is relative (pseudo) polyzoospermia. Absolute (real) and the total sperm count more than 600 millions. This is caused by overactivity of the seminiferous tissue, with normal function of the accessory glands. In these patients there is a relatively high percentage of cases with head to head agglutination in the semen and sperm agglutinins in the blood.

Relation to fertility

Oligozoospermia

A sperm count of less than 20 millions per ml is the cause of a low fertility, even if the total count should be within normal limits (Mcleod and Gold (1953). In the human, only a few ml. semen usually only the first sperm rich part of the ejaculate, will come into contact with the cervical mucus. A great deal of the ejaculate, depending on the total volume, will flow back out of the vagina, it is not important how many spermatozoa are lost in the reflux semen. Therefore it is the density of the sperm cells, especially in the first ml, that is decisive. In most semen samples with low density, the subfertility is increased by the presence of other factors, an accompanying low motility or high percentage of morphologically abnormal sperm cells or both.

Polyzoospermia

In relative (pseudo) polyzoospermia subfertility is the rule. This is caused by the deficiency of normal plasma constituents in these cases. Real polyzoospermia does not decrease the chances for pregnancy, although there are indications that in many instances the pregnancy will end in an abortion.
MOTILITY

As long as the tightly packed spermatozoa are in the epididymis and in the ampulla of the ductus deferens, they remain immobile. As soon as they are expelled by ejaculation, entering the secretions from the prostate and the seminal vesicles, they respond to the change in environment with an outburst of motility. With regard to the motility three different factors are distinguished. The qualitative motility gives the percentage of spermatozoa that are in any way not immobile. The qualitative factor gives the degree of motility of which at least four grades have to be distinguished (1) progressive forward propulsion; (2) weak forward movement; (3) movement on the spot without displacement; (4) Immobility, As a third factor more and more importance has gained regarding the type of motility. (a) in the normal type of motility the tail beat, generated - from the middle piece and also from a small part from the tail structure, (b) if the tail is a weak, forward movement without rotation, (c) Swimming in circular orbits in one plane only (yawning spermatozoa) is probably caused by a restricted activity of fibrillar system in the tail (Van duijn et al. 1966).

Normal figures for the motility are that their should be at least 40 percent of the sperm cells with normal rotation, forward propulsion (grade 1a) within two hours after ejaculation.

In many patients the motility in the first part of a split ejaculate is better than in the complete semen (Eliasson, 1972).

Motility was rated as follows:
> Linear forward progression
> Slowly progressive
Non progressive
Non motile

Motility was rated as % of motile sperms
A low motility of the spermatozoa is called as asthenozoospermia.

Relation with fertility
Good forward propulsion of the spermatozoa is not only necessary for reaching the ovum but also to penetrate through the corona and to enter the egg cell. However, low motility in the semen is not conclusive of a deficiency in this respect. In all cases of asthenozoospermia the motility should also be studied in the cervical mucus.

Real disturbances in motility are more important in a negative sense for the fertilizing capacity of the semen than low sperm count or poor morphology.

MORPHOLOGY
The morphology of the sperm head is determined by four factors.
1. The size (small, normal, big.)
2. The relation between length and width (round, oval elongated.)
3. The shape of the head near the mid piece (normal or pear shaped)
4. The contour (smooth or irregular).

Teratozoospermia: A high percentage of teratoforms may be the result of fixation or staining (Hellinga et al. 1973). If technical faults are eliminated, the cause has to be found in the spermatogenetic tissue, because the morphology of the heads does not change during the passage of the sperm cells through the efferent seminal tracts. As soon as the condition of the spermatogenic tissue is damaged by general factors (fever, malnutrition) or by a
local anomaly (varicocele) the number of teratoforms of several kinds and often also the tapering forms increase, together with a decrease in the density and in the motility of the spermatozoa. At the same time, the number of round cells in the semen, the desquamated cells, tend to increase. This is the "stress pattern of the testes" (MacLeod, 1962). Often this seminal pattern is seen without any known cause. It is considered as a sign of poor condition of the seminal tissue and the increase in output of FSH which is most often found in those cases could be seen as a result of non consumption.

Relation to fertility

In patients with teratozoospermia the fertility is definitely lowered, but it is uncertain whether this caused by the high percentage of abnormal head forms or by the accompanying decrease in density and motility.

Patients with crooked, bent, broken or coiled tails but still a percentage of normal spermatozoa in their semen have been of proven fertility, although probably subfertile. Patients with a high percentage of short tails are to all probability sterile. This is certain of those with the combination of thickened mid pieces and short tufted tails.

OTHER CELLS IN THE SEMEN

In human semen a number of round cells are always present. These are of different nature: leucocytes, desquamated cells of the seminal epithelium, also called exfoliated cells, epithelial cells of the walls of the seminal ducts, sometimes cells from the vagina.

Leucocytes

A few leucocytes are normally present. If so, a count should be made with counting chamber. The range of normality lies below 1x10⁹/L. For normal
semen this means that there are less than 3-5 leucocytes to every 100 spermatozoa.

Relation to fertility

The presence of pus cells is in itself not a hindrance for fertilization. If leucocytes are added to normal semen the motility is not decreased. However, the presence of leucocytes suggests the existence of a local inflammation and this may well be accompanied by a decreased function of the infected accessory gland, and thus by a decrease in fertility.

DESQUAMATED CELLS

There is still some controversy about the frequency of finding unripe germinal cells in the semen. It is agreed that spermatids are often present; these cells are easy to recognize. About spermatocytes and spermatogonia there is disagreement.

SPERMATIDS

Spermatids as seen in colored slides of human semen are about the size of lymphocytes. The cell is round and dense, the protoplasm stains heavily blue or red. Not more than 3-5 are seen against 100 spermatozoa in normal semen.

Relation to fertility

All in all we may assume that the finding of a great number of spermatids in a semen sample points to the presence of other factors of which it is known that they go with a decrease in fertility.

SPERMATOCYTES AND SPERMATOGONIA

According to Joel (1953), in stained slides spermatocytes have a diameter of 11-19µ, with a large, single, mostly round nucleus. The protoplasm is clear with small granules and a light perinuclear zone. Spermatogonia are smaller, 5-
12u and also round, the nucleus is small and darkly staining. The protoplasm is darkly eosinophilic. Pathology: Cells resembling spermatocytes and Spermatogonia are often found as a transitory occurrence in acute distress situations as fever, ischemia, intoxications.

PROSTATIC CELLS

Normally there are no prostatic cells in the semen.

Relation to fertility: None.

ERYTHROCYTES (HEMOSPERMIA)

Normally there are no erythrocytes in the semen.

Relation to fertility

In hemospermia the motility of the spermatozoa and thereby the fertilizing capacity is said to be decreased or even absent in most cases. This is especially true for the rare persistent form, in which both the motility and the fertility have been known to improve after surgical or anti-inflammatory treatment. In the far more frequent transitory cases of hemospermia a decrease in fertility has not been proven.

SPERM FUNCTION TESTS

(a) Sperm cervical mucus interaction The ability of spermatozoa to penetrate the cervical barrier is an important aspect of sperm function that correlates with the fertilizing potential of human spermatozoa in vivo and vitro. In conventional Kremer assay cervical mucus is collected from the female partner during the periovulatory stage of the cycle and carefully loaded into the capillary tube. One end of the tube is then sealed while the open end is inserted into the reservoir of male partners semen. After an incubation period of 30 min.
to 1 hr. at 37°C, the capillary tube is removed from the semen and the concentration of spermatozoa counted at intervals along the tube (1.4 and 7 cm). All this information along with measurements of sperm concentration is used to compute the cervical mucus penetration score. Cervical mucus penetration tests provide important information on the first stage of sperm transport to the site of fertilization. What happens to human spermatozoa between the colonization of cervix and initiation of fertilization is largely unknown. Thereafter, the functional assessment of human spermatozoa rests entirely on their competence to participate in fertilization process itself.

Fertilization is a complete cascade of events and the next step in evaluation of sperm will be to assess interaction between sperm and zona pellucida, induction of acrosome reaction and fusion with vitelline membrane of oocyte.

**EVALUATION OF OBSTRUCTIVE CAUSES OF MALE INFERTILITY**

Patients having normal testicular size and consistency and low ejaculate volumes, sperms density, sperm motility and forward progression should be evaluated to exclude obstructive pathology and varicocele.

**RADIOLOGICAL**

(A) Deferentiovesiculography - It is used to study the pathology of seminal duct or prostate by instilling a dye tri-iodate hydrosoluble methylglucamin salt at 70% through a puncture at vas. It can identify.

1. Vas deferens
2. Ejaculatory ducts
3. Seminal vesicles
(B) Transrectal ultrasonography

Randal et al 1993 have used transrectal ultrasonography to detect ejaculatory duct obstruction and have advocated this as a good procedure and concluded that ejaculatory duct dysfunction has been under diagnosed in the past. This procedure can also be used for the examination of prostate, seminal vesicle.

(C) Scrotal ultrasonography

This is a reliable method for identification of varicocele although venography is the most specific but invasive.

Hydrocele - is sharply depicted as an anechoic fluid collection around the testis between the two layers of the tunica vaginalis.

Hematocele - in comparison presents a complex fluid collection of variegated echogenicity.

Infection - results in an altered echo pattern of the epididymis, which is thickened, and also of the testis if involved. These areas are usually hypoechoic with ill defined margins. A secondary hydrocele or an abscess, usually extratesticular, may be in association. The latter appears as a localized cystic area of complex echogenicity.

Testicular tumors - appear as intra-testicular hypoechoic areas, occasionally with mixed echo patterns, having irregular but well defined margins, surrounded by normal testicular parenchyma.

Cystic areas and calcification may also be encountered.
**Immunological Tests:**

Immunological factor was studied by observing the agglutination of sperms in seminal fluid by testing for antisperm antibodies in the serum of patient.

**Antisperm antibodies:**

Serum dilution (0.5ml in each test tube) is done with normal saline 1:10 dilutions are done. One tube is set with only normal saline to serve as control. After liquefaction of semen, an equal amount (0.5ml) is mixed in each tube. All test tubes are incubated for 1 hour at 37° C The semen is examined on a slide in microscope and it is examined for loss of motility and agglutination of sperms to form clumps. This is taken as positive for presence of antisperm antibodies.

**ENDOCRINE EVALUATION**

Following 3 hormones were assessed by ELISA.

Hormones assessed and their normal values

<table>
<thead>
<tr>
<th>HORMONE</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>1.0-14.0 miu/ml</td>
</tr>
<tr>
<td>LH</td>
<td>7-7.4 miu/ml</td>
</tr>
</tbody>
</table>

Testosterone, T3, T4. TSH and Prolactin

To compensate for the pulsatile release of the gonadotropins, following method for collection of sample has been recommended and used.

Blood samples were taken at 20 minute intervals from the same or different vein and mixed together and analyzed. A normal FSH in presence of semen abnormalities is a good guide to the presence of obstructive pathology.

If serum LH and FSH concentrations are increased the diagnosis is primary testicular failure. These men generally have low testosterone levels,
small testis and azoospermia, but may have oligospermia and normal testosterone levels.

In some infertile men LH and testosterone levels are normal but FSH levels are elevated this is because FSH signifies the state of seminiferous tubules which are more sensitive to damage than leydig cells. A selective increase in FSH can also signify a FSH secreting pituitary tumor. Consequently patients with azoospermia having elevated levels of FSH fall into 3 categories on testicular biopsy-seminiferous tubule hyalinization, sertoli cell only syndrome and germinal cell arrest - all untreatable conditions.

Thus finding of elevated FSH with azoospermia usually is an indication to proceed no further in investigation or therapy.

The estimation of LH and testosterone level in plasma provides evidence that intertubular areas are also involved in the pathological process. As damage to seminiferous tubules becomes severe, an increasing proportion of patients show an elevated FSH and a low testosterone.

Finding of a low testosterone should also prompt search for hypogonadism in which case Gonadotropin level would also be low. It has already been mentioned that normal FSH with oligozoospermia can be because of testicular cause but should also prompt investigation to rule out obstruction in the male genital tract.

Serum prolactin levels are measured in men with sexual dysfunction, decreased libido or delayed adolescence because these symptoms are common with prolactin producing pituitary tumors.
Elevated testosterone levels are usually a consequence of increased serum hormone binding globulin level, as in hyperthyroidism. Testosterone production may be increased however in patients with LH producing pituitary tumors or HCG producing neoplasms or with mutation in androgen receptor that disrupts testosterone negative feedback.

**MALE ACCESSORY GLAND INFECTION:**

Following criteria recommend by WHO was used

**History and Physical signs:**

- a history of urinary symptoms (dysuria, urethral discharge, hematuria, increased frequency or difficulty in voiding)
- a history of epididymoorchitis
- a history of painful ejaculation.
- a history of sexually transmitted disease, thickened, tender or cystic epididymes on clinical examination.
- thickened vasa deferentia on clinical examination.
- Postinfectious, trauma scars on initial examination.
- lymphadenopathy
- abnormal prostate on rectal examination
- Palpable seminal vesicle on rectal examination

**Urinary or Prostatic signs**

- Increased leukocytes on urine analysis
- significant bacteriuria (>10⁵/ml) on urine analysis

**Ejaculate signs**

- abnormal appearance of semen
- abnormal viscosity of ejaculate
 Elevated WBC (>10⁶/ml) in semen sample

Any combination of 2 or more signs, symptoms from the 2 categories were sufficient for diagnosis. In the absence of any signs or symptoms there had to be at least 2 ejaculate signs for diagnosis.

TESTICULAR BIOPSY

Evaluation of testicular biopsy is a direct method of evaluating the state of seminiferous tubules in male with infertility. Studies by Zuckermann et al. showed a direct relationship between seminiferous tubules and sperm counts. Rodriguez et al. showed direct relationship between sperm counts and elongated spermatids on biopsy.

Testicular biopsy is also useful to demonstrate partial or complete obstruction. In the former, the late spermatid count per tubule cross section is inappropriately high. Analysis of testicular biopsy specimen show the direct relationship between germ cell count, and serum FSH and testosterone production and are thus useful for diagnosing Sertoli cell only syndrome, klinefelter's syndrome. Testicular biopsy can identify the type of testicular damage. Evaluation of seminiferous epithelium is performed at low magnification to assess the overall state of spermatogenic process. Each individual cell type is sought and some assessment of this number is made in comparison to normal values. Abnormal cell types are sought. The degree of testicular development is compared to the chronological age of the patient and his pubertal status.

Categorization

There is no universally accepted categorization but the following is recommended.
## Categorization of Testicular Biopsy Findings

<table>
<thead>
<tr>
<th>Category</th>
<th>Appearance</th>
<th>Semen FSH levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Obstructive azoospermia</td>
<td>Near normal histology</td>
<td>Normal</td>
</tr>
<tr>
<td>2 Hypospermatogenesis</td>
<td>All stages of spermatogenesis Present. Number of germ cell depleted. Peritubular fibrosis in severe depletion</td>
<td>Normal or elevated</td>
</tr>
<tr>
<td>3 Sertoli cell only syndrome</td>
<td>No germ cell. Sertoli cells only</td>
<td>elevated</td>
</tr>
<tr>
<td>4 Germinal cell arrest</td>
<td>Cessation of spermatogenesis at Pituitary spermatocyte or spermatogonia stage</td>
<td>elevated</td>
</tr>
<tr>
<td>5 Seminiferous tubule hyalinization</td>
<td>Fibrotic or hyaline outline of tubules</td>
<td>elevated</td>
</tr>
<tr>
<td>6 Immature test</td>
<td>Testicular development retarded in relation to chronological age</td>
<td>Low</td>
</tr>
</tbody>
</table>