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 Reporductive Medicine clinic of the Department of Medicine of this college.

Male partners of 51 couples complaining of infertility was 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 begun with history, the patients History was noted down in the following format.

**HISTORY**

Name : Date of appointment:

Occupation :

Religion : Address :

Age and date of birth :

Wife's name :

Wife's age :

Socioeconomic status :

How long have you been married?

Are you using/used any contraceptive method (specify)?

How long you have been trying to have a baby?

Have you been married before?

Has your wife been married before?
Have either of you achieved a conception either with present partner or with another partner? Yes No
If Yes Present partner Another partner

Wife

Are you currently taking any form of medication? No Yes (If yes please specify:

**Family history**

How many brothers do you have? Number None

How many sister do you have? Number None

Are you aware of any member of your family including your parents having had any difficulty in starting family?

If you are, please specify:

History of any of the following disease

Asthma or other allergy

(If other please specify:

Bronchitis, Orchitis, epididymitis, Prostatitis, Gonorrhoea, Urethritis, Varicocele, Hydrocele, undescended testes, injury to testes, urogenital tuberculosis, Pneumonia

Any other disease of the chest

Diabetes mellitus

High blood pressure

Frequent headaches

Mumps

(If yes how old were you when you had it and do you remember if the disease affected your testicles?

Hernia:

(If yes please say where it occurred:

Chicken pox: Varicose veins:

Haemorrhoids: Back pain:
Any other disease not listed above: H/O Radiation:
Have you ever had a major surgical operation?
Have you ever had an operation of any sort associated with your penis or testicles?
If yes please specify:
Have you had any condition in the past two to three years which caused you to have a fever or a significant increase in body temperature (e.g. flu)?
Do you suffer from recurrent pain in the testicles?
(If yes please indicate the frequency of occurrence and state whether the pain is an ache or a sharp pain:
Do you feel a burning sensation in your penis when you urinate?
What on average is your frequency of ejaculation?
Are you aware of any difficulties at intercourse on either your part or that of your wife or partner?
Indicate your weekly intake of alcohol.
Do you smoke?
If yes specify what, and if cigarettes specify the number per day and duration.
In your daily work are you regularly exposed to heat or chemicals?

**Developmental History and Exposures:**

Developmental history is critical as this can indicate problems with formation of the genitalia that may suggest androgen deficiency or resistance which can present with hypospadias, bifid scrotum, or more marked forms of ambiguous genitalia. The parents should be questioned regarding the nature of his mother's pregnancy, duration, complications and whether
any medication were taken. In utero exposure to diethylstilbestrol (DES) can cause epididymal cyst and an increased incidence of cryptorchism. Late descent of the testes as an infant, or incomplete descent, can indicate a partially cryptorchid state with risk for impaired spermatogenesis as an adult.

**Sexual history:**

The sexual history provides an important information regarding erectile function, frequency of intercourse and sexual techniques. **Erectile dysfunction is usually the first clue to hypogonadism in the adult male.** Onset and duration of impotency and presence or absence of noctural or morning erection with a full bladder can help to evaluate for psychogenic versus organic cause. Loss of morning erections correlates well with an organic cause. Sexual techniques can provide clues for both evaluation of erectile dysfunction and also for infertility in cases in which coitus may be poorly timed or fail to provide deposition of sperm in the vagina. With the infertile male, a history of previous pregnancies can suggest an acute change rather than chronic process and increase the likelihood of a reversible condition. Also, the partner's history should be determined to confirm that she has received adequate evaluation as a potential cause for infertility of the couple.

And lastly patients should be askeed for history of urinary symptoms. These patients have a higher prevalence of azoospermia and abnormal semen quality, particularly abnormal sperm morphology. As many as 27% men with such a history present abnormalities in expressed prostatic fluid and semen, suggestive of chronic male accessory gland infection. A history of urinary symptoms is also more common in men with varicocele.
PHYSICAL EXAMINATION

Physical examination included general examination and genital examination and systemic examination.

GENITAL EXAMINATION was noted down by filling out the following form for each patient in a manner. Already mentioned on detail in the review of literature.

(1) Penis
   Normal Size.
   Foreskin - Retractable (Yes / No)

(2) Testes
   absent
   Normal size
   Consistency
   firm
   soft
   subnormal size
   rudimentary
   firm to soft
   not accessible

Other changes:
(undescended testes, fixed, hydrocele, varicocele, cicatricial changes)

(3) Epididymis
   Normal
   Thickened
   Hardened
   Enlarged

Any other pathological findings

(4) Prostate
(5) Secondary sexual characteristics

Examination of the patient should be made with the doctor sitting and the patient standing before him.

TECHNIQUES

The penis:

First note the size of the penis and find out if a phimosis is present, by drawing back the prepuce. If there are complaints about the erection, search for hard spots or ridges (induratio penis plastica). The is not uncommon to find presence of a hypospadias.

SCROTUM
Abnormalities of the scrotum concerns the shape, the skin or the contents. Especially in obese men the scrotum may be wider above than below, of the infantile type. On the other hand, some patients may have a very long scrotum. Both these anomalies in shape are often seen together with subfertility. The scrotal skin should be examined for the presence of psoriasis, eczema, lymphangioma.

VASA DEFERENTIA

Let the contents of the scrotum above the right testis roll between thumb and second finger of the left hand. After some practice, it is easy to distinguish between the rather soft veins and arteries and the much harder cord that is the ductus deferens. Follow the ductus deferens from as high as possible to the cauda epididymis and search for irregularities, hard spots or interruptions. Thereafter repeat this for the left side using the right hand.

EPIDIDYMIDES

palpate the epididymis from the head to the tail, note if the epididymis is lying closely to or distant from the testis. This is especially of importance if the testis is small. if there is room between the testis and the epididymis, it is probable that the testis has atrophied, otherwise a congenital hypooplasia is more probable. Search for irregularities in the consistency of the epididymis. Small cysts are often found above the testis, without clinical significance. Sometimes a number of small cysts are felt in the head of the epididymis and this is of more importance. It may be a cystic degeneration with obstruction at several points. These cysts may also give rise to the production of antibodies (Hamerlynck, 1970).

TESTES
The position of the testes in the scrotum has to be stated. Normally they are lying immobile in the scrotal sac. In obese men they have the tendency to retract into the subcutaneous tissue or even into the external inguinal ring. This may be a cause of disturbance in spermatogenesis.

The testes have to be measured, which can be done in two different ways. One method is, to measure first the longest diameter of the testis with the aid of caliper. This will add to the measurement only a small error, which can be neglected, then one measure of the width, which is somewhat less reliable. One has to note a largest and a smaller width, because the girth of a testis is not a is note a circle, from these three measurements the volume of the testis can be computed fairly accurately. Complicated formula have been presented for this purpose, less accurate but more suitable for practical use is the relation between the largest diameter (the length) and the volume of the testis as worked out by Hynie, the mean volume of a testis lies between 20 and 30 ml.

The accuracy of measuring the diameter of a testis depends on the consistency. If the testis is rather soft, it is arbitrary how much pressure should be applied with the calipers. This can make quite a difference, especially for the largest diameter. This disadvantage can be overcome and even made to yield profit by taking two 'extreme', measurement. One with as much pressure on the calipers as can be applied without hurting the patient; this is the minimal longitudinal dimension. Thereafter the testis is compressed, firmly on the transverse diameter with thumb and fingers of the other hand and now a second measurement of the length is taken without any pressure of the calipers; this is the maximal longitudinal dimension. The difference between the
minimal longitudinal dimensions normally is less than 10 mm in
testis of normal size. If the consistency is too soft, the difference
is 10 mm or more.

For the largest diameter the normal figures range from 40 to
50 mm. The mean width of a normal testis lies between 20 to
30 mm and the error of measurement will only be small. Thus
the figures for a normal testis will read: 51/43 x 26, meaning
that the maximal longitudinal dimension is 51 mm, the shortest
43 thus the consistency is normal as the difference is less than
10 mm. The mean width is 26 mm. For a testis of normal size
but soft consistency this will read 54/40 x 25. Too small a
testis will show a figure as 39/32 x 20.

**ABNORMAL CONTENTS OF THE SCROTUM**

Apart from the presence of a hydrocele or a spermatocele, it
is of great importance to examine for the presence of a
varicocele. This is rarely found on the right side, sometimes on
both sides but most often on the left side only. The examination
should always be done with the patient standing upright. If the
patient is lying down, the varicocele may disappear completely.
Let the contents of the scrotum above the testis roll through the
fingers, then ask the patient to press by blowing on the back of
his hand. the varicocele is noted as small (not visible from the
outside,) it is most important to note whether the varicocele gets
bigger in pressing because this indicated that reflux occurs. One
should try to describe the degree of increase as accurately as
possible, for instance: from small to moderate; or small increase
in size but not to moderate.

**PROSTATE**

The next stage is the rectal examination, this can be done
either with the patient lying on his back, or standing and
bending forwards. The size and consistency of the prostate should be noted especially whether there are irregularities, hard knots, or other signs of past or present inflammation. This rectal examination and pressure on the prostate gives an embarrassing sensation to the patient and it is recommended to put a generous amount of oil, or preferably some ointment on the glove, a dry glove will make this examination unnecessarily painful. If a prostatitis is suspected, one has to ask the patient whether the sensation is merely disagreeable or really sharply painful.

Normally the seminal vesicles cannot be palpated rectally, except by examiners with unusually long fingers. One should not confuse the side lobes of a butterfly shaped prostate with the seminal vesicles. If one or both of these glands can be felt easily behind the prostate, there is definitely something wrong.

SECONDARY SEX CHARACTERISTICS:

After having completed this examination of the patient’s genital organs, one should give some attention to the secondary sexual characteristics. Of the secondary hair it is noted whether the upper limit of the pubes is horizontal, or of the male type. Is there hair on the breast? Is the beard fully developed, or are there smooth zones on the cheeks? Furthermore, one should never forget to look for the presence of gynecomastia. It may also be of importance to note the voice and the mannerisms of the patient.

INVESTIGATIONS:

First part of investigation included routine investigations done in the following manner

<table>
<thead>
<tr>
<th>Examination</th>
<th>In what Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood HB TLC DLC, ESR</td>
<td>All patients</td>
</tr>
</tbody>
</table>
VDRL, B, sugar  
B. urea  
S. Creatinine  
Radiological  
chest X ray  
X ray skull  
USG Abdomen  

All patients  
As needed  
As needed  
All patients  
As needed  
As needed  

SEmen Examination was generally based on recommendations of the WHO laboratory manual for the Examination of human semen and semen cervical nucleus interaction.

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 continence 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 rigourously 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 form 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 his 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.
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 at usual ejaculatory to low in examination after abstinence might 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 feel you
   (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 drug (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 Cooper and Littre, 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.

**PATHOLOGY :**

**Low Volume**: If the volume of an ejaculate is less than 1.5 ml there are several possible causes.
1. In complete collection of the ejaculate. Repeated examination after re-instruction of the patient is necessary to exclude this factor.

2. Too short a period of continence. After an ejaculation, the seminal vesicles need a time to be fully repleted. In most men the volume of the ejaculate is again at its upper limit after about three to four days of abstinence.

3. The manner of producing the orgasm. The manner of producing the orgasm for the collection of the semen does not have much influence. When masturbation specimens are compared with ejaculates produced by interrupted coitus, the difference is negligible with sporadic exceptions. However, if the ejaculate is produced by a normal coltus, whereafter the wife expresses the semen (reflux method) part of the ejaculate may well be retained in the vagina.

4. Incomplete orgasm. Psychological inhibition may cause a low volume as well as other abnormalities in the semen. One has to ask the patient for this possibility, and repeat the examination. The best way to get information about the orgasm is to ask the patient how many pulsations he notices. In some men the orgasm is of very short duration with only one or two pulsations and this may well be the cause of a low volume. If the patient notices four pulsations or more, and there is nevertheless a low volume a disturbance in the function of the seminal vesicles is more probable.

The disturbance may range from a small ejaculation with only a few droplets of semen plasma, to a slight inhibition of the contractions with much influence on the volume.

5. Abnormal function of the seminal vesicles. This may be caused by a congenital anomaly, possible in different degrees.
(A) High Volume: A volume of more than 6ml usually points to an overdevelopment of the seminal vesicles.

One has to make sure that there is only seminal plasma and no urine in the ejaculate. Some patients have a disturbance in the normal reflexes during orgasm, causing admixture with urine.

Relation to fertility

Low volume: Although usually a volume of less than 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 in the fertilizing capacity because there are more factors involved.

High volume: It is often said that too high a volume (polyspermy) 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 requesterted by diluting the semen. Be this as it may, one should realize that the greatest number of spermatozoon is to be found in the first 1 or 1.5 ml of the ejaculate. This first fraction is requested against the eternal 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.

**PH**

The initial PH depends mainly on the relation between the alkaline secretion of the seminal vesicles and the acid secretion of the prostate. After ejaculation the PH tends to decrease due to the formation of lactic acid from glucose and fructose, especially if the motility is good.

The normal PH ranges from 7.4 to 8.5 if measured within one hour after ejaculation.

**PATHOLOGY :**

(a) **Low pH.** A low pH points to a disturbance in the relation between the secretion of the seminal vesicles and the prostate, usually because of a deficiency of the former. Consequently a low pH is often seen in semen specimen with a low volume. The extreme example is the patient with congenital absence of the Wolffian ducts, a pH below 7.0 can be considered as a confirmation (Kremer, 1967).

(b) **High pH.** A pH above 8.6 is considered as abonomal, although there is no valid reason for this according to some authors.

**RELATION TO FERTILITY**

An abnormally low pH, although in itself 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.

**PATHOLOGY**

Low viscosity. Semen with a low viscosity tends to spread in the vagina. This dilution along the walls may interfere with the formation of a vaginal pool and thus lessen the fertilizing capacity of the semen. This is a theoretical conception that has not been proven.

**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.

**FRUCTOSE AND GLUCOSE**

Fructose: Mann published in 1945 that in animals the reducing substance in the semen, hitherto held to be glucose, was mainly fructose. This was rapidly confirmed for human semen. The fructose in the semen is exclusively produced by the seminal vesicles.

In normal males, there is a wide variation in the fructose content of semen from 6 to 30 mmol/l. The content of fructose is mainly dependent on the production of testosterone. At first a close relationship between the activity of the cells of Leydig and the content of fructose was postulated (Nowakowski) and Schirren, 1956). However, seminal fructose is now considered as an unreliable parameter for androgenicity.

It is not clearly understood why seminal fructose diminishes greatly after sexual continence of more than two weeks (Schirren, 1963).
In semen with a normal number of motile spermatozoa the fructose decreases gradually to about half the initial value after four to five hours. This process of fructolysis is not present in azoospermia nor in necrozoospermia. Some andrologists lay a great value on the estimation of the fructolysis as a parameter for normality of the spermatozoa (Peterson and Freund, 1971).

Glucose - The normal values for glucose lie between 0.01 and 0.2 umol/l.

PATHOLOGY:

Low fructose. Too low a content of fructose in the semen is not at all rare in patients who visit the fertility clinic because of a childless marriage. This is encountered less frequently in men of proven fertility. The cause is always to be found in the seminal vesicles. In most patients the lack of fructose is irreversible and resistant to treatment with androgens. The cause is most often can acquired (post inflammatory) deficient function of the vesicles or sometimes a congenital anomaly.

Low glucose. Glucose is absent in congenital absence of the Wolffian ducts. It is sometimes absent in otherwise normal semen. The background of these cases has not been studied as yet.

High fructose. If a patient has abnormally high content of fructose in the semen, it may be that the prostatic fluid is lacking and that the production of fluid by the seminal vesicles is low.

Glucose. In the laboratory of the Department of Obstetrics and Gynecology of the Vrije University at Amsterdam (Head Dr. H. van Kessel) glucose is not increased in every patient with diabetics. Sometimes a high content of glucose is found (4.0 mmol/L or more) without any explanation.
RELATION TO FERTILITY.

Low fructose. It is often stated that a lack of fructose in the semen is accompanied by low fertility.

High Fructose
Low glucose no data available.
High glucose

PROSTATIC ACID PHOSPHATASE

The content of the seminal plasma should be above 200×10^6 u/l.

PATHOLOGY

In patients with gross abnormalities of the seminal vesicles, as in the congenital absence of the Wollfian ducts, the content of prostatic acid phosphatase lies above one million u/l (Hellinga et al. 1971). In normal patients such high figures are sometimes found with normal function of the seminal vesicles; no case for this anomaly is known.

A low content is often found in combination with low motility and this is presumed to be caused by a diminished function of the prostate.

RELATION TO FERTILITY

There is no sound argument that low content of this compound goes with low fertility.

PROSTAGLANDINS:

Several prostaglandins are present in the seminal plasma. The seminal vesicles are the source of origin (Eliasson, 1969).

Pathology

Already in 1947 it was found, other still by bioassay, that semen from men has a lower prostaglandin concentration than semen of proven fertility. This seems to depend especially on the concentration of prostaglandin E compounds.
Relation to fertility

From the investigations of Bygdeman et al. (1970) the conclusion seems to be warranted that E compounds have some relationship with fertility. The nature of this relationship and whether prostaglandin E is in itself of importance for the reproductive processes, is still uncertain.

THE SPERMATOZOA

SPERM COUNT

Formerly it was presumed that a density of less than 60 millions per ml were too low but now put lat 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 reinstruction of the patient will reveal this possible factor. Further more, too frequent ejaculations and too short a period of continence will result in oligozoospermia in the 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 firotic, caused by some severe illness or inanition in the past. In still other cases, a situation develops where the germinal tissue dissappears 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 spermatogenec tissue, usually leads to oligasthenozoospermia. If there is disorganisation 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 mort than 600 millions. This is caused by overactivity ofr 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 200 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.

**Technique:**

Sperm count was done by diluting the semen to 1:20 with semen diluting fluid in a WBC pipette and counted on Neuber’s hemocytometer after the sperm are allowed to settle and fixed in 10% formalin.

If no spermatozoa are found in motility examinations were made at x 150 and x 600 on 5-10 microscopic fields in each of 2 wet smears. If no sperms are visualized in 50-100 low power fields the semen was centrifuged for 10 minutes and sediment centrifuged and again visualized before declaring the semen azoospermic.

**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 motirity three different factors are distinguished. The qualitative motility gives the percentae 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 distinguishing (1) progressive forward propulsion; (2) weak forward movement; (3) movement on the spot without displacement; (4) Immobility, As a third factor more and more importanceis has ganed to regarding the type of motility. (a) in the normal type of motility the tail beat, generated from the midpiece and also for 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)\(^4\).

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

In many patents the motility in the first part of a split ejaculate is better than in the complete semen (Eliasson, 1972) Technique: Two seperate drops of raw semen are placed on a glass slide and the slide was placed in a incubator at 37 degree for 10 minutes.

This is because at room temperature motility has been shown to be greater than at lower temperature 20\(^\circ\) C. Also examining motility at body temperature is optimal. This has been recommended by various authors (Fruend 1974, Janick and Macleod.
5-10 microscopic fields were examined (at x 150 and x 600 in each of these 2 wet smears percentage motility was rated.

Motility was rated as follows % with

* Linear forward progression
* Slowly progressive
* Non progressive
* Non motile

Motility was rated as % of motile sperms

Pathology:

A low motility of the spermatozoa is called as asthenozoospermia. This may be quantitative, with too low a percentage of motile sperms. Or it may be qualitative, if the degree of motility is insufficient. Abnormal types of motility are named as such stiff tail beat; yawning sperms. Decreased motility may have its cause either in the spermatozoa themselves or in the contents of the seminal plasma theoretically the possibilities are as follows.

1. Asthenozoospermia without any other noticeable abnormality in the seminal pattern, in 9 out of 10 cases is an artifact. This is true as well for qualitative as for quantitative deficiencies. The cause may be a faulty technique: too quick cooling (cold shock), use of an unclean glass jar; moisture; mixture; mixture with acid vaginal secretion; use of a preservative or condom. Repeated examination after reinstatement of the patient is necessary in all cases of asthenozoospermia.

2. If the asthenozoospermia is caused by a deficient function of the seminal vesicles, this may be accompanied by a low volume and a low content of fructose. Also the acid phosphatase may be too high, the pH too acid if the
participation of the prostate fluid dominates over the content of fluid from the vesicles. An increase of epithelial cells in the second part of a split ejaculate is an indication that the seminal vesicles are involvd.

3. If an insufficient function of the prostate is the cause of the asthenozoospermia these may be a high viscosity and sometimes a low content of prostatic acid phosphatase. If the cause is a silent prostatitis leukocytes will be found in the first part of split ejaculate.

4. If none of the above named factors can be found, the fault is most probably in the spermatozoa themselves. Sometimes this can be seen in the coloured slides, when a high percentage of abnormal didpieces is encountered or a great many abnormal tails. Tis can only be established on faltlessly fixed and stain slides.

5. There still remain a number of cases of asthenozoospermia where no cause can be detected along the lines mentioned above. Doepfmer (1969) found that in some patients with unexplained necrozoospermia the testicular spermatozoa, obtained by taking testicular biopsy, did not show any movement if brought in saline or some other nutritive solution. Testicular spermatozoa will usually show some degree of movement in these circumstances. He used this method to distinguish a certain group of patients with "congenital necrozoospermia".

**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 motilitly 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.

**VITALITY**

Living cells cannot be stained by supravital stains. These
substances are not able to penetrate the cell membranes of living
tissue. This is used to find out immobile sperm cells are dead or
still alive. In normal semen samples at least 70 percent of the as
permatozoa do not take the stain. This is expressed as the
semen having a high vitality.

**Pathology:**

In normal circumstances there is a relation between the
percentage of immobile spermatozoa and the percentage of
sperm cells that take the vital stain (Hammen, 1945), found the
percentage of stained sperm cells dependant on the pH, increasing
in acid enviornment with a rearrence of motility after increasing
pH to alkaline. Being motile alive or dead and taking the vital
stain are apparently not identical factors.

**Relation to fertility**

The vitality of spermatozoa in fresh semen may be of
importance with the respect to the prognosis of astheno or
necrozoostermia. With a high percentage of stained and thus
possible dead sperms the chances for a successful treatment are
negligible. unfortunately the opposite is not true.

**Normal Date**

Normal spermatozoa do not clump together. When they
meet and touch each other when moving around, there is no
sign of adhesion.
Pathology:

In certain semen samples, there appears this peculiar phenomenon that is called agglutination. The spermatozoa stick together either head to head or tail to tail, sometimes in a mixed pattern. This is not yet present in the fresh semen; it develops and increases in the course of time, usually within one hour. In some patients practically all the spermatozoa in the semen are agglutinated in the end, although the motility is not lost. In the tail to tail type the heads can be seen at the periphery of the clump, swinging frantically from side to side. In the head to head type, the tails can be seen soving outside of the nucleus formed by the agglutinated heads. In the serum of these patients sperm agglutins are present.

Sometimes the spermatozoa are not only adherent to each other, but also to the round cells in the semen, usually the leucocytes. If in the centre of a clump or agglutinated spermatozoa a leucocyte can be seen, this is called pseudoagglutination. In this case there are no sperm-agglutins in the blood.

In samples with a high viscosity the spermatozoa may end up, after losing their motility, lying aloingside each other in strings or threads throughout the semen. This string - agglutination is the presence of antibodies in the seminal plasma. The titre of the antibodies is higher when tested in the blood, that in the semen.

As with all immunoglobulin antibodies (Ig), in spermagglutinins several types can be distinguished, of which IgM is involved. In tail to tail agglutination usually mainly IgG is present.

In some patients, there is a high titre of spermagglutinins in the blood and only a small degree of agglutination in the semen. It is probable that in those cases the antibody is IgM (Rumke,
1974), IgA spermagglutinin also occurs, formed locally, but rarely in a high titre.

Relation to fertility

Although the agglutination and other autoimmunological factors are recognized to lower the fertility, a follow up study by Rumke et al (1974) has shown that a certain pregnancies still do occur. That this happens more in patients with a normal number of sperm cells than in those with oligozoospermia is understandable. But there is also a significant reverse relation between the titre of the spermagglutinins in the blood serum and the pregnancy rate.

Formation of antibodies as an autoimmunological phenomenon and especially the presence of spermagglutinins in the blood serum is now generally accepted as a cause for subfertility or even fertility. Extensive studies have been made all over the world.

MORPHOLOGY

a. Head forms

In a well stained slide the normal sperm head appears to be oval with smooth contours. In living semen it can be seen that human sperm heads have a large and a small diameter. In coloured slides the heads are lying as a rule with the larger diameter visible and described as so called "front position". A normal head measures 4.5x2.3x1.5 micrometer with a surface size of 8.5 um2. The distal part is more or less clear. This is the cytoplasm, sometimes called acrosome.

The head of the spermatozoa is occupied largely by the nucleus, which is the dense and darkly staining proximal part of the head. This nucleus is filled with closely packed chromatin, consisting of desoxyribonucleoprotein. The finer structures have been revealed with the electron microscope, but for our purpose
it is not necessary to give such an ultrafine description. The proximal part of the head, the borderline with the midpiece, appears as a slightly bent line.

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 midpiece (normal or pear shaped)
4. The contour (smooth or irregular).

Because these factors may be present independently from each other, the number of possible appearances of a sperm head theoretically is $3 \times 3 \times 2 \times 2 = 36$. However, the study from Van Dulijn, Jr. et al. (1972) has shown that the percentage of normal sperms is a more sensitive criterion for the evaluation of an ejaculate than the percentage of abnormal sperm heads. This is also the opinion of many other andrologists [Elisons, 1971; Schirren, 1971].

Therefore it is not necessary to note separately all 35 possible sub-groups of abnormal sperm heads. Only a few special kinds of morphology have to be distinguished because of their clinical significance.

1. **Size**. The size of a normal oval sperm head is about 8.5 um2 less than 7.0 um2 is too small; more than 13.0 um2 is too large. This leaves a fairly large range of normality.

2. **Shape**. In a normal oval sperm head the length this about 4.5 um and the width 2.6 um with a ratio of 1:7 if the ratio is 2:0 or more, the sperm head is too elongated; with a ratio of 1:4 or less, the head is too round. Still these heads, in
common with pear-shaped head forms, are not considered to be pathological, unless the deviation is extreme.

Spermatic stain analysis of human sperm acrosomes

The acrosome reaction of the ejaculated sper cell occurs either spontaneously of at the surface of the zona pellucida after binding to the ZP3 receptor protein. The sperm head enters the oocyte by endocytosis, decondensed, and forms the pronucleus, which shortly thereafter participates in syngametic approximation with the oocyte pronucleus. Failure to fertilize is thought to occur when the sperm lacks acrosomal enzymes (i.e. the round-headed syndrome or prematurely loses acrosomal enzymes because it undergoes a spontaneous acrosome reaction. 

3. Contours. All these heads are only rated as normal if the contours are smooth and regular,

4. Double heads are also observed.

Pathology:

Every semen sample contains a certain percentage of non-oval sperm heads, some abnormal midpieces and abnormal tails, it is impossible to state the exact limit where normality ends and abnormality begins. Not only because there is always a subjective factor involved in the evaluation, but also because it is not known for certain for every type of non-oval head form whether this is to be considered as abnormal.

ABNORMAL FUNCTION OF THE TESTES

1. The normally sized sperm head may be oval, round elongated or pear shaped; these types are all considered to be normal as long as the contours are smooth.

2. Too small sperm heads, if the size is below 7.0 um2. Extreme examples are the pinheads and the microstrongylostrongyluspermatozoa.

3. Too large heads if the size is above 13.0 um².
4. Large, elongated and tapering head form, the ominous tapering heads described in papers of MacLeod.

5. Amorphous heads are those with irregular contours or with too densely staining nuclei; the last named type is sometimes called abnormal acrosome.

6. Double heads with only one tail.

Even in normal semen a few abnormal head forms are always present. However, sometime a special type of abnormal head form is present in great numbers forming a characteristic seminal pattern with more or less pathological significance. **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 headforms or by the accompanying decrease in density and motility.

**Midpiece**

The length of a normal midpiece is about the same as the head, with a diameter of about 0-5 micron. The midpiece should be straight with a central implantation in the sperm head. A small droplet of protoplasm on the midpiece near the sperm head is not considered as abnormal.

**Pathology**

If there is a droplet of protoplasm on the midpiece of considerable size, this is in bulls and other animals a sign of immaturity, possibly caused by a decreased function of the epididymis. It is uncertain whether this also applies to the human.

**Relation to fertility**

Bent midpieces and more especially the crooked ones near the head tamper with the forward propulsion of the spermatozoa they are therefore to all probability to be considered as interfering with fertility.

A great many bent and crooked midpieces cause a qualitative and quantitative decrease in motility and consequently a decrease in fertilizing capacity.

An abnormality that goes with complete sterility is the elongation and thickening of the midpieces as first decreased by Williams (1950).

**Tail**

The tail has about ten time the length of a normal sperm head, between 30 and microns. The tail should be straight or only slightly bent. The sheath, a fibrous structure, and other
particulars are not visible separate in the normal microscope.

**Pathology** Tails that are crooked, grossly bent or broken, are considered as being abnormal. Not rare is the occurrence of coiled tails, either surrounding the head or just behind the sperm head. In some patients the percentage of coiled tails increases with the period of abstinence.

**Relation to fertility** 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 midpieces and short tufted tails.

**Technique**: Rated from examination of 200 spermatozoa. A smear was made on a slide, dried in air and fixed with 10% formaline, for a minute, rinsed in distilled water and stained for 2 minute in hematoxylin.

---

**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 $1 \times 10^9$ /L. For normal semen this means that there are less than 3-5 leucocytes to every 100 spermatozoa.

**Pathology**

If there are more than $1 \times 10^9$/L leucocytes, a split ejaculate
should be examined. In patients with a prostatitis the leucocytes are found in the first part, in vesiculities in the second part and in posterior urethritis in both parts of the split ejaculate.

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

Pathology. More than 5 spermatides per 100 spermatozoa are often encountered in semen with a low sperm count. They appear in abundance in relation with pathological conditions, as severe transitory illness, disappearing spontaneously within two to three months. This phenomenon has been studied by Barton and Wiesner (1950) and more in detail by Macleod (1950) who found a close relation between the appearance of nucleated round cells, the increase of abnormal headforms and the decrease
in motility and density. He called this the "stress pattern" of the
testes.

Too many mononucleated spermatids are sometimes
encountered without gross abnormalities in the semen, but the
presence of multinucleated spermatids is always regarded as a
disturbance of spermatogenesis.

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 11-19u, 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 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.

Pathology: There are two possibilitis for finding prostatic cells.
The first is after treatment with estrogens. This will not occur
often, as the treatment in itself causes ejaculatory impotence.
Second: these cells are found once in a while as an artifact, if
the patient has produced the semen by massage, after he has
been examined by the doctor, this including palpation of the
prostate. They appear as clusters of small cells, with inconspicuous
nuclei. If the semen investigation is repeated with preceding rectal examination the cell will be absent.

**Relation to fertility**: None.

**ERYTHROCYTES (HEMOSPERMIA)**

Normally there are no erythrocytes in the semen.

**Pathology**: A red or brown colour of the semen is suspicious of the presence of erythrocytes or blood. The diagnosis should be established by microscopic examination but also by the reaction with benzidine.

There may be an external source of origin, a fissure in the frenulum or in the prepuce. If reflux semen is delivered or semen produced by coitus interruptus, it may come from the vagina, for instance during menstruation. If these possibilities have been ruled out, it is likely that there is an internal cause. A fractionated ejaculate should now be examined.

If the erythrocytes are present in the first part of the split ejaculate, the chance is great that the blood comes from the prostate and the patient has to be referred to the urologist. The cause may be calculus, prostatitis or a malignant tumour.

Most often the erythrocytes will be found in the last portion. This indicates that the seminal vesicles are the site of origin. In 19 out of 20 cases, this a harmless situation and the anomaly will disappear spontaneously within a few weeks or months. Therefore immediate drastic measures as vesiculography and such are not needed. If the hemospermia persists or recur more than once, the cause must be sought by further, examination by the urologist.

**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.

**Some definitions in summary**

Normality criteria for semen sample:

- **Spermatozoa concentration**
  - > 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.

**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. In diagnostic terms, the only system available for determining weather human spermatozoa can ascend the female recovery. 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. These are complicated tests and will not be discussed here as they fall beyond the scope of the present study.

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

(1) Vas deferens : Enlarged lumen, irregular edged, dilated areas and beaded appearance is usual tubercular obstruction.

(2) Ejaculatory ducts : Unilateral of B/L agenesis can be seen but more commonly provoked by chronic prostatitis that strangles the ejaculatory ducts or provoked by verumontanitis.

(3) Seminal vesicles - Hypoplasia, sclerosis atony (vesiculitis can be seen.

Findings should be correlated with history, seminogram evidence of urinary tract infection or pus cells in semen, Red cells in semen.

(B) Transrectal ultrasonography

Randal et al 1993\(^8\) 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 abcess, usually extratesticular, may be in association. The latter appears as a localised cysitc are of complex echogenicity.\textsuperscript{69}

*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 clarification may also be encountered.\textsuperscript{69}

**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 upto dilutions are done. One tube is set with only normal saline to serve as control. After liquifaction of semen, an equal amount (.5ml) is mixed in each tube. All test tubes are incubated for 1 hour at 37\degree C. The semen is examined on a slide in cicroscope 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>1.0-14.0miu/ 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 analysed. 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 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 oligo-azoospermia 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 recommded 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/trama 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 (>10⁹ /ml) on urine analysis

**Ejaculate signs**
* abnormal apperence 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 contents and elongated spermatids on biopsy⁷⁰.

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 counts and serum FSH and testosterone production and are thus useful for diagnosing Sertoli cell only syndrome, Klinefelter's syndrome⁷¹. 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 Primary spermatocyte of spermatogonia stage</td>
<td>Normal or 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>

### Diagnostic Categories:

Following categories were made -

```
Sexual or ejaculatory dysfunction  →  abnormal  →  sexual dysfunction

Semen Analysis
  └── Normal
     ├── Tests for immunological factor  →  negative
     │    └── No Demonstrable cause
     └── Abnormal
         └── Etiological factor  →  Yes  →  Etiological diagnosis
             └── No identifiable  →  Descriptive diagnosis
```

76
PATIENT WORK UP PLAN
Each Patient was worked up according to the following

<table>
<thead>
<tr>
<th>Sexual and or ejaculatory function</th>
<th>Inadequate</th>
<th>Sexual and/or ejaculatory dysfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adequate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Characteristics of spermatozoa</td>
<td>normal</td>
<td>Seminal Plasma normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No demonstrated cause</td>
</tr>
<tr>
<td></td>
<td>abnormal</td>
<td>Isolate seminal Plasma abnormalities</td>
</tr>
<tr>
<td>Antibodycoated</td>
<td>Immunological Causes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>abnormal</td>
<td></td>
</tr>
<tr>
<td>Causal Factor</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Causal Factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semen Analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iatrogenic Factor</td>
<td>Yes</td>
<td>Iatrogenic Causes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic and/or Environmental factors</td>
<td>Yes</td>
<td>Systemic Causes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital Factor</td>
<td>Yes</td>
<td>Congenital Causes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acquired Testicular Factor</td>
<td>Yes</td>
<td>Acquired Testicular Damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varicocele</td>
<td>Criteria for Infection Yes</td>
<td>Male accessory gland infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone: Low FSH: not Elevated Prolactin Elevated</td>
<td></td>
<td>Endocrine Causes</td>
</tr>
</tbody>
</table>

SEmen Analysis

<table>
<thead>
<tr>
<th>ABNORMAL SEMEN ANALYSIS</th>
<th>Step 1</th>
<th>Etiological and or Descriptive Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>Normal +/or Normal Testicular biopsy</td>
<td>Male genital tract Obstruction</td>
</tr>
<tr>
<td></td>
<td>Raised FSH + Low Testosterone, and/or Corroborative testicular Biopsy</td>
<td>Primary testicular Failure</td>
</tr>
<tr>
<td>No specific diagnosis Made by Step 1 or 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testicular Biopsy</td>
<td>Normal Spermatogenesis (With Normal Semen Analysis)</td>
<td>Primary testicular Failure</td>
</tr>
<tr>
<td></td>
<td>Hypospermatogenesis</td>
<td>Look for cause</td>
</tr>
<tr>
<td></td>
<td>Maturation Arrest</td>
<td>Maturation arrest</td>
</tr>
<tr>
<td></td>
<td>Sertoli cell only</td>
<td>S. cell only syndrome</td>
</tr>
<tr>
<td></td>
<td>Testicular Fibrosis</td>
<td>Look for Mumps Trauma, Infections</td>
</tr>
</tbody>
</table>