SEMENAL FLUID ANALYSIS

Semen (or seminal fluid) is a fluid that is emitted المنبعث from the male genital tract and contains sperms, sugar and protein substances that are capable of fertilizing female ova. Semen analysis, include two major tests:

A- Macroscopic or Physical examination: That includes; ❶ Volume, ❷ Color, ❸ Viscosity (Time to liquefaction), and ❹ pH.

B- Microscopic examination: That includes; ❶ Sperm count, ❷ Motility and Vitality, ❸ Morphology, and ❹ proportion of white cells.

A- Macroscopic or Physical examination:

Physical Examination is carried out after liquefaction of semen that occurs usually within 20-30 minutes of ejaculation.

1) Volume:

Volume of ejaculated semen sample should normally be more than 2 ml. It is measured after the sample has liquefied. Volume less than 2.0 ml is abnormal, and is associated with low sperm count. Excess Volume more than 5 ml. could also mean diluted amount of sperm present in ejaculum.

2) Color:

Normal semen is viscous and **opaque-white** (milky) or **opaque gray-white** in color. After prolonged abstinence (أمتناع), it appears **slightly** yellow. But In case of an inflammatory purulent appears **yellow**. Sometimes appear brown in color in cases of bleeding from a blood capillaries.

3) Viscosity:

Normal semen is thick and viscous immediately following ejaculation, it becomes liquefied within (10 to 30) minutes at (37 ^{O}C) by the action of proteolytic enzymes secreted by prostate and turned into a watery consistency, helps sperm to move. It is considered abnormal if liquefaction does not occur within 60 minutes.

The viscosity of the sample is assessed by filling a pipette with semen and allowing it to flow back into the container. Normal semen will fall drop by drop. If droplets form 'threads' more than 2 cm long, then viscosity is increased.

Increased semen viscosity affects sperm motility and leads to poor invasion of cervical mucus; (It results from infection of seminal vesicles or prostate).

Report is written as follows:

Liquefaction within 30 min. at 37 ^{O}C

Or in case of delayed liquefaction is written:

Viscous after 1 hr. incubation at 37 ^{O}C

4) pH:

A drop of liquefied semen is spread on pH paper (of pH range 6.4-8.0) and pH is recorded after 30 seconds. Normal pH is **(7.2 to 8.0)** after 1 hour of ejaculation.

The portion of semen contributed by seminal vesicles is **Alkaline**, while portion from prostate is **Acidic**. ① Low pH (< 7.0) with absence of sperms (Azoospermia) suggests obstruction of ejaculatory ducts or absence of vas deferens. ② High pH is usually associated with low semen volume ***[as most of the volume is supplied by seminal vesicles (No prostatic fluid)].***

B- Microscopic Examination:

The most important test in semen analysis for infertility is microscopic examination of the semen, which include:

1) Sperm Count:

The sperm count is done after liquefaction and the total number of spermatozoa is reported in **millions/ml** or **(10^6/ml),** and there are two methods for sperm count**:**

First is direct method:

This method is done by taking a drop of semen on clean slid and covered with a cover slide and immediately examined under 40× objective lens and each five sperm per microscopic field represents one million sperm per ml or cm^3.

Second using Haemocytometer:

In this method sperm count is done after liquefaction in a counting chamber **(Haemocytometer)** following dilution with diluting fluid and the total number of spermatozoa is reported in **millions/ml or (10^6/ml).**

Note: Semen specimen is incubated at 37^{O}C after collection to insure liquefaction.

Diluting fluid:

Sodium bicarbonate formalin (1 ml formalin + 5 gm. sodium bicarbonate)

Procedure:

1. Semen is diluted 1:20 with diluting fluid, (Take 1 ml of liquefied semen in a graduated tube and fill with diluting fluid to 20 ml mark. Mix well), or by using WBC diluting pipette just the same way in WBC total count, and a coverslip is placed over the counting chamber.
2. Counting chamber is filled with the well-mixed diluted semen sample using a Pasteur pipette. The chamber is then sit for 10-15 minutes for spermatozoa to settle.
3. The chamber is placed on the microscope stage. Using the **40× objective** and iris diaphragm lowered sufficiently to give sufficient contrast, number of spermatozoa is counted in 4 large corner squares. Spermatozoa whose heads are touching left and upper lines of the square should be counted considered as 'belonging' to that square.
4. Sperm count per ml is calculated as follows:
   Sperm count = Sperms counted × correction factor × 1000 ÷ Number of squares counted × Volume of 1 square
5. Normal sperm count is ≥ 20 million/ml (i.e. ≥ 20×10^6/ml).
6. Sperm count < 20 million/ml may be associated with infertility in males.
   Few millions less than 10 called Oligospermia.
   Zero sperm count (**absence of any sperm in the semen)** called Azoospermia.

Count / ml = N/4 *10 *1000* 20

N : The total count in 4 squares

10 : Volume factor

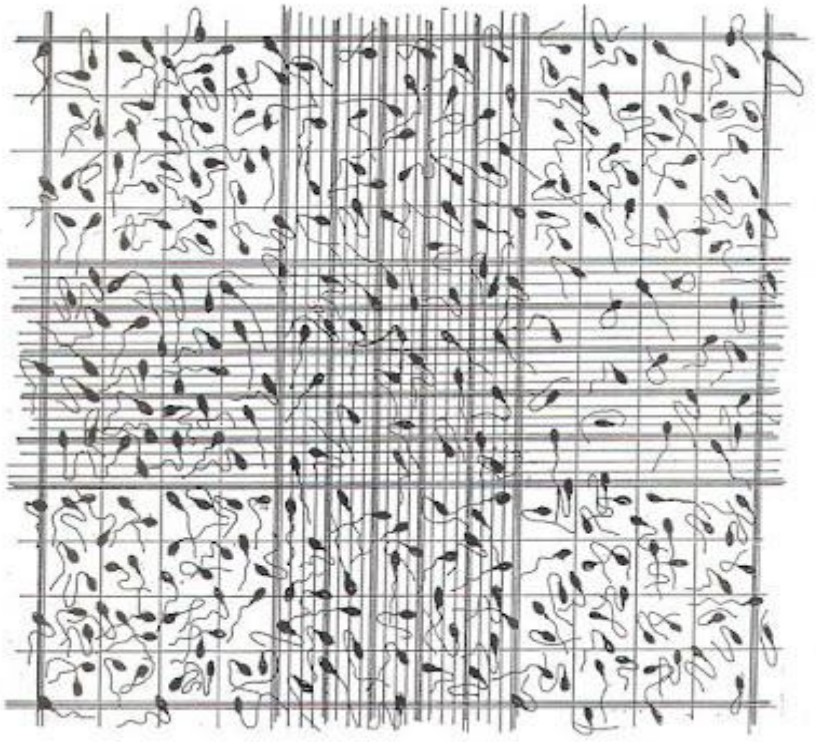
1000 : Generally the sperm count by ml or cm^3 so multiply by 1000 to convert mm^3 to ml or cm3

20 : dilute factor

Count /ml = N * 50,000

Some common abnormal results in semen count:

1. **Azoospermia**: complete absence of sperm from the ejaculate, present in about 1% of all men and 10%–15% of infertile men.
2. **Aspermia**: complete absence of seminal fluid emission upon ejaculation.
3. **Oligospermia**: low sperm count, defined by the World Health Organization (WHO) as concentrations less than 15 million sperm/ml.
   Oligospermia is further classified as:
   (a) Mild: concentrations 10–15 million sperm/ml.
   (b) Moderate: concentrations of 5–10 million sperm/ml.
   (c) Severe: less than 5 million sperm/ml.



Sperm count in haemocytometer

2) Motility Or (Movement):

The first laboratory assessment of sperm function in a wet preparation is sperm motility (ability of the sperms to move). Sperm motility is essential for penetration of cervical mucus, traveling through the fallopian tube, and penetrating the ovum. Only those sperms having rapidly progressive motility are capable of penetrating ovum and fertilizing it.

For a normal result, more than 50 percent of sperm must move normally an hour after ejaculation, the sperm motility divided into:

A) **Active**; Rapidly progressive spermatozoa (moving fast forward in a straight line),

B) **Sluggish**; Slowly progressive spermatozoa (slow linear or non-linear, i.e. crooked or curved movement),

C) Non-progressive spermatozoa (movement of tails, but with no forward progress),

D) **Immotile** spermatozoa (no movement at all). (WHO criteria).

Sperms of grades (C) and (D) are considered to be poorly motile **(Asthenospermia).**

Procedure:

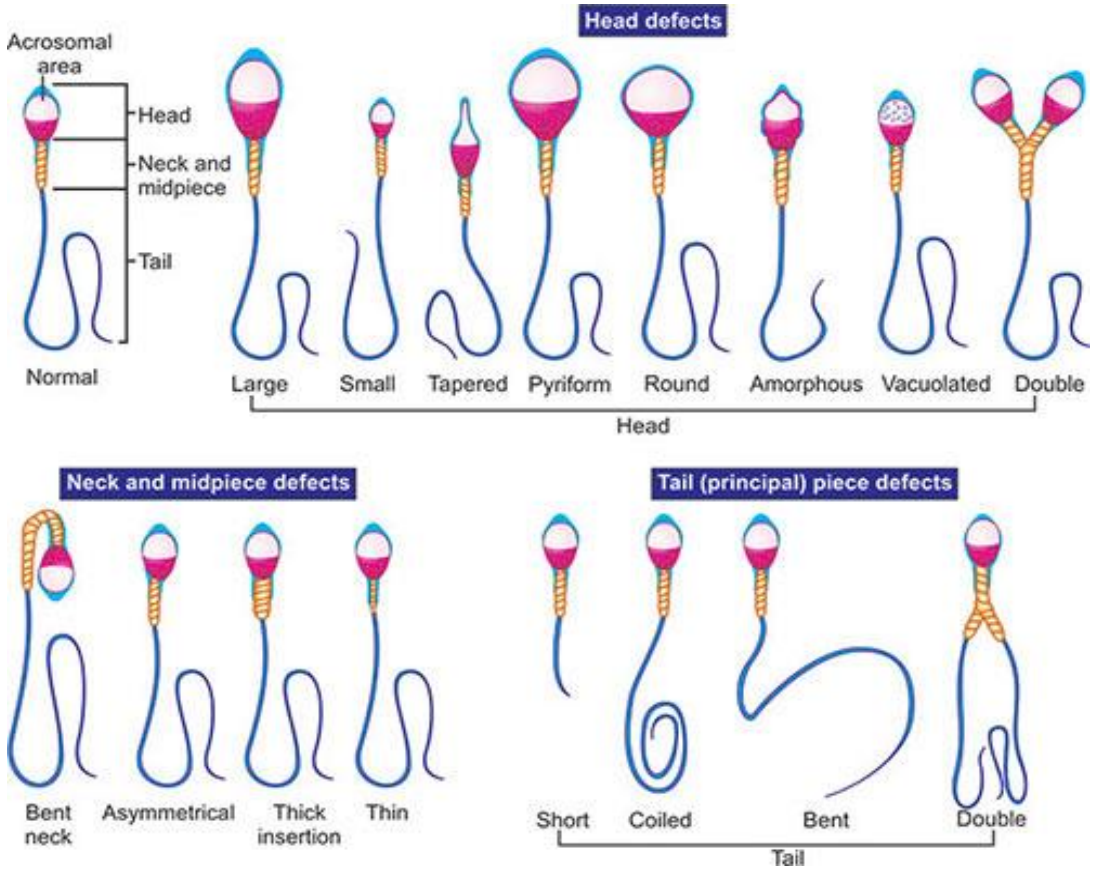
A drop of semen is placed on a glass slide, covered with a coverslip that is then ringed with petroleum jelly to prevent dehydration, and examined under **40×** objective. At least 200 spermatozoa are counted in several different microscopic fields. Result is expressed as a percentage **(%).**

Normal percentage is **≥ 50%** of sperms show rapid progressive and slow progressive motility.

3) Sperm Morphology:

A spermatozoa consists of three main components: (Head, Neck, and Tail). Tail is further subdivided into midpiece (principle) piece, and end piece. The defects in morphology that are associated with infertility in males include ① Defective mid-piece (causes reduced motility), ② Incomplete or absent acrosome (causes inability to penetrate the ovum), and ③ Giant head (defective DNA condensation).

Normal results; more than 50% of spermatozoa should be normal morphology **(WHO, 1999).**



Procedure:

A smear is prepared by spreading a drop of seminal fluid on a glass slide, stained, and percentages of normal and abnormal forms of spermatozoa are counted. The staining techniques used are hematoxylin-eosin, and Rose Bengal-toluidine blue stain. At least 200 spermatozoa should be counted under oil immersion. Percentages of normal and abnormal spermatozoa should be recorded.

Teratozoospermia, also known as **Teratospermia** (وجود نطف مشوهة الشكل بنسبة عالية), is a semen alteration in which there is a large number of spermatozoa with abnormal morphology. Or (It is a condition characterized by the presence of sperm with abnormal morphology that affects fertility in males).

4) Proportion of white cells:

In microscopic examination also investigating the presence of white blood cells (Pus cells) in cases of; Gonorrheal infection, inflammation of the prostate, and sometimes a parasite like ***Trichomonas vaginals*** also found which is transmitted through sexual intercourse (S.T.D; Sexually transmitted disease).

Round cells on microscopic examination may be **white blood cells** or **immature sperm cells**.

It is very important to differentiate between them by using a special stain (peroxidase or Papanicolaou) is required to differentiate between them.

- Presence of large number of **immature sperm cells** indicates spermatogenesis dysfunction at the testicular level.
- Presence of White blood cells more than (1 million/ml) indicate presence of infection, and semen culture & sensitivity is required.

Culture of seminal fluid:

Like any other body secretions semen specimens are cultured on the following media;

1- Blood Agar
2- Chocolate agar
3- MacConkey agar

Culture is done at first and other tests handled after to avoid contamination by streaking a loop on plates.

Blood Agar and Chocolate agar are incubated anaerobically in candle jar provided with **CO2** ratio of **(5-10 %)** for anaerobic bacteria, and MacConkey agar is incubated aerobically for aerobic bacteria.

The most important types of bacteria that are found in seminal fluid are:

- Neisseria gonorrhoeae (Intracellular Gram neg. diplococci; Shown in figure below).
- Staphylococcus aurous
- Mycoplasma
- Coliforms

Antibiotic sensitivity test is performed after culture on identified bacterial growth to determine sensitive and resistant antimicrobial drugs.