

BODY FLUID:-

SEMEN

- ❖ Semen is a thick, yellowish white, glairy, opalescent, secretion having a characteristic odour known as seminal odour. It is also called seminal fluid, fluid that is ejaculated from the male reproductive tract and that contains sperm cells, which are capable of fertilizing the female eggs.
- ❖ Semen also contains other liquids, known as seminal plasma, which help to keep the sperm cells viable.
- ❖ The total volume of semen for each ejaculation of a human male averages between 2 and 5 ml. Each ejaculation contains normally 200 to 300 million sperm.
- ❖ The pH of semen(also referred to as seminal fluid) is slightly alkaline, ranging between pH 7.2 and 7.4.

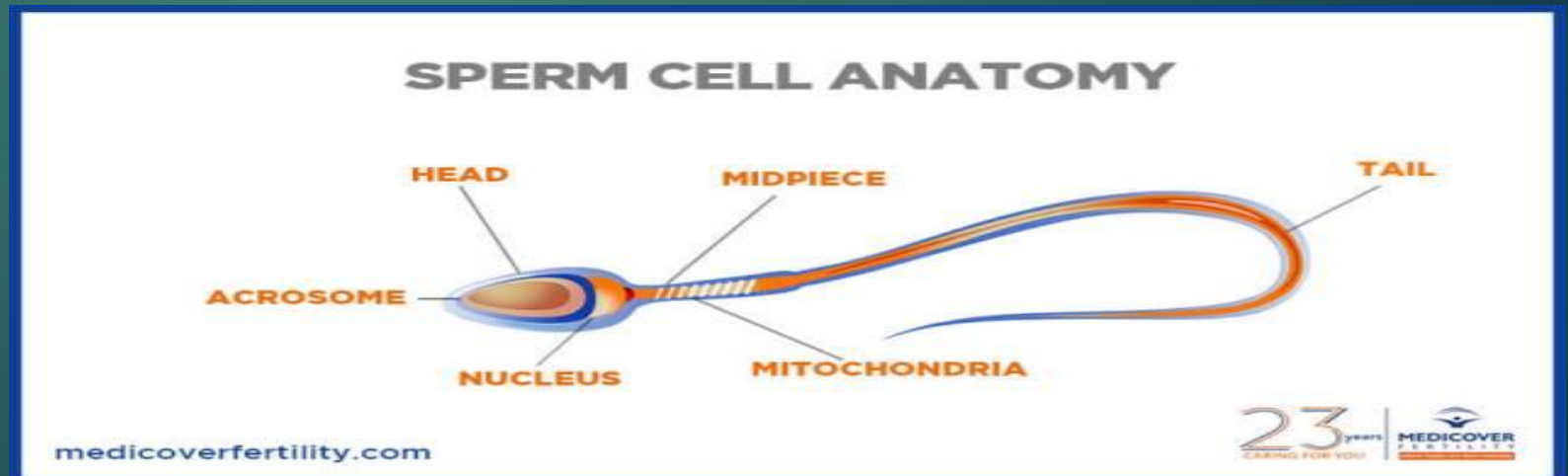
COMPOSITION OF SEMEN

- ❖ Semen is made up of several substances, including sperm cells, enzymes, carbohydrates, minerals, organic compounds, and vitamins.
- ❖ The seminal fluid is produced by four glands: the testes, seminal vesicles, the prostate, and the bulbourethral gland .
- ❖ The **seminal vesicles** produce a yellowish viscous fluid rich in fructose and other substances that makes up about 70% of human semen.
- ❖ The **prostatic secretion**, influenced by dihydrotestosterone, is a whitish (sometimes clear), thin fluid containing proteolytic enzymes, citric acid, acid phosphatase and lipids.
- ❖ The **bulbourethral glands** secrete a clear secretion into the lumen of the urethra to lubricate it.

The three important components of Semen are produced by two organs.

1. **Testes** produce spermatozoa that can be considered as confirmatory evidence for the presence of semen.

2. **Prostate**, on the contrary, produces two very important proteins, namely, Acid phosphatase enzyme and prostate specific antigen.



COLLECTION & PRESERVATION OF SEMEN

- ❖ Collection of semen stain has much precaution. Semen converts in to brittle after drying. So if cloths or other articles are not handled properly or folded it then spermatozoa are breakdown in to pieces.
- ❖ Preservation of semen stain always performed after complete dryness of stain. If stain is wet then bacterial growth is started and putrefaction may occur due to presence of protein in semen.
- ❖ Any infection in testis due to presence of some bacteria also secretes liquid like seminal stain from testis which creates doubt about semen.
- ❖ It is always preserved in air bag not in airtight bag or plastic bag.
- ❖ If stain found on immovable or small article, then preserved whole article or cloth and sent sample to FSL.
- ❖ If stain present on immovable article then sharp knife or scalpel is used to scratch the stain and packed in clean glass bottle.
- ❖ Seminal stain may be present on pubic hair so it is also preserved in bag or clean plastic tube.

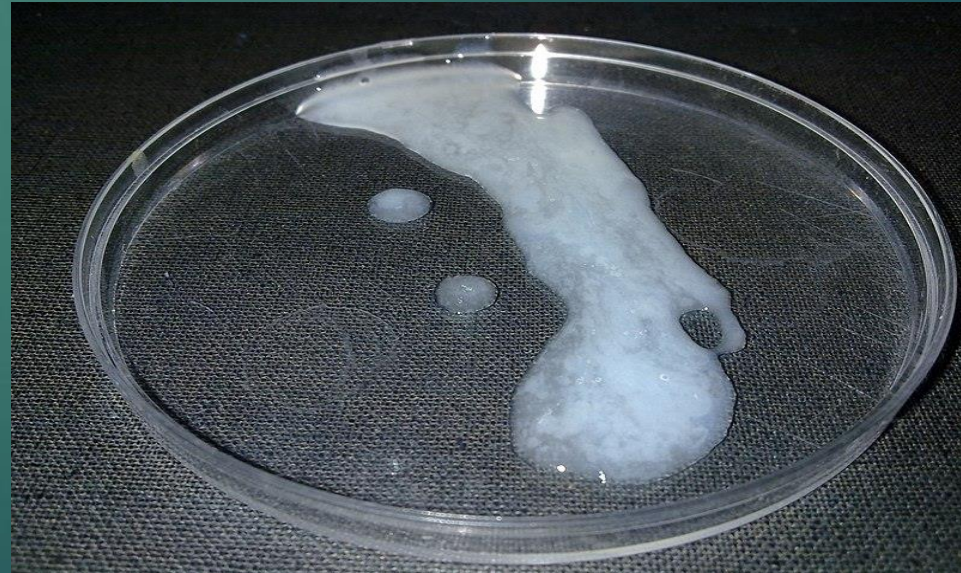
- ❖ If seminal stain present on any body part then take cotton swab is taken, dipped in saline water. Then cotton applies on body and prepare cotton swab. After that drying the cotton swab and preserved in bag.
- ❖ Never touch sample with bare hand because it can contaminate and spoil the specimen.
- ❖ In fresh seminal stain, live spermatozoa are present so keep safe the cloth and send properly to FSL.

LOCATION

- ❖ Stains will usually be found on the clothing of the victim or the perpetrator , particularly the undergarments. stains may also be found on bedding, mattresses, car seats etc.
- ❖ Clothing and articles suspected to contain seminal stains should be seized quickly as possible because an attempt may be made to remove the traces by washing.
- ❖ Often, seminal stains are readily visible on a fabric because they exhibit a stiff, crusty appearance. Fresh semen has a characteristic odour.
- ❖ The female victim of an assault may have a considerable quantity of semen in or around the vagina. In such cases vaginal swabs should be obtained through the medical examiner.
- ❖ Controlled samples should be collected from both the suspect and the victim.

SEMEN AS A FORENSIC EVIDENCE

- ❖ As with other forensic exhibits, the investigation of semen is also carried out in a specific way using various tests.
- ❖ First, the screening tests are performed in order to identify whether the questioned stain is semen or not. Once the preliminary screening tests are positive, a more detailed confirmatory analysis is carried out to establish that the stain is indeed semen.
- ❖ Further exhaustive analysis of seminal stains is performed in order to individualize the stain to a particular individual.



FORENSIC EXAMINATION OF SEMEN

VISUAL AND PHYSICAL EXAMINATION

NAKED EYE EXAMINATION

- ❖ A dry seminal stain is grayish-white in color with irregular map like appearance.
- ❖ The stain area appear stiffened.
- ❖ Seminal stains may mistaken for starch stains.

ULTRAVIOLET LIGHT EXAMINATION

- ❖ Seminal stains fluorescence bluish-white in color. Sometimes can show false positive results because some other stains can also gives fluorescence like saliva, milk etc.
- ❖ Invisible or old stains can be visible under UV light.



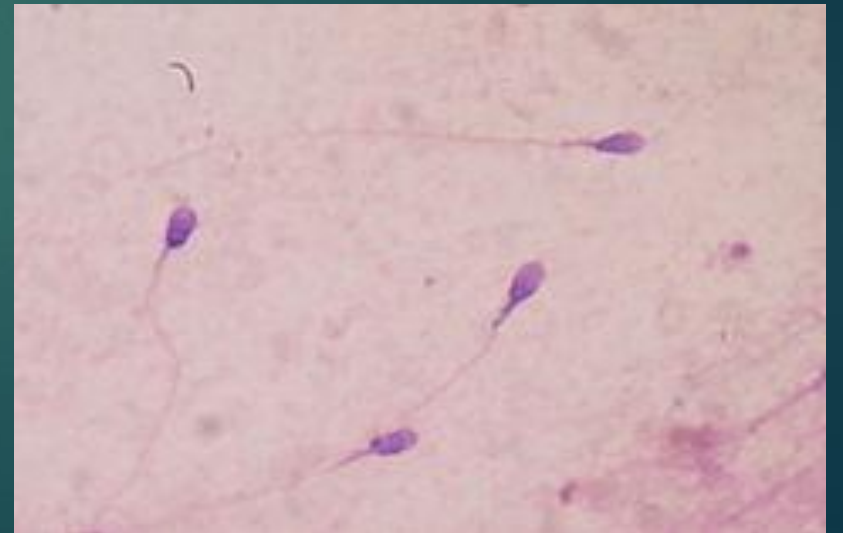
MICROSCOPIC EXAMINATION

Semen can be conclusively identified by the presence of spermatozoa in the stain. When stain is subjected to a microscopic examination, spermatozoa can be identified as having been derived from semen. Spermatozoa contain gram positive protein, the presence of spermatozoa will be confirmed by microscopic appearance of purple bodies.

Microscopic detection of spermatozoa is also considered as confirmatory evidence for presence of semen in suspected stain.

PROCEDURE :

- Stain should be washed in acidulated water in a test tube.
- The test tube should be subjected to ultrasonic oscillation for the separation of spermatozoa from dried stain.
- Then , it should be fixed with dil. sulphuric acid.
- Appearance of *purple bodies* under the microscope can be observed.



PRESUMPTIVE TEST OF SEMEN

1. ACID PHOSPHATE TEST

Most commonly used presumptive to test for detecting semen is called acid phosphatase (ap) test.

- ❖ To perform the test, a drop of the reagent sodium alpha-naphthylphosphate is added to presumptive stain followed by drop of fast blue b. a positive result off this test is a colour **change to dark purple**.
- ❖ But some fruit juices, contraceptive creams and vaginal secretions will also produce a purple colour with this test. However, we can be clear by colour change time. with these substance the colour changes much slower compared to semen. semen will produce the colour change very rapidly, fresh stains may take up to 10 seconds, older stains may take up to 30 seconds, and always less than one minute.
- ❖ NOT A CONFIRMATORY TEST FOR SEMEN

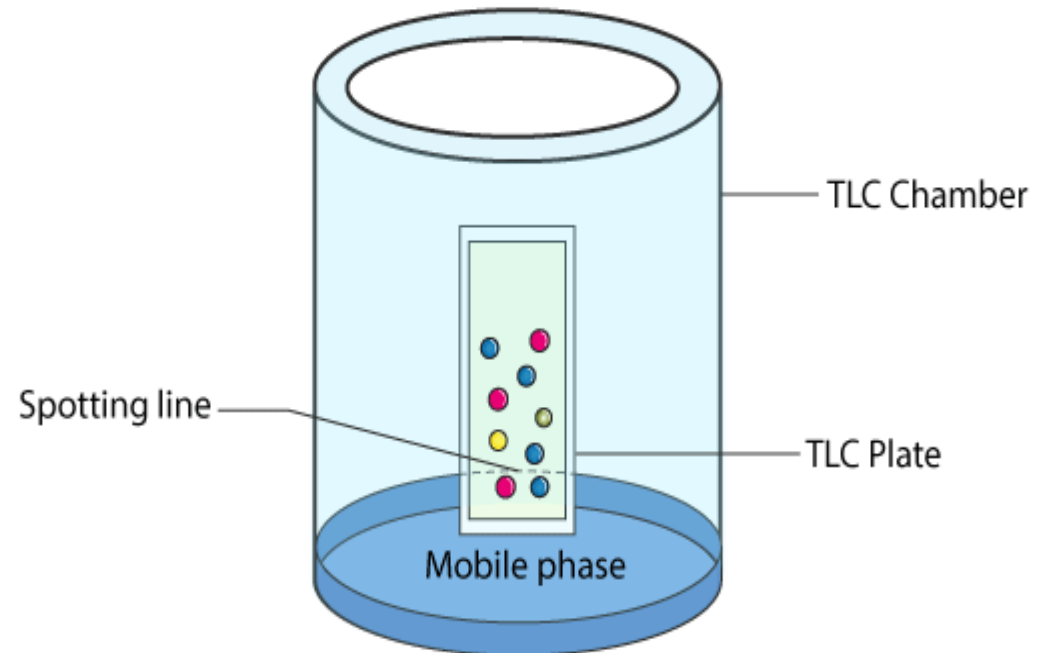


2. THIN LAYER CHROMATOGRAPHY (TLC)

- ❖ This method is use to detect of choline and spermine;
- ❖ In TLC, it is possible to detect choline and spermine simultaneously in seminal stain.
- ❖ It is possible to detect choline and spermine in old seminal stain even after lapse of 5 years by this method.

Two types of reagent use in TLC are
Dragendroff's reagent
Potassium iodoplatinato Reagent

THIN LAYER CHROMATOGRAPHY



CONFIRMATORY TESTS

1. CROSS OVER ELECTROPHORESIS

- ❖ Seminal material can be identified by demonstrating the presence of p30, a semen specific protein.
- ❖ One method of doing this is cross over electrophoresis
- ❖ An extract of suspected stain is placed on the cathod well of gel plate and anti p30 is placed in anode well.
- ❖ Electrophoresis is carried out of 20 min at 200V, forcing two components together.
- ❖ When the antigen p30 meets the antibody, a precipitin band is formed.
- ❖ The presence of precipitin band within an extract of unknown stain proves that the stain contain seminal material, anti-p30

CRYSTAL TESTS FOR SEMEN

2. FLORENCE TEST :

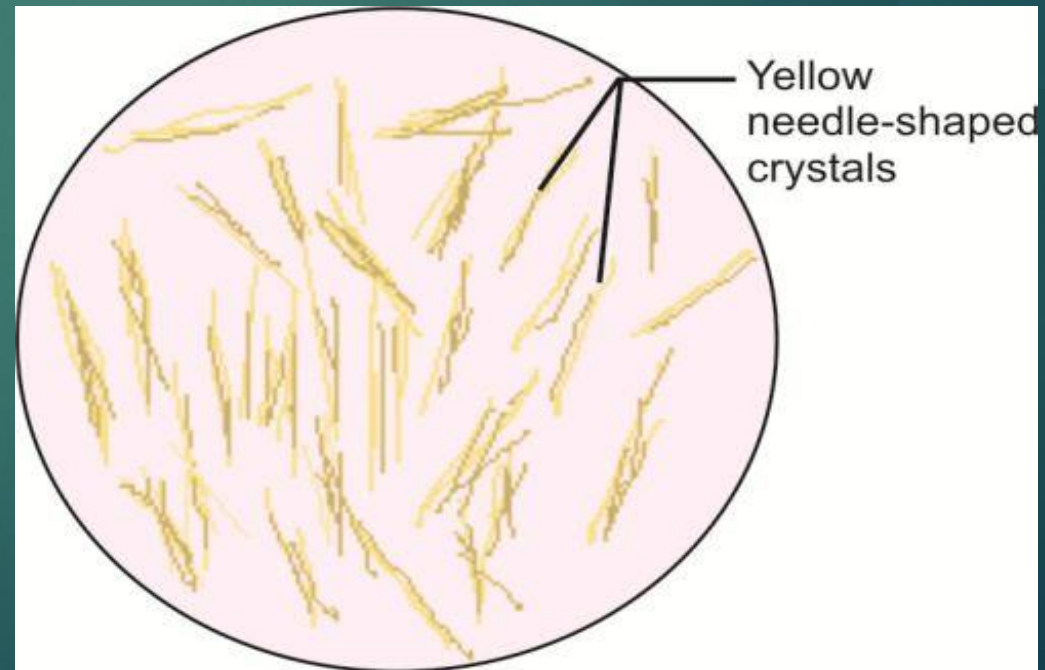
- This test was discovered by Dr.Florence in the year 1886.
- When Florence reagent (PotassiumIodide+Iodine+Water) is applied to the slide it produces *rhomboidal shape dark crystals* of choline periodide.
- Similarly, any tissue or biological material containing sufficient high choline concentration would give positive Florence Test.



3. BARBERIO'S TEST

Test was invented by Barberio in 1905

Questioned stain+ Picric acid → yellow needle shape spermine picrate crystals



FORENSIC IMPORTANCE OF SEMEN AS AN EVIDENCE

- 1-Semen analysis is an important laboratory test and should be thought of in the same way as any other diagnostic assay is used in determining treatment plans for infertility.
- 2-Semen analysis is very important in assault cases as it is very useful for detection of semen in cloths.
- 3-Isolation and identification of seminal stain found on various suspected area such as cloths, bed sheet, pillow, blanket, etc.
- 4-Analysis of various protein present in semen or seminal stain.
- 5-Seminal stains are examined generally in rape cases, Sodomy, Bestiality, Buccal coitus and in cases of Sexual perversions.
- 6-Detection of sperm cell in Vagina and other body parts.
- 7-It is useful because of the relative quantity of spermatozoa and epithelial cells can be assessed. This determination becomes important during subsequent DNA analysis because spermatozoa contain male DNA while most epithelial cells in a male–female sexual assault will contain female DNA from the complainant.