Seminal Fluid Analysis

Semen, also known as seminal fluid, is an organic fluid that contains spermatozoa (sperms the reproductive cells). It is secreted by the sexual glands and other sexual organs of male and can fertilize female ova.

In humans, seminal fluid contains several components besides spermatozoa: proteolytic and other enzymes as well as fructose are elements of seminal fluid to help in the survival of spermatozoa, and provide a medium through where they can move or swim. Basic amines such as spermine and spermidine are responsible for the smell of semen. These alkaline bases buffer the acidic environment of the vaginal canal, and protect DNA inside the sperm from acidic denaturation.

Also a complex homones mixture including several mood-altering hormones (e,g, testosterone, oestrogen, follicle-stimulating hormone, luteinizing hormone, prolactin and several different prostaglandins) can be found in semen.

There are only two tests ordered for semen analysis:

1. **Semen analysis:**

This test helps in diagnosis of male infertility and other diseses, before semen analysis, ejaculation should be avoided for 2–3 days. The semen sample can be collected in a sterile glass container; sample must be analyzed within 1–2 hours. It is recommended that the results of the tests do not vary by more than 20% so some laboratories may need to repeat the tests for more accuracy. Storage and temperature can effect on results.

Semen analysis includes:

- **Appearance and quality of human Semen**

Normally, seminal fluid is clear to milky white in color, thick and sticky (viscous) in consistency, has a pH (acidity) level between 7.8 and 8.0, and contains few or no white blood cells (leukocytes). Blood in the semen sample can cause a pink or reddish color, called hematospermia, and may indicate a medical problem. The volume of semen is different and around 3.4 ml.

- **Sperm count**

This test involves microscopic examination of semen by application of one drop of semen on a clean slide covered with cover slip then examined under 10X and 40X, count 10 to 20 fields, total sperms number obtained by measuring the total volume of semen, multiplying the volume in ml by the number of sperm (in million per ml) to get the total number of sperm. When using the 40X objective, if there are a lot you can count them in a quarter of a field of view and multiply by four. Also the following tests must be done:

a) Morphology (sperm shape and structure; associated with sperm health).
b) Motility (percentage % of sperms that show movement).
c) Mobility (Total motile count or total number of moving sperm; motility describes how much the sperm are moving.

Sperm motility classified into four classes which are:

☒ Rapidly progressive– swimming rapidly, generally maintaining a consistent direction
☒ Slowly progressive– moving slowly with some forward progression
☒ Non-progressive– thrashing about but not going anywhere or going in circles
☒ Immotile– not moving.

Evaluating morphology is easier using stained seminal slides. There are several defects including head, neck, mid-piece, and tail defects. Head defects include large, small, round, double heads, Pin head or micro head sperm should not be counted. Neck defects include bent tails, thick or thin mid-piece. Tail defects include short, double, broken and coiled tails.

The World Health Organization **(WHO)** has the following values for normal semen analysis:

Total volume—greater than 2 ml

Concentration—at least 20 million sperm per Morphology—at least 15% normal sperm

Motility—greater than 50% sperm with forward movement, or 25% with rapid movement within 1 hour of ejaculation

White blood cells—fewer than 1 million per ml

Further analysis (sperm mixed antiglobulin reaction **MAR** test) shows adherent particles in less than 10% of sperm.

2. Seminal fluid culture:

Seminal fluid culture usually starts with stained sperm slide to identify bacterial infection by gram stain; then routine methods can be used using the seminal sample collected in sterile conditions. This test mostly ordered during acute and recurrent genital tract bacterial infections for males.

Swab and wound culture

Before taking any swab sample; place should be disinfected by iodine, alcohol or any other disinfectant. Swabs should be cultured as soon as possible (maximum one hr. or should be kept in refrigerator) to keep bacterial isolates alive even if they are fastidious.

Most common media used in the routine laboratories work are blood agar and macConkyṡ agar, while clinical laboratories use nutrient agar for antibiotic sensitivity test using antibiotic disks arranged as a circle away from edge and each other with one centimeter. Blood agar is used as enrichment media for pathogenic bacteria while macConkyṡ agar is used for gram negative bacteria isolation usually enterobacteriacae family.

Allergy skin Tests

Allergy testing involves a skin test to find out what allergen (Antigen that induces allergy or hypersensitivity), this allergen or antigen can trigger (starts) the allergic response in a person. Skin tests are usually done because they are rapid, and generally less expensive than blood tests. Skin test is done by placing a drop of a solution containing allergen on the skin, and many needles pricks allow the solution to enter the skin. If a red, itchy area form (called a wheal), it means that the person is allergic to that allergen. This is called a positive reaction.

Laboratory tests for fungal infection

For diagnosis of a fungal infection, skin, hair and nail tissue is collected for microscopy and culture (mycology).

Note: Exposing the site to long wavelength ultraviolet radiation (Wood lamp) can help identify some fungal infections of hair (*Tinea capitis*) because the infected hair fluoresces green.

Types of specimen collection for fungal testing:

1. Scrapings of scale, best taken from the leading edge of the rash after cleaning skin with alcohol.
2. Skin stripped off with adhesive tape, which is then stuck on a glass slide.
3. Hair which has been pulled out from the roots.
4. Brushings from an area of scaly scalp.
5. Nail clippings, or skin scraped from under a nail.
6. Skin biopsy.
7. Moist swab from a mucosal surface (inside the mouth or vagina) in a special transport medium.
8. A swab should be taken from pustules in case of secondary bacterial infection.They are transported in a sterile container or a black paper envelope.

Direct microscopy of skin scrapings and nail clippings

Potassium hydroxide (KOH) stain is a commonly-used method because it is inexpensive and easy to perform. Nail clippings or scrapings are placed in a drop of KOH and examined under a microscope for the presence of fungal elements. Periodic Acid-Schiff (PAS) staining can also be used for Histopathology of biopsy

Microscopy can identify a dermatophyte by the presence of:

1. Fungal hyphae (branched filaments or mycelium).
2. Arthrospores (spores)
3. Arthroconidia (specialised external spores)
4. Spores inside a hair (endothrix) or outside a hair (ectothrix).

Fungi are sometimes difficult to find, especially if the tissue is very inflamed, so a negative result does not mean no fungal infection.

Infections with yeasts can be identified by the presence of:

1. Yeast cells, which dividing by budding.
2. Pseudohyphae (branched filaments similar to those of a dermatophyte) forming a pseudomycelium.

Culture of fungi:

Growing the fungus in culture may take several weeks, incubated at 25-30ºC. The specimen is inoculated into a medium such as Sabouraud's dextrose agar containing cycloheximide and chloramphenicol. The cycloheximide is left out if a mould requires identification. Cases that need culture include cryptococcosis, aspergillus, candidosis, and histoplasmosis. Negative culture may be due to treatment before samples collection, a delay before the specimen reached the laboratory and the organism may grow very slowly.