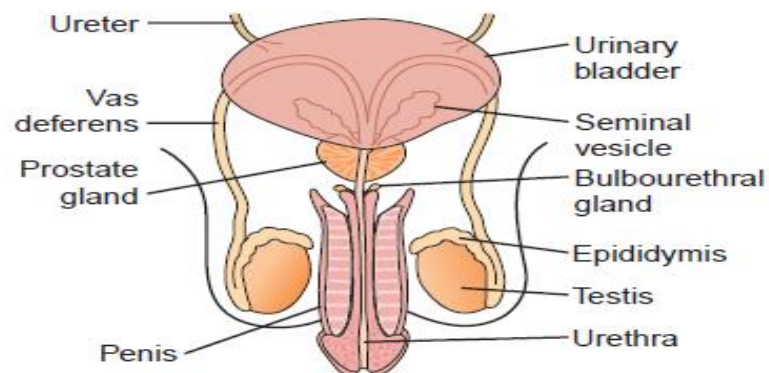
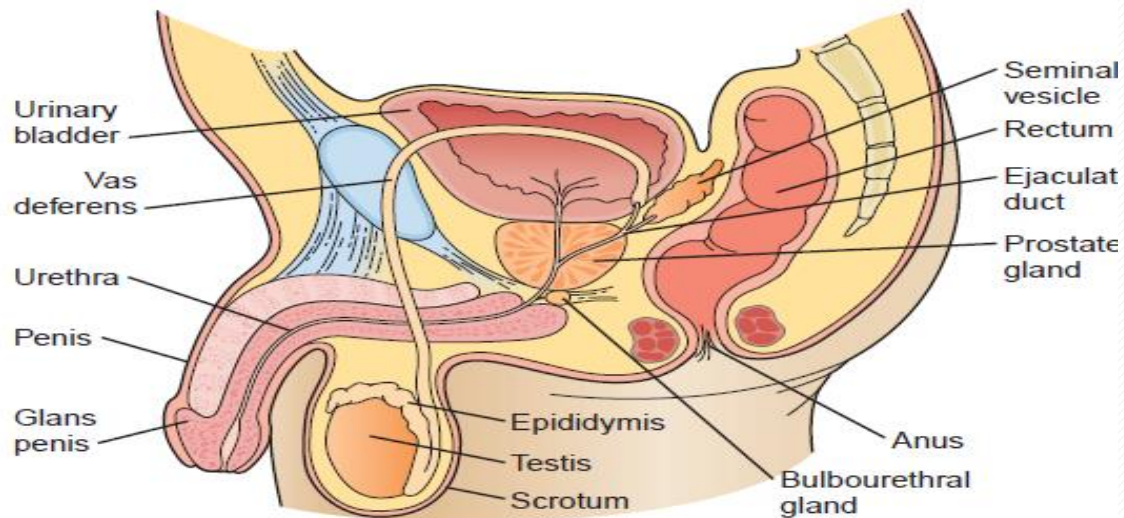


Semen

Reference Books:

- **Urinalysis and body fluids** (Susan King Strasinger- Marjorie Schaub De Lorenzo) Fifth edition

Diagram of the male genitalia



Summary of Semen Production

Seminiferous tubules of testes	Spermatogenesis
Epididymis	Sperm maturation
Ductus deferens	Propel sperm to ejaculatory ducts
Seminal vesicles	Provide nutrients for sperm and fluid
Prostate gland	Provide enzymes and proteins for coagulation and liquefaction
Bulbourethral glands	Add alkaline mucus to neutralize prostatic acid and vaginal acidity

Composition of Semen

- Semen is composed of four fractions that are contributed by the **testes, epididymis, seminal vessels, prostate, and bulbourethral glands**. Each fraction differs in its composition, and the mixing of all four fractions during ejaculation is essential for the production of a normal semen specimen.

Spermatozoa	5%
Seminal fluid	60%–70%
Prostate fluid	20%–30%
Bulbourethral glands	5%

Specimen Collection

The variety in the composition of the semen fractions makes proper collection of a complete specimen essential for accurate evaluation of male fertility. The majority of sperm are contained in the first portion of the ejaculate. Patients should receive detailed instructions for specimen collection.

Specimens are collected following a period of sexual abstinence of from 2 to 3 days to not longer than 5 days. Specimens collected following prolonged abstinence tend to have higher volumes and decreased motility.

- The laboratory should provide warm sterile glass or plastic containers.
- The specimen should be kept at room temperature and delivered to the laboratory within 1 hour of collection.
- Laboratory personnel must record the time of specimen collection and specimen receipt.
- Specimens awaiting analysis should be kept at 37°C.
- Specimens should be collected by masturbation.

Semen Analysis:

The semen analysis for fertility evaluation consists of both macroscopic and microscopic examination. Parameters reported include appearance, volume, viscosity, pH, sperm concentration and count, motility, and morphology.

Appearance:

- Normal semen has a gray-white color, appears translucent, and has a characteristic musty odor. Increased white turbidity indicates the presence of white blood cells (WBCs) and infection within the reproductive tract.
- Varying amounts of red coloration are associated with the presence of red blood cells (RBCs) and are abnormal.
- Yellow coloration may be caused by urine contamination, specimen collection following prolonged abstinence, and medications.

Liquefaction

- A fresh semen specimen is clotted and should liquefy within 30 to 60 minutes after collection: therefore, recording the time of collection is essential for evaluation of semen liquefaction.
- Analysis of the specimen cannot begin until after liquefaction has occurred.
- Failure of liquefaction to occur may be caused by a deficiency in prostatic enzymes

Volume

- Normal semen volume ranges between 2 and 5 ml. It can be measured by pouring the specimen into a clean graduated cylinder. Increased volume may be seen following periods of extended abstinence.
- Decreased volume is more frequently associated with infertility and may indicate improper functioning of one of the semen-producing organs, primarily the seminal vesicles.

Viscosity

Specimen viscosity refers to the consistency of the fluid and may be related to specimen liquefaction. Incompletely liquefied specimens are clumped and highly viscous. The normal semen specimen should be easily drawn into a pipette and form droplets that do not appear clumped or stringy when discharged from the pipette. Normal droplets form a thin thread when released from the pipette. Droplets with threads longer than 2 centimeters are considered highly viscous. Ratings of 0 (watery) to 4 (gel-like) can be assigned to the viscosity report.

Viscosity can also be reported as low, normal, and high. Increased viscosity and incomplete liquefaction impede sperm motility.

pH

The normal pH of semen is alkaline with a range of 7.2 to 8.0. Increased pH is indicative of infection within the reproductive tract. A decreased pH is associated with increased prostatic fluid. Semen for pH testing can be applied to the pH pad of a urinalysis reagent strip and the color compared with the manufacturer's chart.

Sperm Concentration/Count

The actual number of sperm present in a semen specimen is a valid measurement of fertility. Normal values for sperm concentration are commonly listed as greater than 20 million sperm per milliliter, with concentrations between 10 and 20 million per milliliter considered borderline. The total sperm count for the ejaculate can be calculated by multiplying the sperm concentration by the specimen volume. Total sperm counts greater than 40 million per ejaculate are considered normal (20 million per milliliter 2 ml). In the clinical laboratory, sperm concentration is usually performed using the Neubauer counting chamber.

- Normal count (**Normozoospermia**).
- No sperm at all in semen (**azoospermia**).
- Very low sperm count, less than 10 million sperm per ml.
- low sperm count, less than 20 million sperm per ml
(**oligozoospermia**).
- NO ejaculate (**Aspermia**).

Sperm Motility

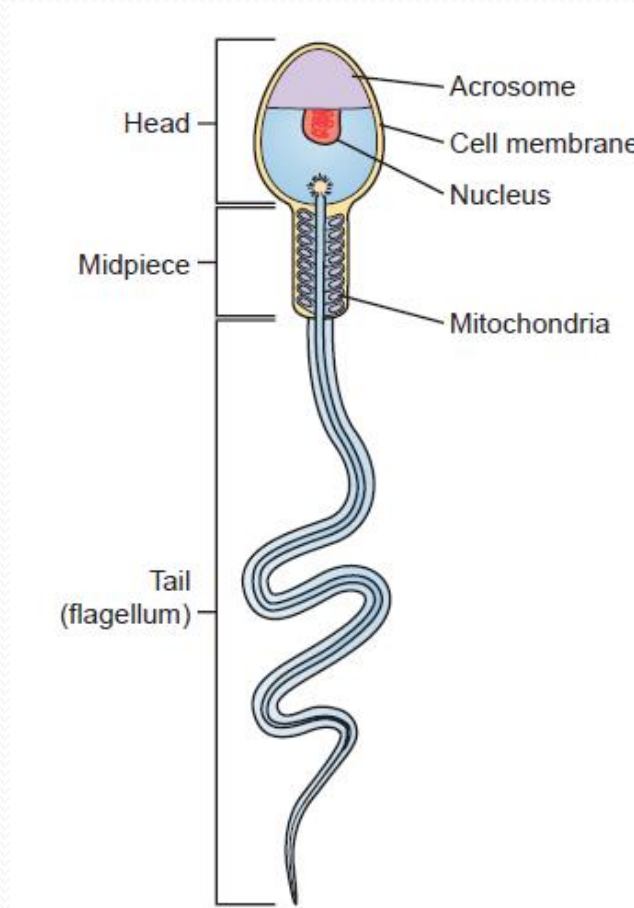
- The presence of sperm capable of forward, progressive movement is critical for fertility. clinical laboratory reporting of sperm motility (determining the percentage of motile sperm and the quality of the motility).
- Assessment of sperm motility should be performed on well mixed, liquefied semen within 1 hour of specimen collection.
- The WHO uses a rating scale of a, b, c, d (see next table). Interpretation states that within 1 hour, 50% or more sperm should be motile in categories a, b, and c, or 25% or more should show progressive motility (a and b).

Sperm Motility Grading

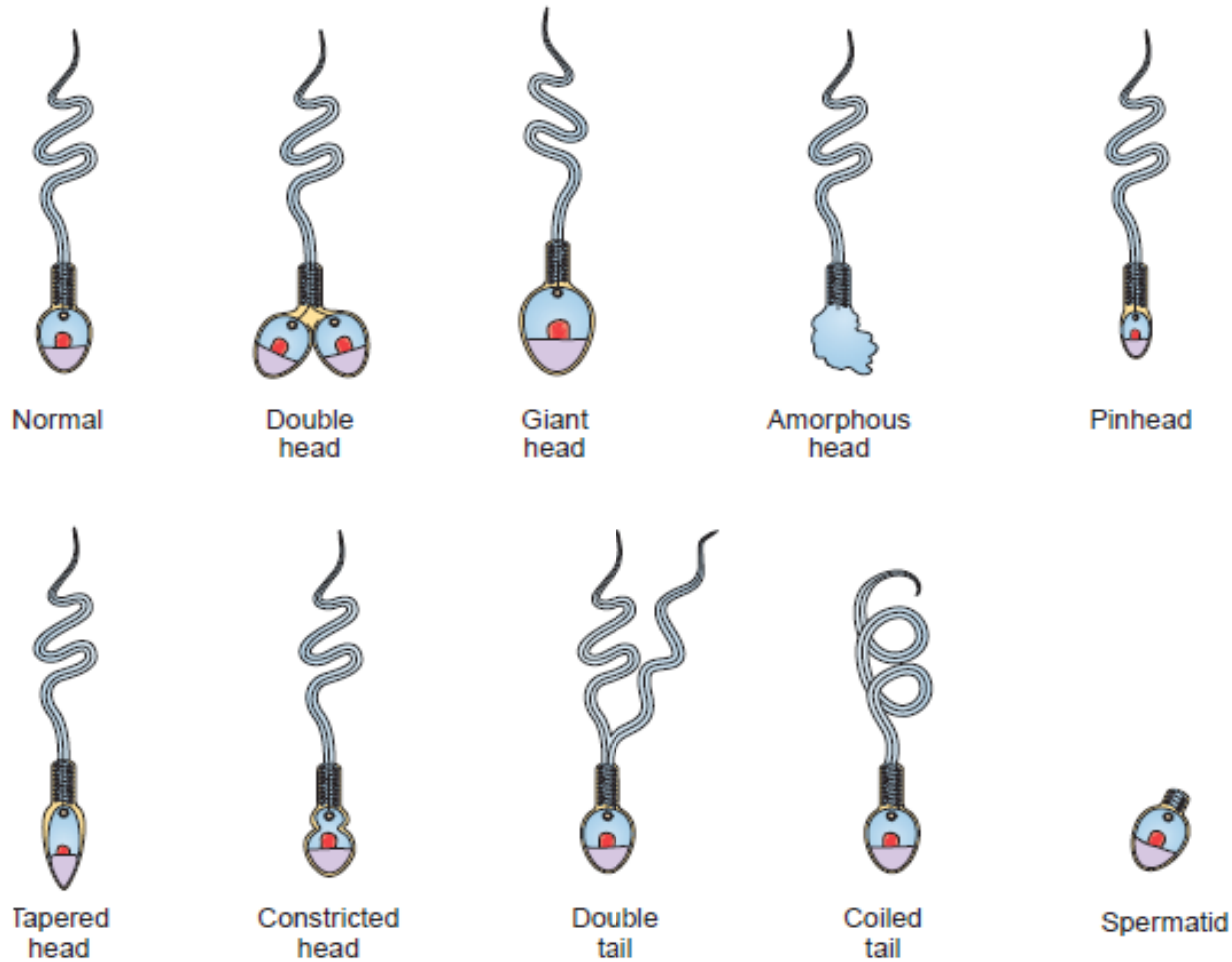
	Grade	WHO Criteria
4.0	a	Rapid, straight-line motility
3.0	b	Slower speed, some lateral movement
2.0	b	Slow forward progression, noticeable lateral movement
1.0	c	No forward progression
0	d	No movement

Sperm Morphology

- The normal sperm has an oval-shaped head approximately 5 μ m long and 3 μ m wide and a long, flagellar tail approximately 45 μ m long.
- Routinely identified abnormalities in head structure include double heads, giant and amorphous heads, pin heads, tapered heads, and constricted heads. Abnormal sperm tails are frequently doubled, coiled, or bent. An abnormally long neckpiece may cause the sperm head to bend backward and interfere with motility.



Normal spermatozoa structure.



Abnormalities of sperm heads and tails

Additional Testing

- The most common are tests for sperm viability, seminal fluid fructose level, sperm agglutinins, and microbial infection.

Sperm Viability

- Decreased sperm viability may be suspected when a specimen has a normal sperm concentration with markedly decreased motility. Viability is evaluated by mixing the specimen with an eosin-nigrosin stain
- preparing a smear, and counting the number of dead cells in 100 sperm. Living cells are not infiltrated by the dye and remain a bluish white color, whereas dead cells stain red against the purple background.
- Normal viability requires 75% living cells and should correspond to the previously evaluated motility.

Seminal Fluid Fructose

- Low sperm concentration may be caused by lack of the support medium produced in the seminal vesicles. This can be indicated by a low to absent fructose level in the semen.
- Specimens can be screened for the presence of fructose using the resorcinol test that produces an orange color when fructose is present.
- A normal quantitative level of fructose is equal to or greater than 13 mol per ejaculate. This can be determined using spectrophotometric methods. Specimens for fructose levels should be tested within 2 hours or frozen to prevent fructolysis.

● **Antisperm Antibodies**

- Antisperm antibodies can be present in both men and women. They may be detected in semen, cervical mucosa, or serum and are considered a possible cause of infertility.
- The presence of antibodies in a male subject can be suspected when clumps of sperm are observed during a routine semen analysis. The presence of antisperm antibodies in a female subject results in a normal semen analysis accompanied by continued infertility. The presence of antisperm antibodies in women may be demonstrated by mixing the semen with the female cervical mucosa or serum and observing for agglutination.

Microbial and Chemical Testing

- The presence of more than 1 million leukocytes per millimeter indicates infection within the reproductive system, frequently the prostate. Routine aerobic and anaerobic cultures and tests for *Chlamydia trachomatis*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* are most frequently performed.
- Additional chemical testing performed on semen may include determination of the levels of neutral -glucosidase, zinc, citric acid, and prostatic acid phosphatase. Just as decreased fructose levels are associated with a lack of seminal fluid, decreased neutral -glucosidase suggests a disorder of the epididymis. Decreased zinc, citrate, and acid phosphatase indicate a lack of prostatic fluid

Additional Testing for Abnormal Semen Analysis

Abnormal Result	Possible Abnormality	Test
Decreased motility with normal count	Viability	Eosin-nigrosin stain
Decreased count	Lack of seminal vesicle support medium	Fructose level
Decreased motility with clumping	Male antisperm antibodies	Mixed agglutination reaction and immuno-bead tests Sperm agglutination with male serum
Normal analysis with continued infertility	Female anti-sperm antibodies	Sperm agglutination with female serum