Semen Analysis

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Objectives

- At the end of the sessions students will able to determine and explain
  - General consideration of Semen
  - Indication and sample collection of semen
  - Volume and gross appearance of ejaculates
  - Microscopic evaluation of semen
  - Biochemical tests of semen

INTRODUCTION

- Semen is a mixture of fluids and cells
- It is composed of four fractions that are contributed by epididymis, seminal vessels, prostate and bulbourethral glands.
- Each fraction differs in its contribution
- But the mixing of all four fractions is essential for the production of a normal semen.

Formation of the sperm cell

- Sperm is produced in ***seminiferous tubules*** located in the testes.
- Germ cells located in the epithelial cells of the seminiferous tubules are responsible for the production of ***spermatozoa***
- The phenomena is aided by specialized ***Sertoli cells*** that provide support and nutrients for the germ cells that undergo mitosis and meiosis (spermatogenesis).

- When spermatogenesis is complete, the immature sperm (non-motile) enter to the epididymis.
- In the epididymis, the sperm mature and develop flagella and remain stored until ejaculation.
- During propelling the sperm cell will combine to seminal fluids inside ductus deferens
- The seminal vesicles produce the majority of the fluid present in semen (60% to 70%).

- The fluid contains a high concentration of fructose that are metabolized for the energy needed for the flagella to swim in the female reproductive tract.

- Approximately 20% to 30% of the semen volume is acidic fluid produced by the prostate gland.

- The acidic fluid contains high concentrations of acid phosphatase, citric acid, zinc, and proteolytic enzymes responsible for both the *coagulation* and *liquefaction* of semen
- The bulbourethral glands contribute about 5% of the fluid volume in the form of a thick, alkaline mucus that helps to neutralize acidity from the prostate secretions and the vagina.

Composition of Semen in volume

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- Spermatozoa= 5%
- Seminal fluid= 60%–70% (Spermatozoa become mobile when exposed to seminal fluid)
- Prostate fluid =20%–30%
- Bulbourethral glands fluid = 5%

Significance of semen analysis

- Soundness evaluation and investigation of fertility problems
- For selection of donors for artificial insemination
- To pass soundness decision for breeding program.
- To predict the fertilizing capacity of a semen
- To asses post vasectomy

Collection and Handling of Semen

- There are three commonly-used techniques for collecting semen:
  - Use of an artificial vagina
  - Manipulation and
  - Electroejaculation.
- The technique used depends on the species being collected and the disposition of the individual male.

- Semen is fragile and susceptible to damage and killing by several environmental conditions.
- When collecting and handling semen it is critical to avoid exposing sperm to two types of insults:

1. ***Exposure to toxic chemicals:***
   - Keeping collection equipment clean and free of spermicidal element.
   - Best to use deionized water for cleaning

2. Thermal stress:

- Sperm are sensitive to environmental temperature.
  - It has to be examined at temp near to body T^o and stored at -196 ^OC in liquid nitrogen
  - Handle semen with care because it may contain infectious pathogens.

Tests for semen

- There are several Macroscopic and Microscopic evaluations which give useful diagnostic information about the semen sample

Macroscopic

- Appearance
- Odour
- Liquefaction
- Volume
- Viscosity
- pH

Microscopic

- Motility
- Morphology
- Concentration
- Viability

Macroscopic Evaluation

1. Appearance and odour

- Normal semen has a gray-white color, appears translucent, and has a characteristic musty odor.
- Yellow coloration may be caused by urine contamination, specimen collection following prolonged abstinence, and medications.
- Increased white turbidity indicates the presence of white blood cells (WBCs) and infection within the reproductive tract.
- If blood is present it may appear pink to orange

2. Liquefaction and Viscosity

- **Liquefaction** is the breakdown of the gel portion of the seminal plasma by the help of the enzyme known as Fibrinolysin
- Normal semen liquefy with in 30-60 minutes after collection.
- Failure to liquefy is due to deficiency in prostatic fluid
- **Viscosity** is inversely related to specimen liquefaction
- Increased viscosity and incomplete liquefaction will impeded sperm motility

3. Volume

- Normal volume vary from species to species
- **Increased volume:** following periods of extended abstinence
- **Decreased volume**: associated with infertility; may indicate improper functioning of one of the semen producing organ.
- **Aspermia** – no semen production at all

	Bovine	Shoat	Swine	Horses	Man
Volume (ml)	4-6	1-2	225	60	2-5

4. pH

- Semen has a narrow pH range from 6.4-8.0.
- It can be measured by immersing lithmus paper or spread a drop of liquefied semen on the paper.
- When the pH is over 8.0 this may be due to infection in RT.
- Decreased pH; associated with increased prostatic fluid
- When the pH is below 6.4 and the semen is found to contain no sperm, this may indicate dysgenesis (failure to develop) of the vas deferens, seminal vesicles or epididymis.

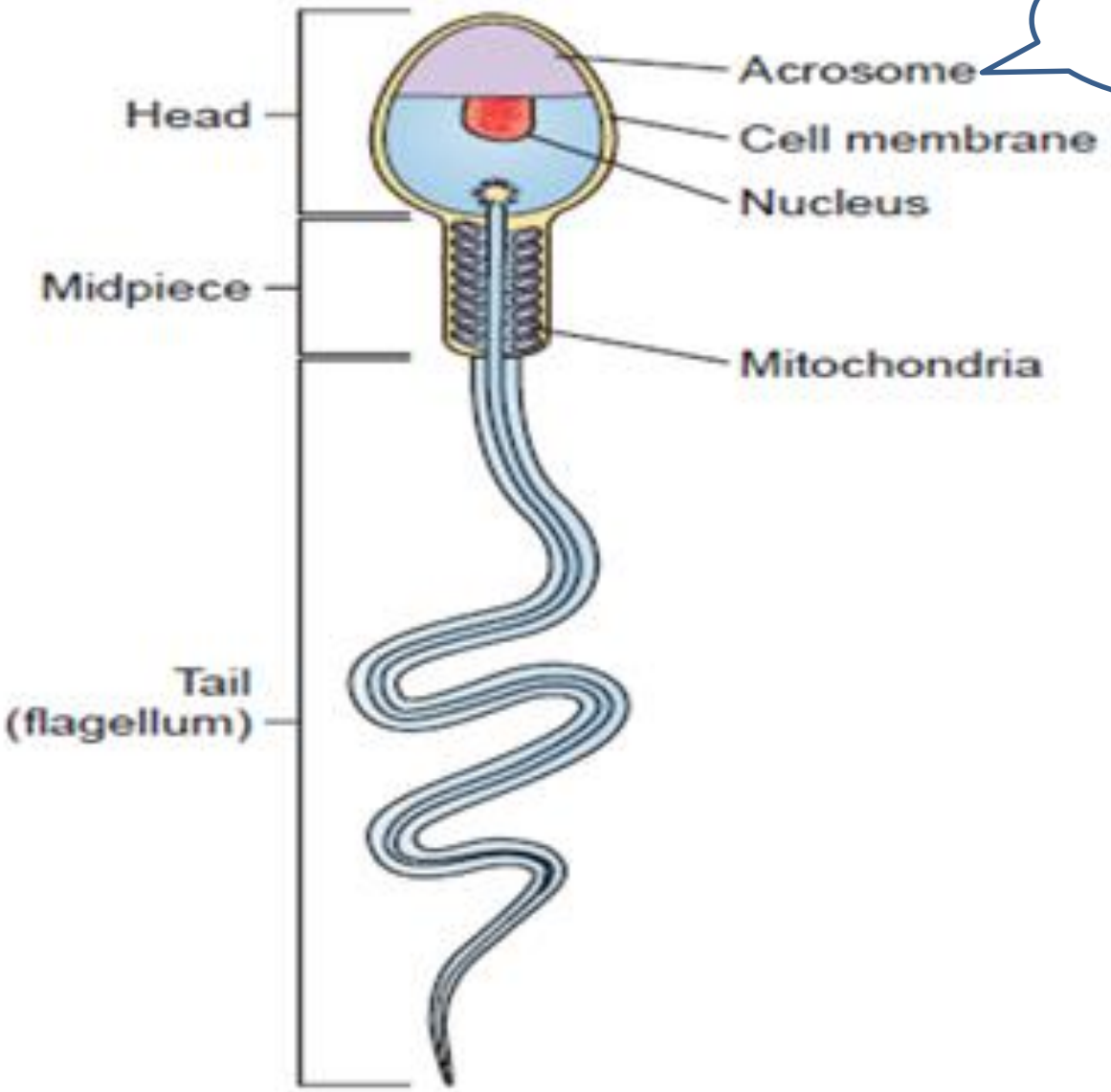
Microscopic Examination

- It is performed to obtain estimates of sperm morphology, motility, concentration, viability and some times wave pattern
- It is done by placing 10µl of thoroughly mixed, liquefied semen on a Microscope slide and coverslip with a 22x22mm size

- The quality of sperm motility is affected by temperature
  - So great care must be taken to ensure that the slides and coverslips, as well as the pipette tips are kept around 37°C
  - The assessment must start as soon as the flow stops. if this is >1 minute, a new wet prep must be made

1. Morphology

- Sperm morphology is evaluated from a thin smeared and stained slide under oil immersion.
- Staining can be performed using Carbol fucshin, Wright's, Giemsa, HE stain which is a matter of laboratory preference.
- The normal sperm has an oval-shaped head, midpiece located between head and tail and flagellated tail required for motility



For penetration of Ovum

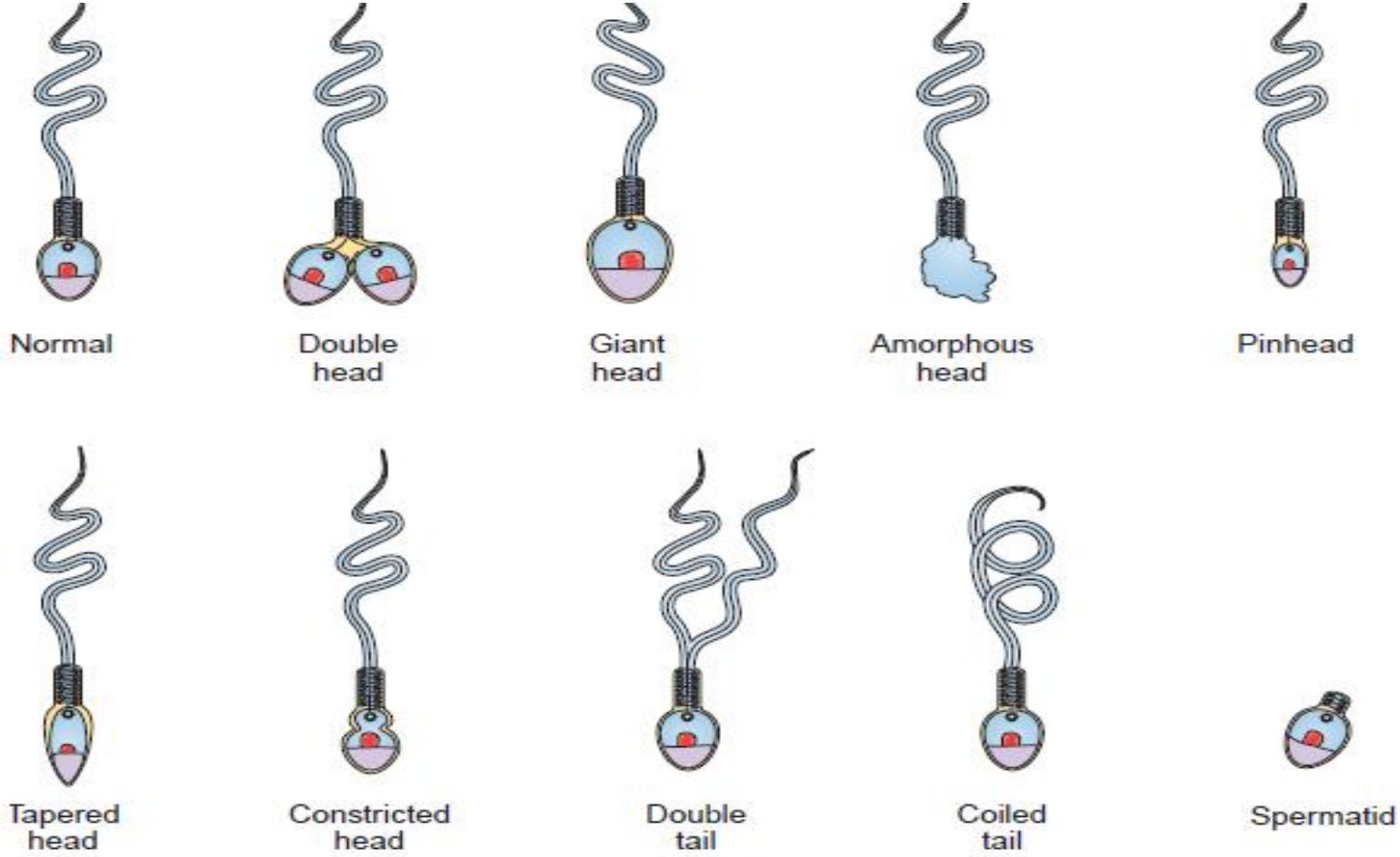
Normal spermatozoa structure.

- **Reporting morphology of spermatozoa**
  - Examine the preparation for normal and abnormal spermatozoa using the 40X objective
  - Use the 100X objective to confirm abnormalities.
  - Count 100 spermatozoa and estimate the percentage showing normal morphology and the percentage that appear abnormal.
  - In normal semen, at least 50% of spermatozoa should show normal morphology.

The following abnormalities may be seen:

- **Head**
  - Greatly increased or decreased in size.
  - Abnormal shape and tapering head (pyriform)
  - Acrosomal cap absent or abnormally large.
  - Bifurcated heads.
- ***Tail***
  - Absent or markedly reduced in length.
  - Double tail.
  - Bent or coiled tail.

Abnormalities of sperm on head and tail



2. Motility assessment

- The sperm cells capable of forward and progressive movement is critical for fertility.

- Because once presented to the cervix, the sperm must propel themselves through the cervical mucosa, uterus, fallopian tubes, and ovum for fertilization.

- Traditionally, clinical laboratory reporting of sperm motility has been a subjective evaluation and determining the percentage of motile sperm and the quality of its motility.
- Assessment of sperm motility should be performed on well mixed, liquefied semen soon after specimen collection.

- The motility movement can then be estimated after evaluating approximately 20 high-power fields.
- Motility is evaluated by both **speed** and **direction**.
- Grading can be done using a scale ranging from 0 to 4,
  - **4** indicating rapid, straight-line movement and
  - **0** indicating no movement.
- A minimum motility of 50% with a rating of 2 is considered as normal.

Motility assessment - types

Grade	Motility type	Percentage
4	Rapid and straight-line motility	80-100%
3	Slower straight line speed with some lateral movement	60-80%
2	Slow forward progression with noticeable lateral movement	40-60%
1	No forward progression but slow lateral movement	20-40%
0	No movement	0-20%

3. Sperm Concentration/Count

- Even though fertilization is accomplished by one spermatozoon, the actual number of sperm present in a semen specimen is a valid measurement for fertility.
- Normal values for sperm concentration varies among species.
- The total sperm count for the ejaculate can be calculated by multiplying the sperm concentration in ml by the specimen volume.

	Cattle	Shoat	Swine	Horses	Man
Sperm conc. (10^9/ml)	**1–1.2**	**3.0**	**0.2**	**0.15**	**0.02**
Sperm/ejac. (10^9/ml)	**4–7**	**4**	**45**	**9**	**0.04**

- Sperm concentration is usually performed using the Neubauer counting chamber.
- The sperm are counted in the same manner as cells in RBC counting.

- Dilution of the semen is essential because it immobilizes the sperm prior to counting.

- The traditional diluting fluid contains ***sodium bicarbonate (5g)*** and ***formalin (1ml) to 100 ml of water*** which immobilize and preserve the cells

- However, good results can also be achieved using saline and distilled water.

4. Viability

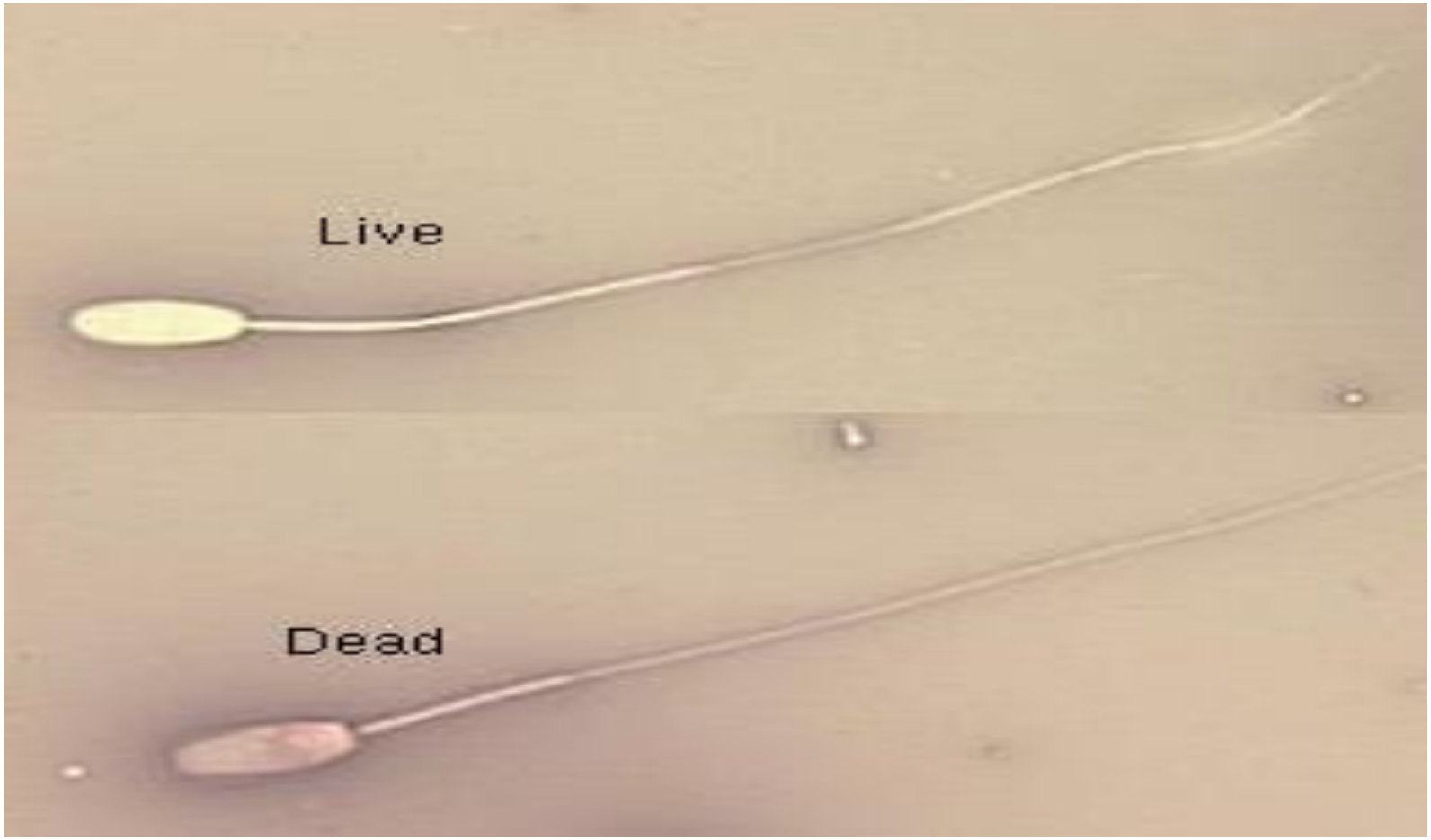
- Decreased sperm viability may be suspected if there is a marked decreased of motility.
- Viability is evaluated by mixing the specimen with an **eosin- aniline stain**.
- The membranes of dead sperms are damaged and can easily take up eosin stain.

- The viable sperms do not allow the stain to penetrate leaving a colorless (bluish) sperm.
- Normal viability requires 75% living cells and should correspond to the evaluated motility.

Procedure

- Mix one drop of semen with 1 drop of 0.5% eosin solution on a slide.
- Make a smear using mixture
- After 2 minutes examine microscopically using 40X objective
- Count 100 sperm cells and compute the percentage of viable and non-viable spermatozoa.
  - Viable spermatozoa remain unstained
  - Non-viable spermatozoa stain red.

Viable and Nonviable spermatozoa demonstrated by the eosin stain



5. Wave pattern

- It is an alternative test for motility
- It is determined by placing a thick drop of semen on a slide under a microscope with low power and reduced light.

- The result is reported as
  - ✓ Very good (4) - Dark, distinct waves moving rapidly
  - ✓ Good(3)- Waves apparent, but with moderate motion
  - ✓ Fair(2) - Waves barely distinguishable
  - ✓ Poor(1) - No waves, but motile sperm are present
  - ✓ Very poor(0)- No waves and no sperm motility

Semen biochemistry

- ***Acid phosphatase:*** marker for prostatic function and important in rape cases
- ***Citric acid:*** can indicate prostatic function - low levels may indicate dysfunction or a prostatic duct obstruction
- ***Zinc:*** marker for prostatic function - *colorimetric assay*
- ***Fructose:*** marker for seminal vesicle function, and is a substrate for energy metabolism – *spectrophotometric assay*

QUESTIONS