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## **Lecture title: Methods of Semen Evaluation**

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**Summary:** Many methods are used for semen evaluation, classical and advanced methods.

### **1. Macroscopic semen Evaluation (Gross evaluation)**

- Volume,
- Color,
- Odor,
- Appearance,
- pH, and
- presence of contaminants, such as blood cells, puss cells, hair or other debris

### **2. Microscopic semen Evaluation**

- Density,
- motility,
- viability,
- morphology, Morphometric characteristics of sperm are one of the most important indicators of fertility, sperm with normal morphology have a significant effect on fertility both in vivo and in vitro
- concentration. Sperm concentration per mL of semen can be determined by counting the sperm

### **3- Advance semen Evaluation**

- Plasma Membrane Integrity,
- Acrosome Integrity,
- Mitochondrial Integrity,



- Chromatin Integrity,
- Reactive Oxygen Species (ROS),
- Sperm Cytometric Analysis,
- Sperm Transcriptome Assay (Gene Expression),

- ✓ Assessment of male fertility is based on the evaluation of sperm.
- ✓ To evaluate semen we need to analyze sperm quality parameters as fertility indicators.
- ✓ Classical and advanced methods for semen evaluation.
- ✓ Advanced techniques, like computer-assisted sperm analysis (CASA) and flow cytometry have been used for analysis.

1- Volume of ejaculate dependent on:

- Species
- Breed
- Age
- Environment.
- Feeding
- Housing
- Method and frequency of semen collection
- Time of the year

2- Color

<u>Abnormal Color</u>	Abnormal due to
Brownish	Orchitis due to blood pigments
Dark red to pink blood	Hemorrhage in male reproductive tract



Yellow green	Pseudomonas aerogenosa infection - pu This color appears on keeping semen for some time after collection
Light brown	Contamination with faeces / dung
Dull and dirty white	Increased number of spermatogenic cells
Yellow	Presence of urine
Chunk clots/Curdy appearance	Infection

3- Odor: Examined also as routine laboratory practice,  
Normally odor-neutral  
urine odor,  
putrid odor,  
and ejaculates with a species-specific fecal odor should be discarded.

4- PH 6.5 – 7.0

## 2. Microscopic semen Evaluation

1- Density

- Dense Semen
- Middle Sperm.
- Rare

2- Motility

Mass and progressive motility



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### 3- Viability

1. Eosin–nigrosine staining      classical
  - Depending on the permeability of sperm, it lets the stain enter cells and stain it.
  - Dead sperm stains due to damaged plasma membrane, whereas the live sperm will not stain and remain colorless.
  - Nigrosin stains the background.
  - Several other stains have been widely used for sperm viability assessment, including fast–green and eosin, and opal–blue and eosin.
- 2- SYBR-14 and PI advance method analysis by flow cytometry or fluorescent microscope.

### References:

- 1- Tanga BM, Qamar AY, Raza S, Bang S, Fang X, Yoon K, Cho J. Semen evaluation: methodological advancements in sperm quality-specific fertility assessment - A review. Anim Biosci. 2021 Aug;34(8):1253-1270. doi: 10.5713/ab.21.0072. Epub 2021 Apr 23. PMID: 33902175; PMCID: PMC8255896.
- 2- Cenariu, M., Pall, E., Borzan, M., Bogdan, L. (2018). Advanced Techniques of Bovine Semen Analysis. Bulletin UASVM Veterinary Medicine 75(1). DOI: <https://doi.org/10.15835/buasvmcn-vm:004317>