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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 is dependent on:

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

2- Color

Abnormal Color	Abnormal due to
Brownish	Orchitis due to blood pigments
Dark red to pink blood	Hemorrhage in the male reproductive tract
Yellow green	Pseudomonas aerogenosa infection - pus 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

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