### **Semen Evaluation**

#### Gross evaluation of semen

- After collection the semen should first be observed grossly. A rough estimation of the concentration can be made based on the opacity (or lack of) and the color of the semen. Very concentrated samples look like heavy cream while very dilute samples have the appearance of watered-down skim milk. Yellow-tinted semen can result from urine contamination and this can be substantiated by smell or by use of a blood urea nitrogen test strip. Additionally, semen contaminated with urine will have rapidly declining or often no motility when examined microscopically. Conversely, a light-yellow or gold appearance is also associated with very highly concentrated semen and the presence of riboflavin, which is a common finding in many Jersey and some Angus bulls. Red- or brown-colored semen is due to the presence of blood or blood pigments and the source of this contamination must be determined.

#### Volume

#### Factors affecting the volume of ejaculate in bull:

• Age: The volume of the ejaculate increase from puberty till reach the maximum volume about 2 year post maturity then begin to decline after 8 years of age.

• Breed: Dairy bulls tend to produce larger ejaculate than do beef bulls.

- Body size: The greater the body size the greater the volume.
- Nutrition: Well-nourished bull give larger volume than the bad nourished one.
- Season: The volume in the summer is larger than it1 the winter.

• Frequency of collection: The volume decreased by increasing the number of the ejaculates at short interval. However when two ejaculates are collected at a short interval the second is usually larger, this is due to increased sexual excitement.

• Sexual excitation and proper preparation of bull (teasing): Sexual stimulation of the bull before the actual mounting result in higher volume and higher sperm cell concentration.

• Method of semen collection: Samples collected by electroejaculator usually have larger volume than those collected by manual manipulation larger than those collected by A V.

## **Evaluation of semen motility**

- After quickly evaluating the semen grossly, a small "standing" drop is placed on a prewarmed slide and evaluated under low-power microscopy for gross motility. Thick, dark, rapidly oscillating swirls are indicative of excellent motility (defined as high-velocity or high-speed motility), a high percentage of sperm that are progressively motile, and a sample of high concentration. This type of sample would typically be classified as "very good." A sample that displays slower moving swirls is classified as "good." A "fair" sample displays no swirls, but significant individual sperm movement. A "poor" sample has no or very little movement/oscillation. Because the concentration of a sample impacts the gross motility designation, individual motility should be assessed if there is any question about the validity of a motility rating based on gross motility. Individual motility can be assessed and, depending on the concentration, a coverslip over either the previously examined droplet or a diluted droplet (diluted with warmed sodium citrate solution). Individual motility is classed "very good" if greater than 70%, "good" if 50–69%, "fair if 30–49%, and "poor" if less than 30%.

## **Evaluation of sperm morphology**

- Accurate evaluation of sperm morphology begins with the preparation of a stained sample of diagnostic quality and the use of a bright-field microscope that has at least  $100 \times$  oil immersion objective ( $1000 \times$  magnification). Additionally, one must make a commitment to the careful examination of at least 100 sperm cells.

- Primary abnormalities are testicular in origin or, more specifically, occur during spermatogenesis, whereas secondary abnormalities are epididymal in origin.

## **Preparation of a slide**

- Evaluation of sperm morphology depends on the preparation of a good semen smear. Begin with a clean warm slide, realizing that some "new" slides may be contaminated with detergents, etc. that interfere with staining. A vital stain, eosin-nigrosin, is currently recommended for field use, due to its ease and consistent staining properties. The eosin portion will penetrate dead sperm cells, staining them pink (red is dead) and leaving live cells unstained (white) against the dark background provided by the nigrosin component.

# **Sperm concentration**

- **Definition:** The number of the sperm cells per unit volume and it is usually expressed by  $n/mm^3$ .

**Importance:** Sperm concentration is very important routine examination because it gives **good indication about the spermatogenic activity of the testis and it is very important for calculating the dilution rate during semen processing.** 

# Methods:

# 1) Density

**2) Opacity tube:** This method depends on comparing the opacity of the examined sample with the opacity of known concentration (matching).

**3)** Colorometric, photometric, absorptiometer and spectrophotometric method: The sperm cells absorb the light and subsequently the amount of the light passed through the semen sample to the photo cell is inversely related to the sperm cell concentration. Compare optic density with standard curve.

**4) Direct cell account (Haemmocytometer):** The haemocytometer has two counting chambers; each chamber formed from four peripheral chambers used four counting WBCs and one central chamber used for counting RBCs counting.



**5)** Computer assisted semen analyzer (CASA): The CASA is an automated reproducible high-performance sperm analysis instrument that can provide precise and accurate information about 16 clinical parameters within 75-second. These parameters include count, motility (A+B+C), morphology, velocity, and functional sperm.

Characteristic	Species				
	Bull	Ram	Stallion	Boar	Dog
Volume (ml)	4 (2-10)	1.0 (0.5-2.0)	60 (30-250)	250 (125-500)	10 (2-19)
Fractionated	N	Ν	Y	γ	Y
Density (x 10 <sup>6</sup> /ml)	1250 (600-2800)	2000 (1250-3000)	120 (30-600)	100 (25-1000)	125 (20-540
Motility (motile sperm, %)	> 70	> 90	> 60	> 60	> 85
Normal spermatozoa (%)	> 75	> 85	> 60	> 60	> 90

# SEMEN EVALUATION (EXAMINATION)

