Semen analysis and assessment

A) Macroscopic examination:

Consist of directly observing and examining the ejaculate in the tube to rule out any defect due to volume, color, odor or dirt.

1. Volume :

This is a parameter that depends on the function of the semen vesicle and sex glands, plus other factors such as age, species, training… etc, and varies from 1 – 8 ml. most bulls provide about 6 ml ejaculate .

2. Color :

This depends on the amount of spermatozoa:

*Milky or creamy white color: semen is good quality.

*Watery milk color: semen is poor quality.

*Yellow color: semen contaminated with urine.

*Pink color: presence of blood.

3. Odor:

In good condition; semen has small similar to fresh milk. The smell of urine indicates that the semen is contaminated with it. If the smell is unpleasant, some kind of disease is suspected in the testicles or elsewhere in the reproductive tract.

4. Appearance :

Its opacity, which depends on the concentration of spermatozoa, is assessed.

5. pH :

Is determined using a paper strip between the range (6.4 – 6.9). Above (6.9) is indicated of low semen quality.

B) Microscopic examination:

Is done under a bright field microscope to analyses the: masal and individual motility of the spermatozoa, the morphology and concentration.

1. Masal mortality:

It is determined by:

* placing a drop of semen on a slide.

*Observing it under a bright field microscope with little magnification.

*The presence of waves and eddies throughout the whole drop is evaluated and is given a classification from 0 to +++, as below:

a) Very good: lots of dark waves moving rapidly (+++).
b) Good: less dark waves than the previous are observed, with moderate movement (++).
c) Normal: clear waves with very slight movement (+).
d) Poor: no waves and the spermatozoa are immobile (0).

2. Individual motility:

This parameter can be analyses more objectively with automated systems such as (CASA) system. However, it is also determined by bright field microscope examination by placing a drop of semen on the slide may be diluted with saline or sodium citrate (0.9 %). A cover slip is then placed over it and observation performed under a bright field microscope with maximum magnification. The

semen is classified according to the type of movement of the individual sperm, as follows:

*Very good: ≤ 70 progressive individual motility.

*Good: 50 – 69 individual motility.

*Normal: 30 – 49 individual motility.

*Poor: ≥ 29 individual motility.

3. Sperm viability, acrosome and morphological abnormality:

Are determined by microscopic observation of a smear of semen subjected to special staining fluids, usually (***Eosin-nigrosin***) . The method involves:

- Placing a drop of approximately 10 micro liters of pure semen on a prepared slide (cleaned and degreased at a temperature of 36 – 37 c° on the heat plate).
- The drop of semen mixed gently with a drop of eosin-nigrosin, which must be at the same temperature as the semen (using the pipette tip).
- Another slide, prepared as above, is supported on the edge of the drop until the liquid begins to spread over the slide by capillary action .It is then spread evenly, firmly and softly .
- The assessment of sperm viability, acrosomic and morphological abnormality is performed under a bright field microscope at X100 magnification.
- A total of 200 sperms are counted and the percentage of non-viable cells, abnormal acrosomes and malformations of the sperm(colored) are calculated
- The minimum value is 70% normal acrosomes.
- The abnormalities observed are classified as primary or secondary.
- This classification is most widely used in the literature, but not the most accurate.

- Primary malformations are by definition, those that originate within the testes during spermatogenesis.
- Secondary malformations are those that originate within the epididymis.
- There is much still to be studied in this field .
- In general: The maximum number of head abnormalities allowed in the ejaculate is between 15 and 20 %.
- Acrosome and tail abnormalities are acceptable up to 25% .
- In no case should less than 70% of normal spermatozoa in the ejaculate of a breeding bull be accepted.

4. Concentration :

This is number of spermatozoa\ml, and is calculated by counting the sperm with respect to the dilution and volume, in a counting chamber (Burcker or similar) under a bright field microscope. Another way to determine this semen parameter is examination with an automatic system, such as CASA system or use of a spectrophotometer.

The characteristics values of bovine <u>semen for normal fertility</u>:

1- Progressive motility: > 50 %.
2- Concentration: > 500 million \ ml.
3- Sperm vitality: > 50 %.
4- Abnormal head: < 20 % (normally 8-12 %).
5- Proximal cytoplasmic droplets: < 4 %.
6- Distal cytoplasmic droplets: < 4 %.
7- Abnormal middle pieces : < 15 %.
8- Double tails: < 4 %.
9- Crooked tails: < 3 %.