Semen Evaluation

1- **Macroscopical examination (Volume, color, density, Viscosity , PH).**
2- **Microscopical examination (Individual sperm motility , total motility" or "mass motility).**

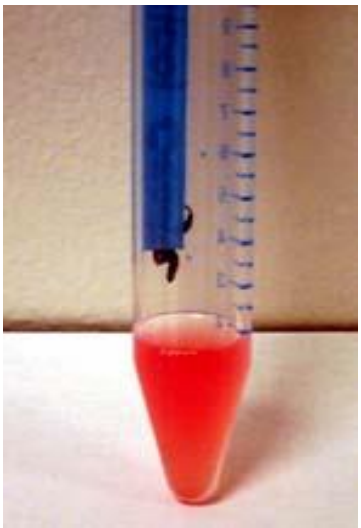
1- Macroscopical examination:

A- Semen color:

- **Bull**: creamy white.
- **Ram** and **buck**: creamy white and thick.
- **Boar**: milky.
- **Stallion**: greyish white and thin.

Abnormal colors:

- **Yellow**: release from presence **pus** or **urine** in semen.
- **Red**: release from new **injury** or **bleeding** in reproductive system .
- **Deep brown**: release from **old bleeding or hemolysis** in RBC.
- **Green**: contamination the seminal fluid by **feces**.
- **Watery color**: there are **no sperms only plasma**, this case called **azospermia**.



B- Volume:

Animal species	Ram	Bull	Stallion	Boar
Vol./ml	**0.5 – 2**	**5 - 15**	**40 - 200**	**250 - 400**

C- Density:

Too thin: from azoospermia or oligospermia.

D- PH:

Normal reaction of semen is alkaline 7.4 at the time of ejaculation.

2- Microscopical examination:

A- Mass motility is examined.

- Mix the semen sample with a wooden stick, as motile sperm cells will try to swim upward and dead cells will settle to the bottom.
- For gross motility use 2 wooden sticks to place a drop of semen on a warm slide.
- Do not use a cover slip and examine the cells under a 10X objective.

numerical	Descriptive scale	Wave motion
0	Very poor	No waves, sperm are immotile
1	poor	No waves, weak rotatory movement
2	fair	No waves, strong rotatory movement (< 50% motile sperms)
3	good	Slow wavy motion (50-80% motile sperms)
4	Very good	Rapid distinct wavy motion (~90% motile sperms)
5	excellent	Very rapid vigorous wavy motion with eddies (>90% motile)

B- Individual sperm motility:

- Individual motility checks for the forward progressive movement of the sperm cells.

- Make the sample by placing a drop of diluent (saline or Na citrate) on a warm slide.
- Place a small amount of semen into the saline.
- Then place a warm cover slip on the drop.
- Examine the sample under high dry (40X) power.
- You must examine the sample quickly as the motility changes very rapidly with heat, light, and cold.

motile sperms%	Descriptive value	Individual motility
0-20	Very poor	1/5 of sperms motile
20-40	poor	2/5 of sperms motile
40-60	fair	3/5 of sperms motile
60-80	good	4/5 of sperms motile
80-100	Very good	5/5 of sperms motile

Individual sperm motility:

A-**Normal**: progressive forward motility

B- **Abnormal**: defects in tail or head of sperm

1- Progressively backward motility - defect in tail.

2- Circular motility –defect in tail.

3- Oscillatory motility – defect in head.

$$\text{Abnormal sperms\%} = \frac{\text{No. of abnormal sperms}}{\text{Total sperms No.}} \text{ X } 100$$

- **sperm concentration:** is the number of sperms in 1ml of raw semen.

Species	Sperm cell concentration	Species	Sperm cell concentration
Bull	$800\times10^6 - 1\times10^9$ / ml	Stallion	$50\times10^6 - 200\times10^6$ / ml
Buffalo-bull	$600\times10^6 - 1\times10^9$ / ml	Boar	$100\times10^6 - 200\times10^6$ / ml
Ram	$2\times10^9 - 4\times10^9$ / ml	Dog	$50\times10^6 - 100\times10^6$ / ml
Camel-bull	$500\times10^6 - 600\times10^6$ / ml		

Hemacytometer

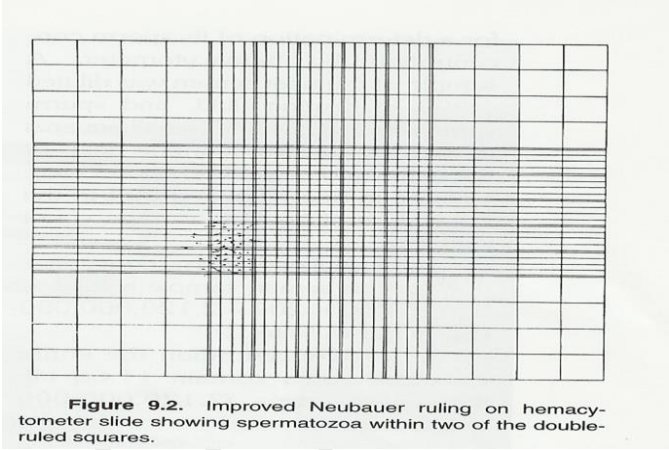


Figure 9.2. Improved Neubauer ruling on hemacytometer slide showing spermatozoa within two of the double-ruled squares.

$$\text{Sperm conc. /ml} = \frac{\text{No.of sperms counted in five medium squares}}{80} \times 400 \times 200 \times 10$$

80=No. of small squares counted in five medium squares.

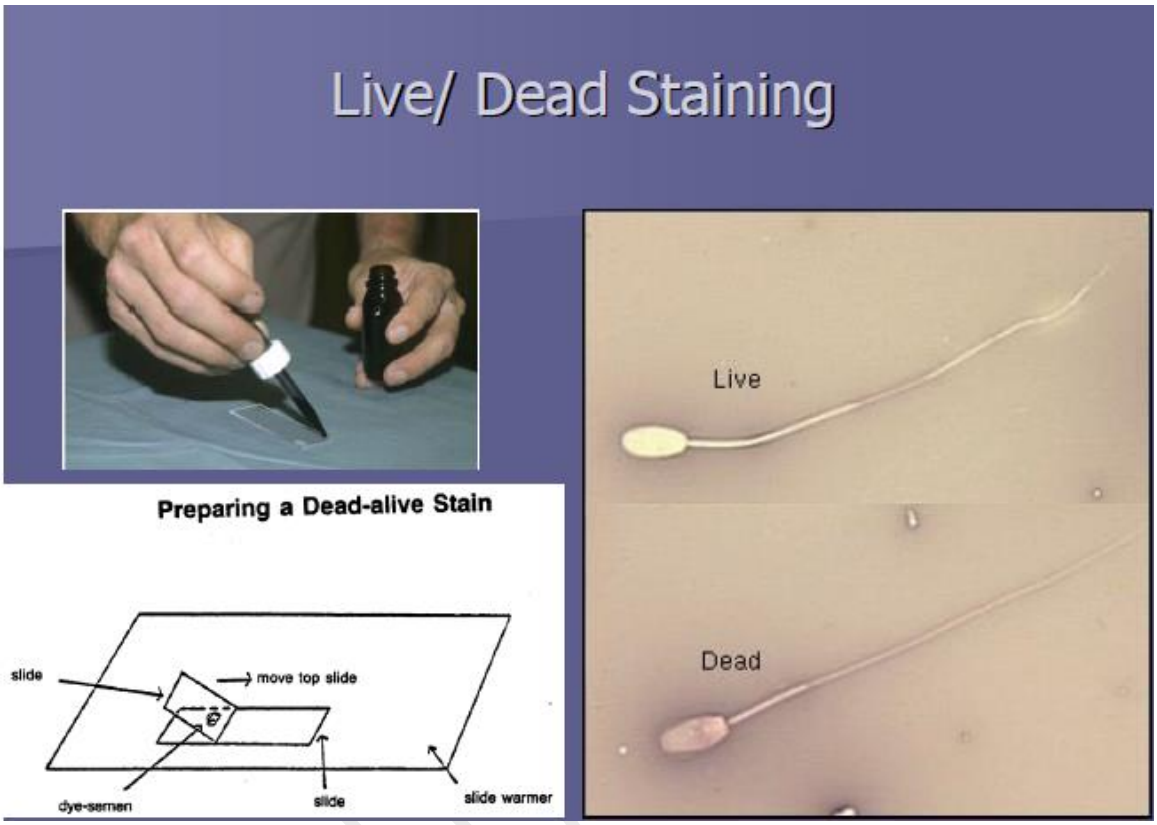
400= The total number of small squares.

200= Dilution factor.

10=Volume coefficient.

live and dead percentage: this can be done by staining the semen with vital stains such as eosin-nigrosin stain, that cannot stain the live sperms but stains the dead sperms.

$$\text{Sperms viability\%} = \frac{NO.\text{ sperm live}}{\text{total sperm } NO.} \text{ X } 100$$



Sperm abnormalities or defects

The abnormal spermatozoa classified as:-

1 – Primary abnormalities: - arise from defects in spermatogenesis inside testes; this type affected the animal fertility (double forms, knobbed sperm defects, decapitated sperm, diadem defect, pear-shaped heads, narrow at the base, small abnormal heads and free abnormal heads).

2- Secondary abnormalities:-

Occurs in epididymis related with the degree of spermatozoa maturation (protoplasmic droplets). this type not affected fertility.

3- Tertiary abnormalities:-

Occurs in any part of mature spermatozoa outside of testes and epididymis, as after ejaculation during handling of the semen such as time of collection, smear, dilution of semen. (free heads, detached acrosome, bent or coiled tail, terminally coiled tail).

Wrinkled acrosome:- this may reflect a nuclear problem which prevents zona attachment by the sperm cell. It is a rare condition.

Pyriform and tapered heads:- the nuclear material is poorly distributed. The defect may be subtle.

Giant or small heads:- This nuclear problem. If the head is twice normal size the cell is a giant cell.

Diadem defect:- With this you see invaginations in the nucleus, mostly by the post nuclear cap. The pit lacks DNA. The condition may be associated with stress in bulls and may come and go as stress changes.

Dense proximal prtoplasmic droplets:- this arises in the epididymis and indicates maturation problem.

Dag defect:- This is a sterilizing defect that occurs in the epididymis so is it is actually a secondary abnormality, but it is a major defect. The condition is inherited and the axoneme is disrupted (fibrils and helix). You see split, shattered, or fractured midpiece. The tail may coil and the motility is low.

Coiled mainpiece:- The mainpiece is coiled within the plasma membrane.

Bent tails:- the bend in the tail may include a droplet which may be in the membrane.

Physiologic (distal)droplet - some consider this a minor defect, but in fact it may be a major defect. These cells do not freeze well because the water in droplet crystalizes and ruptures the cell membrane.

Midpiece

1- central fibrils
2- 9 double inner fibrils
3- 9 coarse outer fibrils
4- Mitochondria
5- plasma membrane

Terminologies of SA

- Azospermia (absence of sperm)
- Oligospermia (low sperm count <15 million/ml)
- Asthenozoospermia (poor sperm motility)
- Necrozoospermia"dead" sperm
- Teratozoospermia (abnormal sperm morphology<4% spermatozoa)

- Hypospermia – low semen volume < 1.5 ml
- Hyperspermia – high semen volume > 6.0 ml
- Polyzoospermia – high sperm concentration, >200M/ml
- Aspermia – no semen volume
- Pyospermia – leukocytes present in semen,
- Hematospermia – red blood cell present in semen