

Breeding Soundness Examination (BSE) and semen collection

1. Breeding soundness examination

- **Indications**

1. Diagnosis of infertility –identify and eliminate infertile males (Judgment depending on circumstance of breeding service)
 2. To ascertain whether the animal will have a level of fertility that is adequate for breeding – Artificial insemination or natural mating
- 2nd purpose more relevant in food animals– pre-introduction
 - BSE estimates fertility levels to be expected from a given male for selection
 - These decisions are important to financial efficiency of the farm
 - **Reduced costs and disease risk** as well as increase

- There are many components to a bull breeding soundness examination.

1. History and health status

this might involve reviewing the health status of the herd of origin if the examination is being carried out prior to purchase, reviewing the results of previous laboratory tests that may have been carried out on samples collected from the bull in the past

- Collecting samples and commissioning more laboratory tests
- Reviewing vaccination and
- Other treatment history
- Reviewing previous breeding performance.

2. Body condition score

- ⌘ This is assessed on a five point scale with 1 being very thin and 5 obese.
- ⌘ Bulls that are obese may suffer from poor semen quality due to deposition of fat in the scrotum leading to thermo-regulation problems

3. Physical examination

This begins at a distance assessing

- Size relative to age
- Body condition
- Conformation
- Locomotion and
- Noting signs that might be consistent with the presence of any infectious or contagious disease.

A more detailed examination may be required particularly to assess:

- The eyes,
- The teeth,
- The legs and feet and, the reproductive
- apparatus.

⌘ The eyes should be examined for lesions that could compromise vision such as cataract, corneal opacity, or carcinoma.

⌘ The incisor/dental pad alignment should be checked for evidence of any significant.

3. Examination of the reproductive tracts

- **Penis and prepuce**

- ‖ The prepuce will be examined for any evidence of constriction or discharge.
- ‖ Dog and boar - possible after manual stimulation
- ‖ Check for – externally visible lesion, erection, protrusion, orientation
- ‖ Palpation of the prepuce and of the more proximal parts of the penis (e.g. the sigmoid flexures of ruminants) for Adhesions, trauma and the presence of tumors

- ‖ The penis will be palpated to ensure that it is

- **Scrotum and Spermatic Cord Contents**

- Check scrotal skin for signs of trauma or ectoparasites
- palpation of scrotal structures (presence, size, texture, tone, evenness of the testes, its free immovability in scrotum, temperature, sensitivity)
- Tail of the epididymi turgidity – sperm reserve
- The ductus deferens should be palpated throughout the scrotal neck and (particularly in rams)
- The spermatic cord should be palpated up to the level of the inguinal ring for the presence of abdominal contents (scrotal hernia) or abnormalities of spermatic vasculature.



Figure 21



Figure 22



Figure 23

Measurement of scrotal circumference

This gives a good estimate of daily sperm producing ability which otherwise requires evaluation of serial semen samples

Scrotal circumference of mature depends on **body weight** and may fluctuate with **season**

Small rams >28 cm are acceptable; Large rams > 34cm acceptable

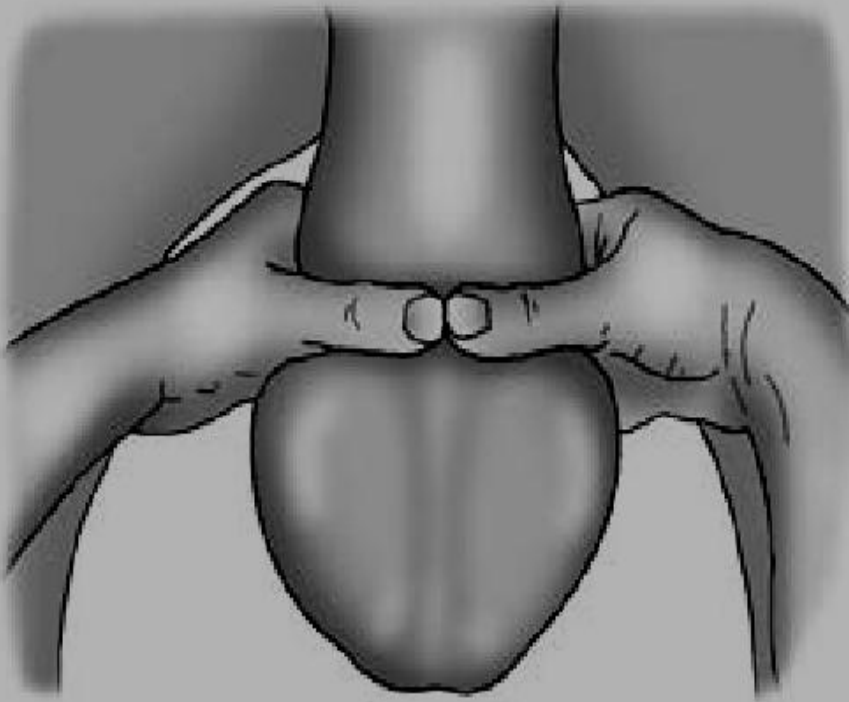


Figure 11.2 Palpation of the testes – see text for details.

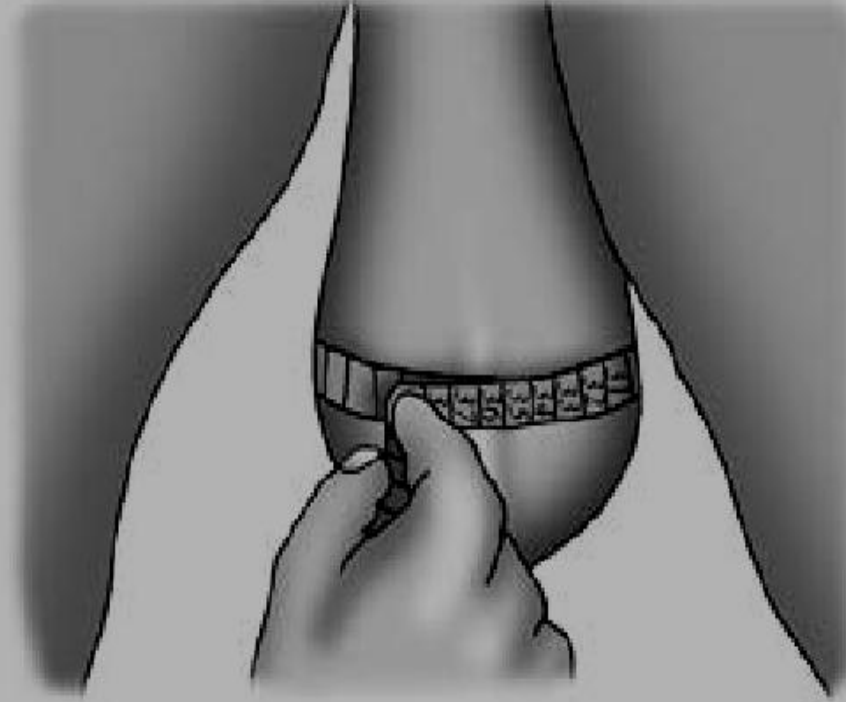


Figure 11.3 Measurement of the scrotal circumference.

- The three common descriptors for scrotal shape are:

- **Straight (slab) sided scrotum** (Fig 18) – may be associated with small testicles and excessive fat in neck of scrotum
- **Normal scrotum** (Fig 19) – a pendulous scrotum with a well defined neck is ideal for thermoregulation of testicles
- **Wedge-shaped scrotum** (Fig 20) – this is associated with smaller testicles and excess fat in the neck which will be detrimental to thermoregulation and semen quality



Figure 18



Figure 19



Figure 20

There is a clear relationship between scrotal conformation and semen quality and in one retrospective study of 958 bulls in Canada 72% of bulls with pendulous scrotums had satisfactory semen quality whereas only 27% of bulls with straight-sided and 1% of bulls with wedge-shaped scrotums had satisfactory semen quality.

- **Accessory sex glands**

- In the bull and the stallion, the prostate, vesicular glands and ampullae can be palpated per rectum
- Small animals – US or X ray examination

Score	Description	Semen	Action
1	Firm and springy	Likely to be acceptable	Meets standard
2	Slightly softer	Adequate semen	Meets standard
3	Poor tone	Probably unacceptable	Evaluate semen
4	Flaccid/ atrophied	Unlikely to be adequate	Classify as unsound

Age	Minimum scrotal circumference
Yearling	32 cm
2-year-old	34 cm
> 2 years old	38 cm (clear pass)
	36 cm (provided testis tone and turgor of epididymal tail are normal)



4.Semen collection and examination

- The best quality samples can be obtained by teasing the bull with a cow that is in estrus and then using an artificial vagina
- The use of electro-ejaculation will also usually result in an acceptable quality sample being obtained more quickly and safely.
- A few bulls will fail to respond satisfactorily to electro-ejaculation and no sample will be obtained.
- An assessment will be made of ejaculate volume and density
- Then sample is viewed using a microscope to assess sperm motility and the number of abnormal or damaged sperm.

5. Libido and serving assessment

- watching the bull's behavior to allow an assessment to be made of his libido (how keen he is to serve cows) and of his ability to serve.
- It is particularly important during this part of the examination to watch carefully for any **penile deviations** which may **prevent intromission** (the penis entering the vagina) and for evidence of an ejaculatory thrust.

- **Circumstances should be taken in to account**
 - Boars and Dairy bulls, will often be willing to mount females that are not in estrus, or even to mount other males, castrates or dummy animals.
 - Rams and beef bulls, although usually of high inherent libido, commonly refuse to mount an estrous female in the presence of a **human observer**
 - Animals that are **stressed by recent transport** are also often unwilling to mount straight away

- **Libido test** - a single pre-stimulated bull is introduced to one restrained heifer. Libido is scored according to the number and vigour of mating attempts (in 5 - 10 minutes and Scored 0 - 10 or other).

Table 11.1 Libido score for bulls

Score	Behaviour
0	Bull shows no sexual interest in presence of cow in oestrus
1	Passing interest only, sniffs perineum, no attempt to mount
2	Interest in cow shown on more than one occasion
3	Active pursuit of cow with persistent sexual interest, no mounting
4	Single mount/mounting attempt but no service
5	Two mounts/mounting attempts but no service
6	More than two mounts/mounting attempts but no service
7	Single service, no further sexual interest
8	Single service with continued sexual interest including mounting
9	Two services, no further sexual interest
10	Two services followed by persistent sexual interest including mounting

- **Serving capacity test** - four to six bulls are admitted to restrained, estrogenized heifers and the **numbers of mounts and effective services** are recorded
- bulls' performance in the **two tests is highly correlated**, so they have now been combined into a single test: **Service test** - takes in to account the **type and age of animals** under examination

Reasons for failing BSE

- Immaturity,
- Poor body condition
- Lameness
- Health status
- Penile damage or deviation
- Inadequate testicular size

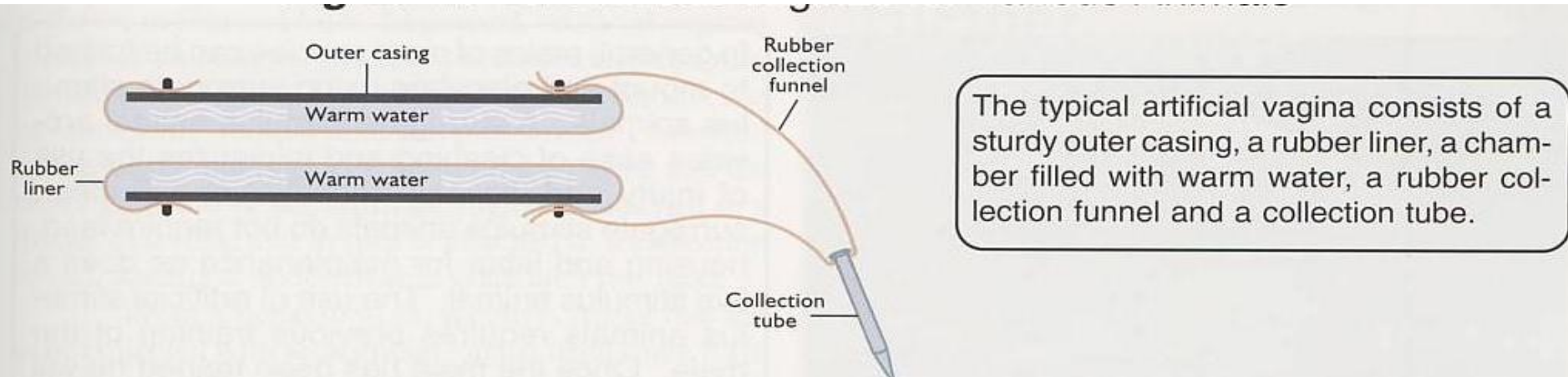
2. Semen Collection

Methods of semen collection

1. Artificial vagina-cattle, horse
 2. Electro-ejaculator -Shoats, wild animals
 3. Gloved hand masturbation-dogs etc...
 4. Post-mortum semen collection-all spp
- ⌘ Collect from tail of epididymis can be used in any species – experimental

Cont...

- **Sperm collection by Artificial Vagina**



- The AV consists of a strong **outer rubber cylinder** containing a **latex liner**. At one end of the AV a **latex extension cone** carrying a **graduated collecting tube** is attached. The **length of the AV** should be adjusted so that the animal ejaculates into the extension cone, thereby producing semen that is as free as possible of particulate or bacterial contamination. The **space between the outer cylinder and latex liner** is filled with warm water, so that the temperature in the lumen of the AV is **between 45°C and 48°C. (bull)** The main stimulus to ejaculation are the temperature (bull, ram) of the AV and its pressure (stallion, boar) upon the penis. A little inert **non spermicidal**

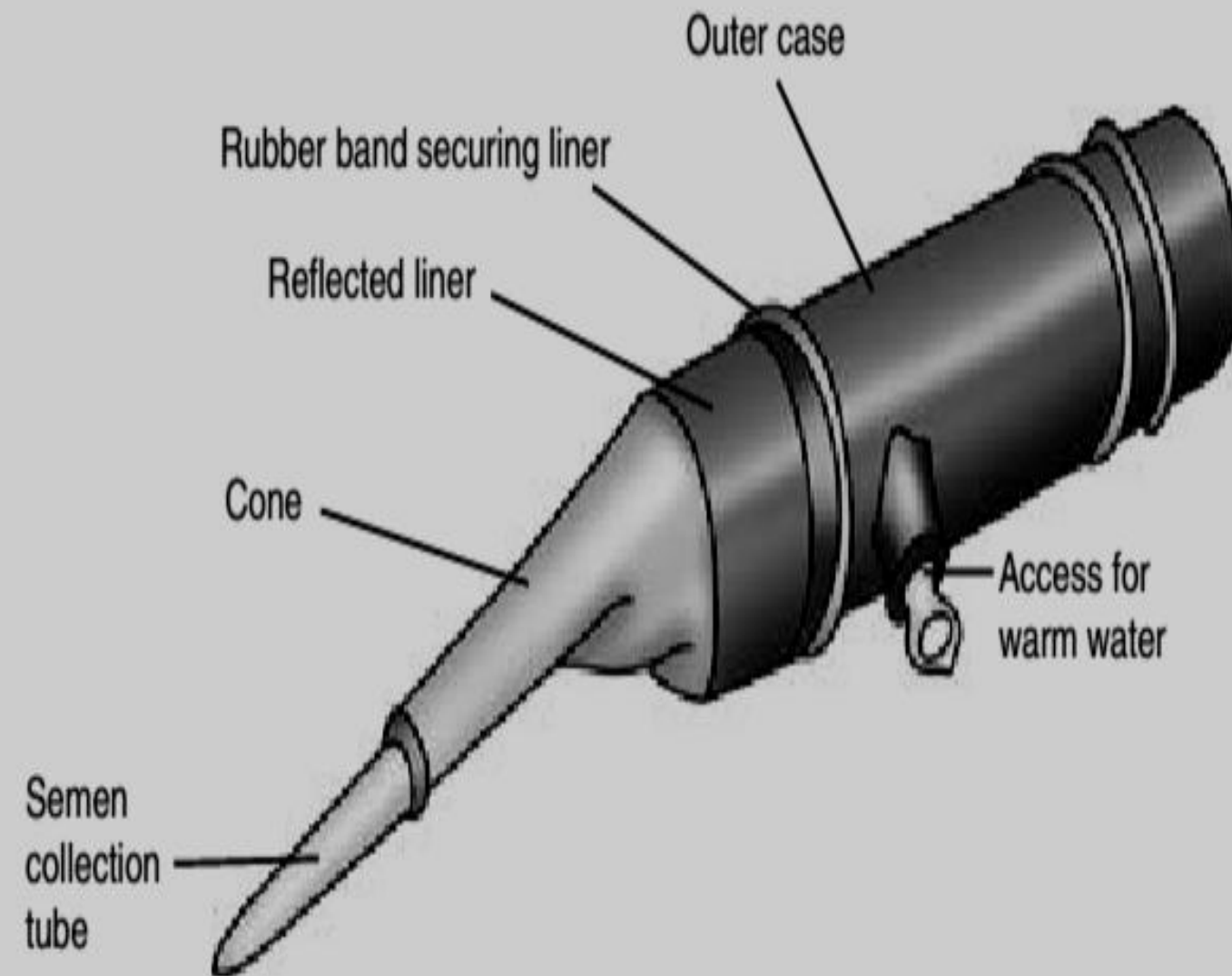


Figure 11.9 Artificial vagina for semen collection from the bull.



The artificial vagina for the stallion consists of a leather outer casing (C) equipped with a port to drain water (arrow). The collection vessel (CV) and the protective covering (PC) are shown. Ideally, ejaculation takes place in the collection cone (CC) so that most of the semen will drain directly into the collection vessel. (Artificial vagina courtesy of Northwest Equine Reproduction Laboratory, University of Idaho, www.avs.uidaho.edu/nerl/)



The artificial vagina for the bull consists of a black casing (C), a rubber liner (RL), a collection cone (CC) and a collection vessel (CV). Water is placed between the casing and the liner. The proper temperature is critical for successful ejaculation in the bull. While not shown in the photograph a protective covering is placed over the cone and collection vessel to prevent cold shock of the semen.



The artificial vagina for the boar consists of a bulb that can apply pressure to the artificial vagina. High pressure is obligatory for stimulation of the glans penis and ejaculation in the boar. The artificial vagina for the boar also consists of an outer casing (C), a liner (L) and a protective covering (PC) that houses the collection vessel. (Photograph courtesy of Minitüb Germany, www.minitüb.de)



The artificial vagina for collection of semen from rams and bucks consists of a rubber casing (C) with a valve (arrow) through which water can be added or emptied, a rubber liner and a collection vessel (CV). The protective covering (PC) is shown above the artificial vagina. (Photograph courtesy of Minitüb Germany, www.minitüb.de)

In general, males of most species can be trained to mount and ejaculate using surrogate stimulus animals. A surrogate stimulus animal provides ease of cleaning and minimizes the risk of injury and disease transmission. Further, surrogate stimulus animals do not require feed, housing and labor for maintenance as does a live stimulus animal. The use of artificial stimulus animals requires previous training of the male. Once the male has been trained he will generally mount the "dummy" readily. The size can be adjusted easily to accommodate various males. Mobile surrogate stimulus animals are used for collection of semen in bulls because the location can be changed with ease.



- **Procedure in bull - similar in other species**

1. Safely restrain the estrus female or dummy
 2. Lead bull to female/dummy but don't let it mount (repeat few times)-penis erection begins and dripping of accessory secretion - good semen
 3. Finally the bull is led up to the cow for mounting with the collector standing to the right of the shoulder of the bull
 4. Small preliminary thrusts occur as the bull locates the vulva and during this, **collector grasps prepuce (not penis) with the left hand and deflects the penis to the right of the hindquarters of the cow, allowing it to find the entrance to the AV.**
 5. The bull will then normally make the ejaculatory thrust into the AV
- The entire procedure must be carried out quietly and methodically, keeping the bull under continual careful observation

- **Sperm collection by Electroejaculation**

- 1) Ram semen is most commonly collected by this method
- 2) EE is placed into the rectum and is used to stimulate the **sacral plexus, hypogastric nerve and parasympathetic outflow via the pudendal nerve**
- 3) Apply **low initial voltages and progressively increase up to the threshold needed for ejaculation**
- 4) **Risk of hind limb stiffening** due to stimulation of the sciatic and obturator nerves





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Semen examination

- Semen samples vary from individual to individual and from ejaculate to ejaculate
- These variable parameters are:-

1. Volume

Each spp of domestic animals have normal volume range

Ex, Cattle = 10 ml

Horse = 75 ml

Pig 200 ml

Sheep/goat = 1 ml

2. Contamination/color of semen

- Normal color for spp
- Ex, Cattle creamy white
Horse yellowish white

Abnormal constituents/contaminants →
blood, pus, tissue debris, etc

Examination should be done both
macroscopically and microscopically

3. Sperm motility

- Motility of sperm is evaluated in two ways
- **A. Mass motility** – Examine the edge of a drop of semen under the microscope and score swirling movement of semen under low magnification.

Ex, 0 = No visible movement

1 = Slight shimmering (tail moving but no progressive movement)

2 = Slow swirling bands of high opacity and low amplitude (poor progressive movement)

3 = Moderate swirling bands of high opacity (moderate progressive movement)

4 = Rapidly swirling bands of high opacity and high amplitude (excellent progressive movement)

- B. Individual sperm motility:** Dilute the sperms and estimate the proportion of sperms that progressively move across the field of vision under medium objective/magnification.
- Sperms that quiver on a single spot are not moving → this shows Brownian motion.

Determination of Proportion of motile sperm-

Here the proportion of immotile sperms give a rough estimate of the proportion dead sperms.

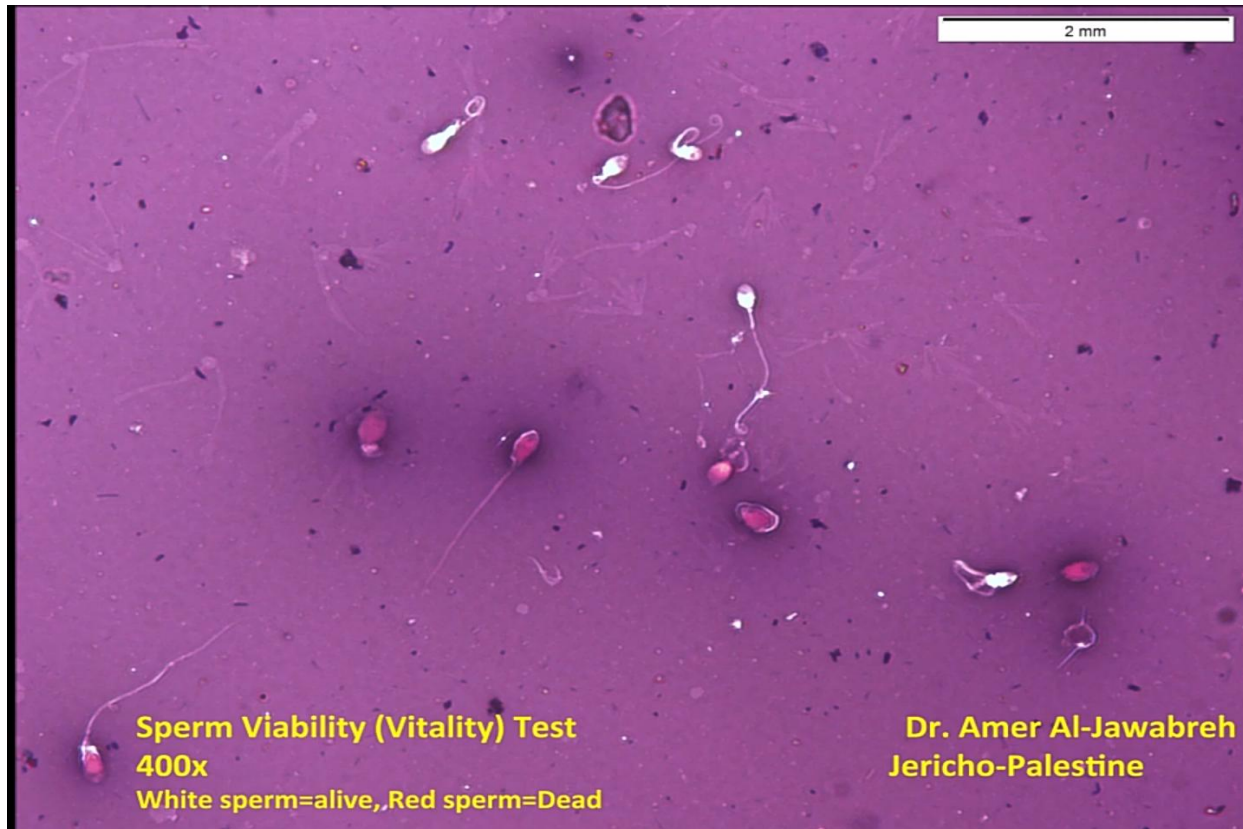
- The incidence of dead sperms low in fresh semen
- May rise after prolonged sexual abstinence
- Higher in semen of males with lower fertility
- An estimation of proportion of dead sperm is made using vital stains
- Negrosin-oesin staining technique
 - \ Oesin stains only dead sperms

Semen examination

- Assessment of abnormal sperm cells
- Sperms have a characteristics form and size
- Variation from **typical pattern** affecting all parts may occur
- This severely reduces fertility.
- Some atypical sperms maybe active and not detectable on **sperm motility test**

Semen examination

- Method:- Re-examine the **negrosin-oesin** smears and count the number of morphologically atypical sperms in a total of 100 sperms



Semen examination

Other methods include :-

Assessment of acrosomal damage

- Make a smear of diluted semen
- Air dry the smear
- Fix in buffered formol
- Geimsa stain for 90 minutes
- Examine under 100 objective

Semen extension

- → dilution of semen to certain concentration or cells/ml
- It serves to reduce the cell concentration to workable values → a number of inseminations from a single ejaculate
- A semen **diluents** or **extender** is also necessary to for **semen preservation**

Semen extension

A semen extender:-

- Provides an energy substrate
- Stabilizes pH
- Provides osmotically controlled environment (buffering)
- Protects against cooling damage

Semen extension

Most diluents thus contain:-

- Salt
- Sugars
- Buffer
- Egg yolk/milk → protect chilling injury
- Glycerol → protect chilling injury
- antibiotics

Semen Cryopreservation

- After dilution at ambient temperature semen should be cooled over 1-2 hours to 0-5°C
- Glycerol may be added at ambient of at 5°C
- Equilibration of sperm with glycerol holding time leads to membrane modification for optimum cryo-survival

Semen Cryopreservation

Semen packing

1. Pellet method:- Small drop of diluted semen is frozen on the surface of liquid CO₂ (dry ice). Cooling rate is 20-30°C/min → maximum 100°C and pellet volume is 0.3-0.8 ml. Good for pigs.

2. Straw method:- Use of plastic (polyvinyl) straws with a length of 133 mm and internal diameter of 2.5 mm (0.5 ml) or 1.7 mm (0.25ml).

Semen Cryopreservation

Freezing rate:- For peak survival cool at the rate of 10-60°C/min.

- At the rate of 50°C is good.
- The critical range of temperature is from 0°C to -45°C
- Below -45°C cooling can be more rapid and it is possible to plunge the straws into the liquid N₂ at -50°C instead of N₂ vapor.

Semen Cryopreservation

Storage:-

- Straws and pellets should remain in the liquid N₂.
- Small sample volume and large surface area lead to rapid change in temperature.
- **Thawing**
- Straws and pellets are thawed by immersing them into water bath at 37-50°C for few seconds.