

Semen Collection

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Methods of semen collection

1. Artificial vagina-cattle horse
2. Electro-ejaculator -Shaots, wild animals
3. Gloved hand masturbation-dogs etc
4. Post-mortum semen collection-all spp
5. Manual expression-birds rabbits ets

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

Semen examination

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

Semen examination

- **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)

Semen examination

- 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)

Semen examination

- Other methods include :-
- **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.

Semen examination

- 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

Semen examination

- An estimation of proportion of dead sperm is made using vital stains
- Negrosin-oesin staining technique
 - Oesin stains only dead sperms
 - Negrosin provides a dark and suitable background to view pale and pink 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.