

Semen processing (Semen Handling)

Including:

1. Dilution (Extension).
2. Package of semen.
3. Preservation (Storage).
4. Insemination (Usage).

1. Dilution or Extension:

It is addition of suitable media to the semen for certain reasons:

- a. Increases the semen volume leading to inseminating larger number of females.
Note: each inseminating dose should contain at least 12×10^{12} progressive motile sperms.
- b. Supply proper substances to protect spermatozoa from sudden variations in temperature, also nutrients supplements.
- c. Improving spermatozoa fertilization ability especially by decreasing capacitating time.

Capacitation of sperms: it is cellular changes that spermatozoa undergo in the female reproductive tract that are necessary before the acrosome reaction and fertilization can take place.

✓ Diluters or extenders constituents and properties:

- i. Should be isotonic solution with semen (e.g.; Sodium Citrate).
- ii. Has buffering capacity (e.g. Sod. Citrate; Tris....etc).
- iii. Able to protect the sperm from cold shock injury during the cooling (Lecithin and lipoproteins derived from egg yolk).
- iv. Must be providing nutrient agents (e.g.; egg yolk, milk, sugars....etc).
- v. Protect semen from microbial contamination by involving some antimicrobial agents (e.g.; gentamycin, tylosin, and pen-strept....etc).
- vi. Sperms should be protected from freezing and thawing effects by adding glycerol to the media.

Extenders components

Each extender used in semen dilution should be composed of two portions; which are **certain** and **additive** components.

A. **Certain components;** including the followings:

- 1) **Water** to dissolve the solid materials of diluters.

- 2) **Source of energy**; e.g.: monosaccharide, aminoacids, and glycerol. Monosaccharide's served as energy source while disaccharides synergized with glycerol to protect sperms from cold shock injury.
- 3) **Isotonic solutions and Buffers**; e.g. Sod. Citrate, phosphate, and Tris this materials aid to prevent pH changing, breaking of solid lipid granules from egg-yolk and facilitate transportation of sperms in female genital tract (swimming media) and protect sperms from heavy metallic ions injuries (Mg & Ca⁺⁺).
- 4) **Organic compounds**; Lecithin and lipoproteins protect sperms cell membrane integrity during semen processing, lecithin and lipoprotein majorly derived from egg-yolk. Egg-yolk concentration should be around and not rather 20% because it contain also phenyl alanine and tyrosin amino acids which have toxic effects on spermatozoa.

B. Additive components; including the following:

- 1) Antibiotics.
- 2) Enzymes; **Catalase** to protect sperms from yielded H₂O₂ produced from amino acids lysis and **Amylase** to decrease capacitating time required.
- 3) Oxytocin hormone; increases contraction of female genital tract muscles to facilitate reaching of sperms to fertilization site (ampulla).
- 4) Vitamins; like Vitamin B1 synergized with antibiotics action.

Factors affecting dilution rate calculation:

- ✓ Species of animal; diluting rate of **bull** semen ranged from **1:15 to 1:50** while for **ram and buck** semen ranged from **1:3 to 1:5**.
- ✓ Semen properties; progressive motility percentage and sperm concentration.
- ✓ Inseminating dose.
- ✓ Temperature of semen preservation. In deep-freezing preservation with optimum conditions approximately 20 – 30% of sperms die.

2. Package of semen or semen canning:

The common forms of semen packaging are; **Straw, Ampoule, Vial, Tube and Pellet**. Each package should be clean, dried and sterilized before using and label with some data such as, name of producer center, country of origin, name of bull, date of manufacturing, breed of bull.....etc.

3. Semen preservation:

Semen can preserve in **three ways** in order to use in artificial insemination which are:

- a. **Above freezing point (4 – 37 C°):** diluted semen preserved at **20 – 37 C°** should be used within **few hours** and may extend to **24 hours** post incubation. While when preserve the diluted semen in refrigerator at **4 – 5 C°**, it may remain with acceptable fertilization rate about **3 – 7 days**. The lowering of temperature should be gradually not suddenly to prevent cold shock injury.
- b. **Below freezing point (Deep-freezing):** term of deep – freezing is a preservation of diluted semen in alcohol, dry ice (solid CO₂; -79C°) and liquid nitrogen (-196 C°).

Note: The sudden changes in temperature degrees of semen has adverse effect on the spermatozoa viability (cold-shock), so presence of lipoproteins, lecithin (from egg-yolk) and glycerol avoid this shock.

➤ **Steps of deep-freezing technique**

- I. Dilution of crude semen.
- II. Cooling of diluted semen from 37 C° into 5 C° gradually.
- III. Addition of Glycerol (5 – 7%) to the diluted semen at 5 C°, which either in one-step or two-steps dilution methods during equilibrium period.
Equilibrium period: it is a required time to equal the concentration of glycerol in both sides of sperm cell membrane (in and outside), at 5 C° temperature, the time required is about 2 – 4 hours. During this period semen packed in straws by semi or full-automated filling machine.
- IV. Transfer of packed semen to freezing in liquid nitrogen until requested (for years).

- c. **Dry freezing by chemicals:** either by drying in vacuum following slow freezing or by dry freezing in Freon and Heptane mixture.

4. Usage of semen: liquid semen (cooled-semen) can use immediately to insemination by usage of intra-uterine catheter by same way of uterine treatment.

In case of deep frozen semen, especially in straw form, semen should be thawed prior to inseminating by specific inseminating-gun.

Thawing: it is a process of re-solute of frozen semen for using either in insemination or evaluation, perfect thawing should took place at 37 C° for about 45 – 120 seconds. Factors affecting thawing time are; volume of semen package and temperature of thawing media.