ANDROLOGY

LECTURE-4

METHODOLOGY OF DEEP FREEZING OF SEMEN

Advantages of Frozen Semen:

- Maximum utilization of semen from a sire
- Long-term preservation, even after bull's death
- Easy international transport
- Eliminates need for maintaining breeding bulls

Ideal semen extender should have the following characteristics:

1) **Isotonic**: The extender should be isotonic.
2) **Contains essential minerals**: The extender should contain minerals to maintain sperm viability.
3) **Contains energy sources**: The extender should contain energy sources like glucose and fructose.
4) **Contains cyroprotective agents**: The extender should contain agents like glycerol, dimethyl sulfoxide (DMSO), dimethyl acetamide (DMA), and ethylene glycol.
5) **Contains substances to eliminate metabolic residues**: The extender should contain substances like buffers and tris to eliminate metabolic residues.
6) **Preserves sperm membrane integrity**: The extender should preserve the plasma membrane integrity and acrosome membrane integrity of spermatozoa.
7) **Contains antibiotics**: The extender should contain antibiotics like penicillin and streptomycin to control bacterial contamination.
8) **Maintains pH**: The extender should maintain a pH of 6.8-7.2.
9) **Contains antioxidants**: The extender should contain antioxidants to reduce oxidative stress.
10) **Provides anti-freezing shock**: The extender should provide anti-freezing shock.

Cryoprotectants, such as glycerol, play a crucial role in **protecting spermatozoa during freezing and thawing** by preventing the formation of damaging ice crystals.

I. **Water Removal:** Glycerol penetrates the sperm cells, allowing some intracellular water to escape. This reduces the total amount of water inside the cell, which is important because less water means fewer opportunities for ice crystals to form during freezing.

II. **Ice Crystal Formation**: When freezing occurs, the remaining water inside the cell freezes into smaller ice crystals instead of larger ones. Smaller ice crystals are less likely to puncture and damage cell membranes, thus preserving cell integrity.

III. **Lower Freezing Point**: Cryoprotectants lower the freezing point of the solution, which helps prevent ice formation at higher temperatures and allows for a more controlled freezing process.

IV. **Hydrogen Bonding:** Cryoprotectants form hydrogen bonds with water molecules, limiting their movement and further minimizing the risk of ice crystal formation both inside and outside the cells.

V. **Vitrification:** Instead of forming ice, cryoprotectants can help create a glassy state (vitrification) during freezing. This transition minimizes cellular damage by maintaining a stable structure without crystalline ice formation.

Cryoprotectant helps in Thawing

1. Osmotic Balance: Cryoprotectants help manage osmotic pressure during thawing. They allow for a controlled release of intracellular water, preventing osmotic shock that can occur when cells rapidly swell or shrink.

2. Antioxidant Properties: Some cryoprotectants also possess antioxidant properties, which reduce oxidative stress and reactive oxygen species (ROS) that can cause additional damage during thawing.

Preparing Tris-Egg Yolk-Glycerol Diluent

Tris (tris(hydroxymethyl)aminomethane) is a widely used organic compound in biochemistry and molecular biology, primarily known for its role as a buffering agent.

1. Prepare Buffer Solution:

•Dissolve Tris base in distilled water(1 litre)

•Adjust **pH** to between 6.8 and 6.9 using hydrochloric acid

2. Sterilize Buffer:

Autoclave the buffer at 100°C for 15 minutes.

Allow the buffer to cool to room temperature.

3. Prepare Diluent:

•Combine 1 liter of cooled buffer with 200 ml of egg yolk.

•Add antibiotics as per schedule and glycerol in the appropriate amount.

4. Mix and Check pH:

•Mix thoroughly and check that the pH is between 6.7 and 6.8.

5. Storage:

Transfer the diluent into sterile containers and label accordingly for storage.

Semen Collection Procedure

- Collection done in the morning for optimal quality
- Bulls washed, dried, and prepuce cleaned
- Semen collected using an artificial vagina (AV)
- 20-minute refractory period after each collection for second collection

Evaluation of Semen

- After collection, semen is evaluated for:
  - Volume
  - Color and consistency
  - Presence of foreign matter
  - Mass activity (scored on +1 to +5 scale)
- **Concentration**: Estimated using photometer or calorimeter
- Diluted with diluent (1:1 ratio)
- **Motility**: >70% initial motility required for further processing

Minimum Requirements for Fresh Semen:

- Volume: ≥ 2.5 ml
- Concentration: ≥ 500 million sperm
- Initial motility: ≥ 70%

FREEZING AND STORAGE OF SEMEN

1. **Freezing Process**
   - Semen diluted with Tris-egg yolk-glycerol diluent
   - Loaded into straws or pellets
   - Sealed, and frozen by gradual cooling (from 5°C to -196°C)
   - Stored in liquid nitrogen at -196°C
2. **Thawing Process**
   - **Ampoule**: Thawed in warm water, then cut
   - **Pellet**: Thawed in sodium citrate solution (3% at 20°C)
   - **Straw**: Thawed in water bath at 37°C

FILLING AND SEALING OF SEMEN STRAWS

- **Manual Filling Method**:
  - **Tools**: Vacuum pump, filling comb, rubber tube, straw clips, PVA powder.
  - **Process**:
    1. Straws (15 medium/20 mini) clipped together.
    2. Cooled to +5°C in cold handling cabinet.
    3. Semen drawn into straws by vacuum pump.
    4. Air space created to allow expansion during freezing.
- **Manual Sealing Method**:
  - PVA powder used to seal straw ends (4-5 mm penetration).
  - Seal formed by dipping straw ends into the powder.
  - Straws placed in water bath at 20°C for 10 minutes to firm the seal.
- **Machine Filling and Sealing**:
  - Automatic machines like MRS-1, MRS-3, and MRS-5 for large-scale operations.

High capacity (up to 12,960 straws per hour).

PRINTING (IDENTIFICATION) OF STRAWS

- **Printing**:
  - Details (bull number, breed, batch no., ejaculate number) printed on straws.
  - **Capacity**: 11,160 straws per hour.

RACKING

- **Racking Process**:
  - Straws are arranged on a freezing rack at room temperature (20°C).
  - Racking is done swiftly to prevent temperature fluctuations

EQUILIBRATION OF SEMEN

- **Purpose**: Stabilize semen with the diluent before freezing.
- **Conditions**:
  - Stored at +5°C for 4 hours (cattle) or 3 hours (buffalo).
  - Pre-freeze motility checked, semen showing ≥60-70% motility selected for freezing.
  - **Test Freezing**: Done to check semen's freeze-thaw viability.

FREEZING OF SEMEN

- **Freezing Methods**:
  1. **Manual Freezing**:
     - Semen exposed to liquid nitrogen vapour at -130°C to -150°C.
     - Then submerged in liquid nitrogen at -196°C.
  2. **Bio Freezer**:
     - Automatic freezing program controlled by computer.
     - Semen racks placed in bio freezer, cooled from +5°C to -140°C in 9 minutes.

Impotentia Coeundi

Infertility in Males

- **Definition**: Temporary loss of fertility in males characterized by reduced viable spermatozoa.
- **Categories of Infertility**:
  - **Impotentia Coeundi**: Reduced sexual libido & inability to copulate.
  - **Impotentia Generandi**: Inability to fertilize due to pathology of testis, mesonephric duct, or accessory sex glands.

Potency

- **Definition**: Physical capability of the male to perform normal roles in copulation (erection, mounting, intromission, ejaculation).
- **Libido**: Willingness and eagerness to mount and service.
- **Factors Influencing Copulatory Behavior**:
  - Testosterone and CNS mechanisms play a key role.
  - **Sexual Cues**: Visual, olfactory, auditory, tactile cues.
  - **Mating Behavior**: Genetic, modified by environmental and physical factors.

Factors Causing Impotentia Coeundi (Loss of Potency)

- **Environmental Causes**: Impact libido and sexual desire.
- **Injuries**: Joint, muscle, bone, tendon, and nerve injuries.

Diseases: Penis and prepuce diseases.

Environmental Factors Affecting Libido

Factor	Effect on Libido	Details
Nutrition	Reduced sexual desire in malnourished animals	Deficiencies in TDN, vitamins, proteins, phosphorus, cobalt.
Overfeeding/Obesity	Laziness, joint issues, lack of libido	Excessive roughage in bulls and rams may hinder copulation.
Systemic Diseases	Loss of sexual desire due to severe debilitation	Examples: Pneumonia, tuberculosis, severe mange, parasitism.
Age	Young and old animals show reduced libido	Older animals: Testosterone decline, arthritis.
Management	Improper handling reduces libido	Harsh training, poor environment, overuse may affect libido.
Psychic Factors	Mental reluctance to breed	Genetically low sexual desire, spinal lesions may cause impotence.

Joint, Muscle, Nerve, Bone, and Tendon Pathology

CONDITION	SYMPTOMS	IMPACT ON LIBIDO
Coxitis (Hip Inflammation)	Short stride, adduction of limb, rupture of round ligament	Common in dogs and boars, may cause inability to copulate.
Gonitis (Knee Inflammation)	Short, stiff gait, joint capsule enlargement	Common in bulls, inhibits mounting.
Joint Lesions	Pain, reluctance to copulate, often in fetlock/phalangeal joints	Affects libido due to pain during mounting.
Spinal Disease	Stiff gait, pain over vertebrae, unsteady walking, hind limb paralysis	Spondylosis in bulls can lead to reduced libido.
Spastic Syndrome	Muscle spasms, crampiness in hind limbs	Common in certain breeds (e.g., Holstein) affecting copulation.

Prognosis and Treatment for Physical Pathologies

- **Prognosis**: Depends on the severity, species, and age of the animal.
  - **Spinal injuries**: Poor prognosis in large animals.
  - **Hip dislocation**: Poor prognosis.
- **Treatment**:
  - Sexual rest and good footing for injured animals.
  - **Electro Ejaculator** for those unable to copulate.
  - **Surgical treatment** for intervertebral disc prolapse in dogs.
  - **Glucocorticoids** for arthritis treatment.
  - **Spastic Syndrome**: Antispasmodics and tranquilizers.
  - **Regular hoof trimming** for male animals with foot-related issues.

Hormonal Treatment for Libido Issues

- **Testosterone**: Used to improve libido.
  - **Dosage**:
    - **Bull**: 100-500 mg
    - **Stallion**: 100-500 mg
    - **Ram**: 50-100 mg
    - **Boar**: 50-100 mg
    - **Dog**: 10-50 mg
  - Repeated every 5-10 days.
- **Human Chorionic Gonadotrophin (hCG)**: Stimulates testosterone production.
  - **Dose**: 3000-4500 IU for large animals, 100-500 IU for dogs.
  - Administered at 4-7 day intervals.

Condition	Cause/Species Affected	Treatment/Management
Inability to protrude penis	Congenital anomalies (e.g., short penis, retractor muscle issues)	Examination, electroejaculator for diagnosis.
Persistent frenulum	Hereditary	Surgical removal of tissue band.
Adhesions (Sigmoid flexure)	Bulls, Rams (trauma)	Antibiotics, sexual rest, possible surgical intervention.
Ruptured penis	Bulls (coitus-related injury)	Antibiotics, surgery 4-10 days post-injury, sexual rest.
Deviations of penis (Corkscrew)	Hereditary	Surgical correction, culling recommended.
Phimosis	Narrow preputial opening	Surgical widening of preputial orifice.
Paraphimosis	Penis can't retract	Manual reduction, surgery if needed.
Balanoposthitis	Infections/trauma	Anti-inflammatory meds, antibiotics.
Penile tumors	Benign or malignant growth	Surgical removal if required.
Defects in penis	Congenital or traumatic	Surgical correction based on defect severity.

Summary:

- **Adhesions, phimosis, paraphimosis, and deviations** are often treatable surgically, but many defects (e.g., **short penis**, **hereditary defects**) require **culling**.
- **Bulls with short penis** should be culled to prevent genetic spread.
- **Traumatic injuries** like **ruptured penis** require **prompt treatment** and **sex rest** to prevent long-term damage.
- In cases of **tumors**, **surgical removal** is generally the solution.

Tumors of the Penis and Prepuce

- **Bulls**:
  - *Transmissible fibropapilloma* (virus):
    - Single/multiple, firm, cauliflower-like growths
    - Common in bulls (9-18 months)
    - Causes: Injury during mounting, hemorrhage, refusal to copulate
    - *Treatment*:
      - Spontaneous recovery in 4 months
      - Wart vaccine
      - Semen from affected bulls should not be used
      - Surgical removal under pudendal nerve block

- **Stallions**:
  - *Squamous cell carcinoma*: Low malignancy
  - *Differentiation*: Granulomas due to *Habronema* larvae
  - *Treatment*: Surgical removal or amputation if needed

•Commonly affects the eyes, particularly the limbal conjunctiva (the border between the cornea and sclera).
•Other frequent sites include the genital areas, such as the sheath around the penis, and the lips, nose, and anus.

- **Dogs**:
  - ***Transmissible venereal tumor (TVT)*:**
    - Spread by coitus
    - Incubation: 5-6 weeks
    - *Treatment*:
      - Surgery (early intervention)
      - Vincristine: 0.025 mg/kg IV, repeat weekly

Impotentia Generandi

- **Definition**: Reduced ability to fertilize; normal sexual desire and ability to copulate, but failure of fertilization or early embryonic death in females.

Categories of Impotentia Generandi

- **Normal Semen Production**: Infertility despite normal sperm production.
- **Abnormal Semen Production**: Sperm production is abnormal, leading to infertility.

Impotentia Generandi with Normal Semen Production

- **Causes**:
  - **Brucellosis, Vibriosis, Trichomoniasis, IBR-IPV Virus, Mycoplasma**: Normal semen but causes infertility in females (failure of fertilization, early embryonic death).
  - **Abnormal Acrosomes** (Knobbed spermatozoa): Caused by defective spermatogenesis due to issues with the Golgi apparatus.
  - **Acrosomal Defects**:
    - Detected using Nigrosin-Eosin, India ink, or Giemsa staining.
    - Affects sperm's ability to penetrate and fertilize the ovum.
  - **Cytogenetic Studies**: Genetic/chromosomal defects in sperms at meiosis may cause infertility.
  - **DNA Content**: Infertile bulls may have lower DNA content in sperm nuclei than fertile bulls.

Impotentia Generandi with Abnormal Semen Production

- **Causes**:
  - **Sufficient sperm quantity not deposited properly during coitus**.
  - **Acrosomal Defects** in spermiogenesis, e.g., autosomal recessive defect in Friesian bulls.

Pathology of Testis

- **Common Testicular Pathologies**:
  - **Testicular Hypoplasia**: Congenital/hereditary, affects sperm production.
  - **Testicular Degeneration**: Acquired, impacts spermatogenesis.

Hereditary Defects of Spermatozoa

- **Defects Include**:
  - **Dag Defect**: Coiled, folded, or split tails. Lowered fertility, confirmed by electron microscopy.
  - **Returned Tails and Narrow Heads**: Genetic defect in Jersey bulls, lowers motility.
  - **Knobbed Acrosome**: Enlarged acrosomal cap (6-8 times normal size). Causes sterility.
  - **Corkscrew Defect**: Midpiece of sperm appears like a corkscrew. Seen in aged bulls; due to testicular degeneration.
  - **Diadem Defect**: Small red-colored spots along sperm nuclear ring; motility not impaired but fertility reduced.
  - **Pseudodroplet Defect**: Irregular thickening in mid-piece of sperm. Lowered fertility.
  - **Decapitated Sperm**: Loose heads and tails in >50% sperm; affects fertility in Guernsey breed.

Inbreeding, Hybridization, and Intersexes

- **Inbreeding**:
  - Results in reduced fertility and increased abnormal seminiferous tubules.
  - Cytogenetic disturbances (e.g., sticky chromosomes, giant cells).
- **Hybrids (Mule Infertility)**:
  - Hybrid infertility occurs when parent species have significant chromosomal differences (e.g., mule - horse and donkey cross).
- **Free martinism**:
  - Infertility in male cotwins of female freemartin. Testicular changes may occur.
- **Intersexes**:
  - **Male Pseudohermaphroditism**: Common in goats, pigs, occasionally in cattle, horses.
  - **True Hermaphroditism**: Rare in domestic animals.
  - **Male Tortie Cats**: XXY chromosomal constitution, sterile.

Tumor Type	Age Range	Clinical Features
Seminomas	>7 years	Slow growth, soft, possible hemorrhage
Sertoli Cell Tumors	Older dogs	Feminization, gynecomastia, hair loss, testicular atrophy
Leydig Cell Tumors	Older dogs	Small, benign, produce androgens

Testicular Tumors in Dogs

Maceration in Pregnancy

Definition: Maceration refers to the breakdown of fetal tissues that occurs when a fetus is retained in the uterus for an extended period after abortion or stillbirth.

Key Points:

1. Timing: Maceration typically occurs during the second half of pregnancy, often as a result of abortion or stillbirth.

2. Causes: It can happen when there is a failure to expel the fetus due to uterine inertia (lack of contractions) and intrauterine infections.

3. Pathophysiology:

I. Bacteria can enter the uterus through a dilated cervix, leading to infection.
II. This results in fetal emphysema and tissue breakdown, where soft tissues are digested through putrefaction and autolysis, ultimately leading to a mass of fetal bones within the uterus.

4. Symptoms: Affected individuals may not exhibit severe systemic illness but may experience:

I. Febrile condition (fever).
II. Anorexia (loss of appetite).
III. Depression.
IV. Vague signs of intermittent straining.
V. Foul, purulent vaginal discharge containing small bones.

5. Prognosis for Fertility: The prognosis for future fertility is very poor following maceration.

6. Treatment: Induction of parturition is necessary and can be achieved using:

I. **Valethamate bromide (Epidosin):** Administered at 80 mg IV.
II. **Double prostaglandin injection (Inj Vetmate**): Administered at 500 µg IM.
III. This is followed by manual removal of the fetus after cervical dilation.

Mummification in Pregnancy

Definition: Mummification refers to the process where a dead fetus remains in the uterus and undergoes dehydration, leading to the formation of a mummified mass.

Types of Mummification

1.Papyraceous Type:

Characterized by shriveled, dried, parchment-like fetal membranes.

Fluids are absorbed, and the uterus contracts around the fetus, molding it into a dry, contorted mass.

2.Hematic Type (more common in bovines):

Involves the accumulation of a chocolate-like blood mass between the uterus and chorion (including the fetus), usually occurring during the last trimester of pregnancy.

Causes

I. Genetic or chromosomal abnormalities
II. Placental defects
III. Infectious agents
IV. Abnormal hormonal concentrations
V. Drugs
VI. Compression or torsion of the umbilical cord
VII. Uterine torsion

Diagnosis

I. Transrectal Palpation: Manual examination to assess uterine contents.
II. Ultrasonographic Examination: Imaging technique to visualize the fetus and membranes.

Treatment

•Administration of PGF2α (Prostaglandin F2 alpha) injection to lyse the corpus luteum, which results in the expulsion of the mummified fetus within 2 to 4 days.

Aspect	Mummification	Maceration
Cervical Status	Cervix is closed, preventing bacterial entry.	Cervix is open, allowing bacteria to enter.
Hormonal Environment	Maintained by a persistent corpus luteum (CL) producing progesterone.	Hormonal changes lead to decreased progesterone levels; the CL may regress.
Fetal Tissue Condition	Fetal tissues become **shriveled and dry**, appearing parchment-like due to fluid absorption.	Fetal tissues undergo putrefaction, becoming soft and decomposed due to bacterial activity.
Presence of Infection	Generally occurs in a sterile environment; no infection present.	Often associated with infection (e.g., septic metritis) due to open cervix.
Fluid Accumulation	Minimal to no fluid accumulation in the uterus; the uterus appears small and dry.	Significant fluid accumulation due to necrotic tissue and infection, leading to a larger uterine size.
Clinical Signs	No foul-smelling discharge; may have irregular, inert fetal mass.	Foul-smelling mucopurulent discharge is common, indicating infection.
Pathological Process	Involves desiccation and drying of tissues without decomposition.	Involves decomposition of tissues through bacterial action, leading to necrosis.
Species Affected	Commonly observed in various species, including cattle, goats, dogs, and cats.	Also seen in multiple species but often noted in cattle and dogs.