ANDROLOGY

LECTURE-3

Semen Collection in Bulls

Introduction to Semen Collection

- Proper and clean collection of semen is critical in artificial insemination.
- Key goals: collect microbe-free semen with spermatozoa of good vigour, resistance, and livability.
- Quality of semen depends on the bull and the collection method.

Semen Collection Methods in Bulls

1. Collection from Vagina

2. Ampullary Massage Technique

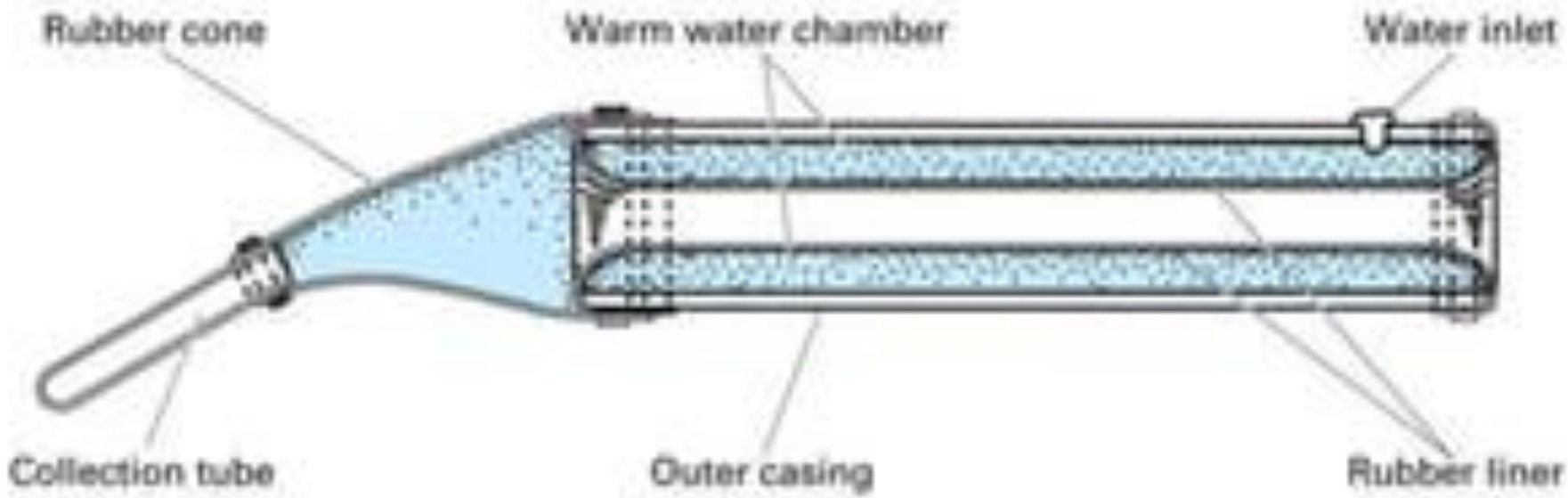
3. Electroejaculation Method

4. Artificial Vagina (AV) Method

Method	Advantages	Disadvantages
Vaginal Method	Simple, low-cost method	Poor semen quality, risk of contamination.
Ampullary Massage	Useful for bulls with low libido or physical incapacity	Poor concentration, requires skill, possible inflammation.
Electroejaculation	Useful for bulls with low libido or unable to mount	Painful, lower semen quality, higher contamination.
Artificial Vagina (AV)	Best quality semen, most natural simulation	Requires skilled personnel, can be affected by temperature.

Parts of Artificial Vagina (AV)

PART	DESCRIPTION
Rubber Hose/Cylinder	Outer tube of the AV (varies in size based on species).
Inner Liner	Inserted inside the rubber cylinder to form a smooth surface.
Director Cone	Guides semen into the collection vial.
Collection Vial	Holds the collected semen.
Insulation Bag	Prevents semen shock due to temperature variations.


Rubber cone
Warm water chamber
Water inlet
Collection tube
Outer casing
Rubber liner

Species	Artificial Vagina Hose	Artificial Vagina Liner	Artificial Vagina Cone
Cattle	Length: 35 cm	Length: 54 cm	Length: 24 cm (Upper: 5.0 cm, Lower: 1.5-2.0 cm)
Buffalo	Length: 30 cm	Length: 54 cm	-
Stallion	Length: 54 cm	Length: 30 cm	-
Sheep	Length: 20 cm	Length: 30 cm	-
Goat	Length: 15 cm	Length: 30 cm	-

Semen Collection in Stallion:

1.Vaginal Method:
The stallion mounts a mare, and a rubber tube is used to collect semen from the vagina into a sterile container.
2.Tail-End Sample:
Similar to the vaginal method, with semen collected into a sterile bottle.
3.Condom/Breeder's Bag:
A sterilized rubber bag covers the erect penis. After ejaculation, semen is transferred to a pre-warmed sterile tube for processing.
4.Artificial Vagina (AV):
The most effective method, using specially designed models like Missouri or Colorado. AVs are adjusted for temperature **(45-48°C)** and pressure to ensure semen quality while maintaining hygiene. Semen collection is optimized at the stallion's Daily Sperm Output (DSO), stable after 7-10 days of regular collection.


High transparency
durable no leakage

Semen Collection in Boars:

1. Gloved Hand Method

2. Artificial Vagina:

a. Length: 12.5 cm, diameter: 4.5 cm
b. Inner liner diameter – 40 cm
c. Temperature of AV: 45-50°C
d. Ejaculation continues for 15 mins
e. Sperm rich fraction present in 3^{rd} or 4^{th} minute

3. Electro-ejaculation method:

a. Electric current (12-20 V, 5-10 sec interval) passed through rectum
b. Ejaculate is heavily contaminated

Semen Collection in Dogs:

1. Digital pressure/massage

a. Ejaculation in three fractions:
   i. First fraction – clear fluid with few sperms
   ii. Second fraction – Actual semen
   iii. Third fraction – Only prostatic fluid
b. Different fractions collected in different digital cups

2. AV method

a. Length of AV – 19-20 cm, Diameter – 5 cm
b. Temperature: 40-42°C

Semen Evaluation

Semen Evaluation and Quality Assessment for Artificial Insemination

Introduction to Semen Evaluation

- **Semen evaluation** is not the sole indicator of bull fertility.
- **Suitability for artificial insemination** depends on multiple semen characteristics.
- Semen evaluation provides insight into sexual function and fertility potential.
- **Semen cannot be evaluated based on a single trait**—multiple factors must be assessed.
- **Semen tests** are essential for assessing semen quality before use.
- **Semen**: A suspension of spermatozoa in seminal plasma, discharged during mating.

Composition of Semen

- **Spermatozoa**:
  - Produced in the **testes** and stored in the **epididymis** (10% of total semen volume).
- **Seminal Plasma**:
  - A mixture of secretions from **seminal vesicles**, **Cowper's glands**, **prostate**, **ampullae**, and **epididymis**.
  - **Seminal vesicle secretions** constitute **55%** of total semen volume.
  - Nourishes sperm cells during ejaculation.

Factors Affecting Semen Characteristics

- **Breed** of the bull.
- **Frequency of semen collection**.
- **Age** and **health** of the bull.
- **Seasonal effects** (e.g., heat stress).
- **Sexual excitement** before collection.
- **Handling and collection techniques**.

Semen Quality and Fertility

- Factors like **aging, cold shock, and preservation** can deteriorate semen quality.
- **Energy sources in spermatozoa** limit their lifespan.
- **Semen examination** helps diagnose genital or testicular conditions.
- **Semen quality** is a key indicator of male fertility.
- **Semen quality variation** is minimal between ejaculates of the same bull.

Semen Storage and Handling

- **After collection**, place semen in a **water bath at 37°C**.
- Avoid **overheating**, **rapid chilling**, or **shaking** semen.
- Prevent **sunlight exposure** to maintain semen vitality.

Precautions to Maintain Semen Quality

1. **Cleanliness**: Ensure **AV (artificial vagina)** and collection containers are free of contaminants (e.g., alcohol, petroleum jelly, antiseptics).
2. **Pre-Collection**: Keep **dirt** and **urine** out of the artificial vagina.
3. **Post-Collection**: Place semen in a **37°C water bath** immediately.
4. **Temperature Control**: Avoid **overheating** or **rapid cooling**.
5. **Avoid Excessive Shaking**: Prevent damage to spermatozoa

MACROSCOPIC SEMEN EVALUATION

- **Parameters:**
  - Volume
  - Colour
  - Consistency/Density
  - Presence of Foreign Materials
  - Gross Motility

- **Volume Determination**: Measured immediately after collection.
- **Factors Affecting Volume**:
  - Species
  - Individual variation
  - Age, body size, and reproductive health
  - Frequency of service
- **Increased Volume**: Seen with age up to 6-8 years.
- **Decreased Volume**:
  - Young males
  - Overused males
  - Incomplete ejaculation
  - Bilateral seminal vesiculitis

Note:

- **Aspermia**: No sperm
- **Hypospermia**: Reduced sperm volume
- **Hyperspermia**: Increased sperm volume

Semen Volume by Species:

Species	Average Volume (ml)	Range (ml)
Bull	4	1-15 (2-8)
Stallion	70	30-250
Ram/Buck	1	0.7-3
Boar	250	125-400
Dog	10	1.25-30
Cat	0.04	0.01-0.12
Fowl	0.75	0.25-2.0
Man	2-6	-
Elephant	50-100	-

COLOUR OF SEMEN

- **Normal Colour**:
  - **Bull/Buck**: Milky white, creamy, opaque.
  - **Buffalo**: Whitish (lighter than bull semen).
  - **Stallion/Boar/Dog**: Pearly white to grey, translucent.
- **Deviations**:
  - **Yellow**: Normal in some bulls (due to riboflavin).
  - **Brownish**: Orchitis (blood pigments).
  - **Dark Red/Pink**: Hemorrhage.
  - **Yellow-Green**: Pseudomonas aeruginosa infection.
  - **Light Brown**: Dung contamination.
  - **Dull White**: Increased spermatogenic cells.
  - **Yellow**: Presence of urine.

- **VISCOSITY AND DENSITY**
- **Viscosity**: Depends on sperm concentration.
  - High concentration → Creamy, viscous.
  - Low concentration → Watery, less viscous.
- **Specific Gravity of Bull Semen**: 1.036 (positive correlation with sperm concentration).
- **Density Grading**:
- **Pathological Conditions Affecting Consistency**:
  - **Epididymitis**: Less milky semen.
  - **Catarrhal Conditions**: Thick viscous semen.

Colour	Density Grade
Creamy	DDDD
Milky	DDD
Thin Milky	DD
Translucent	D
Watery	O

PRESENCE OF FOREIGN MATERIALS

- **Sources of Foreign Materials**:
  - **Animal**: Dung, pus, urine, hair, dust.
  - **Environment**: Sand, bedding, insects.

AV (Artificial Vagina): Water, lubricant jelly, dusting powder.

GROSS MOTILITY:

- **Assessment**: A drop of semen placed on a warm slide and observed with naked eye for wave motion.

* **Wavy Motion**: Indicates live sperm.

Grading Based on Gross Motility:

- **Acceptable Grades**: 4 and 5.
- **Mass Activity**: Collective movement or wave motion of sperm.

Grade	Description	Percentage of Active Sperm
5	Very vigorous, rapid waves, extreme eddies	90-100%
4	Vigorous, rapid waves, abrupt eddies	70-80%
3	Rapid movement, slow waves	50-60%
2	Oscillatory or rotary movement	30-40%
1	Stationary, weak movements	10-20%
0	Immotile sperm	0%

INDIVIDUAL MOTILITY

- **Assessment**: Movement of individual sperm under a phase contrast microscope after dilution (physiological saline, 3% sodium citrate, Tris buffer, or Tris-egg yolk extender).
  - **Progressive Motility**: Rapid forward movement (key factor for fertility).
  - **Types of Movement**:
    - **Circular**: Cold shock.
    - **Reverse**: Movement in reverse direction.
    - **Oscillatory**: Jerky movement.

***Grading of Progressive Motility*:**

- **Ideal Motility**: 70% progressive motility.
- **Effect of Temperature**: Cold or heat shock can reduce motility.

Grade	Progressive Motility (%)
1	90-100%
2	70-80%
3	50-60%
4	30-40%
5	0-20%

Estimation of Sperm Concentration

Importance of Estimating Sperm Concentration:

- Determines the number of females that can be inseminated.
- Aids in diluting fresh semen and ensures sufficient concentration in frozen semen samples.

Methods of Estimating Sperm Concentration

1. Visual Examination
2. Cell Volume Method
3. Colorimeter
4. Photometer
5. Opacity Tubes
6. Computer Assisted Semen Analyzer (CASA)
7. Haemocytometer Method

Visual Examination:

1.Based on the appearance of semen (e.g., creamy, cloudy).
2.Not reliable for medium/low concentration samples.
3.Useful in bulls and rams.

Cell Volume Method:

1.Centrifugation separates sperm from seminal plasma.
2.Measures packed sperm volume.
3.Accuracy is limited due to interference from other materials.

Colorimeter:

1. Measures optical density of semen samples.
2. Converts light transmission into sperm concentration.
3. Requires initial standardization and monthly calibration.

Photometer for measuring sperm concentrations

- **Photometer**
  - Advanced form of colorimeter.
  - Displays concentration, dilution rate, and number of doses.
  - Needs calibration every 2 weeks with haemocytometer.

Opacity Tubes

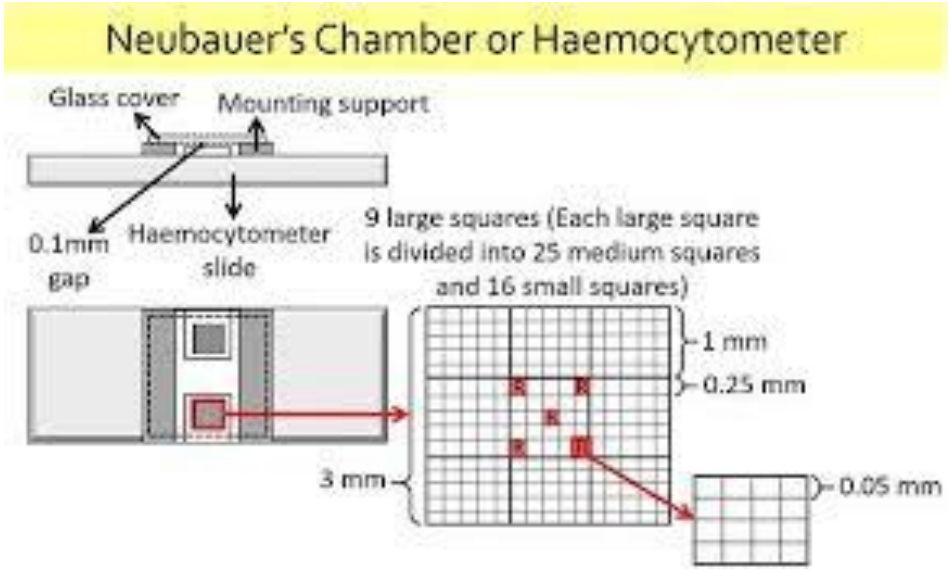
- Brown's opacity tubes used to estimate concentration.
- Dilute semen with formal saline (1:100) and compare with opacity tubes.
- Formula for concentration:
  - **Concentration (sperm/cmm) = Opacity tube number × dilution rate × 100 × 5**

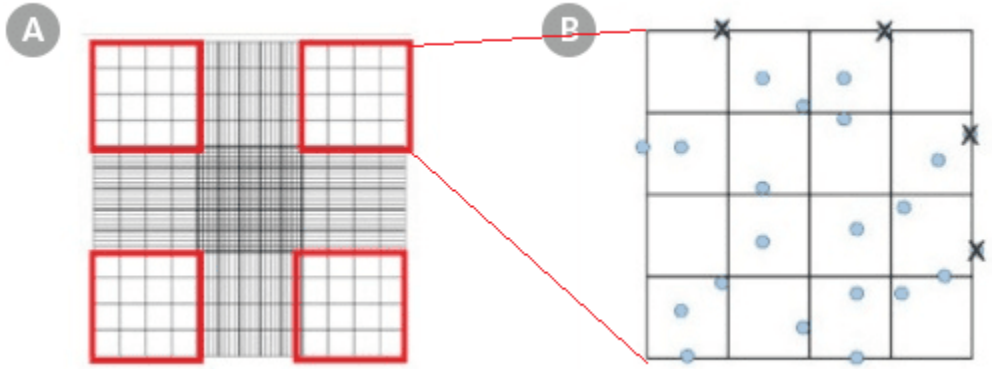
Computer Assisted Semen Analyzer (CASA)

- Most advanced method, but expensive and not commonly used.

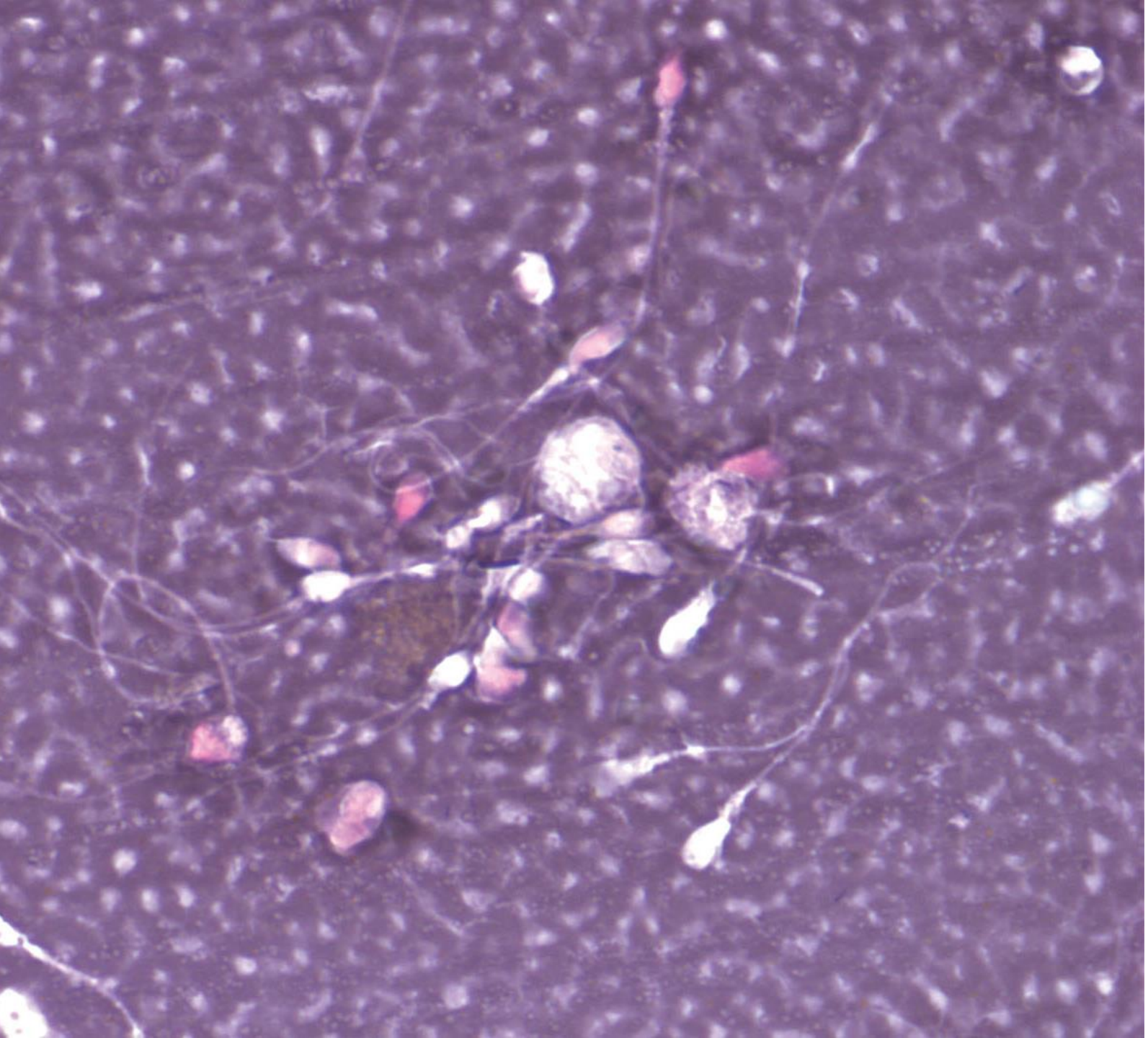
Haemocytometer Method

- Gold standard for accuracy.
- Requires skill, and is time-consuming, not suitable for large sample processing.





Terminology	Explanation
Normozoospermia	Normal sperm concentration
Oligozoospermia	Reduced sperm concentration
Polyzoospermia	Increased sperm concentration
Azoospermia	Zero sperm concentration



- **Eosin-nigrosin staining**: Eosin stains dead sperm pink (membrane intact in live sperm).
- **Nigrosin**: Background stain.
- **Rose Bengal Stain**: Used for more detailed morphology assessment

Interpretation of Spermiogram:

- **Up to 20% abnormalities** are acceptable in bulls (7.5% major, 12.5% minor).
- **Hereditary defects**: <5% allowed.
- **Specific minor abnormalities**: <10% allowed.
- **Abnormalities >15% major or >30% total** may indicate poor breeding potential.

BIOCHEMICAL TESTS:

- **pH**: Bull semen = 6.8, Stallion = 7.4
- **Methylene Blue Reduction Test (MBRT)**: 3-5 min = Good, >9 min = Poor
- **Fructolysis**: Higher fructolysis = better semen quality
- **Pyruvate Utilization**: Increased oxygen uptake = high fertility
- **GOT**: Presence indicates sperm damage (post-thawing)
- **Hyaluronidase**: Indicates acrosomal damage
- **Resazurin Test**: Good semen reduces resazurin quickly (pink in 1 min)
- **Buffering Capacity**: Better semen resists pH changes
- **Phosphatase Test**: High levels correlate with better quality semen
- **Millovanov's Test**: R > 5000 indicates good motility resistance

Note: Glutamate Oxaloacetate Transaminase. This enzyme is also known as Aspartate Aminotransferase (AST) : used as a marker for cellular damage

SEMEN PRESERVATION METHODS

Semen preservation is critical for artificial insemination (AI) programs in cattle and buffalo. The preservation method depends on the temperature of storage, which includes:

1. **Room Temperature Preservation (18–25°C)**
2. **Refrigeration (4–6°C)**
3. **Ultra-Low Temperature Preservation (-79°C to -196°C)**

Extender	Composition	Duration of Preservation	Fertility (%)
Illini Variable Temperature (IVT) Diluent	Sodium citrate, Sodium bicarbonate, Glucose, Potassium chloride, Sulphanilamide	3–6 days	76% (non-return basis)
Cornell University Extender (CUE)	Sodium bicarbonate, Sodium citrate, Potassium chloride, Glucose, Glycine, Catalase, Citric acid	3–6 days	70–80%
Milovanov's Method (Carbonate-Phosphate Method)	Potassium dihydrogen phosphate, Sodium citrate, Glucose, Penicillin G sodium, Streptomycin sulfate, Sulphanilamide	3–6 days	-
Coconut Milk Extender (CME)	Sodium citrate, Penicillin G sodium, Dihydrostreptomycin, Polymixine B, Catalase, Mycostatin, Egg yolk, Coconut milk	7 days	-

Extenders for Refrigeration Preservation:

- ***2. Preservation at Refrigeration Temperature (4–6°C)***
  - **Common method** before frozen semen technology.
  - **Duration**: Semen can be used for insemination up to 72 hours.

Extender	Composition	Duration of Preservation	Fertility (%)
Egg Yolk Phosphate Extender (EYP)	Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Egg yolk	72–96 hours	Good fertility in field tests
Egg Yolk Citrate Extender (EYC)	Sodium citrate dihydrate, Distilled water, Egg yolk	72 hours	Clear semen, widely used for bull and buffalo semen
Caprogen	Sodium citrate, Glucose, Glycine, Glycerol, Egg yolk	4–5 days	-
Kampschmidt (Egg Yolk Glucose Bicarbonate Extender)	Dextrose, Sodium bicarbonate, Sulphamethazine, Egg yolk	72–96 hours	45% fertility for Zebu, 40% for buffaloes
D2 Dilutor	Sodium bicarbonate, Glucose, Fructose, Egg yolk	4–6 days	45% fertility for Zebu, 40% for buffaloes

3. Ultra-Low Temperature Preservation (-79°C to -196°C)

Modern method: Freezing semen for long-term storage.

Extenders for Ultra-Low Temperature Preservation:

Extender	Composition	Duration of Preservation	Fertility (%)
Tris-Egg Yolk-Glycerol Diluent	Tris, Citric acid, Fructose, Glycerol, Penicillin G sodium, Dihydrostreptomycin, Egg yolk	Long-term	-
Sodium Citrate Extender	Sodium citrate, Penicillin G sodium, Dihydrostreptomycin, Egg yolk, Glycerol	Long-term	-
Milk Extender (For Freezing)	Boiled milk, Sulphanilamide, Penicillin G sodium, Dihydrostreptomycin	Long-term	-
Lactose-Egg Yolk Extender	Lactose, Egg yolk	Long-term	Better survival with higher molecular weight sugars

Important Points about Extenders

- **Illini Variable Temperature (IVT) Diluent**: Uses CO_2 to reduce sperm metabolism.
- **Cornell University Extender (CUE)**: CO_2 produced from glycine breakdown, no need for external CO_2.
- **Caprogen**: Developed for 5°C storage, contains catalase to prevent hydrogen peroxide buildup.
- **Milk Extender**: Heated milk and egg yolk protect sperm from cold shock and extend semen lifespan.
- **Tris-egg yolk-glycerol diluent**: Ideal for freezing sperm at ultra-low temperatures, widely used in advanced countries.

Other Important Extenders

- **D5 Dilutor**: Used for buffalo semen, provides good preservation.
- **Glycine Dilutor**: Not suitable for buffalo semen, affects sperm morphology.

- **Coconut Milk Extender (CME)**: Can store semen for 7 days, but limited by coconut milk availability.

Fertility and Duration of Storage Summary

Method/Extender	Preservation Temperature	Duration	Fertility
IVT Diluent	Room temperature (18–25°C)	3–6 days	76%
CUE	Room temperature (20–24°C)	3–6 days	70–80%
Milovanov's	Room temperature	3–6 days	-
Coconut Milk	Room temperature	7 days	-
EYP (Egg Yolk Phosphate)	4–6°C	72–96 hours	Good fertility
EYC (Egg Yolk Citrate)	4–6°C	72 hours	Good fertility
Caprogen	4–6°C	4–5 days	-
D2 Dilutor	4–6°C	4–6 days	40–45%
Tris-Egg Yolk-Glycerol	Ultra-low temp (-79°C to -196°C)	Long-term	-
Milk Extender	Ultra-low temp (-79°C to -196°C)	Long-term	-

In conclusion:

- **Room temperature preservation**: Generally used for short-term storage, effective in resource-limited areas.
- **Refrigeration**: Suitable for 72-hour preservation, widely used.
- **Ultra-low temperature**: Ideal for long-term storage and international semen transport.

PACKING SYSTEMS OF FROZEN SEMEN

Ampoule Method

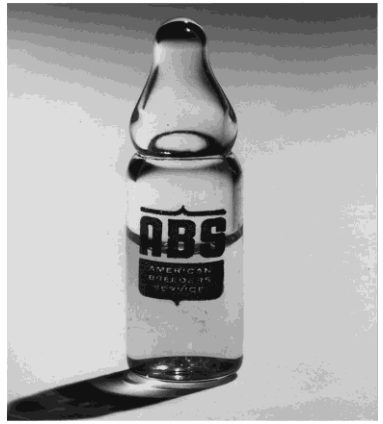
- Developed by Macpherson (1954) and Vandemark & Kinney (1954)
- Semen volume: 0.5-1.0 ml
- Ampoules sealed over flame, leaving 0.5 ml air space
- Freezing process:
  - Initial cooling in alcohol or acetone bath at 5°C
  - Rate of cooling: 1-2°C/min to -15°C
  - More CO_2 to reach -79°C at 4-5°C/min
- Stored in solid CO_2 (-79°C) or liquid nitrogen (-196°C)
- Thawing: Ampoule in warm water, cut, semen drawn into catheter

Merits:

- Contamination avoided
- Identification possible (bull number, etc.)

Demerits:

- Lower freezability and fertility
- Space-consuming in storage
- 8-10% semen loss during thawing
- Glass catheter use for AI has drawbacks



Pellet Method

- Developed by Nagase and Niwa (1963)
- Semen volume: 0.1-0.2 ml
- Freezing: Semen diluted with lactose, glycerol, egg yolk
- Media for dilution:
  - Lactose: 75.3 ml
  - Glycerol: 4.7 ml
  - Egg yolk: 20 ml
- Cooling on solid CO_2 (-79°C)
- Stored in liquid nitrogen

Thawing:

- Thawed in 3% sodium citrate solution with 1.5% fructose at 20°C

Merits:

- Economical
- Less storage space required

Demerits:

- Identification difficult
- **Risk of contamination during storage**
- Pellets may break during handling, leading to sperm loss
- Moderate freezability
- Tedious thawing process

- **Straw Technique**
  - Developed in Denmark (1940)
  - First frozen by Adler (1960) using liquid nitrogen vapour
  - Cassou modified it in 1965 (medium straws), then in 1968 (mini straws)
  - "**French straws**" made of polyvinyl chloride (PVC) commonly used

Minimum Standards
For
Production of Bovine Frozen Semen
2022

DEPARTMENT OF ANIMAL HUSBANDRY & DAIRYING

MINISTRY OF FISHERIES, ANIMAL HUSBANDRY & DAIRYING

GOVERNMENT OF INDIA

KRISHI BHAWAN, NEW DELHI

REVISED - 2022

11 (F) Colour Specifications of straws:

All semen stations shall follow the following colour codes for filling of semen in straws:

Table: 3 **Colour Specifications**

Breed	**Colour**
Holstein	Pink/Rose
HF Crossbred/Frieswal/ Karanswiss	Pistachio Green (light green)
Jersey	Yellow
Jersey Crossbred	Salmon
Indigenous cattle	Orange
Sunandini	Blue
Buffalo	Grey
Gir	Purple
Sahiwal	Orange
Red sindhi	Putty
Rathi	White
Tharparkar	Light yellow

- The new color code will come into existence from 1st April 2023
- If any of above mentioned colour is not available, then transparent straws shall be used.

- **Types of Straws**:
  - Sealed with steel, glass, or plastic beads (German straw)
  - Identification possible by color, bull ID, breed, etc.
- **Merits:**
  - Rapid thawing due to increased surface area
  - Better sperm survival
  - Easier AI (smaller diameter)
  - Efficient delivery into uterus
  - Easier identification (color-coded)
  - Reduced space and easier storage
- **Demerits**:
  - Sealing can be difficult
  - Use of glass catheters has drawbacks

Type of Straw	Length (mm)	Diameter (mm)	Volume (ml)
French medium	135	2.8	0.5
French mini	135	2.0	0.25
German straw	65	2.8	0.25

Say,
Volume of ejaculate = 5 mL
Concentration/mL = 1 X 10^9/ ml
Motility = 80%
Then, No. of motile spermatozoa = 0.8 X 10^9/ ml
Total no. of motile spermatozoa = 0.8 X 10^9/ ml X 5 mL = 4 X 10^9

Dose needed per straw = 20 X 10^6 motile spermatozoa
No. of straws that can be made = 4 X 10^9 / 20 X 10^6 = 200 straws

Volume per straw (French mini) = 0.25 mL
Total volume needed (Diluted semen) = 200 X 0.25 mL = 50 mL

Semen was already diluted 1:1, i.e. 5 mL semen + 5 mL diluent = 10 mL total
Volume of diluter to be added = 50 – 10 = 40 mL