Department of Animal Nutrition, Genetics & Breeding
Course Title: Reproduction of Farm Animals & Biotechnology
Course Code: ANGB 456
Level-4, Semester-II
Credit Hours: 1, Contact Hour: 2

Course Content (Practical)

Methods of reproductive sample collection. Study on semen collection of different farm animals. Evaluation of semen: Enumeration of spermatozoa. Evaluation of semen: Physical test & p^H. Evaluation of semen: Mass motility estimation and individual count of sperm movement. Evaluation of semen: Methylene blue reduction test. Evaluation of semen: Live and dead cell count. Evaluation of semen: Normal and abnormal count of spermatozoa (Morphology). Management practices of breeding animals. Preparation of diluents and extension of semen. Production of frozen semen. Artificial Insemination in farm animals. Study on Oocyte collection, *in vitro* maturation, *in vitro* fertilization and *in vitro* culture. Routine activities at AI center. Visit to different livestock farms, AI center and biotechnology lab.

Experiment 1: Study on Methods of Reproductive Sample Collection

Objectives:

To becomes familiar with reproductive organ.

- Male and female reproductive organ
- Semen (Sperm)
- Ovum
- Embryo
- Oviduct or uterine fluid
- Embryo
- Follicular fluid

Male and female reproductive organ 30% formalin is used to preserve the reproductive organ.

Semen collection: There are four recognized methods of semen collection:

i. Artificial vagina (AV method)
ii. Electro-ejaculate method
iii. Massage method
iv. Collection of semen from vagina of female when she is breed

Preparation for ovary collection:

Physiological saline of .9% NaCl was prepared for washing with 1 liter distilled water. Within 5hours the organ will start degraded. Saline was sterilized in autoclave and store in a refrigerator for future use. On the day of collection 1000mg of Gentamycine added per liter of saline solution. The solution was warm at ($25\text{-}30^{0c}$) and was put in a thermos box to maintain this temperature during transportation / transporting the ovaries from slaughter house to the laboratory. DPBS: Dulbecos phosphate Buffer solution was prepared then it was sterilized in autoclave and stored in refrigerator for further use.

Embryo Collection: There are three methods of embryo collection:

i. Surgical method
ii. Non-surgical method
iii. Laparoscopic method

Oviduct or uterine fluid/ follicular fluid:

At each collection fluid from each surface follicle. Fluid was prepared, centrifuged at 3000rpm for 30 mintues at 4^{o}c. The top portion liquids were collected and again centrifuged for 15min at same seen RPM and temperature. The supernatant was collected and filtered through 45 micro-mili pore and transfer in a sterilized glass beaker. For heat inactivation at 65^{o}c for 30 minutes in a water bath and were stored in a deep freeze.

Experiment 2: Study of Semen Collection

Objective: To become familiar with the methods and equipment used in the collection of semen and to practice the proper method of collection semen from the dairy bull.

Methods of semen collection: There are 4 recognized methods of semen collection in farm animals-

1. Artificial vagina methods (A.V method)
2. Collection of semen by means of electro ejaculation method
3. Collection of semen by palpation or massage method.
4. Collection of semen from vagina just after mating

Collection of semen by palpation or massage palpation by massage: Palpation by massaging the ampullae through the rectal walls may be used to obtain semen. This method of collection requires special training to be used successfully and the semen is after contaminated with dirt and urine.

Collection of semen by means of electrical stimulation: Ejaculation may about by electrical stimulation Bunn and his co-workers (1930) first used this method for sheep. This is a convenient method of obtaining semen from crippled bulls that are unable to serve the artificial vagina. It is also useful for collecting semen from beef or range bulls (unaccustomed to handling) where possible however, due to stimulation provided in advance of collection. The artificial vagina is much preferred.

Briefly the method involved the introduction of an electrode probe which has a number of contact rings (wires) into the rectum. Electric excitation of the ejaculatory nerve centers is produced by means of an alternating current of 5. 10. or 15 volts. The impulsive type of stimulation does away with the necessity of a second electrode fastened or inserted into the longissimus dorsi muscle.

Collection of semen from the vagina just after mating: This may be done with a sponge, pipette, spoon or some similar object. This method is not recommended because the semen is contaminated with mucous, urine and there is an increased danger of spreading genital diseases.

Collection of semen by A.V method: Equipment necessary for semen collection by A.V method.

A. Artificial vagina set.
1. Rubber jacket or cylinder
   i. 2-3 inch in diameter and 14-18 inch long for bull.
   ii. 1-1.5 inch in diameter and 6 inch long for buck or goat
   iii. 6-8 inch in diameter and 30 inch in long for stallion
2. Rubber inner liner
3. Rubber cone
4. Graduated collection vial
5. Water inlet
6. Air inlet
7. Exhaust pumper
8. Protector
9. Rubber bands

B. Vaseline/lubricant (K-Y Jelly)
C. Water (110^0-115^0 F)
D. Gumboot
E. Apron
F. Thermometer
G. Dummy
H. Water heater device
I. Glass rod

Procedure:

1. A satisfactory collection chute is essential for properly restraining of the animal used as dummy. It is also helpful for safe guarding and holding the bull. Before placing the dummy in collection chute it was properly examined and cheeked. Dummy was properly in the collection chute.
2. The parts of artificial vagina was cleaned and sterilized and assembled in the artificial vagina.
3. Approximately 2/3 of the space between the inner liner and robber cylinder was filled with warm at about 11o-115^{o}F
4. A.V was pumped for raising the air pressure inside the vagina.
5. A.V was lubricated by using k.y Jelly with the help of the sterilized glass rod.
6. Collection vial also used at the end of the rubber cone and it was protected by protector.
7. The temperature of the inside of the vagina was examined by the thermometer inserting through the open end of the vagina. It should normally be 40- 44^{o}c for bull and 39^{o}c for buffalo bull.
8. Prior to collection the bull was sufficient excited by bringing the bull to the dummy and collection was performed form the right side by holding the A.V in the right hand and open end of the A.V was being held down word.
9. One or two false mount was not a approached by the collection, to allow the bull sufficiently stimulated and ensure good quality semen collection.
10. When the bull properly mounted, the penis was directed to the A.V by grasping the sheath with left hand. The A.V was kept at an angle of 45^{o} end carve was taken to touch the exposed portion of the penis.
11. Immediately after collection the A.V was turned away keeping the open end upward to allow dropping of all semen to the vial.
12. The collection vial was removed from the A.V and it was kept in a hot water (100^{o} F) barn at body temperature for further investigation.

Result: The amount of collection semen was …….. C.C

Comment: By performing this experiment we gathered a new experience and knowledge and technique about collection.

Experiment no. 03: Evaluation of Semen: Enumeration of Spermatozoa (Concentration/Total Count)

Objectives:

1. To know semen fertility as well as fertility of donor.
2. To know the optimum dilution ratio.
3. To know the technical knowledge.

Method of enumeration of spermatozoa:

There are three methods of enumeration of spermatozoa:

i) Hemo-cytometer method
ii) Photoelectric calorimeter or nephelometer method.
iii) Karrels scale method

Hemo-cytometer Method:

Objective: To calculate the number of spermatozoa per ml of collected semen sample and to draw a conclusion about its future use in artificial insemination.

Apparatus and reagents:

i) A Compound microscope
ii) Hemo-cytometer set
a) Standard & red cell dilution pipette or semen dilution pipette.
b) Counting chamber
c) Cover slip
d) Dilution fluid
e) Semen sample
f) Cotton

Composition of dilution fluid:

i) Distilled water: 50 ml
ii) 2% eosin (for color): 1 ml

iii) 3% Nacl solution: 1 ml (used for killing the cell)

Procedure: The steps in making a sperm concentration count by means of a hemo-cytometer are:

i) Semen sample was mixed well by slowly inverting vial several times.
ii) 0.5 mm of semen was drawn in to standard red dilution cell pipette
iii) Dilution fluid was drawn in to standard red cell pipette up to 101 m.m mark.
iv) The pipette was a agitated by grasping it between the thumb and fore finger and it was rotated in one plane by eight knot motion for 3 minutes to ensure thorough mixing.
v) The first 4 or 5 drops were discarded (to get properly diluted semen from the bulb)
vi) A cover slip was placed over the ruled field of a cytometer slide and a drop was allowed to run under the cover slip.
vii) The count was made under low magnification approximately (10×25). Five large double ruled squares were counted over the field. This would give a total 80 small squares, then the number of spermatozoa per ml of semen to be calculated by using the following formula:

$$\text{Total no. of sperm per ml semen} = \frac{C \times 400 \times d \times 1000}{S}$$

Here: C= No of sperm counted in a given no. of small quarter
S= No. of small square counted
d= Dilution ratio: 200: 1

Normal concentration of spermatozoa in different species

SI No	Species	Concentration
1	Cattle bull	1200(800-1400 million/ml)
2	Buffalo bull	800 (600-1200 million/ml
3	Stallion	250 (200-600 million/ml
4	Ram and buck	3000 (2000-4000 million/ml
5	boar	250 (200-500 million/ml)
6	Dog	250 (125-500 million/ml)
7	Man	150 (100-200 million/ml)

S. No.	Terminology	Explaination
1	Normozoospermia	Normal sperm concentration
2	Oligozoospermia	Reduced sperm concentration
3	Polyzoospermia	Increased sperm concentration
4	Azoospermia	Zero sperm concentration

Result: The number of spermatozoa of the supplied semen sample was 1020 million/cc.

Comment: The supplied semen sample was normal bull semen .

Calculation

Volume of 1 small square= Length × width × depth

$=1/20 \times 1/20 \times 1/10\ mm^3$

$=1/4000\ mm^3$

Volume of 80 small square=$80/4000\ mm^3 = 1/50\ mm^3$

Now, $1/50\ mm^3$ contain 52 sperm

1.....52x 50 sperm

1 cc.....52×50×1000 sperm

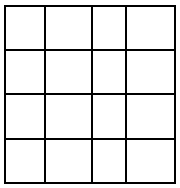
= 2600000 sperm

Dilution ratio= 1: 200

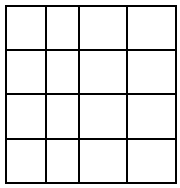
Total no. of sperm= 2600000× 200

= 520000000

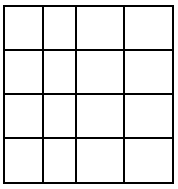
= 520 million



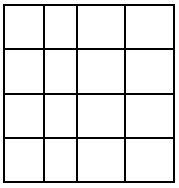
Upper left= 15 sperm



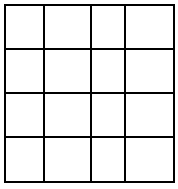
Upper right= 6 sperm



Middle=13 sperm



lower left= 4 sperm



lower right= 14 sperm

Total= 52 sperm

Experiment no. 4: Study on the evaluation of semen by physical test and P^H

Objectives

1. To become familiar with the appearance of good quality semen.

2. To know about different physiological test of semen evaluation.

3. To know the viability of semen by physical observation.

Physical test: The following points should be followed for evaluation of semen:

Volume: Volume is measured directly with the help of graduated collection vial into which semen is collected using (AV method). Just after collection the amount of semen is measured. The normal volume of semen in each ejaculation of different farm species is as follows:

Species	Volume (ml)
Bull	5.0-8.0
Cock	0.2-0.8
Ram/Buck	.8-1.2
Stallion	100
Boar	100-150

Color: The color of semen is observed in collection vial and recorded. The colors of semen are the following types:

- White to cream color - good
- Clear or thin in appearance – few sperm

a) Usual color:

- Milky white
- Yellowish: Yellow color of semen comes from the lipochrome pigment which is secreted from the ampulla.
- Cream color
- Milky

b) Unusual color:

- Watery: Poor concentration of spermatozoa.
- Pinkish or reddish: Due to mixture of fresh blood.
- Deep red or brownish: Due to degenerative blood tissue.
- Greenish: Due to purulent, death cell and blood fluid etc.
- Yellow: Due to mixture of pus-urine.

Consistency: The consistency of semen is observed by inclining and moving of the collection vial with care. Uniform consistency indicates good quality semen and high consistency indicates higher spermatozoa. Consistency of sperm are following type:

i. Thick creamy: Excellent quality.
ii. Thin creamy: Very good quality.
iii. Thick milky: good quality.
iv. Thin milky: Poor quality.

Uniformity: When any clots are formed in semen, this semen is called non-uniformity.

Transparency: It gives following idea about the quality of semen:

i. Opaque: Excellent quality.
ii. Clear: Poor quality.
iii. Cloudy: Bad quality.

Odour: Good quality semen has seminal odour and bad quality semen has unusual odour.

Specific gravity: Usually the specific gravity of semen is higher than 1.0 and approximately same as milk. The specific gravity of semen range 1.036±0.0086.

Specific gravity = weight of semen (gm)/ volume (ml)

P^H: Nitrogen paper should be used for semen p^h estimation. Normal p^H of different species are all follows:

Species	p^H
Bull, Buck. Ram	6.5-7.2
Boar, Stallion	7.04

Animal tag no.	Breed	Date	Volume	Color	Consistency	Transparency	Uniformity	Odour	PH

Comments: Physical test of both semen were usual. So both the semen can be used for further use.

Experiment no. 05: Evaluation of Semen: Mass Motility Estimation and Individual Count of Sperm Movement

Objectives:

1. To study the motility rating as determined from microscopic examination of the semen.
2. To become familiar with the appearance of good quality semen.

Procedure (Mass motility):

1. A clean dry glass slide was warmed approximately to 100^{o}F (38.7^{o}c).
2. The semen sample was mixed properly by slowly inverting the vial 2 or 3 times (do not agitate vigorously).
3. One drop of semen sample was placed on the slide and was spread on the warm slide.
4. Examine under microscope at low magnification (4X).

There are several systems of motility of rating. The following system using '0-5' is usually recommended.

Scale	Grade	Character
Five	Excellent (+++++)	80% or more then spermatozoa are in very vigorous motion. Swirls and eddies are form caused by the movements of the sperm. Movements are so vigorous that it is impossible to observe individual sperm fresh semen.
Four	Very good (++++)	About 70-80% of the sperm are in vigorous motion. Waves and eddies are form a dropped rapidly.
Three	Good (+++)	About 50-70% of the spermatozoa are in motion waves and eddies are form but dropped rapidly.
Two	Fair (++)	About 30-50% of the sperm are in motion waves and eddies are very slowly across the field.
One	Poor (+)	About less than 30% of the sperm are in motion. No waves and eddies are form the movements are weak and oscillatory not progressive.
Zero	non motile	No motile sperm are observe

Result: The motility of the supplied sample was 70- 80% Hence, the sample fall under the grade very good in the scale four.

Recommendation: The sample used for artificial insemination.

Individual count of sperm movement:

Just after collection the semen was diluted with buffer solution and inclined slowly. Then steps were taken from the procedure of mass estimation. When examined under microscope movement of the sperm was observed with care. Motility was observed by following types of movement.

i) Progressive/Forward/Rapid
ii) Oscillatory
iii) Rotatory

Several fields were observed and the number of different movement of the spermatozoa were counted and tabulated.

Field No	Motile sperm			Non motile	Total
	Progressive	Oscillatory	Rotatory		
1	18	9	25	5	57
2	30	10	20	4	64
3	25	13	18	3	59
4	50	30	20	2	102
5	40	20	4	1	65
	163	82	87	15	347

Results: Therefore, on the basis of above observation

$$\% \text{ of motile sperm} = \frac{\text{No. of motile sperm}}{\text{No. of total sperm}} \times 100$$

$$= (332/347) \times 100$$

$$= 95.67\%$$

$$\% \text{ of progressive motile sperm} = \frac{\text{No. of progressive motile sperm}}{\text{No. of total sperm}} \times 100$$

$$= \frac{163}{347} \times 100$$

$$= 46.97\%$$

$$\% \text{ of Oscillatory motile sperm} = \frac{\text{No. of Oscillatory motile sperm}}{\text{No. of total sperm}} \times 100$$

$$= \frac{82}{347} \times 100$$

$$= 23.63\%$$

$$\% \text{ of Rotatory motile sperm} = \frac{\text{No. of Rotatory motile sperm}}{\text{No. of total sperm}} \times 100$$

$$= \frac{87}{347} \times 100$$

$$= 25.07\ \%$$

$$\% \text{ of Non motile sperm} = \frac{\text{No. of Non motile sperm}}{\text{No. of total sperm}} \times 100$$

$$= \frac{15}{347} \times 100$$

$$= 4.32\ \%$$

Comments: Motility of the supplied semen sample was 95.67%.

Recommendation: The sample used for artificial insemination.

Experiment no. 6: Evaluation of Semen: Methylene Blue Reduction Test

Principle: The test was first developed by Walton and Edward in 1938. According to them, it was possible to estimate of the spermatozoa, semen quality on the basis of metabolic estimate of the spermatozoa. Motile and viable spermatozoa in physical condition take oxygen at rapid rate. As a result H^+ concentration in the medium reacts with methylene blue to form leucomethylene, a colorless medium. It is a relative estimation of the quality of a given semen sample.

- This test is based on the principle that the hydrogen ions are liberated during sperm metabolism which will reduce the blue colored methylene blue into colorless leucomethylene blue.
- The hydrogen ions are released due to the dehydrogenase enzyme present in active sperm.
- The time taken to change the color is directly related with the motility and concentration of the spermatozoa.
- More the motility and concentration will lead to more the hydrogen ion release which will cause less time taken by the methylene blue to colorless leucomethylene blue.

Apparatus and Reagents:

i. Egg yolk citrate
ii. Methylene blue dye
iii. Semen sample
iv. Mineral oil/Paraffin oil
v. 10 ml vial
vi. 1.0 ml pipette
vii. 0.1 ml pipette
viii. Water bath (110-115^o F)

Procedure:

1. A methylene blue solution was prepared by dissolving 5gm methylene blue powder in 100 ml of 3.6% sodium citrate buffer.
2. 0.2 ml of semen was diluted with 0.8 ml egg yolk citrate diluter in a 10 ml vial and was thoroughly mixed.
3. 0.1 ml methylene blue solution was added and was mixed well.
4. The tube was sealed with half inch (1/2″) layer of mineral oil to prevent entry of air in to the vial.
5. The vial was placed in hot water bath at 110-115^{o} F (46.5 ^{o} C).
6. Required time was observed for sample to loss the blue color. When blue color will be lost by 3 minutes or less than 3 minutes, indicate excellent quality semen. The blue color will be lost by reaction with 3-6 minutes with good quality semen, 6-9 minutes in medium quality semen and more than 9 minutes in poor quality semen.

Result: Color did not disappear within 9 minutes in the supplied semen sample. So the supplied semen sample was poor quality semen.

Experiment no. 07: Evaluation of semen: Live and dead cell count

Objectives: To become familiar with differential staining technique and determining the percentage of live or dead sperm in the semen.

Discussion: A high percentage of live, progressively motile, vigorous spermatozoa are necessary for good quality semen. The dead and alive sperm is based upon the difference between dead cells and live cells in absorbing certain dyes. In this test the sperm that are dead at the time of the slide is made will absorb the stain and appear blue. Those that are alive will not absorb the stain and will remain while.

Apparatus and Reagents:

1. Microscope
2. Two glass slides
3. Stain (3 stain available)
4. Hot plate (150-200^{o} F)
5. Glass rod

6. Semen sample
7. Cotton

Composition of Stain: There are three alternative stains, among which any one may be used.

I) Fast green eosin blue stain :
   i) Fast green – 2 gm
   ii) Eosin blue – 0.8 gm
   iii) Phosphate buffer – 100 ml

II) Eosin Aniline blue stain:
   i) Eosin blue – 1 gm
   ii) Aniline blue – 0.4 gm
   iii) Phosphate buffer – 100 ml

III) Eosin Nigrosin stain:
   i) Eosin blue – 5 gm
   ii) Nigrosin – 1 gm
   iii) Sodium citrate buffer – 100 ml

Procedure:

i) A clean, dry slide was taken and 1 or 2 drops of stain was placed on the middle of the slide with the help of a glass rod.
ii) A small amount of semen was mixed with the stain
iii) A second slide was drawn over the semen on the first slide and thus the smear was made.
iv) Excess stain was removed by wiping the edges of slides with cotton.
v) The smear was dried rapidly by placing them on a hot plate at 150-200^{o} F temperature (65-93^{o} C).
vi) The slide was placed under the microscope, live and dead sperms were counted from randomly selected field. The sperms were counted under 25 x objective.

The dead sperms were those which absorbed dye and appeared blue color under microscope and those alive did not absorb stain; that was showed white in color. A spermatozoa which was partially stained such as nucleus only was counted as dead.

The total number of 333 sperms was counted and percentage of dead and live sperms was counted by the following formula:

$$\% \text{ of dead sperm} = \frac{\text{Number of dead sperm}}{\text{Total number of sperm}} \times 100$$

$$\% \text{ of live sperm} = \frac{\text{Number of live sperm}}{\text{Total number of sperm}} \times 100$$

Grading of Semen in Respect of Dead and Live Sperm:

i) Excellent quality semen: Contains 5-10 % dead sperm
ii) Good quality semen: Contains 11-20 % dead sperm
iii) Poor quality semen: Contains 21-30 % dead sperm
iv) Very Poor quality (reject): More than 30 % dead sperm.

Result: % of dead sperm = 14.4 %, % of live sperm = 85.5%

Comment: The supplied semen sample was contained 14.4 % dead spermatozoa. So the supplied semen was good quality semen.

Experiment no. 08: Evaluation of Semen: Normal and Abnormal Count of Spermatozoa (Morphology)

Introduction: The relation of sperm morphology to fertility in the bull has been widely studied. An excessive number of abnormal sperm will lower the probability of the semen for fertilization. High quality semen should not contain more that 5 to 15% abnormal sperm, average semen contains 10 to 20% abnormal sperm generally and in poor semen as high as 30 % or more of the sperm may be abnormal in morphology.

Objective:

i) To study the morphology of spermatozoa.
ii) Determine the percentage and type of abnormal spermatozoa found in semen.

Different types of sperm abnormality: All normal sperm consists of head, mid piece, (main or principal piece) and end piece. All abnormalities can be categorized into three:

i) Head abnormality
ii) Mid piece abnormality
iii) Tail abnormality

I. Head abnormality:

A) Size:
   i) mega headed
   ii) Narrow headed

B) Shape:
   i) Pyriform headed
   ii) Tapering headed

C) Number:
   i) Double headed
   ii) Deheaded sperm

II. **Mid Piece abnormalities:**

i) Double mid piece
ii) Absence of mid pieces.
iii) Long mid piece
iv) Short mid piece
v) Beaded mid piece

III. **Tail abnormalities:**

i) Coiled tail
ii) Crooked tail
iii) Double tail
iv) Tailless
v) Very long tail
vi) Short tail

Causes of abnormalities: Abnormality occurs due to

i) Defective spermatogenesis
ii) Excess semen collection
iii) X-ray
iv) Temperature shock

Equipment and reagents:

1. Compound microscope
2. Two glass slides
3. Rose Bengal stain
4. Staining rack
5. Cotton
6. Semen sample
7. Buffer (Any physiological buffer such as citrate buffer)
8. Beaker full of distilled water

Composition of Rose Bengal stain:

i) Rose Bengal powder – 3gm
ii) Distilled water – 99ml
iii) Formalin (40% formaldehyde) – 1ml

Procedure:

1. Two drops of buffer was placed on a clean dry glass slide.
2. One drop of mixed semen was added in buffer.
3. The buffer with semen was spread by covering with another slide.
4. The smear was dried in the air
5. The smear was stained with Rose Bengal stain for 5 minutes. Then remove/rinse of excess stain by dipping slide in distilled water.
6. The smear was dried in the air
7. The slide was placed on the stage of microscope and counted in high magnification.
8. Generally a total of 333 sperms were counted used random fields on different parts of the slide and was recorded in the table.

Result:

% of normal spermatozoa = 87 %

% of head abnormal spermatozoa = 0.9 %

% of mid piece abnormal sperm = 3.6 %

% of tail abnormal sperm = 9.0 %

% of total abnormal sperm = 13.5 %

Grades of semen according to percentage of abnormal sperm:

i. Abnormality < 10 percent: High quality semen
ii. 11-20 percent abnormality: Good quality semen
iii. Greater than 20 percent abnormality: Poor quality semen
iv. Greater than 30 percent abnormality: Rejected grade.

Comment: The supplied semen sample was good quality semen according to grading.

Experiment 9: Study on the Management of Breeding Male of Artificial insemination center at Savar

Objectives: To know the standard management system of breeding male (Bull & Buck)

Management

Management refers to the day to day care of the animals. It's includes housing, feeding, cleaning etc.

Housing: Housing means provide shelter to the animals.

Objectives

i. For better feeding, breeding, care and management of animals.
ii. To protect the animal from adverse weather insect and wild animals etc.

Type of housing:

a) Loose housing barn
b) Convental barn
    a) Single row
    b) Double row

A. **Floor space requirement:**

Animal	Floor space
Bull	Length (20-24) ft * width (12-14)ft
Buck	Length (4-5)ft * Width (3-4)ft

B. Feeding: Feeding is a process of acquainting feed to the animals. For 100 kg body wt. of bull require 6kg green per day. 80.5kg concentrate/day. Feed should be supplied twice a day.

C. Cleaning: It is practiced to remove the dust & dung from the animal house as well and animal body coat to protect the animal from the exo-parasite. It should be practiced twice a day.

D. Exercise: It is practiced to retain the body fitness of the animal for the production of semen. If the animal become fatty semen production is hamper. For better result exercise should be practice every day.

E. Semen collection: Semen collection is the main objective for the rearing of the breeding male (bull and buck). It is very difficult task to collect semen. Semen should be collected once. Sometimes twice per week from the bulls and buck.

Management of the breeding male at Savar AI center-

1. House-
   No. of the bull shed-
   Length –feet/ shed
   Width –feet/ shed

Shed 01	Shed 02	Shed 03	Shed 04	Shed 05	Shed 06

2. Feeding
   Roughage-
   Concentrate- kg/day

Practice twice a day (morning & afternoon)

3. Cleaning- Twice a day morning and afternoon
4. Exercise
5. Semen collection : once/ bull/ week
   Volume-…ml

Experiment no. 10: Introduction with Reproductive biotechnology laboratory and initiation of *in-vitro* production of mammalian embryo.

Objectives:

a) To know the requirements and setting a reproductive bio-technology laboratory.
b) Accustom with *in-vitro* production of mammalian embryo development experiment.

The requirements and setting a reproductive bio-technology laboratory.

1. Refrigerator.
2. Rack for chemicals.
3. Laminate air flow.
4. CO2 incubator.
5. Inverted microscope.
6. Dryer.
7. Wash corner.
8. Water bath
9. Centrifuger.
10. Microscope.
11. P^H meter.
12. Magnetic stirrer.
13. Weighing balance.
14. Ovary transfer bag.

Accustomed with *in-vitro* production of mammalian ovary:

In-vitro production of embryos: Literally *in-vitro* includes in glass or out-side the animal. IVP of embryo include *in-vitro* maturation, fertilization and culture of the embryo. It is the technique in which immature oocyte mature outside the body with appropriate culture media in a glass and test tube of form zygote and finally transfers the zygote in new culture media for development of zygote to mature stage from blastocysts. *In-vitro* production includes:

a) IVM of oocyte.
b) IVF of oocyte.
c) IVC of Oocyte

Steps of *in-vitro* production:

Collection of ovary
↓
Preparation of the ovary
↓
Collection of coc_s
↓
Grading of coc_s
↓
In-vitro maturation
↓
Checking
↓
In-vitro fertilization
↓
Checking
↓
in-vitro culture
↓
Checking
↓
Blastocyst

Procedure:

1. At first the goat ovary was collected and then put the ovary into the saline solution.
2. The coc_s was collected from the ovary.
3. The collected coc_s was graded.
4. After grading coc_s the mature oocyte are transferred for maturation.
5. For maturation droplet dish was kept in incubator for (22-48 hrs)
6. After checking the mature oocyte was fertilized with capacitating sperm.
7. Then fertilization takes place and zygote is produce.
8. After the completion of IVF the zygote incubated for 5 hrs and then introduces a new culture media for the development a mature stage from blastocyst stage embryo.

It takes usually 7-9 days.