सत्यमेव जयते

# Minimum Standard protocol of Semen Production

*and*

# Standard Operating Procedures for Artificial Insemination

in

# Ovine/Caprine species.

**Department of Animal Husbandry and Dairying**
**Ministry of Agriculture and Farmers' Welfare**
**Government of India**

**2019**

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**Dr. Suresh S. Honnappagol**
Animal Husbandry Commissioner

भारत सरकार
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GOVERNMENT OF INDIA
MINISTRY OF AGRICULTURE & FARMERS WELFARE
Department of Animal Husbandry, Dairying & Fisheries
Krishi Bhawan, New Delhi-110 001

**Dated the 15th January, 2018**

## Message

Livestock development is a labour intensive activity requiring a close attention throughout the year. With the growing demand for various products, Animal Husbandry can provide good opportunity, particularly to the small and marginal farmers and the landless to improve their livelihood security. The rural families can certainly take advantage of animal husbandry provided it is made economically viable and the necessary infrastructure is available to support the programme. Thus, there is a need for reorienting the livestock husbandry policy accommodating greater involvement of the private sector and local communities.

Keeping the aforesaid objectivity, the NLM Division of this Department has developed a National Action Plan for Small Ruminants i.e. Sheep and Goat. Through this it is aimed for 2-3% higher growth rate with added production capacity to boost up Nation's income through exports. To achieve this, the sector needs strengthening from various aspects covering breeding, genetics, nutrition, infrastructure management, skill development, processing and exports etc. Accordingly, appropriate strategies and various interventions have been suggested in this National Action Plan document.

I hope the implementation of this National Action Plan in true objectivity will enable achieving the objectives of Nation's priorities of doubling farmers' income by 2022.

**(Suresh S. Honnappagol)**

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Joint Secretary
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Dated, 15th February, 2019

## FOREWORD

Sheep and goats are important species of livestock, for India. They contribute greatly to the agrarian economy, especially in areas where crop and dairy farming are not economical, and play an important role in the livelihood of a large proportion of landless as well as small and marginal farmers.

The population of sheep is 65 million and goats 135 million, in the country as per livestock census 2012. Despite top world population and production potential, productivity of these animals is very low at present. To increase the productivity of these animals, means, increase in production of mutton, Chevon, Milk and Wool, there by augment the income and livelihood issues of farmers too.

Recently department has developed a 'National Action Plan' which would mainly concentrated on breed upgradation, through Artificial Insemination wherein, establishment of semen stations included as one of the main activity. Also, Department is implementing "Genetic Improvement schemes for Sheep and Goats", on pilot basis, in selected districts of 8 different states on selected breeds.

In order to improve quality of Caprine/Ovine breeding programmes now Department of Animal Husbandry and Dairying, is bringing out a "Minimum Standard Protocols for Semen Stations and Standard Operating Procedures for Artificial Insemination". This will lead to improvement in productivity and breed up gradation of our indigenous Sheep and Goat, and skilled manpower.

I am confident that these documents will be useful in bringing desired changes in genetic up gradation programmes being implemented by various stakeholders.

(Dr. O.P. Chaudhary)

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## ACKNOWLEDGEMENT

In order to achieve the goals of National Action Plan and to improve quality of Caprine/Ovine breeding programmes Small Ruminants Unit of National Livestock Mission, Department of Animal Husbandry and Dairying, has come up with a technical document, which could be a milestone in the sector development and enhancement of productivity of small ruminants like sheep and goat.

I would like to mention the strong support and directions of our beloved Secretary (ADF) Shri Tarun Shridhar and Animal Husbandry Commissioner Dr. Suresh S. Honnappagol and Chairman of Technical committee constituted for this purpose.

I would also take this opportunity to convey my gratitude to Dr. O. P. Chaudhary, Joint Secretary (NLM), for his continuous support and insightful suggestions to enable us for framing this document.

I would also like to acknowledge the valuable contribution of the Technical Committee with the participation of scientists/experts from the following institutions and convey my sincere thanks to all of them for their inputs through know how and experience without that the documents could not be developed.

1. ICAR-CSWRI, Avikanagar /CIRG, Mukdum /CSBF,Hisar
2. Veterinary College, Tirupati/TANUVAS,Tamilnadu/COVAS,Kerala/Veterinary College Hebbal, Veterinary college Palambur and J&K.
3. Gujarat Sheep and wool development corporation/Kerala Livestock Development Board/ Sheep development Boards, Karnataka and Telengana
4. Nimbkar Agricultural Research Institute, Phalton
5. International Livestock Research Institute (ILRI)

I would like to mention the efforts of entire team behind this initiative especially Dr. Sulekha S.L, Assistant Commissioner (Poultry, SR &MP), and Dr. Debalina Mitra, Livestock Officer (SR), for their dedicated efforts to make this document happen.

**(Dr. Sujit Kumar Dutta)**
**Deputy Commissioner (Poultry, SR &MP)**

# INDEX

## Annexures

# Minimum Standard protocol of Semen Production in Caprine and Ovine species

## 1. Introduction

Reproduction is critical to attainment of profitability in any livestock enterprise, including sheep and goat rearing. The breeding program on the farm will play a key role in the attainment of reproductive efficiency. Artificial Insemination accelerates the rate of genetic gain within a flock, maximizes the number of offspring from a desirable sire, enables genetic exchange over wide geographical areas, and also allows use of genetic material from incapacitated sires or those no longer alive if their semen had been preserved. Artificial Insemination with frozen semen has been proved to be the best tool worldwide for genetic improvement through dissemination of superior germplasm. This objective can be achieved only if the frozen semen used in AI programme conforms to the quality standards. For production and distribution of quality semen, it is most important that the rams/bucks used in AI programme satisfy quality norms, must be disease free and semen is harvested and processed in accordance with the standard protocols. The least protocols required for production of quality production of Caprine or Ovine semen are covered in this document. Failure to observe these guidelines could lead to production of poor quality semen making it unfit for export/distribution to AI centers.

## 2. Objectives of Official Sanitary Control of Semen Production are to:

a) maintain the health of animals on a semen station at a level which permits the international distribution of semen with a negligible risk of infecting other animals or humans with pathogenic agents transmissible by semen;

b) ensure that semen is hygienically collected, processed and preserved

## 3. Lay out of Semen Production Center

a) A pre-entry isolation facility

b) Animal accommodation areas (including one isolation facility for sick animals) and this should be species specific, where relevant;

c) Semen collection facility,

d) Semen laboratory,

e) Semen storage area and

f) Administration offices;

## 4. Requirements of Semen Production Center

a) SPC should have the official approval by Department of Animal Husbandry Dairying and Fisheries, Ministry of Agriculture, Government of India.

b) The centre should be under the supervision and control of the Veterinary Services, which will be responsible for regular audits, at an interval of no more than 12

months, of protocols, procedures and records on the health and welfare of the animals in the centre and on the hygienic production, storage and dispatch of semen.

c) The centre should be under the direct supervision and control of a Veterinary Officer

d) Personnel at a centre should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms.

e) Protective clothing and footwear should be provided to the personnel of the center.

f) Only animals associated with semen production should be permitted to enter the centre.

g) The entry of visitors should be strictly controlled.

## 5. Bio-security

There should be separate staff and separate bio-security arrangements for semen station and breeding flock, if any.

The risk of disease spread has grown manifold with increasing number of animals maintained at the semen production center. With the expected higher risk, implementation of strict bio-security measures at the semen stations assumes greater significance. Every semen station should have a well defined Bio-security protocol put in place across all its activities, in line with the Bio-security guidelines for sheep and goat farms attached as Annexure I

## 6. Standards/Criteria for selection of High Genetic Merit (HGM) Breeding Rams/Bucks

Before procuring new lambs/kids/bucks/rams for a semen station, a thorough physical examination should be conducted by an accredited Official / Veterinarian to ensure that the animals are free from abnormality and do not display clinical symptom(s) of any infection or any contagious diseases.

**A good ram/buck should meet the following pre-requisites, to select for semen collection;**

a. Anatomical and Structural Soundness

b. Breeding Soundness

c. "True to breed" criteria – It differs from breed to breed. The breed characteristics described by NBAGR may be followed. List of registered breeds of Sheep and Goat as on January 2019,is attached as ***Annexure XIII***

d. Body Condition Score-Bucks should have a body condition score of 3–3.5 (out of 5). Model Body Condition Score Card, is attached as ***Annexure II***

e. Scrotal Circumference-Since the scrotal circumference is positively correlated with semen production capacity, preferably it should be >25 cm in mature bucks.

f. Records like, individual buck/ram data, lifetime information data, pedigree information, progeny/ offspring performance, performance of relatives, etc. can also be taken into consideration.

***Note:***

For every new animal procured, the measurement of scrotal circumference and body weight should be initiated immediately. Standards for scrotal circumference and weight gain index for various breeds shall be fixed by initiating age wise recording of scrotal circumference once in three months and body weight once a month, by the semen stations.

## 7. Quarantine

A quarantine period of minimum **28 days** is compulsory before bringing new bucks/rams into a semen station. Only after favorable results from the health control point, the bucks/rams shall be admitted to the semen station. Relevant definitions are given in ***Annexure- III***

a) In the quarantine station, new animals shall be housed for a minimum of 28days in a place, which is effectively separated and away from the facilities occupied by resident animals. Manpower deployed and all equipment used in handling, feeding, watering and cleaning the new animals shall not be shared with the resident flock(s)

b) Each new animal in quarantine station shall be tested against major contagious diseases before its entry to resident flock as per the OIE guidelines. All tests shall be done by an accredited agency or disease diagnostic laboratory, as indicated in ***Annexure- IV***

c) Before entry into quarantine station, animals shall be examined for any clinical signs of diseases especially Para-tuberculosis.

d) During quarantine period, the breeding animals shall be vaccinated against the major diseases as per the scheduled protocol, if any.

e) Once the quarantine period is over, all eligible animals shall be introduced to the rearing station.

## 8. Testing programme for ram/bucks /teasers in the semen station

**All rams/bucks and teasers resident in the semen collection facilities should be tested at least annually for all the diseases prevalent or reported in the Country.**

The following diseases, should tested, as per OIE guidelines, with negative results:

a) Brucellosis;

b) Ovine epididymitis;

c) Maedi-visna and caprine arthritis/encephalitis;

d) Tuberculosis (for goats only);

e) Contagious agalactia

f) Peste des petits ruminants

g) Contagious caprine pleura pneumonia

h) Bluetongue and

i) Para tuberculosis

Testing protocols for these diseases are given in ***Annexure-IV.***

**As per OIE guidelines; the breeding bucks/rams should be free from above mentioned diseases.** The animals in the rearing station and the resident flock should go through periodical testing and vaccinations as per the scheduled list.

## 9. Vaccination Schedule

The breeding bucks/rams shall be vaccinated against scheduled disease protocol. However, vaccinations against bacterial diseases shall be done only if there is an outbreak or prevalence of a particular disease. Vaccination schedules for Sheep and Goat are attached as Annexure-V & Annexure-VI.

## 10. Culling of bucks/rams and Disposal of Semen Doses due to Specific Diseases

The semen station must remove bucks/rams (within 48 hours), which are positive for Brucellosis, TB and JD.

| Diseases | Bucks/rams | Semen doses |
|---|---|---|
| Brucellosis | Castrate & remove | FS doses in stock to be discarded since the last negative test |
| JD | Culled | FS doses in stock to be discarded since the last negative test |
| TB | Culled | FS doses in stock to be discarded since the last negative test |
| CCPP | Culled | FS doses in stock to be discarded since the last negative test |
| PPR | Culled | FS doses in stock to be discarded since the last negative test |
| Maedi-visna and caprine arthritis/encephalitis | Culled | FS doses in stock to be discarded since the last negative test |
| Bluetongue | Culled | FS doses in stock to be discarded since the last negative test |
| Ovine epididymitis; | Culled | FS doses in stock to be discarded since the last negative test |

In a Semen Station, Culling can also be done to those bucks/rams, which have completed the reproductive period or stipulated semen doses, whichever is achieved earlier, as per the approved breeding programme. In addition, the bucks/rams with poor libido, poor semen quality, incurable lameness, etc. shall also be culled.

## 11. Housing and Management of Ram/Buck

### 11.1 General considerations

a. Ram/Buck sheds shall have spacious individual pens with adequate loafing area, manger and water trough with access to drinking water all time.

b. Appropriate roofing with suitable materials and adequate shade around the buck or ram shed shall be provided.

c. Inside the Semen Station, there shall be an isolation shed for separating ailing / sick animals for treatment.

d. Scientific feeding schedule should be followed for the Ram/Buck.

e. The floor should be sterilized at least once a year by a blowlamp or by burning straws.

f. Disinfectants like **formalin or phenyl** based compounds **shall not be used** in Ram/Buck sheds. Alternatively, compounds containing Gluteraldehyde shall be used. Weekly spraying of Sodium Carbonate (4%) solution shall also be practiced.

### 11.2 Management of Ram/Buck

The objective of daily care of breeding animals (Ram/Buck) is to ensure a satisfactory state of cleanliness. For proper management, the following points shall be considered:

a) The Ram/Buck shall be kept under hygienic conditions at all times.

b) The coat of the Ram/Buck shall be kept clean and generally short.

a) The hooves shall be regularly trimmed.

b) Ram/Buck shall be brushed and groomed regularly, and where necessary, special attention shall be given to the underside of the abdomen, a day prior to semen collection.

c) External cleaning and trimming of preputial hair should be done before semen collection. Cleaning of the prepuce with sterile normal saline solution may be done, if, CFC is more.

d) In the event of obvious soiling, careful cleaning of the preputial orifice and the adjoining areas with soap or a detergent is recommended; followed by thorough rinsing and drying.

## 12. Semen Collection

a) The floor of the mounting area should be clean and provide safe footing. A dusty floor should be avoided.

b) On the day of collection, before collecting semen, the ram or buck shall be properly washed and cleaned. After that, the prepuce shall be cleaned externally with normal saline and a sterilized paper napkin or sterilized cloth napkin soaked in normal saline to remove any sand or dust particles. For each buck/ram a separate napkin shall be used.

c) The person responsible to carry out preputial wash must use disposable gloves and separate sterilized nozzle for each buck/ram to avoid transmission of infection from one buck/ram to another.

d) Semen collection should be individualized based on the buck/ram.

General guidelines on semen collection techniques, are attached as ***Annexure-XIV***

e) Sexual preparation (number of false mounts and restraint) of the buck/ram may be done considering the individual behavior of the buck/ram and not generalized. For this purpose, the sexual behavior of the individual buck/ram shall be studied and documented

f) As a general rule, buck/ram shall be sexually prepared by giving two / three false mounts followed by restraint. The gap between two ejaculates shall be half an hour to one hour depending on the buck/ram. Second ejaculate shall be taken with proper preparation of buck/ram, if required.

g) The hindquarters of the teaser, whether a dummy or a live teaser animal, should be kept clean. A dummy should be cleaned completely after each period of collection. A teaser animal should have its hindquarters cleaned carefully before each collecting session. The dummy or hindquarters of the teaser animals should be sanitized after the collection of each ejaculate. Disposable plastic covers may be used.

h) The hand of the person collecting the semen should not come into contact with the animal's penis. Disposable gloves should be worn by the collector and changed for each collection

i) The artificial vagina should be cleaned completely after each collection where relevant. It should be dismantled, it's various parts washed, rinsed and dried, and kept protected from dust. The inside of the body of the device and the cone should be disinfected before re-assembly using approved disinfection techniques such as those involving the use of alcohol, ethylene oxide or steam. Once re-assembled, it should be kept in a cupboard which is regularly cleaned and disinfected.

j) Appropriate Caprine /Ovine specific AVs can be used.

k) The lubricant and the rod used to spread the lubricant should be clean and should not be exposed to dust between successive collections.

l) The artificial vagina should not be shaken after ejaculation, otherwise lubricant and debris may pass down the cone to join the contents of the collecting tube.

m) When successive ejaculates are being collected, a new artificial vagina should be used for each mounting. The vagina should also be changed when the animal has inserted its penis without ejaculating.

n) The collecting tubes should be sterile, and either disposable or sterilized by autoclaving or heating in an oven at 180°C for at least 30 minutes. They should be kept sealed to prevent exposure to the environment while awaiting use.

o) After semen collection, the tube should be left attached to the cone and within its sleeve until it has been removed from the collection room for transfer to the laboratory

p) The entry of visitors and staff / laborers (other than those not involved in semen collection) shall be strictly prohibited in the collection arena at the time of semen collection.

q) Protective clothing (barn coat) and gumboots shall be used by the veterinarians and personnel during semen collection. Gumboots and barn coat should be washed immediately after completion of semen collection work.

r) **Semen stations must follow the norm of minimum number of ejaculates per collection and minimum number of collections per ram/buck per week as per their breeding programme.** However, an average ram or buck may give 0.5ml to 1.5ml of semen per ejaculate. Some animals can do this 2 or 3 time each day and perhaps every day of the week.

s) After semen collection, the tube should be left attached to the cone and within its sleeve until it has been removed from the collection room for transfer to the laboratory.

## 13 Handling, processing & freezing of semen

### 13.1 Premises of semen processing laboratory.

a. Sufficient trees shall be planted and lawns prepared around the semen station to reduce dust.

b. The ceiling and walls of the laboratory shall be made up of non-porous materials. All cracks and crevices shall be sealed to control pests and insects.

c. Entry of persons to the laboratory, other than laboratory personnel, shall be strictly restricted. Airlock system or anti-room shall be provided to avoid direct entry to the semen-processing laboratory.

d. Laboratory windows shall preferably be made of double sheet glass with fixed aluminum frame. The glass panes shall be plastered with sun control films to avoid direct sunlight. The doors shall be kept closed, especially during dilutor preparation and semen processing.

e. Preferably cassette type or, split type air conditioners fitted with air purifying system with remote temperature control mechanism should be installed to maintain the room temperature at 20C - 22C. The number of ACs to be fixed to sustain this temperature shall depend on the size of the processing room. Maintaining this temperature is most important to achieve the best results when single step dilution method is followed for freezing semen.

f. The flow of air from AC must not be towards the front side of the Laminar Air Flow Unit.

g. Adequate number of thermometers shall be kept in a few places in the laboratory to check the room temperature.

h. Alternatively, central cooling with 10 to 15 air exchanges should be fixed, especially for the semen-processing laboratory. This helps to control the bacterial load in the semen-processing laboratory and in removing obnoxious odour. The processing laboratory should ideally maintain around 55% relative humidity.

i. Sink drains shall be decontaminated routinely with a disinfectant. Sink shall not be placed in the semen processing room.

j. The floors shall be preferably made up of vitrified tiles. Floors and horizontal surfaces shall be cleaned and mopped with a disinfectant solution, as dirt and dust, which settle on these surfaces, are the main sources of contamination.

k. Unwanted furniture, equipment and materials shall not be kept in the laboratory as they only provide additional area for dust and spores to collect.

l. Appropriate number of germicidal UV lights (2470 A) with respect to area of laboratory, laminar airflow unit, apron and laboratory footwear cabinet may be fixed with a common operating switch outside the laboratory. These lights shall be switched "ON" at least 8 hours prior to commencement of work in the laboratory and shall be switched "OFF" before beginning work. The date of installation of the UV lights shall be noted to facilitate replacement as the life of UV tube is of 2000 hours. A logbook should be maintained for timely replacement of UV lights.

m. The laboratory shall be fumigated twice a week with **Cold Fumigant,** using humidifier.

n. Monitoring laboratory environment by bacterial load test should support fumigation. The bacterial load shall be measured every week to monitor pollution of the laboratory atmosphere.

o. The work platform, the parts of equipment and other items to be handled during processing of semen, shall be cleaned with 70% alcohol or Glutaril (Qualigen). It is advisable to repeat cleaning schedule after completing processing of semen.

p. Clean laboratory footwear, apron, hand gloves, mask and caps shall be compulsorily put on while working in the laboratory.

q. Eating, drinking, smoking, etc. shall be prohibited in the laboratory and unnecessary conversation should be discouraged. Besides, entry of persons shall be strictly restricted.

r. Long exposure of semen to ultraviolet rays, visible light in direct sunlight and white florescent light causes chromosomal damage and hence, direct exposure to such sources of light shall be avoided. Hence, there shall be provision for indirect or diffused lighting inside the semen processing room. Care shall also be taken not to switch on tube lights in CH cabinet and laminar air flow unit (LAFU). However, at the time of filling and sealing of straws in LAFU, diffused light could be used.

## 13.2 Equipment

a) The exteriors of all equipment and furniture shall be cleaned weekly. The equipment shall be kept covered by plastic covers when not in use.

b) The pre-filter of Laminar Airflow unit shall be cleaned weekly.

c) Water bath should be inside the Laminar Air Flow.

d) Routine servicing and DOP testing twice a year will ensure efficiency of HEPA filters. Alternatively, culture plate test shall be carried out at frequent interval to assess bacterial load of the air passing through the filters.

e) Digital photometer / Computer aided Spectrophotometer shall be validated with Haemo- cytometer readings for sperm concentration twice a year separately for sheep and goat.

f) The automatic semen straw filling and sealing machine shall be thoroughly cleaned, immediately after use.

g) The microscope lens shall be gently cleaned daily with a piece of cotton soaked in a mixture of ethyl and methyl alcohol (1:1) or a mixture of 80% ethyl alcohol and 20% ether)

h) The bio-freezer shall be defrosted and thoroughly cleaned and dried, immediately after use.

i) Incubators to maintain artificial vagina shall be cleaned and disinfected with 70% alcohol.

j) Single distilled water shall be used in autoclave and thermo-controlled water bath.

The water bath shall be cleaned and filled with single distilled water on a regular basis.

k) The thermometer kept immersed in water bath shall be cleaned daily to have precise temperature reading or water bath fitted with digital display temperature indicator should be used.

l) The Liquid Nitrogen containers returned / received from foreign countries/places and contagious disease prone areas shall be disinfected thoroughly with 4% soda solution and finally with 1 to 4% formaldehyde.

m) The refrigerator meant for storing eggs, antibiotics and buffer shall not be used for storing vaccines and other materials. All such materials shall be stored at a place away from semen laboratory. The refrigerator used for storing eggs, etc. shall be sterilized every week using alcohol swab.

n) All equipment used in semen processing should be covered under Annual Maintenance Contracts.

o) **The following equipments should be validated by Veterinary authority of semen station/Universities/ICAR:**

i. Standard Thermometer
ii. Water Bath
iii. Weighing Balance
iv. Incubator
v. Autoclave
vi. Hot Air Oven
vii. Slide Warmer
viii. Micropipettes
ix. PH Meter
x. Phase Contrast Microscope

p) **The following equipment calibration needs to be certified by Manufacturer/ supplier:**

i. Cold Handling Cabinet
ii. Laminar Air Flow Units
iii. Biological Freezer
iv. Filling, Sealing and Printing Machine

v. Photometer

vi. Triple distillation unit, etc;

A List of equipment needed for semen collection and freezing are listed as ***Annexure VII***

## 13.3 Personnel Hygiene

Clothing, skin and hair of laboratory personnel are the sources of contamination. Hence, all should wear laboratory aprons and footwear all the time while they are in the laboratory. Hands shall be washed with soap and water and rinsed with 70% iso-propyl alcohol, before commencing work in the laboratory. The buck or ram attendants must undergo test for TB every year. Other staff working in farm should be tested for TB once in two years. Restricted entry inside the semen processing room and freezing room shall be strictly adhered to.

## 13.4 Semen Extenders

a) All receptacles used should have been sterilized.

b) Buffer and extenders should be prepared in a separate classified zone.

c) Prolonged storage of purified water is not recommended because water purity deteriorates progressively over a period of time as heavy metals leach from some glass and plastic storage vials / containers.

d) Glassware, collection tubes, etc. shall not be handled from their rim / mouth.

e) Pipetting shall be done away with; instead, adjustable micropipettes and disposable tips shall be used.

f) After adding all the components of buffer viz. TRIS, Citric Acid, Glycerol and Fructose in double, preferably triple distilled water, it should be sterilized again. If buffer is prepared on the previous day and stored in the refrigerator, then antibiotics are to be added next day in the morning after warming it at 34 C.

g) Buffer solutions employed in diluents prepared on the premises should be sterilized by filtration (0.22 µm) or by autoclaving (121°C for 30 minutes) or be prepared using sterile water before adding egg yolk (if applicable) or equivalent additive and antibiotics.

h) If the constituents of diluents are supplied in commercially available powder form, the water used should have been distilled or demineralised, sterilized (121°C for 30 minutes or equivalent), stored correctly and allowed to cool before use.

i) Whenever ***milk, egg yolk or any other animal protein*** is used in preparing the semen diluents, it should be free from pathogenic agents or sterilized; milk heat-treated at 92°C for 3–5 minutes, eggs from SPF flocks when available. When egg yolk is used, it should be separated from eggs using aseptic techniques.

Alternatively, commercial egg yolk prepared for human consumption or egg yolk treated by, for example, pasteurization or irradiation to reduce bacterial contamination, may be used. Other additives should also be sterilized before use.

j) The eggs used for making diluents must be **fresh**. The eggs shall be stored in refrigerator after wiping with dry cotton. Just before preparation of dilutor, eggs shall be wiped with 70% isopropyl alcohol. To avoid Mycoplasma infection, eggs shall be purchased from known sources.

k) Goat semen is different from that of other domestic species in its limited tolerance to the inclusion of egg yolk in the freezing medium, and this tolerance depends on the presence of enzymes in the seminal plasma that react with egg yolk, producing toxic compounds to the spermatozoa. Moreover, the goat is a seasonal breeder that shows variations in semen quality throughout the year, and those variations may affect semen freezability; hence in freezing protocols, if requires, washing can be included.

Details in ***Annexure VIII***

l) The required quantity of yolk shall be separated from albumin on sterile (autoclaved) standard filter papers (Whatman No.1/ Borosil) and yolk membrane shall be punctured using sterile glass rod, Pasteur pipette or sterile straws under the Laminar Air Flow Unit.

m) Diluents should not be stored for more than 72 hours at +5°C before use. A longer storage period is permissible for storage at -20°C. Storage vessels should be stoppered.

n) A mixture of antibiotics should be included with a bactericidal activity at least equivalent to that of the following mixtures in each ml of frozen semen:

- gentamicin (250 µg), tylosin (50 µg), lincomycin–spectinomycin (150/300 µg);
- penicillin (500 IU), streptomycin (500 µg), lincomycin-spectinomycin (150/300 µg);
- amikacin (75 µg), divekacin (25 µg).

o) The names of the antibiotics added and their concentration should be stated in the international veterinary certificate.

Model composition of extenders are given in ***Annexure-XV***

## 13.5 Evaluation& Processing

a) The tube containing freshly collected semen should be sealed as soon as possible after collection, and kept sealed until processed.

b) As soon as the neat semen is received, it shall be kept in a thermo-controlled water bath at 34o C under Laminar Air Flow Unit, after recording the volume of semen.

c) After examination of sperm concentration and initial motility, semen samples shall be primarily diluted with dilutor maintained at 34°C.

d) After initial dilution of semen in the ratio of 1:1, the semen should be extended further after 7 minutes of cooling at 20˚C with dilutor maintained at the lab temperature. The semen samples should not get accumulated for long time in water bath, which may reduce their viability.

e) Sperm concentration shall be checked preferably by a digital photometer with auto dilutor, manufactured by a reputed company. The photometer shall be calibrated separately taking 20 readings each for sheep and goat semen, at least once in six months, with haemocytometer readings. Semen samples showing less than 500 million / ml sperm concentration shall be discarded.

f) Basic semen analysis can be done as per the Guidelines attached as ***Annexure IX***

g) Semen samples selected for freezing should have minimum 70% initial progressive motility. **Final dilution of semen, with a minimum of 40 million progressively motile spermatozoa per frozen thawed dose of 0.25 ml for laparoscopic method of AI and a minimum of 100 million progressively motile spermatozoa per dose of 0.25 ml for cervical or intra-cervical method, shall be done in appropriate flasks with the dilutor maintained at 34°C.**

h) Filling and sealing of semen shall be done under Laminar Air Flow Unit using sterile straws, filling nozzles and fresh rubber tubing. Rubber tubing shall be used once only. Reuse of rubber tubes is not recommended. Considering the advantages that French Mini Straws have over French Medium straws, the semen stations shall use French Mini straws.

i) If sealing powder is used, care should be taken to avoid its being contaminated.

j) The freezing should be carried out as per the recommended protocols for freezing sheep and goat semen.

Cryopreservation of Sheep/Goat semen details attached as ***Annexure X&XI.***

## 13.6 Storage and Identification of frozen semen

a) Semen for export should be stored in straws separately from other genetic material not meeting the requirements of this chapter with fresh liquid nitrogen in sterilized/sanitized flasks before being exported.

b) Semen straws should be sealed and code marked in line with the international standards of the International Committee for Animal Recording (ICAR).

c) Prior to export, semen straws should clearly and permanently be identified and placed into new liquid nitrogen in a new or sterilized flask or container under the supervision of an Official Veterinarian. The contents of the container or flask should be verified by the Official Veterinarian prior to sealing with an official numbered seal before export and accompanied by an international veterinary certificate listing the contents and the number of the official seal.

d) Information pertaining to buck/ram number, breed, name of the institution/organization, year, batch number (as per the day of the year), ejaculate number, etc., shall be printed on straws, preferably after their filling and sealing. After printing, the ink gets instantly dried. If filled straws are printed and racked, the actual number of straws can be easily counted. While printing and racking, the room temperature shall be maintained at 20°C to 22°C.

### 13.7 Post -Thaw motility

After freezing, the semen straws shall be stored in a separate container. Post-thaw motility of semen should be examined at 24 hours (after freezing). Differences in observations shall be updated and recorded for the purpose of accepting a particular batch of semen doses. Whenever there is any doubt, post-thaw motility shall be examined by two experienced persons. Preferably, the person involved in evaluation of neat semen, shall not check the post thaw motility. For a minimum concentration of 40 million live spermatozoa per dose, minimum acceptable post thaw motility shall be 40-50%.

## 14. Quality Checks for frozen semen

This includes,

(i) Quarterly testing of random samples from each batch for bacterial load using standard plate count (The standards for acceptable Colony Forming Units (CFUs) in processed semen is 5000 per ml as per OIE norm. If the bacterial load exceeds the OIE limit, the semen doses are to be discarded.) The frozen semen samples should not have uncountable CFUs as they may have pathogenic organisms. Therefore, semen showing crowded CFUs should be subjected to testing for pathogenic organisms by an outside laboratory.

(ii) Hypo osmotic swelling test (HOST) - for all bucks/rams at least once in a quarter shall be mandatory

(iii) Incubation test - for all bucks/rams at least once in a quarter shall be mandatory

(iv) Acrosome integrity test by Giemsa staining-at least once in a quarter shall be mandatory. Alternatively, wet smear of semen shall be examined using DIC(Differential interference contrast) microscope.

(v) Percent Intact Acrosome - all bucks/rams to be covered once a quarter

(vi) Sperm Concentration – randomly two samples per week each for sheep and goat

A summary of quality tests to be conducted for frozen semen and their cut-off values are given in the following table:

| S. No. | QC Parameters | Cut- off Values |
|---|---|---|
| 1 | Bacterial Load (FSD) | 5000 CFUs /ml |
| 2 | HOST | = 40% |
| 3 | Incubation / Thermo resistance Test | Standard drop in motility by 10% after every 30 minutes |
| 4 | Acrosome Integrity test (Fresh Semen) | = 70% |
| 5 | PIA (post thaw) | = 65 % |
| 6 | Sperm Concentration | 40 million live spermatozoa per dose (0.25 ml) |

Validation of photometer shall be done once in 6 months by checking at least 20 samples each for sheep and goat. Neat semen shall be examined at an interval of every six months for morphological abnormalities. Morphological examination of sperms of young animals must be carried out (at least six samples at weekly intervals) before introducing them in the flock. Semen should not be used if the sample contains a total abnormality of more than 20% and head and mid-piece abnormality (alone) of 7%.

Quality checking of semen straws, drawn randomly from the long storage containers once in three months, should be done as a part of quality assurance.

## 15. Information System

In order to facilitate the information system, all the bucks/rams maintained by the semen station must be identified appropriately. The semen stations shall use suitable software to record data pertaining to various activities and also should have online facility for the same. The semen stations producing more than one million doses may introduce software that can identify and trace the bucks/rams and their ejaculates, production, storage and dispatch of semen **(barcode system).**

a) Volume of semen, density, motility, sperm concentration, dilution rate, total extended volume, post-thaw motility (24 hrs after freezing), and total number of doses produced, etc. shall be maintained. Pre-freeze and post-thaw motility shall be checked for new and problematic ram or buck.

b) Miscellaneous information regarding actual reason(s) for not donating semen, undesired percentage of gross morphological defects, semen pH, presence of dirt, dust, blood, pus, etc. in semen samples shall be noted and recorded.

c) Details of semen supplied to various agencies, including post-thaw motility at the time of dispatch, shall be recorded.

d) Fertility data of ram/buck, conception rate, records of the progeny associated with any genetic defect, percent male / female born, etc. shall be noted and recorded.

e) Report on microbiological examination of semen samples shall be maintained.

f) Record of all quality tests for neat and frozen semen samples shall be maintained.

## 16. Semen Storage

To avoid accidental spread of diseases, the semen station shall follow the procedure of preserving semen doses for at least 30 days after production. Frozen semen doses produced at least 30 days prior to the date of dispatch should only be supplied for AI.

Frozen semen doses after checking post-thaw motility, if found acceptable, shall be kept in temporary storage for 7 days.

After temporary storage, the semen goblets shall be transferred to the bulk storage containers with proper recording of position in the canisters. After each dispatch, records redefining the position of remaining doses shall be updated.

Two reference samples to be kept from dispatched semen and retained for six months or a screen shot of randomly selected sample should be stored and a soft copy of which should be given to the customer

The goblets containing the semen should be well identified and precaution should be taken to see that each goblet has sufficient space for liquid nitrogen.

Mini straws need special care and should not be exposed above liquid nitrogen even for a short time (10 seconds) as they get warm faster and any exposure causes irreversible damage to sperm viability.

Liquid Nitrogen shall be replenished at regular intervals depending on the liquid nitrogen evaporation rate of the container.

## 17. Cleaning and Sterilization

All the items to be washed shall be initially cleaned with running tap water and soaked in warm neutral detergent for at least 30 minutes. These items will then be thoroughly cleaned under running tap water using a brush. Filling nozzles shall be cleaned with pressure using 20 ml syringe. These materials shall be rinsed thoroughly with de-ionized water (5 to 7 changes) to completely remove detergent residues and other impurities.

**Appropriate procedure for sterilization of different materials, used in the semen station, is attached as *Annexure XII.***

## 18. Quality Control of Consumables

### 18.1 Chemicals

The chemicals of only highest purity of either, Analytical Reagent (AR) or Guaranteed Reagent (GR), from reputed manufacturing companies shall be used. Whenever a new chemical is to be introduced in the routine process, it is recommended to examine the post-thaw revival rates after conducting few spilt ejaculate trials (maintaining a control) with the new chemical. Assay of chemicals shall be >99%, having less impurities.

### 18.2 Straws

1. Straws manufactured by reputed companies are safer to use for production of quality semen. While buying straws, package volume and microbial load in straws shall be checked randomly from the consignment. In addition, some empty straws should be placed in filling and sealing machine and the machine should be run to see the sealing quality of the straws. In case of any foul smell, it should be presumed that the straws are manufactured from poor plastic which could be toxic to the spermatozoa and can even result in reduced motility on long storage.
2. The factory plug should not be loose. The factory seal should be impenetrable and the seal formed should be homogeneous and compact.
3. The straws should be intact (without cracks/dents, etc.) during and after freezing/ thawing.
4. The movement of straws along the printing machine should be free and print should be clear and sharp. Print should not fade as a result of freezing and subsequent thawing.
5. The use of dark colored straws should be avoided, as they are not transparent enough. Due to this, it is difficult to distinguish between filled / semi-filled straws.
6. Movement of the factory plug should be free.
7. Straws should be routinely checked for microbial load.

**Note:** The semen stations should avoid purchase of consumables on lowest quotation basis. For example: To produce top quality semen, it is better to use AR / GR reagents manufactured by reputed companies whose products are reliable. This is true with other consumables also.

## 19. Minimum Manpower Requirement of Semen Production Center

| Designation | Up to 10 lakh doses | >10-25 lakh Doses | >25-50 lakh Doses | >50lakh doses | Mega Semen Station, 10 Million doses |
|---|---|---|---|---|---|
| General Manager | 1 | 1 | 1 | 1 | 1 |
| QCO/QAO | 1 | 1 | 1 | 1 | 1 |
| Vet. Officer | 1 | 2 | 3 | 3-4 | 5-6 |
| Agriculture Officer | 1 | 1 | 1 | 1 | 1 |
| Data Mgmt. Officer | -- | 1 | 1 | 1 | 1 |
| Accts. & Adm. Officer | -- | 1 | 1 | 1-2 | 1-2 |
| Office Assistant | 1 | 2 | 3 | 5 | 6-7 |
| Livestock Assistant | 1 | 2 | 3 | 4 | 5 |
| Agri. Assistant | | | | | |
| Lab Technician | 1 | 2 | 3-4 | 5-6 | 8-10 |
| Vehicle/Tractor Driver | 1 | 2 | 3 | 4 | 5 |
| Lab Attendant | 2 | 3 | 3-5 | 7-8 | 10-12 |
| Buck/ram Attendant | 1 person per 10 animals | | | | |
| Agri. Laborers | 15-20/100 acres depending on mechanization level | | | | |

**The minimum man power structure suggested above is meant only for semen/fodder production. For other activities, manpower may be positioned as per the need.**

For dispatch of semen, facility should be created preferably away from semen station and operated by other person/s not responsible for semen production.

The GOI / Department of AH / Livestock Boards / NGO / Private agencies / Union and Federation shall review the requirement of manpower position for each semen station and finalize the staff structure for recruiting additional manpower. After recruitment, all new persons shall be trained at any of the recognized institutes. Once trained, they shall continue to work in the semen station at least for five years.

## 20. Refresher training / visit to other semen lab:

Technical exposure of semen station personnel working in the semen lab must be arranged compulsorily once in two to three years at reputed institutions like CSWRI, Avikanagar, CIRG, Makdoom, Universities etc.

As semen production activity is an extremely technical work, job rotation of personnel could be detrimental in maintaining the quality of semen. Therefore, personnel working in a semen station should not be transferred at least for five years. If it is inevitable, in the interest of carrying out good work, it should be essential that a proper replacement is identified at least six months in advance and is trained in semen production technology.

# Annexure-1

## Biosecurity Guidelines - Basic Tenets can be applied to State Sheep/Goat Farms

### A. EXECUTIVE SUMMARY

### 1. What is Biosecurity

1.1. Broadly speaking, biosecurity is a set of measures for protecting a population from infectious and contagious diseases at the national, regional and farm level.

### 2. Why is it important in a sheep and goat farm

2.1 Biosecurity is proactive and focuses on routine, day to day on-farm activities to protect the health of the herd by limiting the transmission of infectious agents that can cause disease in a farm or herd. Infectious agents are generally invisible, and can be moved from place to place in organic matter and on a wide range of materials that are frequently present in farming environments.

2.2 Biosecurity focuses on reducing the risk of disease, on the assumption that infectious agents could be present thereby contributing to improved animal health, improvement in day to day production, savings in production cost and increased profitability.

2.3 Biosecurity practices can also minimize the risk of exposure to zoonotic disease for the farmers, their families and their workers and reduce food safety risks potentially inherent in certain activities undertaken on the farm.

### 3. Major routes for disease ingress and pathogen transmission

3.1 Livestock

a. transfer of livestock between different production groups/areas

b. dead livestock disposal

c. dirt, manure or contaminants

3.2 People

a. farm personnel and family members living on site

b. contractors, maintenance personnel, neighbours, service personnel, visitors

c. dirt/ manure/ contaminants carried on hands, boots, clothing, hair, etc.

3.3 Vehicles and equipment

a. dirt/ manure/ contaminants carried on cars, trucks, tractors, weighing scales, husbandry equipment (plants, dips & vaccination guns, etc)

3.4 Feed and water

a. raw materials

b. post-production contamination or spoilage during transport and storage

c. faecal and urine contamination from the same species or other species

3.5 Pests and weeds

a. poisonous/ invasive plants

b. feral animals

c. domestic animals

d. rodents

e. insects

**4. Implementation:** Keeping the above in mind, it is felt that Farm-level biosecurity is all about a series of management practices designed to minimize, prevent or control the introduction of infectious pathogens onto a farm, spread within a farm and export of these pathogens beyond the farm, which may have an adverse effect on the economy, the environment and human health. Thus these guidelines are proposed to act as roadmaps for keeping a high level of Farm-level biosecurity. These guidelines have been prepared keeping in mind the operations and management in the Central Sheep Breeding Farm, Hisar, Haryana. It should be clear though that no single biosecurity measure/ plan provide an answer to preventing all diseases. They are not "Must Do" in a farm. These are guidelines on which any farm can dwell upon to develop their own biosecurity measures that suit their needs. All farms are advocated that they have their own set of Standing Operations Procedures while taking these guidelines into consideration. It is also necessitated that these guidelines be made aware to all personnel involved in all activities in the farm and that they are reviewed and revised from time to time.

They are structured under following heads:

**I. Movement of livestock, people and vehicles**

1. Livestock

1.1 Introduction of new animals

1.2 Re-entry of farm animals

1.3 Transportation of farm animals

2. Feed

3. People

4. Vehicles

5. Tools and Equipment

6. Unwanted entries

**II: Overall biosecurity within the farm**

1. Create a diagram of the farm layout

2. Facility management

3. Perimeter and interior fencing

4. Management of Feed, Water and Bedding

5. Animal Health Management

6. Management of Equipment and tools

7. Cleaning and disinfection of facilities and on-farm equipment

8. Carcass and waste management

9. Pasture management

10. Personnel management

11. Monitoring and record keeping

12. Response Plan for Disease Outbreaks

13. Animal welfare and biosecurity

**5.** Though detailed elsewhere in the guidelines, should there be an indication of any widespread infection or outbreak of disease, or suspicion of a notifiable disease, etc.; the nearest RDDL should also be informed to collect samples / material as per their norms and protocols.

**6.** The following may also be reported in the event of the above at following e-mail addresses:-

ahc-dadf@nic.in, jslh-dadf@nic.in, jspf-dadf@nic.in, jcpoul@nic.in, jclh-ahd@gov.in, acsrmp-dadf@gov.in

**7.** As mentioned, these guidelines have been prepared keeping in mind the operations and management in the Central Sheep Breeding Farm (CSBF), Hisar, Haryana. The CSBF was established in 1969-70 in collaboration with the Government of Australia under Colombo Plan. The farm has been established with the objectives of production of a large number of improved cross bred rams for distribution to the sheep raising areas of India; Setting up extension and Training programmes to ensure the best use of the ram produced and Development of suitable management system and requisite facilities for breeding and rearing under Indian conditions, using purely Indian Resources. So far, over 2011 acclimatized breeding rams, 508 Beetal bucks and 1765 breeding ewes of exotic breeds and their crosses have been supplied till 2014-15.

The farm also imparts six day Training on Machine Sheep Shearing which includes machine shearing techniques, wool grading and maintenance of shearing machine, six day Training on Sheep/ Goat Management and Production, one day Training on Sheep/ Goat Production and Health. A separate training programme may also be organized if a full batch of about 30 trainees is sponsored by the State.

Further details may be obtained from the Director, Central Sheep Breeding Farm, Hisar, Haryana at telephone 01662-264329, telefax 01662-264263 or at email hisar_csbf@ yahoo.com and face book page at www.facebook.com/sheepgoatindia.com.

## B. INTERVENTION I: MOVEMENT OF LIVESTOCK, PEOPLE AND VEHICLES

The essence of any biosecurity plan is to prevent any ingress of disease into the farm or farm premises. Taking this into cognizance, the following are envisaged.

**1. Livestock:** Manage the introduction and movement of livestock in a way that minimizes the risk of introducing or spreading infectious disease.

1.1 Introduction of new animals: Newly purchased livestock entering the farm present a high risk activity for the unintentional introduction of disease agents, weed seeds or pests.

**Recommendations:**

a. Check animals for health status before purchasing. Pre-purchase inspection or veterinary inspection/certification would be helpful

b. Segregate, observe and treat (as required) newly introduced animals. Hold new stock in quarantine (isolation in separate pens) for 24 hours to ensure they have had time to empty out prior to release from quarantine and remember to provide clean drinking water all the time.

c. Newly arrived sheep/ goats should be routinely dewormed. This should be done as per deworming plan of the farm in order to avoid anthelminthic resistance in the animals.

d. Complete all disease testing, treatments, procedures and vaccinations before animals are released from isolation.

e. Quarantine paddocks or pens should be as near as possible to the farm entrance and well away from other stock. As a minimum, a double fenced 3 metre gap should be provided between newly arrived animals and resident stock.

f. Raise as many replacement stocks as possible on the farm, and only add new animals from off farm sources when necessary.

1.2 **Re-entry of farm animals:** Knowing the health status of animals that are re-entering the farm (e.g., animals attending livestock shows, etc.) enables to minimize the risk of introducing and spreading disease to the existing herd.

**Recommendations:**

a. Consider testing returning animals prior to introduction or reintroduction, in consultation with herd veterinarian. Tests used to determine disease status can include serology, culture, and fecal egg counts.

b. In any case, the animal should remain in isolation until the test results are known. Observe no clinical signs of disease are noticed during the isolation period. Have a plan for animals with positive test results; e.g., treat, do not buy or discard/dispose.

c. Prevent any sharing of feeding or watering equipment, penning, handling facilities or equipment between isolated animals and resident animals unless they are first cleaned and disinfected.

d. Complete all disease testing, treatments, procedures and vaccinations before sheep/ goats are released from isolation.

1.3 **Transportation of farm animals:** When taking animals to shows, melas and sales, remember that the farm stock can also transmit disease(s) to other animals by mixing or by coming into contact with pens, vehicles, people and equipment.

**Recommendations:**

a. No livestock shall be dispatch from the farm until authorised by the relevant authority.

b. Ensure that a mandatory veterinary inspection of all animals attending any show/ mela is conducted prior to unloading, and that any animal with evidence of being diseased is not unloaded.

c. Ensure that animals that have recently parturited/ aborted (i.e., within the last two weeks) or may parturate at the time of the show are excluded due to risk of transmission of infectious abortion diseases.

d. Transport animals in a vehicle that has been cleaned and disinfected prior to use. Ideally, this vehicle is dedicated exclusively to the farm's use.

e. Prevent direct contact and limit proximity with other animals and livestock in transit and on-site.

f. Supply bedding and feed from own farm. Ensure a clean supply of water onsite.

g. Bring feeders, water buckets, and grooming and handling equipment from home farm.

h. Limit handling of farm's animals by others.

i. Animal that are sold and moved out of the farm should be transported as per Transportation Rules taking into account their welfare. [Refer Transport of Animals, Rules, 1978]

**2. Feed:** Feed has the potential to be a source of contamination, infection or infestation. It can carry/ harbour disease agents, chemicals residues, weed seeds and/or pests. Incorrectly stored feeds can also deteriorate, grow unwanted disease agents (such as mould) or become contaminated via pests and vermin.

**Recommendations:**

a. Purchase feed from suppliers who produce quality feeds that are labelled and comply with regulations for feed designated for ruminant feeding. Ensure that it is transported in a clean carrier.

b. Take feed and forage samples from each batch. Label and store them to allow testing at a later date for quality and for the presence or absence of toxins, if necessary.

3. **People:** People entering the farm mainly are farm workers, family members, visitors and service providers. It is to be ensured that their movement and activities do not compromise with the animal and human health.

**Recommendations:**

a. Ensure that all farm workers are aware and understand of the biosecurity practices on the farm and are prepared to implement them and also to comply with any changes to the plan and practices.

b. In the event of visit of any service providers or visitors, ensure that all farm employees are made aware of the same. Similarly, all visitors and service providers are also to be made aware in advance, prior to their visit, of the biosecurity practices that will apply and come prepared.

c. On arrival, all farm visitors/ service providers are to record their visit in the visitors' register. They are then briefed of the layout of farm, which areas they are permitted to access, and what biosecurity practices need to be applied in that location.

d. Ensure that visitors and service providers access only areas of the farm that are necessary.

e. Permit contact with animals only when necessary.

f. All who enter and work on, or visit the farm wash and/or sanitize their hands upon entry and exit, when moving between farm zones, and when approaching or leaving certain identified risk areas of the farm premises such as isolation/ sick pens, etc.

g. Hands should also be washed or sanitized before and after any contact with animals, especially those that are diseased or of unknown health status, following contact with any potentially contaminated material, such as deadstock, aborted fetuses, placentas or manure.

h. Encourage the use of Personal Protective Equipment (PPE) when visitors move onto farm property. Visitors/ service providers are to be explained of the use of PPE, and how to put it on and remove it. It is to be ensured that all discarded PPEs or any potentially contaminated materials are disposed off in garbage cans with sealable/ proper lids provided. Personal cleanliness and general hygiene should be encouraged amongst the visitors and service providers.

i. Meet with farm workers and their family members at least twice yearly to discuss the usefulness and effectiveness of each of the practices in the biosecurity plan. Basics such as not leaving the animal area without cleaning any contamination such as animal excreta from their clothes, or not leaving the animal area without cleansing and disinfecting their shoes may be highlighted in such sessions.

j. Maintain a visitors' register with all required information duly entered on entry and exit from the farm.

**4. Vehicles:** All parts of a vehicle can carry disease causing organisms, pests and weeds seeds. Without restricting parking and vehicle movements within the property, it would be difficult to control and monitor the spread of diseases, pests and weeds.

**Recommendations:**

a. Restrict access of off farm vehicles. Vehicles that travel from farm to farm should not be allowed to enter the farm unless they have been cleaned and disinfected in such a way that any contamination that may be present on the undercarriage or exterior of the vehicle will not be deposited in the farm.

b. Multiple, unsecured entry points to the farm make it difficult to control and manage entry of vehicles to the farm. Encourage entry into the farm via one or two routes only.

c. Animal transportation vehicles should be loaded and unloaded at the periphery of the farm and animals can then be led to the isolation area.

d. Feed trucks should also unload or load in designated areas preferably near the feed godowns without entering the main farm area. Ensure that trucks used to transport feed or silage have not been used for any purpose that presents a biosecurity risk to the herd and that they have been suitably cleaned before use.

e. Cleaning and disinfection is the principle biosecurity tool for reducing vehicle-related disease risk. Provide a wash area for vehicles that need to enter the farm. If possible, use a high pressure wash down facility located well away from crops or livestock for cleaning vehicles and equipment. Ensure that the run-off from vehicle wash is directed away from production areas of the farm.

f. Should the wash area be not possible, ensure that vehicles that enter the farm pass through a wheel dip constructed at the entry point to the farm.

g. Maintain a vehicle register with all required information duly entered on entry and exit from the farm.

**5. Tools and Equipment:** Tools and equipment can carry diseases, pests and weeds seeds. The risk for disease spread is higher when equipment is borrowed, lent or bought second-hand from other properties. It is to be ensured that movement and usage of equipment and machinery do not compromise animal and human health in any way, not only within the farm but also of other farms in the area.

**Recommendations:**

a. Ensure that any personnel, equipment or machinery do not leave the farm until authorised by the relevant authority.

b. Minimise lending and borrowing of equipment between properties. If lent, ensure it is cleaned before and after use.

c. Clean and disinfect tools and equipment before and after use on livestock or between different batches or herds of animals.

d. Clean and disinfect second-hand, borrowed or lent equipment before and after use.

e. Have dedicated tools, clothing and footwear available for use in specific areas like production areas or in isolation areas where sick or quarantined animals are kept.

**6. Unwanted entries:** Pests, stray animals, predators and wildlife represent a pool of unique risks to Sheep and Goat farms. They are difficult to fully control, but do require attention in the biosecurity plan of the farm as they may be a high risk source of contamination for certain common diseases. Predation from wild dogs, birds of prey and other animals may also pose an issue for young, weak or incapacitated livestock. Pest species may also include insects such as locusts and flies, deer, vermin, etc.

**Recommendations:**

a. **Access of stray animals like dogs, cats and other livestock to farm area and specifically to manure, placentas, dead-stock and other potential sources of contaminated material should not be allowed.**

b. Ensure farm buildings are in good repair and that feed stores and godowns are vermin proof.

c. Ensure boundary fences are secure. Regularly undertake property inspections to assess possible biosecurity breaches and/or potential for breaches. Correct where necessary.

d. Remove or contain anything that is likely to attract vermin, insect pests or wild animals. Fencing off the domestic waste disposal areas (rubbish dumps), will assist in reducing scavenging by feral and domestic animals and prevent livestock, feral animal and wildlife access.

e. Coordinate with the families of farm workers, neighbours and other local community members and groups to maximize the effectiveness of actions to control the pest animals.

f. Ensure a rodent control programme in the farm to avoid possible spread of disease and loss due to contamination at feed stores, etc.

g. An integrated fly control programme may be developed.

## C. INTERVENTION II: OVERALL BIOSECURITY WITHIN THE FARM

To help achieve a good level of biosecurity, the following management practices are also recommended to be added to the biosecurity plan.

**1. Farm layout:** Having a diagram of the layout of the farm would be a good idea as this can aid in how one can approach to tackling the biosecurity concerns of the farm. Having a map or diagram of the farm would help in assessment of the farm's operations, people on the farm, identify where risk points exist, people's activities, and facilities and how they are maintained, locate areas for housing animals with different disease status, storage areas for feed, equipment, etc.

Areas that could be highlighted on the farm layout include:

- Access points
- Gates and barriers
- Staff residential area
- Farm buildings, including barns, sheds, service areas, farm office and utility areas
- Pens and isolation areas
- Animal loading and unloading area
- Feed storage area
- Manure storage area
- Deadstock pickup area or compost location
- Driveways and lanes
- Parking areas
- Fuel delivery/storage area
- Paths and walkways
- Pastures
- Wells and other water sources

**2. Facility management:** Most practices that are contained in biosecurity plans for Sheep and Goat farms are designed to reduce the risk of disease transmission between animals and from people and their tools, equipment and vehicles to animals. In addition to these activities that act more directly on the disease risks, there are also important options to consider in developing a plan. The design and construction of facilities that house Sheep and Goat can be modified to support other biosecurity practices and/or to address risks directly.

**Recommendations:**

a. Design floor surfaces to be more easily cleaned.

b. Use of smooth or non-porous materials can be considered so that adherence of both organic materials and pathogens to surfaces is reduced.

c. Design the facility so that there is less distance involved while carrying and removing manure or other sources of contamination such as soiled bedding from sick area, etc.

d. Keep ease of access for cleaning equipment in mind while designing barns, or divisions between pens, etc.

e. Moving new introductions or sick animals for quarantine provides a mechanism for these animals to spread any disease organisms to other members of the flock. Identify and accordingly design pathways for movement of animals within the farm, in addition to scheduling the order of animal movements, and cleaning and disinfection between uses, where appropriate.

**3. Perimeter and interior fencing:** Fencing is used to maintain separation between resident animals and other animals on the farm, and between the herd and livestock on adjacent farms. Fencing also serves to separate certain animals from the rest of the herd under preplanned circumstances.

**Recommendations:**

a. Install and maintain perimeter fencing to ensure that animals do not wander to uncontrolled areas and to restrict interaction with wildlife or contact with neighboring livestock or other livestock on the farm.

b. Install and maintain interior fencing that is appropriate for its biosecurity purpose. For example, when pasturing animals of differing health status in adjoining pastures, consider a buffer zone between the pastures.

c. Regularly inspect for fencing faults such as gaps, loose wires or washouts and swiftly maintain adequate boundary fence.

**4. Management of Feed, Water and Bedding:** Feed, water and bedding serve to support the health of farm animals and therefore the flock's resistance to disease. Adequate and quality supplies are required, and storage is secure from contamination

**Recommendations:**

a. Ensure that both farm-grown and purchased feed be free of toxins that may naturally occur or that may form in storage.

b. Assessment of the quality and nutritional value of the feed is important to ensure a complete, healthy ration.

c. Purchase feed from known suppliers who produce quality feeds and ensure that it is transported in a clean carrier.

d. Store feed in a secure, clean facility that limits degradation of feed and prevents access by wildlife, rodents, pests, dogs and cats.

e. Design and position feeders to prevent fecal and other contamination by the animals while in use. If feeders become contaminated, remove and dispose of the feed, and then clean and disinfect the feeders before use.

f. Clean fresh water in adequate volume should be made available to all stock at all times. Water should be tested at least annually to ensure its cleanliness and safety. Its source location and facility should be checked to ensure that there is no contamination from surface water or runoff or from material such as bones, faeces, plant matter or carcasses.

g. Design and position water bowls, troughs and other waterers to prevent fecal and other contamination by animals while in use.

h. Dispose of contaminated water when found and clean and disinfect the waterer(s) before the next use.

i. Bedding material purchased should be of good and clean material. It should be stored in a protected location such that it remains dry and uncontaminated. As much as possible, bedding material should be secure from contamination by pests, dogs and cats, and rodents. Bedding material in use should be judged by its moisture and cleanliness, cleared regularly, and replaced by dry, clean product.

j. Remove and replace bedding from hospital and isolation pens regularly. Discarded bedding should be moved to an area that does not have animal access.

**5. Animal Health Management:** Improvements in animal health should be one of the goals in implementing an on-farm biosecurity plan. The concept of improving animal health, welfare and biosecurity on-farm, is all about managing risk. Disease in animals can be caused by infectious agents (such as viruses, bacteria, fungi, protozoa and prions), parasites (such as gastro-intestinal worms, ticks, lice, flies, fleas, etc.), chemicals and poisons, nutritional issues, injuries and inherited genetic problems. For most of these causative classes of disease there are preventive measures that can be taken to minimise the impacts to livestock.

**Recommendations**:

a. Ensure all farm workers are aware of the importance of early detection and reporting of unusual animal deaths or animals exhibiting signs of sickness.

b. Monitor the flock's disease status through routine diagnostic testing (e.g. fecal egg counts, serological testing) and including post mortem examinations for unexpected or excessive livestock deaths.

c. Vaccination programs to control or prevent disease within the flock.

d. Metaphylactic / prophylactic medication programs to control or prevent disease within the flock (e.g. deworming, foot bathing).

e. Decision plan for isolating sick animal including release from isolation. This includes resident animals, new introductions and returning animals.

f. Do not bring young stocks which are more vulnerable to disease onto paddocks or pasture vacated by older animals (which are more disease resistant and will probably include disease carriers) without a reasonable stand-down period, such as 7 days between grazings by different stock classes.

g. Proper storage of medications and vaccines.

h. Proper disposal of animal health medications and vaccines, including used needles and syringes. *[Refer Bio-Medical Waste (Management & Handling) Rules, 1998 under Environment (Protection) Act, 1986]*

i. Ensuring that the animal health management programme comply with the requirements of any relevant public and regulatory programs, including environment, food safety, animal health and animal welfare.

j. Record the timing of vaccinations and other preventative measures being used on the herd/flock management calendar and where necessary, matched to seasonal conditions or management operations.

k. Farm workers are to be educated when to sound alert for call on the nearest veterinarian or veterinary authorities in the event of abnormal deaths, unfamiliar disease, rapid spread of disease in flock, or any abnormal behavior in the herd.

l. It is to be noted that under OIE Terrestrial Code some animal diseases are notifiable – which means one have a legal responsibility to report them to animal health authorities. *[Refer Infectious and Contagious Diseases Act, 2009]*

**6. Management of Equipment and tools:** Equipment, tools and vehicles that are brought onto the farm are moved into it only if necessary. If possible, dedicate equipment and tools to one activity or area.

**Recommendations:**

a. Purchase own equipment whenever feasible.

b. Service equipment, tools and vehicles that are brought onto the farm are cleaned and disinfected before arrival and, if they are used, they are cleaned and disinfected between uses.

c. Ideally, purchase and use a dedicated set of equipment (shovels, forks, scrapers, buckets, etc.) for use in each of the following areas:

   i. isolation area for new arrivals or returning animals

   ii. isolation areas for sick animals

   iii. kidding pens

d. Dedicated equipment may also be used for other facilities outside the above areas, to perform each of the following tasks:

   i. manure and soiled bedding handling

   ii. deadstock management

   iii. feed management

   iv. clean bedding management

e. If dedicated equipment for identified risk areas or individual activities is not feasible then they have to be cleaned and disinfected properly after each use and when moving from one area facility to another.

f. Ensure that those responsible for cleaning and disinfection know what type of soap or detergent and disinfectant solutions are required for each task for maximum effectiveness.

g. Clean and disinfect feeders and waterers regularly, based on use and experience, and whenever contamination with manure, urine and/or other potentially contaminated materials occurs, and whenever they are being prepared for use by other sheep/ goats of differing health status.

h. Store equipment that has been cleaned and disinfected in a clean environment.

**7. Cleaning and disinfection of facilities and on-farm equipment:** Cleaning is a constant activity on a livestock farm and disinfection is needed under certain circumstances when required to reduce the risk of disease transmission.

**Recommendations:**

a. Ensure that pen areas, feeders, waterers, equipment and vehicles are cleaned to remove organic material that can harbour disease pathogens or other contaminants.

b. Disinfection is required to eliminate pathogens. However, chemicals used to disinfect are not effective if the surface has not been previously thoroughly cleaned of organic matter.

c. Set an appropriate interval for cleaning. For example, cleaning and disinfection should be completed before or after a significant management event such as clipping or shearing, removal of manure and/or bedding.

d. Cleaning and disinfection is conducted prior to and after use, as well as in the routine maintenance of equipment and facilities. Focus on the facilities that house the herd e.g. barn surfaces, including floors, pens, railings, walkways, etc., and the tools, and equipment used to manage the herd or individual animal such as buckets, forks, shovels, feeders; water troughs etc.

e. Ensure effective cleaning and disinfecting of vehicles that transport animals, especially those from other locations; and other vehicles, such as visitors' and service providers' vehicles, especially those that have driven on other farms.

f. Special attention should also be given to clean and, where possible, disinfect pens or areas deadstock, aborted fetuses and placentas are discovered, areas such as the quarantine areas, kidding areas and hospital pen. Also include pathways used by animals of different disease status and/or susceptibility that pose a risk of disease transmission.

g. Some other considerations that will influence the risk management decisions to disinfect are the density of animals, level of contamination, and health status of animals.

**8. Carcass and waste management:** Dead animals and waste are a high risk source for some diseases. The life cycle of many pests involves them being shed in urine or faeces and the contaminated pasture being re-ingested. Some animals are super-shedders whose waste is highly infectious.

**Recommendations:**

a. Select disposal areas to avoid the potential spread of contaminants by water, wind or animals.

b. Secure and contain disposal areas where possible to prevent access by livestock, feral and domestic animals and wildlife.

c. Dispose of carcasses aborted fetuses, placentas and waste in a segregated area, where possible, taking into account environmental and public considerations.

d. Ensure herd contact with manure is minimized. Manure is a high disease contamination risk for most common diseases and may be a source of weeds if not composted thoroughly.

e. Remove manure on a regular schedule, taking care not to contaminate animals of differing disease status. Clean up spills immediately.

f. Clean and disinfect equipment used to collect and move manure after use and, if possible, dedicate it to that purpose.

g. Bio-waste such as leaf material or fallen fruit, etc. can attract or harbour pests and diseases. It is important to break the life cycle of insect pests. Collect all plant waste that shows signs of pests or disease and dispose of it by deep burial or burning, well away from water sources, nursery and production areas.

h. Ensure government requirements for carcass, effluent and waste management are adhered to where applicable.

**9. Pasture management:** Understanding the relationships between stocking density, carrying capacity, the condition of land and the health of livestock is essential. Managing the farm's pasture within the livestock management system, how rotation of livestock through a grazing cycle, when certain areas may become deficient in certain nutrients and what practices are undertaken (such as faecal testing, etc.), and how they are performed is also essential.

**Recommendations:**

a. If having own pasture for grazing, consider zoning different areas of the pasture land and ensure that certain zones are allowed for grazing. In other words, rotate the livestock through a grazing cycle in the different zones of the pasture.

b. Set up a gastrointestinal parasite control program that manages pasture contamination, uses anthelmintics appropriately, and monitor animals for internal parasites.

c. Avoid placing animals of differing susceptibility or immune status in the same zone/ pasture.

d. Prevent young and vulnerable livestock from immediately being allowed to graze in zones or pastures where older animals have recently grazed.

e. Invasive weed spread can reduce the productivity of land due to competition with native grasses and improved pastures. Poisonous plants can have an impact on the productivity of the herd/flock through poor health or even death to livestock in some cases. Make a list of the poisonous plants that occur in the area. Knowledge of local poisonous plants is vital to managing animal health and livestock production.

f. In combating infestations of weeds on farms, chemicals that may have toxic effects on livestock, are often used. These chemicals can also present a risk if they turn up as a residue in the human food chain. Such chemicals have with-holding periods associated with their use, to prevent them ending up in the food chain.

g. Ensure that there is no misuse of chemicals which can also lead to the development of resistance by pests, potentially creating new biosecurity risks and management challenges. Be sure to follow the instructions on the label and observe withholding periods after treatments.

**10. Personnel management:** In addition to adverse effects on the agricultural economy, diseases and pests can have negative effects on the environment and on human health. The benefits of implementing on-farm biosecurity practices are therefore not only significant in improving animal health and welfare, or more secure financial health for the farmers, but are equally important to ensure protection of health of farm workers and other service providers as well.

**Recommendations:**

a. Ensure family members, farm workers, visitors and service providers understand zoonotic diseases and take full precautions to protect themselves.

b. Ensure all personnel working on-farm are vaccinated for identified risk diseases (e.g. tetanus) and, where necessary, vaccinate livestock against zoonotic diseases (e.g. leptospirosis).

c. Provide clean clothing and footwear or suitable disposable clothing and footwear for farm

workers, service providers and visitors who need to enter the farm, more especially to those who work in the risk areas such as quarantine/ sick areas, etc.

d. Ensure that all farm workers and visitors understand the protocols and the use and change/disposal of protective clothing, footwear and gloves at the farm and that these are strictly followed.

e. The farm workers should be aware of personal hygiene. All who enter and work on, or visit the farm, wash and/or sanitize their hands upon entry and exit, especially when moving between the quarantine area, or after handling animals that are diseased or of unknown health status, following contact with any potentially contaminated material, such as deadstock, aborted fetuses, placentas, manure, etc.

f. It is to be ensured that the family members of the farm workers are also aware of the biosecurity practices in the farm and any change in the procedures is communicated to them as well.

**11. Monitoring and record keeping:** It is essential to have a system of maintenance of information which can then be used to improve the effectiveness of biosecurity practices. It is also only by regularly reviewing records for production, biosecurity operations, animal health events, diagnostic test results, etc can one determine the current flock status and accordingly develop strategies and initiate interventions and changes for the betterment of the farm.

**Recommendations:**

a. Animal health records should be maintained together with other flock production and farm management records. Viewing production records together with movement, disease surveillance and diagnostic records, health and treatment records, etc. will provide a more complete understanding of flock performance; this, in turn, will enable valuable analysis of the impact of biosecurity practices to be done.

b. Analysis of the farm records with respect to disease and treatment rates, productivity, diagnostic testing and results of certain practices are useful when looking ahead to future seasons.

c. Records if reviewed regularly are useful for farmers to set goals for the health and productivity of the flock.

**12. Response Plan for Disease Outbreaks:** Part of biosecurity planning is also to have a farm-based plan for response to a disease outbreak or the suspicion of an outbreak on the farm or in the region. A response plan is needed to guide farm activity in rapidly-developing and large scale changes in health status.

**Recommendations:**

a. The plan is developed in advance, and will include preparatory steps to be taken before an

outbreak occurs, identification of potential trigger points and enhanced biosecurity protocols to be initiated on the farm under specific circumstances.

b. Identifying the types of disease emergencies or trigger points that may require a response is vital.

c. One must know what they are to do in each of these emergency situations.

d. It is also important that a recovery plan be in place as the next action following execution of the response plan. While recovery efforts are often disease-specific and therefore difficult to plan in advance, one needs to know what is required to be done in order to return to full production once the disease emergency has been successfully managed.

**13. Animal welfare and biosecurity:** Maintaining high standards of animal welfare also assists in delivering husbandry and hygiene practices that align with good biosecurity. It demonstrates a commitment from the farm/ industry to manage risk related to animal welfare to our consumers and trading partners. It is of utmost importance that animals should be free from stress for which overcrowding should be avoided. Cleanliness, availability of quality feed and water should also be ensured. While transporting animals, it is essential that they are managed in a way that reduces stress and minimises any risks to animal welfare. *[Refer Transport of Animals, Rules, 1978]*.

# Appendix-I

## Herd Health Management Programme

A herd health management program identifies the key components required for appropriate disease prevention, control, and treatment for each farm.

Following is the Annual Health Calendar for Sheep & Goat adopted by the Central Sheep Breeding farm, Hisar, Haryana. This can be altered as per regional variations and State Policy under consultation with local Veterinarian.

**Table: Annual Health Calendar (Sheep & Goat) adopted by Central Sheep Breeding Farm, Hisar, Haryana**

| | | | | |
|---|---|---|---|---|
| 1 | VACCINATION | | | |
| a). | Sheep Pox Vaccine<br>Cost per dose :- Rs 1.00 | Live Attenuated | December / January | All Breed<br>At 4 Months<br>Annually |
| b). | Goat Pox Vaccine<br>Cost per dose :- Rs 2.00 | Live Attenuated | October / November | All Goat<br>At 4 Months<br>Annually |
| c). | Multi Component Clostridial Vaccine<br><br>Cost per dose :- Rs 1.97 | Inactivated | August/October | All stock, New Born Lambs(Sheep &Goat)<br>1. At 1 month & Booster Dose after 21days.<br>2. Repeat after 9 months. |
| d) | Biovac (FMD +HS)<br><br>Cost per dose :- Rs 5.21 | Oil Adjuvant | October/June | Sheep & Goat<br>1. At3 months.<br>2. Repeat after 9 months. |
| e) | Contagious Ecthyma Vaccine (Farm Produce) | Formalized | Feb./March | Lambs & Kids<br>1. At 2 months. |
| f) | PPR<br>Cost per dose :- Rs 1.00 | Live Attenuated | October / November | All sheep& Goat<br>1. At 6months & Repeat after 3 years. |
| g) | Reverine 1 Br.melitensis.<br>Cost per dose :- Rs 46.00 | Live Attenuated | Before Breeding | All Sheep& Goat<br>1. At 3-6 months & Booster dose is not necessary. |
| 2. | DEWORMING: | | | |
| a). | Broad Sepectrum Anthelmentic | Ivermectin / Closantel / Albendazole | | At every two rotation of months/medicines |

| | | | | |
|---|---|---|---|---|
| b). | Narrow Spectrum Anthelmentic | Praziquantel | April/May | All Lambs , Kids & Weaners<br>1. At 2&Repeat at 4-6 months. |
| c). | Anti Coccidial treatment | Sulphamethazine + Trimethoprim | June/July | Young animals & at the time diarrhoea<br>1. At 2-3months mix with feed. |
| 3) | ECTOPARASITIC INFESTATION | | | |
| a). | Dipping | Ectomin / Butox | Sept/Oct/Mar/Apr<br>. | Post-Shearing |
| 4) | LAMB/KID CARING | | | |
| a). | Naval dressing with Povidone /Betadine Immediately after birth. | | | |
| b) | Colostrum feeding to entire young stock. | | | |
| c). | Antibiotic treatment during change of climate/ weather | | | |
| 5) | IMMUNOSTIMULENT | In. Lemasol | With vaccination (Twice in a year) | |

*For optimum benefits of vaccination, deworm animals at least 15 days before vaccination.*

*Multivalent vaccines may be used after judicious planning so that effective coverage is achieved by farmer and is also economical.*

*Ensure that deworming protocol used does not give rise to Anthelminthic Resistance.*

Similarly screenings against various diseases is also advocated. Following is adapted from Annual Goat Health Calendar adopted by Central Institute for Research on Goats, Makhdoom, Mathura, Uttar Pradesh.

**Table: Suggested screenings for diseases.**

| Diseases | Period | Recommendations |
|---|---|---|
| Brucellosis+ | Once in a year | Positive animals need to be euthanized and buried |
| Johne's Disease* | 6 months/ Once in a year, | Positive animals are to be removed from herd/ flock |
| Mycoplasmosis | Once in a year | Treatment with specific drugs |
| Mastitis | Early milking stage | Treatment with specific drugs |
| Endo- parasites | Regular screening of faecal samples | Monitor worm load (EPG/OPG) of the animals to decide time of deworming. |

*+ Screening of adult sheep/ goats especially breeding rams/ bucks and breedable females. From aborted animals submit 2 serum samples (Zero day i.e., day of abortion / still births and 21 days after abortion / still birth).*

**Preferably one month after lambing/ kidding.*

***Note: The above schedules and programmes are general guidelines and may be modified pertaining to local conditions and Veterinarian recommendations.***

# Appendix-II

## List of Notifiable Sheep & Goat Diseases Listed under the Prevention and Control of Infectious and Contagious Diseases in Animals Act, 2009

1. Caprine arthritis/encephalitis
2. Contagious agalactia
3. Contagious caprine pleuropneumonia.
4. Enzootic abortion of ewes (ovine chlamydiosis)
5. Maedi-visna.
6. Nairobi sheep disease.
7. Ovine epididymitis *(Brucella ovis)*.
8. Paste des petits ruminants.
9. Salmonellosis *(S. abortusovis)*
10. Scrapie.
11. Sheep pox and goat pox

# Annexure-II

## Body Condition Score Card

| Sl. No. | | Backbone | Short Ribs |
|---|---|---|---|
| 1. |   | The bones from a sharp narrow ridge. Each vertebra can be easily felt as a bone under the skin. There is only a very small eye muscle. | The end of the short ribs is very obvious. It is easy to feel the squares shape of the ends. Using fingers spread 1 cm apart, it feels like fingernail under the skin practically no covering. |
| 2. |   | The backbones from a narrow ridge but the points are rounded with muscle. It is easy to press between each bone. There is a reasonable eye muscle | The ends of the short ribs are rounded but it is easy to press between them. Using fingers spread 0.5 cms apart the ends feel round like finger ends. They are covered with flesh but it is easy to press under and between them. |
| 3. |   | The vertebrae are only slightly elevated above a full eye muscle. It is possible to feel each rounded bone but not to press between them. | The ends of short ribs are well rounded and filled in with muscle. Using 4 fingers pressed tightly together, it is possible to feel the rounded ends but not between them. They are well covered and filled in with muscle. |
| 4. |   | It is possible to feel most vertebrae with pressure. The back bone is a smooth slightly raised ridge above full eye muscles and the skin floats over it. | It is only to feel or sense one or two short ribs and only possible to press under them with difficulty. It feels like the side of the palm, where maybe one end can just be sensed. |
| 5. |   | The spine may only be felt (if at all) by pressing down firmly between the fat covered eye muscles. A bustle of fat may appear over the tail | It is virtually impossible to feel under the ends as the triangle formed by the long ribs and hip bone is filled with meat and at the short rib ends cannot be felt. |

# Annexure-III

## Definitions for use in the Health Protocol

| | |
|---|---|
| **Bucks/Rams** | Adult male Goat/Sheep used for collection of semen. Teasers and other animals of different age group, which are resident in the semen station, are also subjected to similar disease testing, vaccination and medications for maintaining their health status. |
| **Buck kid/ Ram lamb** | A male sheep or goat, which has not yet reached sexual maturity. |
| **Known health status** | Animals originating from a semen station or rearing station that is strictly complying with the guidelines mentioned in the MSP. |
| **Unknown health status** | Animals originating from village or farm where all the animals of the farm or the village have not been tested against the MSP diseases |
| **Rearing station** | A farm where young animals/lamb/kid, coming from quarantine station are reared till they attain sexual maturity and subsequently get shifted to semen station. A series of clinical and laboratory examinations, vaccinations and medications etc…are undertaken during the stay of these animals in the rearing station to maintain their health status. |
| **Quarantine station** | A farm where ram/buck/lamb/kid, are isolated and examined to assess the health status before shifting to the semen station or rearing station. A series of clinical and laboratory examinations, vaccinations and medications etc. are undertaken during quarantine. |
| **Semen station** | A farm along with semen processing facilities where adult bucks/rams are housed for semen collection and processing. A series of clinical and laboratory examinations, vaccinations and medications etc. are undertaken during the stay of bucks/rams in the semen station to maintain their health status. |

# Annexure-IV

## Details of the tests to be conducted at Semen Station

| Sl No | Disease | Test | Sample | Tested by Officers of | Frequency of testing |
|---|---|---|---|---|---|
| 1 | Brucellosis | ELISA | Serum | Semen station CDDL /RDDL/NDDB | Annually |
| 2 | TB* | DTH-Tuberculin PPD | Intra-dermal on the buck or ram | Semen station CDDL /RDDL/NDDB | Annually |
| 3 | Ovine epididymitis; | Indirect ELISA | Serum | Semen station CDDL /RDDL/NDDB | Annually |
| 4 | Maedi-visna and caprine arthritis/encephalitis; | ELISA | Serum | Semen station CDDL /RDDL/NDDB | Annually |
| 5 | Bluetongue | ELISA | Serum | Semen station CDDL /RDDL/NDDB | Annually |
| 6 | Contagious agalactia | ELISA | Serum | Semen station CDDL /RDDL | Annually |
| 7 | Peste des petits ruminants | Competitive ELISA/PCR | Serum | Semen station CDDL /RDDL | Annually |
| 8 | Contagious caprine pleura pneumonia | CFT | Serum | Seme n station CDDL /RDDL | Annually |
| 9 | Para -Tuberculosis (In Goats only) | ELISA | Serum | Semen station CDDL /RDDL | Annually |

# Annexure-V

## Vaccination Schedule – Sheep

(ref: CSWRI, Avikanagar)

| Sl. No. | Name of Disease | Initial vaccination | | Annual Vaccination |
|---|---|---|---|---|
| | | I$^{st}$Injection | Booster Injection | |
| 1 | Peste-des-petits ruminants (PPR) | At 3 Months age | Not required | Once in 3 years in the month of December |
| 2 | Enterotoxaemia (ET) | At 3-4 Months age | 3-4 weeks later of 1st Injection | Twice in a year, preferably in June-July before the onset of monsoon and in December month |
| 3 | Foot & Mouth Disease (FMD) | At 3-4 Months age | 3-4 weeks later of 1st Injection | Twice in a year (June-July and November-December) |
| 4 | Sheep Pox | At 3-4 Months age | 3-4 weeks later of 1st Injection | Repeat annually in December-January |
| 5 | Hemorrhagic Septicemia (HS) | At 3-4 Months age | 3-4 weeks later of 1st Injection | Once in a year, preferably in June-July before the onset of monsoon |
| Deworming: Preferably in August-September (not required under stall feeding) | | | | |
| Dipping : All the rams in the month of April, preferably 2 weeks after the shearing | | | | |

# Annexure-VI

## Vaccination Schedule –Goat

(ref: CIRG,Makdoom)

| Sl No | Name of disease | Initial Vaccination | | Annual vaccination |
|---|---|---|---|---|
| | | I$^{st}$ Injection | Booster Injection | |
| 1 | Peste -des -petits ruminants (PPR) | At 3 months age | not required | Confer immunity for 3 years |
| 2 | Enterotoxaemia (ET) | At 3-4 months age | 3-4 weeks later of I$^{st}$Injection | Annual vaccination (two dose with one month interval) |
| 3 | Foot & Mouth Disease (FMD) | At 3-4 months age | 3-4 week later of I$^{st}$ injection | Every 6 months interval |
| 4 | Goat Pox (GP) | At 3-4 months age | 3-4 weeks later of I$^{st}$ Injection | Annual vaccination |
| 5 | Hemorrhagic Septicemia (HS) | At 3-4 months age | 3-4 weeks later of I$^{st}$ Injection | Annual vaccination |

**Note:**

1) primary vaccination should be maintained serially and for each vaccination a minimum interval of 15-21 days should be followed
2) Thorough checking of labels and instructions of vaccine administration should be followed.
3) There should be an approved vaccination programme specific to each semen station.

# Annexure-VII

## List of equipments needed for semen collection and freezing

### Semen collection

1. Animal handling facilities
2. Artificial vagina
3. AV liners
4. Service crate for restraining teaser female
5. Liquid paraffin or any non-spermicidal lubricant
6. Graduated semen collection cups of 5 ml capacity
7. Surgical gauze
8. Test tubes of 15ml capacity for dilution and measuring the sperm concentration
9. Micropipettes
10. Incubator for storing the ready to use Artificial vagina
11. Hot air oven and autoclave for sterilization
12. Glass marker pens and pencils

### Semen examination

- Trinocular microscope with 10x, 40x and 100x objectives and stage warming facility
- Microscope slides and cover slips
- Haemocytometer or photometer to measure sperm concentration
- Basic sperm staining dyes for sperm viability and other structural and functional integrity
- Pipettes of different capacities
- Computer assisted semen analyser (Optional)

### Semen dilution

- Analytical weigh balance (preferable three digit)
- A.R. grade glycerol
- Buffers, sugars and antibiotics for short and long-term preservation

- pH meter
- Osmometer (Optional)
- Measuring cylinder (25, 50 and 100ml capacity)
- Whatman circular filter paper of 12 cm diameter (Grade 1)
- Triple glass distilled water
- Low speed centrifuge to clarify the semen extender
- Glass beakers of varying capacity
- Temperature controlled magnetic stirrer with bars

## Short-term preservation

- Refrigerator
- Semen shipper or wide-mouth thermos flasks with ice cubes
- Screw capped glass vials (5ml or 10 ml capacity)
- Glass beaker of 100 and 250ml capacity to hold the glass vials in foam
- Warm water (35 degree Celsius)
- Thermometers
- Aluminium foils
- Parafilm
- Adhesive taps
- Glass markers

## Cryopreservation/ Long-term preservation

- French mini or medium straws
- Liquid nitrogen transport and storage dewars
- Plastic goblets for holding frozen semen
- Cold handling cabinet
- Glass tray with warm water (35 degree Celsius)
- Thermometer (range of 30 to -200 degree Celsius)
- Programmable cell freezer
- Long handled stainless steel forceps
- Straw filling, sealing and printing machines
- Scissors

# Annexure-VIII

## Use of egg yolk as a component of Goat semen extender- Washing procedure with Ringer Solution

When we use egg yolk as a component of a goat semen extender, there are reports of reduction in the conception rate. An enzyme (phosphotidase) produced by the bulbo-urethral glands of the male goat catalyses the hydrolysis of lecithins in egg yolk to fatty acids and lysolecithins, which are toxic to the spermatozoa. The presence of phosphotidase in the seminal plasma of the goat means that media containing egg yolk cannot be used for semen extension. In that case, Washing goat spermatozoa in a physiological solution may be preferred. The ingredients and procedure of making washing solution is described below

| | Ingredients | Amount to Make |
|---|---|---|
| | 100 ml | 1 liter |
| NaCl | 0.86 gm | 8.6 gm |
| KCl | 0.03 gm | 0.3 gm |
| CaCl2.2H20 | 0.033 gm | 0.33 gm |
| H20 to | 100 ml | 1000 ml |

a. Weigh out the ingredients listed above.

b. Add 50 ml H2O (or 500 ml of H2O, if making up a liter) to the powdered ingredients.

c. Mix until all the ingredients are dissolved.

d. Add H2O to bring the total volume to 100 ml (or 1000 ml, if you are making a liter).

# Annexure-IX

## Guidelines for Basic Semen Analysis

Three basic characteristics should be addressed when evaluating semen and estimating Sperm viability:

1. sperm concentration;
2. motility; and
3. Morphology.

### Sperm concentration

Concentration is most accurately estimated with specialized equipment, such as a spectrophotometer. Counting can also be done manually, under the microscope, using a Haemocytometer.

### Motility

The movement of the sperm should be checked: first, because movement indicates that the sperm are alive; and second, because motility is correlated with fertility. Two types of motility are usually evaluated – Mass motility and Individual motility.

### Mass motility

1. Place a drop of diluted semen on a pre-warmed slide (37°C) and examine sperm at 10X under a standard or phase-contrast microscope.
2. Look for general movement of the sperm with rapidly moving waves and individual swirls of sperm within the waves.

### Individual motility

1. Place, on a pre-warmed slide, a drop of semen diluted (1:10) in saline solution, citrate or extender. When CASA equipment is used, chambers of a special design are needed (Makler chambers).
2. Position a cover slip over the mixture and examine under 40X magnification.
3. Estimate the proportion of individual sperm that are moving progressively forward (so-called "progressive forward motility"). This can be done by randomly picking ten or more

sperm in different areas of the slide, counting those with forward motility and dividing by the total.

4. Although motility and its correlation with fertility may vary by species, the following Figures of Post Thaw Motility can be used as a general guideline:
   - > 70 percent = Excellent
   - 50 to 60 percent = Very good
   - 40 to 50 percent = Good
   - 30 to 40 percent = Satisfactory
   - < 30 percent = unsatisfactory

## Morphology

Abnormally shaped or damaged sperm are less likely to be capable of fertilization than normal sperm are (Berndtsonet al.,1981). Mixing the semen with a stain (e.g. eosin-nigrosin) highlights the sperm so that abnormalities can be readily identified under a microscope.

Two kinds of abnormalities can be defined: primary abnormalities, which are assumed to have occurred in the testes; and secondary abnormalities, which arise in the epididymis or ejaculate.

The proportion of normal sperm should be > 70 percent.

1. Place a drop or stripe of stain on a warmed microscope slide.
2. Add a small amount of semen.
3. Mix the semen and the stain with another slide and then use the narrow edge of the second slide to smear the mixture across the first slide.
4. Cover the mixture with a cover slip and examine under 1000 X magnification (oil immersion).
5. Examine the sperm for abnormalities, including the following:
   - abnormally shaped (tapered or pear-shaped) or sized (too large or small) heads;
   - missing or stump tails;
   - coiled or bent tails;
   - detached or creased (folded-over) acrosome;
   - clumping of multiple sperm; and
   - plasma droplets on tails.
6. Count at least 100 sperm and calculate the proportion (%) of abnormalities.
7. Discard semen if the proportion of abnormalities is too high (more than 30 percent)

# Annexure-X

## Cryo-preservation of SHEEP semen

### Freezing

1. The collected semen should contain about 4 x 109 sperm per ml and should be maintained at 37 °C.
2. Evaluate semen visually and microscopically. Semen should be white and quite viscous.
3. Select only those ejaculates with mass motility above 4 and less than 30 percent abnormal sperm.
4. Evaluate sperm concentration and determine final volume for a concentration of 40 x 106 sperm/ml.
5. Dilute the semen to the proper volume using a "one-step" or "two-step" procedure. (Paulenzet al. (2002))

### One-step procedure

a. Add the entire sample the volume of One-Step Diluent (300 Mm Tris, 28 mM glucose, 95 mM citric acid, 2 percent [v:v] glycerol, 15 percent egg yolk, 1mg/ml of streptomycin sulfate and 0.06 mg/ml of benzyl penicillin) required to obtain the desired final sperm concentration.

b. Cool to 4 °C within one hour and maintain for at least 1.5 hours.

### Two-step procedure

a. Add Diluent A to the semen at 30 °C to obtain 60 % of the final volume (Diluent A consists of 25.75 g of lactose in 250 ml bi-distilled water + 20 percent egg yolk).

b. Cool progressively to +4 °C over two hours (0.2 °C/minute).

c. Prepare Diluent B: Reconstitute milk from a non-fat powder source (4 g into100 ml bi-distilled water) and adjust pH to 6.6 with a Tris solution (20 g of trisodium-citrate-5.5 H2O into 70 ml H2O); then mix nine volumes of the resulting solution with one volume of glycerol.

d. Add Diluent B up to final volume (Diluent B consists of Diluent A +11 % glycerol).

e. Add Diluent B in three equal parts, over 30 minutes, at 4 °C up to the final volume.

f. Keep the semen for 90 minutes at +4 °C.

6. Fill 0.25 ml plastic straws with semen.

7. Place straws horizontally in liquid nitrogen vapour at -75 °C for eight minutes.

8. Transfer directly into liquid nitrogen at -196 °C and store.

## Thawing

1. Thaw straws in a water bath at 37 °C for 30 seconds.

2. Assess semen viability: mix one volume of sperm to four volumes of a sodium citrate solution (20 g of tri-sodiumcitrate-2 H20 in 70 ml bi-distilled water) at 38 °C and estimate the proportion of motile sperm after five minutes and after two hours: only sperm with more than 30 percent of living spermatozoa at two hours should be used for insemination.

3. Proceed to surgical or non-surgical insemination of pre-synchronized recipients.

# Annexure-XI

## Cryo-preservation of GOAT semen

### Freezing

1. Collected semen should contain about 4 x 109 sperm per ejaculate when sampling occurs in season. Semen should be kept at 32 °C for transfer to the laboratory and processing.
2. Evaluate semen visually for any abnormalities.
3. Wash sperm with a Krebs Ringer Phosphate Glucose Solution (0.9 percent NaCl,1.15 percent KCl, 1.22 percent CaCl2, 2.11 percent KH2PO4, 3.82 percent MgSO4-7H2O, 5.24 percent glucose) by mixing one volume sperm with nine volumes of the washing solution at 28 °C to 32 °C, followed by centrifugation at 500 g for 15minutes at 20 °C.
4. Discard the supernatant, and evaluate the semen (wave motion, concentration).Select only those ejaculates with a mass motility greater than 60 percent.
5. Calculate the final volume (V). Repeat centrifugation under the same conditions at20 °C.
6. Dilute the semen to the proper volume using a one-step or a two-step procedure:

### One-step procedure

a. Add the entire sample to the volume of One-Step Diluent (300 mMTris; 28mM glucose; 95 mM citric acid; 2 percent [v:v] glycerol; 2.5 percent egg yolk;1 mg/ml of streptomycin sulfate and 0.06 mg/ml of benzyl penicillin) required to obtain the desired final sperm concentration ( $\geqslant$ 200 million sperm per ml).

b. Cool to 4 °C within one hour and maintain for at least 1.5 hours.

### Two-step procedure

a. Prepare Diluent A: 80 ml of a sodium citrate solution (194 mg glucose +3.52 g sodium citrate +1.05 g streptomycin +50 000 IU penicillin in 100 ml distilled water) supplemented with 20 ml egg yolk.

b. Add V/2 of Diluent A to the pelleted sperm at 20 °C.

c. Cool to +4 °C within 30 minutes (at 0.5 °C/minute).

d. Add V/2 Diluent B (Diluent A + 14 percent v:v glycerol) in three successive steps with ten minute intervals. Diluent should also be at +4 °C.

7. Fill 0.25 ml plastic straws with semen.
8. Freeze straws in liquid nitrogen vapour for five minutes.
9. Plunge directly into liquid nitrogen and store.

## Thawing

1. Thaw straws in a water bath at 37 °C for 30 seconds.
2. Assess post-thaw motility.
3. Proceed to insemination of does

# Annexure-XII

## Appropriate procedure for sterilization of different materials, used in the semen station:

### 1. Laboratory and other areas

Cold fumigation solution is ideal for fumigation of laboratory and other areas. It should be done as per SOP.

### 2. Artificial Vagina (AV)

a) Cone from the AV and water from AV jacket shall be removed before washing.

b) Cones and AVs shall be cleaned thoroughly with a soft sponge brush under running tap water and then soaked in warm neutral cleaner for about 30 minutes, followed by proper rinsing in warm and clean water and then three times rinsing with double distilled water.

c) For sterilization, fully assembled AVs shall be autoclaved at 5 p.s.i. pressure for 20 minutes. During sterilization, the valve of AV shall be kept open. Alternatively, use AV sterilizer (using double distilled water in the sterilizer) for proper sterilization of AVs.

d) Finally AVs shall be stored overnight in an incubator at 45o C.

e) To achieve best cleaning effect, AVs shall be cleaned immediately after use, preferably by non-spermicidal neutral detergent.

### 3. Glassware

a) The glassware shall be washed thoroughly with running tap water and soaked in warm, non-spermicidal neutral detergent solution for about 30 minutes.

b) Using appropriate nylon brush, the glassware shall be cleaned and rinsed with running tap water. The collection tubes shall be brushed at least 3 times and thoroughly cleaned and rinsed with distilled water.

c) Finally the glassware shall be rinsed three times with double distilled water and allowed to dry by keeping them inverted on a blotting paper or a drying stand made of SS/ plastic.

d) The open end/s of the dried glassware shall be covered with aluminium foil and sterilized in hot air oven at 160°C for one hour or at 180°C for 30 minutes. One item should be wrapped with newspaper and its mild charring will indicate proper sterilization.

## 4. Rubber wares

The washing and cleaning procedure of rubber wares is similar to that of glass ware. Care shall be taken to clean the rubber wares with sponge brush instead of nylon brush. Plastic tips shall be cleaned by water jet with force using a syringe. Sterilization technique, however, differs owing to the thermo-sensitivity of the rubber items. Thermo-resistant rubber wares shall be sterilized by autoclaving at 3- 4 p.s.i. for 10 minutes. (The rubber tubing for semen filling shall not be reused).

## 5. Distilled Water

Fresh triple glass distilled water or Milli-Q purified water shall be autoclaved at 15 p.s.i. for 15 minutes and used for preparation of the dilutor.

## 6. Buffer

Buffer shall be sterilized by autoclaving at 5 p.s.i. pressure for 20 minutes. After autoclaving, buffer shall be cooled and stored in refrigerator.

## 7. Bacteriological Media

It is to be autoclaved at 15 p.s.i. pressure for 15 minutes.

## 8. Filter Papers

A bunch of clean filter papers of standard brand like Whatman No. 1 (thrashed to remove dirt, if any) shall be wrapped in thick cotton cloth for sterilization in an autoclave at 5 p.s.i. pressure for 20 minutes.

Summary of Sterilization of various items

***a) Autoclave***

| Sr.No. | Item | Pressure (p.s.i.) | Time (Min.) |
|---|---|---|---|
| 1. | Artificial Vagina | 5 | 20 |
| 2. | Buffer | 5 | 20 |
| 3. | Plastic Tips | 5 | 20 |
| 4. | Filter Papers | 5 | 20 |
| 5. | Buck or ram Apron | 5 | 20 |
| 6. | Thermo-resistant Rubber wares | 3-4 | 10 |
| 7. | Bacteriological Media | 15 | 15 |
| 8. | Distilled Water | 15 | 15 |
| 9. | Surgical Equipment | 10 | 10 |

*b) Hot Air Oven*

| Sr.No. | Item | Temperature | Time (min.) |
|---|---|---|---|
| 1. | Glass wares | 160o C / 180o C | 60/30 |
| 2. | Filling Nozzles | 160o C / 180o C | 60/30 |

*c) AV Steriliser*

Wherever Autoclave is not used, AVs and rubber cones shall be sterilised using AV sterilizer. After sterilizer starts boiling, 30 minutes vapour sterilisation shall be done.

# Annexure-XIII

**Registered Breeds of Sheep** (ICAR- National Bureau of Animal Genetic Resources, as on January 2019)

| Sl. No. | Breed | Home Tract |
|---|---|---|
| 1 | Balangir | Orissa |
| 2 | Bellary | Karnataka |
| 3 | Bhakarwal | Jammu and Kashmir |
| 4 | Bonpala | Sikkim |
| 5 | Changthangi | Jammu and Kashmir |
| 6 | Chokla | Rajasthan |
| 7 | Chottnagpuri | Jharkhand |
| 8 | Coimbatore | Tamilnadu |
| 9 | Deccani | Andhra Pradesh and Maharashtra |
| 10 | Gaddi | Himachal Pradesh |
| 11 | Ganjam | Orissa |
| 12 | Garole | West Bengal |
| 13 | Gurez | Jammu and Kashmir |
| 14 | Hassan | Karnataka |

| Sl. No. | Breed | Home Tract |
|---|---|---|
| 15 | Jaisalmeri | Rajasthan |
| 16 | Jalauni | Uttar Pradesh and Madhya Pradesh |
| 17 | Karnah | Jammu and Kashmir |
| 18 | Kenguri | Karnataka |
| 19 | Kilakarsal | Tamilnadu |
| 20 | Madras Red | Tamilnadu |
| 21 | Magra | Rajasthan |
| 22 | Malpura | Rajasthan |
| 23 | Mandya | Karnataka |
| 24 | Marwari | Rajasthan and Gujarat |
| 25 | Mecheri | Tamilnadu |
| 26 | Muzzafarnagri | Uttar Pradesh and Uttarakhand |
| 27 | Nali | Rajasthan |
| 28 | Nellore | Andhra Pradesh |
| 29 | Nilgiri | Tamilnadu |
| 30 | Patanwadi | Gujarat |
| 31 | Poonchi | Jammu and Kashmir |
| 32 | Pugal | Rajasthan |

| Sl. No. | Breed | Home Tract |
|---|---|---|
| 33 | Ramnad White | Tamilnadu |
| 34 | Rampur Bushair | Himachal Pradesh |
| 35 | Shahbadi | Bihar |
| 36 | Sonadi | Rajasthan |
| 37 | Tibetan | Arunachal Pradesh |
| 38 | Tiruchi Black | Tamilnadu |
| 39 | Vembur | Tamilnadu |
| 40 | Katchaikatty Black | Tamilnadu |
| 41 | Chevaadu | Tamilnadu |
| 42 | Kendrapada | Odisha |
| 43 | Panchali | Gujarat |

**Registered Breeds of Goat** (ICAR- National Bureau of Animal Genetic Resources, as on January 2019)

| S.N. | Breed | Home Tract |
|---|---|---|
| 1 | Attapady | Kerala |
| 2 | Barbari | Uttar Pradesh and Rajasthan |
| 3 | Beetal | Punjab |
| 4 | Black Bengal | West Bengal |
| 5 | Changthangi | Jammu and Kashmir |
| 6 | Chegu | Himachal Pradesh |
| 7 | Gaddi | Himachal Pradesh |
| 8 | Ganjam | Orissa |
| 9 | Gohilwadi | Gujarat |
| 10 | Jakhrana | Rajasthan |
| 11 | Jamunapari | Uttar Pradesh |
| 12 | KanniAdu | Tamilnadu |
| 13 | Kutchi | Gujarat |
| 14 | Malabari | Kerala |
| 15 | Marwari | Rajasthan |
| 16 | Mehsana | Gujarat |
| 17 | Osmanabadi | Maharashtra |
| 18 | Sangamneri | Maharashtra |

| S.N. | Breed | Home Tract |
|---|---|---|
| 19 | Sirohi | Rajasthan and Gujarat |
| 20 | Surti | Gujarat |
| 21 | Zalawadi | Gujarat |
| 22 | Konkan Kanyal | Maharashtra |
| 23 | Berari | Maharashtra |
| 24 | Pantja | Uttarakhand and Uttar Pradesh |
| 25 | Teressa | Andaman & Nicobar |
| 26 | Kodi Adu | Tamil Nadu |
| 27 | Salem Black | Tamil Nadu |
| 28 | Sumi-Ne | Nagaland |
| 29 | Kahmi | Gujarat |
| 30 | Rohilkhandi | Uttar Pradesh |
| 31 | Assam Hill | Assam and Meghalaya |
| 32 | Bidri | Karnataka |
| 33 | Nandidurga | Karnataka |
| 34 | Bhakarwali | Jammu and Kashmir |

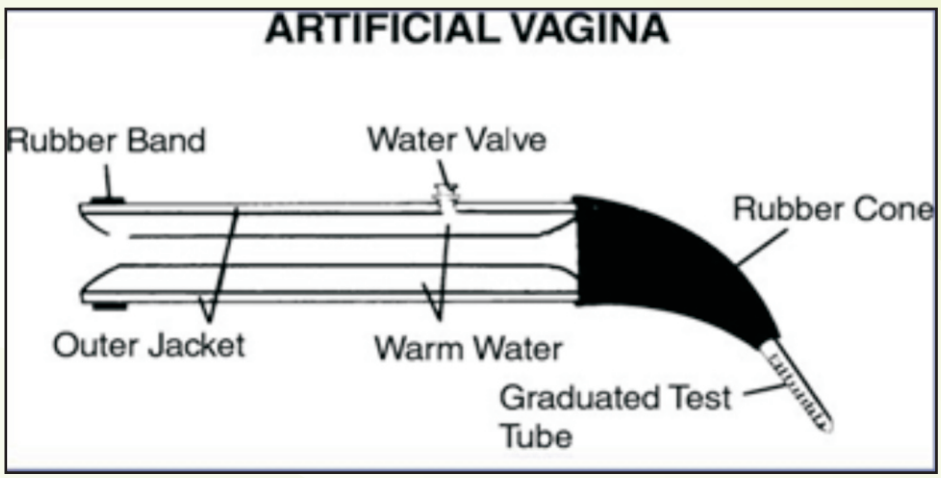
# Annexure-XIV

## General guidelines for Semen Collection Technique

The methods commonly used for collection of Ram and Buck semen are the artificial vagina (AV) and the electro ejaculator (EE).

**The most commonly used and easiest procedure is the use of the AV.**

The AV method is painless, quicker and does not stress the animal at all. It has an inner rubber liner (containing water at a temperature of 100°F) placed between the liner and the hose. The warmer water simulates the vagina of a doe. A latex rubber collection cone is placed in the AV and a graduated collection tube is placed on the end of the cone.




The buck is collected for semen evaluation or processing by allowing him to mount a doe in heat, another buck, or a wether. The usual procedure is to use a teaser doe that is in heat. This can be a natural heat or one induced by a prostaglandin product.

A doe in heat usually stands better for a buck than a wether or another buck. She emits a smell when in heat that causes the buck to give a better ejaculate. The doe is usually tied or held and the buck allowed to go through his courting behavior. The buck is allowed a few false mounts, and then the person with the AV collects the ejaculate by directing the penis into the AV. The test tube containing the ejaculate should be protected from direct sunlight and cold temperatures.

**Electro ejaculators:** Electro ejaculators come in a variety of shapes and sizes and are used when animals are not trained for AV collection, are physically unable to mount, or a suitable mount is not available. Electro-ejaculation involves the solid restraint of the sire and a rectal probe, through which a steadily increasing current is passed until ejaculation occurs. Not every sire will give semen by this method. If the sire is not handled gently, or too much power used, he will usually urinate in the semen collection glass, which renders the semen unusable. Thus, electro-ejaculation is only performed when absolutely necessary and then with considerable care.

# Annexure-XV

## Model composition of extenders:

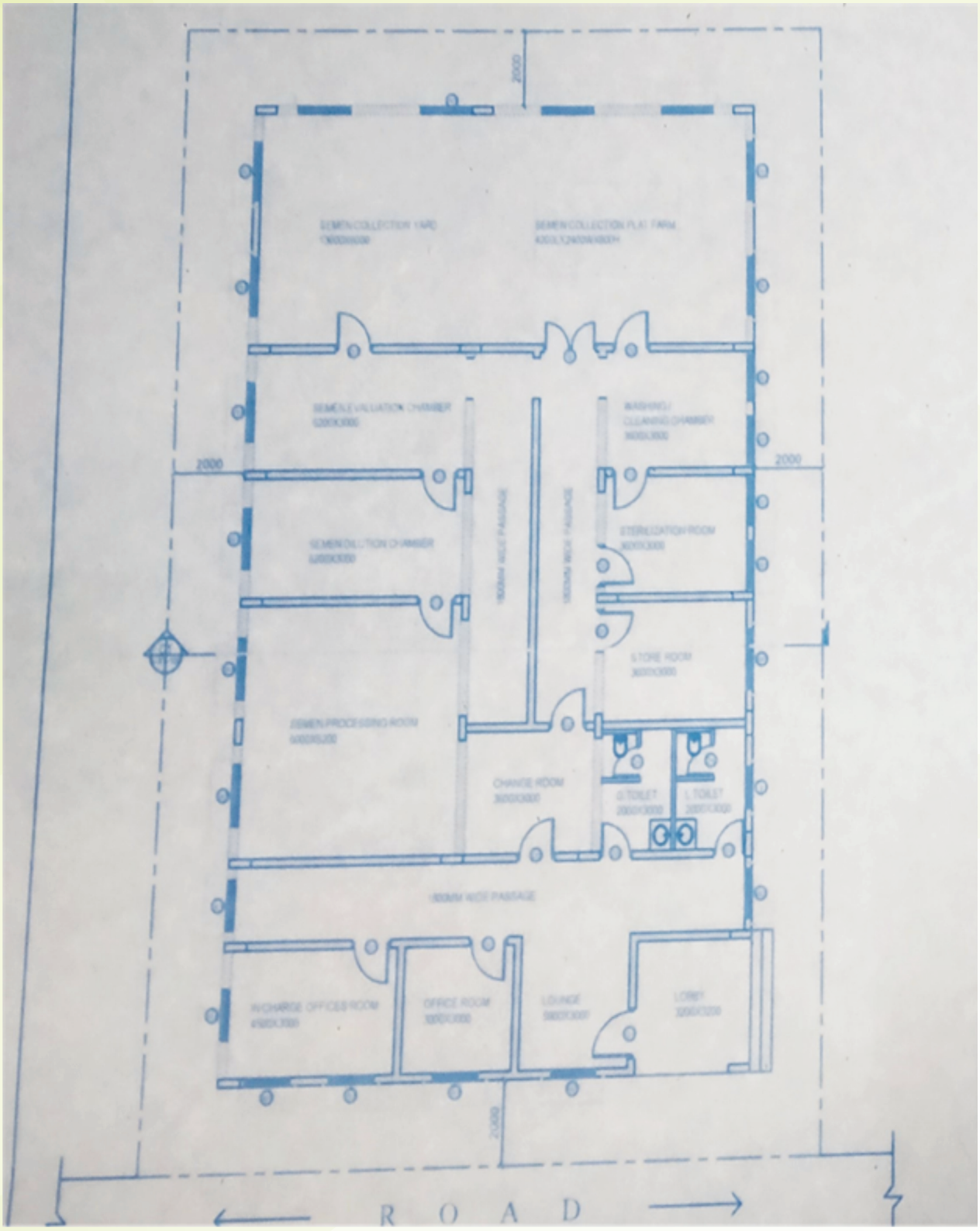
Semen of rams and bucks is routinely extended immediately after collection at 37°C and cooled slowly to prevent cold shock, resulting in sperm damage.

## Extending Formulas

| Formula | Diluter A (w/o glycerol) | Diluter B (with glycerol) |
|---|---|---|
| **Tris Egg-Yolk** | | |
| Tris (g) | 12.1 | 12.1 |
| Citric Acid, monohydrate (g) | 6.7 | 6.7 |
| Glucose or Fructose (g) | 5 | 5 |
| Penicillin (units/ml) | 1000 | 1000 |
| Streptomycin (ug/ml) | 1000 | 1000 |
| Glycerol (ml) | --- | 70 |
| Egg Yolk (ml) | 100 | 100 |
| Distilled H2O to final volume (ml) | 500 | 500 |
| **Milk-Glucose** | | |
| Milk Powder (g) (1% fat) | 50 | 50 |
| Glucose (g) | 0.97 | 0.97 |
| Penicillin (units/ml) | 1000 | 1000 |
| Streptomycin (ug/ml) | 1000 | 1000 |
| Glycerol (ml) | --- | 70 |
| Distilled H2O to final volume (ml) | 500 | 500 |

After extension, semen is cooled at 5°C at approximately 0.5°C/min.

## Plan Layout - SPC, Hebbal




## Semen Production Center, Veterinary College, Hebbal

# Standard Operating Procedures

*for*

# Artificial Insemination

*in*

# Caprine and Ovine Species

## Introduction

The main value of Artificial Insemination (AI) is in its judicious application to livestock improvement and to control venereal diseases that may occur in natural breeding. The practical possibility of being able to store frozen semen almost indefinitely gives scope for the maximum possible use of the best sires. The successful use of commercial (AI) requires a high standard of technical efficiency at the (AI) centre. The use of a very small number of rams/bucks on a very wide scale does give opportunity for widespread dissemination of harmful genes and spread of infectious agents should not be overlooked. Vigilant tracking of each consignment of imported frozen semen is required to ensure their freedom from reproductive and other infectious diseases.

## 1. Objective of AI

To deliver an effective dose of semen to the ewe's/doe's uterus, artificially. The Minimum Standards aim at Quality AI delivery to breedable does and ewes. AI services can be provided through AI centre where farmers of all villages and hamlets coming under the ambit of an AI Centre will bring their animals or at the farmers' door step through Mobile AI Technicians as per the breeding policy of the concerned State.

## 2. Breeding Policy

Ensure that the AI technicians follow the breeding policy of the State and gather information relevant to breeding goals envisaged in the breeding policy.

## 3. Quality of frozen and liquid semen

Frozen semen shall be from semen stations which have been following the minimum standards laid down by the Government of India for semen production and processing.

## 4. Semen storage and distribution

### 4.1 Storage and Distribution of Frozen semen Straw (FSS):

**AI Service Providers should:**

a. Store frozen semen doses in a well-ventilated, all weather safe storage area.

b. Ensure a proper and foolproof identification system for each semen container, canister, and goblet so that a buck's/ram's semen can be traced with ease.

c. While transferring semen doses, goblets should be well identified and precaution should be taken to see that each goblet has sufficient space for liquid nitrogen. Frozen semen should not be exposed above liquid nitrogen as it may cause irreversible damage to sperm viability.

d. All transfers of frozen semen straws into goblets should take place under liquid nitrogen, in a polystyrene / thermocol box.

e. Liquid Nitrogen should be replenished in both storage and distribution containers at regular intervals to ensure proper level of liquid nitrogen

f. Details of semen doses supplied to various AI technicians at the time of dispatch should be recorded. After each dispatch, records redefining the position of remaining doses should be updated.

**4.2 Storage and Distribution of Liquid Semen:**

**AI Service Providers should:**

a. Store liquid semen containers, vials in a refrigerator at 5 degree Centigrade

b. Ensure a proper and foolproof identification system for each liquid semen container /vial so that a buck's/ram's semen can be traced with ease.

c. Ensure that the liquid semen containers /vials are not being exposed to outside temperature unnecessarily or repeatedly as it may cause irreversible damage to sperm viability.

d. Details of semen doses supplied to various AI technicians at the time of dispatch should be recorded. After each dispatch, records redefining the position of remaining doses should be updated.

**4.3. Liquid Nitrogen procurement, storage & delivery**

- A bulk Liquid Nitrogen (LN) sourcing, storage and delivery facility should be available.
- A schedule of LN replenishment to all AI centres on fortnightly / monthly / quarterly basis, whichever is convenient depending on the field conditions shall be worked out by each service provider and shall be adhered to in the interest of maintaining quality of semen. A log book shall be maintained for all such schedules at different locations/ starting points of supply routes.
- The AI centre should have a bigger container for Liquid Nitrogen and Frozen Semen Straws (FSSs) storage, preferably of 35 litre capacity, and a small portable LN container, preferably 2-3 litre capacity, to carry the straws to the place where the AI is carried out.
- The LN containers in the AI centre shall be protected sufficiently to avoid damage to container.

- AI centre should have a dip stick with critical level marks and a ready reckoner for assessing the LN levels and quantity of LN in litres.
- Supply of LN should be either through portable LN tankers of 500 to 2000 litre capacities with gravitational flow or through the LN delivery pump and not by pouring LN from one container to another container.

### 4.4 AI guns, sheaths and AI accessories

Stainless steel AI guns, AI sheath, syringe and/or micropipette shall be of good quality and make. AI accessories like forceps and scissors shall be made of good quality stainless steel. Thermos flask and thermometer shall be of good quality.

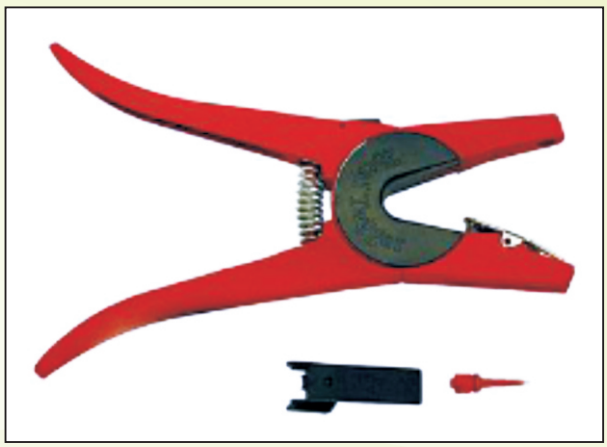
## 5. Animal Identification

Every animal receiving AI shall be identified with an Ear Tag with a unique number and a barcode. These numbers shall enable generation of reports concerned to the individual animal and the associated information through an information system.

Only polyurethane laser printed ear tags having a 12 digit number and a bar code shall be used. The numbering system followed shall be unique with the last digit of the number being a "check digit" to ensure that no two animals are tagged with the same number. The ear tag shall be applied inside the ear of animals, in the center of the ear lobe with the female part of the tag inside the ear.

**Figure. 1: Ear Tag**

**Figure. 2: Ear Tag Applicator**



## 6. Qualifications, experience or training necessary to perform this procedure

Operators should be familiar with the correct techniques and the anatomy and physiology of the ewe before attempting this procedure. Artificial Insemination should only be carried out by a veterinarian or trained AI technician.

## 7. Selection of Ewes/Does

1. Ewes/Does must be individually identified by ear tag or other permanent marking prior to use for AI purpose. Only mature, in oestrus, Ewes/Does may be used. Animals under 15 months of age or undersized animals should not be used. They do not need to have had a kid/lamb but should be sexually mature. Ewes/Does must be in good body condition and good general health as assessed by a competent Veterinarian.

2. Only non-pregnant Ewes/Does should be used. An accurate history of NO mixing with rams/bucks must be available, or Ewes/Does must be pregnancy tested prior to the commencement of insemination. Ewes/Does showing vaginal discharge (other than oestrus discharge) should not be used. The oestrus status of Ewes/Does used for insemination is significant. More successful penetration of the cervix is possible in oestrus Ewes/Does.

3. The Ewes/Does must be restrained properly, with hind legs over a rail of suitable height to prevent lateral or forward movement.

**An indicative list of reproductive parameters of sheep and goat is annexed as annexure (XVII)**

## 8. Standard Operating Procedures for Artificial Insemination Delivery in Sheep & Goat

The Standard Operating Procedures that should be followed by AI technicians in carrying out AI and handling semen doses is as follows. AI services providers should ensure that every AI technician has a copy of this SOP and he keeps it with him for reference whenever he goes for insemination work.

### 8.1. Details of Procedure:

**The procedure requires**

- Synchronization of estrus within the flock of ewes/does. (For insemination of a group of does on the same day, the estrus cycle of the group needs to be manipulated and synchronized so that ovulation occurs at a set time. Otherwise this process is optional in does)
- Identification of the ewes/does in estrus
- Insemination of those ewes/does.

### 8.2 Methods of Oestrus Synchronization

### 8.2.1 Standard procedures for Oestrus Synchronization by using Sudden Ram/Buck Introduction effect

I) There shall be no ram/buck in the flock or within sight, smell or hearing of the ewes/does for at least three weeks to use this method of estrus synchronization.

ii) Then a vasectomised/castrated ram/buck or an intact ram/buck with an apron shall be introduced in the flock, the ewes/does will exhibit oestrus because of the pheromones secreted by the buck/ram within the next 5 or 6 days and may be inseminated at this time.

### 8.2.2 Standard procedures for Oestrus Synchronization by Hormonal method

### A. Progesterone based protocol:

I) A sponge or CIDR (Controlled Internal Drug Release Dispenser) impregnated with the hormone progesterone or with synthetic derivatives of progesterone known as progestogens (melengestrol acetate (MGA), altrenogest or norgestomet) shall be introduced into the vagina of the ewe/doe.

These agents mimic the action of the corpus luteum (CL), a progesterone secreting structure located on the ovary. Elevated levels of progesterone released

by the CIDR prevent the ewe/doe from coming into estrus until the device is removed.

ii) After CIDR removal, the ewe/doe will come into heat about 36-72 h later and can be bred by AI. CIDRs can be administered for varying periods, typically ranging from 9 to 21 days.

**B. Prostaglandin-based protocols :**

Prostaglandin F2 alpha (PGF2), a naturally occurring hormone is effective for estrous synchronization only if females are normally cycling and have a Corpus Luteum (CL) present on their ovaries. The CL is the ovarian structure that produces progesterone in the female, keeping her out of estrous.

This method typically involves administration of 2 PGF injections, the first given on Day 1 and the second on Day 10. Ewes/does will come into heat after either the first or second injection and can be bred by AI at either time. Most of the treated ewes/does will show estrus 36-96 h after the second PGF injection.

**C. Combined methods utilizing both progesterone- and prostaglandin-based treatments:**

A CIDR-PGF protocol involves a short, 6-day CIDR treatment. A CIDR is inserted on Day 1 in the morning and six days later is removed. An injection of PGF must be given either at the time of CIDR removal. If PGF is given at the time of CIDR removal, ewes/does will come into estrus 36-72 h later and can be bred by AI.

**D. Combined methods utilizing both progesterone- and PMSG:**

i) A sponge or CIDR impregnated with the hormone progesterone shall be introduced in to the vagina of the ewe/doe and shall be removed after 17 days.

ii) An intramuscular injection of PMSG (Pregnant Mare Serum Gonadotropin) shall be given to the ewe/doe at the time of sponge/CIDR removal.

iii) All the ewes/does will exhibit oestrus simultaneously around 30 hours after removal of the sponge/CIDR.

The Standard Operating Procedures that should be followed by AI technicians in carrying out AI and handling semen doses is as follows. AI services providers should ensure that every AI technician has a copy of this SOP and he keeps it with him for reference whenever he goes for insemination work.

## 8.3 Procedure for Artificial Insemination in Sheep

1. Cervical AI or trans-cervical AI:
2. Laparoscopic intra-uterine AI (where semen is deposited directly into the uterus)

**An indicative list of things required for AI is annexed as annexure (II)**

### 8.3.1 Standard Operating Procedure for Cervical/trans-cervical method

1. The ewe in oestrus shall be separated from the flock. Ewes detected to be in oestrus in the morning shall be inseminated in the evening and those detected in the evening shall be inseminated in the next morning.
2. Lift the hind legs of the doe/ewe in a 45° angle towards the direction of sunlight.
3. The inseminator should gently introduce a lubricated speculum into the vagina to determine the stage of oestrus. If a large quantity of vaginal mucus is preventing the correct location of the cervix, the assistant should tilt the animal down to allow the mucus to run out of the vagina with the aid of the speculum.
4. Locate the cervix using the speculum with the help of sunlight or AI light.
5. The inseminator shall try to pass the insemination gun/pipette as deep as possible into the cervix (Generally,0.5 to 1cm) and deposit the semen. The assistant should hold the doe/ewe firmly. Ensure that no urine or faecal matter contaminates the tip of the AI gun.
6. The ewe should then be gently lowered and returned to the flock while keeping it calm.
7. The speculum should be properly disinfected/ sterilized between two inseminations.

### 8.3.2 Standard Operating Procedure for Laparoscopic method

1. The ewe in oestrus shall be separated from the flock. Ewes detected to be in oestrus in the morning should be inseminated in the evening and those detected in the evening should be inseminated in the next morning or FTB (Fixed Time Breeding) can be adopted.
2. For 12- 24 hours prior to the scheduled procedure, the ewe must be denied food and water.
3. The ewe shall be anesthetized and placed on a surgical table and restrained by use of a cradle, with the head pointed down.
4. Portions of the abdominal area of her belly shall be scrubbed and sterilized.

5. Semen can be deposited directly into the uterus, via the technique of laparoscopy.
6. Following insemination, the incisions shall be stapled or sutured and dressed with an antibiotic ointment.
7. Following skin closure of the abdominal wounds, the female is promptly released from the cradle, and she can return to feed immediately.

### 8.4 Procedure for Artificial Insemination in Goat

In goat Cervical AI or trans-cervical AI procedure should be performed as per the process depicted below: An indicative list of things required for AI is annexed as annexure (II)

1. The doe in estrus shall be separated from the flock. Does detected to be in estrus in the morning shall be inseminated in the evening and those detected in the evening shall be inseminated the next morning.
2. Lift the hind legs of the doe in a 45° angle towards the direction of sunlight.
3. The inseminator should gently introduce a lubricated speculum into the vagina to determine the stage of estrus. If a large quantity of vaginal mucus is preventing the correct location of the cervix, the assistant should tilt the animal down to allow the mucus to run out of the vagina with the aid of the speculum.
4. Locate the cervix using the speculum with the help of sunlight or AI light.
5. The inseminator shall try to pass the insemination gun as deep as possible into the cervix (Generally0.5 to 1cm) and deposit the semen. The assistant should hold the doe firmly. Ensure that no urine or fecal matter contaminates the tip of the AI gun.
6. The doe should then be gently lowered and returned to the flock while keeping it calm.
7. The speculum should be properly disinfected/ sterilized between two inseminations.

## 9. Standard Operating Procedures for (AI) Technicians

### 9.1. Semen Handling for A.I. with Frozen Semen Straw (FSS)

1. Keep the liquid Nitrogen container with semen straws in a location that allows seeing into the neck tube of the container, and ease in withdrawing & replenishment of semen and liquid nitrogen. The surrounding should be well ventilated, dry and dust free.
2. Clean (AI) gun, scissors and other accessories whenever they get soiled or at least once a week with hot water and air dry them. Sanitize the (AI) gun and the scissor

with Isopropyl alcohol after drying. The AI equipments should be kept always clean after each insemination. Surgical spirit and soaps are lethal to semen, hence should not be used to clean equipments.

3. Measure the liquid nitrogen level of the containers weekly with the help of measuring scale provided with liquid nitrogen container. Maintain the record of measurements to monitor its evaporation rate. Replenish the liquid nitrogen when needed.

4. Use small liquid nitrogen container to carry the semen straws to the field. Maintain the liquid nitrogen level above the straw level.

5. Carry the required semen doses in the liquid nitrogen container to farmer's / farm supervisor's door step. Never carry semen straws in pocket / thermos-flask / polythene bags filled with water/ ice etc.

6. Maintain an accurate semen inventory to lessen the risk of semen exposure.

7. Always attach the paper tag provided with each goblet to the requisite canister of the container to identify the type of semen in each canister.

8. A skilled operator will take less than 2-3 minutes to inseminate each animal. The professionalism and skill of the inseminator are vital components for success. The surgical approach, cleanliness, recognition of internal and uterine abnormalities, and risk assessment of infection and disease must be observed.

## 9.2. Semen Handling for AI with liquid semen

1. Avoid exposure of semen to sunlight and do not take sample outside the semen shipper.
2. Immediately close the shipper after pipetting the semen.
3. Maintain an accurate semen inventory to lessen the risk of semen exposure.
4. Use semen shipper packed with ice to carry the liquid semen to the field. Maintain the proper ice level in semen shipper.

## 9.3. Procedure for Thawing

1. Thaw straws in a water bath at 37 °C for 30 seconds: Set the temperature of water in the receptacle to 37°C using a thermometer. Lift the required canister and remove the required straw with the help of forceps. Plunge the straw for 30 seconds in the water in the receptacle. Wipe the straw with tissue paper or a clean cloth.

2. May assess post-thaw motility under microscope

3. Load the straw in an AI gun, cut the sealed end of the straw and prepare the AI gun for insemination by placing a sheath over it and proceed to inseminate the does/ewes.

## 10. Post Insemination Advice to Farmer/ Farm Supervisor

1. Ask farmer/ farm supervisor to keep the animal under observation for next 12-24 hrs.
2. Inform the farmer/ farm supervisor to save the animal from scrub rams/bucks during the remaining part of present heat.
3. If signs of heat persist even after 18-24 hrs, repeat (AI), otherwise observe for heat symptoms after 18-21 days and also after 36-42 days.
4. If animal does not repeat heat at 18-21 days intervals for two consecutive times, check for pregnancy diagnosis after 2 months from the date of insemination.

## 11. Post Insemination follow-up by the Veterinary /AI Technician

1. Follow each and every animal inseminated after around 21 days to find out whether it has repeat heat.
2. Follow each and every animal inseminated for pregnancy diagnosis after 1 months and record the date and result of pregnancy diagnosis in the register.
3. Follow each and every pregnant animal and record kidding/lambing details of the animals inseminated in the register.
4. Maintain all records related to artificial insemination, pregnancy diagnosis, and kidding/lambing
5. Advise farmer/ farm supervisor on proper heat detection, feeding, management and healthcare of animals.

## 12. Methods of Pregnancy Detection

**1. Non return to estrus:**

The non-return to estrus is among the oldest and commonly used methods for pregnancy detection in small ruminants. It is not expensive, practical and yields information about pregnancy at a very early stage after the mating ($17^{th}$-$21^{st}$ day). The non-return to estrus evaluation is not recommended during the late breeding period and for animals, synchronized during the anoestrus. Very often, pregnant goats also exhibit signs of estrus. Such animals could be hardly differentiated. Hence the technique is not deemed reliable for detection of pregnancy.

2. **Abdominal Inspection and palpation; live weight monitoring**

The inspection of the abdominal region, trans-abdominal palpation of the uterus and fetuses and body weight increase are techniques for pregnancy diagnosis in goats with a low precision, applicable only during the second half of gestation. The live weight increase during pregnancy is rather variable, and therefore not enough reliable traits.

3. **Recto-abdominal technique:**

Rectal palpation, a routine technique for pregnancy diagnosis in large animals, is not pertinent to sheep and goats due to specific anatomic features. However pregnancy in small ruminants can be detected by recto abdominal palpation technique by palpation with both hands. The method includes palpation of the uterus through the rectum with one hand, while the other presses the abdominal wall. Pregnancy is diagnosed on the basis of cervical dilatation, altered position of the uterus, palpation of placentomes or parts of the fetus, asymmetry of uterine horns and impossibility for palpation of ovaries

4. **Vaginal biopsy:**

For diagnosing pregnancy in does and ewes after 40 days post breeding, histological evaluation of vaginal biopsies has high accuracy rate. In pregnant animals, vaginal mucosal cells and nuclei were half of the size than those in non pregnant animals, which have polygonal and squamous cells in more than 10 layers. Pregnant ewe's vaginal epithelium has few layers of columnar, cuboidal and prismodial cells. For biopsy samples must be taken from the anterior vagina.

5. **Radiography:**

Radiography can be used to detect pregnancy and multiple births with high accuracy once the fetal skeleton is mineralized. Fetal skeleton is often radio opaque after 65 days of gestation. Uterine enlargement suggestive of pregnancy may be detected earlier than this but cannot be differentiated from hydrometra or pyometra. Under field conditions, this technique is not practical for examining large number of ewes and does, but may be useful for an individual animal when ultrasound equipment is not available.

6. **Ultrasonic techniques:**

Ultrasonography as a method for monitoring of the reproduction status in small ruminants is becoming increasingly important and popular. In living tissues ultrasonic techniques can be used to examine subsurface structures. One of the most important and beneficial features of ultrasound for pregnancy diagnosis is its safety to the operator and patient. All three types of ultrasonographicals viz., amplitude-depth (A-scan), Doppler and Real time B-scan ultrasonics can be used to diagnose pregnancy in ewes and does under field conditions.

6.1 A-Scan ultrasonic techniques: It involves the principles of echo amplitude or amplitude depth versus time. Diagnosis of pregnancy is based on detection of fluid filled uterus. These units are sensitive at a depth of 10 to 20 cm. A-scan ultrasound applied to the flank region has proven to be reliable from 50 to 120 days of gestation in sheep and goats.

In standing ewe or doe, the transducer is placed on the lower right flank in front of the udder. Clipping of hair or wool of this area facilitates optimal contact. A-scan technique may be of particular importance in areas where transport or electricity may not be available.

6.2 Doppler ultrasonics: Diagnosis of pregnancy by the Doppler ultrasonics involves the principle of detecting the movements as an indication of pregnancy such as fetal heart beat, fetal circulation and fetal movements. Fetal heart beat and fetal pulse which are faster than maternal pulse or fetal movement are taken as positive criteria of pregnancy. In ewes and does, external application of the ultrasonic Doppler has been used for detection of pregnancy and approaches an accuracy of 100% during last half of gestation but is not effective 50 days or earlier.

Doppler ultrasonics - rectal: For diagnosing pregnancy, intra rectal Doppler technique is superior to the external technique during early second trimester. It may also be used 25 to 30 days post-breeding but it is best to use between 35 to 40 days of gestation. Fetal viability can be detected but accurate detection of multiple fetuses is difficult with Doppler technique.

6.3 B-mode ultrasonic scanning: Real time ultrasound produces a two dimensional image on a screen which can be photographed by a Polaroid camera. It produces a moving image of the uterus, fetal fluids, fetus, fetal heart beat and placentomes. For optimal image visibility, examinations should be performed away from direct sun light. The scanning is performed on the standing ewe and doe. Trans abdominal scanning should be ideally performed between 40 to 75 days of gestation. Various uterine pathological conditions such as hydrometra, pyometra and fetal mummification can be distinguished from pregnancy by real time ultrasonics. By measuring width of the fetal skull, fetal age in ewe and doe can also be determined by the use of real time ultrasonics at 40-100 days of gestation.

6.4 Hormone assay:

Another method of pregnancy diagnosis in small ruminants is by measuring concentrations of steroid hormones such as estrone sulfate and progesterone at specific times post breeding. Sensitive tests like radioimmunoassay (RIA) and Enzyme linked immunosorbant assay (ELISA) have allowed detection of these hormones in the blood, milk and urine.

6.5 Estrone Sulphate detection: Sheep and goat placenta produces estrone sulfate. In sheep plasma estrone sulfate can be detected around 70 days after conception and in does 40-50 days post breeding. A positive estrone sulfate test indicates a viable fetus.

6.6 Progesterone test: Plasma progesterone concentration may be tested by RIA or ELISA 18 days post breeding in ewes and 19-23 days post breeding in does with high accuracy.

## 7. Analysis of Pregnancy Associated Proteins:

The detection of pregnancy in goats could be done by immunological analysis of some specific proteins, such as the early pregnancy factor. Specific antigens are detected by radioimmunological (RI?) or immunoenzymatic (ELISA) techniques using monoclonal or polyclonal antibodies between the 5th and 60th gestation days.

7.1 Pregnancy associated glycoproteins: The detection of pregnancy associated glycoproteins (PAGs) in blood plasma or milk samples is a reliable method for early pregnancy diagnosis in goats.

7.2 Pregnancy-specific protein B: Detection of Pregnancy-specific protein B (PSPB) is established in sheep and goats and could serve for purpose of diagnosis of pregnancy.

# Annexure-XVI

## REPRODUCTIVE PARAMETERS OF SHEEP AND GOAT

| Sl No. | Parameters | Sheep | Goat |
|---|---|---|---|
| 1 | Breeding age | variable for different breeds | variable for different breeds |
| 2 | Comes to heat after kidding | 21 days after | 21 days after |
| 3 | Length of pregnancy | 147 days (ranges between 144 and 152 days) | 147 days (ranges between 144 and 152 days) |
| 4 | Male female ratio | 1:33 | 1:20 |
| 5 | Estrous period is repeated | 16-17 days in ewes (range 14 -19 days) | 19-21 days in does (range 17-24 days) |
| 6 | estrous period lasts | about 24 -36 hours | about 34 -38 hours |

# Annexure-XVII

## List of things required for AI

### AI with Liquid semen

Oestrus synchronization is mandatory for AI in sheep either with liquid semen or frozen semen.

- Glass speculum/cylinder of 200mm length, 1.8mm thickness (open at both ends with smooth edges)
- Headlight
- Insemination glass pipettes (0.2ml only)
- Handypette pipette Aid to inseminate with glass pipettes

### Laparoscope aided AI with frozen semen

- Cradle to restrain the ewes
- Wool clipper
- Razor with blades
- Stool for inseminator
- Biological Incubator
- Laparoscope with accessories like Carbon dioxide and gas inflator
- Local anaesthesia (lignocaine 2%)
- Xylazine sedative
- Silk suture (No 1) with needles for skin
- BP handle with curved surgical blade
- Sterile guaze or swabs
- Normal saline and 70 percent alcohol for disinfection of laparoscope and trocars
- Antiseptic solutions and dressing creams
- Frozen semen
- Semen thawing kit
- Semen thawing kit
- Tissue paper
- Ovine Aspic and AI assembly

## Transcervical AI with frozen semen

- Cradle to restrain the ewes
- Modified AI gun (Guelph) with insemination neeedle for transcervical AI
- Vaginal speculum
- Miniature light source
- Atraumatic long forcep to grasp cervical fold
- Measuring scale to measure depth of penetration of insemination needle
- Lubricant
- Mild detergent to clean the perineum