ཀྲུལ་ཡོངས་ཨོ་ཚས་ཞིབ་འཚོལ་དང་གོང་འཕེལ་ལྷེ་བ།

NATIONAL DAIRY RESEARCH AND DEVELOPMENT CENTRE
DEPARTMENT OF LIVESTOCK
MINISTRY OF AGRICULTURE & FORESTS
YUSIPANG, THIMPHU

STANDARD OPERATING PROCEDURES (SOP) FOR BOVINE FROZEN SEMEN PRODUCTION

NDRDC Publication (2018): 1

Dr. Dorji and Mr. Mohan Raj Gurung
NDRDC, Yusipang
Tel: +975 17116985 (PD) / 17116976 (PABX) / Post Box: 1058

Suggested Citation:
Dr. Dorji and Mohan Raj Gurung (2018), Standard Operating Procedures (SOP) for Bovine Frozen Semen Production. National Dairy Research & Development Centre, Department of Livestock, MoAF, Yusipang, Thimphu, Bhutan

Layout, Design and edition:
Lokey Thapa
Yuka Nakanishi

Contact Address
Specialist Head/Program Director
National Dairy Research & Development Centre,
Yusipang, Department of Livestock
Ministry of Agriculture and Forests

PO Box: 1058
Office Phone: +97517116985, 17116985 (PABX) Lab: 17116986
Email:ndrc@moaf.gov.bt

Foreword

I am pleased to note that National Dairy Research & Development Centre is coming up with the Standard Operating Procedures (SOP) for Bovine Frozen Semen Production. This is necessary to operate the National Semen Processing Laboratory following set standards.

Semen production is a specialized job and requires a high level of technical and professional skill. Semen doses are required to be produced with minimum microbes and a very high percentage of progressively motile sperms in each dose of 20 million sperms.

Application of Artificial Insemination (AI) technology through use of frozen bovine semen has been the driving force for steady increase in exotic blood level of cattle population as well as milk production in the country. With increased awareness and access to mortable road, the numbers of AI Outreach Station has reached to 109

The development of the Standard Operating Procedures is timely as the Centre embarks on technological advances in cattle breeding including initiation of Embryo Transfer (ET) technology. The SOP represents current recommended good practices that will guide staff and ensure the provision of high quality services to beneficiaries. The SOPs have been largely guided by existing national policies and guidelines.

The Standard Operating Procedures required for quality semen production is detailed in this manual. The SOP provides general guidelines for all the activities for production of quality Bovine Frozen Semen. This manual has been prepared primarily for the Semen Processing Laboratory which intends to expand and modernize semen production facilities and produce diseases free semen for artificial breeding. The manual also provides specific guidelines and minimum standards for management of bulls, collection and processing of semen, quality control and bio-security measures

The Department appreciates NDRDC for their proactive initiatives in coming up with this document. It is hoped that the SOP developed will be a useful guide to meet minimum semen processing standards as per World Organization for Animal Health (OIE).

Tashi Delek!

Dr. Tashi Samdup
DIRECTOR GENERAL
DEPARTMENT OF LIVESTOCK

Table of contents

Abbreviations

AI:	Artificial Insemination
AIT:	Artificial Insemination Technician
AR:	Analytical Reagent
AV:	Artificial Vagina
BAFRA:	Bhutan Agriculture & Food Regulatory Authority
BPF:	Bypass Protein Feed
BGC:	Bovine Genital Campylobacteriosis
BQ:	Black Quarter
BSE:	Breeding Soundness Examination
BVD:	Bovine Viral Diarrhoea
CF:	Crude Fiber
CFU:	Colony Forming Unit
CP:	Crude Protein
DM:	Dry Matter
DSP:	Daily Sperm Production
ELIZA:	Enzyme Linked Immunosorbent Assay
FMD:	Foot and Mouth Disease
GR:	Guaranteed Reagent
HF:	Holstein Friesian
HOST:	Hypo Osmotic Swelling test
HS:	Haemorrhagic Septicaemia
IBR:	Infectious Bovine Rhinotrachieties
ICAR:	International Committee for Animal Recording
IM:	Initial Motility
JD:	Johne's disease / Paratuberculosis
LAFU:	Laminar Air Flow Unit
LN_2:	Liquid Nitrogen
MA:	Mass Activity
MoAF:	Ministry of Agriculture & Forest, Royal Government of Bhutan
MS:	Minimum Standards
NaCl:	Sodium Chloride
NBIN:	National Bovine Identification Number
NCIS:	National Cattle Identification and Recording System
NDRC:	National Dairy Research Centre, Yusipang
OIE:	Office International des Epizooties / World Organization for Animal Health
PCR:	Polymerase Chain Reaction
PD:	Pregnancy Diagnosis
PT:	Progeny Testing
PTM:	Post Thaw Motility
SID:	Single Intradermal test
SOP:	Standard Operating Procedures
SPC:	Semen Processing Centre
TB:	Tuberculosis
TDN:	Total Digestible Nutrients

Introduction

Artificial Insemination (AI) has proved to be the best tool worldwide for genetic improvement through dissemination of superior Germplasm. Frozen semen in 0.5 ml or 0.25 ml straws has become the universally accepted unit of storage and transfer of bovine genetics. AI has contributed enormously to the genetic improvement of dairy cattle in the last few decades. This objective should be achieved only if the frozen semen used in the AI programme conforms to the prescribed quality standards.

Semen production is a specialized job and requires a high level of technical and professional skill. Semen doses are required to be produced with minimum microbes and a very high percentage of progressively motile sperms in each dose of 20 million sperms. A large number of sophisticated equipment are required to be handled on one side and strict bio security measures are required to be put in place on the other. The end users are also now looking for quality. The frozen semen is no longer perceived as a dose to impregnate cattle, but perceived as a source of genetics to produce better quality animals.

For production of quality frozen semen, it is most important that the Donor bulls used satisfy certain norms, adequate care is taken in maintaining their disease free status and semen is harvested and processed in accordance with standard protocols.

The Standard Operating Procedures (SOP) required for quality semen production is detailed in this manual. The SOP provides general guidelines for all the activities for Production of quality Bovine Frozen semen. Failure to observe these guidelines would lead to production of poor quality semen making them unfit for distribution to AI centres. Thus, this SOP on Production of bovine frozen semen provides measures:

- ✓ To maintain the health of donor bulls on the Semen Processing Centre (SPC) at a level which permits the production of quality semen having negligible risk of infecting other animals or humans with pathogenic organisms that can be transmitted by semen.
- ✓ To ensure hygienic collection, processing and storage of frozen bovine semen

Selection criteria and procurement of Semen Donor bulls

One of the key factors affecting productivity is the genetic ability of cattle for milk production, which is an inherited character. Other factors like health, nutrition and management of the animal provide an enabling environment. Quality semen donor bulls contribute significantly in enhancing the genetic potential of its progeny for economically important traits like milk production, fat% and protein%, fertility, body conformation etc. Therefore, proper selection of donor bulls with high and transmittable genetic potential to its progenies for milk production and other important traits is very important.

Pedigree and Genetic merit

i. Selection of donor bulls should be done through methods like progeny testing (PT) and pedigree selection.
ii. Semen donor bull selection should follow prescribed Minimum Standard Protocols and Standard Operating Procedures for PT through Government approved PT Program.
iii. Progeny testing is only possible in breeds having large population size.
iv. On account of the small size of population of Siri / Nublang / Thrabum (native cattle breed of Bhutan) and Mithun (Bos frontalis), PT program was never tried in Bhutan. Since it is very essential and high time to initiate progeny testing scheme, effort is

underway since October 2017 to start progeny testing scheme and in active phase of implementation in two pilot Dzongkhags.

v. Till Progeny testing through PT Program is in place, selection of donor bulls of different cattle breeds available in the country should be based on the pedigree selection (table 2).

vi. Selecting the best donor bulls based on the performance of their parents (Dam's Standard lactation yield) forms the basis of pedigree selection.

vii. The Lactation yield of the dam should be recorded once a month continuously for 11 times or until the animal becomes dry.

viii. Standard Lactation Yield of the milk recorded animal should be calculated using the Test Interval Method (A4) described at Section 2.1.5.1 of the International Agreement of Recording Practices published by International Committee for Animal Recording (ICAR).

ix. Other important factors like health, nutrition, management, temperament, phenotypic, genotypic character of the donors should be also taken into consideration.

x. Dam's milk yield for F1 crosses will be as that of the dams of indigenous breed (siri cows).

xi. For import of donor bulls, frozen semen & embryos; the standards for import of Germplasm as prescribed in Livestock Rules and Regulation 2017 and in import permit of bovine Germplasm" issued by Bhutan Agriculture & Food Regulatory Authority (BAFRA), Ministry of Agriculture & Forest (MoAF), Royal Government of Bhutan (RGoB) and as revised from time to time should be followed.

Table 1: The Standards and Specifications for the selection of potential semen donor bulls through pedigree selection

Breed	Dam's Lactation yield (Kg)		
	First	Best	Fat %
Pure Jersey	3000	3750	5
Jersey Cross- F2	2800	3500	4.5
Brown Swiss – F2	2400	3000	4.5
Siri / Nublang	400	500	5
Pure Holstein Friesian	4500	5600	3.5
HF Cross- F2	4000	5000	4

Physical Examination

i. Before procuring new bull calves / bulls for semen production, a thorough physical examination should be conducted by a qualified Veterinarian to ensure that the bulls are free from abnormality and do not display clinical symptom(s) of any infection or any contagious diseases.

ii. Scrotal circumference and weight gain index recording should be performed once in three months and once a month, respectively. For every new bull procured, the measurement of scrotal circumference and body weight should be initiated immediately.

iii. Prior to introduction of new bulls for semen collection, breeding soundness examination (BSE) of bulls including physical examination of bulls, overall appearance, reproductive tract, semen evaluation, measurement of scrotal circumference etc should also be carried out.

Karyotying and testing for genetically transmitted diseases

i. Once testing facilities are in place, donor bulls should be subjected to karyotyping to rule out any chromosomal abnormality and genetic disease testing to eliminate bulls having genetic diseases.

Table 2: Specific tests can be conducted for genetically transmitted diseases as given in the Table below.

Breed	Tests to be carried out
Indigenous cattle	Factor XI deficiency syndrome, Bovine Leukocyte Adhesion Deficiency (BLAD), Citrullinemia
Jersey & Jersey Crossbreds	Factor XI deficiency syndrome, Bovine Leukocyte Adhesion Deficiency (BLAD), Citrullinemia
HF & HF crossbreds	Factor XI deficiency syndrome, Bovine Leukocyte Adhesion Deficiency (BLAD), Citrullinemia, Deficiency of Uridine Monophosphate Synthase (DUMPS)

Identification of the Bull

Each Donor bull should be ear tagged with laser printed National Bovine Identification Number (NBIN) under the National Cattle Identification and Recording System (NCIS). The NBIN is unique, eight digit number, where the first 2 digits are Dzongkhag / District code, followed by six digits animal's unique identifying number. The NBIN will remain with the animal for its lifetime. The uniform numbering system would link each identified donor bull to its birthplace or premises of origin.

Quarantine

i. A quarantine period of minimum 60 days is compulsory before bringing new bulls into the Semen Processing Centre (SPC). Only after favourable results from the animal health Centre, the bulls should be admitted to the semen station. Relevant definitions related to health protocol & quarantine is given in Annexure 1.

ii. In the quarantine station, new animals should be housed for a minimum of 30 days in a place which is effectively separated and away from (preferably at a distance of 5 km) from resident bulls.

iii. Manpower deployed and all equipment used in handling, feeding, watering and cleaning the new bulls should not be shared with the resident herd.

iv. Each new animal in quarantine station will be tested against major contagious diseases like Tuberculosis (TB), Johne's disease / Paratuberculosis (JD), Brucellosis, Campylobacteriosis and Trichomoniasis before its entry to resident herd. All tests should be done by an accredited agency or disease diagnostic laboratory.

v. During quarantine period, the bulls should be vaccinated against Foot and Mouth Disease (FMD), Haemorrhagic Septicaemia (HS), Black Quarter (BQ), Theileriosis and Anthrax.

vi. However, vaccinations against bacterial diseases should be done only if there is an outbreak or prevalence of a particular disease.

vii. Once the quarantine period is over, the bulls will be introduced to the donor bull rearing area.

viii. The Quarantine Guidelines for maturing & adult donor bulls under different situations is given in Annexure 2.

Management of Donor Bulls

Donor bulls selected are considered highly valuable units of semen production. The donor bull is expected to produce large number of frozen semen doses of good quality which will result in optimum pregnancy rate in the field. For maximizing use of the selected bull, good management practices are essential as detailed below;

Feeding

i. The management of fodder and feed for donor bulls is not as complicated as that of dairy cattle. The donor bulls being ruminants require fodder of good quality and in adequate quantity for their maintenance. In order to balance the nutrient requirements, it is essential to feed quality cattle feed.
ii. Supplementary feeding of 30 to 100 gm of mineral mixture / day / bull is essential to meet the physiological requirements.
iii. Water is one of the most important component of nutrients and it should be made available all the time.
iv. The requirement of Feed, Fodder, mineral supplements, water etc is detailed in Annexure 3.

Housing

i. The type of housing decides the comfort level, quality and quantity of semen production by donor bulls.
ii. Bull sheds should be permanent structure having spacious loafing area.
iii. Single bullpens having loafing area are suitable and ideal for donor bulls. If individual bull housing is not practiced, adequate space should be provided.
iv. The bulls should be kept free and each bull should have adequate loafing area (minimum 10 M^2).
v. The sheds should be airy and allow breeze.
vi. In places where the climate is cool most of the year, walls are recommended. However attention should be given to ensure adequate ventilation and avoiding stale air in the sheds.
vii. The sheds should have feeding manger and water trough with access to drinking water all time.
viii. The sheds should be in East West direction to avoid direct sunlight on bulls since testicular degeneration due to intense sunlight on testis / scrotums have been reported.
ix. In case the sheds are in north South direction, measures should be taken to provide adequate shade to bulls to protect from direct sunlight.
x. Trees to protect from direct sunlight should surround the sheds.
xi. The roof should be made of asbestos or suitable materials.
xii. The flooring of sheds should be non-slippery cement concrete with adequate grooves or strong wooden planks.
xiii. Strong smelling disinfectants like formalin or phenyl based compounds should not be used in the bull sheds. Alternatively, compounds containing gluteraldehyde, colloidal iodine or chlorine compounds like hypo chlorate should be used.
xiv. Weekly spraying of Sodium Carbonate (4%) solution should be practiced.
xv. The floor should be sterilized at least once a year by a blowlamp or by burning straws.
xvi. At one corner of the farm, there should be an isolation shed for separating ailing / sick animals for treatment. The isolation shed should be away from the main housing of bulls.

xvii. Bull once diagnosed suffering from infectious diseases should be removed immediately from bull shed for safety of other bulls.

xviii. In addition there should be facility for quarantine of new bulls. This facility should be preferably at least 1 km away from main housing. All housing facilities should have arrangements for feeding and supply of water.

xix. There should be separate staff and separate bio-security arrangements for semen donor bulls and female herd.

Exercise

i. As per the published literature, the effect of exercise of bulls on the semen quality is varying.

ii. However it is preferable to exercise the bulls at least on alternate days for about 45 minutes to 1 hr.

iii. During the exercise, it would be possible to observe the gait and note any lameness / foot problems.

Vaccination

i. Considering the physiology of spermatogenesis, it is preferable to avoid any type of vaccination as vaccination induces febrile reaction and thereby affect the quality of semen. However keeping in view the prevailing animal disease outbreaks, it is recommended to plan a vaccination schedule and implement strictly. Wherever possible it is recommended to minimize the number of vaccinations each year using combination vaccines like HS & BQ. In case of FMD, it is preferable to use oil adjuvant vaccine.

ii. The site of vaccination should be washed with 2% Savlon/ Dettol solution prior to vaccination to avoid formation of abscess due to secondary infections. The spot of injections should also be cleaned with surgical spirit / tincture of iodine

iii. Ensure use of correct vaccine and correct dose and route. The instructions of manufacturer should be followed and the information batch number, date of expiry of vaccine should be recorded at the time of vaccination.

i. The donor bulls should be vaccinated against FMD, HS, BQ, Theileriosis and Anthrax.

ii. Vaccinations against HS and BQ should be carried out in the areas having incidence of these diseases.

iv. In case of Theileriosis, Exotic and crossbred bulls should be vaccinated once in their lifetime. However, if tick infestation is high, the exotic and crossbred bulls should be vaccinated against Theileriosis once in three years.

v. If ecto parasites are common, measures should be taken to reduce parasites by use of safe sprays like Butox.

vi. Vaccinations against bacterial diseases should be done only if there is an outbreak or prevalence of a particular disease.

vii. To reduce lay off time, the bulls should be vaccinated on the rest day or the day after completing semen collection. Sexual rest may not be required unless febrile condition is noticed.

viii. Ring vaccination for all cloven footed animals including swine against FMD within a radius of 3 to 5 km around the Farm is very important. This will help to establish a ring of immunity and control outbreaks in the Farm and minimize losses.

Donor Screening / testing

i. As per OIE guidelines, the Donor bulls should be tested against regularly and free from diseases like Tuberculosis, Johne's disease, Brucellosis, IBR, BVD, Campylobacteriosis and Trichomoniasis.

ii. Though Johne's disease is not a sexually transmitted disease but from the herd health point of view, bulls found positive should be removed and therefore it has been included in the protocol.

iii. The laboratory should have the authority for immediate disposal (within 48 hours) of bulls that test positive for diseases (like Brucellosis, TB, JD, BVD), poor libido, poor semen quality, incurable lameness etc.

iv. Bulls found positive for Campylobacteriosis and Trichomoniasis should be isolated and treated.

v. Transfer those bulls which have completed 3 years of productive period or 30 thousand semen doses, whichever is achieved earlier.

vi. The detail Disease screening and health management of Donor bulls should be referred from Annexure 4.

Table 3: The disposal of donor and semen due to specific diseases should be carried out as per Table below

Diseases	**Donors**	**Semen doses**
Brucellosis	Castrate & dispose	To be discarded since the last negative test
TB	Remove	To be discarded since the last negative test
JD	Remove	To be discarded since the last negative test
IBR/IPV	Remove	To be discarded since the last negative test
BVD	Remove	To be discarded since the last negative test
Campylobacteriosis	Treat and retain	To be discarded since the last negative test
Trichomoniasis	Treat and retain	To be discarded since the last negative test
FMD	Retain	Last one month doses to be discarded

Healthcare & Hygiene Management of Donors

i. Daily care of donor bulls should be practiced to ensure a satisfactory state of cleanliness and under hygienic conditions at all times.

ii. The hooves of the bulls should be trimmed at periodical intervals to prevent over growth and lameness. Particularly bulls of exotic breeds like HF are more prone for hoof problems.

iii. Where pure HF bulls are maintained, it is recommended to arrange a separate footbath for bulls. The bulls should be made to stand in the footbath for at least half an hour at weekly intervals.

iv. The footbath should be filled with 0.5% copper sulphate solution. Alternately 1% formalin solution can also be used either for footbath or spray on the hooves.

v. The coat of the bulls should be kept clean and generally short.

vi. The donor bulls should be washed with water on alternate days (during summer) to keep them clean. It is recommended to groom with coir / nylon brush every day to keep the skin in shining and glowing condition.

vii. Bulls should be brushed and groomed regularly and special attention should be given to the underside of the abdomen a day prior to semen collection.

viii. The length of the tuft of hairs at the prepucial orifice, which is invariably soiled, should be cut to about 2 cm. The hair should not be removed altogether, because of its protective role. If cut too short, it may cause irritation of the prepucial mucosa.

ix. Cleaning of the prepuce with sterile normal saline solution may be done every ten days if the microbial load is within the prescribed limits. Cleaning prior to the day of collection should be practiced if the microbial load in frozen semen is beyond the prescribed limit.

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

Semen Processing Laboratory

i. Sufficient trees should be planted and lawns prepared around the Laboratory premises to reduce dust.

ii. The ceiling and walls of the laboratory should be made up of non-porous materials.

iii. All cracks and crevices should be sealed to control pests and insects.

iv. The floors should be preferably made up of vitrified tiles.

v. Airlock system or anti-room should be provided to avoid direct entry to the semen processing laboratory.

vi. Laboratory windows should preferably be made of double sheet glass with fixed aluminium frame. The glass panes should be plastered with sun control films to avoid direct sunlight.

vii. The doors should be kept closed, especially during diluents preparation and semen processing.

viii. Internal temperature control mechanism should be installed to maintain the room temperature at 20°C - 22°C as maintaining temperature is most important to achieve the best results.

ix. 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.

x. The processing laboratory should ideally maintain around 55% relative humidity.

xi. The flow of air from AC must not be towards the front side of the Laminar Air Flow Unit (LAFU).

xii. Adequate number of room thermometers should be kept in separate places in the laboratory to check the room temperature.

xiii. Only authorized personnel should be allowed access into the processing room. Entry of persons other than laboratory personnel should be strictly restricted.

xiv. No staff from the semen collection yard / AV preparation room and visitors should be allowed in the processing room.

xv. Clean laboratory footwear, apron, hand gloves, mask and caps should be compulsorily used by all staff while working in the laboratory.

xvi. Reusable clothing materials should be washed, cleaned and sterilized on a regular basis.

xvii. Sink drains should be decontaminated routinely with disinfectants.

xviii. Floors and horizontal surfaces should be cleaned and mopped with a disinfectant solution as dirt / dust, which settle on these surfaces, are the main sources of contamination.

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

xx. Appropriate number of germicidal UV lights (2470 A) with respect to area of laboratory, LAFU, apron and laboratory footwear cabinet may be fixed with a common operating switch outside the laboratory. These lights should be switched 'on' at least 8 hours prior to commencement of work in the laboratory and should be switched 'off' before beginning work. The date of installation of the UV lights should 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.

xxi. The laboratory should be well fumigated once a month with Cold Fumigant, using humidifier and aerated prior to commencement of work.

xxii. Fumigation should be supported by monitoring laboratory environment by bacterial load test. The bacterial load should be measured every quarter to monitor pollution of the laboratory atmosphere.

xxiii. The work tables / surfaces / LAFU, parts of equipment and hands of operator should be sterilized by use of 70% alcohol prior to commencement of work. The cleaning schedule should be repeated after completing processing of semen.

xxiv. Eating, drinking, smoking, etc. should be prohibited in the laboratory and unnecessary conversation should be discouraged.

xxv. Long exposure of semen to ultraviolet rays, visible light in direct sunlight and white florescent light causes chromosomal damage. Thus direct exposure to such sources of light should be avoided. There should be provision for indirect or diffused lighting inside the semen processing laboratory.

xxvi. Care should also be taken not to switch on tube lights in cooling chamber and laminar air flow unit. However, at the time of filling and sealing of straws in the cooling chamber, diffused light could be used.

Semen Collection Yard

i. The floor of the collection yard should be made of concrete.

ii. Preferably the mounting area should have sand and limestone mixture for proper footing of donor bulls. Alternatively, good quality rubber mat (with interlocking) at the mounting area is required for adequate cushioning effect.

iii. After semen collection, the area must be thoroughly cleaned and odourless disinfectant solution sprayed.

iv. A dusty floor should be avoided to prevent dust falling on the AV / semen samples.

v. The entry of visitors and staff/labourers (other than those involved in semen collection) should be strictly prohibited in the collection yard at the time of semen collection.

Preparation of Donor Bulls

i. The preparation of the bulls scheduled for collection should begin on previous day collection.

ii. The prepucial hair should be trimmed to 2 cm lengths. Long prepucial hair causes adhering of the dung leading to spoiling the ejaculate. If trimmed too closely, it will cause irritation leading to frequent masturbation / infection of prepuce.

iii. The underside of abdomen should be washed on previous day to ensure freedom from dung, urine and dust.

iv. An hour prior to collection, the underside of bulls should be brushed with a grooming brush to remove adhering dust/grass.

v. The donor bulls should be properly washed and cleaned at the waiting yard before brought to semen collection yard.

vi. After the animals are brought to collection yard, the prepuce / prepucial opening should be sprayed with 0.9 % normal saline and wiped dry using sterilized napkin or disposable tissue paper to remove any dirt / dust particles.
vii. Disposable hand gloves and separate sterilized napkins or disposable tissue papers should be used for each bull to avoid transmission of IBR infection from one bull to another.
viii. The bull attendants should wear lab coats kept at the collection yard. The bull attendants working closely with bulls like grooming, semen collection etc. should also wear protective foot wear to avoid injuries.
ix. Bulls are ready for semen collection and freezing procedures after they have passed a complete physical examination, the required health tests and a breeding soundness evaluation.

Pre collection stimulation

i. Proper sexual stimulation of bulls prior to collection will help to collect semen in good quality and quantity.
ii. Sexual preparation (number of false mounts and restraint) of Donors should be carried out considering the individual behaviour of the bulls and not generalized. For this purpose, the sexual behaviour of the individual bulls should be studied and documented.
iii. A common applied practice is to allow the bull to perform two false mounts followed by 1-2 minutes of active restraint and again one false mount before semen is finally collected.
iv. Use of bull aprons during false mount will avoid the penis touching hindquarter of the dummy.

Frequency of Collection

i. For effective use of donor bulls such as maintaining reproductive ability for a long time the most appropriate frequency of semen collection for Bull is 2 ejaculations per day every 2 - 4 days.
ii. The norm of minimum two ejaculates per collection and minimum two collections per bull per week for taking at least 90 collections and 180 ejaculates annually from each adult bull is advised. However, a maximum number of collections per bull would depend on the individual performance of the bull.
iii. Semen must be collected from a bull having normal libido. The gap between two ejaculates should be around 30 minutes depending on the refractory period of the bull.
iv. Second ejaculate should be taken with proper preparation of bulls.

Hygiene and precautions for Semen collector

i. Before entering the collection yard, the semen collector should change dress and footwear.
ii. Wear protective semen collection clothing (lab coat and cap) and gumboots.
iii. Before every collection, wash hands with 0.1 % Savlon or use disposable gloves or do both.
iv. Use a new pair of disposable gloves for each collection. If not, wash and disinfect hands after each semen collection.
v. Use a sterilized napkin to wipe hands and change napkin after each collection.
vi. Semen Collector should not enter the AV room during collection and the semen processing laboratory.

vii. Wash the footwear and coat on weekly basis after completion of semen collection work.

Artificial Vagina (AV) and its preparation

i. The AV method for semen collection is used almost exclusively in semen processing laboratories for practical reasons and because it produces physiologically normal semen samples.

ii. The components of a bovine AV include Outer Rubber Cylinder / Rigid Casing, Inner Rubber Sleeve / Liner, Rubber Cone (receiving semen) and Graduated Semen Collection Tube.

iii. Prior to collection all the parts are cleaned, sterilized and assembled into artificial vagina.

iv. The inner sleeve / liner is put into the outer cylinder and both the ends of inner sleeve are reflected over the outer rubber cylinder forming a watertight space between the outer cylinder and the inner liner.

v. The rubber cone with the attached graduated semen collection tube is then slipped over one of the ends of this water jacketed barrel and then tightly secured with rubber bands.

vi. Water at 40.5 – 42^{o}C is filled in through the water valve / outlet to ½ to ¾th full. Bulls for semen collection require an AV temperature of 40.5 to 42°C. Thus while preparing the AV, the temperature, which is a critical factor in stimulating ejaculation should be maintained at 105 –107°F (40.5 – 42°C). However, Temperatures up to 118°F (48°C) may assist collections in untrained young bulls.

vii. Pressure is adjusted by adding / blowing air through air valve until the AV liner protrudes slightly from the ends. AV pressure is also an important factor for obtaining ejaculates of optimum quality.

viii. The friction necessary for the bull to thrust is provided by pressure of AV and the surface of the liner. Generally smooth liners are preferred for young bulls and slightly roughened surface (rough liner) may be necessary for older bulls.

ix. The open end of sterilized AV should be covered with aluminium foil / plastic wraps, which is removed at the time of semen collection.

x. Appropriate size AV (8-14") should be used for cattle and AV cone should be of top quality Neoprene rubber.

xi. Use of lubricant should be avoided. If it is extremely essential to use lubricant, separate sterilized glass rods should be used for smearing K-Y Jelly or sterilized non-spermicidal lubricant such as white Vaseline / vaginal jelly to the upper 1/3rd of the AV.

xii. An insulation bag / AV jacket is attached to AV hose to protect the ejaculated semen from sudden drop of temperature / avoid cold shock and also protect the collection tube from breakage.

xiii. Immediately after collection, the AVs should be thoroughly cleaned by non-spermicidal neutral detergent.

xiv. Separate AVs should be used for each mounting. The AV should be changed even if the bull has inserted its penis without successful ejaculation. The same AV should not be used twice.

Technique of Semen Collection

i. Collection of semen is best done by trained personnel with proper sterilized and prepared AV.
ii. Two personnel are required for bull semen collection by AV; the bull handler and the semen collector who carries the AV.
iii. Teamwork between bull handler and semen collector is essential to assure correct bull identification, use of correct sexual preparation procedure and safety of employees.
iv. At the time of collection both the collector and attendant should avoid sudden moves.
v. Sterilized bull aprons should be used to avoid penis touching hind quarter of the dummy during collection.
vi. Check temperature of the AV before collection. Ideal temperature is 40.5 to 42ºC with Pressure of 45 to 55 mm of Hg. Younger bulls requires higher pressure.
vii. Semen should be obtained from bulls with normal libido and collection should be attempted only if the bull is proper sexually prepared.
viii. The bull after pre-collection stimulation is brought near the rear of a suitable teaser / dummy.
ix. Since semen collection by AV imitates natural breeding, the bull must mount teaser / dummy.
x. When the bull mounts and when the penis is completely separated from the sheath, the collector must grasp the penis through the sheath and direct it to the opening of the lubricated AV (serve the AV).
xi. Care should be taken not to touch the exposed part of the penis as this stimulates the bull to ejaculate. Also, touching of the penis by the collector causes shying of the bull and the bull will not readily mount.
xii. During collection, the AV is held at an angle of 55^{o} with the horizontal plane of the teaser or dummy. This is because the penis of bull enters the vagina of cow at that angle.
xiii. When the bull has mounted, it will make seeking motions and thrust into the AV. The thrust should be vigorous to ensure an ejaculate has been collected.
xiv. While taking collection always ensured that AV is not thrust on penis of bull, instead penis should be guided to AV.
xv. To reduce possible loss of sperm do not remove the AV from the penis too quickly after the bull has ejaculated.
xvi. After the bull dismounts the AV is taken off the penis and kept in an upright position. This allows the ejaculate to flow from cone to collection tube, which is then detached from the cone and taken to laboratory for examination.
xvii. Hold the AV before and after semen collection in such a way that the lubricating jelly / debris after collection is not mixed with the ejaculate.
xviii. The AV should not to be shaken after ejaculation; otherwise lubricant and debris may mix with the semen samples.
xix. Ensure that there is an interval of minimum 15 minutes between two collections from the same animal.
xx. As soon as the first ejaculate is taken, the bull apron should be removed and dipped in the plastic bucket filled with detergent lotion. For second ejaculate, a fresh apron should be tied to the bull.
xxi. Avoid distractions during semen collection. The entry of visitors to the collection yard during collection should be prohibited.

xxii. The semen collector should understand the individual bull's sexual behaviour and physiology.
xxiii. The semen collector and bull attendants should not be changed frequently.
xxiv. Never mistreat or abuse a bull at the time of semen collection. Too high or too low temperature of AV, forcing of the AV on penis, grasping of the penis instead of sheath are painful conditions leading to sexual suppression.
xxv. Immediately after completion of semen collection, dip the AV in a container having neutral detergent solution for cleaning.
xxvi. Gumboots, lab coats, aprons should be washed regularly after completion of semen collection work.

Handling of neat semen

i. The Collection tube containing the freshly collected semen should be capped with aluminium foil as soon as it is transferred through pass box to the laboratory. The collection tube should be kept capped until processed.
ii. As soon as the neat semen is received it is observed for colour, consistency and presence of visible contaminants.
iii. The volume of semen along with the breed and bull number is recorded.
iv. Keep the neat semen in a temperature controlled dry bath at 36 - 37^0C near the pass box.
v. The undiluted semen should not come in contact with cold surface of glass tubes.
vi. The semen sample should not be allowed to cool down below 25^0C before dilution.
vii. Examination and Evaluation of Semen quality is necessary for assessing the potential fertility of a semen sample.
viii. Examination of semen is carried out immediately as per the procedure detailed below.

Examination & Evaluation of semen quality

Macroscopic/ Physical tests

I. Volume

i. The volume of semen is directly measured from graduated semen collection tube / vial.
ii. The capacity to produce spermatozoa per gram of testicular tissue (Daily Sperm Production; DSP) is well correlated to scrotal circumference measurement in young bulls.
iii. The volume of semen per ejaculation differs markedly according to factors such as animal species, age of animal, collection method, frequency of ejaculation, etc.
iv. Volume of semen per ejaculation in older male animals is usually greater than in young animals.
v. A high protein diet causes an increase in volume of semen per ejaculate.
vi. Ejaculates with a volume of > 2 ml should be rejected.

Colour

i. Semen of bulls resembles whole milk in colour & its white opaqueness is entirely due to the dense mass of sperm cells present.
ii. Varying shades of yellow is considered to be due to presence of Riboflavin (Vitamin B_2).
iii. Abnormal colour of semen denotes certain pathological conditions of genitalia.
iv. Ejaculates having abnormal colour should be discarded as detailed in Table below;

Table 4: showing abnormal colour and reasons

Abnormal colour	Reasons
Yellowish	Presence of pus or urine
Dark yellow and thick	presence of pus
Dark yellow and thin	presence of urine
Pinkish/reddish	presence of fresh blood
Deep red/ brownish	degenerated blood tissue
Greenish	Purulent degeneration

Consistency or cloudiness

i. The normal bull semen is creamy or milky in consistency depending upon concentration of sperms.
ii. Ejaculates with abnormal consistency should be rejected
iii. The consistency of semen based on spermatozoa concentration is given in Table below;

Table 5: Semen consistency and concentration

Consistency	Concentration of sperm (Million/ml)
Thick creamy	Over 2000
Creamy	1500-2000
Thin creamy	1000-1500
Milky	500-1000
Watery	Below 100

Microscopic Examination

Mass activity (MA)

i. A drop of normal neat semen immediately after collection is placed (by use of a micropipette and a pipette tip) on a warm glass slide without cover slip.
ii. It is viewed using phase contrast microscope under low power objective (10X) with warm stage heated to 37^{o}C and the type of movement is observed (swirls / waves).
iii. The mass motion of the spermatozoa is affected by concentration, percentage of progressively motile cells and the speed / vigour of sperm motion. If one or more of these factors is comprised the swirling of mass motion will be suppressed.
iv. MA is graded on a scale of 0 to 4 depending on the vigorosity of the waves as detailed in table 4
v. Ejaculates without any wave motion (grade 1 & below) should be rejected as static ejaculates that show no MA seldom freeze well.

Table 5: mass mortality and grading scale

Grade	Mass Activity
0	No activity
1 (+)	Movement slightly vigorous but no waves or eddies.
2 (++)	Wave formation with slight whirls, which moves slowly across the field
3 (+++)	Rapid & vigorous waves with whirls/eddies which change with great rapidity
4(++++)	Extremely rapid movement and curing of swirls and eddies.

Initial motility (IM)

i. A drop of neat semen immediately after collection is placed on warm slide with cover slip on a warm stage heated to 37^{o}C.

ii. Examine using phase contrast microscope under low power objective (20X), at a magnification of 200 times (10X eyepiece and 20X objective) to estimate percentage of motile cells (movement of individual sperms).

iii. The IM grading is from 0-100 % as detailed in Table 6

Table 6: Initial mortality and grading

Grade	Initial Motility
0	No initial motility
20%	Mostly weak and oscillatory
40%	Rapid or vigorous
60%	Very rapid and vigorous
80%	Mostly vigorous and progressive
100%	Highly vigorous and progressive

iv. Ejaculates with less than 60% motile cells should be rejected as semen samples selected for freezing should have a minimum of 60% IM.

Sperm concentration

The concentration of spermatozoa in an ejaculate can be determined by the following methods:

Visual examination of density

i. By visualizing the consistency / cloudiness of semen sample as described in macroscopic tests.

ii. Although a rough prediction of sperm concentration can be determined by visual evaluation of semen sample, exact concentrations must be known to determine the number of doses of semen to be frozen.

Photometer/spectrophotometer

The sperm concentration can be assessed using photometer / spectrophotometer. Photometers / spectrophotometer are designed to measure the percentage of light transmitted through a Light absorbing media under evaluation. The percentage of transmission is then translated to the sperm concentration. The photometer / spectrophotometer should be calibrated by hemocytometer readings. Sperm concentration should be checked using SDMS spectrophotometer with auto dilutor, manufactured by a reputed company.

CASA Systems

i. Visual microscopic analysis of individual progressive motility is somewhat subjective even when performed by very skilled people and becomes tedious when large numbers of sample must be analyzed. Computer Assisted Semen Analysis (CASA) systems have the potential to increase objectivity of analysis and reduce worker fatigue.

ii. CASA Systems use computer programmed digital analysis of microscope fields of moving sperm.

iii. Several characteristics of motility are quantified, and sperm concentration is also determined. These systems require appropriate dilution of semen samples before filling of one or more commercially supplied disposable counting chambers with a depth of 20 microns. A counting chamber is placed under a microscope and up to 7 microscope fields are analyzed in just seconds per field. Data can be stored and provided on customized printed forms.

Neubauer Haemocytometer

Haemocytometer is the most ideal standard method for determination of cell concentrations and for calibration of electronic systems of cell counting. The method is very reliable and inexpensive, but will take about 10 minutes per sample and involves tedious visual counting of sperm. The haemocytometer which is used for RBC count is also used for sperm count.

i. The semen sample immediately after collection should be kept in water-bath at $37^{o}C$.
ii. 0.1 ml of semen is drawn into the micropipette. The exterior of the pipette should be whipped with filter paper and semen should be mixed thoroughly with 19.9 ml of 4% NaCl solution.
iii. Charge the haemocytometer with diluted semen for counting spermatozoa under low power objective (20X).
iv. Count spermatozoa in 5 large squares (containing 16 small squares) of Neubauer Haemocytometer.
v. The spermatozoa number counted in five large squares is summed up; this number is multiplied by 10^7 for estimation of sperm concentration.
vi. The average Sperm concentration in bovine species in ($1200X10^6$ /ml) with a range of 300 to 2000 million / ml.
vii. Semen samples showing less than 500 million / ml sperm concentration should be discarded.

Live and dead count

i. One drop of semen is mixed with 2 -3 drops of Eosin-Nigrosin stain on a glass slide.
ii. A film / smear is made from the mixture on a glass slide using coverslip.
iii. The film / smear after proper drying is observed under oil immersion (100X)
iv. Living spermatozoa appear unstained while dead spermatozoa are stained pink with Eosin against brownish/purple background of Nigrosin.
v. Eosin component of the stain will penetrate the injured or broken cell membrane, causing the cell to become pink. Sperm cells with intact membrane look colourless/ white against the dark background provided by Nigrosin component of the stain.
vi. Several fields at random should be examined and counted. Partially stained sperms should be included with the totally stained cells, representing the number of dead cells in the sample.
vii. Total / Differential counts are made of about 200 to 400 of the living (clear) and dead (pink) sperms, and from this percentage live and percentage dead is calculated.
viii. Live % = No. of live cells counted / Total No. of cells counted.
ix. Dead % = No. of dead cells counted / Total No. of cells counted.
x. Any semen sample containing more than 20% dead cells is discarded.

Percentage of abnormal spermatozoa

i. The procedure for determining abnormal spermatozoa in the semen sample is similar to that of live and dead spermatozoa count (eosin Nigrosin staining).
ii. The abnormalities of bull spermatozoa consist of major and minor abnormalities, the detail of which is in **Annexure 5**.
iii. Ejaculates with more than 20% abnormal sperm cells (Major Minor) should be rejected.
iv. The minimum standards for frozen bovine semen certified fit for use in the field is summarized in Table 7.

Table 7: Parameters and minimum standards of semen for processing

Parameters	Minimum standards for processing
Volume of neat semen	>2 ml
Colour of neat semen	Creamy, yellowish, Milky
Purity of neat semen	Neat and Clean
Mass Activity of neat semen	++ & above
Initial Motility of neat semen	60 % & Above
Concentration of neat semen	>500 X 10^6 / ml
Live % of neat semen	80% & above
Concentration / straw	20 X 10^6 / dose
PTM of frozen semen	> 40%

Printing of Straws

i. Information regarding breed of donor bull, NBIN, date of Manufacture, name of the organization, country etc. should be printed on the straws.
ii. The straws should be printed before filling and sealing. After printing the ink gets instantly dried.
iii. While printing straws, the room temperature should be maintained at 20^{o}C.
iv. The laboratory should follow the following printing format / abbreviations:
Cattle Breed: J = Jersey, BS = Brown Swiss, N = Nublang, M = Mithun
NBIN of Donor: Eight digit ID # of the bull as per NCIS
Date: Date of Production / manufacture (DD / MM /YYYY)
Name of Centre & Location: NDRC, Yusipang
Country of origin: BHT = Bhutan

Concentration of spermatozoa per straw & diluents addition

i. The average number of sperm provided per dose in the last 5 decades has been 10 million progressively motile sperm post-thaw.
ii. Since approximately 50% of sperm die in the freezing process, it is necessary to double the number of sperm per dose; i.e., if 10 million progressively motile sperm post-thaw is the goal, the number of sperm per dose should be 20 million.
iii. Soon after receiving the semen it should be evaluated with the mandatory macroscopic and microscopic tests as detailed above.
iv. After having done the tests, the selected semen samples should be primarily diluted with extender maintained at 37^o C.
v. Final dilution of semen keeping a minimum of 20 million spermatozoa per dose should be done in appropriate flasks with the diluents maintained at 37^o C.
vi. Keep the extended semen in the flask inside the Cold Handling Cabinet before printing the straws.

Automatic Filling and sealing of straws

i. Automatic Filling and sealing of semen should be done in the Cold Handling Cabinet / Cooling Chamber / Unit using sterile straws, filling nozzles and fresh rubber tubings.
ii. Considering the advantages the French Mini Straws has over French Medium straws, the laboratory should use French Mini straws.
iii. Unused straws should be repacked (air-tight) under LAFU before storage.

Cooling of semen & Equilibration

i. Distribute / arrange the Filled and sealed straw evenly on the Distribution Block and Freezing Rack inside the Cooling Chamber.
ii. Switch on the Cooling Machine about 20 - 30 minutes after the filled sealed semen straws have been placed inside the Cooling Chamber.
iii. The temperature will gradually fall from room temperature to $4^{o}C$.
iv. To avoid cold shock, semen should be first diluted / extended and then cooled gradually from room temperature to $4^{0}C$ over a period of several hours. Slow cooling is necessary for better liveability of the spermatozoa.
v. It is found that semen stored at 4 - $5^{0}C$ for few hours before freezing gives better post thawing motility and fertility. This pre freeze storage period is termed as Equilibration time.
vi. It was believed that during this period the sperm cells became permeated with glycerol and an ionic and osmotic equilibrium is established between sperm cells and its medium. Equilibration period allows spermatozoa to acquire better resistance to freezing stress. Addition of sugars like fructose or arabinose helps to reduce the equilibration time.

Vapour freezing:

i. After the equilibration period, the filled / sealed semen straws are vapour frozen (-$85^{o}C$ for 8 minutes) in the Bio Auto Freezer.
ii. After vapour freezing, the semen straws are plunged in semen storage containers containing LN_2 for 24 hours.
iii. The detailed procedures / steps for operating and using different laboratory equipments for semen evaluation, processing and cryo preservation of quality semen is detailed in Annexure 6.

Post thaw motility (PTM)

i. After freezing, the semen straws should be stored in a separate Container.
ii. PTM is done to assess the semen quality after thawing.
iii. PTM of semen should be examined at 24 hr after processing.
iv. Samples of each frozen semen batch from each bull are thawed and the percentage of cells exhibiting normal progressive motility is assessed.
v. Differences in observations should be updated and recorded for the purpose of accepting a particular batch of semen doses.
vi. A single straw (sample) is removed with pre cooled forceps from the goblet.
vii. The straw is held firmly and shaken vigorously once or twice to remove excess LN_2 from the cotton plug to prevent popping of the straws.
viii. The straw should be fully immersed in warm water at $37^{0}C$ for 30 seconds.
ix. After thawing period, dry the straw with tissue paper or towel. This is because water is lethal to the sperm cells.

x. Cut the straw at both ends (near laboratory & manufacture seals level) by holding the straw horizontally with a pair of scissors.
xi. Few drops of semen are put on a glass slide with cover slip and observe using phase contrast microscope under low power objective (20X) to find out the PTM.
xii. Whenever there is any doubt, PTM should be examined by two experienced persons. Preferably, the person involved in evaluation of neat semen, should not check the PTM.
xiii. For a minimum concentration of 20 million per dose, minimum acceptable post thaw motility should be 40%.
xiv. Semen doses below 40% motility should be discarded.

Semen Storage

i. After checking PTM, if found acceptable, frozen semen doses should be transferred and stored by fully immersing in LN_2 Storage Containers with proper identification / recording at the Semen Bank.
ii. The frozen semen is kept in the goblets which are available in different capacities and sizes. The bigger goblets hold 360 medium straws, the small goblets 100 straws and even smaller 25 straws. Polythene goblets are suitable than aluminium materials.
iii. The goblets containing frozen semen are put in canisters under LN_2. A thick paper chit showing the date of production, Donor NBIN, breed, No. of straws / doses, and the PTM% is placed in the goblet along with the straws. Another paper chit with the same information as above is attached on the hooked handle of the canister for future identification and reference.
iv. Mini straws need special care and should not be exposed above LN_2 even for a short time (10 seconds) as they get warm faster and any exposure causes irreversible damage to sperm viability.
v. Before distribution the frozen semen should undergo a quarantine period of one month during which microbial load is studied in the batch of semen using standard procedure. During the quarantine period all semen which does not fulfil the required standard should be discarded.
vi. To avoid accidental spread of diseases, the semen processing laboratory should follow the procedure of preserving semen doses for at least 30 days after production.
vii. Semen doses produced before 30 days and which have passed the microbial load tests should only be supplied for field use.
viii. After each dispatch, records redefining the position of remaining doses should be updated.
ix. LN_2 should be replenished at regular intervals (weekly) depending on the LN_2 evaporation rate of the Containers / Semen Tanks.

Information management

i. The Laboratory should install suitable software so that manual data entry can be avoided.
ii. The following records and information should be precisely maintained; Volume of semen, motility, sperm concentration, dilution rate, total extended volume, post-thaw motility (24 hrs), total number of doses produced etc
iii. 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 should be maintained.

iv. Details of semen supplied to AI centres, Government Nucleus Farms, Regional Livestock development Centres (RLDCs) and other agencies including PTM at the time of dispatch should be maintained.
v. Fertility data of bulls, conception rate, records of the progeny associated with any genetic defect, percent male / female born, etc should be also kept.
vi. Report on microbiological examination of semen samples should be maintained.
vii. Records of all quality tests for neat and frozen semen samples should be documented.

Quality Checks for frozen semen

i. Quarterly testing of random samples from each semen straw batch for bacterial load using standard plate count should be carried out. The standards for acceptable colony forming unit (CFU) in processed semen is 5000 CFU per ml as per OIE norm.
ii. If the bacterial load exceeds the OIE limit, the semen doses should be discarded.
iii. 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.
iv. Hypo osmotic swelling test (HOST) for all bulls at least once in a quarter is mandatory.
v. Incubation test for all bulls at least once in a quarter is mandatory.
vi. Acrosome integrity test by Giemsa staining for all bulls at least once in a quarter should be mandatory. Alternatively, wet smear of semen should be examined using DIC microscope.
vii. Percent Intact Acrosome of all bulls should be done once a quarter.
viii. Quality checking of semen straws, drawn randomly from the long storage containers once a year, should be done as a part of quality assurance.
ix. A summary of quality tests to be conducted for frozen semen and their cut-off values are given in the Table 8

Table 8: Quality control parameters and cut off values

Sl #	QC Parameters	Cut- off Values
1	Bacterial Load (FSD)	5000 CFUs /ml
2	Hypo Osmotic Swelling Test (HOST)	≥ 40%
3	Incubation / Thermo resistance Test	Standard drop in motility by 10% after every 30 minutes
4	Acrosome Integrity (Fresh Semen)	≥ 70%
5	Percent Intact Acrosome (PIA)	≥ 65 %
6	Sperm Concentration	20 million spermatozoa per dose (0.25 ml French Mini straw)

Maintenance of Laboratory Equipment

i. The exteriors of all equipment and furniture should be cleaned weekly.
ii. The equipment should be kept covered by plastic covers when not in use.
iii. The pre filter of laminar unit should be cleaned weekly. Routine servicing and DOP testing twice a year will ensure efficiency of HEPA filters. Alternatively culture plate test to assess bacterial load of the filter should be carried out.
iv. Spectrophotometer should be recalibrated with Haemocytometer readings for sperm concentration once a year. Validation of spectrophotometer should be done once a year based on at least 10 samples.

v. The automatic semen straw filling and sealing machine should be thoroughly cleaned immediately after use.
vi. The microscope lens should be gently cleaned daily with a piece of cotton soaked in a mixture of ethyl and methyl alcohol (1:1).
vii. The Bio Freezer should be defrosted and thoroughly cleaned and dried immediately after use.
viii. Incubators to maintain AV should be cleaned and disinfected monthly with 70% alcohol.
ix. Single distilled water should be used in autoclave and thermo controlled water bath. The water bath should be cleaned and filled with single distilled water on a regular basis. The thermometer kept immersed in water bath should be cleaned weekly to have precise temperature reading.
x. The LN_2 Containers returning / received from foreign countries and other contagious disease prone areas should be disinfected thoroughly with 4% soda solution and finally with 1 to 4% formaldehyde. Excess LN_2 available as a result of filling the containers with canisters and goblets, should never be brought inside the laboratory.
xi. There should be two refrigerators, one for storing diluents, antibiotics and buffer and the other outside the laboratory for storing vaccines and other materials.
xii. The refrigerators should be sterilized every week using alcohol swab.

Diluents

i. All disposable and reusable supplies coming in contact with semen and diluents must be sterile and devoid of toxins and pyrogen.
ii. 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.
iii. Glass wares, collection tubes, etc. should not be handled from their rim / mouth.
iv. Pipetting should be done away with, instead adjustable micropipettes and disposable tips should be used.
v. After adding all the components of diluents in double, preferably triple distilled water, it should be again sterilized. If buffer is prepared on the previous day and stored in the refrigerator then antibiotics are to be added on the next day morning after warming it at 34°C.
vi. Antibiotics in diluents: Combination of antibiotics, in diluents, which can control Mycoplasma like Tylosin, Lincospectin and Gentamycin may be used. Alternatively, a combination of Penicillin and Streptomycin should be used.
vii. Only fresh semen extender/diluents should be used because changes in the pH of stored extender are considered to be responsible for the deterioration of some nutrient components.

Personnel Hygiene

i. Clothing, skin and hair of laboratory personnel / visitors are sources of the contamination. Hence not only the personnel but also visitors should wear laboratory aprons and footwear all the time while they are in the laboratory.
ii. Hands should be washed with soap and water and rinsed with 70% alcohol, before commencing work in the laboratory.
iii. All personnel working in the semen processing laboratory must undergo test for TB every year.

Cleaning and Sterilization of Laboratory Equipment

i. All the items to be washed should be initially cleaned with running tap water and soaked in warm neutral detergent for at least 30 minutes.
ii. These items will then be thoroughly cleaned under running tap water using a brush.
iii. Filling nozzles should be cleaned with pressure using 20 ml syringe. These materials should be rinsed thoroughly with de ionised water (5 to 7 changes) to completely remove detergent residues and other impurities.
iv. Procedure for sterilization of different materials used in the laboratory is annexed in Annexure 7.

Quality Control of Consumables

The Laboratory should avoid purchase of consumables on lowest quotation basis. To produce top quality semen, it is better to use Analytical Reagent (AR) or Guaranteed Reagent (GR) reagents manufactured by reputed companies whose products are reliable. This is true with other consumables also.

Chemicals

i. Chemicals of only highest purity of either, AR or GR, from reputed manufacturing companies should be used.
ii. 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.
iii. Assay of chemicals should be >99%, having less impurities.

Straws

i. Straws manufactured by reputed companies are safer to use for production of quality semen.
ii. While buying straws package volume and microbial load in straws should be checked randomly from the consignment.
iii. 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.
iv. The factory plug should not be loose. The factory seal should be impenetrable and the seal formed should be homogeneous and compact.
v. The straws should be intact (without cracks / dents etc.) during and after freezing / thawing.
vi. The movement of straws along the printing machine should be free and print should be clear and sharp. The Print should not fade as a result of freezing and subsequent thawing.
vii. The use of dark coloured straws should be avoided as they are not transparent enough. Not only is filling / racking inconvenient, it is also difficult to distinguish between filled / semi-filled straws.
viii. Movement of the factory plug should be free.
ix. Straws should be routinely checked for microbial load.

Human Resource

i. The Centre should review the manpower position in the semen processing laboratory and propose for recruitment of additional manpower wherever it is necessary.
ii. After recruitment, the personnel should be trained in semen production technology at recognized institutes.
iii. Once trained, they should continue to work in the Laboratory for minimum of five years.
iv. Refresher training / technical interaction of personnel working in the semen lab should be arranged compulsorily once in three years in recognized institutions.
v. Semen production work is highly technical thus frequent transfer of personnel would be detrimental in maintaining the quality of semen.
vi. Before initiating any transfers, it is essential that a proper replacement is identified at least a year in advance so that he / she is trained and posted in the laboratory to learn the skills. Only then the semen station personnel should be transferred.
vii. The Human Resource requirement at the Semen Processing Laboratory is summarized in the Table 9.
viii. The manpower structure suggested above is meant only for semen processing laboratory. For other activities, manpower may be positioned as per the need.

Table 9: Available human resource

Sl #	Designation	No. of Personnel
1	Sector Head / VO / QCO	1
2	Laboratory Officer	2
3	Lab Technician	1
4	Lab Assistant	1
5	Lab Helper / Semen Collector	1
6	Bull Attendant	1 person per 7- 8 bulls

Bio-security

i. The risk of disease spread grows manifold with increasing number of donor bulls maintained for frozen semen production.
ii. With the expected higher risk, implementation of strict bio security measures at the laboratory assumes greater significance.
iii. The laboratory should have a well defined Bio security measures put in place across all its activities.
iv. The motorable road access to laboratory should have tyre wash which should be filled with 4% solution of washing soda (Sodium Carbonate). The size of tyre wash should be at least 8.5 metres long, 3.5 metres wide and 20 cm deep to ensure dipping of rear tyre of tractor. This is required to avoid entry of viral diseases into cattle sheds.
v. At the place of entry to Laboratory, a footbath and hand wash should be provided.
vi. The footbath should have 5% Potassium Per magnate or 4% soda solution.
vii. The Hand wash should be practiced using hand wash spirits or 70% alcohol.
viii. All the staff as well as visitors to Laboratory should use foot bath as well as hand wash before entering.
ix. All the staff and visitors to Laboratory should use bio security gears like lab coat, disposable cap, Mouth masks and disposable shoe covers before entering the Laboratory.

x. The bull sheds as well as pathways should be sprayed with 4% soda solution at monthly intervals to reduce chances of viral diseases.

xi. Bio security sign boards / notice must be displayed at the entrance to Laboratory premises.

xii. The semen processing laboratory and its complex / premises should have proper fencing and gates to prevent / restrict entry of visitors / stray animals.

Summary flow chart for frozen semen processing

1. Preparation of Diluents in the Laminar Air Flow Unit
- 100 ml of BIOXCEL diluents in 400 ml DD H_2O
- Diluted BIOXCEL kept in water bath (37^0C)
- Add Oxy-free II

↓

2. Semen collection in AV

↓

3. Transferred in Dry bath (37^0C)

↓

4. Evaluation of semen
- Macroscopic examination (volume, colour, consistency)
- Microscopic examination (MA, IM, Live &dead count, sperm concentration, abnormal sperm %)

↓

5. Evaluated / Suitable semen diluted with BIOXCEL at 37^0C

↓

6. Auto Filling & sealing of semen straws at room temp (21^0C)

↓

7. Gradual cooling from 21 to 4^0C (4 - 5 hours) in Cooling Chamber

↓

8. Vapour freezing (–85^0C for 8 minutes) in Bio Auto Freezer

↓

9. Plunging in LN_2 (–196^0C)

↓

10. PTM inspection

↓

11. Storage in Semen Bank

Annexure 1: Definitions used in the Health Protocol	
Bull	Adult male cattle used for collection of semen. Teasers and other animals in the semen stations are also subjected to similar disease testing, vaccination and medications for maintaining their health status.
Bull Calf	Male cattle 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.
MSP diseases	MSP diseases are the set of diseases, the causative organism of which should not be present in the semen or preferably in the bull. These diseases include Bovine Brucellosis, Tuberculosis (TB), Paratuberculosis (JD), Bovine Genital Campylobacteriosis, Trichomoniasis and Foot and Mouth Disease (FMD).
Quarantine station	A farm where bulls or bull calves are isolated and examined to assess the health status before shifting to the donor bull rearing area. A series of clinical and laboratory examinations, vaccinations and medications etc. are undertaken during quarantine.
Rearing station	A Unit where bull calves or young bulls, 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 bull calves in the rearing station to maintain their health status.
Semen station	A Unit along with semen processing facilities where adult bulls are housed for semen collection and processing. A series of clinical and laboratory examinations, vaccinations and medications etc. are undertaken during the stay of bulls in the semen station to maintain their health status.
Unknown health status	Animals originating from Govt. Cattle Farms or village where all the animals of the farm or the village have not been tested against the MSP diseases

Annexure 2: Quarantine Guidelines for Semen Donors

1. Quarantine of adult bulls of unknown health status		
Quarantine period	Minimum 60 days or long enough to allow at least two tests for MSP diseases to be performed during quarantine with a minimum interval of 30 days between the two tests. In case of TB & JD, interval between two tests should not be less than 62 days	
Shifting of bulls from the quarantine	Within 30 days from the date when the last test was performed and all bulls were found negative	
Action on finding a positive result	Brucellosis, TB, JD, Bovine Genital Campylobacteriosis, Trichomoniasis	Cull / remove the positive bull and put all the remaining bulls under extended quarantine
Extended quarantine	For a period of minimum 60 days or long enough to allow at least two tests for the diseases mentioned above to be performed, from the day last positive bull was culled / removed. Perform one test within the last 30 days of the extended quarantine.	
Action on finding a positive during extended quarantine	During Quarantine, if the bulls are housed and managed Individually: Remove only the positive bulls In groups (not more than 3 animals in each group): Remove all bulls in the group in which positive was detected Free and not in groups: Remove all the bulls	

2. Quarantine of adult bulls of known health status	
Quarantine period	Minimum 30 days or long enough to allow at least one test for all MSP diseases
Shifting of bulls from the quarantine	Within 30 days of the last negative test
Action on finding a positive result	Same as in 1 (above)
Extended quarantine	For a period of minimum 30 days from the day last positive bull was culled / removed. Perform one test within the last 30 days of the extended quarantine.
Action on finding a positive during extended quarantine	Same as in 1 (above)

3. Quarantine of adult bulls procured from Government Cattle Farms	
Quarantine period	Minimum 30 days or sufficient to allow at least one test for MSP diseases
Shifting of bulls from the quarantine	Within 30 days of the last negative test
Action on finding positive result	Same as in 1 (above)
Extended quarantine	For a period of 30 days from the day last positive bull was culled / removed. Perform one test within the last 30 days of the extended quarantine
Action on finding a positive during extended quarantine	Same as in 1 (above)

4. Quarantine of calves above 2 months of age		
Quarantine period	Same as in 1 (above)	
Shifting of calves from quarantine	Within 30 days of negative results	
Action taken on finding positive calf	TB, JD	Remove the positive calf and put all the remaining calves under extended quarantine
	Bovine Genital Campylobacteriosis & Trichomoniasis	Tests conducted only on calves older than 6 months Remove positive calf and put all remaining calves under extended quarantine
	Brucellosis	Remove +ve calf irrespective of age & put all remaining calves under extended quarantine OR If +ve calf is < 9 months old, isolate the calf till it is 9 month old and retest. Calf positive at retesting should be removed
Extended quarantine	For a period of minimum 60 days from the day last positive calf was removed. Perform one test within the last 30 days of the extended quarantine.	
Action on finding a positive during extended quarantine	Same as in 1 (above)	

Annexure 3: Feeding Management of Semen Donor Bulls

The management of feed and fodder for donor bulls is not as complicated as that of dairy cattle if the requirements for maintenance is adequately met. However the regularity and consistency in the quality is an important factor. Scientific feeding schedule shall be followed for the donor bulls. A general guideline on requirement of Feed, Fodder, mineral supplements, water etc for growing and adult donor bulls is detailed below;

Fodder

i. The bulls being ruminants require fodder of good quality and in adequate quantity for their maintenance. It is estimated that a young growing bulls requires 2.5 to 3.0 % of body weight as dry matter (DM) per day and 10 to 12 % crude protein (CP) with 65% total digestible nutrients (TDN), Yearling bulls need DM 2.0 to 3.0 % of the body weight per day while Adult bull DM intake is 1.2 to1.4 % of body weight per day. The DM requirements includes concentrate feed (compounded cattle feed).

ii. The quality of fodder of any crop varies considerably according to age of crop, soil conditions, irrigation etc. Hence proximate analysis of different fodder crops cultivated to determine nutrient quality and optimum time of harvest of fodder is recommended.

iii. Except for very tender and succulent fodders, most fodders should be chaffed before feeding. The blades of the chaff cutter should be adjusted to ensure the size of the cut fodder is at least 50 mm. Too small size of fodder is not recommended. The dry fodder (Hays / Straws) should be chaffed preferably along with green fodder. The dry fodder can also be fed separately.

iv. Feeding of good quality hay is recommended instead of feeding paddy straw or maize. The hay is more nutritious and palatable. Wherever it is possible to feed large quantity of hay, the green fodder can be reduced to 5 to 10 kg / bull / day.

v. Feeding of silage during lean seasons @ 10 to 15 Kg. / day / bull is recommended. During silage preparation, adequate precautions should be taken to avoid growth of molds and aerobic condition.

vi. Routine quality analysis of feed and fodder for arriving at a balanced ration should be carried out.

Feed

i. To balance the nutrient requirements, it is essential to include cattle concentrate feed.

ii. The Concentrate feed should be purchased from a reputed manufacturer using good quality ingredients free from toxic elements, moulds and fungi.

iii. The concentrate feed can be in the form of mash or pellets, however feeding of cotton seed cake should be preferably avoided.

iv. The concentrate feed should be supplied to bulls mixed with small quantity of green / dry fodder to stimulate salivary glands.

v. The proximate analysis of the feed at regular intervals should be done to ensure the nutrient quality of the feed.

vi. The requirements of nutrients for providing fodder and feed to the growing and adult donor bulls is detailed in Tables below.

Mineral mixture

i. Supplementary feeding of 30 to 100 gm of mineral mixture / day / bull is essential to meet the physiological requirements. 50 gm mineral mixture (bulls up to 200 kg), 70 gm mineral mixture (bulls 200 to 350 kg) and 100 gm mineral mixture (bulls above 350 kg) is recommended.
ii. The quantity varies upon the body weight of the bull and mineral profile of the soil used for fodder cultivation. If data on mineral profile of the area is available, it should be used for formulating appropriate mineral mixture.
iii. The ingredients of the mineral mixture should be free from animal origin. The mineral mixture without salt should be preferably purchased.
iv. Salt in the range of 10 to 15 gm / day / bull can be added to the mineral mixture.
v. The mineral mixture should be thoroughly mixed with concentrate feed to avoid wastage.

Potable water

i. Fresh Water is one of the most important component of nutrients and it should be made available all the time.
ii. The water should be clean, free from organic matter and potable. The water should be under shade and accessible all the time.
iii. If River water / pond water should be used, adequate treatment to eliminate contaminants and biological material should be practiced.

Table 10: Summary of Summer & Winter feeding of Semen Donor bulls & ET Donor cows

	Donor bulls		Thrabum ET Donors		JerseyX ET Donors	
Particulars	Summer	Winter	Summer	Winter	Summer	Winter
Green grass (Kg)	20 - 25		21		19	
Silage (Kg)		10		10		7
Hay (Kg)	1	2	1	1.5	1	2
Paddy straw (kg)	0	1.5	0	1.5	0	1.5
Concentrate (Kg)	2.75	2.75	2.1	2.45	2.1	2.45
Salt (Kg)	0.03	0.03	0.03	0.03	0.03	0.03

Table 11: Compound cattle feeding for cattle

Compound cattle feed	**Cow (300 - 400 kg)**	**Cow (400 - 500 kg)**	**Young bulls (200 – 500 kg)**	**Adult bulls (500 – 700 kg)**
For Maintenance	2.0 kg	2.5 – 3.0 kg	2.5 – 3.0 kg	2.5 – 3.0 kg
For Milk production (per litre)	400 gm	400 gm		
For Pregnancy (last two months)	2.0 kg	3.0 kg		

If 15 to 20 kg good quality cultivated green fodder is available for feeding cows, then compound feed for body maintenance need not be given. Donor bulls should be given good quality roughage with sufficient concentrates. Too much roughage feeding should be avoided as it makes the bull paunchy and slow in donating semen. In addition, large concentrate allowance may make the bull too much fatty and less virile.

Table 12: Daily Nutrient requirements of growing and mature donor bulls

Body wt (kg)	gain/day (g)	DM/day(kg)	CP (g)	TDN (kg)	Ca (g)	P (g)	Vit A (1000 IU)
i. Growing bulls							
100	750	2.8	390	1.9	11	8	4
150	750	4.3	460	2.7	15	11	6
200	750	5.7	530	3.4	18	14	8
250	750	7	610	4	21	16	10
300	750	8.2	680	4.6	23	17	13
350	750	9.3	760	5.2	24	18	15
400	700	10.2	820	5.7	25	19	17
450	600	10.4	875	5.8	26	20	19
500	400	10	885	5.6	26	20	21
550	250	10	845	5.6	25	19	23
600	100	9.8	800	5.5	24	18	26
ii. Maintenance of mature donor bulls							
500	-	8.3	640	4.6	20	15	21
600	-	9.6	735	5.4	22	17	26
700	-	10.9	830	6.1	25	19	30

Table 13: Daily Ration for Semen Donor Bulls

Body wt (kg)		Calf starter (kg)	CF (kg)	BPF (kg)	Hay (kg)	Green Fodder(kg)
i. Growing bulls						
100		2	-	-	0.5	6-8
150		-	-	2	0	8-10
200		-	-	2	0.5	15
300		-		2	1	ad lib.
400	a)	-		2	3	ad lib.
	b)	-	2.5	-	3	ad lib.
500 - 600	a)	-	-	2.5	2-4	ad lib.
	b)	-	3	-	2-4	ad lib.
ii. Mature Donor bulls						
500 - 700	a)	-	2.5	-	2-4	ad lib.
	b)	-	-	2	2-4	ad lib.

*BPF: Bypass Protein Feed

Table 14: Nutrients available in feed & fodder

Particulars	Calf starter	CF	BPF	Green fodder	Hay
DM %	90	90	90	20-25	90
CP %	22-23	18-19	22-23	5-6	5-6
TDN %	70	62-64	65-68	55-60	55

Annexure 4: Disease screening and health management of Donor bulls

Bovine Tuberculosis

Screening Test	Delayed Hypersensitivity: Single Intradermal (SID) test
Reagent	Bovine tuberculin PPD
Testing at	On site, where animals are housed
Result criteria	As per *OIE* norms **Positive:** Increase in skin thickness 4 mm or more, or presence of clinical signs viz. exudation, necrosis, pain, inflammation of lymphatic duct of that region or lymph node, 72 h post inoculation **Negative:** Increase in skin thickness < 2 mm & without clinical signs as listed above **Inconclusive:** Increase in skin thickness > 2mm & less than 4mm, absence of above clinical signs, 72 h post inoculation. Bull with inconclusive result should be immediately isolated. Only if the animal is -ve during the testing in isolation, it should be brought back to the station
Eligible animals	All animals above 2 months of age
Frequency of testing	Positive herd: Minimum 60 days after culling of last +ve animals Negative herd: 6 Monthly (± 1 week) after last whole herd -ve testing
Action on finding positive bull	Animal: Immediate isolation & removal from the herd (within 2 days) Semen: Destroy frozen semen doses of +ve animals since the last -ve test
Tuberculosis free herd (OIE)	Herd found -ve on 2 consecutive tuberculin tests at an interval of 6 months, 1st test performed 6 months after culling of last affected animal
Quarantine	Duration: Minimum 90 days. Test schedule: Two tuberculin tests, minimum interval of 60 days between tests

Johne's Disease (JD) or Paratuberculosis

Screening test	Delayed Hypersensitivity SID test (Skin test)
Reagent	Johnin PPD
Testing at	On site, where animals are housed
Result criteria	**Positive:** Increase in skin thickness of 4mm or more (discrete circumscribed swelling) with clinical signs as in 1., 72 h post inoculation **Negative:** Increase in skin thickness < 2 mm & without clinical signs as listed above **Inconclusive:** Increase in skin thickness > 2mm & less than 4mm, absence of above clinical signs, 72 h post inoculation. Bull with inconclusive result should be immediately isolated. Only if animal is -ve during the testing in isolation, it should be brought back to the station
Eligible animals	All animals above 2 months of age
Frequency of testing	Positive herd: Minimum 60 days after culling of last +ve animal Negative herd: Six monthly (± 1 week) after last whole herd -ve testing

Action on finding positive bull	Animal: Immediate isolation and remove from herd (within 2 days) Semen : Destroy frozen semen doses of +ve animals since last -ve test
JD negative herd	Herd found -ve on two consecutive Johnin tests carried out at an interval of 6 months, the first being performed 6 months after culling of the last affected animal. If frequency of testing is > 2 in a year, the testing should establish that all animals in the herd have been -ve for the last 6 months beginning from 6 months after culling the last affected animal.
Quarantine	Duration: Minimum 90 days Test schedule: Two Johnin tests, min interval of 60 days between tests

Bovine Brucellosis	
Screening Test	ELISA, RBPT+CFT
Sample	Serum
Eligible animals	All above 1 year, In females 14 days after calving or abortion
Frequency of testing	Positive herd: 30 - 60 days after culling of last +ve animal Negative herd: Six Monthly after last whole herd -ve testing Negative herd (optional): Where disease maintains a very low profile (< 1 % +ve) quarterly / 6 monthly sample collected to minimize losses
Action on finding positive bull	Animal: Immediate isolation and remove from herd after castration (within 2 day). Semen: Destroy frozen semen doses of +ve animal since last -ve test
Brucellosis free herd (OIE)	Herd found -ve on two consecutive annual tests
Quarantine	Duration: Minimum 30 days Test schedule: Two tests, Serum ELISA, interval of 30 days between tests. Only -ve animals to be allowed to mix with the rest of the herd
Additional testing at sexual maturity	Serum ELISA before bulls are used for semen collection and distribution for AI

Infectious Bovine Rhinotracheitis (IBR)	
Screening test	ELISA / Real time PCR
Sample	Serum
Eligible animals	All animals
Frequency of testing	Positive Herd: Whole herd test, 30 - 60 days after culling of +ve animals. Six monthly, after the herd become -ve. Negative Herd: Exactly 1 year (± 1 week) after last whole herd testing Where disease has been maintaining a low profile (< 5 % positive) quarterly or six monthly sample could be collected to minimize losses
Action on finding positive bull	Immediately isolate and remove from herd after castration (within 2 days)
IBR free herd (OIE)	Whole herd tested negative on two consecutive occasions at an interval of 2 to 12 months between tests
Quarantine	Duration: Minimum 30 days Testing: Two tests, Serum ELISA, interval of 21 days between tests

Additional testing at sexual maturity	Serum ELISA before bulls are used for semen distribution in field AI program

Bovine Viral Diarrhoea (BVD)

Screening test	Virus isolation, PCR, ELISA
Sample	Blood
Eligible animals	All animals
Frequency of testing	Positive herd: As per OIE norms Negative herd: Exactly 1 year (± 1 week) after last whole herd -ve testing
Action on finding positive bull	Animal: Immediately isolate and remove from herd Semen: Destroy by incineration frozen semen doses of +ve animal since last –ve test
Quarantine	Duration: Minimum 30 days Testing schedule: As per OIE norms
Additional testing at sexual maturity	Virus isolation before bulls used for semen distribution to AI programme

Bovine Genital Campylobacteriosis (BGC)

Screening test	Bacterial isolation & identification
Sample	Preputial washing / semen
Eligible animals	All animals above 6 months of age
Prevention	Annual sheath lavage
Frequency of testing	Positive herd: 30 days after treatment / culling of +ve animals Negative herd: Annual (± 1 week) after last whole herd -ve testing
Action on finding a positive bull	Animal: Immediately isolate & remove from herd (within 2 days) Semen: Destroy semen doses of +ve animal since last -ve test
Quarantine	Duration: Minimum 30 days Test schedule: One tests if age is less than 6 months, else 3 consecutive tests at weekly intervals.
Bovine Genital Campylobacteriosis free herd	All animals are negative on two consecutive annual testing

Bovine Trichomoniasis

Screening test	Agent isolation & identification
Sample	Prepucial washing
Eligible animals	All male animals above 6 months of age
Prevention	Annual sheath lavage
Frequency of testing	Positive: Min 0 days after treatment / culling of +ve animals Negative: Annual (± 1 week) after last whole herd -ve testing
Action on finding positive animal	Animal: Immediately isolate & remove from herd (within 2 days) Semen: Destroy frozen semen of +ve animal since last –ve test
Quarantine	Duration: Minimum 30 days. Test schedule: One test, if age is less than 6 months, else 3 consecutive tests at weekly intervals
Additional testing at sexual maturity	Protozoa isolation before bulls are used for semen distribution for AI
Trichomoniasis free herd	All animals are -ve to two consecutive annual testing

Foot and Mouth disease (FMD) outbreak management

Immediate action to be taken	Immediate disinfection of premises and fomites Destruction of contaminated feed & fodder by burning
Frozen semen doses of FMD infected animal	Destroy frozen semen collected from infected animal up to one month prior to onset of outbreak
Action to be taken on FMD infected animal	Isolate the affected bull immediately. Affected bull is treated and rested for 90 days after recovery from clinical symptoms No semen collection from any infected animal during the infection and up to 3 months after last case has recovered in the farm
Animals in the farm not affected by FMD	No semen collection from healthy bulls during the outbreak and no semen collection up to one month after the last case have recovered
Semen distribution	If frozen semen is from the same area where FMD is recorded, suspend semen distribution till 30 days after last case has recovered
FMD outbreak in areas surrounding the Semen Processing Laboratory	
Ring vaccination	Arrange immediate ring vaccination within a radius of 10 Km around the focus of infection starting from the perimeter towards the focus
Disinfection	Disinfection of the roadsides adjacent to the farm on a daily basis
Movement of fodder	Stop all fodder movement through areas of infection
Animal movement	Stop animal movement of farm animals through areas of infection

Table 15: Summary of the Tests to be conducted on Donor bulls

Sl #	Disease	Test	Sample
1	Brucellosis	ELISA	Serum
2	TB	DTH-Tuberculin PPD	Intra-dermal on the bull
3	JD	DTH- Johnin PPD	Intra-dermal on the bull
4	Trichomoniasis	Agent identification	Prepucial washings/ semen
5	Bovine Genital Campylobacteriosis	Agent identification	Prepucial washings
6	FMD	ELISA	Serum

Annexure 5: Detail Abnormalities of Bull Spermatozoa

1. Morphology of Normal Spermatozoa

i. The Spermatozoa consists of Head, Neck and Tail.

ii. The shape of a normal sperm in most domestic animals is flattened and ovoid. The major portion of the Head consists of Nucleus surrounded by a thin layer of cytoplasm. The anterior half of Head is enveloped by Acrosome. The posterior head cap or post acrosomal sheath encloses the posterior half.

iii. A definite transverse line, called Equatorial segment, separates the two head caps from each other.

iv. The Neck is very short and furnishes the connection between the Head and the mid piece.

v. The Tail consists of mid piece, Principal piece and End piece.

2. Major abnormalities:

The major abnormalities of bull spermatozoa are as below;

i. **Proximal Protoplasmic droplets:** Small spherical masses of cytoplasm 2-3 microns in diameter commonly found on the tails of small percentage of ejaculated spermatozoa. The droplets are found in either of the two positions on the mid piece, surrounding the neck and proximal mid pieces region (Proximal droplet), or surrounding the mid piece just proximal to the annulus (Distal droplets). With eosin nigrosin stain, the droplets usually appear as shriveled white, opaque retractile body.

ii. **Pyriform / Tapered head:** A classical pyriform head is distinctly pear shaped; the acrosomal region is full and rounded, while the post acrosomal regional is narrow. Tapered head shape differs from pyriform shape in that the entire nucleus appears narrow in both the acrosomal and post acrosomal region. Tapered head often appear to be smaller than normal or elongated.

iii. **Nuclear vacuoles (Pouches, Creaters, Diadem):** A narrowed mouthed invagination of the nuclear membrane into the nucleoplasm nuclear pouches. A sparkling, round or elongated white spots predominantly in the form of string of a beads like a necklace at the level of acrosome - post acrosomal sheath junction **(Diadem defect)**. On differential interference contrast microscopy, this defect appears as surface-oriented crater quite uniform in size about 0.6 microns in diameter **(Creaters)**. **Vacuoles** are seen as clear sports or holes through the nucleus.

iv. **Knobbed Acrosomes/Acrosome Defects:** In ram semen, Knobbed Acrosomes resembles a knob or projection from the apex of the spermatozoa. The most typical appearance of this abnormality in bull semen is a flattening or indentation of the apex of the Acrosome. Knobbed Acrosome defect is described as a refractile or dark staining area or eccentric thickening at the apex of affected spermatozoa. A few cells do show a small bead like protrusion at the sperm apex.

v. **Micro cephalic / Macro cephalic heads / Giant Head:** Micro cephalic denote any sperm head that, when compared to the normal population of sperm cells in a smear, is clearly smaller than normal. Macro cephalic / giant head denotes any sperm head that is clearly larger than the heads of normal population of sperm cells.

vi. **Deformities of Mid piece:** It denotes bend or folded mid piece. A bend in the distal region of the mid piece in the form of letter J or there may be a second bend in the opposite direction above the first bend.

vii. **Dag defect**: Folding or coiling of the mid piece with the axis of the main fold in the distal half of the mid piece. The major feature of this defect is fracturing or shattering of axonemal elements at any point in the mid piece associated with disrupted arrangement of mitochondrial sheath. In Mid piece defect, mitochondrial sheath is smooth & complete as opposed to the rough disrupted mitochondrial distribution seen in Dag defect.

viii. **Under developed double form**: These are defective spermatozoa with either double heads or double mid piece or double tails.

Minor abnormalities

i. **Distal Protoplasmic droplet:** Small spherical masses of cytoplasm 2-3 microns in diameter are commonly found on the tails of small percentage of ejaculated spermatozoa. The droplets are found in either of the two positions on the mid piece, surrounding the neck and proximal mid pieces region (Proximal droplet), or surrounding the mid piece just proximal to the annulus (distal droplets). With eosin nigrosin stain, the droplets usually appear as shriveled white, opaque retractile body.

ii. **Detached Head / Head Normal without Tail:** Spermatozoa with normal head but without tail

iii. **Bent/ coiled Tail:** Spermatozoa with tail that is bent or tightly coiled

iv. **Abaxial tail / Accessory tail:** Abaxial tail defect is abaxial attachment of sperm tail to the base of nucleus, where as Accessory tail defect is a short stump or structures coming from the base of the sperm head.

Annexure 6: Procedures on operation / use of different Equipments used in the laboratory

DIC (Differential Interference Contrast) Microscope

i. Switch on the DIC Microscope.
ii. Set the light intensity control to “3” position for required brightness.
iii. Slide the knob to “BF” Bright field position.
iv. Set the objective to 10 X for Mass Activity.
v. Set the objective to 20 X for Initial Motility.
vi. Place the sample on the glass slides under the microscope.
vii. Adjust the focus with the Focusing drive course.
viii. Fine adjust with the Focusing Drive fine.
ix. Examine the sample and grade the findings.
x. After evaluations turn off the microscope.
xi. Clean the microscope and cover it.

xii. For screen display on the laptop screen

- Connect the camera to the Laptop.
- Slide the knob of the DIC to camera view, “close eye”.
- Click on the “Zeiss” icon on the Laptop screen.
- Click on live processing.
- The image of the microscope will appear in the laptop screen.

Warm stage and warm plate

i. Switch on the Warm stage.
ii. Set the temperature to 37^{o}C by pressing the set button.
iii. Press up and down arrow to adjust temperature to 37^{o}C.
iv. Place clean glass slides and cover slip on the warm stage.
v. Place the warm plate on the microscope stage.

Semen Diluter

i. Switch on the Semen Diluter.
ii. Select "Size" for big syringe 5000 µl.
iii. Select "Size" for small syringe 100 µl.
iv. Set "Volume" of NaCl 0.9% for big syringe to 3960 µl by pressing the button (▼).
v. Set "Volume" of fresh semen for small syringe to 40 µl by pressing the button (▼).
vi. Set "Speed" for Big syringe at 5.
vii. Set "Speed" for Small syringe at 2.
viii. Dip the pipette of big syringe to beaker containing 0.9 % NaCl solution.
ix. Dip the pipette of small syringe to fresh semen with micropipette tip.
x. Press the hand button or Press run or (▲) button on the machine to draw in / remove the required volume of the samples.
xi. Samples are drawn in the big and small syringes.
xii. Press the hand button or (▼) button on the screen to draw the required volume of NaCl & semen sample into the Cuvette.
xiii. The Sample (inside the Cuvette) is ready for loading in the SDMS 5 machine.
xiv. After completing the above procedures, Press Prime button to rinse and clean the system.
xv. Turn off the switch.

Spectrophotometer SDMS5

i. Turn on the spectrophotometer (SDNS5) at least 30 min prior to commencement of semen evaluation.
ii. Enter Date and press OK.
iii. Select Method 8 (Bull + Calculation) and press OK.
iv. Place a fresh Cuvette filled with 4 ml of 0.9% Nacl in the photometer.
v. Calibrate to 0 measurement and press OK.
vi. Enter Bull ID and press OK.
vii. Enter Ejaculation Volume of Semen in ml and press OK.
viii. Enter IM % and press OK.
ix. Enter million of Spermatozoa / dose (20 million) for 0.25 ml straw.
x. Place thoroughly mixed / diluted semen sample corvette in the photometer.
xi. Read spectrophotometer at the ratio of 1: 200 (40 µl semen: 3960 µl NaCl).
xii. Press M for measuring. The analysis takes about 30 seconds.
xiii. Press OK for printing the result.
xiv. The number of doses of frozen semen and the final extender volume to be added to the neat semen will be calculated.
xv. Add the diluents/extender as per the result.
xvi. A fresh cuvette is used for each sample / ejaculate.
xvii. Photometer SDM5 is used to determine the number of doses that can be produced from an ejaculate and automatic calculation of extender volume required.

Straw Printer Easy Coder 2

i. Switch on the Straw Printer.
ii. Put on the computer.
iii. Select the Easy coder Icon on the desktop.
iv. Select set print Format.
v. Enter straw information like Breed, Donor NBIN, Production Date, Centre & Location, and Country of origin.
vi. Name and save the print format as Jersey/ Mithun / Nublang / B Swiss.
vii. Selecting the Donor bull ID will give the details saved previously.
viii. Set the spacing if all information is clear on the straw.
ix. Enter the number of Straws to be printed.
x. Press Start Print to Sample print 2 to 3 straws
xi. Load the Straws to the Straw Printer.
xii. Press F1 on the Straw Printer.
xiii. The straw printing will commence and print the straws as per command.
xiv. When all straws are printed, click "Complete Printing job" on the computer screen.
xv. Click on Program and Close to exit.
xvi. Switch of the Straw Printer and the Computer.

Cool Handling Cabinet

i. Switch on the Cool Handling Cabinet about 20 to 30 minutes after loading the filled / sealed semen straws inside the Cool Handling Cabinet.
ii. Set the temperature at 3 stages (one time setting).
    a. Room temperature 25^{o}C to 10^{o}C for 2 hours.
    b. 10^{o}C to 4^{o}C for 4 hours.
    c. 4^{o}C for 1 hour.
iii. The above setting is done only once a year.
iv. Switch off the machine after use.

Auto Straw Filling and Sealing Machine

i. Attach Vacuum Tubing (long tube) and Suction Nozzle fixed with Rubber Washer (small rubber tubes) on the Rt side of the machine.
ii. Pull the Vacuum Tubing into the Clamping Range of the Suction Valve (Rt. Side).
iii. The above steps (i & ii) is on the Vacuum Suction part of the machine on the Rt side and is done once a week.
iv. Attach the Vacuum Tubing (long tube) with Filling Nozzle fixed with Rubber Washer on the Lt. side of the machine.
v. Pull the Vacuum Tubing into the Clamping Range of the Filling Valve (Lt Side).
vi. The above steps (iv & v) is on the Filling part of the machine on the Lt side and is done during each processing.
vii. Switch on the Auto Filling and Sealing Machine by Pressing green "O-I" switch.
viii. Set the machine vacuum pressure at 21.8 (displayed on the screen and pre set already).
ix. Load the printed straws in the Straw Hopper.
x. Connect the Filling Tubing to the Plastic Semen Cone containing extended semen.
xi. Press "Start" button indicated with blinking green light to fill / seal the straws.
xii. Open the Lever to release the straws from the Straw Hopper.
xiii. Straws will be automatically filled and sealed by ultrasonic sealing unit.
xiv. After completion of filling / sealing of the required semen straws; remove the Filling Nozzle and Rubber Tubing from the machine.

xv. Switch Off the machine and cover it with the plastic cover provided.
xvi. The Automatic Semen Straw Filling and Sealing Machine should be thoroughly cleaned immediately after use.

Bio Auto Freezer & Supply Tank

i. Switch on the Bio Auto Freezer.
ii. Open the Tap /valve (by rotating the tap) of the Supply Tank (200 litres) to release LN_2.
iii. Press / turn on the switch by turning the Red yellow device downwards.
iv. Set the Machine to -85^{o}C (pre set)
v. Turn on the fan.
vi. Turn the nozzle by turning it sideways.
vii. Wait till the temperature reach -85^{o}C which is preset.
viii. Transfer the equilibrated semen straw with the freezing racks from cooling chamber to the Bio Auto freezer.
ix. Vapor freeze the straws for 8 minutes at -85^{o}C.
x. After completion of Vapour freezing; Collect the straws and transfer in the pre cooled goblet.
xi. Plunge the goblet containing vapour frozen straws into the semen Storage Tank containing LN_2.
xii. Close the valve / tap of the LN_2 Supply tank.
xiii. Turn off the Auto Freezer by rotating the Red / Yellow switch upwards till it is positioned sideways / horizontal.
xiv. Switch off the machine.

Laminar Flow unit

i. Switch on the Laminar Flow Unit by pressing on “Mains”.
ii. Switch on the Fluorescence light by pressing “FL”.
iii. Prepare the diluents (100 ml BIOXCEL & 400 ml of DD water).
iv. Transfer to the conical flask containing the prepared Diluents into the Water Bath at 37 ^{o}C.
v. Switch off fluorescence light by pressing “FL”
vi. Switch on UV light for 10-15 minutes by pressing “UV”
vii. Switch off the unit by pressing “Mains”

Dry Bath

i. Switch on the Dry Water Bath.
ii. Set the temperature to 37^{o}C by pressing ▲ and ▼ button and pressing “set” when required temperature is reached.
iii. It takes 15 minutes to come to the set temperature.
iv. Dry Bath is ready for use.

Water Bath

i. Switch on the water Bath by pressing the big button.
ii. Set the temperature by pressing set button and rotate the temperature setting button to temperature at 37^{o}C.
iii. It takes 15 – 20 minutes to come to the set temperature.
iv. Water Bath is ready for use.

Distillation set

i. Connect water pipe from tap to the distillation set.
ii. Switch on the Unit.
iii. Connect the outlet pipe to the storage container to collect the distilled water.
iv. Pour the distilled water in conical flask, cover the flask with aluminum foil and keep in Sterilizer for sterilization.
v. Switch off the machine after use.

Autoclave

i. Switch on the Autoclave.
ii. Set the temperature to 105 ^{o}C by pressing ▲ and ▼ button and pressing "set" when required temperature is reached.
iii. Set the time (1 – 2 hrs).
iv. Put the required glass wares, distilled water, filling nozzle (needles) etc in the autoclave.
v. Tightly close the lid by turning the handle on top.
vi. Press start to run the program.
vii. After sterilization Switch off the Machine.

Hot Air Oven

i. Switch on the Hot Air Oven by pressing the Big Push Button.
ii. Set the temperature by pressing set button and rotate the temperature setting button to temperature at 70^{o}C.
iii. Hot Air Oven is ready for use.
iv. Press stop after using.
v. Switch off the Machine.

AV Cabinet

i. Switch on the Machine by pressing on the Main switch.
ii. Set the temperature to 40 ^{o}C by pressing ▲ and ▼ button and pressing "set" when required temperature is reached.
iii. Put the AV inside.

Annexure 7: Procedure for Sterilization of different materials used in the laboratory

Laboratory and other areas

Cold fumigation solution is ideal for fumigation of Laboratory and other areas. It should be done as per detailed below;

i. Materials like Fumigator (Aerosol formation disinfector) and Formaldehyde Solution (35 – 39 % w/v) are required for fumigation.
i. Review of the microbial count indicates the necessity of fumigation.
ii. Calculate the area in cubic meter which is to be fumigated.
iii. Calculate the formaldehyde solution using dosage 22 ml of 34 – 40 % w/v of the formaldehyde solution per M^3.
iv. Decide the no. of fumigators to be used (based on area).
v. Put total quantity of formaldehyde solution in the fumigator.
vi. See the doors and windows are closed and sealed properly.
vii. Switch off exhausting fans & other fans; switch all the fumigators from outside switch.
viii. Evaporate full formaldehyde solution and switch off fumigators after completing fumigation (about 2 h).
ix. Ensure that all the dampers of exhaust area, ducts are shut off during fumigation.
x. Switch on the exhaust fans / air handling systems after 2 hours of fumigation.
xi. Inspect the room for the absences of formalin fumes before entry in the fumigated rooms.
xii. Expose the settle plates and record the microbial count.
xiii. Review the microbial count so as to establish the effectiveness of fumigation.
xiv. Maintain a register to record observations on microbiological load in semen processing laboratory.

Artificial Vagina (AV)

i. Cleaning of AV is done immediately after use with non-spermicidal detergent and wetting agent. Soap is not recommended as it is difficult to rinse off besides harmful to sperms. Thus to achieve best cleaning effect, non-spermicidal neutral detergent should be used to clean AV immediately after use.
ii. Cone from the AV and water / air from AV jacket should be removed before washing.
iii. The dismantled rubber parts are cleaned first in cold water containing non-spermicidal detergent with a soft brush and then in warm water.
iv. The dismantled AV parts are then rinsed in distilled water.
v. Cleaned AV liners & cones are immersed in 70% alcohol for 5 minutes for sterilization. The above procedure requires dismantling/assembling of AV every time.
vi. For cleaning and sterilization without fully dismantling AV; after removing cone and collection vial / tube; the AV with liner intact and Cone are washed first in cold water containing non-spermicidal detergent with a soft brush and then in warm water. Alternatively, the AV with liner intact and Cone should 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 lastly rinsed three times with double distilled water.
vii. For sterilization, fully assembled AV should be autoclaved at 5 psi pressure for 20 minutes. Alternatively, Sterilization can be done in boiling water for 45 minutes or in steam for 20 minutes.

viii. During sterilization of AV, valves should be kept open. Alternatively, use AV sterilizer (using double distilled water in the sterilizer) for proper sterilization of AV.
ix. The cleaned & sterilized AV parts are covered with aluminum foils / plastic wraps and kept / stored overnight in incubator / warm cabinet / AV cabinet at 45^{o} C for next day's use.

Glass wares

i. The glass wares should be washed thoroughly with running tap water and soaked in warm, non-spermicidal neutral detergent solution for about 30 minutes.
ii. Use appropriate nylon brushes to clean the glass wares and rinsed with running tap water.
iii. The collection tubes should be brushed at least 3 times and thoroughly cleaned and rinsed with distilled water.
iv. Finally the glass wares should be rinsed three times with double distilled water and allowed to dry by keeping them inverted on a blotting paper.
v. The open ends of the dried glass wares should be covered with aluminum foil and sterilized in hot air oven at 160^{o} C for 1 hour or at 180^{o} C for 30 minutes.

Rubber wares

i. Washing and cleaning procedure of rubber wares is similar to that of glass wares.
ii. Care should be taken to clean the rubber wares with sponge brush instead of nylon brush.
iii. Plastic tips should be cleaned by water jet with force using a syringe.
iv. Sterilization technique, however, differs owing to the thermo-sensitivity of the rubber items.
v. Thermo-sensitive rubber wares should be packed and sealed in specific polythene bags and sterilized in Ethylene Oxide gas sterilizer.
vi. Thermo-resistant rubber wares should be sterilized by autoclaving at 3 - 4 psi for 10 minutes.
vii. The rubber tubing for semen filling should not be reused.

Distilled Water

i. Triple glass distilled water or Milli-Q purified water should be autoclaved at 15 psi for 15 minutes and used for preparation of the dilutor.
ii. The Triple glass distilled water or Milli-Q purified water stored for more than 7 days should not be used for diluents preparation.

Buffer, Bacteriological Media and Filter papers

i. Buffer should be sterilized by autoclaving at 5 psi pressure for 20 minutes.
ii. After autoclaving buffer should be cooled and then antibiotics added under laminar air flow unit and then it is stored in refrigerator.
iii. Biological media should be autoclaved at 15 psi pressure for 15 minutes.
iv. Bunch of clean filter papers of standard brand like Whatman No 1 (thrashed to remove dirt, if any) should be wrapped in thick cotton cloth for sterilization in an autoclave at 5 psi pressure for 20 minutes.

Summary of Sterilization

Autoclave

Sl #	Items	Pressure (psi)	Time (min.)
1.	Artificial Vagina	5	20
2.	Buffer	5	20
3.	Plastic Tips	5	20
4.	Filter Papers	5	20
5.	Bull 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

Hot Air Oven

Sl #	Items	Temperature	Time (min.)
1.	Glass wares	$160^{o}C/180^{o}C$	60/30
2.	Filling Nozzles	$160^{o}C/ 80^{o}C$	60/30

AV Sterilizer

If Autoclave is not used, AV and rubber cones should be sterilized using AV sterilizer. After sterilizer starts boiling, 30 minutes vapor sterilization should be done.

Ethylene Oxide Gas Sterilizer

The exposure time for gas sterilization is inversely proportional to the gas concentration under which the equipment is exposed in the sterilizer. Commonly used concentration of ethylene oxide is 900 mg per litre of cubic space for a period of six hours. All items sterilized with gas must be aired adequately before use. A minimum exposure of 72 hours at room temperature with adequate ventilation is recommended.

SEXED SEMEN: IS IT A REALITY IN BHUTAN?

What is sexed semen?

Semen having X or Y bearing sperm to produce progenies of a desired sex either female or male (with about 80-90% accuracy) is known as sexed semen. Sex sorting technology was developed by the United States Department of Agriculture (USDA) researchers in Livermore, California, and Beltsville, Maryland. The technology was patented as "Beltsville Sperm sexing technology". The commercialization of sexed semen started in United States in 2001 with a license granted to Sexing Technologies (ST), Texas. At present, ST commercially produces sex sorted semen in many countries of Europe, USA, Canada, Mexico, Brazil, China, Japan and other countries.

How Sexed Semen is produced?

- Among several methods for semen sexing, flow cytometry based sorting has emerged as most efficient with purity of more than 90%.
- X-chromosome (female) contains about 3.8% more DNA than the Y-chromosome (male) in cattle. This difference in DNA content is used to sort the X- from the Y- bearing sperm. Sperm sorting procedure is as follows:
- Dilute sperm to a very low concentration and stain them with a fluorescent dye and send through the flow-cytometer
- As sperm pass through the internal laser beam, larger X chromosome, emit slightly more light than Y male sperm
- Laser detectors measure the amount of fluorescence and assign positive or negative charges to each droplet containing a single sperm(Figure 1).

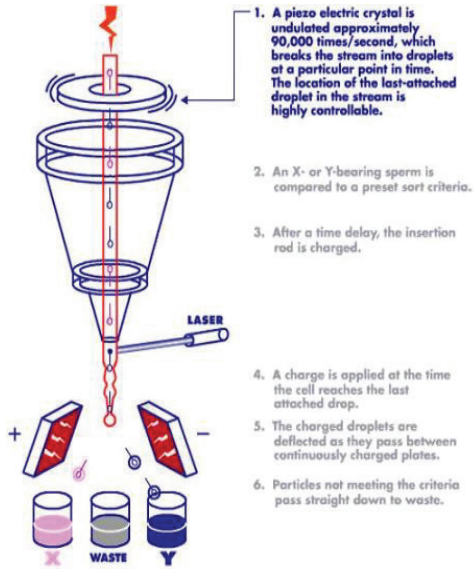

Figure 1.Sex sorting using flow cytometry
(Courtesy: Sexing Technologies, USA

Advantages of using sexed semen

- Producing mostly female calves helps the farmers to save resources that would have been shared with unwanted males.
- Production of more female calves: increase supply of replacement heifers
- Opportunity to sell surplus heifers to other farmers/farms
- Speed up genetic improvement through:
  - ❖ Increasing efficiency of progeny testing Scheme(PTS)
  - ❖ Increasing efficiency of embryo transfer and In Vitro Fertilization(IVF) schemes

Limitations of using sexed semen

- High cost of sex sorting machine (USD 250,000 or Nu17.5M per Machine(University of Florida, USA, 2018)
- Low sorting efficiency and speed
- Require highly skilled person to operate sex sorting machines
- Damage to the sperm due to shear force, electrostatic charge & droplet formation ·

Implementation limitations

- High cost of sexed semen $23 or Nu.1600/ dose compared to $4.7 or Nu.329/ dose for imported conventional semen
- Sperm concentration of sexed semen ranges between 2-4 million/dose whereas it is 20 million/dose in conventional semen. Accordingly, conception rate with sex sorted semen is 10-15% less than the conventional semen. This factor is critical considering low AI coverage in Bhutan (about 17%) owing to difficult terrain

Sexed semen availability and field trials in Bhutan

Sexed semen is not produced in Bhutan due to high investment cost. It is imported regularly from western countries. Trials were conducted in a random samples of animals at Govt. as well as village farms. Conception rate with sex-sorted semen is 49% in heifers and 37.5% in cows (NDRDC, 2018). Sex ratio is about 92% (female) in Govt. farms.

Recommendations for use of sexed semen in Bhutan

Sexed semen has low sperm numbers per dose and compromises sperm viability. Hence, considering their high fertility rate of heifers, it is recommended that sexed semen should be used only in virgin heifers for better conception rate.

NATIONAL DAIRY RESEARCH & DEVELOPMENT CENTRE
DEPARTMENT OF LIVESTOCK
MINISTRY OF AGRICULTURE AND FORESTS
YUSIPANG, THIMPHU, BHUTAN, POST BOX: 1058
17116985 (Direct), 17116986 (Lab), 17116976 (PABX)
www.ndrc.gov.bt