National Dairy Plan Phase I

Manual on

Semen Production

Project Implementation Plan: Volume IV C

Project Management Unit

(located in NDDB)

Table of Contents

Foreword **1**

1. Introduction **3**

1.1. What is a manual and why it is needed? 3

1.2. Whom is this manual for? 3

1.3. Semen Production: A Background 3

1.4. Rationale of the Programme 4

1.5. Objectives of the Programme 5

2. Semen Production Project: An Overview **5**

2.1. Area of Operation 5

2.2. Snapshot of Project Activities 5

3. Project Sub-activities/Steps **8**

3.1. Evaluation and Selection of Bulls 8

3.2. Identification of bulls 10

3.3. Quarantine 11

3.4. Rearing of young bulls 11

3.5. Training of bulls 12

3.6. Main Semen Station 12

3.7. Housing and Management of bulls (as mentioned in Appendix III) 13

3.8. Animal Health Protocols in Semen Production 13

3.9. Collection Arena and Semen Collection (as mentioned in Appendix III) 14

3.10. Evaluation and processing of semen (as mentioned in Appendix III) 14

4. Management of the Frozen Semen Station **15**

4.1. Project Management 15

5. Managing Procurement **16**

6. Fund flow Mechanism and financial management **16**

7. Project Monitoring and Evaluation **16**

7.1. Information Disclosure 17

7.2. Grievance Redressal Mechanism (GRM) 17

Annex I: Grievance Redressal mechanism under NDP I **19**

Appendix III Minimum Standards for Production Of Bovine Frozen Semen **21**

List of figures

Figure 2.1: Schematic representation of the Technical programme..... 7
Figure 3.1: Scrotal Circumference..... 9
Figure 3.2: Measurement Error..... 9
Figure 3.3: Ear Tag..... 10
Figure 3.4: Tag Applicator 10
Figure 3.5: Ear tagged Animal 11

Abbreviations

AI	:	Artificial Insemination
AIT	:	Artificial Insemination Technician
AV	:	Artificial Vagina
BMC	:	Bulk Milk Cooler
BAIF	:	Bharatiya Agro Industries Foundation
BQ	:	Black Quarter
BV	:	Breeding Value
CCBFs	:	Central Cattle Breeding Farms
CEO	:	Chief Executive Officer
CFSP & TI	:	Central Frozen Semen Production and Training Institute
CFU	:	Colony Forming Unit
CL	:	Corpus Luteum
CMU	:	Central Monitoring Unit
CRI	:	Calf Rearing In-charge
DADF	:	Department of Animal Husbandry, Dairying & Fisheries
DC	:	District Coordinator
DCS	:	Dairy Cooperative Society
DIC	:	Differential Interference Contrast
DNA	:	Deoxyribonucleic Acid
EIA	:	End Implementing Agency
ELISA	:	Enzyme Linked Immunosorbent Assay
FMD	:	Foot and Mouth Disease
FSH	:	Follicle Secreting Hormone
FUR	:	Fund Utilization Report
GoI	:	Government of India
GRM	:	Grievance Redressal Mechanism
GRO	:	Grievance Redressal Officer
HEPA	:	High-Efficiency Particulate Air
HF	:	Holstein Friesian
HS	:	Haemorrhagic Septicemia
IBR	:	Infectious Bovine Rhinotracheitis
IBRD	:	International Bank for Reconstruction and Development
IDA	:	International Development Association
INAPH	:	Information Network for Animal Productivity & Health
JD	:	Johne's Disease

LN	:	Liquid Nitrogen
LRP	:	Local Resource Person
MAIT	:	Mobile Artificial Insemination Technician
MC	:	Management Committee
MMT	:	Million Metric Tonne
MoA	:	Ministry of Agriculture
MoU	:	Memorandum of Understanding
MRT	:	Milk Ring Test
MT	:	Metric Tonne
NDDB	:	National Dairy Development Board
NGO	:	Non Government Organisation
NPCBB	:	National Project for Cattle and Buffalo Breeding
NS	:	Natural Service
OIE	:	World Organisation for Animal Health
PC	:	Project Coordinator
PD	:	Pregnancy Diagnosis
PDA	:	Personal Digital Assistant
PIP	:	Project Implementation Plan
PMC	:	Project Management Cell
PMU	:	Project Management Unit
PT	:	Progeny Testing
PTM	:	Post Thaw Motility
RBP	:	Ration Balancing Program
SOPs	:	Standard Operating Procedures
TB	:	Tuberculosis

Foreword

The demand for semen doses for artificial insemination has been growing in the country. In the last five years, the demand for frozen semen doses has increased by an average of 12 percent per year. In 2004-5, fifty seven semen stations produced 37.2 million frozen semen doses, whereas in 2009-10, forty nine semen stations produced 65.9 million doses. The demand for semen doses in the country is projected around 100 million doses by 2016-17 and 140 million doses by 2021-22.

The consumers are also now looking for quality. The semen by many is no longer perceived as a dose to impregnate their animals, but perceived as a source of genetics to produce better quality animals.
It is realised that while one thinks about expanding the semen production facility in terms of increasing bull housing and semen processing facilities, one has also to think about putting in place quality control and bio-security systems at semen stations. One has to think about creating separate quarantine and rearing facilities and creating disease free zone around semen stations.

Considering the need for expanding the semen production facilities in the country, under NDP, it is planned to assist a select existing semen stations to expand and modernize their facilities to produce high quality diseases free semen doses.

This manual has been prepared primarily for the semen stations which intend to expand and modernize their semen production facilities and produce quality diseases free semen doses. It would help equip them with knowledge and skills to produce high quality semen doses.

The manual provides specific guidelines and minimum standards for management of bulls, collection and processing of semen, quality control, bio-security measures etc.

It is expected that the manual for Semen Production programme will be a useful guide for the people involved in semen production and other people directly or indirectly involved in implementation of NDP.

1. Introduction

1.1. What is a manual and why it is needed?

1.1.1. A manual is a reference book which presents information that is necessary for operating or implementing a particular system, project etc. It is written to give technical assistance to the people so that they can have sound guidance while implementing a project. A manual basically tells what one is supposed to do, how one should go about it, when and where and by what means one should execute a particular task, with whom and with whose support should one implement a project etc. It is needed as it becomes the guiding document according to which the project should be implemented.

1.2. Whom is this manual for?

1.2.1. This manual has been prepared primarily for the agency which would be involved in frozen semen production and for those who would be directly or indirectly concerned with the expansion of semen production activities under NDP I.

1.3. Semen Production: A Background

1.3.1. A low percentage of breedable animals inseminated is one of the reasons for the low productivity of dairy animals in the country. Though many agencies (mainly government and cooperatives) have set up facilities for semen production, there is a need to improve quality of semen doses they produce and expand the scale of their operation.

1.3.2. Under NDP I, it is envisaged that in order to achieve the desired genetic change in the population to increase the milk production at the pace required, the percentage of breedable animals inseminated should be increased from the current level of about 20% to 35% by 2016-17. And to inseminate 35%

of breedable animals, the number of AIs to be performed annually would go up from the current level of 50 million to over 95 million, and the frozen semen production in turn would need to be raised from the current level of 66 million doses to 100 million doses by 2016-17.

1.3.3. Presently, there are 49 semen stations – 36 in the government sector, two managed by NDDB, nine in the cooperative sector, and two in the private sector and NGO sector- spread over 20 states producing annually 66 million doses. Top ten semen stations are producing 50% of the total doses and top 25 semen stations about 85% of the total doses.

1.3.4. 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.

1.4. Rationale of the Programme

1.4.1. The annual demand for milk is projected to reach around 200 MMT by 2021-22. Given the present productivity levels of our bovines and the resource constraints, it is impossible to meet this demand through domestic production unless productivity of our bovines increases. A three pronged strategy is planned to achieve this target. Increase the proportion of animals under AI from the existing 20% to 35%, strengthen the frozen semen production infrastructure to produce about 100 million doses to meet the requirement of this increased AI coverage, and produce the required high genetic merit bulls through appropriate genetic improvement programmes.

1.5. Objectives of the Programme

1.5.1. The main objectives of the Frozen Semen Production Programme are:

a. Produce good quality disease free frozen semen doses from bulls of High Genetic Merit, to meet the demand of semen doses for AI.

b. Achieve the target of producing 100 million doses of disease free frozen semen to achieve AI coverage of targeted 35% breedable female animals by 2016-17.

2. Semen Production Project: An Overview

2.1. Area of Operation

2.1.1. It is envisaged that the project will focus on a select existing semen stations graded 'A' or 'B' by Central Monitoring Unit (GoI) and owned by state governments, central government, Cooperatives, and NGOs. The emphasis would be on increasing the semen production by renovating the existing semen collection and processing facilities, adding /replacing laboratory equipment and instruments, creating infrastructure for bull housing, quarantine and rearing facilities to induct new bulls of those breeds which are in demand. Simultaneously, all efforts would be made to enhance sperm harvesting from each bull and streamlining and synchronizing various steps in semen production line to get top quality frozen semen doses.

2.2. Snapshot of Project Activities

2.2.1. A schematic representation of various activities of semen station is given in

2.2.2. Figure 2.1. The major activities that a semen station undertakes include:

a. Selection of bulls for semen production;

b. Identification of selected bulls;

c. Quarantine, rearing and training of bulls selected for semen production;

d. Induction of trained, disease free and genetically normal bulls for semen production;

e. Scientific management of bulls;

f. Maintenance of semen collection arena and collection of semen from bulls;

g. Evaluation of semen ejaculates and processing of qualified semen samples;

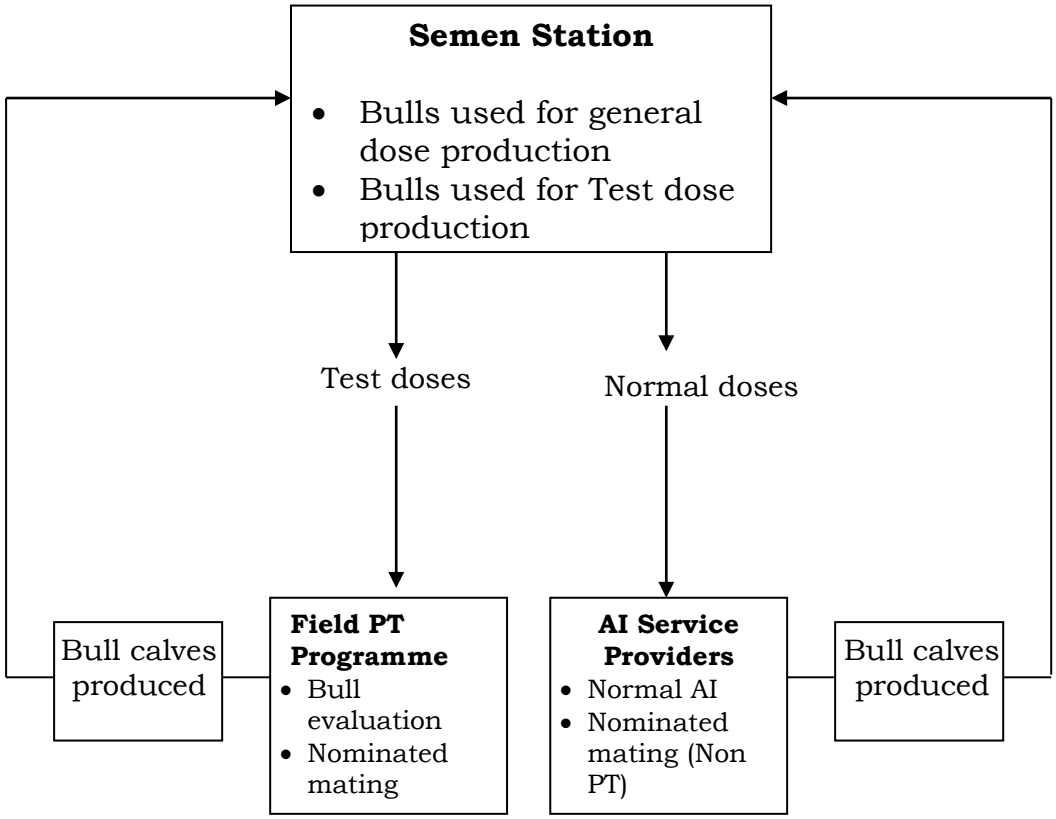
h. Freezing and cryopreservation of semen doses;

i. Quality Assurance and quarantine of semen doses;

j. Distribution of semen doses for field AI after undertaking the required quality checks;

k. Receive feedback on fertility etc.

Figure 2.1: Schematic representation of the Technical programme



3. Project Sub-activities/Steps

The End Implementing Agencies (EIA) implementing the frozen semen production programme under NDP-I shall follow the "Minimum Standards for Production of Bovine Frozen Semen" prescribed by DADF, GoI, as provided at Appendix III.

3.1. Evaluation and Selection of Bulls

3.1.1. Eligible young bulls born and procured from either PT projects or non PT areas shall be subjected to thorough physical examination by a qualified veterinarian to ensure that bulls do not show any clinical symptom of disease(s). He/She shall also carry out breeding soundness examination of bulls including measurement of scrotal circumference.

3.1.2. The size of the testis is correlated with sperm production of the bull and age of puberty of offsprings. It is important to take measurement of scrotal circumference as it is an indirect measure of testicular mass; it is associated with sperm production as well as with parenchymal health of the testicular tissues. Bulls with small testes produce less sperm, have a delayed puberty in their daughters and sons and have earlier testicular degeneration.

3.1.3. The scrotal circumference is measured with the help of a specialised tape at the widest point of the scrotum ensuring that the testes are in scrotum by pulling them down and kept together. The following pictures show the correct method of measurement:

Figure 3.1: Scrotal Circumference

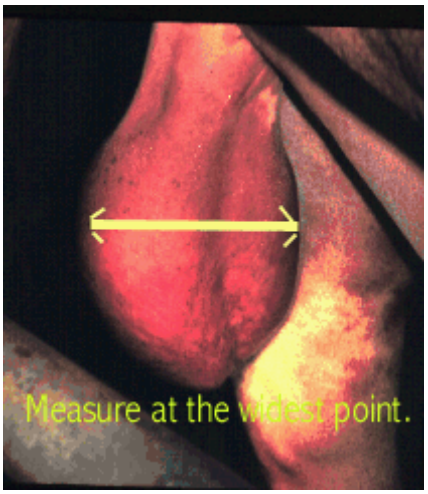
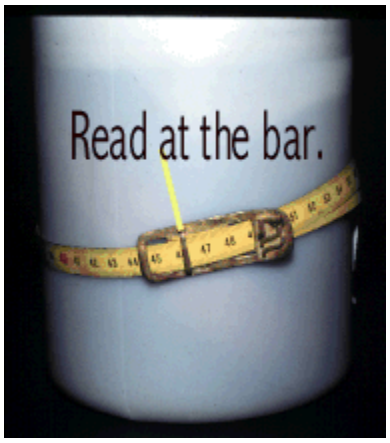


Figure 3.2: Measurement Error



3.1.4. Errors could occur if there is fat in the scrotum, a hernia or there is hydrocoele. Errors could also occur, if testes are pushed apart, when a measurement is taken.

3.1.5. The bulls shall also be subjected to karyotyping to rule out any chromosomal abnormality and genetic disease testing to eliminate bulls having genetic diseases.

3.1.6. The young bulls shall have to pass various health tests: TB, JD, Brucellosis, Campylobacteriosis and Trichomoniasis.

3.2. Identification of bulls

3.2.1. All bull calves/bulls maintained at a Quarantine Station/Rearing station/Semen Station shall be identified by applying recommended plastic ear tags.

3.2.2. Only polyurethane laser printed ear tags having a 12 digit number and a bar code shall be used. The numbering system followed shall be unique with the last digit of the number being a "check digit" to ensure that no two animals are tagged with the same number.

Figure 3.3: Ear Tag

Figure 3.4: Tag Applicator

3.2.3. The ear tag shall be applied inside the ear of animals, in the center of the ear lobe with the female part of the tag inside the ear.

Figure 3.5: Ear tagged Animal

3.2.4. If the ear tag falls off, a new ear tag shall be applied immediately and the information shall be immediately updated in the concerned registers and in the computer.

3.3. Quarantine

3.3.1. Each frozen semen production station shall have standard facility for quarantine of bull calves/bulls, about five kilometres away from the main semen station. It should be;

a. Constructed so that contact with other livestock is prevented;

b. Such that it can be easily cleaned and disinfected.

3.3.2. Quarantine procedures for bull calves shall be followed.

3.4. Rearing of young bulls

3.4.1. Each frozen semen production station shall have standard facility for rearing of bull calves. The Rearing facility;

- Must not have any direct contact with the main semen station;

- Must be constructed so that contact with other livestock is prevented;

- Must be such that it can be easily cleaned and disinfected.

3.4.2. Rearing facility receives animals from Quarantine Station, keeps them till they reach sexual maturity and then they are shifted to Semen Station.

3.5. Training of bulls

3.5.1. Main semen station receives mature bulls from Rearing facility and trains them for semen collection.

3.5.2. Trainee bulls need to be taught how to mount on a dummy animal, donate semen in an AV and dismount, with the help of an attendant and a semen collector. Young bulls learn by seeing older bulls donating semen.

3.5.3. They should be treated gently and firmly so that they have no fear of the dummy animal, bull attendant and semen collector.

3.5.4. Semen samples collected from the bulls under training shall be examined for semen profile including sperm morphology. The bulls will be qualified for regular semen collection once their **s**emen samples are cleared by the Quality Control lab.

3.6. Main Semen Station

The main semen station must:

- Have bull housing facilities having individual pen with adequate loafing area and are separated from the semen collection and processing facilities;

- Have bull isolation facilities;

- Have semen collection and processing facilities;

- Have semen quarantine facilities;
- Be constructed so that contact with other livestock is prevented;
- Be such that all bull housing, semen collection, semen processing and semen quarantine facilities can be easily cleaned and disinfected.

3.7. Housing and Management of bulls (as mentioned in Appendix III)

3.8. Animal Health Protocols in Semen Production

3.8.1. Disease reporting

- The bull attendant/labour who observes any abnormal health event like high fever, off feed, symptoms of diseases like FMD etc would report the same to the veterinary officer who would in turn confirm it (in consultation with the unit head) and report the occurrence to the nearest veterinary officer and the District Animal Husbandry Officer of the State Animal Husbandry Department. The recording in the INAPH/ SSMS system would be done by the supervisor/veterinary officer.
- Reports of such occurrences would also be provided in a compatible format to the district veterinary authorities on a regular basis from the INAPH/ SSMS system.
- Adequate training would be provided to the veterinary officer/ supervisor to equip them adequately on various aspects of disease reporting.

3.8.2. Bio-security measures at semen station

- The bull attendants, labourers and veterinary officers who are in close contact with the bulls would need to follow certain hygienic practices that would minimize the spread of infection.

- Standard practices to be followed in the event of a confirmed or suspected outbreak of diseases like FMD, etc.

- Adequate training should be provided to the labourers, bull attendants and veterinary officers to equip them adequately on various aspects of biosecurity protocols required at the semen station.

3.9. Collection Arena and Semen Collection (as mentioned in Appendix III)

3.10. Evaluation and processing of semen (as mentioned in Appendix III)

3.10.1. Semen freezing

- After printing, straws are arranged on freezing racks using ramp meant for Mini straws. These racks are kept in Cold Handling Cabinet at 4-6° C for 4-5 hours for equilibration. The cabinet should be maintained at the prescribed temperature during this period so that sperms are not exposed to temperature fluctuation.

- Then racks are transferred into chamber of freezing unit (Bio-freezer for bovine semen) controlled by a computerized programmer. Pre-decided freezing protocol on the programmer takes care of freezing of semen doses. Freezing completes at -150 °C.

- Frozen semen doses are now taken from freezing unit and plunged into liquid nitrogen (LN) for cryo-preservation.

3.11 Semen storage/ dispatch (as mentioned in Appendix III)

3.12 **Information System (as mentioned in Appendix III).**

4. Management of the Frozen Semen Station

4.1. Project Management

4.1.1. Under the Project, PMU (located at NDDB) will provide funds to the identified Frozen Semen Stations for strengthening their infrastructure – addition/ renovation of quarantine, rearing, bull housing, semen collection and processing facilities, addition/replacement of laboratory equipment and instruments, farm machinery and equipment and induction of new bulls, in order to increase its capacity for production of disease free quality semen and to assist it to achieve grade 'A'.

4.1.2. An appropriate agreement would be signed with the respective End Implementing Agency (EIA) owning the semen station:

a. To ensure that the funds are used for the purpose, for which these are provided, following World Bank's procurement guidelines.

b. To ensure that all equipment, instruments and bulls procured and buildings or structures constructed are as per the standard requirements/ specifications.

c. To ensure that the Minimum Standards as prescribed by the DADF, GoI are strictly adhered to.

d. To ensure that PMU (located at NDDB) representative is included in the Project Management Committee of the Semen Station and/or in the Agency's Board.

e. To ensure that the agency regularly provides information to PMU (located at NDDB) on all aspects as prescribed.

f. PMU (located at NDDB) or its appointed representative would continuously monitor the activities of the station.

5. Managing Procurement

- Procurement management practices to be followed by the EIA are described in the Procurement Manual, Vol. III of PIP.

6. Fund flow Mechanism and financial management

- Fund flow mechanism and financial management practices to be followed by the EIA is described in the Financial Manual, Vol. II of PIP.

7. Project Monitoring and Evaluation

- PMU (located at NDDB) representative on the Project Management Committee of the Semen Station and/or on the Agency's Board will ensure that all terms and conditions of the Agreement signed are adhered to by the agency in utilization of funds.

- PMU (located at NDDB) would continuously monitor the progress of the work and activities of the station through periodic visits.

- Agency will be required to provide information on the activities of the station regularly.

- The Frozen Semen Stations shall maintain all documents and use SSMS (Semen Station Monitoring System) for collecting information online from semen processing laboratory, semen inventory, quality control and dispatch. The information shall be analysed for generating different reports and giving feedback to all the concerned in the project.

7.1. Information Disclosure

7.1.1. The EIA will have a website containing *suo moto* disclosures of the sub project related information, details of the activities, area(s) where the activities are being implemented, procurement plan etc. It will also regularly post the progress of the project and the particulars of the person who may be contacted in the EIA for seeking further information.

7.2. Grievance Redressal Mechanism (GRM)

7.2.1. "A grievance would usually refer to some form of dissatisfaction by a stakeholder, which needs to be redressed in order to continue smooth implementation of the project". The project will evolve a system for redressal of grievances that may arise in the course of implementation. The GRM will be structured in a manner so that it can be monitored, as it provides important feedback on the project activities.

7.2.2. The EIA would have a designated officer as 'Grievance Redressal Officer' (GRO) to deal with grievances. His contact number/ mailing IDs and address etc would have to be displayed on the web site of the EIA and at other relevant locations such as notice boards.

7.2.3. Each GRO would need to:

- Maintain a computerised database of Grievances (*through a unique identification number*), acknowledgements and information about their disposal.
- Monitor the progress of disposal of the grievances.
- Fix time limit for disposal of the Grievances.
- Deal with each Grievance in a fair manner.

- Determine an appropriate periodicity when internal / external meetings would be held to implement the GRM in an efficient manner.

7.2.4. The procedure to be followed for grievance handling is given at **Annex I**.

Annex I: Grievance Redressal mechanism under NDP I

For addressing grievances arising under NDP I, following grievance redressal mechanism be adopted.

Appointment of Grievance Redressal Officer

1. The EIA under NDP I shall designate an officer as 'Grievance Redressal Officer' (GRO) to deal with all matters relating to grievances.
2. The EIA should display at a prominent place/ notice board the name of GRO with location, contact numbers/ mailing IDs and address along with the specific visiting hours for hearing / receiving the grievances.

Grievance/Complaint Submission:

1. While complaint is made, it can either be made orally or in writing:
    - The name of the individual or organization, address and telephone number (if any) of the complainant.
    - A brief description of the matter which is the source of the grievance, including copies of any relevant and supporting documents.
    - Relief sought
2. Grievances may also be submitted in the Complaint Box kept at reception of the office of the EIA. The Complaint Box should be opened on daily basis by the GRO. Complaint can also be sent by post.
3. A complaint made through electronic means (e-mail, fax) should also be accepted and replied, if requested, should be sent through e- mail also.
4. In case the complainant is not satisfied with the response at a certain level, He/She/she will be free to approach the next level.

Grievance Redressal Procedure:

1. Every application received should be tagged with a reference number. The grievance system should be continuous for the whole year.
2. Every application or petition should be acknowledged through standard acknowledgement slips or a copy of the receipt which should be dispatched to the complainant within 3 days of receipt of complaint or handed over to person at the time of receipt for complaints submitted in person.
3. Every application should carry such a slip for future reference indicating the name, designation and telephone number of the official who is processing the case. The time frame in which a reply will be sent should also be indicated.
4. The complainant should be quickly informed of the action taken by way of redressal within proposed response time.
5. A record of all complaints received and action taken till disposal should be maintained.
6. A reply to any grievance must cover all points raised and not address the grievance partially. If there is any follow- up action, it must be pursued.
7. No grievance is to be rejected without having been independently examined. At a minimum, this means that an officer superior, to the one who delayed taking the original decision or took the original decision that is cause for grievance, should actually examine the case as well as the reply, intended to be sent to the complainant. If a complaint is rejected, the reasons for such rejection must be made explicit and should be intimated to the complainant within the time frame.

Grievance redressal mechanisms will consider the vulnerability of gender, Scheduled Caste/Scheduled Tribe and other vulnerable populations.

Appendix I

Minimum Standards

For
Production of Bovine Frozen Semen

MINIMUM STANDARDS FOR PRODUCTION OF BOVINE FROZEN SEMEN

Artificial Insemination with frozen semen has been proved to be the best tool world wide for genetic improvement through dissemination of superior germplasm. This objective can be achieved only if the frozen semen used in AI programme conforms to the quality standards. For production and distribution of quality semen, it is most important that the bulls used in AI programme satisfy quality norms, bulls are disease free and semen is harvested and processed in accordance with the standard protocols. The least protocols required for production of quality semen are covered in this manual. Failure to observe these guidelines could lead to production of poor quality semen making it unfit for distribution to AI centres.

1. Standard for Genetic Merit of Breeding Bulls

Bulls procured should be the ones produced following prescribed Minimum Standard Protocols and Standard Operating Procedures for Progeny Testing (PT) through Government approved Progeny testing (PT) Programmes. If such bulls are not available and if there are no PT programmes for certain breeds, the procurement of bulls should be based on the dam's Standard lactation yield. Breed wise dam's lactation yields are given below. Preferably, the Lactation yield would be arrived at by recording the animal once a month continuously for 11 times or until the animal becomes dry. 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).

Breed	Dam's Lactation yield (Kgs)		
	First	**Best**	**Fat %**
Pure HF	4500	5600	3.5
Pure Jersey	3000	3750	5.0
Sahiwal	2400	3000	4.0
Red Sindhi	2000	2500	4.5
Gir	2400	3000	4.5
Kankrej	2000	2500	4.5
Tharparkar	2000	2500	4.0
Hariana	1600	2000	4.0
Rathi	1600	2000	4.0
Ongole	1100	1600	4.0
Deoni	800	1000	4.0
Khillar	380	500	4.0
Dangi	400	530	4.0
Amritmahal	400	500	4.0
HF Cross- F2	4000	5000	4.0
Jersey CB- F2	2800	3500	4.5
Sunandini	2500	3000	3.5
Murrah	2400	3000	7.0
Mehsana	2400	3000	7.0
Nili Ravi	2400	3000	7.0
Jaffrabadi	2800	3500	8.0
Surti	1600	2000	7.0
Banni	2400	3000	7.0
Bhadawari	1300	1600	8.0
Pandharpuri	1300	1600	7.0

Dam's milk yield for F1 crosses will be as that of the indigenous dam's i.e. Gir, Sahiwal, Kankrej, Red Sindhi, etc.

For import of bulls and embryos, the standards for import of germplasm as prescribed in the "Guidelines for export / import of bovine germplasm" issued by DADF, MoA, GoI and as revised from time to time shall be followed.

2. Physical Examination

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

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

Prior to introduction of new bulls for semen collection, breeding soundness examination shall also be carried out.

3. Karyotying and testing for genetically transmitted diseases

It is necessary that all animals be karyotyped to rule out any chromosomal defects. Specific tests may also be conducted for genetically transmitted diseases as given in the table:

Breed	**Tests to be carried out**
Indigenous cattle and buffaloes	Factor XI deficiency syndrome, Bovine Leukocyte Adhesion Deficiency (BLAD), Citrullinemia
HF and HF crossbreds	Factor XI deficiency syndrome, Bovine Leukocyte Adhesion

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

4. Quarantine

A quarantine period of **minimum 60 days*** is compulsory before bringing new bulls into a semen station. Only after favourable results from the health control point, the bulls shall be admitted to the semen station. Relevant definitions are given in Annexure- 1

a) In the quarantine station, new animals shall be housed for a minimum of **60** days in a place which is effectively separated and away from (preferably at a distance of 5 km) the facilities occupied by resident bulls. Manpower deployed and all equipment used in handling, feeding, watering and cleaning the new bulls shall not be shared with the resident herd(s).

b) Each new animal in quarantine station will be tested against major contagious diseases before its entry to resident herd e.g. TB, JD, Brucellosis, Campylobacteriosis and Trichomoniasis. All tests shall be done by an accredited agency or disease diagnostic laboratory as indicated in Annexure- 2.

During quarantine period, the bulls shall be vaccinated against FMD, HS, BQ, Theileriosis and Anthrax. However, vaccinations against bacterial diseases shall be done only if there is an outbreak or prevalence of a particular disease.

Once the quarantine period is over, all bulls shall be introduced to the young bull rearing station.

**The procedure and duration for quarantine in different situations is given in Annexures- 3A, 3B, 3C & 3D.*

5. Testing of Bulls

Testing protocols for bulls against Tuberculosis, Johne's disease, Brucellosis, Campylobacteriosis and Trichomoniasis are given in Annexures- 4 to 8. As per OIE guidelines, the breeding bulls should be free from above mentioned diseases. 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 MSP. The bulls in the rearing station and the resident herd should go through periodical testing and vaccinations as per the schedule listed in the manual.

6. Vaccination Schedule

The bulls shall be vaccinated against FMD, HS, BQ, Theileriosis and Anthrax. However, vaccinations against bacterial diseases shall be done only if there is an outbreak or prevalence of a particular disease.

Theileriosis – Exotic and crossbred bulls shall be vaccinated once in their lifetime.

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

The semen station shall arrange for carrying out ring vaccinations for all cloven footed animals including swines against FMD within a radius of 10 km around the semen station. Vaccinations against HS and BQ shall be carried out in the areas having incidence of these diseases.

7. Culling of Bulls and Semen Doses due to Specific Diseases

Diseases	Bulls	Semen doses
FMD	Retain	Last one month's doses to be discarded, refer Annexure- 9
Brucellosis	Castrate & remove	FS doses in stock to be discarded since the last negative test
TB	Remove	FS doses in stock to be discarded since the last negative test
JD	Remove	FS doses in stock to be discarded since the last negative test
Campylobacteriosis	Treat and retain	FS doses in stock to be discarded since the last negative test
Trichomoniasis	Treat and retain	FS doses in stock to be discarded since the last negative test

The semen station must remove bulls (within 48 hours) which are positive for Brucellosis, TB and JD. Bulls found positive for Campylobacteriosis and Trichomoniasis shall be isolated and treated. Besides, the semen station shall cull those bulls which have completed eight years of productive period or 3 lakh semen doses, whichever is achieved earlier. In addition, the bulls with poor libido, poor semen quality, incurable lameness, etc. shall also be culled.

8. Housing

Bull sheds shall have spacious individual pens with adequate loafing area, manger and water trough with access to drinking water all time. Adequate shade around the bull shed shall be provided. The roof shall be made of asbestos or suitable materials. During summer, cooling system with sprinklers and fans is required particularly for the buffaloes and exotic bulls. Disinfectants like **formalin or phenyl** based compounds **shall not be used** in the bull sheds. Alternatively,

compounds containing Gluteraldehyde shall be used. Weekly spraying of Sodium Carbonate (4%) solution shall also be practiced. The floor should be sterilized at least once a year by a blowlamp or by burning straws. At one corner of the farm, there shall be an isolation shed for separating ailing / sick bull(s) for treatment. Bull(s) once diagnosed suffering from infectious diseases shall be removed immediately from semen station for safety of other bulls.

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

9. Management of Bulls

The objective of daily care of bulls is to ensure a satisfactory state of cleanliness. For proper management of bulls, the following points shall be considered:

a) The bulls shall be kept under hygienic conditions at all times.

b) The coat of the bulls shall be kept clean and generally short. The hooves shall be regularly trimmed.

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

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

e) 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 can be practiced if the microbial load in frozen semen is beyond the prescribed limit.

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

g) Scientific feeding schedule shall be followed for the bulls. A general guideline is attached as Annexure- 10. Semen station shall carryout routine quality analysis of feed and fodder for arriving at a balanced ration.

10. Semen Collection

a) Ideally, the floor of the collection yard shall be made of concrete layer at a depth of one foot from the ground level. Mixture of sand and limestone shall be used to fill up to ground level and pressed firmly. If it is not possible to renovate the entire collection arena, at least the mounting area shall have sand and limestone mixture for proper footing of bulls. Alternatively, good quality rubber mat (with interlocking arrangement) or coir mat shall be put into concrete groove of the mounting area for adequate cushioning effect. After collection, the area must be thoroughly cleaned and odorless disinfectant solution (Colloidal iodine) be sprayed. A dusty floor shall be avoided to prevent dust falling on the AV / semen samples.

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

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

d) Semen collection should be individualized based on the bull

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

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

g) Sterilized bull aprons shall be used to avoid penis touching hindquarter of the dummy.

h) Before every collection, the semen collector shall either wash his hands with 0.1% Savlon solution or use disposable gloves or do both. The semen collector shall not touch the penis.

i) Preferably veterinarians shall take semen collection. If semen is collected by staff, a veterinarian shall remain present to supervise the collection process. While taking collection, it shall be ensured that AV is not thrust on penis of bull, instead penis should be guided to AV.

j) Immediately after collection, the AVs shall be thoroughly cleaned by non-spermicidal neutral detergent. Separate AVs shall be used for each ejaculation. The AV shall be changed even if the bull has inserted its penis without successful ejaculation. The same AV shall not be used twice. The AVs

shall always be kept inverted and the collection tube shall be covered with felt / water jacket (plastic bottle filled with warm water at 34^{o} C) to avoid cold shock. The open end of sterilized AVs shall be covered with aluminium foil, which would be removed at the time when bull is ready for giving semen.

k) Appropriate size AVs, ranging from 8-14", shall be used for cattle and buffaloes to ensure semen is ejaculated in cone. For buffaloes, goat AVs can also be used. The cone shall be of top quality Neoprene rubber.

l) Use of lubricant shall be avoided. If it is extremely essential to use lubricant, separate sterilized glass rods shall be used for smearing K-Y Jelly on each AV.

m) The AV shall not to be shaken after ejaculation; otherwise lubricant and debris may mix with the semen samples.

n) As soon as the first ejaculate is taken, the bull apron should be removed and dipped in the plastic tub filled with detergent lotion. For second ejaculate, a fresh apron should be tied to the bull

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

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

q) Semen stations must follow 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. However, a maximum number

of collections per bull would depend on the individual capacity of the bull.

11. Handling, processing & freezing of semen

11 (A) Premises

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

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

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

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

e) Preferably cassette type or, split type air conditioners fitted with air purifying system with remote temperature control mechanism should be installed to maintain the room temperature at 20°C - 22°C. The number of ACs to be fixed to sustain this temperature shall depend on the size of the processing room. Maintaining this temperature is most important to achieve the best results when single step dilution method is followed for freezing semen. The flow of air from AC must not be towards the front side of the Laminar Air Flow Unit. Adequate number of thermometers

shall be kept in a few places in the laboratory to check the room temperature.

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

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

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

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

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

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

k) Fumigation should be supported by monitoring laboratory environment by bacterial load test. The bacterial load shall be measured every week to monitor pollution of the laboratory atmosphere.

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

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

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

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

11 (B) Equipment

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

b) The pre-filter of Laminar Airflow unit shall be cleaned weekly. Routine servicing and DOP testing twice a year will ensure efficiency of HEPA filters. Alternatively, culture plate test shall be carried out at frequent interval to assess bacterial load of the air passing through the filters.

c) Digital photometer / Computer aided Spectrophotometer shall be validated with Haemocytometer readings for sperm concentration twice a year separately for cattle and buffalo (20 samples each).

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

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

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

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

h) Single distilled water shall be used in autoclave and thermo-controlled water bath. The water bath shall be cleaned and filled with single distilled water on a regular basis.

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

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

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

l) The following equipment should be validated by NABL certified laboratories:

i. Standard Thermometer

ii. Water Bath

iii. Weighing Balance

iv. Incubator

v. Autoclave

vi. Hot Air Oven

vii. Slide Warmer

viii. Micropipettes

ix. pH Meter

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

i. Cold Handling Cabinet

ii. Laminar Air Flow Units

iii. Biological Freezer

iv. Microjet Ink Printer

v. Filling & Sealing Machine

vi. Photometer

vii. Triple distillation unit, etc;

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

11 (C) Personnel Hygiene

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

11 (D) Diluents

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

b) All disposable and reusable supplies coming in contact with the semen and dilutor must be sterile and devoid of toxins and pyrogens.

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

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

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

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

g) Antibiotics in diluents: A combination of Penicillin and Streptomycin shall be used in diluents. However, it is better to use a combination of Gentamycin, Tylosin and Lincospectin (GTLS), if available, which can control Mycoplasma.

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

i) The required quantity of yolk shall be separated from albumin on sterile (autoclaved) standard filter papers (Whatman No.1/ Borosil) and yolk membrane shall be punctured using sterile glass rod, Pasteur pipette or sterile straws under the Laminar Air Flow Unit. Only fresh semen extender/dilutor shall be used because changes in the pH of stored extender are considered to be responsible for the deterioration of some nutrient components. Day old extender should not be used.

11 (E) Evaluation & Processing

a) The tube containing the freshly collected semen should be capped with aluminium foil as soon as it is placed in the pass box before transferring to the laboratory. The collection tube shall remain capped until processed.

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

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

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

d) Sperm concentration shall be checked preferably by a digital photometer with auto dilutor, manufactured by a reputed company. The photometer shall be calibrated separately taking 20 readings each for cattle and buffalo semen, at least once in

six months, with haemocytometer readings. Semen samples showing less than 500 million / ml sperm concentration shall be discarded.

The volume of straws should be determined as it may vary from batch to batch. While determining the dilution rate as per the photometric reading, the actual volume of mini straw should be fed to the photometer. Straw volume of randomly drawn straws from a day's production should be checked as part of quality assurance and documented.

e) Semen samples selected for freezing should have minimum 70% initial progressive motility. Final dilution of semen, keeping a minimum of 20 million spermatozoa per dose, shall be done in appropriate flasks with the dilutor maintained at 34^{o} C.

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

g) Unused straws shall be repacked (air-tight) under Laminar Air Flow Unit before storage. Immediately after use, all the glass ware, rubber ware, plastic tips and other reusables shall be immersed in neutral detergent solution (to be kept in a plastic tub near the Laminar Air Flow Unit).

h) The freezing should be carried out as per the recommended protocols for freezing cattle and buffalo semen. After freezing gets over, the straws should be collected from the racks using scoop tongs. The operator should wear woollen gloves with leather gloves over it to avoid frost injury

11 (F) Colour Specifications:

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

Breed	**Colour**
Holstein	Pink/Rose
HF Crossbred	Pistachio Green (light green)
Jersey	Yellow
Jersey Crossbred	Salmon
Indigenous cattle	Orange
Sunandini	Blue
Buffalo	Grey

If any of above mentioned colour is not available, then transparent straws shall be used.

11 (G) Printing of Straws

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

All semen stations shall follow the following printing abbreviations:

Jersey	–	JY	Farm No. / Name
Holstein	–	HF	Breed
HF Cross	–	CB HF	Name of Institute
Jersey Cross	–	CB JY	Batch No. / Date of Prodn.
Sunandini	–	SUN	
Sahiwal	–	SAH	
Red Sindhi	–	RS	
Kankrej	–	KANK	
Gir	–	GIR	
Tharparkar	–	THAR	
Rathi	–	RATHI	
Hariana	–	HAR	
Ongole	–	ONGL	
Deoni	–	DEONI	
Khillar	–	KHLR	
Dangi	–	DANGI	
Amritmahal	–	AMHL	
Murrah Buffalo	–	MBF	
Surti Buffalo	–	SBF	
Jaffrabadi Buffalo	–	JBF	
Mehsana Buffalo	–	MSNB	
Nilli Ravi Buffalo	–	NLRVB	
Banni Buffalo	–	BBF	
Bhadawari Buffalo	–	BDBF	
Pandharpuri Buffalo	–	PNPB	

11 (H) Post thaw motility

After freezing, the semen straws shall be stored in a separate container. Post-thaw motility of semen should be examined at 24 hours (after freezing). Differences in observations shall be updated and recorded for the purpose of accepting a particular batch of semen doses. Whenever there is any doubt, post-thaw motility shall be examined by two experienced persons. Preferably, the person involved in evaluation of neat semen, shall not check the post thaw motility. For a minimum concentration of 20 million per dose, minimum acceptable post thaw motility shall be 50%. Semen doses below 50% progressive motility shall be discarded.

11 (I) Quality Checks for frozen semen

This includes (i) Quarterly testing of random samples from each batch for bacterial load using standard plate count (The standards for acceptable colony forming units (CFUs) in processed semen is 5000 per ml as per OIE norm. If the bacterial load exceeds the OIE limit, the semen doses are to be discarded.)

The frozen semen samples should not have uncountable CFUs as they may have pathogenic organisms. Therefore, semen showing crowded CFUs should be subjected to testing for pathogenic organisms by an outside laboratory.

(ii) Hypo osmotic swelling test (HOST) - for all bulls at least once in a quarter shall be mandatory (iii) Incubation test - for all bulls at least once in a quarter shall be mandatory (iv) Acrosome integrity test by Giemsa staining - for all bulls at least once in a quarter shall be mandatory. Alternatively, wet smear of semen shall be examined using DIC microscope (v) Percent Intact Acrosome - all bulls to be covered once a quarter (vi) Sperm

Concentration – randomly two samples per week each for cattle and buffalo.

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

Sr. No.	**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 Mini straw)

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

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

11 (J) Information System

In order to facilitate the information system, all the bulls maintained by the semen station must be identified by ear tags/ cold branding.

The semen stations shall use suitable software to record data pertaining to various activities and also should have online facility for the same. The semen stations producing more than one million doses may introduce software that can identify and trace the bulls and their ejaculates, production, storage and dispatch of semen (barcode system).

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

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

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

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

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

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

11 (K) Semen Storage

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

After checking post-thaw motility, if found acceptable, frozen semen doses shall be kept in temporary storage for 7 days. After temporary storage, the semen goblets shall be transferred to the bulk storage containers with proper recording of position in the canisters. After each dispatch, records redefining the position of remaining doses shall be updated.

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

The goblets containing the semen should be well identified and precaution should be taken to see that each goblet has sufficient space for liquid nitrogen. Mini straws need special care and should not be exposed above liquid nitrogen even for a short time (10 seconds) as they get warm faster and any exposure causes irreversible damage to sperm viability.

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

12 Biosecurity

The risk of disease spread has grown manifold with increasing number of bulls maintained at the semen production center. With the expected higher risk, implementation of strict biosecurity measures at the semen stations assumes greater significance. Every semen station should have a well defined Biosecurity protocol put in place across all its activities.

13 Cleaning and Sterilisation

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

13.1 Laboratory and other areas

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

13.2 Artificial Vagina (AV)

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

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

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

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

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

13.3 Glassware

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

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

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

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

13.4 Rubber wares

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

13.5 Distilled Water

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

13.6 Buffer

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

13.7 Bacteriological Media

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

13.8 Filter Papers

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

14 Summary of Sterilization

a) Autoclave

Sr.No.	Item	Pressure (p.s.i.)	Time (Min.)
1.	Artificial Vagina	5	20
2.	Buffer	5	20
3.	Plastic Tips	5	20
4.	Filter Papers	5	20
5.	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

(The rubber wares can withstand above pressure and duration provided the quality is good)

b) Hot Air Oven

Sr.No.	Item	Temperature	Time (min.)
1.	7.2.4.1. Glass wares	160° C / 180° C	60/30
2.	7.2.4.2. Filling Nozzles	160° C / 180° C	60/30

c) AV Steriliser

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

15 Quality Control of Consumables

Chemicals

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

Straws

1. Straws manufactured by reputed companies are safer to use for production of quality semen. While buying straws, package volume and microbial load in straws shall be checked randomly from the consignment. In addition, some empty straws should be placed in filling and sealing machine and the machine should be run to see the sealing quality of the straws. In case of any foul smell, it should be presumed that the straws are manufactured from poor plastic which could be toxic to the spermatozoa and can even result in reduced motility on long storage.

2. The factory plug should not be loose. The factory seal should be impenetrable and the seal formed should be homogeneous and compact.

3. The straws should be intact (without cracks / dents, etc.) during and after freezing / thawing.

4. The movement of straws along the printing machine should be free and print should be clear and sharp. Print should not fade as a result of freezing and subsequent thawing.

5. The use of dark coloured straws should be avoided, as they are not transparent enough. Due to this, it is difficult to distinguish between filled / semi-filled straws.

6. Movement of the factory plug should be free.

7. Straws should be routinely checked for microbial load.

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

16 Manpower Requirement for semen production

Designation	**Up to 10 lakh doses**	**>10-25 lakh Doses**	**>25-50 lakh Doses**	**>50 lakh doses**	**Mega Semen Stn. 10m doses**
General Manager	1	1	1	1	1
QCO/QAO	1	1	1	1	1
Vet. Officer	1	2	3	3-4	5-6
Agriculure Officer	1	1	1	1	1
Data Mgmt. Officer	--	1	1	1	1
Accts. & Adm. Officer	--	1	1	1-2	1-2
Office Assistant	1	2	3	5	6-7
Livestock Assistant	1	2	3	4	5
Agri. Assistant					
Lab Technician	1	2	3-4	5-6	8-10
Vehicle/Tractor Driver	1	2	3	4	5

Designation	Up to 10 lakh doses	>10-25 lakh Doses	>25-50 lakh Doses	>50 lakh doses	Mega Semen Stn. 10m doses
Lab Attendant	2	3	3-5	7-8	10-12
Bull Attendant	1 person per 7- 8 bulls				
Agri. Labourers	15-20/100 acres depending on mechanization level				

The manpower structure suggested above is meant only for semen/fodder production. For other activities, manpower may be positioned as per the need. For dispatch of semen, facility should be created preferably away from semen station and operated by other person/s not responsible for semen production. The GOI / Department of AH / Livestock Boards / NGO / Private agencies / Union and Federation shall review the requirement of manpower position for each semen station and finalise the staff structure for recruiting additional manpower. After recruitment, all new persons shall be trained at any of the recognized institutes. Once trained, they shall continue to work in the semen station at least for five years.

Refresher training / visit to other semen lab: technical exposure of semen station personnel working in the semen lab must be arranged compulsorily once in two to three years at reputed institutions like CFSP&TI - Hessarghatta, KLDB - Mattupatty, etc. As semen production activity is an extremely technical work, job rotation of personnel could be detrimental in maintaining the quality of semen. Therefore, personnel working in a semen station should not be transferred at least for five years. If it is inevitable, in the interest of carrying out good work, it should be essential that a proper replacement is identified at least six months in advance and is trained in semen production technology.

Annexure- 1

DEFINITIONS FOR USE IN THE HEALTH PROTOCOL

Bull	Adult male cattle or buffalo used for collection of semen. Teasers and other animals resident in the semen stations are also subjected to similar disease testing, vaccination and medications for maintaining their health status.
Bull Calf	A male cattle or buffalo 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 semen station or rearing station. A series of clinical and laboratory examinations, vaccinations and medications etc. are undertaken during quarantine.
Rearing station	A farm 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 farm 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 village or farm where all the animals of the farm or the village have not been tested against the MSP diseases

Annexure- 2

Details of the tests to be conducted			
Disease	**Test**	**Sample**	**Tested by officers of**
Brucellosis	ELISA	Serum	CDDL/RDDL/ NDDB/PD_ADMAS
TB*	DTH- Tuberculin PPD	Intra-dermal on the bull	Semen station/ CDDL/RDDL/ NDDB
JD*	DTH- Johnin PPD	Intra-dermal on the bull	Semen station/ CDDL/RDDL/ NDDB
Trichomoniasis	Agent identification	Preputial washings / semen	CDDL/RDDL/ NDDB
Bovine Genital Campylobacteriosis	Agent identification	Preputial washings	CDDL/RDDL/ NDDB
FMD	ELISA	Serum	PD-FMD, Mukteshwar and its laboratories/ NDDB

*** TB and JD testing at Quarantine Station as well as Rearing Station shall be performed by the officers of the Semen Station. However, the testing at the Semen Station shall be done by the Officers of the CDDL/RDDL/NDDB.**

Quarantine Guidelines

Annexure- 3A

<table>
<tr><th colspan="3">A. Quarantine of adult bulls of unknown health status</th></tr>
<tr><td>Quarantine period</td><td colspan="2">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 and JD the interval between the two tests should not be less than 62 days.</td></tr>
<tr><td>Shifting of bulls from the quarantine</td><td colspan="2">Within 30 days from the date when the last test was performed and all bulls were found negative.</td></tr>
<tr><td>Action on finding a positive result</td><td>Brucellosis, TB, JD, Bovine Genital Campylobacteriosis, Trichomoniasis</td><td>Cull / remove the positive bull and put all the remaining bulls under extended quarantine.</td></tr>
<tr><td>Extended quarantine</td><td colspan="2">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.</td></tr>
<tr><td>Action on finding a positive during extended quarantine</td><td colspan="2">During Quarantine, if the bulls are housed and managed
Individually - Remove only the positive bull.
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.</td></tr>
</table>

B. 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 Annex- 3A
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 Annex- 3A

C. Quarantine of adult bulls to be shifted between the farms managed by the same administration ■ For shifting between semen stations for semen production ■ From a rearing station that implements Quarantine (Annexure-3D) before allowing entry of calves for rearing	
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 a positive result	Same as in Annexure- 3A
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 Annexure- 3A

<table>
<tr><th colspan="3">D. Quarantine of calves above 2 months of age</th></tr>
<tr><td>Quarantine period</td><td colspan="2">Minimum 60 days or sufficient to allow at least two tests for each of the MSP diseases to be performed with a minimum interval of 30 days between the tests. In case of TB and JD the interval between the two tests should not be less than 62 days.</td></tr>
<tr><td>Shifting of calves from quarantine</td><td colspan="2">Within 30 days of negative results.</td></tr>
<tr><td rowspan="3">Action taken on finding positive calf</td><td>TB, JD</td><td>Remove the positive calf and put all the remaining calves under extended quarantine.</td></tr>
<tr><td>Bovine Genital Campylobacteriosis and Trichomoniasis</td><td>Tests conducted only on calves older than 6 months. Remove the positive calf and put all the remaining calves under extended quarantine.</td></tr>
<tr><td>Brucellosis</td><td>Remove the positive calf irrespective of age and put all the remaining calves under extended quarantine.
OR
If the positive calf is less than 9 months old, isolate the calf till it is 9 month old and retest. Calf positive at retesting should be removed.</td></tr>
<tr><td>Extended quarantine</td><td colspan="2">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.</td></tr>
<tr><td>Action on finding a positive during extended quarantine</td><td colspan="2">Same as in Annexure- 3A</td></tr>
</table>

Annexure- 4

Disease testing and management of Bovine Tuberculosis in Semen Station

Name of test	Delayed Hypersensitivity – Single Intra Dermal (SID) Test
Reagent used	Bovine tuberculin PPD
Manufacturer	IVRI, Izatnagar
Testing done	On site, where animals are housed
Result criteria	***Positive:*** Increase in skin thickness of 4 mm or more, or presence of clinical signs viz. exudation, necrosis, pain, and inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation. ***Negative***: Increase in skin thickness less than 2 mm & without clinical signs viz. exudation, necrosis, pain, inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation. ***Inconclusive***: Increase in skin thickness more than 2mm & less than 4mm, absence of above clinical signs, 72 hours post-inoculation. Bull with inconclusive result should be immediately isolated. Only if the animal is negative during the testing in isolation, it should be brought back to the semen station.
Eligible animals	Animals above 2 months of age
Action to be taken on Positive animal	Immediate isolation and removal from herd (within 2 days)

Frozen semen doses of the positive animal	Destroy frozen semen doses of the positive animal since the last negative test.
Positive herd testing	Testing not before 42 days after culling of last positive animal.
Negative herd testing	Six monthly (± 1 week) testing after last whole herd negative testing.
TB free herd	Herd found negative on two consecutive tuberculin tests carried out at an interval of 6 months, the first being performed 6 months after the culling of last affected animal. If frequency of testing is more than two in a year, the testing should establish that all animals in the herd have been negative for the last 6 months beginning from 6 months after culling the last affected animal.

Annexure- 5

Disease testing and management of Paratuberculosis (JD) in Semen Station

Name of test	Delayed Hypersensitivity – Single Intra Dermal (SID) Test
Reagent used	Johnin PPD
Manufacturer	IVRI, Izatnagar
Testing done	On site, where animals are housed
Result criteria	***Positive:*** Increase in skin thickness of 4 mm or more, or presence of clinical signs viz. exudation, necrosis, pain, and inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation. ***Negative***: Increase in skin thickness less than 2 mm & without clinical signs viz. exudation, necrosis, pain, inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation. ***Inconclusive***: Increase in skin thickness more than 2mm & less than 4mm, absence of above clinical signs, 72 hours post-inoculation. Bull with inconclusive result should be immediately isolated. Only if the animal is negative during the testing in isolation, it should be brought back to the semen station.
Eligible animals	Animals above 2 months of age
Action to be taken on Positive animal	Immediate isolation and removal from herd (within 2 days)
Frozen semen	Destroy frozen semen doses of the positive animal

doses of the positive animal	since the last negative test.
Positive herd testing	Testing not before 42 days after culling of last positive animal.
Negative herd testing	Six monthly (± 1 week) testing after last whole herd negative testing.
JD negative herd	Herd found negative 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 more than 2 in a year, the testing should establish that all animals in the herd have been negative for the last 6 months beginning from 6 months after culling the last affected animal.

Annexure- 6

Disease testing and management of Bovine Brucellosis in Semen Station

Name of test	Enzyme Linked Immunosorbent Assay (ELISA)
Sample required	Serum
Eligible animals	All animals. However, animals up to 9 months of age may have maternal antibodies.
Action to be taken on the positive animal	Immediate isolation and removal from herd after castration (within 2 days)
Frozen semen doses of the positive animal	Destroy frozen semen doses of the positive animal since the last negative test.
Positive herd testing	Testing 30 to 60 days after culling of last positive animal.
Negative herd testing	Six monthly (± 1 week) testing after last whole herd negative testing.
Brucellosis free herd	Herd found negative on two consecutive annual tests. If the frequency of testing is more than one in a year, the testing should demonstrate that the herd has been negative for the last one year

Annexure- 7

Disease testing and management of Bovine Genital Campylobacteriosis (BGC) in Semen Station

Name of test	Agent –Identification
Sample required	Preputial washing/ semen
Eligible animals	Animals above 6 months of age
Positive animal	Immediate isolation and removal from herd (within 2 days)
Frozen semen doses of the positive animal	Destroy frozen semen doses of the positive animal since the last negative test.
Positive herd testing	Minimum of 30 days after treatment/culling of last positive animal.
Negative herd testing	Annual (± 1 week) testing after last whole herd negative testing.
Bovine Genital Campylobacteriosis free herd	All animals are negative on two consecutive annual testing.

Annexure- 8

Disease testing and management of Bovine Trichomonosis in Semen Station

Name of test	Agent –Identification
Sample required	Preputial washing
Eligible animals	Animals above 6 months of age.
Action to be taken on Positive animal	Immediate isolation and removal from herd (within 2 days)
Frozen semen doses of the positive animal	Destroy frozen semen doses of the positive animal since last negative test.
Positive herd testing	Minimum of 30 days after treatment/culling of last positive animal.
Negative herd testing	Annual (± 1 week) testing after last whole herd negative testing.
Bovine Trichomonosis free herd	All animals are negative on two consecutive annual testing.

Annexure-9

Management of Foot & Mouth Disease (FMD) in Semen Station

FMD outbreak in semen station	
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 has recovered.
Semen Sale	If frozen semen sale is from the same campus of the SS where FMD is recorded, suspend semen sale till 30 days after the last case has recovered.
FMD outbreak in areas surrounding the SS	
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 semen station through areas of infection.

Annexure - 10

Feeding Growing and Mature Bulls

Daily nutrient requirements of growing and mature bulls *

Body wt (kg)	**gain/day (g)**	**DM/day (kg)**	**C.P. (g)**	**TDN (kg)**	**Ca (g)**	**P (g)**	**Vit. A (1000 IU)**
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
Maintenance of mature breeding 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

Daily ration for Bulls

Body wt.		Calf starter	C.F.	B.P.F.	Hay	Green Fodder
(kg)		**(kg)**	**(kg)**	**(kg)**	**(kg)**	**(kg)**
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	a)	-	-	2.5	2-4	ad lib.
	b)	-	3	-	2-4	ad lib.
600	a)	-	-	2.5	2-4	ad lib.
	b)	-	3	-	2-4	ad lib.
Mature breeding bulls						
500	a)	-	2.5	-	2-4	ad lib.
	b)	-	-	2	2-4	ad lib.
600		----------do----------				
700		----------do----------				

Note : 1) Mineral mixture should be supplemented as follows :

- 50 g mineral mixture for bulls up to 200 kg body weight
- 70 g mineral mixture for bulls between 200 to 350 kg body weight.
- 100 g mineral mixture for bulls above 350 kg body weight

2) Fresh water should be made available 24 hrs.

Green fodder requirement of 10 mature bulls would be approx. 125 MT per year, which can be grown in 1 hectare of land by intensive farming.

*** Source:** Ranjhan, S.K (1980). Animal nutrition & feeding practices in India,
2nd Ed., p196-212

7.2.5. Nutrients available in feed & fod

	Calf starter	**C.F.**	**B.P.F.**	**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