सत्यमेव जयते

Minimum Standard Protocol

for Semen Production and Standard Operating Procedures for Artificial Insemination using Liquid Semen in Pig



Ministry of Fisheries, Animal Husbandry and Dairying
Department of Animal Husbandry and Dairying



Minimum Standard Protocol

for Semen Production and Standard Operating Procedures for Artificial Insemination using Liquid Semen in Pig

Ministry of Fisheries, Animal Husbandry and Dairying
Department of Animal Husbandry and Dairying

राजीव रंजन सिंह उर्फ ललन सिंह
RAJIV RANJAN SINGH ALIAS LALAN SINGH

सत्यमेव जयते

पंचायती राज मंत्री
और मत्स्यपालन, पशुपालन एवं डेयरी मंत्री
भारत सरकार
Minister of Panchayati Raj and
Minister of Fisheries, Animal Husbandry and Dairying
Government of India

DO. No.....MIN PR&FAHD/20.26



MESSAGE

Pig rearing has long been a source of livelihood, nutrition and financial security for millions of rural, tribal and marginal households, especially in the North-Eastern States and tribal belts across the country. The pig is among the most efficient converters of feed into quality animal protein. It reaches market weight early, produces large litters and needs relatively modest land and capital. This makes piggery a strong option for income generation, self-employment and women's empowerment.

India's pig population was about 9.06 million in the 20th Livestock Census (2019) largely held by smallholders rearing pigs in backyard systems. Despite this potential, productivity remains low. Many farmers still rely on natural mating with poor-quality boars leading to inbreeding, slow genetic progress and higher disease risk. Diseases such as African Swine Fever have further shown the need for safer breeding practices and stronger biosecurity.

Artificial Insemination (AI) using liquid semen is one of the most practical solutions to these challenges. A single superior boar can serve many sows, taking quality genetics to the farmer's doorstep without moving live animals across regions. This improves litter size and growth, lowers the cost of maintaining breeding boars and reduces disease transmission associated with natural service.

For AI to succeed, quality must be assured at every step, from boar selection and semen collection to processing, storage, transport and insemination. This publication lays down the Minimum Standard Protocol and Standard Operating Procedures needed to establish uniform, technically sound semen laboratories and AI services across the country. It will guide State Governments, veterinary professionals and AI technicians in delivering reliable, scientific and hygienic breeding services.

I congratulate the Department of Animal Husbandry & Dairying and the ICAR–National Research Centre on Pig for this timely effort. I am confident that it will strengthen the pig sector, raise farmers income and contribute to nutritional security and the vision of a Aatmanirbhar and Viksit Bharat.

(Rajiv Ranjan Singh)

Room No. 234, B- Wing, Krishi Bhawan, Dr. Rajendra Prasad Road, New Delhi-110 001 (India),
Ph. +91 11-23380780/81/82 Fax: +91 11- 23380783 Resi. 011-23782781/82

प्रो. एस. पी. सिंह बघेल
राज्य मंत्री
मत्स्यपालन, पशुपालन एवं डेयरी
एवं
पंचायती राज मंत्रालय
भारत सरकार

सत्यमेव जयते



Prof. S. P. Singh Baghel
Minister of State
Fisheries Animal Husbandry & Dairying
and
Ministry of Panchayati Raj
Government of India

MESSAGE

The livestock sector is a pillar of rural livelihoods, and piggery holds special promise for small and marginal farmers, landless families and tribal communities. Pigs need little space, adapt well to local resources and give quick returns, making pig farming a dependable source of supplementary income for many households.

The sector, however, still faces gaps. Superior breeding boars are scarce in the field, and scientific breeding practices are not widely adopted. AI infrastructure is limited, and farmers often lack access to quality semen and trained technicians. As a result, many pig farmers cannot benefit from genetic improvement, and productivity remains low compared with its potential.

The Government of India, through the Department of Animal Husbandry & Dairying and schemes such as the National Livestock Mission, is committed to promoting breed improvement and organised, quality-assured breeding services. Liquid semen AI is well suited to pigs. It makes wider use of superior boars possible, lowers the cost of breeding and helps improve litter size, growth rate and overall farm profitability.

The publication "Minimum Standard Protocol for Semen Production and Standard Operating Procedures for Artificial Insemination using Liquid Semen in Pig" comes at the right time. It sets uniform standards for laboratory infrastructure, semen production, quality control, biosecurity, transport and insemination. These standards will help States set up dependable semen laboratories and train skilled AI service providers, so that quality genetics reaches the last mile.

I appreciate the efforts of all the scientists, officers and experts who prepared this document. I hope State Governments, veterinarians, AI workers and progressive farmers will make full use of it. Its wide adoption will improve reproductive efficiency, raise farmers' income and support the sustainable growth of the pig sector.

(Prof. S. P. Singh Baghel)

Office : Room No.199-J, 1st Floor, Krishi Bhawan, New Delhi-110001
Residence : 7, K, Kamraj Lane, New Delhi - 110011
Phone : 011-23380378, 23380379, E-mail : mos-fahd@dahd.nic.in

नरेश पाल गंगवार, भा.प्र.से.
NARESH PAL GANGWAR, IAS
सचिव
SECRETARY

सत्यमेव जयते

भारत सरकार
मत्स्यपालन, पशुपालन एवं डेयरी मंत्रालय
पशुपालन एवं डेयरी विभाग
Government of India
Ministry of Fisheries, Animal Husbandry & Dairying
Department of Animal Husbandry & Dairying
218, A-Wing, Krishi Bhawan
New Delhi-110001



Message

Pig farming plays an important role in improving livelihoods, nutritional security and income generation, particularly for small and marginal farmers, tribal communities, women and rural entrepreneurs. With its high reproductive efficiency, rapid growth rate and relatively low investment requirements, piggery has immense potential to contribute towards rural economic development and diversification of the livestock sector.

Genetic improvement is one of the most effective means of enhancing productivity in pig production. Artificial Insemination (AI) using quality liquid semen enables wider dissemination of superior germplasm, reduces dependence on breeding boars and strengthens scientific breeding programmes. The expansion of AI services, however, requires standardized infrastructure and technically sound operational practices to ensure quality, uniformity and reliability.

The Department has therefore brought out this publication, **"Minimum Standard Protocol for Semen Production and Standard Operating Procedures for Artificial Insemination using Liquid Semen in Pig."** Besides providing comprehensive guidance on semen production, quality evaluation, preservation, transportation, biosecurity and AI procedures, the publication lays down the Minimum Standard Protocol for establishing Pig Semen Collection and Processing Laboratories. It provides guidance on laboratory planning, infrastructure, equipment, quality assurance and operational procedures, enabling States and institutions to establish technically sound laboratories based on uniform national standards. Such standardization will ensure consistency in semen quality and strengthen AI services across the country.

I am confident that this publication will serve as a valuable reference for State Animal Husbandry Departments, veterinary institutions, breeding centres, AI technicians, researchers and other stakeholders engaged in pig development. Its effective implementation will strengthen scientific breeding programmes, accelerate genetic improvement, enhance productivity and contribute towards making pig farming a more profitable and sustainable enterprise.

I compliment the officers of the Department, technical experts and collaborating institutions whose collective efforts have made this publication possible.

(Naresh Pal Gangwar)

e-mail : secyahd@nic.in, Tel.: +91-11-23382608, +91-11-23385360
Website : https://dahd.nic.in

डॉ. नवीना बी. महेश्वरप्पा
Dr. Naveena B. Maheswarappa
पशुपालन आयुक्त
Animal Husbandry Commissioner
Tel. : 011-21401453

सत्यमेव जयते

भारत सरकार
मत्स्यपालन, पशुपालन एवं डेयरी मंत्रालय
पशुपालन एवं डेयरी विभाग
कृषि भवन, नई दिल्ली–110001
Government of India
Ministry of Fisheries, Animal Husbandry & Dairying
Department of Animal Husbandry & Dairying
Krishi Bhawan, New Delhi-110001
Website : https://dahd.nic.in



FOREWORD

Pig production is an important livelihood activity for rural, tribal and smallholder farming communities. However, the sector continues to face challenges related to limited availability of superior breeding males, low adoption of scientific breeding practices, inadequate AI infrastructure and difficulties in maintaining the quality of semen during storage and transport. Artificial Insemination offers a practical solution to these constraints by enabling wider use of superior boar germplasm, improving breeding efficiency and supporting genetic upgradation at the farmers' doorstep.

The publication "Minimum Standard Protocol for Semen Production and Standard Operating Procedures for Artificial Insemination using Liquid Semen in Pig" has been developed to provide practical, field-oriented and technically sound guidance for promoting artificial insemination in pigs. It explains the complete process from selection and management of boars to semen collection, semen evaluation, use of extenders, semen preservation, transportation, heat detection and actual insemination procedure. The document also includes laboratory hygiene, quality control, biosecurity, disease testing and post-insemination follow-up measures, which are essential for ensuring successful AI outcomes.

Promotion of liquid semen in pig AI has special importance because liquid semen is widely used in commercial pig AI systems and can support field-level services when maintained under appropriate temperature and handling conditions. The protocol provides guidance on semen, quality checks, dose preparation and transport precautions, which are crucial for maintaining semen fertility and ensuring better conception results.

This document will be beneficial for State Animal Husbandry Departments, veterinary officers, laboratory personnel, AI technicians, extension workers, pig breeding centres and farmers. It will help in strengthening technical capacity, improving semen quality, standardizing AI services and ensuring hygienic and scientific breeding practices in piggery.

I hope this publication will support the expansion of AI services in pigs, improve reproductive performance, enhance productivity and contribute to better income generation for pig farmers. By promoting scientific breeding practices and quality liquid semen-based AI, the Department moves closer to strengthening piggery as a profitable and sustainable livestock enterprise.

Naveena 6/8/26

(Naveena B. Maheswarappa)

डॉ. मुथुकुमारसामी बी., भा.प्र.से.
Dr. Muthukumarasamy B., IAS
संयुक्त सचिव
Joint Secretary

सत्यमेव जयते

भारत सरकार
मत्स्यपालन, पशुपालन एवं डेयरी मंत्रालय
पशुपालन और डेयरी विभाग
Government of India
Ministry of Fisheries,
Animal Husbandry & Dairying
Department of Animal Husbandry & Dairying
190-A, F-Wing, Krishi Bhawan,
New Delhi-110001



PREFACE

The promotion of scientific breeding technologies is a key priority of the Department of Animal Husbandry & Dairying for enhancing livestock productivity and improving farmers' income. Artificial Insemination (AI) using liquid semen provides an efficient and economical approach for accelerating genetic improvement in pigs, expanding access to superior germplasm and strengthening organized breeding services across the country.

This publication, **"Minimum Standard Protocol for Semen Production and Standard Operating Procedures for Artificial Insemination using Liquid Semen in Pig."** has been developed as a standardized technical guide for promoting quality-assured pig breeding services. In addition to prescribing Standard Operating Procedures for Artificial Insemination, it lays down the Minimum Standard Protocol for establishing Pig Semen Collection and Processing Laboratories. The document provides guidance on laboratory planning, infrastructure, equipment, semen production, quality assurance, biosecurity and operational procedures, enabling States and institutions to establish technically sound laboratories with uniform standards and consistent practices across the country.

The protocol is intended to serve as a practical reference for State Animal Husbandry Departments, veterinary officers, semen production laboratories, AI technicians and other stakeholders involved in pig breeding programmes. Its adoption will strengthen technical capacity, improve service quality and facilitate systematic genetic improvement of the national pig population.

I place on record my sincere appreciation to the Dr Vivek Kumar Gupta, Director, ICAR–National Research Centre on Pig, for providing technical leadership in the preparation of this publication. I also acknowledge the valuable contributions of Dr. Sunil Kumar, Dr Rafiqul Islam, Scientist, ICAR–National Research Centre on Pig; Dr.Sujit Dutta, Joint Commissioner, DAHD; Dr.Pramod Kumar Kaushik, Assistant Commissioner, DAHD and Dr. Chandra Prakash Dixit, Livestock Officer, DAHD, whose expertise, coordination and dedicated efforts have been instrumental in developing this protocol.

I am confident that this publication will facilitate wider adoption of scientific AI practices, establishment of standardized pig semen laboratories and strengthening of breeding programmes, thereby improving reproductive efficiency, productivity and the livelihoods of pig farmers while contributing to the sustainable growth of the pig sector in India.

(Dr. Muthukumarasamy B.)

E-mail : js-nlm@dahd.nic.in, Tel.: +91-11-23382354 (O)
Website : https://dahd.nic.in

Content

1.	Introduction to Artificial Insemination in Pig	**1**
2.	Liquid semen production Laboratory in pig setup	**2**
3.	Material used in Liquid semen production Laboratory in Pig	**7**
4.	Training of Boar	**13**
5.	Semen collection and handling of semen	**14**
6.	Basic Semen Evaluation tests and importance	**17**
6(i).	Estimation of Sperm Motility	**21**
6(ii).	Estimation of Sperm Concentration	**22**
6(iii).	Sperm Viability and Morphology Evaluation	**22**
6(iv).	Estimation of Sperm Acrosomal Integrity	**25**
6(v).	Hyposomotic Sperm Swelling Test (HOST)	**26**
6(vi).	Microbiological Evaluation of Semen	**27**
7.	Extenders used for Liquid semen for A.I.	**29**
8.	Semen Extension Process	**31**
9.	Storage/preservation and Transportation of liquid semen	**33**
10.	Heat Detection	**36**
11.	Time of Artificial Insemination	**38**
12.	Standard operating procedure for performing A.I.	**39**
13.	Post Artificial Insemination Measures	**41**
14.	Diseases transmitted through semen and Diagnosis	**43**
15.	Laboratory Quality Management	**46**
16.	Boar Management	**56**
17.	Biosecurity and Quarantine Procedures	**60**
18.	Standards/Criteria for Selection of High Genetic Merit (HGM) Breeding Boar	**62**
19.	Reproductive Parameters of Pig- Annexure I	**66**
20.	Vaccination and deworming Schedule for Pig - Annexure II	**68**
21.	Body Condition Score Card- Annexure III	**69**
22.	Registered Breeds of Pig- Annexure IV	**70**

1. Introduction to Artificial Insemination in Pig

Artificial insemination is a technique in which semen with live sperms is collected from trained boar, then evaluated, processed and introduced into female (gilt/sow) reproductive tract during proper time of estrus with the help of instruments under hygienic conditions that results in the formation of a viable offspring. The technique is first generation and oldest reproductive biotechnology for rapid genetic improvement which has reached to the farmer's doorstep. Artificial Insemination (A.I.) is used worldwide. However, A.I. in pigs is still in infancy stage. As per recent livestock censuses, consecutive decline trends have been observed in pig population across the country. But a rise in pig population has been observed in Assam state that is mainly use of A.I. in this state as compared to others. Performing artificial insemination in pig is very simple as compared to cattle and buffalo. Artificial insemination in pigs is mainly done using liquid semen while A.I. with frozen-thawed semen is still in infancy stage. Only less than 1 percent of artificial inseminations practiced using frozen thawed semen in commercial application. The remaining 99% is preserved in liquid form at 16–20 °C. Production purpose is only for genetic transfer and conservation which is limited due to the high cryodamage's, complicated process freezing, reduced farrowing rates and litter size.

Advantages of Artificial Insemination in Pig

- Faster genetic improvement can be achieved through A.I. technique by improving the reproduction, production, carcasses, feed conversion and adaptability traits.
- AI can be instrumental in crossbreeding programme at larger scale for synthetic breed developments.
- AI can be done at distant places using semen from superior quality boar. It can reduce the import cost of live animals.
- Sexually transmissible disease can be prevented.
- A.I. overcomes the size differences between male and female.
- Record keeping for production and pedigree sheet gets improved, therefore dam and sire lines can be maintained very well.
- For small and marginal farmers, A.I. is highly cost-effective method of reproduction, because it eliminates the cost of feeding and management of a breeding boar.
- It minimizes the management cost in conjunction with synchronization technique for induction of estrus / heat, where a group / number of sow can be bred at a predetermined, therefore resulting farrowing in more synchronous way.
- Semen evaluation in A.I. allows investigation of low fertile boars at an early stage.
- A.I. provides breakthrough or essential for further assisted reproductive technologies such in vitro fertilization, sperm sexing, transgenic embryo production etc.

A.I. in Pig in India

Pig production is an important secondary occupation for livelihood and nutritional security of the rural people, especially in North-Eastern region of India. Pig rearing has been integral part of their way of life since time immemorial. Approximately 80% of tribal population in Northeastern India is involved in raising pigs on a small scale (free range scavenging system) which are mostly reared under semi-intensive system of management. Pigs are concentrated in the North-Eastern Region where almost more than 40% of the country's total pig population exists. 80% of the pigs are indigenous non-descript type. As per the 20th livestock census, the pig population is 9.06 million and is presently showing a decline trend. One of the reasons behind declining pig population is lack of breedable males for multiplying the population. Only 3-4 or very few males are reared in a village, after which mating doesn't occur at the time of heat. The artificial insemination is the technology by which this problem is solved because the semen for service can be provided at any time and at any place at farmers' doorstep. The technology aims in reduction of cost of keeping & feeding males, genetic improvement, overcoming the size of male and female and it can be done at farmers doorstep. Main constraints for A.I. in developing countries like India are

1. Lack of suitable transportation system for maintaining the semen preservation temperature till at the site of insemination.
2. Lack of sufficient number of A.I. laboratory/centers across the country to supply the liquid semen doses.

Limitations of AI

1. Improper cleaning of the instruments and unhygienic conditions may lead to lower fertility.
2. The market for the boars is reduced while that for the superior germplasm is increased.
3. The selection of the sire should be very rigid in all respects.
4. Preservation and transportation of semen is difficult under severe climatic conditions like prevailing in most parts of India.
5. Initial investment is more and requires infrastructure facilities.

2. Liquid semen production Laboratory setup

A proper lab design sets the stage for high quality as efficient semen dose production. Lab design affects hygienic production and efficient workflows. Lab design details often have to be decided on a case-by-case basis depending on the unique situation encountered at each stud (space, staffing, budget etc.). The major parts of laboratory include semen processing area, sterilization room, storeroom and office. The collection is better to place in conjunction with semen processing area through a pass box. However, if distance from boar shed is far,

then in such situation, making pass box is not good. However, it should be ensured that the semen collection should be hygienic and should be transported as soon as possible to semen processing area.

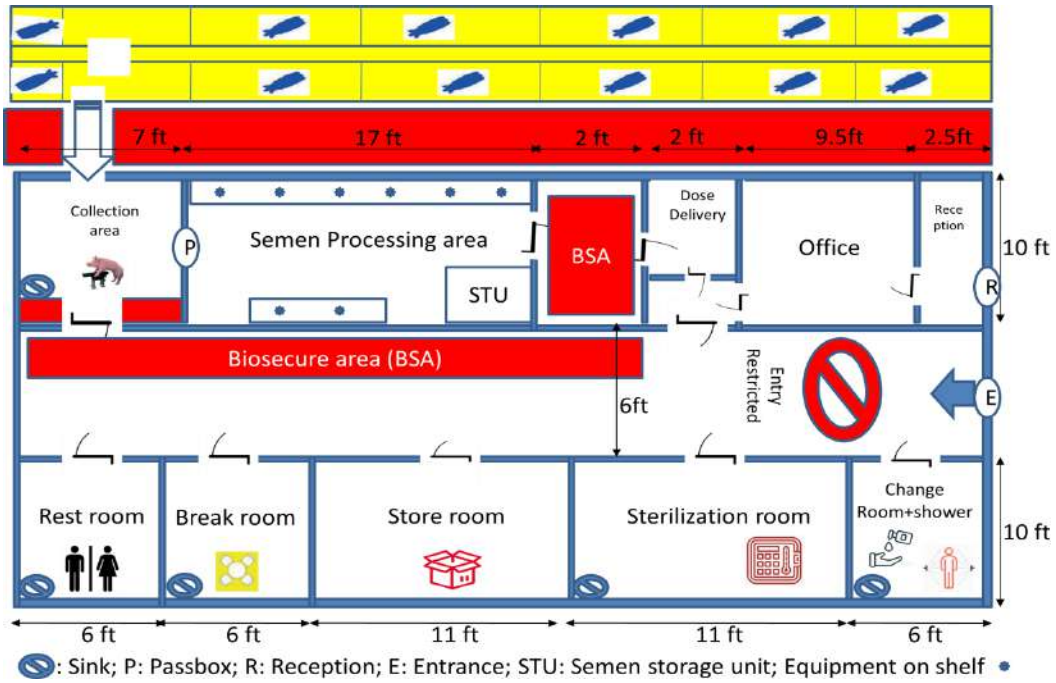


Fig 2.1. Showing minimal essential infrastructure for construction of A.I. Lab. on small scale. The dimensions can be modified as per budget and space availability.

Table 2.1: Principles of A.I. Laboratory Designs

Principle	Explanation
Separation between production lab and cleaning area (wet lab) Supply storage room Semen storage (cool) room / office Break room Sanitary rooms Facility rooms (water, power, compressor, etc.)	The production lab is uniquely used for the evaluation and processing of semen. The layout is set up in a way that this room is easy to clean and disinfect. To prevent contamination of the processed semen, all other activities require rooms separate from the production area.
Place only items/machines essential for Production in lab.	The less items or equipment ease the cleaning process of the lab.
Have short distances between working stations.	Allows for faster processing.
In-line-workflow.	No crossing of working ways from “dirty” (ejaculate arrival, evaluation) to “clean” (extension, filling) areas to avoid cross contamination.
Replace air condition filters regularly.	Less distribution of dirt/bacteria via air.

Lab furniture movable (wheels)/open space to floor.	Allows to clean area beneath.
Avoid storage cabinets in the production lab. Work with mobile carts containing daily supplies.	Cabinets need to be cleaned as well and this is often not done on a regular basis.
All surfaces (ceiling, walls, floor, countertops and furniture) composed of the appropriate materials and constructed for ease of cleaning and compatibility with disinfection.	Facilitate proper cleaning and disinfection.
Allow distance between wires/pipes and the wall / floor.	Facilitates easy access to clean.
No 90° angles between floor and wall.	Facilitates cleaning.
Floor coating without seams and drains.	Facilitates easy cleaning and no gathering of dirt and bacteria.
Power sockets above equipment area.	Facilitates cleaning with no wires on the floor or against the wall or on the horizontal surfaces.
Separate rooms for storage of filled semen doses and lab supplies.	Facilitates cleaning.

Practices for Good Laboratory Hygiene in A.I. Laboratory (Source: DAHD, Govt. of India)

Besides the aforementioned rules to set up a proper lab, a variety of practices help to reduce bacterial load and cross contamination during semen processing:

Premises

a. Sufficient trees should 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 should be sealed to control pests and insects.

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

d. Laboratory windows should preferably be made of double sheet glass with fixed aluminum frame. The glass panes should 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 20ºC - 22ºC. The number of ACs to be fixed to sustain this temperature should depend on the size of the processing room. Maintaining this temperature is most important to achieve the best results. The flow of air from AC must not be towards the front side of the Laminar Air Flow Unit. Adequate number of thermometers should 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 should be decontaminated routinely with a disinfectant. Sink should not be placed in the semen processing room.

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

h. Unwanted furniture, equipment and materials should 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 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 2000 hours. A logbook should be maintained for timely replacement of UV lights. The laboratory should be fumigated twice a week with Cold Fumigant, using humidifier.

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

k. The work platform, the parts of equipment and other items to be handled during processing of semen, should be cleaned with 70% alcohol. It is advisable to repeat cleaning schedule after completing processing of semen.

l. Clean laboratory footwear, aprons, hand gloves, mask and caps should be compulsorily put on while working in the laboratory.

m. Unpack supplies from outer packaging (if possible) prior to entering the laboratory.

n. Long exposure of semen to ultraviolet rays, visible light in direct sunlight and white fluorescent light causes chromosomal damage and hence, direct exposure to such

sources of light should be avoided. Hence, there should be provision for indirect or diffused lighting inside the semen processing room.

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. Make it mandatory to wash hands and disinfect prior entering the laboratory.

Hands should be washed with soap and water and rinsed with 70% alcohol, before commencing work in the laboratory. Restricted entry inside the semen processing room and storage room should be strictly adhered to. No food or beverages in the laboratory (can be consumed in the Rest/break room) should be consumed. Wash hands and disinfect after meals and after visiting the restroom.

Semen

- If semen pass-through window is used, never open both sides at the same time.
- Do not store unnecessary items in the pass-through window.
- Nothing from the barn besides the ejaculate (in a thermos/plastic bag or comparable) should enter the laboratory.
- If people have to transfer from the barn to the laboratory on the same day (not preferred), make it mandatory to shower and change clothes.
- Don't touch anything that comes in direct contact with semen/extender with bare hands.
- Use disposable plastic bags that will contain ejaculate/semen. This will reduce the risk for cross contamination and limit cleaning.
- Remove spilled ejaculate/extender from countertops immediately with paper towel/ tissue and disinfected with alcohol wipes.
- Filter pressurized air prior to use (i.e. pneumatic tube systems, filling/packing machines).
- Dispose of trashed semen and residual extender after production in the toilet or an alternative disposal place outside the production lab or in the sink that is used only for cleaning.
- Sanitize hands in between ejaculates being processed and every time there is any possibility of contamination.

Table 2.2 Estimate of manpower requirement for boar semen production

Designation	10 Boars	>10-20 Boars	>21-30 Boars	>30Boars	>50 Boars
General Manager	1	1	1	1	1

QCO/QAO	1	1	1	1	1
Vet. Officer	1	1	2	2	2
Data Mgmt. Officer	--	1	1	1	1
Accts. & Admin	--	1	1	1	1
Office Assistant	1	2	2	3	3
Livestock Assistant	1	2	2	3	3
Lab Technician	1	2	3	3	2
Lab Attendant	2	2	2	3	3
Boar Attendant	1 person per 10 Boars				

3. Material used in Liquid semen production Laboratory in Pig

In the present chapter, the list of essential equipment's and consumables are shown, the quantity of the equipment's may be modified depending on the capacity of the boars to be maintained. The cost of equipment's is tentative, which may vary depending on the capacity, purchasing firm, quantity and quality of the equipment used/purchased.

Required Equipment and Quantity
Dummy sow (02) • It should be heavy & comfortable to boar. • Should be of adjustable height. • Separate dummy should be used for training. • Dummy can be designed or may be purchased.
Hot air oven (02) • Used for sterilization of glassware, thermos flask and other laboratory consumables.
Double (better tripled) distilled or Ultrapure water filtration (01) • Used for water purification needed for extender preparation and other laboratory use. • Quality of water is one of the critical factor for extender quality. • If not ultrapure, at least double distilled water should be used. • Water qualifying for standard parameters should be used.
BOD Incubator (120-150 liters) (01) • Used for holding semen at particular temperature for particular time.
Autoclave (30/40 lit) (01) • Used for sterilization of extenders, consumables, Buchner's funnel having multilayered miracloth. • If commercial sterilized filters are used, no need to place miracloth in Buchner's funnel.
Air Conditioner (2 units) • Should be in semen processing area of laboratory.

Biosafety Cabinet/Laminar Air Flow (01) • Used to prevent microbial contamination and improving the quality control of laboratory.
• Extension of semen should be carried out under Laminar air flow.
pH meter (01) • For estimation of pH of different solutions, extenders prepared in the laboratory.
Refrigerator (01) • For keeping some chemicals and prepared solution.
Microscope with biotherm (01) • For semen evaluation, phase contrast is best. • Biotherm stage must control temperature for warming glass slides.
Domestic polythene sealing machine (01) • For sealing of semen dose packet. • If commercially available semi-automatic filling and sealing machine is used, then there is no need of Domestic polythene sealing machine. However, domestic polythene sealing machines are very cheap and require very low maintenance.
Analytical weighing balance (01) • For weighing chemicals for the purpose of preparation of extender, stains and other solutions.
Semen Preservation Cabinet/box with temperature 15/17^{o}C (02) • Used for storage or preservation of semen doses. • Smaller boxes can be used to transport the semen doses.
Water bath (1) • A water bath with precise temperature and time control should be purchased.
Magnetic Stirrer with Hot plate (1) • For ready to use extender mixing.

Care and Maintenance of Equipments

a) The exterior 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 intervals to assess bacterial load of the air passing through the filters.

c) If Digital photometer / Computer aided Spectrophotometer are used, these shall be validated with Haemocytometer readings for sperm concentration twice a year (minimum 30 samples).

d) The Semi-automatic semen packet 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) or isopropyl alcohol or absolute ethanol or as per manufactures guidelines for lens cleaning.

f) BOD Incubators for semen holding shall be cleaned and disinfected with 70% alcohol at weekly interval.

g) 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.

h) 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.

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

   1. Standard Thermometer.
   2. Water Bath.
   3. Weighing Balance.
   4. BOD Incubator.
   5. Autoclave.
   6. Hot Air Oven.
   7. Slide Warmer.
   8. Micropipettes.
   9. pH Meter.

j) The following equipment calibration needs to be certified by manufacturer/supplier:

   1. Laminar Air Flow Unit.
   2. Filling & Sealing Machine.
   3. Photometer, if used.
   4. Triple distillation unit, etc;

k) All equipment used in semen processing should be covered under annual maintenance contracts.

Cleaning and Sterilization

All the items to be washed shall be initially cleaned with running tap water and soaked in warm neutral detergent for at least 30 minutes. These items will then be thoroughly cleaned under running tap water using a brush. Filling nozzles shall be cleaned with pressure using 20 ml

syringe. These materials shall be rinsed thoroughly with de-ionized water (5 to 7 changes) to completely remove detergent residues and other impurities. Appropriate procedure for sterilization of different materials, used in the semen station, is given below:

Laboratory and other areas

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

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 thermos flask 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 aluminum 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 charging will indicate proper sterilization.

Rubber ware

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. Disposable plastic tips should be used. Thermo-resistant rubber wares shall be sterilized by autoclaving at 3- 4 p.s.i. for 10 minutes.

Distilled Water

Fresh triple glass distilled water or Ultrapure water shall be autoclaved at 15 p.s.i. for 15 minutes and used for preparation of the extender or as per manufacturer's guidelines.

Bacteriological Media

For microbiological quality testing, prepared media to be autoclaved at 15 p.s.i. pressure for 15 minutes.

Filter Papers

A bunch of clean filter papers of standard brand like Whatman No. 1 (used in stain preparations; 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. No need to sterilize the filter paper used in semen collection for gel separation as they are already sterilized and are of disposable use.

Chemicals and consumables

The chemicals of only highest purity of either Analytical Reagent (AR) or Guaranteed Reagent (GR), from reputed manufacturing companies should be used. Assay of chemicals should be >99%, having less impurities. Chemicals from Sr. 1-11 are needed, if extender needs to prepare in laboratory otherwise readymade/ commercially available extender can be used. Further, the quantity of other consumables is tentative and generic.

Table. 3.1 Consumables used for semen collection, processing, sealing of A.I. doses and for performing A.I.

S.N.	Chemicals and Stains	Qt
1	Glucose	5kg
2	EDTA (di-Na, di-H2O)	1kg
3	Sodium–citrate	1kg
4	Pot. Sod. Tartrate	1kg
5	Sodium–bicarbonate	1kg
6	Potassium chloride	1kg
7	TRIS	1kg
8	HEPES	25g
9	Citric acid	1kg
10	Cysteine	25 g
11	BSA	10g
12	Streptopenicillin, Gentamicin	As req
13	Any commercial extender	As req
14	Giemsa's stain	25 g
15	Eosin and Nigrosin stains	100 g
16	Aniline blue stain	100g
17	Glycerol	250ml
18	Pot. Permanganate crystals	500g
19	Methanol	1 L
20	Formalin	1 L
21	Sod. Chloride	1kg
22	Ethanol	1 L

S.N.	Glasswares/Plasticware	Qt
1	Measuring cylinder (25/ 100/250ml)	5
2	Conical flask (150/250/500ml)	5
3	Reagent bottle (150/250/500/1000ml)	5
4	Glass Funnel (75/100mm)	5
5	Glass beaker (50/100/250/500ml)	5

6	Glass slide	As req
7	Cover slips (18 x18 mm)	As req
8	Slide box	5
9	Tube stand (1.5/2.0/15/50 ml)	5
10	Micro centrifuge tubes (0.5/1.5/2.0 ml)	As req

S.N.	Miscellaneous items	Qt
1	Povidone iodine	1L
2	Miracloth/Filter paper/sterile gauge	10 mt
3	Bucket	2
4	Face masks, Head Covers, Wrist covers	1000
5	Towel	2
6	Detergent & Hanger for glassware's	As req
7	Cotton and Aluminium Foil	2 roll
8	Gumboots	2x5
9	Aprons	5
10	Carry baskets	5
11	Bottle washing brush (Nylon and rubber)	5

Consumables

Particular	Qt	Particular	Qt.
Cold chain boxes	(02)	Makler's chamber	(02)
Semen dose packet	(2000) (100ml)	Thermos Flask; (05)	Narrow 3; Wide Mouth 2
Foam tip Catheter	(2000)	Buchner's Funnel	05(small diameter)
Microtips	(10/100/200/1000ul)	Pipette stand	(02)
Tissue paper roll/ Kimwip	(20)	Wash bottle	(08)
Adjustable Micro pipettes (04)	(10/100/200/1000ul)	Examination hand gloves	10 Pack (Nitrile/Vinyl)
Hemocytometer	02	B-Gel/Lubricant jelly	05)
Sterile Semen filter paper	500	Miracloth	50 Meters

4. Training of Boar

Materials: Dummy, Gloves, Gumboot, Facemask, Apron, head cover.

Preparation for Training of Boars

- Records of individual boars training and success should be kept.
- Remove any source of distraction in the collection area or trainee boar pen.
- Ensure the boar's own pen/collection pen doesn't allow the boar to wander too much.
- Ensure staff safety. Make sure the boar is comfortable with human contact.
- Have experienced and patient staff conduct training.
- Adjust dummy height to the boar's size (~120° angle rear legs-belly).
- With regard to training a boar for semen collection, the key word is patience.
- Ideally, the boar should be trained when he is between 8 and 10 months of age. Training can take 3-4 weeks and occasionally some boars are never trained.
- It is not imperative to collect the semen in difficult boars it is more important to achieve a comfortable secure erection and ejaculation.
- Boar being trained should be exposed to the dummy sow immediately after collection of semen from an already trained boar.

Procedure for Training of AI Boars:

1. Introduce dummy to boar's own pen. To begin, the boar should be moved to the collection room or pen two or three times at daily intervals, with the purpose of getting him acquainted with the dummy sow.
2. Leave in the pen and allow the boar to investigate the dummy by himself, although keep an eye on him.
3. If the boar shows no interest in the dummy encourage by gently but firmly nudging his head towards the dummy. Rub and pat the dummy. Try sitting on the dummy. Talk to the boar all the time. Do not leave an uninterested boar with the dummy for more than 20 minutes. If he has not worked do not treat with food, however, provide vocal support. If the boar mounts the dummy, slowly enter the area and talk to the boar. Stimulate his sides, testes and prepuce and try to collect from the boar, talk and give encouragement all the time. If the boar has worked, it is treated with food and vocal support.
4. Once the boar mounts, he will usually start thrusting movements. At this stage gently but firmly rub the prepuce with a gloved hand. The boar may express some surprise at first and stop thrusting. Persevere, be sensitive, but keep under control. The boar will gain more confidence in the procedures. If the boar dismounts when attempting to

collect, allow his to dismount. Allow him to resettle and remount and start again. Talk and reassure him. Be his friend. If the boar repeatedly dismounts when attempting to collect him from the side. Allow him to mount and stand directly behind him and reach forward to massage the sheath and apply pressure with your legs to his rear. Allow the penis to erect and continue massage. Once the erect is more secure, then move slowly around the side and continue collecting without causing the boar to dismount.

5. Repeat from #4, four times a week until the boar readily allows you to collect semen.
6. If the boar is reluctant to mount the dummy allow him to watch another boar being worked.
7. When a boar has allowed a collection, collect the next day and then rest for the rest of that week.
8. Work boar once weekly for 4 weeks, then place on standard work rotation (minimum rest 4 days, maximum rest 14 days).

5. Semen collection and handling of semen

Gloved hand method is the most satisfactory method for collecting semen from boars.

Materials: Dummy, Gloves, Gumboot, Facemask, Apron, Head Cover, Narrow mouth thermos flask/container, Buchner funnel, Miracloth/double layer of cheesecloth (or sterile gauze) filter/ commercial filter paper (specific for boar semen, non-woven tissue and non-absorbing), $KMnO_4$ Solution, Rubber mat for boar dummy.

Procedure:

1. Introduce the boar to the collection area and let him investigate.
2. Once the boar identifies and investigates the dummy, he should readily mount.
3. After the boar has become interested and mounted the dummy, approach the boar from the rear.
4. With two vinyl gloves on your hand, gently reach around to the boar's prepuce and massage the penis through the prepuce.
5. The boar will then start to thrust and extend his penis out of the sheath. Remove the outer vinyl glove from your hand.
6. Once the boar extends his penis out of the prepuce, the corkscrew-shaped penile tip should be grasped with the fingers with uniform pressure applied. Some boars require a substantial amount of digital pressure, whereas other boars require minimal digital pressure. The boar is only responsive to pressure applied around the corkscrew-shaped tip of the penis. Pressure applied elsewhere on the penis will elicit a negative response

causing most boars to dismount. With the proper pressure applied, the boar will extend his penis and cease thrusting.

7. After a brief pause, the boar will start to ejaculate. A pre-warmed (38°C) insulated thermos is a convenient and economical semen collection vessel.
8. The first few jets of an ejaculate function to flush out the urethra and should be allowed to go on the ground. These jets are usually emitted while the boar is still thrusting. Always discard the first part of the ejaculate (pre-sperm). It is clear, watery fluid and does not contain sperm, but it may have a high bacterial count. After thrusting has ceased, fluid and gel components are ejaculated by the boar.
9. The gel fraction should be filtered out of the ejaculate during collection using gauze or a mesh filter which has been placed over the mouth of the thermos/cup.
10. Collect sperm-rich fraction into pre-warmed container. It will be very chalky in appearance and contain 80-90% of all sperm cells in the ejaculate. Collect this fraction until it changes to a clearer, watery fluid.
11. Once it is certain the sperm-rich fraction is complete, continue collecting but discard the remainder (post-sperm) of the ejaculate.
12. Remove the filter with gel and discard.
13. Cap the collection container and place it in warm container.
14. Be sure to label the container with the boar I.D.
15. Transport the ejaculate to the processing area.
16. Attempt to schedule procedures so that the ejaculate is extended within 15 minutes after collection.

Semen fractions

Ejaculation starts after the thrusting motions are completed. The ejaculation process can be divided visually into four fractions.

Table 5.1: Boar semen fractions

Pre-sperm	20-25ml	Watery + few gelatin	Discard
Sperm-rich	40 to 100 ml	viscous-chalky, milky-white	collected
Sperm poor	70 to 400 ml	opaque, chalky gray	collected
Gelatinous	20 to 40 ml	sticky substance	filtered

Collection frequency:

Table 5.2: Suggested semen collection frequencies for A.I. boars based upon boar age

Boar age (months)	Semen collection frequency
<8	Once every 7–10 days
8–10	Once every 7 days
10–12	Up to three times in a 14-day period
12+	Up to two times in a 7-day period

Handling of semen

The sensitive nature of the sperm cell makes it more important that semen receive the utmost care during collection and handling. Take the following precautions to ensure the maintenance of fertility of the sperm cells and prevent low conception:

- Protect sperm cells from extreme heat or cold. Sperm cells are very sensitive to sudden changes in temperature. The collection equipment and all materials used in extending the semen should be at body temperature (102 °F). During cold weather collections should be made inside a building to protect the semen from the temperature.
- Prevent contact with water and harmful chemical agents. Wash and dry the dummy and the boar's underline if needed. Make certain that the collecting, extension and inseminating equipment and utensils are washed, rinsed and dried according to instructions.
- Avoid exposing semen to direct sunlight.
- Exposure to air should be minimal.
- Handle semen gently. Stir or swirl. Do not shake.
- ID number of the boar and date should be marked immediately after collection.

Table 5.3: General Recommendations for Hygienic Collection

Area	Recommendation
Boar housing	• Ensure dry and clean lying area for boars. • Exchange bedding material regularly. • Regularly execute complete cleaning and disinfection of the whole barn. • Schedules differ depending on factors like bedding material and humidity. Minimum 1x/year.

Boar	• Have the boar enter collection area free from feces, bedding material etc. • Clean and dry his belly/underside if needed. • Trim preputial hair on regular basis.
Collection area/ Dummy	• Keep separated from housing area, if possible. • Clean disinfect and dry after every production day (power wash, detergent, disinfectant). • Emphasize cleaning the underside of the dummy (flip dummy up for cleaning, if possible). • Replace dummies with deep scratches on surface.
Utensils (Cups, gloves, ...)	• Store all material nearby the collection area in a closed cabinet to minimize contamination. • Prepare collection cups (styrofoam and gauze/filter) in a clean environment. • If collection bags with integrated filters are used, consider preparation in the barn. • Don't touch collection filters and inside of the cup/bag with your hands. • Use a warm, insulated flask to hold the collection cup/ bag.
Collection technique	• Refer to the chapter "Semen Collection".
Warming cabinet	• Clean, disinfect and dry after every production day (detergent-dry-alcohol).
Insulated flasks	• Clean, disinfect and dry after every production day (detergent-dry-alcohol).

6. Basic semen evaluation tests and importance

Semen evaluation is not a sure indicator of fertility. The only way to be certain is to test-mate prior to the breeding season. Test-mating does not, however, ensure that the boar will remain fertile for the entire season. Evaluation of boar semen for measures of fertility is an important component to success with artificial insemination (AI). Always keep in mind the two basic principles of semen collection and processing-

1. Use hygienic techniques and
2. Control temperature changes.

Semen evaluation should be conducted as rapidly as possible after collection to ensure that the evaluation is representative of the sample as ejaculated and to preserve the original quality

of the ejaculate. When evaluating semen, keep in mind whether the sperm-rich fraction or the sperm-rich plus sperm-poor fractions are being evaluated. There is a substantial difference between the two collections in terms of sperm concentration and color.

Macroscopic evaluation

Volume

Semen volume should be measured in a prewarmed (102 °F) graduated conical flask/cylinder/beaker just prior to being extended or inseminated. However, the volume may be measured by weighing the ejaculate considering 1 gram equal to 1 mL. Usually, the gel free semen volume is considered for extension purpose. The gel volume should be separately measured. The volume of ejaculate is considered including the volume of gel. The volume normally ranges from 40 to 100 ml for sperm-rich fraction and from 110 to 500 ml for the sperm rich plus sperm-poor fractions. On an average 250 ml is the total volume of boar ejaculate.

Appearance and color

Good quality semen should have a chalky, milky appearance. This shows sperm concentration is high. As sperm concentration gets lower, the semen becomes more transparent. The normal whole boar ejaculated must be of considerable volume (>150 ml) and depending on the concentration, should be a milky white color. Occasionally small amounts of blood, usually originating from the urethra, may be present in the ejaculate, which gives the semen a pinkish hue. This normally does not reduce the fertility or the viability of the ejaculate, but a darker red color associated with a pungent odor should be cause for discarding the collection.

Odor/smell

Good quality boar semen does not have an offensive smell; however, if fluids from the preputial diverticulum or urine have infiltrated, the semen will have a bad smell and should be discarded. The emitting odor is most likely reflective of prepuce fluids (urine) which are generally heavily laden with bacterial and foreign contaminants. Ejaculates must be evaluated for deviating odors and discarded if detected.

Table 6.1: Macroscopic semen evaluation

Factor	Normal	Abnormal
Color	Chalky milky	Red or brown or yellowish (all variations)
Consistency	Creamy, milky	Water-like, flaky
Impurities	None	Blood, pus, urine, feces, others
Odor	Neutral	Urine, feces, putrid

Microscopic examination

A microscope is essential to make an artificial insemination program work, it is useful to verify concentration and motility, as well as to check for abnormal sperm. An expensive microscope is not essential, but one that has two or three power settings ranging from 100x up to 1000x is needed. Accurate sperm counting and other parameter evaluation requires additional equipment. Semen should remain in the collection container during the microscopic evaluation phase. This allows the temperature to gradually decrease and helps prevent damage to sperm cells by light and air. Before removing any semen sample for evaluation, thoroughly mix semen by gently swirling the container.

Motility

Semen sample analysis for motility is highly valued as a measure for identifying samples of poor quality. The measure of motility must be greater than 80% to reach acceptable fertility limits. At low magnification, samples are often graded as good or poor, or some percentage is assigned by subjective viewing. At high magnification, count 10 cells (motile and non-motile cells) in a defined field. This process is repeated in fields 5 to 10 times to get an estimate that is more precise. The computer assisted semen analysis (CASA) machine also measures motility as well and is highly accurate and fast in tracking each sperm cell. Specified procedures are followed for each machine.

Concentration

Concentration is an essential measure of total sperm cells in the ejaculate. This measure is important for determining the number of doses and how many sperm (motile and normal) will be in each dose. The three primary ways of measuring concentration include: hemacytometer, photometer and CASA.

Sperm cell morphology

Sperm morphology and acrosome integrity also affect tools to estimate semen viability and can also provide more information about the ejaculate in terms of its quality than is possible with just a motility evaluation.

Like motility, it appears that a certain percentage of normal morphological spermatozoa are needed in an AI dose to optimize fertility rates. Semen collections with less than 70 % normal morphological sperm can be identified as inferior collections if the semen is used at or below this level.

Acrosome Integrity

Although acrosome evaluations are time consuming and require a more advanced type of

microscope (phase contrast) and technique to operate the scope, some research suggests that acrosome integrity may be a better indication of sperm quality than motility.

Microbiology

Furthermore, routine periodic semen culturing is becoming standard operating procedure in most boar studs and should be conducted in on farm studs to monitor facility sanitation and hygiene processing procedures. Bacteriology cultures are fairly inexpensive and are performed at all diagnostic laboratories. Even though contamination of semen with bacteria is almost inevitable, there is very little scientific information regarding interactions between type and level of bacteria and fertility. However, since bacterial contamination has been associated with decreased storage life, clumping and persistent uterine infections, it is necessary to conduct periodical microbiological tests of semen.

Minimal and limiting Parameters

It is important to remember that some of these measurements are difficult to determine accurately and morphological and acrosome quality should not be approached as absolute values.

Table 6.2. Initial Semen Quality Standards and suggested value limit for semen used within 24 hours after collection

Ejaculate Characteristic	Normal Value	Limit Value
Ejaculate Volume[a]	100-500 ml	50 ml
Total Sperm per Ejaculate (x 10^9)[a]	10-100	10
Progressive Motility[c]	70-95%	62 %
Clumping (% coverage of microscopic field)	0-10 %	25%
Curled Tails	1-2 %	10 %
Morphological Abnormalities[c]	5-10 %	30 %
Acrosome Abnormalities[c]	5-10 %	49 %
Cytoplasmic Droplets	< 5 %	15 %
[a]Data from Larsson K: Current Therapy in Theriogenology, 2nd ed. P.972.		
[b]Pork Industry Handbook no. 136. Semen collection, evaluation and processing in the boar.		
[c]Flowers, 1998.		

Basic performa to be filled for record of each semen collection from each Boar

Boar identification: Breed of Boar:	Age of boar in months: As of date:

Date of examination		Gel-free volume, mL	
Abnormal color		Abnormal odor	
Motility, %		Concentration per mL	
Normal sperm, %		Proximal droplets, %	
Distal droplets, %		Abnormal midpiece, %	
Tail bent at midpiece		Short/twin tails, %	
Entire tail coiled under midpiece, %		Entire tail coiled around sperm head, %	
Loosened neck, %		Tail without head, %	
Head without tail, %		Giant, small, pyriform, head	
Elongated, or twin heads, %		Loosened acrosome, %	
Lost acrosome %		Knobbed, acrosome %	

6 (i) Estimation of Sperm Motility

Sperm mobility is highly valued as a measure for identifying samples of poor quality. Motility is the general ability of sperm to move, normally expressed as a % of total motile sperms while progressive motility is the ability of sperm to move in a forward direction, normally expressed as % of total sperm.

Materials: Compound microscope with biotherm stage, glass slide, cover slip, micropipettes.

Procedure: To measure motility, the following procedures are used for semen samples:

1. Take a clean, grease free glass slide and cover slip and warm at 37°C for 5 minutes.
2. Take a drop of thoroughly mixed semen (7-10 µl) and put on prewarmed slide. Add a cover slip.
3. Evaluate sample at low (100 X) or high (>400 X) magnification for measures of motility on a 0 to 100% basis. It will be observed as for moving cells by differentially counting 10 sperm, distinguishing between moving and immotile (still) cells. Then quickly move to another area of the slide and count 10 more. Do this 10 times and then total the number of motile and non-motile cells. Number of live cells will then be the percent motility.

Interpretation

- Always consider progressive motile sperms for further processing.
- Ejaculate having sperms with circular motility pattern should not be considered.
- Preliminary morphological estimation particularly for sperm tail may be seen while motility estimation.
- Gel mass particles are also seen while motility estimation. The high prevalence of gel particles is not considered good. Therefore, filtration of gel while semen collection should

be undertaken properly.

- After covering with cover slip motility should be estimated as soon as possible because the light coming from optical system of the microscope will give false negative results.
- Fresh ejaculates with progressive motility greater than 80 percent should be considered for further processing.

Note: The CASA machine also measures motility and is highly accurate and fast in tracking each sperm cell. Specified procedures are followed for this machine.

6 (ii) Estimation of sperm concentration

An accurate estimation of concentration will allow extension rates that optimize boar utilization and fertility of extended semen. The ways of measuring concentration include: hemacytometer, photometer and CASA system. The procedure with haemocytometer is described here.

Materials: Microscope with biotherm stage, Hemocytometer counting chamber, Micropipettes, semen sample, Diluting/Immobilizing fluid (prepared by adding 50 g of sodium bicarbonate and 10 ml of 11.6 mol/l (35%) formalin to distilled water).

Procedure:

1. Dilute the semen with diluting fluid and charge the hemocytometer.
2. Cover the hemocytometer, after 5 minutes, the sperm cells settle onto the glass.
3. Observe under 40 X Objective, when using a standard light microscope, the iris diaphragm should be closed to improve sperm visualization.
4. Sperm are counted in $0.1mm^3$. Sperm heads are not counted if they touch the right or bottom triple lines of the squares.
5. Calculations: Both chambers of the hemacytometer are counted. Sperm numbers from each side should be within 10% of each other. If not, clean the hemacytometer and repeat sample addition (it is not necessary to repeat the dilution step). When sperm counts are within 10% average of the two numbers.

   No. of sperm/ml = no. of sperms in $0.1mm^3$ X 10 X Dilution Factor X 1000

6 (iii) Sperm Viability and Morphology Evaluation

Sperm morphology is one of the effective tools to estimate semen viability and can also provide more information about the ejaculate in terms of its quality than is possible with just a motility evaluation.

Materials: Eosin–Nigrosin Working stain, glass slides, Microscope, semen, micropipettes.

Procedure: The staining solution for the one-step technique contained 0.67% eosin Y and 10% nigrosin dissolved in 0.9% sodium chloride in distilled water. Thus dissolve 0.67 g of eosin Y yellow and 0.9 g sodium chloride in 100 ml distilled water under gentle heating. Then add 10 g of nigrosin. Then brought the solution to the boil and allowed to cool to room temperature (20°C) after which filter it through quality filter paper and store in a dark and sealed glass bottle. Before using it, the staining solution should be brought to room temperature.

- Take microscope slides and nigrosin-eosin stain prewarmed to 37°C temperature.
- Place a drop of semen and stain adjustment to each other on prewarmed slide.
- Mix both semen and stain, then make a smear.
- Dry the slide in the air.
- Examine using a bright field microscope.
- White sperms (unstained) are classified as live and those that showed any pink are classified as dead, with the sole exception for sperm with a slight pink or red appearance restricted to the neck region (`leaky necks'), which are assessed as live.
- Count 200 cells in different fields. This is expressed as percent.
- Abnormal forms- coiled tails, bent tail, cytoplasmic droplet, headless, tailless, short tail also counted as percent.

Table: 6.3 Points to review and interventions when having ejaculates with an increased number of abnormal cells

Potential Cause	Intervention
Ambient barn temperatures above 22°C	• Equip barn with cool cells or other devices to lower temperature. • Increase ventilation rate for better heat convection. • Place more sensitive boars/breeds closer to cool cells. • Conduct boar collection, training, feeding during cooler morning hours. • Feed smaller portions more frequent. • Check water supply. • Don't vaccinate during high summer. • If possible, avoid boar transport during high summer (or do early morning/at night).

Elevated body temperature (Treatments/ Vacci nation)	• Split boars in groups for vaccination and apply treatment of groups 4-6 weeks apart. • Apply antipyretics if core temperature rises over 39°C/102°F (take diagnostic sample to rule out PRRS virus infection). • If together with sickness/lameness, apply treatment based on herd veterinarian's recommendation.
Boar age	• Boars below 220 days of age are still developing sperm cell production and therefore may have a lower percentage of normal cells. They can be trained/ collected; however, it is advised not to use their semen until boar is approximately 220 days old. • Boars above 3 years of age typically display a slight increase in abnormal cells.
Mycotoxins	• Check feed for mycotoxins. • Clean troughs prior feeding (avoid mold). • Use mycotoxin binders in feed. • Consult your nutritionist for additional intervention options.
Wrong collection frequency	• Avoid schedules of <1 and >2 collections/week in adult boars. • Avoid schedules of >1 collection/week in boars <12 month of age. • Collect boars at least every 7 days.
Lameness/sickness	• Apply treatment according to herd veterinarian and do not collect boars until recovery.
Pathological testis	• Consult your herd veterinarian for proper treatment. If damage is persistent with no recovery of semen quality, cull the boar.

6 (iv) Estimation of Sperm Acrosomal Integrity

Porcine sperm has distinct acrosomal ridge in contrast to sperm of other domestic species. Acrosomes are differentially categorized into four morphological classes: normal apical ridge (NAR), damaged apical ridge (DAR), missing apical ridge (MAR) and loose acrosomal cap (LAC) as described by Pursel et al. (1972). The sperm-rich fractions of ejaculates with >85% motile sperm and normal apical ridge (NAR) acrosome should be used in the A.I. programme.

Materials: Stock solution of Giemsa's stain, glass slide, cover slip, semen sample, microscope.

Giemsa's stock solution
Giemsa's powder – 3.8 gm (Merck)
Methanol (AR grade) – 375 ml
Glycerol (AR grade) – 125 ml

Working Giemsa's Solution
Giemsa's stock solution = 3ml
Sorenson's phosphate Buffer = 2ml
Distilled Water =35ml

Mix Giemsa's powder in pestle and mortar by adding methanol slowly. Then add glycerol into the mixture in glass amber coloured bottle. Leave the solution for maturation for seven days at 37°C. During the period, the stock solution is mixed well for a few minutes every day by shaking the bottle.

- *Sorenson's 0.1M phosphate Buffer pH-7* (17ml of stock solution A +33ml of stock solution B); A-Dissolve 13.609 g of potassium phosphate, monobasic anhydrous (KH_2PO_4) in 1 L dist. Water; B-Dissolve 14.198 g of sodium phosphate, dibasic, anhydrous (Na_2HPO_4) in 1 L dist. water.

Procedure:

1. Make a smear of semen on clean grease free slide.
2. Fix with glutarldehyde/methanol for 10 minutes.
3. Dry it.
4. Flood with working solution of Giemsa's solution.
5. After 60 minutes, rinse with tap water.
6. Dry it.
7. Observe under Microscope at 100X/Oil immersion.
8. Count the sperms with intact acrosome stained.

Interpretation

Count 200 cells in different fields. This is expressed as percent.

6 (v) Hyposomotic Sperm Swelling Test (HOST)

Materials: Microscope, water bath, micropipettes, HOST medium, microtips.

Preparation of semen specimen

The semen is maintained in a water bath at 37°C and allowed to stabilize for 3 to 5 min. The percentage of motile cells is estimated microscopically at 37°C.

Preparation of Media

Hypo-osmotic solution is prepared by weighing the fructose and sodium citrate as per the formula. Aliquot of 1 ml can be prepared in the test tubes and stored at – 20°C for later use.

- Sodium citrate dihydrate - 7.35gm.
- Fructose - 13.51 gm.
- Dist. water - 1000 ml.

Procedure for HOST

1. Add 1 ml of the specimen containing the spermatozoa with 1 ml of hypo-osmatic solution.
2. The solution is then incubated at 37°C for one hour.
3. Similarly for control 1 ml of semen is incubated in 1.0 ml of normal saline solution (0.9% NaCl) for one hour.
4. A drop is placed on the clean glass slide and covered with cover glass.
5. Observe under microscope at 400 X magnification.
6. A total of 200 sperm is counted in at least 5 different fields. (both in HOS /Normal Saline).

Interpretation

1. The initial response of the sperm to the hypo-osmotic solution is a small enlargement of the tip of the tail or at the junction of the mid piece and tail piece. Later the alterations are characterized by the presence of a swollen area at the tip of the tail, a hairpin curvature of the tail, a shortened and thickened tail, or a swollen area that partly or completely enveloped the curvature of the sperm.
2. There is good correlation between the percentage of motile spermatozoa and spermatozoa that reacted to the HOS test and between the percentages of sperm with intact membranes and HOST reactive sperm.

6 (vi) Microbiological evaluation of semen

Bacterial contamination is a serious and very real deterrent to profitable semen production in swine. It is routinely observed in raw and extended semen produced for artificial insemination (AI) when semen is collected by the gloved-hand technique. Storage temperature favors the survival of bacterial contaminants. Bacterial contamination of porcine semen has been associated with deleterious effects.

Materials: Different media, microbiological facilities, Water bath, pH meter, Laminar airflow, Bunsen burner, Colony counter, Automatic dispenser pipette, Vortex mixture, Test tube screw cap 10ml capacity, Conical flask (1000ml, 500ml, 250ml.), Glass (Borosil) Petri dish of diameter 90 to 100 mm, Incubator system, microscope.

Procedure:

a. **Preparation of plate count agar:** Media should be prepared as per manufacture's guidelines and pH adjustment and serial dilutions should be done.

b. **Inoculation and incubation**

- Take 0.5 ml of each dilution and introduce under sterile conditions (laminar flow table with Bunsen burner) into each of four petri dishes.
- Add to each Petri dish about 15 ml of counting agar cooled to 45 °C. Mix by circular agitation. Allow to solidify on a level surface.
- The time elapsing between the end of the preparation of the initial suspension and the moment when the medium is poured into dishes shall not exceed 15 minutes.
- Invert the prepared plates and place them in the incubator maintained at 37°C for 48-72 hours.
- For control plates: Inoculate and incubate two control plates in parallel with the operations by using 0.5 ml of diluents.

c. **Interpretation of results**

1. ***Examination of control plates***

   In all cases, carry out an initial examination of the control plates to determine whether colonies are present within the medium. If colonies are present, discard plates and plates containing the test sample and recommence the procedure. If colonies are not present proceed to an examination of the plates containing the test sample.

2. ***Counting***

   After 72 hours of incubation at 37°C the colonies are counted by means of the colony counting equipment or using the naked eye. Count only well distinguishable colonies,

which have grown within the medium and on the surface of the medium. Reject any plate in which more than half of the surface is overgrown. Select for the purpose of counting the dilution, which contains between 30 and 300 colony-forming units (CFUs) per dish.

d. Expression of the results

Record the number of CFUs counted. Multiply by the dilution factor. Express the results as the number of microorganism or CFUs per sample or per ml of semen.

e. Method of calculation

N = C X D [Where, N = number of colony forming units per ml or per sample; C = Number of colonies counted; D = dilution factor].

Table 6.4. Causes and intervention of bacterial ejaculate contamination

Potential Cause	Intervention
High bacterial load on boar surface due to dirty housing area.	• Keep boar housing area as dry and clean as possible by removing bedding material more often, increase air flow on ground level, adjusting nipple drinkers to not sprinkle floor. • Clean housing area more frequent. • Clean boars belly/prepucial area with dry paper-towel prior collection.
High bacterial contamination in collection area.	• Clean and disinfect dummy and collection area after every production day with emphasis on underside of dummy. • Work with warm-up pens for boars to pee and defecate prior entering collection area. • Evacuate prepuce in warm-up area. • Remove feces from collection area with shovel in between collections.
Contaminated supplies.	• Store collection gloves, cups and filters clean and dry until usage. • Do not place collection cup on floor. • Do not touch collection filter/inside of collection bag with hands.

Contamination caused by wrong collection technique.	• Refer to instructions made in this section, chapter "semen collection" and "hygiene".
Wrong choice of detergents / disinfectants or wrong application.	• Ensure that agents are effective against suspected bacterial flora. • Use agents according to manufacturer's instructions.

7. Extenders used for liquid semen for A.I.

Semen extender is a liquid solution which is added to semen to preserve its fertilizing ability. It acts as a buffer to protect the sperm cells from their own toxic byproducts and it protects the sperm cells from cold shock and osmotic shock during the chilling and shipping process. Extender should be prepared for each production day in high quality glassware to reduce hygiene risk. Several extenders are tested in literature and categorized depending on time period they preserve the spermatozoa. Instead of using commercial extender, when extender needs to be prepared in laboratory from chemicals, the extender should be prepared in the afternoon before the extension day because after mixing of the chemicals, it needs to be autoclaved, cool to room temperature and then to be placed in BOD incubator at 22°C in evening. However, it is always better to prepare the extender on the same day of use. Volume of the extender needs to be prepared depends on the number A.I. doses to be used for the purpose at farm and to be sold and number of ejaculates to be collected. Further, with a little experience, you will be able to anticipate how much volume will be needed.

Short-term (1-3 days)

Beltsville Liquid (BL-1)

Beltsville thawing Solution (BTS)

Illinois Variable temperature (IVT)

Kiev

Vital(R)

Reading

X-cell(R)

Zorlesco

ZORPVA

Long-term (over 4 days)

Acromax(R)

Androhep(R)

Modena

MR-A(R)

MULBERRY III(R)

Basics activities to prepare an extender

- Extender/diluents should be prepared in a separate classified zone.
- Decide how many liters of extender to be prepared.
- All disposable and reusable must be sterile and devoid of toxins and pyrogens.
- The water used for extender preparation should be ultra purified and free of bacteria.
- Commercial extenders should be used/prepared according the manufacture's guidelines.
- The composition of some commercial extenders is available in literature and the same can be prepared in the laboratory at lower cost with all standard steps.
- Glass ware, collection tubes, etc. should not be handled from their rim / mouth.

Table 7.1: Required water quality for extender preparation

Parameter	Level
Resistivity (MΩ -cm) at 25° C	>18
Conductivity (µS/cm)	< 0.056
Total Organic Carbon (ppb)	<50
Sodium (ppb)	<1
Chloride (ppb)	<1
Total Silica (ppb)	<3

- After adding all the components of extender in double, preferably triple distilled water, it should be sterilized. If extender is prepared on the previous day and stored in the BOD incubator, then antibiotics are to be added next day in the morning at 22^{o}C.
- Antibiotics in diluents: A combination of Penicillin, Gentamicin and Streptomycin should be used in diluents.
- The time until an extender preparation can be used varies between products (refer to manufacturer instruction).
- Use a conductivity or TDS meter or refractometer to check for the correct water-extender ratio prior to using the extender.
- Use a pH meter as an additional check for extender readiness. Determine the appropriate value according to the manufacturer's specifications.
- Ensure that all vats, tubes and pipelines that get exposed to extender are cleaned every time production is completed.

Materials: Commercially extender packet/chemicals required to make extender, weighing balance, spatula, 1L glass bottle/flask, autoclave, aluminium foil, rubber gloves.

Procedure

There are several ways to prepare the extender. Manufactures guidelines should be followed while using commercial extender.

GEPS preparation in Laboratory

- Weigh the required quantity of chemicals.
- Add the chemicals in flask.
- Completely dissolve the chemicals in required double distilled water.
- Cover the flask with aluminium foil.
- Place in autoclave.
- Adjust the temperature (121°C), pressure (15psi) and time (15 minutes).
- After completion of process, open the valve to release the pressure.
- Care should be taken not to open the autoclave until it is cool and pressure is released.
- After this take out the extender, bring it to the room temperature.
- Place the extender in BOD at 22°C.
- Just before extension, add the antibiotics in the extender.
- Then it is ready for use.

8. Semen extension process

Raw ejaculates have a very limited shelf life after collection, so their capacity to fertilize oocytes needs to be preserved by adding semen extender. Usually, extenders preserve semen for up to 3-7 days after collection. The dilution rate of the raw ejaculate (how many semen doses get produced) is dependent on the targeted cell count per AI dose. Semen extender can be considered the "backbone" of every AI dose and should be prepared with care.

Cardinal Rules:

1. Adjust extender temperature to within 1° of semen temperature.
2. Always add the extender to the semen.

Semen extension process

The extension process also varies depending on type of extenders used and guidelines of manufactures. The most common semen extension strategies are the one-and two-phase extension. They differ in temperatures of the extender when added to the raw semen. Recent studies (Schulze et al., 2013) indicate a slight quality advantage of ejaculates extended with the

one step, isothermic method. However, a simplified extension process is described here.

Simplified Processing

a. The thermos flask containing the freshly collected semen should be closed as soon as after collection and it should be placed in the pass box before transferring to the laboratory. If there is no provision of pass box in the laboratory, it should be transferred to laboratory within 30 minutes.

b. As soon as the neat semen is received, it shall be kept in a BOD incubator at 22°C after gross evaluation. Keep aliquot for microscopic evaluation, if the ejaculate qualify the minimum criteria for motility etc., keep it otherwise discard the sample.

c. After examination of sperm concentration and motility, semen samples should be maintained at 22°C for 4 hr. After 4 hr, dilution of semen in the ratio of 3:1 (extender 60 ml: 20 ml semen), the semen should be extended with dilutor maintained at the 22°C temperature.

d. Final dilution of semen is done, keeping a minimum of 3 billion spermatozoa per dose with minimum volume of 80 ml and maintained at 17°C. Filling and sealing of semen shall be done under Laminar Air Flow Unit.

Semen Filling

- Most often, the extended semen gets "filled" in plastic tubes or bags/packets with the help of packaging machines. Due to the cost of the automatic packaging machines and the lower throughput, smaller boar studs typically fill semen doses manually.
- In general, the dose volumes are between 80 and 150 ml, depending on the type of AI to be completed. For conventional AI, the dose volume is recommended to have a minimum of 80 ml.

Dedicated Cells per Dose

The number of cells per dose varies between studs and is influenced by multiple factors. Most common across the globe are counts between 3 and 5 billion cells per dose in 80-100 ml volume. The cell concentration per dose should not exceed about 60 million cells per milliliter as this may result in reduced shelf-life.

9. Storage/preservation and Transportation of liquid semen

The goal of semen packaging during shipment and delivery is to protect the doses against cool or warm ambient temperatures as well as physical damage including from sunlight. The goal is to maintain semen doses at a temperature of 17± 2°C/63±3.6°F with temperature fluctuations not exceeding 1°C/2°F until they are used on farm. No matter how well semen doses are prepared, if they are not carefully transported, their reproductive performance is jeopardized. Whether transported by commercial carrier or in-house staff, the basic goals include gentle handling, stable semen temperature and minimal cost. Label shipping containers with "Live Semen Shipment."

Semen Storage/preservation

Artificial insemination with fresh, extended semen should be practiced routinely on a swine operation before using stored liquid semen. Label the semen bottles/packet to indicate boar identification, plus date and time collected. If semen is to be stored, it should be allowed to cool to room temperature (15-17 °C/70° to 75 °F), which takes about two hours. Do not leave semen in a lighted area. Semen temperature should be lowered at a rate of one degree per hour. Cooling semen too rapidly causes damage to the sperm. Semen should be placed in a commercial liquid semen storage unit set to maintain the correct temperature for the extender used. Most extenders require a storage temperature of 60 to 65 °F. Since temperature varies greatly among storage units, check ahead of time to be sure the appropriate temperature can be maintained. The best method to store semen is to purchase a commercially thermostatically controlled liquid semen unit which keeps the temperature between 60.8 to 64.4 °F. A high-low thermometer should be kept inside either cooling method.

The key points of proper on farm semen storage are listed below:

- Store semen at 17±2°C/63±3.6°F. Avoid using semen stored outside this range.
- Use a surge-protected, fan-assisted cooler that can warm and cool.
- Space preservation unit at least 2.5 cm/1inch from the wall to work effectively.
- Open cooler shelving to allow complete air flow.
- Store doses loosely and horizontally.
- Record semen cooler temperature and who checks the inventory daily.
- Use several digital thermometers with outside readers. Place the sensors at different locations in the cooler (near cooling/heating element, near door and in the middle).
- Use semen within three days after production.
- Transport semen to the breeding barn in an insulated container/thermos flask.

- Only take doses to the barn that will be used within 1 hour. Do not take doses back from the barn to the semen cooler.

Note

- It is always economic, better and advisable to store semen in sterile container at 15-17^{o}C, then on the day of use/AI, check the motility and viability of stored semen, then fill the stored semen in packet/ bottle.
- Semen bottles/packets should be carefully swirled one to two times daily to resuspend sperm cells and to prevent sperm heads from sticking together. Rotation is necessary since sperm metabolizes energy sources and return lactic acid into the extender. If the acid builds up, it could lower the pH and kill sperm. Do not use semen that is past its expiration date. Even with high quality ejaculates, a 5-10% drop in semen motility is expected within the first 24 h of storage. After this time, few observable changes are expected in the semen quality within the limits of the extender. Semen quality should periodically be checked in storage for quality assurance.
- Semen can be successfully stored for three to seven days (depending on extender used); the highest fertility occurs prior to three days of storage.

Semen Storage Checklist

Once boar semen reaches the farm/stakeholder, the farm personnel/stakeholder accepts full responsibility for proper handling and storage.

- Record the arrival of all semen batches.
- Use semen storage units with forced-air circulation that both heat and cool to maintain the crucial 17 °C/63° F.
- Do not warm semen once it has been cooled and equilibrated to 17 °C/63° F.
- Monitor the semen storage unit temperature daily. This is easily done by filling a bottle with 70 ml. of water and placing it near the center of the semen storage unit. Simply insert a thermometer through the bottle stopper to obtain the daily temperature reading. Thermometer readings confirm the accurate operation of the unit's temperature controller and digital readout or it will alert the technician to a possible temperature maintenance problem and when temperature readings are taken, that is a good time to rotate semen doses.
- Do not overload storage units with warm semen.
- Do not open the storage unit during the cooling process.
- Use a high-low thermometer to record the high and low temperatures of the semen storage unit.

- Store semen doses away from ultraviolet light.
- Regularly clean the semen storage unit using a disinfectant such as conflict or a bleach solution.
- Do not return semen doses to the storage unit if their temperature has fluctuated more than 5.4° F. (3° C.). This holds true if the doses are temporarily removed from the storage unit and temperature fluctuations exceed the recommended range. Discard those doses.
- Breeding herd managers should check semen doses for consistent volume (80 ml), test semen for sperm cell concentration and number and check semen motility and morphology. Each dose should have a minimum of 3 billion viable sperm cells.
- Keep the semen storage unit in a temperature-controlled room, such as an office, with even airflow on all sides of the container. Avoid hot corners.
- Plug storage unit into its own electrical circuit to avoid power surges and/or use a UPS surge protector.

Transportation using boxes with controlled temperature:

If around 40 semen doses, each of volume 80 ml need to be transported, the best option is to equip the delivery vehicles with plug-in climate boxes using the vehicle's power supply. The units are able to cool and warm and have an outside display for regular internal temperature checks. A stir fan helps to circulate the conditioned air and creates equal temperatures inside the whole box. When packing the boxes, there should be enough room inside for air to be circulated. Semen transported in such climate boxes does not need special insulated packaging materials such as Styrofoam boxes.

Transportation using boxes with uncontrolled temperature:

If semen needs to be shipped with no temperature controlled boxes/devices, the package can be exposed to extreme and/or fluctuating temperatures. The following strategy can be used to transport the semen doses with mitigation of temperature fluctuations to protect the semen doses:

- All materials such as boxes, foil, gel packs etc. should be maintained at (15-17^{o}C) before use.
- Ensure that semen is at the final storage temperature (15-17^{o}C) prior to packaging.
- Take a Styrofoam box and use insulating foil to cover the inner Styrofoam box (2 box system) or the single Styrofoam box in the 1 box system. The foil can also be used as an inner layer to wrap directly around the doses as well.
- Wrap the doses in foil and place in a single thick wall or double Styrofoam box on the cooled gel packs.

- Place gel packs around the doses to stabilize the transport environment around the doses as they add both volume and temperature regulation to help mitigate fluctuations. Gel packs at cool temperature (15-17°C) should only be placed in direct contact with the semen doses.
- Close the box in air tight and place this box inside another Stryofoam box.
- Close the box and make the packaging airtight.
- Keep semen transport times as short as possible.

Transportation using household thermos flask

If a few doses and that are to be used immediately as soon as possible at a shorter distance, house thermos flask can be used. Thermos flask should be of wider mouth. 7 doses each of 80 ml can be packed in a thermos flask of one liter capacity. Thermos flask should be kept at (15-17°C) with opened lid. Place the doses in the flask and close the lid immediately and transport the doses and use the doses as soon as possible. Jolts should be avoided during transportation.

10. Heat Detection

Accurate heat detection is the key to successful AI. Heat detection needs to be carried out slowly and methodically every day. Twice daily estrus detection is preferable, as it does identify the onset of heat more accurately, but in many cases once a day is adequate. The goal is to recognize when the sow or gilt reaches "standing heat," which is the period when she stands still and rigid when weight is put on her loin. Detection of standing heat is critical because it determines when the animal should be inseminated. Always remember that each female is an individual. To achieve easier detection, boar contact should only be provided when the stock person is present. Keeping good records is essential for observant for signs of heat. Stockpersons must be aware of the full range of signs of early heat to heat because the placement of quality semen at the right time in the right place is essential for successful AI. Not all of the signs of heat will be seen in any one female. Different females exhibit heat in different ways and the "art of the stockperson" is to be able to clearly identify and respond to the various signals displayed.

Signs of Heat

Early heat

This is the period just prior to standing for mating, when the gilt/sow becomes restless and is easily disturbed - duration around one day.

An early heat female may:

- Have swollen and red vulva (more typical in gilts but not consistently in sows).

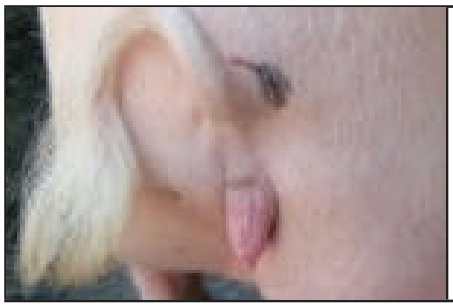
- Climb on gates and walls.
- Produce a watery discharge from the vulva.
- Emit a high -pitched whine.
- Mount other females but do not stand themselves.

Heat

This is the period during which the sow is prepared to stand for mating. A female in heat may:

- Have a normal vulva (swelling and reddening subsides).
- Stands with tail upright and flicking up and down.
- Have poor appetite.
- Become very vocal - repeated grunts or long growls.
- Display pricked ears.
- Show “standing reflex” and “flank fold reflex’.
- Stand with arched back.
- Have glazed eyes.
- Tremble.
- Have a tacky discharge from the vulva.
- Be attracted to stockperson.
- Seek contact with boar - if allowed.
- Stand rigid if mounted by other female.
- Show positive response to “back pressure test” in the presence of the boar.

Table 10.1: Some of the pictorial representations with respect to phases of estrus are shown

	**2 to 4 days** • Before estrus honk, female lose their appetite and appear nervous, thus signaling approaching estrus. **Initial period** • Refusal to stand for a boar and for back-pressure applied by humans.

	**Hours later** • Female will allow only a boar to mount. • Still refuse back-pressure from humans. • vulva red and swollen (in gilts especially). • little is mucus secreted.
	**As estrus advances approximately 4-8 hours** • Exhibit estrous symptoms in response to the boar for about 44 hours, swollen red vulva has noticeably subsided. • To human stimulation for about 20 hours, Mucus is plentiful & color changes from clear to grayish.
	**Late stages** • Female will again refuse to stand for human but will accept boar. After this, female will refuse stimuli from both. • In the last 12 to 24 hours, the sow 'stands' for the boar but increasingly less for the handler. Insemination at this time also gives poor results.

11. Time of Artificial Insemination

The timing of insemination is the single most important factor affecting fertilization rate and overall success of AI. The most critical factor is ensuring that an insemination with fertile semen occurs at -18 to -12 hours before the moment of ovulation. Generally, gilts should be bred 12 hours after standing heat is detected and again 12 hours after the first insemination. Sows should be bred 18-24 hours after detection of standing heat and again 12 hours later. However, these insemination schedules work on most animals, but it may need to adjust timing on those that have extra short or long heat periods. Therefore, an AI strategy has to be developed to optimize insemination timing.

Essential information

- Weaning-to-heat interval varies between sows. Sows showing early heat, say four days after weaning, tend to be in heat longer (3 days) than sows which come on heat on day 6-7 (2 days).
- The weaning to heat interval varies with season and length of lactation.
- Ovulation occurs during the last 1/3 of the standing estrus period.

- Never inseminate a sow/gilt that is not in standing heat.
- 90% of sows weaned on the same day will ovulate in a 12-hour window.
- Unfertilized ova degenerate approximately 8 hours after ovulation.
- Sperm can live within the uterine tract for approximately 24 hours.
- Long-life extenders help prolong sperm life up to the point of insemination.
- Treat each sow as an individual.

Key points for successful timing of insemination

- Accurate twice-per-day standing estrus detection is essential (7am/4pm).
- Twice/day estrus identification allows more accurate heat detection and thus timing of insemination.

Table 11.1 Suggested timings of A.I.

Frequency of estrus detection	Best time to inseminate relative to the first detection of standing estrus	
	Gilts	Sows
Once daily	0 and 24 hours	0 and 24 hours
Twice daily (12 hr apart)	12 and 24 hours	24 and 36 hours

12. Standard operating procedure for performing A.I.

Performing A.I. in pig is very easy in comparison to cattle and buffalo where extensive rectal palpation of genitalia and passing the A.I. gun through the cervix needs extensive training and expertise. Liquid semen AI is performed in female animal in heat after 24-28 hours of onset of heat.

Materials: 1. Semen dose 2. Antiseptic solution 3. Damp paper towel 4. An insemination catheter 5. Lubricating jelly, 6. Cotton.

Procedure of A.I.

Before using the semen, evaluate it under a microscope. Shipment, diluent, storage temperature, fluctuations in temperature and length of time since collection may all affect the shelf life, motility and viability of the semen.

- Before inseminating the female, use a paper towel to clean the vulva.
- Lubricate the tip of the spirette or catheter using any non spermicidal lubricant or a few drops of extended semen. Avoid getting lubricant in the opening of the spirette/catheter.

- Gently guide the spirette/catheter at an angle of 45°, with the tip pointed up, through the vagina to the cervix. The bottle/packet/bag of diluted semen is not attached to the spirette/catheter at this point. Keep the tip pointed up to reduce the chance of coming into contact with the bladder, which could cause a backflow of urine into the spirette/catheter. If this happens, use a new spirette/catheter because urine kills sperm. This is the primary reason the bottle of diluted semen should not be connected to the spirette/catheter until the cervix has been entered. Not connecting the bottle at this point also avoids exposing the semen unnecessarily to extremes of light or temperature.
- After inserting with an angle, make the catheter straight and make forward till it stops to go further.
- Now, use a counterclockwise rotation to insert the spirette/catheter into the cervix. Resistance can be felt by gently pulling back on the spirette. A foam tipped catheter is not always inserted into the cervix but usually is positioned up against the cervix.
- Gently invert the bottle/packet of diluted semen two or three times to mix the semen. Attach the bottle to the end of the spirette and discharge the semen slowly.
- A gentle squeeze to start the process may be needed, but after that the semen should be allowed to be taken up by uterine contractions. This process takes at least 3 minutes. Because of the variation in intensity of uterine contractions, gilts often take longer to inseminate than sows. Depositing the semen too rapidly will cause a backflow of semen out of the vulva. Obviously, semen that flows out onto the ground is wasted.
- A small amount of semen backflow is expected. If an excessive amount of backflow occurs, stop the insemination. Either the semen is being deposited too rapidly or the spirette is not fitted properly into the cervix. A quarter-turn of the spirette might correct this. If semen flow stops, reposition the spirette by a quarter-turn or move the catheter back and forth a bit to reinitiate semen flow. Also, cut a small hole in the semen bottle if flow has stopped because of a vacuum buildup.
- If there is a great deal of resistance to the flow of semen, reposition the spirette because the tip may be lodged against a cervical fold.
- When all of the semen has been deposited into the female, remove the spirette by rotating it clockwise while gently pulling.
- A new spirette/catheter should be used for each insemination to eliminate the possibility of transmission of disease or infection from one female to another.
- Keep the female in quiet surroundings for 20 to 30 minutes. Distress at this time may still disrupt semen transport and fertilization.

13. Post Artificial Insemination Measures

Advice to Farmers Post Insemination:

1. Ask to keep the animal under observation for next 12-24 hrs.
2. If signs of heat persist even after 18-24 hrs, call for a repeat AI, otherwise observe for heat symptoms after 18-21 days and also after 36-42 days.
3. If animal does not repeat heat at 18-21 days intervals for two consecutive times, call AI technician for pregnancy diagnosis after 2-3 months from the date of insemination.
4. If required, keep body of the animal cool by keeping animal in the shed and sprinkling water.
5. If an animal does not conceive even after three consecutive AIs, farmer should be advised to get the animal examined by a veterinarian and follow his advice.

Post insemination follow-up:

1. Follow each and every animal inseminated after around 21 days to find out whether it has repeated.
2. Follow each and every animal inseminated for pregnancy diagnosis after 2-3 months and record the date and result of pregnancy diagnosis in the format provided and send it to the area office on a monthly basis.
3. During pregnancy diagnosis, corroborate the findings with date of insemination and in case of mismatch or otherwise also, ask farmer about the post AI events to know whether animal remained in heat for a long period or came again in heat after the insemination. If yes, after how many days? And whether farmer availed AI services of some other service provider or arranged natural service during the same or subsequent heat.
4. Follow every pregnant animal and record farrowing details of the animals inseminated in the format provided.
5. Maintain all records related to artificial insemination, pregnancy diagnosis and farrowing and money transaction.
6. Advise farmers on proper heat detection, feeding, management and healthcare of animals as suggested by the experts. Also advise on the care and management of animals during advance pregnancy and after calving, including care and management of new born piglets.

Gestation: Gestation ranges from 112 to 116 days depending on the breed, litter size and season. It is often stated as 3 months, 3 weeks and 3 days (114 days). It is important that animal movement and stress are kept to a minimum in the first 5-30 days of pregnancy to prevent early embryonic death.

Care and Management of pregnant animals

The average gestation period of sow is 114 days. There may be variation of ±5 days

- Record the date of mating and find the expected farrowing date.
- Provide a separate sty for each sow.
- Keep the floor clean dry and non-slippery.
- Keep the animal liberally fed and kindly treated.
- Avoid rough treatment like kicking abusing, pushing etc.
- Don't allow the animal to walk a long distance to avoid fatigue.
- Provide bedding of 8-10 cm. chopped straw under covered area.
- Deworm sows 2 weeks before farrowing.

Care and management at the time of farrowing

- The farrowing pens should be thoroughly cleaned before the sow is brought to farrowing pen.
- Appearance of milk in teats when pressed indicates the approach of farrowing time.
- Attend the farrowing throughout. It may last up to 24 hours. Sows generally give birth to successive piglets at an interval of 5-7 minutes with total duration 2.5-3 hrs.
- The time taken for expulsion of litter varies from 1 hour to 5 hours. The interval between the birth of the first and that of successive piglets vary from a few minutes to 3 hours.
- About 30 per cent of piglets are usually born in posterior presentation.
- Generally, placenta is shed in portions only after all piglets are born. Expulsion of placenta is usually within 3 hours after expulsion of fetus.
- Piglets start suckling within 10-15 minutes after birth.
- The sow should be left undisturbed at farrowing and may be helped during emergencies.
- Keep the farrowing pen warm, dry and clean.

Neonatal Care and Management

- Keep piglets to creep area to protect from chilly weather.
- Assist piglets to get the teat and suckle colostrum from their mother's udder.
- Check the udder for agalactia, if yes, fostering should be done.
- Cut the navel cord with sterilized scissors leaving 2.5 cm from the body and disinfect with iodine solution.

- Clip needle teeth.
- Iron injections at the age of 4 and 14 Days is recommended.
- Ear tagging should be done for permanent identification of the pigs.
- Provide veterinary aid whenever necessary.

14. Diseases transmissible through semen and Diagnosis

Artificial Insemination (A.I.) allows the introduction of new genetic material with a reduced risk of disease. There is the potential that pathogenic organisms can be transported in semen. However, the probability of new pathogens moving into a herd with the addition of new genetic material in the form of a live boar from almost any source appears much higher than from semen collected from boars quarantined, isolated and tested periodically for a wide variety of organisms. Swine viruses that can be present in boar semen includes:

- Porcine Respiratory and Reproductive Syndrome Virus (PRRSV)
- Porcine Parvovirus (PPV)
- Pseudorabies Virus
- Porcine rubulavirus (PoRV), also known as La-Piedad-Michoacan paramyxovirus (LPMV)
- Porcine enterovirus (PEV) and porcine teschovirus (PTV)
- African swine fever virus (ASFV)
- Classical swine fever virus (CSFV)
- Foot and Mouth Disease Virus
- Japanese B encephalitis virus (JBEV)
- Porcine circovirus type 2 (PCV2)

Although the potential for disease transfer in semen is not absolutely known with many pathogens, the following classification can be made based on current research.

A. Diseases that can be potentially transmitted through semen but can be prevented by periodic testing of boars:

Leptospirosis, Tuberculosis, Brucellosis, Pseudorabies

Transfer of these diseases in semen can be prevented by isolating boars, periodically testing for these diseases, and only using the boars for semen collection (no natural services). The broad spectrum antibiotics normally added to semen extenders are effective against the bacteria causing leptospirosis, brucellosis, and tuberculosis.

B. Diseases that could be potentially transmitted via semen, although the common mode of transmission is via the oral route:

Parvovirus, Enterovirus, Mycoplasma

Since both parvovirus and enterovirus are common in adult swine, it is important to immunize females before breeding whether by natural or artificial insemination. Mycoplasmas have been isolated from swine semen on rare occasions. The primary isolates have been M. hyorhinis and several mycoplasmas not recognized as pathogens in swine. Boars with high serum titers to M. hyopneumoniae, indicating prior infection, have not had it isolated from semen. Semen may be contaminated during collection due to aerosolized organisms if precautions are not taken. Even if semen is infected, it is doubtful that it will introduce Mycoplasma infection into recipient animals. A greater concern is contamination of the inseminator's clothing and his equipment, so, the greater risk of transmission is direct animal exposure.

C. Disease organisms that should not be present in semen if collection is performed under sanitary conditions.

These diseases are normally spread via oral or fecal transmission.

- Bordetella bronchiseptica (atrophic rhinitis)
- Hemophilus pleuropneumoniae (HPP)
- Hemophilus parasuis (HPS)
- Treponema Hyodysentesiae (dysentery)
- Transmissible Gastroenteritis (TOE)
- Rota virus
- Swine influenza
- Erysipelas
- Pasteurella
- Campylobacter
- Eperythrozoon suis (EPE)

Conditions applicable to testing of boars

Boars should only enter an artificial insemination centre if they fulfill the applicable codes requirements as per WOAH/OIE - Terrestrial Animal Health Codes Manual. The animals should be subject to the test complying with the standards in the Terrestrial Manual.

1. Pre-quarantine

The animals should be clinically healthy, physiologically normal and comply with the WOAH/OIE - Terrestrial Animal Health Codes Manual for following diseases within 30 days prior to entry into isolation at the quarantine station.

a. Porcine brucellosis.

b. Foot and mouth disease.

c. Aujeszky's disease.

d. Teschovirus encephalomyelitis.

e. Transmissible gastroenteritis.

f. Swine vesicular disease.

g. African swine fever.

h. Classical swine fever.

i. Porcine reproductive and respiratory syndrom.

2. Testing in the quarantine station prior to entering the semen collection facilities

Prior to entering the semen collection facilities of the artificial insemination center, boars should be kept in a quarantine station for at least 28 days. The animals should be subjected to diagnostic tests as described below a minimum of 21 days after entering the quarantine station, with negative results. The animals should be subject to the test complying with the standards in WOAH/OIE - Terrestrial Animal Health Codes Manual for following diseases:

a) Porcine brucellosis.

b) Foot and mouth disease.

c) Aujeszky's disease.

d) Teschovirus encephalomyelitis.

e) Transmissible gastroenteritis.

f) Swine vesicular disease.

g) African swine fever.

h) Classical swine fever.

i) Porcine reproductive and respiratory syndrome.

3. Testing programme for boars resident in the semen collection facilities

All boars resident in the semen collection facilities should be tested at least annually for the

following diseases, with negative results, where the compartment/zone or country is not free: The animals should be subject to the test complying with the standards in WOAH/OIE - Terrestrial Animal Health Codes Manual for following diseases:

a) Porcine brucellosis.

b) Foot and mouth disease.

c) Aujeszky's disease.

d) Teschovirus encephalomyelitis.

e) Transmissible gastroenteritis.

f) Swine vesicular disease.

g) African swine fever.

h) Classical swine fever.

i) Porcine reproductive and respiratory syndrome.

15. Laboratory Quality Management

Goal

The primary target of every AI laboratory is to produce consistent semen doses of good quality which will fertilize a ova and produce litter capable of independent existence. The goal of the laboratory process is the efficient evaluation of ejaculates to determine whether they meet the established quality standards as described in Table and the production of doses that satisfy both in-house and customer requirements.

Table 15.1: Expected Results from AI Laboratory Management Program

Trait	Target	Intervention Level
Consistent amount of cells per AI dose	≤+/- 5% off target	> +/- 10% off target
Consistent AI dose volume	≤+/- 1 ml off target	> +/- 2 ml off target
AI dose minimum total motility at expiration date	≥70%	<60%
AI dose minimum progressive motility at expiration date	≥60%	<50%
AI dose maximum amount of abnormal cells (primary and secondary defects)	≤20%	>20%
AI dose maximum amount of cytoplasmic droplets only	≤15%	>15%
AI dose bacterial cell count	<1 cfu/ml	>1 cfu/ml

Laboratory Setup

A proper lab design sets the stage for high quality, efficient semen dose production. Lab design affects hygienic production and efficient workflows. Lab design details often have to be decided on a case-by-case basis depending on the unique situation encountered at each stud (space, staffing, etc.). Laboratory hygiene and major principles and targets of semen production laboratory designs have been given in the chapter "set up of Semen Production Lab".

Evaluation

The foundation of semen dose production is the measurement of raw ejaculate sperm cell numbers as well as the evaluation of two key quality parameters, semen motility and cell morphology. Only ejaculates that meet a set standard of minimum criteria listed in table should be processed. Discard ejaculates that do not meet the minimum standard.

Table 15.2: Thresholds for Semen Evaluation

Semen Variable	Normal Value	Minimum Threshold
Appearance	Milky to creamy consistency	
Color	Gray white to white in color	
Volume	100 – 500 ml	≤ 50 ml
Total no. of sperm cells	20-120 billion	>15 billion
Motile sperm cells (raw ejaculates)	80-95%	To meet minimum required semen motility at expiration date
Progressive motile sperm cells (raw ejaculate)	60-90%	To meet minimum required semen motility at expiration date
Agglutination	0-10%	≤ 30%
Abnormal sperm cells (primary and secondary defects)	10-15%	≤ 20%
Cytoplasmic droplets (as part of abnormal cells)	5-10%	≤ 15%
Maximum motility loss/24 hours	2.5 – 3.0%	≤ 3%
Maximum progressive motility loss/ 24 hours	2.5 – 3.0%	≤ 3%
Motile sperm cells at expiration date		≥ 70%
Progressive motile sperm cells at expiration data		≥ 60%

Cleaning and Disinfection

A cleaning and disinfection plan contains the frequency and the method of cleaning and

disinfection. There are general cleaning and disinfection procedures for barn, laboratory (including ceilings, walls, floors, countertops and cabinets) and boar housing. There are more specific cleaning and disinfection procedures for collection pens and dummies in the barn and for materials and equipment in the laboratory. All cleaning and disinfection procedures follow a set of simple, but important steps:

1. Remove organic material.
2. Clean by using detergent (soaking).
3. Scrub the surface with a brush, sponge or cloth to detach organic matter and dissolve biofilm.
4. Rinse with the objective to dilute and remove detergent, grease and proteins.
5. Dry.
6. Disinfect or sterilize to kill remaining microorganisms.
7. Clean/disinfect or replace cleaning materials (brush/sponge/cloth) after each usage.

Proper cleaning is the most important part of the above-described procedure. Disinfection of surfaces without proper cleaning doesn't kill microorganisms as organic matter will protect them against disinfectants.

The use of detergents and disinfectants require the correct concentration, water temperature and exposure time to be effective. Strictly follow the manufacturer's instructions and assure sufficient rinsing to remove any residue. Furthermore, it is important to use disinfectants that are effective against the bacteria that are found in the environment to be treated. Keep in mind that bacteria can gain resistance against disinfectants over time, which is a good reason to use several equally effective commercially available products.

Cleaning and disinfection of materials is not as simple as it seems. If not executed properly there is a risk that materials get contaminated or that detergent/disinfectant residues get left behind. If material with residues comes in direct contact with semen, the semen quality is compromised. This is a critical point yet it is difficult to monitor which is the reason many boar studs decide to use disposable instead of reusable materials.

Cleaning and disinfection should be made as easy as possible by choosing the right design and materials for the walls, ceilings, countertops and other furniture. Use smooth, solid and non-corrosive materials for walls (i.e. poured epoxy/resin flooring, stainless steel, solid petroleum-based composites and many others), floors and countertops as much as possible. The areas that need daily cleaning (the production lab, the collection area and dummy) should only contain essential items required for production. For example: Store excess consumables in a storage room and not in the laboratory, thereby eliminating the need for laboratory cabinets. Do paperwork in a separate office. Use a wheel cart and only take consumables into the laboratory

that are needed for that day's production. Use stainless steel tables on wheels that are easy to move around the production laboratory or remove the tables entirely for a complete cleaning of walls and floors.

Detergents

There are many choices for detergents. The detergent must be effective in removing organic matter, must leave no residues after rinsing and must be non-toxic. Safe choices would be to use lab ware detergents, cleaning detergents for the feed industry, or even liquid detergents for manual dish washing. Make sure all surfaces and materials are well rinsed after using the detergent. Follow the instructions on the label. Do not use spray bottles with the cleaning product as vapor can easily spread to areas that cannot be rinsed. We recommend preparing a cleaning solution that can be administered on the surface with either a sponge or cloth.

Disinfectants

Disinfection is needed if sterilization is not an option for re-usable materials. At the same time, equipment and work counters have to be disinfected after cleaning at the end of each production day. Table shows a list of active ingredients for disinfectants.

Table 15.3: Activity Levels of Selected Liquid Germicides

Procedure/Product	Aqueous Concentration	Activity Level
Disinfection		
Glutaraldehyde	Variable	high to intermediate
Ortho-phthal-aldehyde (OPA)	0.50%	high
Hydrogen peroxide	3-6%	high to intermediate
Formaldehyde	1-8%	high to low
Chlorine dioxide	Variable	high
Peracetic acid	Variable	high
Chlorine compounds	500 -5,000 ml/L free available chlorine	intermediate
Alcohol (ethyl, isopropyl)	70%	intermediate
Phenolic compounds	0.5 to 3%	intermediate to low
Iodophor compounds	30-50 mg/l free iodine to 10,000 mg/l available iodine 0.1-0.2%	intermediate to low
Quaternary ammonium compounds (QUADS)		low

There are a few disinfectants that work well for boar studs:

- 70% Isopropyl alcohol (preferred).
- Germicidal bleach.

Clean and disinfect all equipment used during semen dose production after each use. This

includes CASA systems, computers, keyboards, dispensers, microscopes, telephones, pipettes, etc. Do not forget to clean and disinfect the walls up to a height of 35 cm/14inch from counter top after each production day. Make sure to disassemble devices as much as possible and clean all parts. Ease of cleaning should be important buying criteria when you purchase equipment. Clean from high surfaces to lower surfaces, use cleaning materials that are disposable. Make a schedule for daily, weekly and monthly cleaning. Contemplate hiring professional cleaners for general rooms such as showers, break room, hallways, restrooms, offices and other general areas so that boar stud staff can focus on the cleaning and disinfection of the production areas.

Internal Quality Assurance and Control

QC monitoring is vital to ensure high semen production standards are being maintained at a boar stud. The semen dose (end product) should continually be monitored on a pre-determined schedule for the number of cells, post-production motility and temperature. Laboratory processes and components that contribute to the end product are also important aspects of a QC monitoring program. This program should include the post-dilution motility testing of prepared extender at the start of the processing day. In general, the objective of internal or external monitoring is that 95% or more of the tested samples of the end product meet the semen dose quality criteria.

Extender Quality Control

Each (prepared) extender has specifications for pH and conductivity. As described previously, both can be tested with measurement devices to ensure that extenders are prepared in the correct way. These measurements should be performed after the prepared extender has been well mixed and is stabilized. The required stabilization time differs between extenders. Keep a log of the instrument calibration and measurements results for reference.

Post-Dilution Motility Evaluation

A post-dilution motility evaluation is performed on each vat of extender, directly after dilution (2-3 samples) and 2 hours after dilution of the same samples. If there are quality problems with the extender, these will be detected early and a new extender can be prepared without a major loss of productivity. When there is more than one vat being used, test each one at the beginning of production. Encountering a problem early-on will allow enough time to prepare fresh extender for later use.

Post-Production Motility Evaluation

A post-production motility evaluation can be used to assess the sperm motility over several days after production. The reduction in motility per 24 hours is a good indicator to what extent the semen dose production has been performed correctly. It also indicates that sperm cell quality is likely to be maintained at a sufficient level until the expiration date. The goal for

total motility is to remain above 70% of the total sperm cells at expiration. Boars with semen quality issues should be identified prior to dilution (i.e. trashed raw ejaculates; on-hold and possible culling if problem persists) or through frequent post production motility evaluation of individual ejaculates (i.e. history of long-term viability issues). The frequency of individual boar testing should be based on a risk assessment. Testing at least every 4th ejaculate per boar is necessary to detect any problems with post-production motility. The number of samples to test is dependent on the total number of doses produced on a production day. The evaluation of a representative number of samples is needed to monitor the quality of the production process for internal reference and should be used to detect and address problems in order to prevent issues in the future. Finally the system has to ensure that produced semen doses still meet the minimum motility values by expiration date.

Monitoring Number of Cells per Dose

The monitoring of sperm cell number needs to be performed to ensure the minimum threshold is being met and to determine the variation between doses. The monitoring needs to be done on a representative number of samples. The preference here is to use accurate equipment such a Nucleocounter® or a Flow Cytometer. This equipment is expensive and is not widely used in commercial AI laboratories. If you don't have the equipment, the testing can be outsourced to a professional 3rd party. As discussed previously, another accurate option is to use a counting chamber (hemocytometer) and microscope. However, this is a time-consuming process and requires a skilled and experienced technician.

Temperature Management

Some extenders may protect against sperm damage due to temperature fluctuations. However, temperature management during collection, evaluation, processing, storage and shipping remain important. Temperature stress should be minimal during the production, storage and shipping of semen doses. Critical temperature management aspects for the semen production process include:

- Monitor ejaculate temperature during collection and transition (time) from collection to dilution. Measure semen temperature at different points during the transition from collection to reception to dilution. This provides an indication of the required temperature for materials and extender.
- Note that ejaculate temperatures may differ between seasons and boar studs. Therefore, frequent measurements are important to adjust material and extender temperatures accordingly.
- Allow a maximum temperature difference of ±2°C/3.6°F from the semen temperature for materials or extender.
- Use a calibrated thermometer or check the thermometer used against a calibrated

thermometer to ensure accuracy of readings.

- Check the temperature setting on the heating device (if present). Note that temperature settings are not always accurate and should be checked with a thermometer regularly.
- Microscopes to evaluate motility should have a heated stage with a temperature set (and checked/confirmed) at 38°C/100°F for semen to express optimal motility.
- After dilution, cool semen gradually to a temperature of 17 ± 2°C/ 63±3.6°F prior to shipping.

Microbiological Testing

Microbiological testing is normally performed by an external specialized laboratory but can also be performed in house. Sample the end product water, extender and environment such as dummies as well as the countertop and sink/drain.

External Quality Control

External quality control of the end product is required for a variety of reasons. It assures that equipment is calibrated correctly and dose quality is within acceptable limits. Routine external monitoring to evaluate cell numbers, motility and morphology at an independent, certified andrology laboratory is one of the cornerstones of a QC program. Preferably, such an andrology laboratory uses a CASA system for motility evaluation and an accurate and validated method to determine the concentration, e.g. by using a Nucleocounter® or flow cytometer. The preferred morphology scoring would be with fixed or stain sperm cells evaluated manually at 1,000x magnification with all specific classifications of abnormalities reported. In Table, the minimum criteria for a good quality semen dose can be found.

Table 15.4: Minimum Criteria Semen Dose at Expiration Date

Semen Variable	Minimum Criteria
Volume	< ±1 ml off target
No. of sperm cells / dose	<± 5% off target
Motile sperm cells	> 70%
Progressive motile sperm cells	> 60%
Loss of (progressive) motility per 24 hours	< 3%
Agglutination	< 20%
Abnormal sperm cells	< 20%
Cytoplasmic droplets (as part of abnormal cells)	< 20%
Bacterial contamination	< 1CFU/ml

Microbiological Testing

Bacteria in semen doses can deteriorate the quality of the semen. Semen extenders contain antibiotics that should inactivate bacteria in the ejaculate. However, if the contamination is large, the antibiotic concentration may not be sufficient to inactivate all bacteria. Antimicrobial resistance in bacteria is another cause of bacterial contamination of semen doses. The microbiology testing of semen doses provides an insight into the hygiene level of semen production. Extender is prepared with purified water and accounts for at least 75% of the semen dose volume. Therefore, it is important to test the water and extender on a routine basis. Most bacteria found in semen doses are from animal, human or environmental origin and thrive best at moderate temperatures between 20°C/68°F and 45°C/113°F under aerobic conditions. Therefore, microbiological testing can be performed best at a temperature of 37°C/99°F under aerobic conditions.

- Microbiological testing should be performed at least 24 hours after extender dilution to give antibiotics time to inactivate bacteria.
- Due to competition, certain bacteria multiply slower and only if others are inactivated. Therefore, testing a portion of the samples at a later age may show bacterial growth, even though growth wasn't observed in fresh samples.
- If bacteria are found on a regular basis: Identification is necessary to find the source of the contamination.
- Testing susceptibility against a panel of antibiotics including the extender antibiotics will give information about resistance development.

HACCP

HACCP stands for Hazard Analysis Critical Control Point. HACCP is a systematic preventive approach used in food production to ensure food safety from biological, chemical and physical perspective. It analyzes hazards in production processes that can cause the finished product to be unsafe and it designs measurements to reduce the risk level. This methodology can very well be used for semen production. The HACCP principles are as follows:

Conduct a hazard analysis: Find the hazards that cause substandard semen dose quality. Think of cold shock, bacterial contamination, errors in extender preparation, water quality, cross contamination, temperature variation, toxic materials, etc.

Identify the critical control points: Analyze each step and all materials used from semen collection to semen packaging, storage and transportation. Examples of critical points in semen production are: Water quality (both chemical and microbiological), extender quality (pH and conductivity), antibiotic effectiveness, contamination points and non-intentional hazardous substances in materials that come into contact with semen such as latex or powdered gloves,

bleached cotton gauze, detergent residues in hoses, etc.

Establish critical limits: For each control point set up critical limits. For example:

a. Microbiological status:

i. Semen dose: <1 CFU/ml.

ii. Countertop surface after cleaning: < 3 CFU/cm^2.

iii. Extender: <1 CFU/ml.

iv. Purified water (for extender preparation): <1 CFU/ml.

v. Source water: Coliform bacteria <1 CFU/100ml.

vi. Dummy surface after cleaning: < 10 CFU/cm^2.

b. Temperatures:

1. Collection cup: 38°C ± 2°C/100°F±3.6°F.
2. Heating plate and block for microscope slides, cover slips and sample tubes: 38°C ± 1°C/100°F±1.8°F.
3. Heated microscope stage: 38°C ± 1°C/100°F±1.8°F.
4. Plastic liners, tubes and bags: assure sufficient warming time if taken out of the storage so the temperature is at least room temperature (20°C/68°F) and not warmer than the diluted ejaculate.
5. Extender: ejaculate temperature before dilution ± 2°C/3.6°F.
6. Cool room: 17°C ± 2°C/63°F±3.6°F.

Monitor CCPs:

- Once you have identified CCPs and established critical limits, perform relevant tests on a representative number of samples. For water used to prepare extender, measure the conductivity. For extender, measure conductivity and pH.
- Check the hygiene of semen collection.
- Take the temperature of ejaculates at arrival in the lab and check the temperature of extender.
- Have the water source (at inlet) analyzed for heavy metals and organic matters frequently (quarterly).
- Test the water, extender and semen doses for microbiological contamination.
- Test the contamination of surfaces after cleaning to check the effectiveness of the sanitization procedures.

- Test the scales used in the lab with standard weights before use.
- Test the temperatures of heating devices every time before production.
- Test the dilution rate of semen with extender.
- Control the speed of dilution of semen with extender.

Establish corrective actions:

Where there is a deviation from established critical limits, corrective actions are necessary. An important purpose of corrective action is to prevent the sales of substandard semen doses which may affect fertility in a negative way. Corrective actions should include the following elements: • Determine and correct the cause of non-compliance • Determine the disposition of the non-compliant product • Record the corrective actions that have been taken Examples: • Purified water with bacterial contamination: o Sanitize the water purification system o Change resins/filters o Cleanout the water storage tank o Check the UV-filters and replace if necessary o Use purchased water to produce extender or consider boiling the water (depending on volume needed) o If contamination returns, you may want to consider re-designing the water purification system by re-circulating water, replacement of the water storage tank, or changing/ upgrading the UV-light capacity. Record the deviation, the corrective actions and the test results in a log/journal after the changes have been completed.

- Establish and verify procedures for ensuring the HACCP methodology is working as intended: Verification procedures may include activities such as review of HACCP plans, CCP records, critical limits (based on monitoring results), number of samples for microbial testing and testing frequency.
- Keep records: Records are important. Test results should be recorded, results should be analyzed and the information obtained be used to improve procedures. This process should result in the resetting of the minimum criteria in order to improve the control you have over production and reduce the hazard risks.

16. Boar Management

Good AI boar performance is the result of good boar management that includes proper boar quarantine and training, high standards in nutrition and excellent hygiene and semen collection practices. Under all circumstances, producers should adhere to the locally applicable laws that regulate management and housing practices (eg. Indian Standard Code of Practice for Pig Housing, Nutrition etc), even if they differ from the recommendations presented in these guidelines.

Table 16.1: General Recommendations for Boar Housing Management

Factor	Quarantine	Production Barn
Housing		
Thermo neutral zone[1]	20°C (68°F)	
Humidity	>40% - <70%	
Gasses (max. ppm per m³ air)	Ammonia: 20 ppm Carbone Dioxide: 3,000 ppm Hydrogen Sulfide: 5 ppm	
Ventilation (in Cubic Feet per Minute (CFM) per head)	• Min (cold): 14 CFM. • Max: 150 CFM.	
Air speed (Feet per Minute)	• 400-800 FPM.	
Housing[2]	• House boars individually. • Maintain visual and/or direct contact with other animals. • House boars at same age/breed close to each other to make visual comparison easier.	
Flooring[2]	• Slopped solid floor. • Insulated lying area. • Slatted floor: Keep dry and clean. • Straw: Exchange at least once a week. Regular mycotoxin tests recommended. Ensure safe source (pig manure free). • Sawdust:-exchange 2-3 times/year (depending on humidity). • Keep lying area dry and free of feces as much as possible.	
Feeding Management		
Water	• Have clean and fresh water always available. • Use nipple drinkers (80-90 cm above floor) or troughs. • Daily demand approx. 17 L (4.5 Gallons) per boar/day. • Minimum flow rate 1 L/min (0.26 Gallons). Test monthly. • Test nipple drinker when cleaning troughs. • Water quality (check twice a year).	

Diet	• Specific for age/weight to maintain required body condition. • Approx. 3000 ME kcal/kg, 16-18% C.P.(0.85% Lysin), 4% fat and 5% fibre. • Generally, 1.5-3 kg of ration on daily basis. • If thermo neutral zone below 20°C, additional 0.08 kg/day with every 1°C decrease. • Average grain particle size 750-900 micron.	
	• Avoid the use of by-products or co-products where mycotoxins may be concentrated. Boar feed should be produced out of tested raw material. • Use of mycotoxin binders may be beneficial. • Check for mycotoxin contamination regularly. • Adding anti-oxidants may be beneficial.	
Feeding strategy	• Feed 2-3 times per day. • Clean troughs prior to feeding. • If automatic feeders are used, check accuracy by weighing and adjusting every 2 weeks.	
	• To adapt to new feed and environment provide a reduced ration (2/3 of the regular amount) for 2-3 days; After the first 2-3 days feed boars in order to maintain a body condition of 2. • At entry weights of approx. 160 kg, it is normal to provide about 2.5 kg feed with 7.9 Mcal/ME feed per day.	• Adjust individual feeding based on visual body conditioning score. • Target is >95% of boars in normal body condition.
Health		
Treatments	• Herd veterinarians should follow local regulations. • Minimize treatments as much as possible as negative effects on semen quality can occur. • Oral application is preferred over injection if available. • Drugs are dissolved in solvents or sterile water. Solvent ingredients may have an adverse effect on semen production and/or quality. Typically, drugs are not tested for these effects. Injections should never be done in the collection pen. • Keep records of all treatments given.	

	• Vaccinations should not start within the first 3 days after arrival. • Some vaccines have a negative effect on semen quality. Apply those vaccines as early as possible to give the boar time to recover prior to semen collection starting. Negative effects might be seen up to 8 weeks after treatment.	• Split herd vaccinations over several groups with time to mitigate potential negative effects on semen quality. • Reduce no. of vaccinations to an absolute minimum.
Diagnostic testing[4]	• Conduct diagnostic testing on diseases and with test methods according to herd veterinarian and local regulations. • Sample as gentle as possible. Consider saliva testing, blood sampling etc. • Contact your herd veterinarian for detailed instructions.	
	• Conduct initial test on the boars within seven days of arrival. • Test 100% of the population at the end of the isolation period.	• Conduct regular tests on common diseases transmittable by semen and mandatory ones.
Clinical monitoring	• Walk boar house daily to check boars and to identify those that did not clean up their feed or show signs of sickness. • Measure and record temperature for every suspicious boar. • In case of fever (≥39°C or ≥102°F) contact your veterinarian immediately and skip collection of the boar that day. A diagnostic work-up on blood samples is recommended. • Get boars up every day at feeding to observe for lameness. • Consult your veterinarian, in case of doubts. • Keep records for any boars that are treated or off-feed.	
Semen collection management of sick animals	• Don't collect lame animals until full recovery and following the conclusion of painkiller treatments. • Don't collect animals that display blood in the semen for 2 weeks. If blood continues to appear after the rest period, medical investigation and proper treatment according to your veterinarian should be done. • Don't collect animals with depressed general condition until recovery.	

Factor	Quarantine	Production House
Semen Collection		
Start collection[5]	• Don't start collection training until 180 days of age or after 270 days of age. • Don't start collection training until at least 5 days after arrival.	• Don't start semen collection for use in AI prior to 220 days of age. • 2 consecutive ejaculates that pass the minimum thresholds should be collected prior to using the doses for AI (2nd good can be used).
Collection frequency	• Conduct collection training 2 consecutive days following 1 rest day and repeat until the boar has successfully been collected 2 consecutive training days. • After successful training, collect boars once every 7 days.	• Collect boars below 12 months of age: 1/week. • Collect boars above 12 months of age: Min.: 1/week Max.: every 3 days. • Response to collection frequency may vary by boar.
Non collects	• Don't collect lame or sick boars until recovery. • Boars with blood in the semen should rest 2 weeks.	
Collection schedule	• During training, first collect the boars with high libido, followed by the more challenging animals.	• Manage collection schedule via proactive planning and scheduling. • Prioritize by index and eligibility (collect highest indexed boars with adequate rest days and good semen quality first).

1 Pig House temperatures below 20˚C/68°F are difficult to realize in some regions or during summer. However, it has been shown that this is the critical temperature threshold prior to negative impacts on semen production.

2 Always follow local legislation concerning housing and bedding requirements for AI boars.

3 Examples for body condition categories should be defined together with herd veterinarian.

4 It should be defined together with herd veterinarian.

5 Differences might appear depending on whether boars are trained on quarantine or in the stud.

17. Bio-Security

Most boar houses serve a large number of sows/farms and disease that can spread via semen (e.g. PRRS, Classical Swine Fever) can cause tremendous economic damage. Therefore, protection of the health status of the boar herd is crucial. Proper location, quarantine and barn management can help to reduce the risk of introducing pathogens to the boar stud and customer sow farms. Table summarizes biosecurity best practices. Additional guidelines and practices should be documented and implemented with support of your local veterinarian.

Table 17.1: Characteristics of a Bio-Secure Boar House

Area	Characteristics of a Bio-Secure Boar House
Facility location	• Establish stud as far away as possible from other pig farms, major roads, etc. • Keep stud protected from access by unintended visitors (lockers, fence).
Staff	• Ensure min. 48 hrs. downtime from other pig contact. • Use shoe disinfection/cover. • Shower in and complete change of clothes and shoes. • Ensure one night downtime if changing from quarantine to production barn.
Visitors	• Only allow essential visitors (maintenance personnel etc.). • Apply the same rules as for barn staff. • Sign in to visitor book (name, reason for visit, when/where last pig contact, confirm bio-security rules). • Keep frequently used (maintenance) tools in stock at the stud. • Clean and disinfect all tools / supplies that need to enter the stud.
Supplies	• Clean and disinfect supplies prior to introduction, fog with disinfectant. • Consider an offsite delivery drop-off location to reduce the amount of outside traffic entering the boar stud. • Remove shipment packaging (i.e. outside cardboard boxes) to enhance the disinfection process.
Vehicles /trailers	• Clean, disinfect and dry prior to approaching the production barn (animal transports). • Set appropriate trailer downtime standards between animal transports.
Other	• Establish rodent and other pest control procedures. • Install positive pressure air filtration of the barn. • Establish independent quarantine of all incoming replacement boars for a set period of time prior to introduction into the herd.

Quarantine Procedures

In all instances, a meticulously devised and vigilantly executed quarantine regimen represents the most efficacious strategy for mitigating risks associated with external pathogen introductions. The quarantine involves the isolation and acclimatization of incoming stock from other animals on the farm. This is important because an animal may not exhibit symptoms of a disease for a period, termed the incubation period, even after the entry of the pathogen. So, following an observation period under veterinary supervision, at an intermediate station, will minimize the risk due to asymptomatic animals and animals in the incubation period. Vaccination against endemic diseases, deworming and testing for diseases like such as brucellosis, PRRS, PCV2, ASF and PPV infections, etc may be performed during the period. Quarantines must be designed as bio-contention units, to avoid the spillover of any undesired pathogen brought by the incoming animals. Typically, this entails constructing the quarantine at a considerable distance from the principal farm units and treating it as an autonomous entity, akin to an external facility. Ideally, a quarantine shed should be located at least 500 meters away from the core zone or main farm unit. The quarantine facility should be equipped with separate clothes and equipment for the staff. A minimum quarantine period of 21 days is recommended. Stocks in quarantine sheds should follow an all-in, allout strategy, where the new animals are brought together and moved out together. Proper disinfection should be followed during each cycle.

Labour management in a quarantine facility:

- Establish a controlled access point for the quarantine shed, preferably with a footbath and hand-washing facilities.
- Restrict entry to authorized personnel only and maintain records.
- Implement a strict biosecurity protocol, including wearing protective clothing, such as coveralls, boots and gloves, when entering the shed.
- Ensure that visitors and vehicles are disinfected before entering the premises.

Cleaning and disinfection of quarantine facility:

- Develop a regular cleaning and disinfection schedule for the quarantine shed.
- Detergent wash is preferred to remove all organic material, including manure and bedding, from the shed before cleaning.
- Use appropriate disinfectants effective against common pathogens, following recommended dilution rates.
- Pay special attention to areas, such as feeders, waterers and walls, during cleaning and disinfection.

Animal management in quarantine facility:

- Keep animals in the quarantine shed separate from the main herd to prevent disease transmission.
- Ensure All-In-All-Out strategy.
- Retain for a minimum of 21 days.
- Perform disease testing.
- Perform vaccinations and deworming.
- Monitor for any signs of illness or abnormal behaviour during the quarantine period.

Waste Management in quarantine facility:

- Implement proper waste management practices, including the safe disposal of manure and other organic waste from the quarantine shed.
- Ensure that waste disposal areas are located away from water sources to prevent contamination.

Record keeping in quarantine facility:

- Maintain accurate and up-to-date records of animal movements, health status, treatments, vaccinations and any other relevant information.
- Regularly review and update records to facilitate traceability and disease management.

18. Standards/Criteria for Selection of High Genetic Merit (HGM) Breeding Boar

In modern commercial breeding programs, most boars of high genetic merit are housed in boar stations, where they are managed for production of semen doses for use with artificial insemination. Boar stations should be typically managed to ensure the highest levels of health and biosecurity. However, on smaller farms, breeding boars may receive insufficient attention in herd health programs. Regardless of the size of the operation, as with other food animal species, boars should be examined for breeding soundness before selection and use in a breeding program. Problems become evident when boars show lack of libido or inability to copulate, or if an increased number of females bred by the boar return to estrus ~3 weeks later. At a minimum, a breeding soundness evaluation should include a history, general physical examination (including genital examination), semen evaluation and behavior evaluation. Selection of boars by visual appraisal is the simplest and oldest method used by the swine industry. However, individual performance testing and later use of computers to incorporate relatives' data and account for environmental variation, resulted in greater rate of improvement for economically

important traits. Examples of molecular genetic tools that have increased improvement for some traits may be applied. Accurate identification of genetic merit is increasingly important with widespread use of AI and resultant greater progeny number per sire. The following criteria should be considered for selection of High Genetic Merit (HGM) breeding boars.

a. History/ Pedigree/Genetic testing.

b. Systemic evaluation and diseases testing in serum/plasma and semen.

c. Physical and Genital Examinations.

d. Behavioral Evaluation.

e. Semen Evaluation.

Selection

When selecting a boar for a breeding program, factors such as origination from a specific disease-free herd, performance, soundness and conformation, age of puberty and other pertinent parameters related to reproduction should be considered. All boars to be used in a breeding program should, at a minimum, be seronegative for CSF, ASF, PCV, PPV, brucellosis, porcine reproductive and respiratory syndrome and pseudorabies (Aujeszky disease). In addition, all boars should be isolated and acclimatized for at least 45–60 days and be tested/retested for diseases naive to the herd before introduction into the herd. If involved in the selection process, boars from large litters that reach puberty early (5½–6 months) tend to produce highly productive daughters who also reach puberty at an early age. Performance parameters such as feed efficiency, backfat and average daily gain are also highly heritable. Boars with 90kg body weight with length not less than 100 cm are preferred for breeding though relaxation can be given to the above traits for Indian breeds as per their conformation. Generally, animals with maximum weight at 6 months of age are preferred for breeding. The breeding boar must have well developed testes, stout body and wide apart strong legs, high vigor with active look and free from diseases and physical defects. Boar should not be used for breeding purposes until it attains 28-30weeks of age. Selection of boar should be done with utmost importance with maximum selection intensity.

Skeletal conformation and examination for current or potential locomotor dysfunction should be assessed. Any unsoundness that may interfere with the boar's ability to approach, mount and successfully breed/ejaculate should be determined. Acute or chronic musculoskeletal conditions may elicit pain that causes the boar to appear uninterested in mounting. Boars are usually selected as breeding prospects at 3–6 months old. The genetic background of the boar should be consistent with the intended use. Selection of boars with heritable defects such as umbilical or inguinal hernias, cryptorchidism, rectal prolapse and poor underlines can be avoided by careful analysis of the source herd production records.

Genital Examinations:

The testicles, epididymides and scrotum should be examined and palpated for size, symmetry (<1 cm difference in diameter), consistency and pathologic changes. An appreciation of normal testicular consistency is necessary to detect subtle changes. The penis and prepuce should be examined for abnormalities during semen collection. Testicular size is directly correlated with genotype, age and weight of boars between 142–282 days of age and 185–375 lb (84–171 kg) body weight. Testicular size increases until ~18 months of age; testicular growth and sperm numbers increase at the greatest rate between 5 and 12 months of age. Because age and testicular weight are important identifiers of early sexual development, boars should be ≥8 months old before use in a breeding program.

Libido/Behavioral Evaluation

Poor libido is likely caused by behavioral rather than endocrinologic problems. Semen collection allows evaluation of boar libido and ability to breed and it provides a sample ejaculate for quality assessment. Boars exhibit precopulatory sexual behavior in response to visual and olfactory stimulation. The boar grunts or barks rhythmically, chomps jaws, salivates and typically engages in head-to-head contact with the sow or dummy, followed by nuzzling her flanks or the side of the dummy to test for voluntary immobilization. These activities should be observed, because aberrant sexual behavior may result in infertility. There are also breed and strain differences; the tendency to be timid, nervous and nonaggressive can be influenced by selection in a breed over several generations and can result in boars with poor libido. Pain from genital lesions or musculoskeletal problems can have a strong negative effect. Libido can also be impaired by an unfamiliar environment, the presence of a feared person, or distractions such as available feed. Once the boar has mounted, erection and protrusion of the penis occur as the boar searches for the vulva and tactile stimulation. Close observation is necessary to notice injuries and lesions of the penis as well as improper erection. Congenital and genetic problems include incomplete erection, penile hypoplasia, masturbation into the diverticulum (ie, “balling up”) and persistent frenulum.

Guidelines for Boar Semen Evaluation

1. Ideally, libido, mating ability, semen quality and breeding results (conception rate and litter size) should be considered in boar evaluation.
2. If a boar produces an ejaculate of low or marginal quality when examined in vitro, additional ejaculates should be assessed at 1- to 2-week intervals to ascertain whether quality has improved over time.
3. Valuable boars with spermiograms that do not improve over 2–3 months are unlikely to ever improve and should be culled.
4. Boars with azoospermia on two complete ejaculates or that are unable to achieve

complete erection should be culled immediately.

5. Those that have penile lesions or blood in the semen should be sexually rested for ≥2–3 weeks and reevaluated.
6. For boars with persistent frenulum or that habitually masturbate in the diverticulum, surgical correction is recommended; however, the progeny should not be kept for breeding, because these conditions are most likely heritable.
7. All results of the fertility examination must be considered in relation to age, disease history, environmental stress, prior breeding usage, breeding system and the techniques of semen collection and handling.

Table 18.1: Suggested Spermiogram Values for Boars used in Artificial Insemination

Parameter	Minimum criteria/Cut off values
Ejaculate volume	≥80–150 mL for sperm-rich fraction and ≥200–400 mL for whole ejaculate
Color	Opaque to white
Total sperm numbers	>35–100 × 10^9 sperm/ejaculate
Concentration	200–500 sperm × 10^6/mL depending on ejaculate volume collected
Sample motility	>70%–90%
Abnormal morphology (including droplets)	< 20%–25%
Cytoplasmic droplets (both proximal and distal)	< 15%–20%

(Source: MSD Veterinary Manual)

19. Reproductive Parameters of Pig

Female		Male	
Puberty	150-180 d	Germ cell division and differentiation.	Fetus (20-40 days gestation)
Breeding age.	210-230d	Testicular descent from the abdomen into the scrotum.	Fetus (60-90 days gestation
Cycle.	Polyestrous year	Mounting behavior displayed.	1-2 months
Duration of cycle.	21 (18-24 d); LP: 13-15d; FP:5-7d	2nd germ cell division and increase in testes to b.wt. ratio.	3 months
Duration of estrus.	Gilt (36-48h); Sow (48-72h)	Sperm appears in seminiferous tubules and erection can occur.	4 months
Time of ovulation.	32-48h after onset of estrus	Puberty begins and sperm appear in ejaculate.	5.5 months
Type of ovulation.	Spontaneous	Testis size, semen concentration & ejaculate volume increases.	6-18 months
Ovulation rate.	Gilt (10-15f); Sow (15-25f)	Sperm transport time in oviduct.	Approx.15-30 min.
Duration of ovulation.	Approx. 4-6h (induced) 1-3 h spontaneous	Capacitation time (oviduct).	4-6 h
Fertility of ovulated egg.	Max. 6-10h	Preserved sperm fertility (oviduct).	Approx. 18-24 h
Lactational anoestrus.	16–40d	Semen preservation temp.	15-17°C
Weaning to Estrus Interval (WEI).	4–6 d	Optimum mating/ insemination time.	32-48 h after onset of estrus.
Major source of P4 in pregnancy.	CL	Spermatogenesis duration.	39d.
Gestational duration.	114 (112-117d)	Pheromone Production.	Saliva.

Parameter	Average (range)	Comments
Pre-attachment period.	2 days in oviduct.	Stress and overfeeding will reduce implantation and increase embryo loss.
Embryonic period.	13-34 days.	--
Attachment starts.	13–14 of pregnancy.	
Implanted and expansion of allantois.	Day 15-20.	
MRP (Exocrine-Endocrine model.	days 11 and 12 after ovulation.	E2, IFNG, IFND
Fetal period.	35-114 days.	Skeletal ossification commences around 35d.
Development of fetal immunocompetence.	From day 70-75.	Embryonic immune reaction: day 30 Immunocompetence: day 70.
Placentation.	Estrogen, Prolactin-luteotropic.	epitheliochorial, diffuse and non-deciduate (or adeciduate).

20. Vaccination and deworming for pig

1. Vaccination Program:

Vaccine	Dose &Route of administration	Age of Primary vaccination*	1st Booster	Revaccination
Classical Swine Fever Vaccine.	1ml, I/M	Post weaning (Weaning is usually done at 45 days).	After 30 days of 1st vaccination.	6-month interval.
FMD Vaccine (Oil adjuvanted trivalent).	2ml, I/M	2 Months.	-	6-month interval.
Porcine Circo virus vaccine.	1ml, I/M	3weeks of age (Single dose vaccine).	-	-
*Consider Lapanized/cell culture vaccination as per manufacturers guidelines.				

2. Deworming Schedule: Deworming of the animals should be done for both end and ecto parasites as per the recommendation of Veterinarian.

For Control of Endoparasite				
Name of dewormer	**Dose rate**	**First dose**	**Second dose**	**Repeat**
Piperazine liquid (45 % w/v).	@100-300mg/kg body weight.	at 3 weeks of age	at 21days of first deworming	at 2 months interval
Albendazole.	@5-7.5 mg/Kg. body weight orally.			
Fenbendazole.	@5-7.5 body orally mg/Kg. weight.			
For Control of Ectoparasite				
Ivermectin	@ 0.3-0.6mg/kg. body weight subcutaneously			

3. General health management for pigs:

Separating the umbilical cord.	The naval or umbilical cord should be cut (keeping 2.5 cm distance from umbilicus) as early as possible after birth.	Disinfected by using an io-dine solution to prevent any secondary infection.
Teeth clipping.	Done on the day of birth.	It is advisable to clip teeth using a suitable tusk clipper.
Iron injection.	Iron supplements.	I/M, on 4th and 14th day of birth.

21. Pig Body Condition Score Card

	Body Condition Score 1	Body Condition Score 2	Body Condition Score 3	Body Condition Score 4	Body Condition Score 5
Appearance	Emaciated	Thin	Ideal	Fat	Obese
Fat Cover	The pig is visually thin, with hips and backbone every prominent and no fat cover over hips and backbone.	The hip bones and backbone are easily felt without any pressure on the palms.	It takes firm pressure with the palm to feel the hip bones and backbone.	It is impossible to feel the bones at all even with pressure on the palm of the hands.	The pig is carrying so much fat that it is Impossible to feel the hip bones and backbone even by pushing down with a single finger.
Pelvic bones	Very prominent. Deep cavity around tail head.	Obvious with slight cover.	Covered but felt pressure.	Only felt with firm pressure. No cavities around tail.	Thick fat cover, impossible to feel bones. Flankfull and rounded.
Loin	Vertebrae are prominent and sharp. Very narrow loin. Hollow flank.	Narrow loin. Flank rather hollow. Slight cover on spine, but prominent vertebrae.	Spine covered and rounded.	Difficult to feel vertebrae. Flank filled.	Thick fat cover, impossible to feel bones. Flankfull and rounded.
Ribs	Individual ribs are very prominent.	Rib cage less apparent but individual ribs easily detected with slight pressure.	Ribs are covered but can be felt with pressure.	Rib cage not visible and difficult to feel.	Thick fat cover, not possible to feel ribs.

(FACT SHEET 12; CPC, Canada)

22. Registered Breeds of Pig in India

S.N.	Breed	Home Tract	Accession number
New Breeds			
1.	Ghoongroo	West Bengal	INDIA_PIG_2100_GHOONGROO_09001
2.	Niang Megha	Meghalaya	INDIA_PIG_1300_NIANGMEGHA_09002
3.	Agonda Goan	Goa	INDIA_PIG_3500_AGONDAGOAN_09003
4.	Tenyi Vo	Nagaland	INDIA_PIG_1400_TENYIVO_09004
5.	Nicobari	Andaman & Nicobar	INDIA_PIG_3300_NICOBARI_09005
6.	Doom	Assam	INDIA_PIG_0200_DOOM_09006
7.	Zovawk	Mizoram	INDIA_PIG_2700_ZOVAWK_09007
8.	Ghurrah	Uttar Pradesh	INDIA_PIG_2000_GHURRAH_09008
9.	Mali	Tripura	INDIA_PIG_1900_MALI_09009
10.	Purnea	Bihar and Jharkhand	INDIA_PIG_0325_PURNEA_09010
11.	Banda	Jharkhand	INDIA_PIG_2500_BANDA_09011
12.	Manipuri Black	Manipur	INDIA_PIG_1200_MANIPURIBLACK_09012
13.	Wak Chambil	Meghalaya	INDIA_PIG_1300_WAKCHAMBIL_09013
14.	Andamani	Andaman & Nicobar	INDIA_PIG_3300_ANDAMANI_09014
15.	Karkambi	Maharashtra	INDIA_PIG_1100_KARKAMBI_09015

(Source: https://nbagr.res.in/pig)



Ministry of Fisheries, Animal Husbandry and Dairying
Department of Animal Husbandry and Dairying

Krishi Bhawan, Dr. Rajendra Prasad Rd, Opposite Rail Bhavan,
Rajpath Area, Central Secretariat, New Delhi-110001

Printed by the Manager, Govt. of India Press, Minto Road, New Delhi-110002