CHAPTER 4.6.

GENERAL HYGIENE IN SEMEN COLLECTION,
processing and storage

Article 4.6.1.

General provisions

The objective of this chapter is to provide recommendations that will reduce the likelihood of introduction and spread of *listed diseases* and contamination of fresh, chilled, or frozen semen of various species of donor animals with potentially pathogenic agents in a *semen collection centre*.

This chapter provides recommendations on:

1. procedures for the collection, processing, and storage of semen of bovine, ovine, caprine, porcine, equine, and cervid donor animals;
2. *biosecurity* measures for the operation of *semen collection centres*;
3. conditions applicable to the management and housing of semen donor animals and teasers.

This chapter provides a comprehensive framework for processes that can be applied to reduce the likelihood of transmission of *listed diseases* in semen. *Veterinary Services* play a key role in identifying, assessing, and managing disease *risk* posed by the collection, processing, and storage of semen from various species of donor animals in a *semen collection centre* and establishing appropriate measures to minimize this risk. The *Veterinary Authority* should provide the regulatory standards and/or oversight to ensure that the recommendations in this chapter, as appropriate, are complied with.

Although this chapter is focused on reducing the probability of transmitting *listed diseases* through international trade of semen, the recommendations in this chapter may also be appropriately applied when semen is collected, processed, and stored for domestic distribution.

Recommendations on animal welfare are applicable to the animals kept within the *semen collection centre*, in accordance with relevant articles in Chapter 7.1. of the *Terrestrial Code*.

Recommendations regarding specific animal health requirements for donor animals to provide assurance of the absence of selected *listed diseases*, infections and infestations are found in Chapter 4.7. and other relevant disease-specific chapters.

For the purposes of the *Terrestrial Code,* the *semen collection centre* is comprised of:

1) animal accommodation facilities;

2) semen collection facilities;

3) semen processing facilities, including mobile laboratories;

4) semen storage facilities;

5) administration offices.

The listed facilities may be on one location or consist of single or multiple facility entities on several locations.

For the purposes of this chapter,

1) ‘biosecure’ refers to the state of a place or facility, in which *biosecurity* is effectively implemented;

2) ‘resident facility’ means a biosecure accommodation facility where donor and teaser animals are kept for the purpose of semen collection;

3) ‘pre-entry isolation facility’ means a biosecure accommodation facility where donor and teaser animals are subjected to testing prior to entering the resident facility;

4) ‘germplasm storage tank’ means a sealable canister for storage and transport of semen, embryos or oocytes.

Article 4.6.2.

General conditions applicable to semen collection centres

The *semen collection centre* should be approved by the *Veterinary Authority*.

For that purpose, the *Veterinary Services* should conduct regular audits of *biosecurity plans*, protocols, procedures and records on the health of the animals in the *semen collection centre* and on the hygienic production, storage and dispatch of semen, at least annually, and request appropriate corrective actions, if needed.

Each facility in the *semen collection centre* should be under the direct supervision of a *veterinarian* who is responsible for ensuring that the health, welfare, and *biosecurity* in the facilities under his/her supervision are implemented, and all documentation is kept current.

*Animal identification, animal traceability,* and movement registration should be in accordance with Chapter 4.2. and Chapter 4.3.

The *semen collection centre* should implement and document processes that ensure identification and traceability of semen from collection to processing and storage and final dispatch from the semen storage facility. Fresh, chilled, or frozen semen products stored and/or dispatched from the semen storage facility should be identified in accordance with the national regulation to allow accurate and transparent identification of the donor animal, where the semen was collected and/or processed, and when it was collected.

Donor and teaser animals should be maintained in animal accommodation facilities separate from animals not associated with the *semen collection centre* or maintained in separate animal accommodation facilities that may have a different *animal health status*.

*Biosecurity plans* should be developed for the *semen collection centre* in accordance with a *risk analysis* and should at a minimum address the following for each facility:

1) Personnel on the *semen collection centre* should be technically competent and apply high standards of personal hygiene, to prevent the introduction of pathogenic agents. Personnel should receive regular training and demonstrate competency of skills applicable to the *semen collection centre* and covering his/her specific responsibilities at the centre, which are documented*.*

2) In general, only donor and teaser animals of the same species should be permitted to the *semen collection centre*. All donor and teaser animals should meet the animal health status as determined by the *semen collection centre* and comply with the regulations set out by the *Veterinary Authority*. If other animals are needed on the *semen collection centre*, such as dogs for herding purposes, these should be kept on the *semen collection centre* and not transferred from one establishment to another and measures to prevent their contacts with *wildlife* should be implemented. Other species may be resident on the *semen collection centre*, provided that appropriate pre-entry tests have been conducted and *biosecurity* is in place to ensure they meet the animal health status as determined by the *semen collection centre* prior to entry. These animals should be kept in separate biosecure animal accommodation facilities that are physically separate from animals associated with semen production.

3) Natural mating should be avoided at least four weeks prior to entry into the pre-entry isolation facility and avoided after entry into the animal accommodation facility or semen collection facility.

4) Measures should be in place to prevent the entry of *wildlife* susceptible to pathogenic agents transmissible to the animals in the *semen collection centre*.

5) The entry of visitors to any part of the *semen collection centre* where *biosecurity* is required should only be allowed if authorised and controlled. Appropriate protective clothing and footwear only for use within the *semen collection centre* facilities should be provided. Footbaths should be provided, where necessary, and regularly cleaned. Records should be kept of all visitors that enter the *semen collection centre*.

6) Appropriate *disinfection* of work areas and equipment should be implemented and documented regularly by trained and competent staff.

7) Control measures should be in place to minimise the entry of insects and rodents.

8) *Vehicles* for the transport of animals, *feed*, and waste and manure removal should be used in a manner which minimises health risks to animals in the *semen collection centre.*

Article 4.6.3.

Recommendations applicable to animal accommodation facilities

Animal accommodation facilities should be designed so that cleaning and *disinfection* measures are easy and efficient to implement. Individual and group housing pens should be kept clean and the bedding renewed as often as necessary to ensure it is dry and clean.

The animal accommodation facilities should include dedicated areas for *feed* storage, for manure storage, bedding storage, and for the isolation of any sick animals. Animal accommodation facilities should be species-specific, where relevant.

There should be a separate pre-entry isolation facility that is managed as a separate biosecure facility for holding animals that are required to complete testing and isolation prior to entry to the resident facility. Procedures for animal identification, blood sampling and vaccination of animals within the *semen collection centre* should be conducted in accordance with relevant recommendations in the *Terrestrial Code*. In the instance where the *Veterinary Authority* has determined that pre-entry isolation facility is not required, pre-entry conditions to enter the resident facility or semen collection facility should be included in the *biosecurity plan* of the *semen collection centre.*

The decision to house animals indoors or outdoors will be determined by the *semen collection centre* in accordance with the *biosecurity plan*. Donor animals and teasers that are housed outdoors or allowed access outdoors, should be accommodated to minimise *vector* attacks and adequately protected from adverse weather conditions. Donor animals and teasers that are housed indoors, should be accommodated to allow for adequate ventilation and proper footing and bedding.

All donor and teaser animal accommodations should be adapted to the needs of the species of donor being collected. Watering and feeding systems should be constructed so that it provides minimum contact between donor animals and can be easily cleaned.

Bedding should be clean and dry, soft, easy to spread and remove. Bedding should be removed regularly and replaced, following thorough cleaning and *disinfection* of relevant surfaces.

*Feed* and bedding material should be kept in a dry place and stored in a manner to prevent access by *wildlife* or pests and stored in conditions that are well monitored.

Manure, litter, and bedding material should be disposed of in such a way as to prevent the transmission of diseases and be in compliance with all relevant health and environmental legislation.

Article 4.6.4.

Recommendations applicable to semen collection and semen collection facilities

The semen collection facility can be co-located with the resident facility and share *biosecurity* to accommodate the same designated *animal health status* of the resident facility. If the semen collection facility is co-located with a resident facility, the semen collection facility should not be used to collect other donor animals not housed in the resident facility. If the semen collection facility is a separate facility, *biosecurity* should be in place to allow only animals of the same *animal health status* to be permitted entry into that facility.

Donors and teaser animals should be kept and prepared in a way to facilitate the hygienic collection of semen. Donor animals should be dry and clean when arriving in the semen collection area.

Donor animals should be collected in the semen collection facility and not collected in the resident facility.

Personnel and visitors should be provided with protective clothing and footwear for use only at the semen collection facilities and worn at all times.

Equipment used for the animals should be dedicated to the semen collection facility or disinfected before being introduced to the centre. All other equipment and tools brought on to the premises should be examined and *disinfected*, if necessary, to minimise the introduction of pathogenic agents.

The semen collection facility and associated equipment should allow for effective cleaning and *disinfection*,where applicable.

The floor of the mounting area should be clean and provide safe footing. When rubber mats are used, they should be cleaned after each collection.

Preputial orifices of donor animals should be clean and free of excessive hair or wool to avoid contamination of the semen. Hair or wool at the preputial orifice should be regularly trimmed as needed but not completely removed to avoid excessive irritation of the preputial mucosa while urinating.

Hair or wool on the hindquarters of teaser animals should be kept short to avoid contamination during the collection process. A teaser animal should have its hindquarters thoroughly cleaned before each collection session. A plastic apron can be used to cover the hindquarters of the teaser animal, but the apron should be replaced with a clean apron or thoroughly cleaned and *disinfected* between donor animals.

A dummy mount, if used, should be made of a material that is easy to clean and disinfect and should be thoroughly cleaned after each collection. Disposable plastic covers may be used.

When used, the artificial vagina should be cleaned completely after each collection. It should be dismantled, washed, rinsed, dried, and protected from dust. The inside of the body of the device and the cone should be *disinfected* before re-assembly using *disinfection* procedures approved by the *Veterinary Authority.*

Lubricant used in the artificial vagina should be new and the equipment used to spread the lubricant should be clean and free of dust.

The artificial vagina should be handled in a manner to prevent dirt and debris from entering.

When successive ejaculates are being collected from the same donor, a new artificial vagina should be used for each collection to prevent any contamination. The artificial vagina should also be changed when the animal has inserted its penis without ejaculating.

All semen should be collected into a sterile receptacle, either disposable or sterilised by autoclaving or heating and kept clean prior to use.

After semen collection, the receptacle should be left attached to the cone within its sleeve or sheath until it has been removed from the collection area to the laboratory.

During collection, the technician should wear disposable gloves and change them between donor animals.

Article 4.6.5.

General principles applicable to semen processing and semen processing facilities

The semen processing facility should be physically separated from other semen collection facilities and may include separate areas for the preparation and cleaning of artificial vaginas, semen evaluation and processing, semen pre-storage and storage.

The semen processing facility should be constructed with materials that permit effective cleaning and *disinfection*, in accordance with Chapter 4.14.

Entry to the facility should be restricted to authorised personnel only.

Protective clothing for use only in the semen processing facility should be provided and worn at all times.

The facility and its equipment should be regularly cleaned and well maintained. Work surfaces for semen evaluation and processing should be regularly cleaned and disinfected.

Only semen from the same species and from donors with the same *animal health status* should be processed at the same time. Semen from donors with a different *animal health status* or from different species may be processed consecutively if appropriate hygienic measures in accordance with the *biosecurity plan* have been implemented.

Semen should be collected in a manner that ensures accurate identification and traceability of collecting tubes from the time of semen collection until storage.

All containers and instruments used for the collection, processing, preservation or freezing of semen should be single-use or be cleaned and disinfected or sterilised before use, depending on the manufacturer’s instructions.

The receptacle containing freshly collected semen should be stoppered or covered in a way to prevent contamination as soon as possible after collection, until processing. During processing, containers containing the semen should be stoppered or covered during times when diluent or other components are not being added.

Equipment used for gender-sorting of sperm should be clean and disinfected between ejaculates in accordance with the recommendations of the manufacturer. Where seminal plasma, or components thereof, is added to sorted semen prior to cryopreservation and storage, it should be derived from animals of the same *animal health status*.

Recommendations regarding the use of diluents for processing semen:

1) Buffer solutions used in diluents prepared on the premises should be sterilised by filtration (0.22 µm) or by autoclaving (121°C for 30 minutes) or be prepared using sterile water before adding egg yolk (if applicable) or equivalent additives, or antibiotics.

2) In the case of ready-to-use commercial extenders, the manufacturer’s recommendations should be followed.

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

4) Whenever milk, egg yolk or any other animal protein is used in preparing the semen diluent, the product should be free from pathogenic agents or sterilised; milk heat-treated at 92°C for 3–5 minutes, eggs from SPF flocks when available. When egg yolk is used, it should be separated from the egg white using aseptic techniques. Alternatively, commercial egg yolk prepared for human consumption or egg yolk treated by, for example, pasteurisation or irradiation to reduce bacterial contamination. Commercial powdered skim milk for human consumption may be used. Other additives should be sterilised before use.

5) Diluent should be stored according to manufacturer’s instructions. Storage vessels should be stoppered.

6) Antibiotics may be added to the diluent to minimise the growth of bacterial contaminants or control specific venereal pathogens that may be present in semen.

 Article 4.6.6.

General principles applicable to semen storage and storage facilities

Semen storage facilities and germplasm *s*torage tanks should allow for easy cleaning and *disinfection*.

The manufacturer’s instructions for the safe *disinfection* of germplasm storage tanks should be complied with.

Movement of germplasmstorage tanks from one semen storage facility to another should be completed under controlled conditions subject to the *biosecurity plan* ofthe *semen collection centre.*

Access to the semen storage facility should be restricted to authorised personnel.

Accurate records should be maintained that identify semen being transferred in, stored, and transferred out of the semen storage facility.

Only new liquid nitrogen should be used to fill or top up germplasm storage tanks.