

CHAPTER 4.6.

COLLECTION AND PROCESSING OF BOVINE, SMALL RUMINANT AND PORCINE SEMEN

Article 4.6.1.

General considerations

The purposes of official sanitary control of semen production are to:

- 1) maintain the health of *animals* on an *artificial insemination centre* at a level which permits the international distribution of semen with a negligible risk of infecting other *animals* or humans with pathogens transmissible by semen;
- 2) ensure that semen is hygienically collected, processed and stored.

Artificial insemination centres should comply with recommendations in Chapter 4.5.

Standards for diagnostic tests are described in the *Terrestrial Manual*.

Article 4.6.2.

Conditions applicable to testing of bulls and teaser animals

Bulls and teaser animals should enter an *artificial insemination centre* only when they fulfil the following requirements.

1. Prior to entering pre-entry isolation facility

The *animals* should comply with the following requirements prior to entry into isolation at the pre-entry isolation facility where the country or *zone* of origin is not free from the *diseases* in question.

- a) Bovine brucellosis – Point 3 or 4 of Article 11.3.5.
- b) Bovine tuberculosis – Point 3 or 4 of Article 11.6.5.
- c) Bovine viral diarrhoea (BVD)

The *animals* should be subjected to:

- i) a virus isolation test or a test for virus antigen, with negative results; and
 - ii) a serological test to determine the serological status of every *animal*.
- d) Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis
If the *artificial insemination centre* is to be considered as infectious bovine rhinotracheitis/infectious pustular vulvovaginitis free (IBR/IPV), the *animals* should either:
 - i) come from an IBR/IPV free *herd* as defined in Article 11.11.3.; or
 - ii) be subjected, with negative results, to a serological test for IBR/IPV on a blood sample.
 - e) Bluetongue
The *animals* should comply with Articles 8.3.7. or 8.3.8., depending on the bluetongue status of the country or *zone* of origin of the *animals*.

2. Testing in the pre-entry isolation facility prior to entering the semen collection facilities

Prior to entering the semen collection facilities of the *artificial insemination centre*, bulls and teaser animals should be kept in a pre-entry isolation facility for at least 28 days. The *animals* should be tested as described below a minimum of 21 days after entering the pre-entry isolation facility, except for *Campylobacter fetus* subsp. *venerealis* and *Tritrichomonas foetus*, for which testing may commence after 7 days in pre-entry isolation. All the results should be negative except in the case of BVD antibody serological testing (see point 2 b) i) below).

- a) Bovine brucellosis

The *animals* should be subjected to a serological test with negative results.

b) BVD

- i) The *animals* should be subjected to a virus isolation test or a test for virus antigen, with negative results. Only when all the *animals* in pre-entry isolation have had negative results, may the *animals* enter the semen collection facilities.
- ii) All *animals* should be subjected to a serological test to determine the presence or absence of BVD antibodies.
- iii) Only if no seroconversion occurs in the *animals* which tested seronegative before entry into the pre-entry isolation facility, may any *animal* (seronegative or seropositive) be allowed entry into the semen collection facilities.
- iv) If seroconversion occurs, all the *animals* that remain seronegative should be kept in pre-entry isolation until there is no more seroconversion in the group for a period of three weeks. Serologically positive *animals* may be allowed entry into the semen collection facilities.

c) *Campylobacter fetus* subsp. *venerealis*

- i) *Animals* less than six months old or kept since that age only in a single sex group prior to pre-entry isolation should be tested once on a preputial specimen, with a negative result.
- ii) *Animals* aged six months or older that could have had contact with females prior to pre-entry isolation should be tested three times at weekly intervals on a preputial specimen, with a negative result in each case.

d) *Tritrichomonas foetus*

- i) *Animals* less than six months old or kept since that age only in a single sex group prior to pre-entry isolation, should be tested once on a preputial specimen, with a negative result.
- ii) *Animals* aged six months or older that could have had contact with females prior to pre-entry isolation should be tested three times at weekly intervals on a preputial specimen, with a negative result in each case.

e) IBR/IPV

If the *artificial insemination centre* is to be considered as IBR/IPV free, the *animals* should be subjected, with negative results, to a diagnostic test for IBR/IPV on a blood sample. If any *animal* tests positive, the *animal* should be removed immediately from the pre-entry isolation facility and the other *animals* of the same group should remain in pre-entry isolation and be retested, with negative results, not less than 21 days after removal of the positive *animal*.

f) Bluetongue

The *animals* should comply with the provisions referred to in Articles 8.3.6., 8.3.7. or 8.3.8., depending on the bluetongue status of the country or *zone* where the pre-entry isolation facility is located.

3. Testing programme for bulls and teasers resident in the semen collection facilities

All bulls and teasers resident in the semen collection facilities should be tested at least annually for the following *diseases*, with negative results, where the country or *zone* where the semen collection facilities are located is not free:

- a) Bovine brucellosis
- b) Bovine tuberculosis
- c) BVD

Animals negative to previous serological tests should be retested to confirm absence of antibodies.

Should an *animal* become serologically positive, every ejaculate of that *animal* collected since the last negative test should be either discarded or tested for virus with negative results.

d) *Campylobacter fetus* subsp. *venerealis*

- i) A preputial specimen should be tested.
- ii) Only bulls on semen production or having contact with bulls on semen production need to be tested. Bulls returning to collection after a lay off of more than six months should be tested not more than 30 days prior to resuming production.

e) Bluetongue

The *animals* should comply with the provisions referred to in Article 8.3.10. or Article 8.3.11.

f) *Tritrichomonas foetus*

- i) A preputial specimen should be cultured.
- ii) Only bulls on semen production or having contact with bulls on semen production need to be tested. Bulls returning to collection after a lay off of more than six months should be tested not more than 30 days prior to resuming production.

- g) **IBR/IPV**
If the *artificial insemination centre* is to be considered as IBR/IPV free, the *animals* should comply with the provisions in point 2 c) of Article 11.11.3.
4. Testing for BVD prior to the initial dispatch of semen from each serologically positive bull
Prior to the initial dispatch of semen from BVD serologically positive bulls, a semen sample from each *animal* should be subjected to a virus isolation or virus antigen test for BVD. In the event of a positive result, the bull should be removed from the centre and all of its semen destroyed.
5. Testing of frozen semen for IBR/IPV in artificial insemination centres not considered as IBR/IPV free
Each aliquot of frozen semen should be tested as per Article 11.11.7.

Article 4.6.3.

Conditions applicable to testing of rams/bucks and teaser animals

Rams/bucks and teaser animals should only enter an *artificial insemination centre* if they fulfil the following requirements.

1. Prior to entering pre-entry isolation facility
The *animals* should comply with the following requirements prior to entry into isolation at the pre-entry isolation facility where the country or *zone* of origin is not free from the *diseases* in question.
- a) Caprine and ovine brucellosis – Article 14.1.6.
 - b) Ovine epididymitis – Article 14.7.3.
 - c) Contagious agalactia – Points 1 and 2 of Article 14.3.1.
 - d) Peste des petits ruminants – Points 1, 2 or 4 of Article 14.8.10.
 - e) Contagious caprine pleuropneumonia – Article 14.4.7., depending on the CCPP status of the country or *zone* of origin of the *animals*.
 - f) Paratuberculosis – Free from clinical signs for the past two years.
 - g) Scrapie – Comply with Article 14.9.8. if the *animals* do not originate from a scrapie free country or *zone* as defined in Article 14.9.3.
 - h) Maedi-visna – Article 14.6.2.
 - i) Caprine arthritis/encephalitis – Article 14.2.2. in the case of goats.
 - j) Bluetongue
The *animals* should comply with Articles 8.3.7. or 8.3.8., depending on the bluetongue status of the country or *zone* of origin of the *animals*.
 - k) Tuberculosis – In the case of goats, a single or comparative tuberculin test, with negative results.
2. Testing in the pre-entry isolation facility prior to entering the semen collection facilities
Prior to entering the semen collection facilities of the *artificial insemination centre*, rams/bucks and teasers should be kept in a pre-entry isolation facility for at least 28 days. The *animals* should be tested as described below a minimum of 21 days after entering the pre-entry isolation facility, with negative results.
- a) Caprine and ovine brucellosis – Point 1 c) of Article 14.1.8.
 - b) Ovine epididymitis – Point 1 d) of Article 14.7.4.
 - c) Maedi-visna and caprine arthritis/encephalitis – Test on *animals*.
 - d) Bluetongue
The *animals* should comply with the provisions referred to in Articles 8.3.6., 8.3.7. or 8.3.8., depending on the bluetongue status of the country or *zone* where the pre-entry isolation facility is located.
3. Testing programme for rams/bucks and teasers resident in the semen collection facilities
All rams/bucks and teasers resident in the semen collection facilities should be tested at least annually for the following *diseases*, with negative results, where the country or *zone* where the semen collection facilities are located is not free:
- a) caprine and ovine brucellosis;
 - b) ovine epididymitis;

- c) Maedi-visna and caprine arthritis/encephalitis;
- d) tuberculosis (for goats only);
- e) bluetongue.

The *animals* should comply with the provisions referred to in Article 8.3.10. or Article 8.3.11.

Article 4.6.4.

Conditions applicable to testing of boars

Boars should only enter an *artificial insemination centre* if they fulfil the following requirements.

1. Prior to entering pre-entry isolation facility

The *animals* should be clinically healthy, physiologically normal and comply with the following requirements within 30 days prior to entry into isolation at the pre-entry isolation facility where the country or *zone* of origin is not free from the *diseases* in question.

- a) Porcine brucellosis – Article 15.3.3.
- b) Foot and mouth disease – Articles 8.6.12., 8.6.13. or 8.6.14.
- c) Aujeszky's disease – Article 8.2.9. or Article 8.2.10.
- d) Transmissible gastroenteritis – Article 15.5.2.
- e) Swine vesicular disease – Article 15.4.5. or Article 15.4.7.
- f) African swine fever – Article 15.1.5. or Article 15.1.6.
- g) Classical swine fever – Article 15.2.7. or Article 15.2.8.
- h) Porcine reproductive and respiratory syndrome – Test complying with the standards in the *Terrestrial Manual*.

2. Testing in the pre-entry isolation facility prior to entering the semen collection facilities

Prior to entering the semen collection facilities of the *artificial insemination centre*, boars should be kept in a pre-entry isolation facility for at least 28 days. The *animals* should be subjected to diagnostic tests as described below a minimum of 21 days after entering the pre-entry isolation facility, with negative results.

- a) Porcine brucellosis – Article 15.3.5.
- b) Foot and mouth disease – Articles 8.6.15., 8.6.16., 8.6.17. or 8.6.18.
- c) Aujeszky's disease – Articles 8.2.13., 8.2.14. or 8.2.15.
- d) Transmissible gastroenteritis – Article 15.5.4.
- e) Swine vesicular disease – Article 15.4.9. or Article 15.4.10.
- f) African swine fever – Article 15.1.8. or Article 15.1.9.
- g) Classical swine fever – Article 15.2.10. or Article 15.2.11.
- h) Porcine reproductive and respiratory syndrome – The test complying with the standards in the *Terrestrial Manual*.

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 country or *zone* where the semen collection facilities are located is not free:

- a) Porcine brucellosis – Article 15.3.5.
- b) Foot and mouth disease – Articles 8.6.15., 8.6.16., 8.6.17. or 8.6.18.
- c) Aujeszky's disease – Articles 8.2.13., 8.2.14. or 8.2.15.
- d) Transmissible gastroenteritis – Article 15.5.4.
- e) Swine vesicular disease – Article 15.4.9. or Article 15.4.10.
- f) African swine fever – Article 15.1.8. or Article 15.1.9.
- g) Classical swine fever – Article 15.2.10. or Article 15.2.11.
- h) Porcine reproductive and respiratory syndrome – The test complying with the standards in the *Terrestrial Manual*.

Article 4.6.5.

General considerations for hygienic collection and handling of semen

Observation of the recommendations described in the Articles below will very significantly reduce the likelihood of the semen being contaminated with common bacteria which are potentially pathogenic.

Article 4.6.6.

Conditions applicable to the collection of semen

- 1) The floor of the mounting area should be clean and provide safe footing. A dusty floor should be avoided.
- 2) The hindquarters of the teaser, whether a dummy or a live teaser animal, should be kept clean. A dummy should be cleaned completely after each period of collection. A teaser animal should have its hindquarters cleaned carefully before each collecting session. The dummy or hindquarters of the teaser animals should be sanitized after the collection of each ejaculate. Disposable plastic covers may be used.
- 3) The hand of the person collecting the semen should not come into contact with the *animal's* penis. Disposable gloves should be worn by the collector and changed for each collection.
- 4) The artificial vagina should be cleaned completely after each collection where relevant. It should be dismantled, its various parts washed, rinsed and dried, and kept protected from dust. The inside of the body of the device and the cone should be disinfected before re-assembly using approved *disinfection* techniques such as those involving the use of alcohol, ethylene oxide or steam. Once re-assembled, it should be kept in a cupboard which is regularly cleaned and disinfected.
- 5) The lubricant used should be clean. The rod used to spread the lubricant should be clean and should not be exposed to dust between successive collections.
- 6) The artificial vagina should not be shaken after ejaculation, otherwise lubricant and debris may pass down the cone to join the contents of the collecting tube.
- 7) When successive ejaculates are being collected, a new artificial vagina should be used for each mounting. The vagina should also be changed when the *animal* has inserted its penis without ejaculating.
- 8) The collecting tubes should be sterile, and either disposable or sterilised by autoclaving or heating in an oven at 180°C for at least 30 minutes. They should be kept sealed to prevent exposure to the environment while awaiting use.
- 9) After semen collection, the tube should be left attached to the cone and within its sleeve until it has been removed from the collection room for transfer to the laboratory.

Article 4.6.7.

Conditions applicable to the handling of semen and preparation of semen samples in the laboratory**1. Diluents**

- a) All receptacles used should have been sterilised.
- b) Buffer solutions employed in diluents prepared on the premises should be sterilized by filtration (0.22 µm) or by autoclaving (121°C for 30 minutes) or be prepared using sterile water before adding egg yolk (if applicable) or equivalent additive and antibiotics.
- c) If the constituents of a diluent are supplied in commercially available powder form, the water used should have been distilled or demineralised, sterilized (121°C for 30 minutes or equivalent), stored correctly and allowed to cool before use.
- d) Whenever milk, egg yolk or any other animal protein is used in preparing the semen diluent, the product should be free of pathogens 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 eggs 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, may be used. Other additives should also be sterilized before use.
- e) Diluent should not be stored for more than 72 hours at +5°C before use. A longer storage period is permissible for storage at -20°C. Storage vessels should be stoppered.

- f) A mixture of antibiotics should be included with a bactericidal activity at least equivalent to that of the following mixtures in each ml of frozen semen: gentamicin (250 µg), tylosin (50 µg), lincomycin-spectinomycin (150/300 µg); penicillin (500 IU), streptomycin (500 µg), lincomycin-spectinomycin (150/300 µg); or amikacin (75 µg), divexacin (25 µg).

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

2. Procedure for dilution and packing

- a) The tube containing freshly collected semen should be sealed as soon as possible after collection, and kept sealed until processed.
- b) After dilution and during refrigeration, the semen should also be kept in a stoppered container.
- c) During the course of filling receptacles for dispatch (such as insemination straws), the receptacles and other disposable items should be used immediately after being unpacked. Materials for repeated use should be disinfected with alcohol, ethylene oxide, steam or other approved *disinfection* techniques.
- d) If sealing powder is used, care should be taken to avoid its being contaminated.

3. Conditions applicable to the storage and identification of frozen semen

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

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

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

4. Sperm sorting

Equipment used for sex-sorting sperm should be clean and disinfected between *animals* according to the recommendations of the licencer of the system. Where seminal plasma, or components thereof, is added to sorted semen prior to cryopreservation and storage, it should be derived from *animals* of same or better health status.

Semen straws containing sex-sorted sperm should be permanently identified as such.
