

CHAPTER 4.5.

GENERAL HYGIENE IN SEMEN COLLECTION AND PROCESSING CENTRES

Article 4.5.1.

General considerations

Observation of the recommendations described in the articles below will very significantly reduce the likelihood of the semen being contaminated with common micro-organisms some of which are potentially pathogenic.

Article 4.5.2.

Conditions applicable to artificial insemination centres

1. The artificial insemination centre is comprised of:
 - a) animal accommodation areas (including one isolation facility for sick *animals*) and a semen collection room, these two premises hereon designated as semen collection facilities; accommodation areas should be species specific where relevant;
 - b) a semen laboratory and semen storage areas;
 - c) administration offices;
 - d) apre-entry isolation facility which is not compulsory in case of horses.
2. The centre should be under the direct supervision and control of a centre *veterinarian*.
3. Only *animals* associated with semen production should be permitted to enter the centre. Other species of livestock may exceptionally be resident on the centre, provided that they are kept physically apart from these *animals*.
4. Donors and teasers on the centre should be adequately isolated from farm livestock on adjacent land or buildings for instance by natural or artificial means.
5. The entry of visitors should be strictly controlled. Personnel at a centre should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms. Protective clothing and footwear for use only on the centre should be provided.
6. Individual semen containers and storage rooms should be capable of being disinfected.
7. The centre should be officially approved by the *Veterinary Authority*.
8. The centre should be under the supervision and control of the *Veterinary Services* which will be responsible for regular audits, at an interval of no more than **6 12** months, of protocols, procedures and records on the health and welfare of the *animals* in the centre and on the hygienic production, storage and dispatch of semen.

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Article 4.5.3.

Conditions applicable to semen collection facilities

1. The semen collection facilities should include separate and distinct areas for accommodating resident *animals*, for semen collection, for feed storage, for manure storage, and for the isolation of *animals* suspected of being infected.
2. Only *animals* associated with semen production should be permitted to enter the semen collection facilities. Other species of *animals* may be resident at the centre, if necessary for the movement or handling of the donors and teasers or for security, but contact with the donors and teasers should be minimised. All *animals* resident at the semen collection facilities should meet the minimum health requirements for donors.
3. The donors and teasers should be adequately isolated to prevent the transmission of *diseases* from farm livestock and other *animals*. Measures should be in place to prevent the entry of wild *animals* susceptible to ruminant and swine *diseases* transmissible via semen.
4. Personnel at the centre should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms. Special protective clothing and footwear for use only at the semen collection facilities should be provided and worn at all times inside.
5. Visitors to the semen collection facilities should be kept to a minimum, and visits should be subject to formal authorisation and control. Equipment for use with the livestock should be dedicated to the semen collection facilities or disinfected prior to entry. All equipment and tools brought on to the premises should be examined and treated if necessary to ensure that they cannot introduce *disease*.
6. *Vehicles* used for transport of *animals* to and from the semen collection facilities should not be allowed to enter the facilities.
7. The semen collection area should be cleaned daily after collection. The *animals'* accommodation should be kept clean.
8. Fodder introduction and manure removal should be done in a manner which poses no significant animal health risk.

Article 4.5.4.

Conditions applicable to semen laboratories

1. The semen laboratory should be physically separated from the semen collection facilities, and include separate areas for artificial vagina cleaning and preparation, semen evaluation and processing, semen pre-storage and storage. Entry to the laboratory should be prohibited to unauthorised personnel.
2. The laboratory personnel should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms during semen evaluation, processing and storage.
3. Visitors to the laboratory should be kept to a minimum, and visits should be subject to formal authorisation and control.
4. The laboratory should be constructed with materials that permit effective cleaning and *disinfection*.
5. The laboratory should be regularly cleaned. Work surfaces for semen evaluation and processing should be cleaned and disinfected at the end of each workday.

Annex 8 (contd)

6. The laboratory should be treated against rodents and insects on a regular basis as needed to control these pests.
7. The storage rooms and individual semen containers should be easy to clean and disinfect.
8. Only semen collected from donors having a health status equivalent to or better than the donors at the semen collection facilities should be processed in the laboratory.

Article 4.5.5.

Conditions applicable to the management of bulls, rams, bucks and boars

The objective is to keep the *animals* in a satisfactory state of cleanliness, particularly of the lower thorax and abdomen.

1. Whether on pasture or housed, the *animal* should be kept under hygienic conditions. If housed, the litter should be kept clean and renewed as often as necessary.
2. The coat of the *animal* should be kept clean.
3. For bulls, the tuft of hairs at the preputial orifice, which is often soiled, should be cut to about 2 cm. The hair should not be removed altogether, because of its protective role. If cut too short, irritation of the preputial mucosa may result because these hairs aid the drainage of urine.
4. The *animal* should be brushed regularly, and where necessary on the day before semen collection, paying special attention to the underside of the abdomen.
5. In the event of obvious soiling, there should be careful cleaning, with soap or a detergent, of the preputial orifice and the adjoining areas, followed by thorough rinsing and drying.
6. When the *animal* is brought into the collection area, the technician should make sure that it is clean, and that it is not carrying any excessive litter or particles of feed on its body or its hooves.

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Annex 8 (contd)

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-mucosal disease (BVD-MD)

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.

Annex 8 (contd)

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-MD antibody serological testing (see point 2b)i) below).

a) Bovine brucellosis

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

b) BVD-MD

i) All *animals* should be tested for viraemia as described in point 1c) above.

Only when all the *animals* in pre-entry isolation test negative for viraemia, may the *animals* enter the semen collection facilities upon completion of the 28-day pre-entry isolation period.

ii) After 21 days in pre-entry isolation, all *animals* should be subjected to a serological test to determine the presence or absence of BVD-MD antibodies.

iii) Only if no sero-conversion 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 sero-conversion 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 3 weeks. Serologically positive *animals* may be allowed entry into the semen collection facilities.

c) *campylobacter fetus* subsp. *venerealis*

i) *Animals* less than 6 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 6 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 6 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 6 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.

Annex 8 (contd)

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-MD

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 6 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 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 6 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-MD prior to the initial dispatch of semen from each serologically positive bull

Prior to the initial dispatch of semen from BVD-MD serologically positive bulls, a semen sample from each *animal* should be subjected to a virus isolation or virus antigen test for BVD-MD. 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, and 4 or 5 of Article 14.8.7.
- 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 2 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.

Annex 8 (contd)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 1c) of Article 14.1.8.
- b) Ovine epididymitis – Points 1d) and 2) of Article 14.7.4.
- c) Maedi-visna and caprine arthritis/encephalitis – Test on *animals* and semen.
- 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 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 8.5.12., 8.5.13. or 8.5.14.
- c) Aujeszky's disease – Article 8.2.8. or Article 8.2.9.
- d. ~~Teschevirus encephalomyelitis – Article 15.5.4. or Article 15.5.6.~~

Annex 8 (contd)

- ed) Transmissible gastroenteritis – Article 15.6.2.
- fe) Swine vesicular disease – Article 15.4.5. or Article 15.4.7.
- ff) African swine fever – Article 15.1.5. or Article 15.1.6.
- fg) Classical swine fever – Article 15.2.5. or Article 15.2.6.
- fh) 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 8.5.15., 8.5.16., 8.5.17. or 8.5.18.
- c) Aujeszky's disease – Articles 8.2.12., 8.2.13. or 8.2.14.
- ~~d. Teschovirus encephalomyelitis – Article 15.5.8. or Article 15.5.9.~~
- ed) Transmissible gastroenteritis – Article 15.6.4.
- fe) Swine vesicular disease – Article 15.4.9. or Article 15.4.10.
- ff) African swine fever – Article 15.1.8. or Article 15.1.9.
- fg) Classical swine fever – Article 15.2.8. or Article 15.2.9.
- fh) 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 8.5.15., 8.5.16., 8.5.17. or 8.5.18.
- c) Aujeszky's disease – Articles 8.2.12., 8.2.13. or 8.2.14.
- ~~d. Teschovirus encephalomyelitis – Article 15.5.8. or Article 15.5.9.~~
- ed) Transmissible gastroenteritis – Article 15.6.4.
- fe) Swine vesicular disease – Article 15.4.9. or Article 15.4.10.
- ff) African swine fever – Article 15.1.8. or Article 15.1.9.

Annex 8 (contd)

hg) Classical swine fever – Article 15.2.8. or Article 15.2.9.

ih) 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 laboratory1. 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 (75µg), divexacin (25µg).

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 of semen

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

Annex 8 (contd)

Semen straws should be sealed and code marked in line with the international standards of the International Committee for Animal Recording (ICAR)¹.

Prior to export, semen straws or pellets 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.

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1. The ICAR international standards on straws are contained in Recording Guidelines - Appendices to the international agreement of recording practices. The text of this document is available at the following web site: www.icar.org

CHAPTER 4.7.

COLLECTION AND PROCESSING OF *IN VIVO*
DERIVED EMBRYOS FROM LIVESTOCK AND
HORSES

Article 4.7.1.

Aims of control

The purpose of official sanitary control of *in vivo* derived embryos intended for movement internationally is to ensure that specific pathogenic organisms, which could be associated with embryos, are controlled and transmission of *infection* to recipient *animals* and progeny is avoided.

Article 4.7.2.

Conditions applicable to the embryo collection team

The embryo collection team is a group of competent technicians, including at least one *veterinarian*, to perform the collection, processing and storage of embryos. The following conditions should apply:

1. The team should be approved by the *Competent Authority*.
2. The team should be supervised by a team *veterinarian*.
3. The team *veterinarian* is responsible for all team operations which include verification of donor health status, sanitary handling and surgery of donors and *disinfection* and hygienic procedures.
4. Team personnel should be adequately trained in the techniques and principles of disease control. High standards of hygiene should be practiced to preclude the introduction of *infection*.
5. The collection team should have adequate facilities and equipment for:
 - a) collecting embryos;
 - b) processing and treatment of embryos at a permanent site or mobile laboratory;
 - c) storing embryos.

These facilities need not necessarily be at the same location.

6. The embryo collection team should keep a record of its activities, which should be maintained for inspection by the *Veterinary Authority* for a period of at least 2 years after the embryos have been exported.
7. The embryo collection team should be subjected to regular inspection at least once a year by an *Official Veterinarian* to ensure compliance with procedures for the sanitary collection, processing and storage of embryos.

Annex 8 (contd)

Article 4.7.3.

Conditions applicable to processing laboratories

A processing laboratory used by the embryo collection team may be mobile or permanent. It is a facility in which embryos are recovered from collection media, examined and subjected to any required treatments such as washing and being examined and prepared for freezing and storage. A permanent laboratory may be part of a specifically designed collection and processing unit, or a suitably adapted part of an existing building. It may be on the premises where the donor *animals* are kept. In either case, the laboratory should be physically separated from *animals*. Both mobile and permanent laboratories should have a clear separation between dirty areas (animal handling) and the clean processing area.

Additionally:

1. The processing laboratory should be under the direct supervision of the team *veterinarian* and be regularly inspected by an *Official Veterinarian*.
2. While embryos for export are being handled prior to their storage in ampoules, vials or straws, no embryos of a lesser health status should be processed.
3. The processing laboratory should be protected against rodents and insects.
4. The processing laboratory should be constructed with materials which permit its effective cleansing and *disinfection*. This should be done frequently, and always before and after each occasion on which embryos for export are processed.

Article 4.7.4.

Conditions applicable to the introduction of donor animals1. Donor animals

- a) The *Veterinary Authority* should have knowledge of, and authority over, the *herd/flock* from which the donor *animals* have been sourced.
- b) The donor *animals* should not be situated in a *herd/flock* subject to veterinary restrictions for OIE *listed disease* or pathogens for relevant species (see Chapter 1.2. of the *Terrestrial Code*), other than those that are in IETS Category 1 for the species of embryos being collected (see Article 4.7.14. and footnote¹).
- c) At the time of collection, the donor *animals* should be clinically inspected by the team *veterinarian*, or by a *veterinarian* responsible to the team *veterinarian* and certified to be free of clinical signs of *diseases*.

2. Semen donors

- a) Semen used to inseminate donor *animal s* artificially should have been produced and processed in accordance with the provisions of Chapter 4.6.
- b) When the donor of the semen used to inseminate donor females for embryo production is dead, and when the health status of the semen donor concerning a particular infectious *disease* or *diseases* of concern was not known at the time of semen collection, additional tests may be required of the inseminated donor female after embryo collection to verify that these infectious *diseases* were not transmitted. An alternative may be to test an aliquot of semen from the same collection date.

Annex 8 (contd)

- c) Where natural service or fresh semen is used, donor sires should meet the health conditions set out in Chapter 4.6. as appropriate to the species.

Article 4.7.5.

Risk management

With regard to *disease* transmission, transfer of *in vivo* derived embryos is a very low risk method for moving animal genetic material. Irrespective of animal species, there are three phases in the embryo transfer process that determine the final level of risk:

1. The first phase, which is applicable to *diseases* not included in Category 1 of the IETS categorisation 1 (Article 4.7.14.), comprises the risk potential for embryo contamination and depends on:
 - a) the disease situation in the *exporting country* and/or *zone* ;
 - b) the health status of the *herds/flocks* and the donors from which the embryos are collected;
 - c) the pathogenic characteristics of the specified disease agents that are of concern to the *Veterinary Authority* of the *importing country* .
2. The second phase covers risk mitigation by use of internationally accepted procedures for processing of embryos which are set out in the IETS Manual². These include the following:
 - a) The embryos should be washed at least ten times with at least 100-fold dilutions between each wash, and a fresh pipette should be used for transferring the embryos through each wash.
 - b) Only embryos from the same donor should be washed together, and no more than ten embryos should be washed at any one time.
 - c) Sometimes, for example when inactivation or removal of certain viruses (e.g. bovine herpesvirus-1, and Aujeszky's disease virus) is required, the standard washing procedure should be modified to include additional washes with the enzyme trypsin, as described in the IETS Manual².
 - d) The zona pellucida of each embryo, after washing, should be examined over its entire surface area at not less than 50X magnification to ensure that it is intact and free of adherent material.

[NOTE: All shipments of embryos should be accompanied by a statement signed by the team veterinarian certifying that these embryo processing procedures have been completed.]
3. The third phase, which is applicable to *diseases* not included in Category 1 of the IETS categorisation 1 (Article 4.7.14.) and which are of concern to the *Veterinary Authority* of the *importing country*, encompasses the risk reductions resulting from:
 - a) post-collection *surveillance* of the donors and donor *herd/flock* based on the recognized *incubation periods* of the *diseases* of concern to determine retrospectively the health status of donors whilst the embryos are stored (in species where effective storage by cryopreservation is possible) in the *exporting country*;
 - b) testing of embryo-collection (flushing) fluids and non-viable embryos, or other samples such as blood, in a laboratory for presence of specified disease agents.

Annex 8 (contd)

Article 4.7.6.

Conditions applicable to the collection and storage of embryos1. Media

Any biological product of animal origin used in the media and solutions for collection, processing, washing or storage of embryos should be free of pathogenic micro-organisms.

Media and solutions used in the collection and storage of embryos should be sterilized by approved methods according to the IETS Manual² and handled in such a manner as to ensure that sterility is maintained. Antibiotics should be added to collection, processing, washing and storage media as recommended in the IETS Manual².

2. Equipment

- a) All equipment used to collect, handle, wash, freeze and store embryos should ideally be new or at least sterilised prior to use as recommended in the IETS Manual².
- b) Used equipment should not be transferred between countries for re-use by the embryo collection team.

Article 4.7.7.

Optional tests and treatments

1. The testing of samples can be requested by an *importing country* to confirm the absence of pathogenic organisms that may be transmitted via *in vivo* derived embryos, or to help assess whether the degree of quality control of the collection team (with regard to adherence to procedures as described in the IETS Manual²) is at an acceptable level. Samples may include:

a) Non-viable embryos/oocytes

Where the viable, zona pellucida intact embryos from a donor are intended for export, all non-fertilized oocytes and degenerated or zona pellucida compromised embryos collected from that donor should be washed according to the IETS Manual² and pooled for testing if requested by the *importing country*. Non-viable embryos/oocytes from the donor should be processed and stored together.

b) Embryo collection (flushing) fluids

The collection fluid should be placed in a sterile, closed container and, if there is a large amount, it should be allowed to stand undisturbed for one hour. The supernatant fluid should then be removed and the bottom 10-20 ml, along with accumulated debris, decanted into a sterile bottle. If a filter is used in the collection of embryos/oocytes then any debris that is retained on the filter should be rinsed off into the retained fluid.

c) Washing fluids

The last four washes of the embryos/oocytes should be pooled (IETS Manual²).

d) Samples

The samples referred to above should be stored at 4°C and tested within 24 hours. If this is not possible, then samples should be stored frozen at -70°C or lower.

Annex 8 (contd)

2. When treatment of the viable embryos is modified to include additional washings with the enzyme trypsin (see paragraph 2c) in Article 4.7.5.), the procedure should be carried out according to the IETS Manual². Enzyme treatment is necessary only when pathogens for which the IETS recommends this additional treatment (such as with trypsin) may be present.

It should be noted that such treatment is not always beneficial and it should not be regarded as a general disinfectant. It may also have adverse effects on embryo viability, for instance in the case of equine embryos where the embryonic capsule could be damaged by the enzyme.

Article 4.7.8.

Conditions applicable to the storage and transport of embryos

1. The embryos for export should be stored in sealed sterile ampoules, vials or straws under strict hygienic conditions at a storage place approved by the *Veterinary Authority* of the *exporting country* where there is no risk of contamination of the embryos.
2. Only embryos from the same individual donor should be stored together in the same ampoule, vial or straw.
3. The embryos should if possible, depending on the species, be frozen, stored with fresh liquid nitrogen in cleaned and sterilized tanks or containers under strict hygienic conditions at the approved storage place.
4. Ampoules, vials or straws should be sealed at the time of freezing (or prior to export where cryopreservation is not possible), and they should be clearly identified by labels according to the standardised system recommended in the IETS Manual².
5. Liquid nitrogen containers should be sealed under the supervision of the *Official Veterinarian* prior to shipment from the *exporting country*.
6. Embryos should not be exported until the appropriate veterinary certificates are completed.

Article 4.7.9.

Procedure for micromanipulation

When micromanipulation of the embryos is to be carried out, this should be done after completion of the treatments described in point 2 of Article 4.7.5. and conducted in accordance with Chapter 4.9.

Article 4.7.10.

Specific conditions applicable to porcine embryos

The *herd* of origin should be free of clinical signs of swine vesicular disease and brucellosis.

The development of effective cryopreservation methods for the storage of zona pellucida-intact porcine embryos is still at a very early stage.

Annex 8 (contd)

Article 4.7.11.

Specific conditions/comments applicable to equine embryos

The recommendations apply principally to embryos from *animals* continuously resident in national equine populations and therefore may be found unsuitable for those from equines routinely involved in events or competitions at the international level. For instance, in appropriate circumstances horses travelling with an *international veterinary certificate* (e.g. competition horses) may be exempt where mutually agreed upon on a bilateral basis between the respective *Veterinary Authorities*.

Article 4.7.12.

Specific conditions/comments applicable to camelid embryos

South American camelid embryos recovered from the uterine cavity by the conventional non-surgical flushing technique at 6.5 to 7 days post-ovulation are almost invariably at the hatched blastocyst stage, and thus the zona pellucida has already been shed. Since the embryos do not enter the uterus and cannot be recovered before 6.5 to 7 days, it would be unrealistic to stipulate for these species that only zona pellucida-intact embryos can be used in *international trade*. It should be noted however that in 2008 the development of cryopreservation methods for storage of camelid embryos is still at a very early stage, and also that pathogen interaction studies with camelid embryos have not yet been carried out.

Article 4.7.13.

Specific conditions/comments applicable to cervid embryos

The recommendations apply principally to embryos derived from *animals* continuously resident in national domestic or ranched cervid populations and therefore may be found to be unsuitable for those from cervids in feral or other circumstances related to biodiversity or germplasm conservation efforts.

Article 4.7.14.

Recommendations regarding the risk of disease transmission via *in vivo* derived embryos

Based on the conclusions of the Research Subcommittee of the Health and Safety Advisory Committee (HASAC) of the IETS¹, the following *diseases* and pathogenic agents are categorised into four categories, which applies only to *in vivo* derived embryos.

1. Category 1

- a) Category 1 *diseases* or pathogenic agents are those for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer according to the IETS Manual².
- b) The following *diseases* or pathogenic agents are in category 1:
 - Aujeszky's disease (pseudorabies) (swine): trypsin treatment required
 - Bluetongue (cattle)
 - Bovine spongiform encephalopathy (cattle)
 - *Brucella abortus* (cattle)
 - Enzootic bovine leukosis

Annex 8 (contd)

- Foot and mouth disease (cattle)
- Infectious bovine rhinotracheitis: trypsin treatment required
- Scrapie (sheep).

2. Category 2

- a) Category 2 *diseases* are those for which substantial evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer according to the IETS Manual², but for which additional transfers are required to verify existing data.
- b) The following *diseases* are in category 2:
 - Bluetongue (sheep)
 - Caprine arthritis/encephalitis
 - Classical swine fever (hog cholera).

3. Category 3

- a) Category 3 *diseases* or pathogenic agents are those for which preliminary evidence indicates that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer according to the IETS Manual², but for which additional *in vitro* and *in vivo* experimental data are required to substantiate the preliminary findings.
- b) The following *diseases* or pathogenic agents are in category 3:
 - Bovine immunodeficiency virus
 - Bovine spongiform encephalopathy (goats)
 - Bovine viral diarrhea virus (cattle)
 - *Campylobacter fetus* (sheep)
 - Foot and mouth disease (swine, sheep and goats)
 - *Haemophilus somnus* (cattle)
 - Maedi-visna (sheep)
 - *Mycobacterium paratuberculosis* (cattle)
 - *Neosporacanium* (cattle)
 - Ovine pulmonary adenomatosis
 - Porcine reproductive and respiratory disease syndrome (PRRS)
 - Rinderpest (cattle)
 - Swine vesicular disease.

Annex 8 (contd)4. Category 4

- a) Category 4 *diseases* or pathogenic agents are those for which studies have been done, or are in progress, that indicate:
- i) that no conclusions are yet possible with regard to the level of transmission risk; or
 - ii) the risk of transmission via embryo transfer might not be negligible even if the embryos are properly handled according to the IETS Manual² between collection and transfer.
- b) The following *diseases* or pathogenic agents are in category 4:
- African swine fever
 - Akabane (cattle)
 - Bovine anaplasmosis
 - Bluetongue (goats)
 - Border disease (sheep)
 - Bovine herpesvirus-4
 - *Chlamydia psittaci* (cattle, sheep)
 - Contagious equine metritis
 - Enterovirus (cattle, swine)
 - Equine rhinopneumonitis
 - *Escherichia coli* 09:K99 (cattle)
 - *Leptospira borg petersenii* serovar *hardjo bovis* (cattle)
 - *Leptospira* sp. (swine)
 - Lumpy skin disease
 - *Mycobacterium bovis* (cattle)
 - *Mycoplasma* spp. (swine)
 - Ovine epididymitis (*Brucella ovis*)
 - Parainfluenza-3 virus (cattle)
 - Parvovirus (swine)
 - Porcine circovirus (type 2) (pigs)
 - Scrapie (goats)

- *Trichostrongylus axei* (cattle)
- *Ureaplasma/Mycoplasma* spp. (cattle, goats)
- Vesicular stomatitis (cattle, swine).
- Equine coital exanthema (EHV-3).

¹ Based on available research and field information, the Research Subcommittee of the Health and Safety Advisory Committee (HASAC) of the International Embryo Transfer Society (IETS) has categorised some diseases based on their relative risk of dissemination by properly processed and handled *in vivo* derived embryos. This chapter that contains the complete list of IETS categorised diseases is shown in Article 4.7.14.

² Manual of the International Embryo Transfer Society.

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