Annex XIII

CHAPTER 4.~~5~~6.

COLLECTION AND PROCESSING OF BOVINE, SMALL RUMINANT AND PORCINE SEMEN

EU position

The EU can support the adoption of the modified chapter.

However, since the article 14.9.8 of the Chapter on Scrapie is not deleted, then the point 1.g) of article 4.6.3 below should be reinstated.

Article 4.~~5~~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.~~6~~5.

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

Article 4.~~5~~6.2.

Conditions applicable to testing of bulls and teaser animals

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

1. ~~Pre-quarantine~~ Prior to entering pre-entry isolation facility

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

   a) Bovine brucellosis~~.~~ ~~The *animals* should comply with~~ point 3 or 4 of Article 11.3.5.

   b) Bovine tuberculosis~~.~~ ~~The *animals* should comply with~~ point 3 or ~~4~~ of Article 11.7.5.

   c) Bovine viral diarrhoea-mucosal disease (BVD-MD)

      The *animals* should be subjected to ~~the following tests~~:

      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 11.13.3.; 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 ~~8.3.6.,~~ 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 ~~quarantine station~~ 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 ~~*quarantine station*~~ pre-entry isolation facility for at least 28 days. The *animals* should be ~~subjected to diagnostic tests~~ tested as described below a minimum of 21 days after entering the ~~*quarantine station*~~ pre-entry isolation facility, except for *Campylobacter fetus* subsp. *venerealis* and *Tritrichomonas foetus*, for which testing may commence after 7 days in ~~quarantine~~ 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 ~~quarantine~~ pre-entry isolation test negative for viraemia, may the *animals* enter the semen collection facilities upon completion of the 28-day ~~quarantine~~ pre-entry isolation period.

ii) After 21 days in ~~quarantine~~ 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 ~~*quarantine station*~~ 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 quarantine pre-entry isolation ~~over a prolonged time~~ 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 quarantine 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 ~~quarantine~~ 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 ~~quarantine~~ 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 ~~quarantine~~ 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 ~~*quarantine station*~~ pre-entry isolation facility and the other *animals* of the same group should remain in ~~quarantine~~ 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~~9~~6., 8.3.7~~10~~7. or 8.3.8~~11~~8., depending on the bluetongue status of the country or *zone* where the pre-entry isolation facility ~~semen collection centre~~ is located ~~of origin of the *animals*~~.

~~3. 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.~~

~~4. 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 11.13.7.~~

~~5~~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 ~~centre is~~ facilities are located ~~of origin~~ 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.

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d) *Campylobacter fetus* subsp. *venerealis*

i) A preputial specimen should be ~~cultured~~ 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~~s 8.3.6., 8.3.7. or~~ 8.3.811., ~~depending on the bluetongue status of the country of origin of the *animals*~~.

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 11.13.3.

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 11.13.7.

Article 4.~~5~~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. ~~Pre-quarantine~~ Prior to entering pre-entry isolation facility

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

a) Caprine and ovine brucellosis = ~~The *animals* should comply with~~ Article 14.1.6.

b) Ovine epididymitis = ~~The *animals* should comply with~~ Article 14.7.3.

c) Contagious agalactia = ~~The *animals* should comply with~~ points 1 and 2 of Article 14.3.1.

d) Peste des petits ruminants = ~~The *animals* should comply with~~ points 1, 2, and 4 or 5 of Article 14.8.7.

e) Contagious caprine pleuropneumonia = ~~The *animals* should comply with~~ ~~Article 14.4.5. or~~ Article 14.4.7., depending on the CCPP status of the country or *zone* of origin of the *animals*.

f) Paratuberculosis = ~~The *animals* should be~~ free from clinical signs for the past 2 years.

~~g) Scrapie~~

~~If the *animals* do not originate from a scrapie free country or zone as defined in Article 14.9.3., the *animals* should comply with Article 14.9.6.~~

EU position

Since the article 14.9.8 of the Chapter on Scrapie is not deleted, the point g) above should be reinstated as follows:

g) scrapie – article 14.9.8

~~H~~g) Maedi-visna = ~~The *animals* should comply with~~ Article 14.6.2.

~~I~~h) Caprine arthritis/encephalitis = In the case of goats, ~~the *animals* should comply with~~ Article 14.2.2.

Ji) Bluetongue

The *animals* should comply with Articles ~~8.3.6.,~~ 8.3.7. or 8.3.8., depending on the bluetongue status of the country or *zone* of origin of the *animals*.

~~K~~j) Tuberculosis = In the case of goats, ~~the *animals* should be subject to~~ a single or comparative tuberculin test, with negative results.

~~l) Border disease~~

~~The *animals* should be subject to a viral agent isolation test with negative results.~~

2. Testing in the ~~*quarantine station*~~ pre-entry isolation facility ~~station~~ 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 ~~*quarantine station*~~ pre-entry isolation facility for at least 28 days. The *animals* should be ~~subjected to diagnostic tests~~ tested as described below a minimum of 21 days after entering the ~~*quarantine station*~~ pre-entry isolation facility, with negative results.

a) Caprine and ovine brucellosis = ~~The *animals* should be subject to testing as described in~~ point 1c) of Article 14.1.8.

b) Ovine epididymitis = ~~The *animals* and semen should be subject to testing as described in~~ points 1d) and 2 of Article 14.7.4.

c) Maedi-visna and caprine arthritis/encephalitis = ~~The *animals* and semen should be subjected to~~ a ~~serological~~ test ~~for antibodies~~ on *animals* and semen.

d) Bluetongue

The *animals* should comply with the provisions referred to in Articles 8.3.~~6~~9~~6~~., 8.3.~~7~~1~~07~~. or 8.3.~~8~~1~~18~~., depending on the bluetongue status of the country or *zone* where the pre-entry isolation ~~semen collection centre~~ facility is located ~~of origin of the *animals*~~.

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 ~~centre~~ facilities ~~is~~ are located ~~of origin~~ 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 8.3.11.

Article 4.~~5~~6.4.

Conditions applicable to testing of boars

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

1. ~~Pre-quarantine~~ 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 ~~*quarantine station*~~ pre-entry isolation facility where the country or *zone* of origin is not free from the *diseases* in question.

a) Porcine brucellosis = ~~The *animals* should comply with~~ Article 15.4.3.

b) Foot and mouth disease = ~~The *animals* should comply with~~ Articles 8.5.10., 8.5.11. or 8.5.12.

c) Aujeszky's disease = ~~The *animals* should comply with~~ Article 8.2.8. or Article 8.2.9.

~~d) Teschovirus encephalomyelitis~~

~~The *animals* should comply with Article 15.6.4. or Article 15.6.6.~~

~~e~~d) Transmissible gastroenteritis = ~~The *animals* should comply with~~ Article 15.7.2.

~~f~~c) Swine vesicular disease = ~~The *animals* should comply with~~ Article 15.5.5. or Article 15.5.7.

~~g~~f) African swine fever = ~~The *animals* should comply with~~ Article 15.1.5. or Article 15.1.6.

~~h~~g) Classical swine fever = ~~The *animals* should comply with~~ Articles 15.3.5. or 15.3.6.

~~i~~h) Porcine reproductive and respiratory syndrome = ~~The *animals* should be subject to~~ the test complying with the standards in the *Terrestrial Manual*.

2. Testing in the ~~quarantine station~~ 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 ~~*quarantine station*~~ 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 ~~*quarantine station*~~ pre-entry isolation facility, with negative results.

a) Porcine brucellosis = ~~The *animals* should comply with~~ Article 15.4.5.

b) Foot and mouth disease = ~~The *animals* should comply with~~ Articles 8.5.13., 8.5.14., 8.5.15. or 8.5.16.

c) Aujeszky's disease = ~~The *animals* should comply with~~ Articles 8.2.12., 8.2.13. or 8.2.14.

~~d) Teschovirus encephalomyelitis~~

~~The *animals* should comply with Article 15.6.8. or Article 15.6.9.~~

~~e~~d) Transmissible gastroenteritis = ~~The *animals* should comply with~~ Article 15.7.4.

~~f~~e) Swine vesicular disease = ~~The *animals* should comply with~~ Article 15.5.9. or Article 15.5.10.

~~g~~f) African swine fever = ~~The *animals* should comply with~~ Article 15.1.8. or Article 15.1.9.

~~h~~g) Classical swine fever = ~~The *animals* should comply with~~ Articles 15.3.8. or 15.3.9.

~~i~~h) Porcine reproductive and respiratory syndrome = ~~The *animals* should be subject to the test complying with~~ 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 ~~*compartment/ zone* or~~ country or *zone* where the semen collection facilities are located is not free:

a) Porcine brucellosis = ~~The *animals* should comply with~~ Article 15.4.5.

b) Foot and mouth disease = ~~The *animals* should comply with~~ Articles 8.5.13., 8.5.14., 8.5.15. or 8.5.16.

c) Aujeszky's disease = ~~The *animals* should comply with~~ Articles 8.2.12., 8.2.13. or 8.2.14. regarding testing every four months.

~~d) Teschovirus encephalomyelitis~~

~~The *animals* should comply with Article 15.6.8. or Article 15.6.9.~~

~~F~~d) Transmissible gastroenteritis = ~~The *animals* should comply with~~ Article 15.7.4.

~~F~~e) Swine vesicular disease = ~~The *animals* should comply with~~ Article 15.5.9. or Article 15.5.10.

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~~G~~f) African swine fever = ~~The *animals* should comply with~~ Article 15.1.8. or Article 15.1.9. Routine test to be applied at least every six months.

~~H~~g) Classical swine fever = ~~The *animals* should comply with~~ Articles 15.3.8. or 15.3.9.

~~I~~h) Porcine reproductive and respiratory syndrome = ~~The *animals* should be subject to~~ the test complying with the standards in the *Terrestrial Manual*.

Article 4.~~5~~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.~~5~~6.6.

Conditions applicable to the collection of semen

1. The floor of the mounting area should be ~~easy to~~ clean and ~~to disinfect~~ 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 animal 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.~~5~~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 ~~must~~ 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 ~~must~~ 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 ~~must~~ 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), divekacin (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 these ~~recommendations~~ requirements of this chapter ~~in~~ with fresh liquid nitrogen in sterilised/sanitised flasks before being exported.

Annex XIII (contd)

Bovine s~~S~~emen straws should be sealed and code marked in line with the guidelines 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 ~~manufacturer's~~ recommendations of the licenser 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. See International Agreement of Recording Practices: available at the following web site: www.icar.org

CHAPTER 4.~~6~~5.

GENERAL HYGIENE IN SEMEN COLLECTION AND PROCESSING CENTRES

EU position

The EU can support the adoption of the modified chapter.

Article 4.~~6~~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 ~~bacteria~~ which are potentially pathogenic.

Article 4.~~6~~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) ~~A *quarantine station*~~ a pre-entry isolation facility which is not compulsory in case of horses ~~may also be attached to either situated on the same premises as a), b) and c) above but isolated from the aforementioned, or be established at a different site to the centre, provided that it is on a different location from that of those two first parts~~.

~~2. The centre should be officially approved by the *Veterinary Authority*.~~

~~3. 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 months, of protocols, procedures and prescribed records on the health and *welfare* of the *animals* in the centre and on the hygienic production, storage and dispatch of semen.~~

4~~2~~. The centre should be under the direct supervision and control of a~~n *Official*~~ centre *v*~~V~~*eterinarian*.

5~~3~~. Only ~~swine~~ *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 ~~the swine~~ these *animals*.

6~~4~~. ~~Swine~~ 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.

~~7~~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.

~~8~~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 months, of protocols, procedures and ~~prescribed~~ records on the health and *welfare* of the *animals* in the centre and on the hygienic production, storage and dispatch of semen.

Article 4.~~6~~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 ~~must~~ 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 ~~must~~ 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 ~~and semen collection areas~~ should be kept clean~~ed and disinfected at least once a year.~~

8. Fodder introduction and manure removal should be done in a manner which poses no significant animal health risk.

Article 4.~~6~~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.

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.~~6~~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 ~~must~~ should be kept clean and renewed as often as necessary.

2. The coat of the *animal* should be kept clean.

3. For bulls, ~~the length of~~ the tuft of hairs at the preputial orifice, which is ~~invariably~~ 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 ~~must~~ 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~~, for such materials are always heavily contaminated~~.

— text deleted

CHAPTER 4.7.

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

EU position

The EU can support the adoption of the modified chapter.

However, the EU would prefer that the wording of article 4.7.14 below concerning IETS categorisation be modified to reflect that the categorisation is endorsed by the OIE.

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*.

~~1~~2. The team should be supervised by a team *veterinarian*.

~~2~~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.

~~3~~4. ~~The team *veterinarian* should be specifically approved for this purpose.~~

~~4~~54. 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~~65. 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~~7~~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~~8~~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.

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 animals

1. 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 footnote[1]).

   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 animals artificially should have been produced and processed in accordance with the provisions of Chapter 4.~~5~~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 ~~subject~~ an aliquot of semen from the same collection date ~~to testing~~.

c) Where natural service or fresh semen is used, donor sires should meet the health conditions set out in Chapter 4.~~5~~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 Manual[2]. These include the following:

   a) The embryos ~~must~~ should be washed at least ten times with at least 100-fold dilutions between each wash, and a fresh pipette ~~must~~ 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 Manual[2].

   d The zona pellucida of each embryo, after washing, ~~must~~ 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 ~~must~~ 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 (Article 4.7.14.) and which are of concern to the *Veterinary Authority* of the *importing country*, encompasses the risk reductions resulting from:

Annex XIII (contd)

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.

Article 4.7.6.

Conditions applicable to the collection and storage of embryos

1. 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 Manual[2] 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 Manual[2].

2. Equipment

a) All equipment used to collect, handle, wash, freeze and store embryos should ideally be new or at least sterilized prior to use as recommended in the IETS Manual[2].

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 Manual[2]) 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 Manual[2] 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 ~~must~~ should be rinsed off into the retained fluid.

c) Washing fluids

The last four washes of the embryos/oocytes should be pooled (IETS Manual[2]).

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.

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 Manual[2]. 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 ~~necessarily~~ 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 Manual[2].

5. Liquid nitrogen containers should be sealed under the supervision of the *Official Veterinarian* prior to shipment from the *exporting country*.

6. Embryos ~~must~~ 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 ~~and~~ ~~pathogenic enterovirus encephalomyelitis~~.

Annex XIII (contd)

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

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 ~~must~~ should be noted 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

The IETS has categorised[1] the following *diseases* and pathogenic agents into four categories, which applies only to *in vivo* derived embryos.

EU comment

The wording above is a simple restatement of what the IETS has categorised. It should be modified to reflect that the categorisation is endorsed by the OIE: "Based on the conclusions of the HASAC[1] of the IETS, the following *diseases* and pathogenic agents are categorised into four categories, which applies only to *in vivo* derived embryos." This is important because as it is written now, no contestation can be made to the lists and categories, even if the Members may disagree with the IETS conclusions.

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 Manual[2].

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

EU comment

Some EU experts are not convinced that the evidence is sufficient to move scrapie to category 1. But no change is proposed, as the heading of the article simply restate the conclusion of the IETS.

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 Manual[2], but for which additional transfers are required to verify existing data.

b) The following *diseases* or pathogenic agents are in category 2:

EU comment

There is an editorial mistake, see above the highlighted correction.

- Bluetongue (sheep)
- Caprine arthritis/encephalitis
- Classical swine fever (hog cholera)
- ~~Scrapie (sheep).~~

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 Manual[2], 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)
- *Neospora caninum* (cattle)
- Ovine pulmonary adenomatosis
- Porcine reproductive and respiratory disease syndrome (PRRS)
- Rinderpest (cattle)
- Swine vesicular disease.

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 Manual[2] 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 borgpetersenii* serovar *hardjobovis* (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)
- *Tritrichomonas foetus* (cattle)
- *Ureaplasma/Mycoplasma* spp. (cattle, goats)
- Vesicular stomatitis (cattle, swine).

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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.

Annex XIII (contd)

CHAPTER 4.8.

COLLECTION AND PROCESSING OF *IN VITRO* PRODUCED EMBRYOS / OOCYTES FROM LIVESTOCK AND HORSES

EU position

The EU can support the adoption of the modified chapter.

Article 4.8.1.

Aims of control

Production of embryos *in vitro* involves the collection of oocytes from the ovaries of donors, *in vitro* maturation and fertilization of the oocytes, then *in vitro* culture to the morula/blastocyst stage at which they are ready for transfer into recipients. The purpose of official sanitary control of *in vitro* produced embryos intended for movement internationally is to ensure that specific pathogenic organisms, which could be associated with such embryos, are controlled and transmission of *infection* to recipient animals and progeny is avoided. The conditions outlined in this chapter are also applicable where the movement of *in vitro* maturing (IVM) oocytes is intended.

Article 4.8.2.

Conditions applicable to the embryo production team

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

1. The team should be approved by the *Competent Authority.*

~~1~~2. The team should be supervised by a team *veterinarian.*

~~2~~3. The team *veterinarian* is responsible for all team operations which include the hygienic collection of ovaries and oocytes and all other procedures involved in the production of embryos intended for international movement.

~~3~~4. ~~The team *veterinarian* should be specifically approved for this purpose.~~

~~4~~54. Team personnel should be adequately trained in the techniques and principles of disease control. High standards of hygiene should be practised to preclude the introduction of *infection.*

~~5~~65. The production team should have adequate facilities and equipment for:

a) collecting ovaries and/or oocytes;

b) processing of oocytes and production of embryos at a permanent ~~site~~ or mobile laboratory;

c) storing oocytes and/or embryos.

These facilities need not necessarily be at the same location.

~~6~~7. The embryo production 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~~8. The embryo production 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 and processing of oocytes and the production and storage of embryos.

Article 4.8.3.

Conditions applicable to the processing laboratories

A processing laboratory used by the embryo production team may be mobile or permanent. It may be contiguous with the oocyte recovery area or at a separate location. It is a facility in which oocytes which have been recovered from ovaries are then matured and fertilised, and where the resulting embryos are further cultured *in vitro*.

Embryos may also be subjected to any required treatments such as washing and storage and quarantine in this laboratory.

Additionally:

1. The laboratory should be under the direct supervision of the team *veterinarian* and regularly inspected by an *Official Veterinarian*.

2. While embryos for export are being produced prior to their storage in ampoules, vials or straws, no oocyte/embryo of a lesser health status should be recovered or processed in the same laboratory.

3. The 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 when embryos for export are processed.

Article 4.8.4.

Conditions applicable to donor animals

Oocytes for the *in vitro* production of embryos are obtained from donors basically in two different ways: individual collection or batch collection. The recommended conditions for these differ.

Individual collection usually involves the aspiration of oocytes from the ovaries of individual live *animals* on the farm where the *animal* resides, or at the laboratory. Occasionally oocytes may also be recovered from individual live donors by aspiration from surgically excised ovaries. When oocytes are recovered from individual live *animals*, the conditions for these donors should resemble those set out in Article 4.7.4.

In these cases the cleaning and sterilisation of equipment (e.g. ultrasound guided probes) is especially important and ~~must~~ should be carried out between each donor in accordance with the recommendations in the Manual of the International Embryo Transfer Society (IETS)[1].

Batch collection involves the removal of ovaries from batches of donors slaughtered at a slaughterhouse/*abattoir* (hereafter '*abattoir*'); these ovaries are then transported to the processing laboratory where the oocytes are recovered from the ovarian follicles by aspiration. Batch collection has the disadvantage that it is usually impractical to relate the ovaries which are transported to the laboratory to the donors which were slaughtered at the *abattoir*. Nevertheless, it is critical to ensure that only healthy tissues are obtained and that they are removed from the donors and transported to the laboratory in a hygienic manner.

Additionally:

1. The *Veterinary Authority* should have knowledge of~~, and authority over,~~ the *herd(s)*/*flock(s)* from which the donor animals have been sourced.

2. The donor animals should not originate from *herds* / *flocks* ~~which~~ that are subject to veterinary restrictions for ~~*listed diseases* of concern (under study)~~ foot and mouth disease, rinderpest and peste des petits ruminants, and neither should the removal of any tissue or aspiration of oocytes take place in an *infected zone*, or one that is subject to veterinary restrictions for ~~*listed diseases* of concern (under study)~~ those *diseases*.

3. In the case of oocyte recovery from live donors, post-collection surveillance of the donors and donor *herd(s) /flock(s)* should be conducted based on the recognized *incubation periods* of the *diseases* of concern to determine retrospectively the health status of donors.

4. In the case of oocyte recovery from batches of ovaries collected from an *abattoir*, the *abattoir* should be officially approved and under the supervision of a *veterinarian* whose responsibility is to ensure that ante-mortem and post-mortem inspections of potential donor animals are carried out, and to certify them to be free of clinical or pathological signs of ~~infectious~~ the *diseases* ~~(under study)~~ listed in point 2 above.

5. Donor animals slaughtered at an *abattoir* should not have been designated for compulsory *slaughter* for a *notifiable disease* and should not be slaughtered at the same time as donors from which ovaries and other tissues will be removed.

6. Batches of ovaries and other tissues collected from an *abattoir* should not be transported to the processing laboratory before confirmation has been obtained that ante- and post-mortem inspection of donors has been satisfactorily completed.

7. Equipment for the removal and transport of ovaries and other tissues should be cleaned and sterilised before use and exclusively used for these purposes.

8. Records of the identities and origins of all donors should be maintained for inspection by the *Veterinary Authority* for a period of at least 2 years after the embryos have been exported. While this may be difficult to achieve in the case of batch collection, it is to be expected that the identities of the *herds*/*flocks* from which the donors originated will be maintained.

Article 4.8.5.

Optional tests and treatments

~~The main~~ A supplementary approach for ensuring that *in vitro* produced embryos do not transmit *disease* is by testing various materials to confirm the absence of pathogenic organisms listed in point 2 of Article 4.8.4. ~~which~~ ~~that are of concern to the *importing country*~~.

Tests may also be used to assess whether quality control procedures being applied in the processing laboratory are of an acceptable standard.

Annex XIII (contd)

Tests may be carried out on the following materials:

a) non-viable oocytes/embryos from any stage of the *in vitro* production line from batches intended for export;

b) samples of *in vitro* maturation medium taken prior to mixing the oocytes with semen for the fertilisation process;

c) samples of embryo culture medium taken immediately prior to embryo storage.

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

Additionally:

1. Semen used to fertilise oocytes *in vitro* should meet the health requirements and standards set out in Chapter 4. ~~5~~6. as appropriate to the species.

   When the donor of the semen used to fertilise the oocytes is ~~no longer living~~ 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 on the spare embryos may be required to verify that these infectious *diseases* were not transmitted. An alternative may be to test an aliquot of semen from the same collection date.

2. Any biological product of animal origin, including co-culture cells and media constituents, used in oocyte recovery, maturation, fertilisation, culture, washing and storage should be free of living pathogens. Media should be sterilised prior to use by approved methods according to the IETS Manual[1] and handled in such a manner as to ensure that sterility is maintained. Antibiotics should be added to all fluids and media as recommended in the IETS Manual[1].

3. All equipment used to recover, handle, culture, wash, freeze and store oocytes/embryos should be new or cleaned and sterilised prior to use as recommended in the IETS Manual[1].

Article 4.8.6.

Risk management

With regard to disease transmission, transfer of *in vitro* produced embryos is a low risk method for moving animal genetic material although the risk is not quite as low as for *in vivo* derived embryos. It should be noted that categorisation of *diseases*/disease agents by the IETS, as described for *in vivo* derived embryos in Article 4.7.14., does not apply in the case of *in vitro* produced embryos. Irrespective of the animal species, there are three phases in the embryo production and transfer process that determine the final level of risk. These are as follows:

1. the first phase comprises the risk potential for ovary/oocyte/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 ovaries/oocytes/embryos are collected;

   c the pathogenic characteristics of the specified disease agents listed in point 2 of Article 4.8.4. ~~(under study) that are of concern to the *Veterinary Authority* of the *importing country*~~;

2. the second phase covers risk mitigation by the use of internationally accepted procedures for the processing of embryos which are set out in the IETS Manual1[1]. These include the following:

   a) after the *in vitro* culture period is finished 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 (in the case of individual collection) or from the same batch (in the case of batch collection) 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, or 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 Manual[1];

   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;

3. the third phase, which is applicable to *diseases* listed in point 2 of Article 4.8.4. ~~(under study) 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 *herds/flocks* based on the recognised *incubation periods* of the *diseases* of concern to determine retrospectively the health status of the donors whilst the embryos are stored (in species where effective storage by cryopreservation is possible) in the *exporting country*. Post-collection surveillance of donors is not, of course, possible in the case of batch collection from an *abattoir*, although surveillance of the *herds/flocks* of origin may be possible;

   b) testing of oocytes/embryos, co-culture cells, media and other samples (e.g. blood) (as referred to in Article 4.8.4.) in a laboratory for presence of disease agents.

Article 4.8.7.

Conditions applicable to the storage and transport of embryos

1. Only embryos from the same individual donor or from the same batch collection should be stored together in the same ampoule, vial or straw.

2. The embryos should if possible, depending on the species, be frozen in fresh liquid nitrogen or other cryoprotectant and then stored in fresh cryoprotectant in cleaned and sterilised tanks or containers under strict hygienic conditions at a storage place.

3. Ampoules, vials or straws ~~must~~ should be sealed at the time of freezing and should be labelled according to the IETS Manual[1].

4. Liquid nitrogen containers should be sealed prior to shipment from the *exporting country*.

5. Embryos ~~must~~ should not be exported until the appropriate veterinary certificates are completed.

Article 4.8.8.

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 4.8.6. and conducted in accordance with Chapter 4.9.

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[1] Manual of the International Embryo Transfer Society.

CHAPTER 4.10.

COLLECTION AND PROCESSING OF LABORATORY RODENT AND RABBIT EMBRYOS / OVA

EU position

The EU can support the adoption of the modified chapter.

Article 4.10.1.

~~**Conditions applicable to the maintenance of laboratory animal colonies**~~

~~Maintenance of laboratory animal colonies of specific genotypes requires intensive breeding management within specialised premises. They may be kept in a gnotobiotic environment, in either a 'germfree' system or a 'barrier' room (usually with defined flora), in a conventional colony, or under undefined conditions. In both the germfree and barrier systems, the animals are raised in a controlled environment according to protocols that attempt to eliminate potential sources of microbiological contamination. The primary difference is that the barrier maintained animals have been inoculated with known (defined) microbes[1] using a cocktail of non-pathogenic flora, whereas germfree animals are kept free from both pathogenic and non-pathogenic microbes.~~

~~A second category is where laboratory animals are kept in closed, conventional colonies within which known pathogens may exist. Here, less rigid colony management protocols are used to control potential sources of contamination, but implementation of simple aseptic precautions (e.g. autoclaving of feed and bedding) should allow animals to be maintained in a microbiologically defined system. Finally, laboratory animals may live in environments with undefined microbiological conditions (e.g. non-restricted colonies, free-ranging animals).~~

~~*Disease* testing and donor animal/embryo handling requirements can therefore be considered as being of three distinct types, depending on the type of colony being dealt with, i.e. defined floral, conventional and undefined. The health status of all colonies should be confirmed quarterly by bacteriological, virological, parasitological, serological and immunohistochemical tests on pre-designated sentinel animals or other representative animals of the colony (e.g. older breeding males which have sired multiple litters).~~

Microbial status of laboratory animal colonies

Colonies of the various species and genotypes of laboratory animals are usually kept within specialised premises and their microbial status depends largely on the system whereby the colony was formed and is maintained. In this Chapter the microbial status of colonies is considered to be of three main types: 'defined', 'conventional' and 'undefined'. Colonies of defined status are those where, at least initially, the animals are totally free of pathogenic and non-pathogenic micro-organisms (i.e. gnotobiotic), although sometimes a cocktail of known, non-pathogenic micro-organisms has been given subsequently. In either case defined colonies are kept in highly controlled environments in barrier maintained rooms, with strict protocols in place to exclude all potential sources of unwanted microbiological contamination. Colonies of conventional status are those where the animals are kept in closed colonies but where known ('specific') pathogens as well as non-pathogenic micro-organisms may exist. While management protocols for conventional colonies may be less rigid than those for defined colonies, they are designed to control potential sources of microbial contamination. Simple aseptic precautions (e.g. the autoclaving of food and bedding) are taken to ensure that the animals do not become infected with any unwanted microflora. Finally, laboratory animals may be kept in microbiologically undefined colonies which are unrestricted and

may include free ranging animals. Details of these different types of colony can be found in the FELASA Report[1].

The health status of defined and conventional colonies should be confirmed at least quarterly by bacteriological, virological, parasitological, serological and other tests on pre-designated sentinel animals or other representative members of the colony. Older breeding males which have sired multiple litters are often selected for this purpose.

The purpose of official sanitary control of laboratory rodent and rabbit embryos intended for movement internationally is to ensure that specific pathogenic micro-organisms, which could be associated with such embryos, are controlled and transmission of *infection* to recipient animals, progeny and colonies, is avoided. Requirements for the management of donors and processing of embryos vary depending on the microbial status of the colony, i.e. whether it is defined (including gnotobiotic), conventional, or undefined.

Article 4.10.2.

Conditions applicable to the embryo ~~production~~ collection team~~/laboratory~~

The embryo collection team is a group of competent technicians including at least one experienced professional to perform the collection, processing and storage of embryos/oocytes.

The following conditions should apply:

1. The ~~embryo production~~ team ~~must~~ should be ~~composed of competent technicians~~ supervised by a~~n experienced embryologist~~ team professional ~~holding a graduate academic degree (e.g. M.S., Ph.D., D.V.M.)~~.

2. The team professional is responsible for all team operations which include verification of colony and donor health status, sanitary handling and surgery of donors, *disinfection* and hygienic procedures. The team professional should be responsible to the institute *veterinarian.*

3. The institute *veterinarian* should be certified or accredited in laboratory animal care and should be specifically *approved* for the purpose of embryo collection for export. It is the responsibility of the institute *veterinarian* to ensure that required health profiling procedures appropriate for the colony status are implemented. He/she is responsible for certifying that the embryo handling procedures and laboratory facilities conform to the requirements laid down in this Chapter.

~~2~~4. Team personnel should be adequately trained in the techniques and principles of *disease* control and in the use of aseptic techniques in embryo handling. ~~Laboratory sanitary procedures must conform with requirements in the IETS Manual[2]~~The zoonotic potential of specific pathogens affecting the various laboratory animal species should be identified and understood so as to avoid contamination of colonies via human vectors, and vice versa.

~~3~~5. ~~The embryo production team must~~ should ~~use all necessary precautions to protect the animals, animal facilities, laboratory and equipment against microbiological contamination. In particular, the zoonotic potential of specific pathogens should be identified and understood by staff members to avoid contamination of colonies via human vectors, or vice versa~~ High standards of hygiene should be practiced to preclude the introduction of *infection* to the donor animals, colonies, facilities, and equipment. Restrictions should be established to prevent free access of personnel into the embryo collection and handling ~~laboratory~~ facilities especially after ~~their exposure~~ such personnel have been exposed to other animal facilities.

6. The 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.

~~4. Proper records must should be maintained for inspection by the chief embryologist (i.e. supervisor).~~

~~Until standardised record sheets are developed for laboratory animals, it is the responsibility of each laboratory to maintain complete animal and embryo records (i.e. embryo collection, cryopreservation data). Information of the type shown in standard IETS record sheets[2] for livestock species should be incorporated, where applicable, and data such as embryo quality grading system, morphological stage at cryopreservation and genotypic identification of the donors should be clearly given in the records.~~

~~5~~7. It is the responsibility of the ~~chief embryologist (i.e. laboratory supervisor)~~ institute *veterinarian* to ensure that ~~the~~ complete animal and embryo~~s are properly stored in sterile, sealed containers (e.g. ampules or straws)~~ records, including records of collection, processing and storage of embryos are maintained. ~~In addition, the containers must be correctly identified using a standard format which includes embryo species/genotype, cryopreservation date, number and stage of embryos, container number and indication of any specialised procedure (e.g. *in vitro* fertilisation, micromanipulation) or condition (e.g. germfree, microbiologically defined)~~ Record sheets of the type shown in the IETS Manual[2] for livestock species should be used where applicable, and data such as genotypic identification of the donors, embryo quality grading, morphological stage and should be given. ~~If appropriate t~~The embryo collection team should keep a record of its activities which should be maintained for inspection by the *Veterinary Authority* for at least 2 years after the embryos have been exported.

8. The embryo collection team, if involved in the *export* of embryos, should be approved by the *Competent Authority* and be subject to regular inspection~~, preferably annually,~~ by an *Official Veterinarian* to ensure compliance with procedures for the sanitary collection, processing and storage of embryos.

Article 4.10.2bis.

Conditions applicable to the processing laboratory

A processing laboratory used by the embryo collection team is a facility in which embryos are recovered from donors (or from their excised reproductive tracts), and from the collection media. Here also the embryos are examined and subjected to any required treatments such as washing, cryopreservation for storage and quarantine pending results of any diagnostic procedures. The processing 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 but in this case should be physically separated from animals.

Additionally:

1. The processing laboratory should be under the supervision of the institute *veterinarian* and be 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 lesser health status should be processed.

3. 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.

Annex XIII (contd)

Article 4.10.2tris.

Risk management

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

1. The first phase comprises the *risk* potential for embryo contamination and depends on:

   a) the *disease* situation in the *exporting country* and/or *zone*;

   b) the microbial status of the colony (i.e. defined, conventional or undefined) 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 Manual[2]. These include the following:

   a) Depending on microbial status of the colony, the embryos should be washed up to ten times with at least 100-fold dilutions between each wash, with a fresh pipette being 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 removal of certain viruses (e.g. herpesviruses) is required, the standard washing procedure should be modified to include additional washes with the enzyme trypsin, as described in the IETS Manual[2].

   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 (apart from the mucin layer in the case of rabbit embryos) free of adherent material.

3. The third phase, which is applicable to *diseases* of concern to the *Veterinary Authority* of the *importing country*, encompasses *risk* mitigation resulting from:

   a) post-collection *surveillance* of the microbial status of the donor colony based on the recognized *incubation periods* of the *diseases* of concern to determine retrospectively the health status of the colony whilst the embryos are stored (in species where effective storage by cryopreservation is possible) in the *exporting country*;

   b) post-mortem testing of the donor(s) or other samples such as blood, embryo-collection (flushing) fluids and non-viable embryos, in a *laboratory* for presence of specified *disease agents*.

Article 4.10.3.

Conditions applicable to the embryo team/institute veterinarian

1. ~~The *veterinarian*, certified in laboratory animal care or laboratory animal accredited, must ensure that the required colony health profiling procedures are implemented, and the results are reviewed and properly recorded before shipment of embryos. He/she is also responsible for confirming that proper animal management/sanitation conditions have been maintained~~ It is the responsibility of the institute *veterinarian* to ensure that required health testing procedures are implemented to demonstrate microbial status of the colony (i.e. defined, conventional or undefined). Colony microbial status should be reviewed by the institute veterinarian before shipment of the embryos.

2. The *veterinarian* is responsible for certifying that the embryo handling procedures and laboratory conditions were maintained in accordance with ~~the IETS Manual[2]~~ Articles 4.10.2. and 4.10.2bis.

3. The *veterinarian* ~~must supervise all quarantine practices to protect against unwanted contamination and spread of *disease*, and to ensure that valid results are generated~~ is responsible for the risk management procedures outlined in Article 4.10.2tris.

4. The *veterinarian* ~~must~~ should authorise all embryo shipments, ensuring that the correct embryo collection records and veterinary certification documents ~~and embryo collection records are~~ have been completed and are included in the shipments.

~~Article 4.10.4.~~

~~**Test programmes for donor animals**~~

~~Sentinel animals in each donor colony should be subjected to routine monthly microbial screening. Testing for specific pathogens is species dependent and will undoubtedly also be influenced by geographic location. Recommendations regarding specific microbial agents to be tested for in mice, rats, cotton rats, hamsters, guinea pigs, gerbils and rabbits have been published elsewhere[3].~~

Article 4.10.5.

Conditions applicable to ~~the embryo/animal handling~~ donors from animal colonies of different microbial status

It should be noted that the conditions applicable to donor animals vary according to the microbial status of the colony from which they originate, i.e. defined, conventional or undefined.

Sentinel animals in each donor colony of defined and conventional status should be subjected to routine microbial screening, preferably monthly, but at least quarterly. Testing for specific pathogens depends on the animal species and may be influenced by geographical location. Recommendations regarding specific microbial agents to be tested for in different laboratory animal species have been published elsewhere[1].

1. Defined microbial ~~conditions~~ status

   a) ~~Germfree and m~~Microbiologically defined colonies (Article 4.10.1.), ~~barrier maintained animals~~ represent the cleanest sources of gametes, and the embryos recovered from these animals can be regarded as pathogen free.

Annex XIII (contd)

b) Since the ~~animals themselves~~ male and female donors are pathogen free ~~or possess defined flora (usually based on random, monthly testing of sentinel animals)~~, dissection of the female reproductive tract and embryo ~~isolation~~ collection procedures ~~can~~ should be performed under aseptic laboratory conditions, ~~and do not require the use of~~ using a biological safety cabinet if appropriate.

c) ~~Strict aseptic procedures should nevertheless be followed and, while embryo washing is not essential to safeguard against any possible air-borne contamination in the laboratory, it is recommended that embryos undergo at least a 3-step washing procedure. In each wash, embryos should be gently agitated in the medium, and the wash volume must constitute at least a one hundred-fold dilution of the volume in which the embryos are transferred~~ Embryo washed as described in point 2 of Article 4.10.2tris is not necessary but it is recommended that embryos are washed 2 or 3 times. In each wash, embryos should be gently agitated in the medium.

~~d) Microbial testing of flush or washing media is not required.~~

~~e~~d) ~~Cryopreserved embryos should be designated, in the appropriate records,~~ The embryos should be recorded as coming from a germfree or microbiologically defined, barrier maintained colony, thus indicating that ~~additional safeguards~~ special *risk* management procedures (Article 4.10.2tris.) for pathogen removal are not necessary. ~~Isolation and health status monitoring of~~ The need to *quarantine* the embryo recipients ~~should be considered but the need to quarantine them is a decision~~ is a matter for the importing ~~laboratory~~ institute.

2. Conventional conditions

a) ~~Animals maintained under these conditions generally represent closed colonies whose~~ Colonies of conventional microbial status are usually closed and their health status is routinely ~~profiled~~ monitored (Article 4.10.1.). ~~They~~ The animals may have been exposed to various pathogens, resulting in *infection* ~~the isolation of infectious agents~~, with positive antibody titres or even active clinical *disease*. ~~. However, prior to embryo collection there should be familiarity with~~ but the pathogen(s) of ~~particular~~ concern in each individual ~~the~~ colony should be well known.

b) Reproductive tracts (uteri, oviducts and/or ovaries) should be removed at a separate site and then taken into the embryo processing laboratory. These procedures should be performed by ~~separate~~ different technicians or, at the ~~very~~ minimum, their protective clothing should be changed between locations. If ~~the~~ animals ~~are to~~ should be handled in the laboratory, the tracts should be dissected out within a biological safety cabinet. This will help protect against the possible shedding of pathogens into the laboratory itself.

c) Once the reproductive tracts have been removed, embryo recovery should be performed under aseptic conditions. ~~Embryos must be inspected (>100x) for the presence of cracks in the zona pellucida and only zona-intact embryos should be kept. They must then be washed using the standard 10-step procedure, described~~ Depending on which, if any, pathogens are known to occur in the colony, embryos should be processed according to the risk management procedures, including washing, as described in Article 4.10.2tris, and in the IETS Manual[2]. ~~This recommendation could be waived in the future if sufficient research evidence from embryo-pathogen interaction studies warranted it.~~

d) Embryos derived from animals that have positive antibody titres or other evidence of specific pathogens should only be transferred into a new colony via a *quarantine* system, using microbiologically defined recipient females. ~~As an additional safeguard,~~ *Quarantine* may also be appropriate if there is any uncertainty about ~~the donor or disease status of the embryos, quarantining of recipients should be applied~~ the microbial status of the donor colony or the donors. In ~~certain~~ situations where the embryos ~~might~~ could have been exposed to bacterial infection ~~(e.g. mycoplasma)~~, they should be cultured in a medium containing ~~an~~ appropriate antibiotic for 24 h ~~pre-freezing, or post-thawing and prior to transfer~~ before cryopreservation, or in the interval between thawing and transfer into recipients.

e) ~~If the embryos were not handled in the recommended manner, this must be indicated on the shipment records, and mandatory quarantining of the recipient dam and offspring should be imposed by the recipient institution until their health status is confirmed. The recipient dam should then be tested post-weaning for pathogens, and introduction of the progeny into the colony should only take place if test results are satisfactory.~~ If the recipient institution does decide to *quarantine* the recipient dam and offspring until their health status is confirmed, the recipients should be tested post-weaning for pathogens of concern, and introduction of offspring into the colony should only take place if the test results are satisfactory.

3. Undefined microbial conditions

a) ~~These animals are derived from either the wild~~ Embryos from free ranging animals or from colonies of unknown health status ~~and embryos from them~~ require ~~maximum precautions~~ the full range of *risk* management procedures that are described in Article 4.10.2tris and in the IETS Manual[2]. ~~The health status of breeder males and donor females should be determined~~ The procedures resemble those used for embryos of livestock as recommended in Chapter 4.7. and Chapter 4.8. of this *Terrestrial Code*. Ideally, the breeder males and donor females should be separated from other animals and tested 15 days before and on the day of breeding (for males) or at embryo collection (for females). Alternatively, the animals could be incorporated into a conventional colony, where, over time, a health history can be documented to reduce the strict monitoring and embryo handling requirements.

b) ~~A b~~Biological safety cabinet should be used for ~~all animal, tissue and embryo~~ handling donors and reproductive tissues, and for processing embryos.

c) Post-mortem testing of the donor females for *diseases* or pathogens of concern to the *importing country* may be appropriate after the embryos/oocytes have been collected. Alternatively if embryos are collected surgically a~~A~~n aliquot of flush fluid from each donor, or a pooled sample, should be tested for the presence of specific pathogens of concern ~~to the *importing country* and laboratory~~.

d) Embryos ~~must~~ should be washed at least 10 times in accordance with the protocols in the IETS Manual[2] ~~(i.e. the 10-step wash, possibly including trypsin treatment in the case of certain herpesviruses)~~ and ~~an aliquot of media from the last four (pooled) washes should be tested for pathogens~~ trypsin treatment should be used if presence of certain pathogenic herpesviruses is of concern.

e) Cryopreserved embryos ~~must~~ should be stored in the exporting laboratory until such time as the necessary *disease* screening of colonies, tissues ~~and~~ or fluids is completed and the ~~certification~~ supporting documents for certification completed and signed by the institute *veterinarian*.

f) On arrival in the *importing country* the ~~All~~ embryos ~~from these animals must~~ should be transferred into ~~a colony via~~ recipients in a quarantine system~~, as discussed above~~. Recipients should be tested at intervals appropriate to recognized *incubation period*s of the *diseases* of concern. In addition to testing ~~the~~ recipients ~~dam~~ after transfer, ~~all~~ the offspring should be tested at 12 weeks of age and~~/or individuals from successive generations should be tested~~ before their introduction into breeding colonies outside the quarantine facility.

Annex XIII (contd)

Article 4.10.5.bis.

Conditions applicable to the storage and transport of embryos

1. Embryos for export should be frozen in fresh liquid nitrogen and then stored in fresh liquid nitrogen in cleaned and disinfected tanks or containers.

2. The embryos 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*. Only embryos from the same donor should be stored together in the same ampoule, vial or straw.

3. 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 according to or similar to the system recommended in the IETS Manual[2]. Identification should include details of the species/genotype of the donors, microbial status (e.g. defined, conventional or undefined), collection/cryopreservation date, number and developmental stage of the embryos, container number and details of any specialized procedure such as *in vitro* fertilization, micromanipulation.

4. Liquid nitrogen storage containers should be sealed under the supervision of the *Official Veterinarian* prior to shipment from the *exporting country*.

5. Embryos should not be exported until the appropriate veterinary certificates are completed.

Article 4.10.6.

~~**Special experimental circumstances**~~ **Procedures for *in vitro* fertilization and micromanipulation**

If embryos are to be ~~cryopreserved following specialised~~ produced by *in vitro* fertilization of oocytes, it is advised that the washed sperm should be used so as to minimize the *risk* of possible pathogen exposure. If embryos are to undergo micromanipulation procedures that involve penetration of the zona pellucida, ~~they must undergo the required washing steps (depending on colony status) before treatment. In the case of *in vitro* fertilisation, to minimise possible pathogen exposure, it is also advised that only washed sperm should be used. Embryos should be washed again before cryopreservation~~ any required *risk* management steps (including washing) should be carried out first, as described in Chapter 4.9.

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1 **Recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit breeding colonies**.- Report of the Federation of European Laboratory Animal Science Associations (FELASA), Working Group on Animal Health accepted by the FELASA Board of Management, November 1992.

2 Manual of the International Embryo Transfer Society ~~(1998)~~.

3 ~~Schiewe M.C., Hollifield V.M., Kasbohm L.A. & Schmidt P.M. (1995) - Embryo importation and cryobanking strategies for laboratory animals and wildlife species. *Theriogenology*, **43**, 97-104.~~