TERRESTRIAL ANIMAL HEALTH STANDARDS COMMISSION

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CHAPTER 4.7.

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

Article 4.7.1.

Aims of control

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

Article 4.7.2.

Conditions applicable to the embryo collection team

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

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

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

3. The team *veterinarian* is responsible for all team operations which include verification of donor health status, sanitary handling and surgery of donors and *disinfection* and hygienic procedures.

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

5. The collection team should have adequate facilities and equipment for:

   a) collecting embryos;

   b) processing and treatment of embryos at a permanent site or mobile laboratory;

   c) storing embryos.

   These facilities need not necessarily be at the same location.

6. The embryo collection team should keep a record of its activities, which should be maintained for inspection by the *Veterinary Authority* for a period of at least two years after the embryos have been exported.

7. The embryo collection team should be subjected to regular inspection at least once a year by an *Official Veterinarian* to ensure compliance with procedures for the sanitary collection, processing and storage of embryos.

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* or *flock* from which the donor *animals* have been sourced.

   b) The donor *animals* should not be situated in a *herd* or *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 International Embryo Transfer Society (IETS) Category 1 for the species of embryos being collected (see Article 4.7.14.).

   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 4.6.

   b) When the donor of the semen used to inseminate donor females for embryo production is dead, and when the health status of the semen donor concerning a particular infectious *disease* or *diseases* of concern was not known at the time of semen collection, additional tests may be required of the inseminated donor female after embryo collection to verify that these infectious *diseases* were not transmitted. An alternative may be to test an aliquot of semen from the same collection date.

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

Article 4.7.5.

Risk management

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

1. The first phase, which is applicable to *diseases* not included in Category 1 of the IETS categorisation (Article 4.7.14.), comprises the risk potential for embryo contamination and depends on:

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

   b) the health status of the *herds* or *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 should be washed at least ten times with at least 100–fold dilutions between each wash, and a fresh pipette should be used for transferring the embryos through each wash.

   b) Only embryos from the same donor should be washed together, and no more than ten embryos should be washed at any one time.

   c) Sometimes, for example when inactivation or removal of certain viruses, such as 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, should be examined over its entire surface area at not less than 50X magnification to ensure that it is intact and free of adherent material.

   ~~*[NOTE: All shipments of embryos should be accompanied by a statement signed by the team veterinarian certifying that these embryo processing procedures have been completed.]*~~

3. The third phase, which is applicable to *diseases* not included in Category 1 of the IETS categorisation[1] (Article 4.7.14.) and which are of concern to the *Veterinary Authority* of the *importing country*, encompasses the risk reductions resulting from:

   a) post-collection *surveillance* of the donors and donor *herds* or *flocks* based on the recognised *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 sterilised 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 sterilised 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 and oocytes

Where the viable, zona pellucida intact embryos from a donor are intended for export, all non-fertilised 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 and 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 and oocytes then any debris that is retained on the filter should be rinsed off into the retained fluid.

c) Washing fluids

The last four washes of the embryos and oocytes should be pooled according to the IETS Manual.

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

Article 4.7.9.

Procedure for micromanipulation

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

Article 4.7.10.

Specific conditions applicable to porcine embryos

The *herd* of origin should be free of clinical signs of swine vesicular disease and brucellosis. The development of effective cryopreservation methods for the storage of zona pellucida-intact porcine embryos is still at a very early stage.

Article 4.7.11.

Specific conditions 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 horses routinely involved in events or competitions at the international level. For instance, in appropriate circumstances horses travelling with an *international veterinary certificate* may be exempt where mutually agreed upon on a bilateral basis between the respective *Veterinary Authorities*.

Article 4.7.12.

Specific conditions 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*. The development of cryopreservation methods for storage of camelid embryos is still at an early stage, and also that pathogen interaction studies with camelid embryos have not yet been carried out.

Article 4.7.13.

Specific conditions applicable to cervid embryos

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

Article 4.7.14.

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

Based on the conclusions of the IETS[4], the following *diseases* and pathogenic agents are categorised into four categories, which applies only to *in vivo* derived embryos.

1. Category 1

   a) Category 1 *diseases* or pathogenic agents are those for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer according to the IETS Manual[2].

   b) The following *diseases* or pathogenic agents are in Category 1:

      – Aujeszky's disease (pigs): 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).

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* are in Category 2:

– Bluetongue (sheep)
– Caprine arthritis/encephalitis
– Classical swine fever.

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 (not a *listed disease*)
– Bovine spongiform encephalopathy (goats) (not a *listed disease* of goats)
– Bovine viral diarrhoea virus (cattle)
– *Campylobacter fetus* (sheep) (not a *listed disease* of sheep)
– Foot and mouth disease (pigs, sheep and goats)
– *Haemophilus somnus* (cattle) (not a *listed disease*)
– Maedi-visna (sheep)
– *Mycobacterium paratuberculosis* (cattle)
– *Neospora caninum* (cattle) (not a *listed disease*)
– Ovine pulmonary adenomatosis (not a *listed disease*)
– 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) (not a *listed disease*)
- Bovine anaplasmosis
- Bluetongue (goats)
- Border disease (sheep) (not a *listed disease*)
- Bovine herpesvirus-4 (not a *listed disease*)
- *Chlamydia psittaci* (cattle, sheep)
- Contagious equine metritis
- Enterovirus (cattle, pigs) (not a *listed disease*)
- Equine rhinopneumonitis
- Equine viral arteritis
- *Escherichia coli* O9:K99 (cattle) (not a *listed disease*)
- *Leptospira borgpetersenii serovar hardjobovis (*cattle*)* (not a *listed disease*)
- *Leptospira* sp. (pigs) (not a *listed disease*)
- Lumpy skin disease
- *Mycobacterium bovis* (cattle)
- *Mycoplasma* spp. (pigs)
- Ovine epididymitis (*Brucella ovis*)
- Parainfluenza-3 virus (cattle) (not a *listed disease*)
- Parvovirus (pigs) (not a *listed disease*)
- Porcine circovirus (type 2) (pigs) (not a *listed disease*)
- Scrapie (goats)
- *Tritrichomonas foetus* (cattle)
- *Ureaplasma and Mycoplasma* spp. (cattle, goats) (not a *listed disease*)
- Vesicular stomatitis (cattle, pigs).