[F1ANNEX D

Textual Amendments

F1 Substituted by Commission Regulation (EU) No 176/2010 of 2 March 2010 amending Annex D to Council Directive 92/65/EEC as regards semen collection and storage centres, embryo collection and production teams, and conditions for donor animals of the equine, ovine and caprine species and for handling semen, ova and embryos of those species (Text with EEA relevance).

CHAPTER III

Requirements applicable to semen, ova and embryos

I. *Conditions for the collection, processing, preservation, storage and transport of semen*

1.1. Where, without prejudice to Directive 2001/82/EC of the European Parliament and of the Council[(1)], antibiotics or a mixture of antibiotics are added with a bactericidal activity at least equivalent to that of the following mixtures in each ml of semen: gentamicin (250 µg), tylosin (50 µg), lincomycin-spectinomycin (150/300 µg); penicillin (500 IU), streptomycin (500 µg), lincomycin-spectinomycin (150/300 µg); or amikacin (75 µg), divekacin (25 µg), the names of the antibiotics added and their concentration shall be stated in the health certificate referred to in the fourth indent of Article 11(2).

1.2. All instruments used for the collection, processing, preservation or freezing of semen shall be either disinfected or sterilised as appropriate before use, except for single-use instruments.

1.3. Frozen semen shall:

(a) be placed and stored in storage containers:

(i) which have been cleansed and disinfected or sterilised before use, or are single-use containers;

(ii) with a cryogenic agent; which shall not be previously used for other products of animal origin;

(b) prior to dispatch or use, be stored in approved conditions for a minimum period of 30 days from the date of collection.

1.4. Semen to be subject for trade shall:

(a) be transported to the Member State of destination in transport containers which have been cleansed and disinfected or sterilised before use, or are single-use containers, and which have been sealed and numbered prior to dispatch from the approved semen collection or storage centres;

(b) be marked in such a way that the number on the straws or other packages coincides with the number on the health certificate referred to in the fourth indent of Article 11(2) and with the container in which they are stored and transported.

II. *Conditions for ova and embryos*

1. Collection and processing of *in vivo* derived embryos

In vivo derived embryos shall be conceived as a result of artificial insemination with semen meeting the requirements of this Directive and shall be collected, processed and preserved in accordance with the following:

1.1. Embryos shall be collected and processed by an approved embryo collection team, without coming into contact with any other batch of embryos not complying with the requirements of this Directive.

1.2. Embryos shall be collected in a place, which is separated from other parts of the premises or holding where the embryo is collected and which shall be in good repair and constructed with materials which permit its effective and easy cleansing and disinfection.

1.3. Embryos shall be processed (examined, washed, treated and placed in identified and sterile straws, ampoules or other packages) in either a permanently sited laboratory or a mobile laboratory, which, as regards susceptible species, is situated in an area in which there has been no outbreak of foot-and-mouth disease for the past 30 days within a 10 kilometre radius.

1.4. All equipment used to collect, handle, wash, freeze and store embryos shall either be sterilised or properly cleansed and disinfected prior to use according to the IETS Manual[(2)], or be single-use equipment.

1.5. Any biological product of animal origin used in the media and solutions for collection, processing, washing or storage of embryos shall be free of pathogenic micro-organisms. Media and solutions used in the collection, freezing and storage of embryos shall be sterilised by approved methods according to the IETS Manual and handled in such a manner as to ensure that sterility is maintained. Antibiotics might be added, when appropriate, to collection, processing, washing and storage media according to the IETS Manual.

1.6. The cryogenic agents used for preservation or storage of embryos shall not be previously used for other products of animal origin.

1.7. Each embryo straw, ampoule or other package shall be clearly identified by labels according to the standardised system according to the IETS Manual.

1.8. **[**[F2]The embryos shall be washed and have an intact *zona pellucida*, or the embryonic capsule in case of equine embryos, before and immediately after washing. In accordance with the IETS Manual, the standard washing procedure shall be modified to include additional washes with the enzyme trypsin where recommended for the inactivation or removal of certain pathogens.**]**

1.9. Embryos from different donor animals shall not be washed together.

1.10. **[**[F2]The *zona pellucida* of each embryo, or the embryonic capsule in case of equine embryos, shall be examined over its entire surface area at not less than 50 × magnification and certified to be intact and free of adherent material.**]**

1.11. Embryos of a batch that has successfully undergone the examination set out in point 1.10 shall be placed in a sterile straw, ampoule or other package marked in accordance with point 1.7 which shall be sealed immediately.

1.12. Each embryo shall, where appropriate, be frozen as soon as possible and stored in a place which is under the control of the team veterinarian.

1.13. Each embryo collection team shall submit for official examination for bacterial and viral contamination routine samples of non-viable embryos or ova, flushing fluids or washing fluids resulting from its activities according to the IETS Manual.

1.14. Each embryo collection team shall keep a record of its activities in respect of embryo collection for a period of two years after the embryos have been the subject of trade or import, including:

(a) the breed, age and individual identification of the donor animals concerned;

(b) the place of collection, processing and storage of embryos collected by the team;

(c) the identification of the embryos together with details of the consignee of the shipment.

Textual Amendments

F2 Substituted by Commission Implementing Regulation (EU) No 846/2014 of 4 August 2014 amending Annex D to Council Directive 92/65/EEC as regards the conditions for donor animals of the equine species (Text with EEA relevance).

2. Collection and processing of ova, ovaries and other tissues, with the aim of producing *in vitro* derived embryos

The conditions set out in points 1.1 to 1.14 shall apply *mutatis mutandis* to the collection and processing of ova, ovaries and other tissues for use in *in vitro* fertilisation and/or *in vitro* culture. In addition, the following shall apply:

2.1. The competent authority shall have knowledge of, and authority over, the holding(s) of origin of the donor animals.

2.2. When ovaries and other tissues are collected at a slaughterhouse, either from individual animals or from batches of donors (batch collection), the slaughterhouse shall be officially approved in accordance with Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption[(3)] and under the supervision of a veterinarian whose responsibility it 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 signs of the relevant contagious diseases transmissible to animals. The slaughterhouse shall, as regards susceptible species, be situated in an area in which there has been no outbreak of foot-and-mouth disease for the past 30 days within a 10 kilometre radius.

2.3. Batches of ovaries shall not be brought into the processing laboratory until *post-mortem* inspection of donor animals is completed.

2.4. Equipment for removal and transport of ovaries and other tissues shall be cleansed and disinfected or sterilised before use and exclusively used for these purposes.

3. Processing of *in vitro* derived embryos

The conditions laid down in points 1.1 to 1.14 shall apply *mutatis mutandis* to the processing of *in vitro* derived embryos. In addition, the following shall apply:

3.1. *In vitro* derived embryos shall be conceived as a result of *in vitro* fertilisation with semen meeting the requirements of this Directive.

3.2. After the *in vitro* culture period is completed but prior to freezing, storage and transport of the embryos, they shall be washed and undergo the treatments referred to in points 1.8, 1.10 and 1.11.

3.3. Embryos from different donor animals, in the case of individual animal recovery, or from different batch collections shall not be washed together.

3.4. Embryos from different donor animals, in the case of individual animal recovery, or from different batch collections shall not be stored in the same straw, ampoule or other package.

4. Processing of micromanipulated embryos

Prior to any micromanipulation which compromises the integrity of the *zona pellucida*, all embryos or ova shall be collected and processed according to the sanitary conditions set out in points 1, 2 and 3. In addition, the following conditions shall apply:

4.1. Where micromanipulation of the embryo which involves penetration of the *zona pellucida* is carried out, this shall be done in suitable laboratory facilities under supervision of an approved team veterinarian.

4.2. Each embryo collection team shall keep records of its activities according to point 1.14, including details of micromanipulation techniques which involve penetration of the *zona pellucida* and which have been performed on the embryos. In the case of embryos derived by *in vitro* fertilisation, the identification of the embryos may be done on the basis of a batch, but shall contain details of the date and place of collection of ovaries and/or ova. It shall also be possible to identify the holding of origin of the donor animals.

5. Storage of embryos

5.1. Each embryo collection and production teams shall ensure that the embryos are stored at suitable temperatures in storage premises referred to in point 1.8 of Section III of Chapter I.

5.2. Frozen embryos shall, prior to dispatch, be stored in approved conditions for a minimum period of 30 days from the date of their collection or production.

6. Transport of embryos

6.1. Embryos to be subject for trade shall be transported to the Member State of destination in containers which have been cleansed and disinfected or sterilised before use, or are single-use containers, and which have been sealed and numbered prior to dispatch from the approved storage premises.

6.2. The straws, ampoules or other packages shall be marked in such a way that the number on the straws, ampoules or other packages coincides with the number on the health certificate referred to in the third indent of Article 11(3) and with the container in which they are stored and transported.**]**

(1) **[**[F1]OJ L 311, 28.11.2001, p. 1.**]**

(2) **[**[F1]Manual of the International Embryo Transfer Society — A procedural guide and general information for the use of embryo transfer technology emphasising sanitary procedures, published by the International Embryo Transfer Society, 1111 North Dunlap Avenue, Savoy, Illinois 61874 USA (http://www.iets.org/).**]**

(3) **[**[F1]OJ L 139, 30.4.2004, p. 206.**]**

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