ANNEX A

CHAPTER I

[F1Conditions for the approval of embryo collection and embryo production teams]

In order to be given approval each embryo collection team must fulfil the following requirements:

(a) the collection, processing and storage of embryos must be carried out either by a team veterinarian or under his responsibility by one or more technicians who are competent and trained by the team veterinarian in methods and techniques of hygiene;

(b) it must be placed under the general supervision and authority of the official veterinarian;

(c) it must have at its disposal permanent or mobile laboratory facilities where embryos can be examined, processed and packed, consisting of at least a work surface, a microscope and cryogenic equipment;

(d) in the case of a permanently sited laboratory, it must have at its disposal:

— a room where embryos can be manipulated which is adjacent to but physically separate from the area used to handle the donor animals during collection,

— a room or area equipped for cleansing and sterilizing instruments and equipment used in embryo collection and manipulation[F1,]

— [F2where micromanipulation of the embryo which involves penetration of the *zona pellucida* is to be carried out, this shall be done in suitable laminar-flow facilities which shall be properly cleaned and disinfected between batches;]

(e) it must have at its disposal in the case of a mobile laboratory a specially equipped part of the vehicle consisting of two separate sections,

— one for the examination and manipulation of embryos which shall be a clean section, and

— the other for accommodating equipment and materials used in contact with the donor animals.

A mobile laboratory shall always have contact with a permanently sited laboratory to ensure the sterilization of its equipment and the provision of fluids and other products necessary for the collection and manipulation of embryos.

Textual Amendments

F2 Inserted by Commission Decision of 8 February 1994 amending Council Directive 89/556/EEC on animal health conditions governing intra-Community trade in and importation from third countries of embryos of domestic animals of the bovine species (Text with EEA relevance) (94/113/EC).

[F2Furthermore, to be approved as a team for the production and processing of embryos derived by *in vitro* fertilization and/or *in vitro* culture, an embryo production team must fulfil the following additional requirements:

(f) the personnel must be trained in appropriate disease control and laboratory techniques, particularly in procedures for working in sterile conditions;

(g) it must have at its disposal a permanently-sited processing laboratory which must:

— have adequate equipment and facilities, including a separate room for recovering oocytes from ovaries, and separate rooms or areas for processing oocytes and embryos, and storing embryos,

— have laminar-flow facilities under which all oocytes, semen and embryos must be processed; however, the centrifugation of semen may be carried out outside the laminar-flow facility, as long as full hygienic precautions are taken;

(h) where oocytes and other tissues are to be collected in an abattoir, it must have at its disposal suitable equipment for the collection and transport of the ovaries and other tissues to the processing laboratory in a hygienic and safe manner.**]**

Textual Amendments

F1 Substituted by Commission Decision of 8 February 1994 amending Council Directive 89/556/EEC on animal health conditions governing intra-Community trade in and importation from third countries of embryos of domestic animals of the bovine species (Text with EEA relevance) (94/113/EC).

CHAPTER II

Conditions relating to the collection, processing, storage and transport of embryos by the approved embryo collection[F2or production] team

1. Collection and processing

(a) Embryos shall be collected and processed by an approved collection team, without coming into contact with any other consignment of embryos not meeting the requirements of this Directive.

(b) Embryos shall be collected in a place which is isolated from other parts of the premises or holding and which must be in good repair and easy to cleanse and disinfect.

(c) Embryos shall be processed (examined, washed, treated and placed in identified and sterile containers) in either a permanent laboratory facility or a mobile laboratory facility, which is not situated in a zone subject to prohibition or quarantine measures.

(d) All implements which come into contact with the embryos or the donor animal during collection and processing shall be disposable or shall be properly disinfected or sterilized prior to use.

(e) Products of animal origin used during collection of the embryos and in the transport medium shall be obtained from sources which present no animal health risk or are to be so treated prior to use so that such risk is prevented.**[**F2All media and solutions shall be sterilized by approved methods according to the recommendations of the manual of the International Embryo Transfer Society (IETS). Antibiotics may be added to the media in accordance with the IETS manual.**]**

(f) Storage flasks and transport flasks shall be properly disinfected or sterilized before the commencement of each filling operation.

(g) The cryogenic agent used shall not have been previously used for other products of animal origin.

(h) Each embryo container and the containers in which they are stored and transported shall be clearly code-marked in such a way that the date of collection of the embryos and the breed and identification of the donor sire and donor dam, as well as the registration number of the team can be readily established. The characteristics and form of this code-marking shall be established in accordance with the procedure laid down in Article 18.

(i) Each embryo shall be washed at least 10 times in a special fluid for embryos which shall be changed each time and which, unless decided otherwise under point (m), shall contain trypsin, in accordance with internationally recognized procedures. Each wash shall be a 100-fold dilution of the previous wash and a sterile micropipette shall be used to transfer the embryo on each occasion.

(j) After the last wash each embryo shall be subjected to microscopic examination**[**F2at a magnification of at least × 50**]** over its entire surface to determine that the '*zona pellucida*' is intact and is free from any adherent material.**[**F2Any micromanipulation which involves penetration of the *zona pellucida* must be carried out in the facilities approved for the purpose, and after the last wash and examination. Such micromanipulation may only be carried out on an embryo having an intact *zona pellucida*.**]**

(k) Each consignment of embryos that has successfully undergone the examination provided for in (j) shall be placed in a sterile container marked in accordance with (h) and which shall be sealed immediately.

(l) 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 and which is subject to regular inspection by an official veterinarian.

(m) In accordance with the procedure laid down in Article 18 a protocol shall be drawn up before the date provided for in Article 20 concerning authorized flushing and washing fluids, washing techniques and, where necessary, enzymatic treatments together with authorized transportation media.

Pending the adoption of a protocol on enzymatic treatments, the national rules on the use of trypsin shall continue to apply, in compliance with the general provisions of the Treaty.

(n) Each collection team must submit routine samples of flushing fluids, washing fluids, disintegrated embryos, non-fertilized ova etc., resulting from its activities for official examination for bacterial and viral contamination. The procedure for collecting of samples, conducting such examinations, together with the standards to be achieved shall be decided in accordance with the procedure laid down in Article 18. If the standards laid down are not achieved the competent authority which granted the official approval to the team shall withdraw that approval.

(o) Each collection team must keep a record of its activities in respect of embryo collection during the 12 months before and 12 months after storage including:

— the breed, age and identification of the donor animals concerned,

— the place of collection, processing and storage of embryos collected by the team,

— the identification of the embryos together with details of their destination if known**[**F1,**]**

— **[**F2details of micromanipulation techniques which involve penetration of the *zona pellucida* or other techniques such as *in vitro* fertilization and/or *in vitro* culture which have been performed on the embryos. In the case of embryos derived by *in vitro* fertilization, the identification may be done on the basis of a batch, but must contain details of the date and place of collection of ovaries and/or oocytes. It must also be possible to identify the herd of origin of the donor animals.**]**

[F2The conditions laid down in subparagraphs (a) to (o) shall apply as appropriate to the collection, processing, storage and transport of ovaries, oocytes and other tissues for use in *in vitro* fertilization and/or *in vitro* culture. Furthermore, the following additional conditions shall apply:

(p) when ovaries and other tissues are to be collected at an abattoir, the abattoir should be officially approved and under the control of an official veterinarian whose responsibility it is to carry out ante-and post-mortem inspection of donors;

(q) materials and equipment coming into direct contact with ovaries and other tissues shall be sterilized before use and after sterilization, used exclusively for those purposes. Separate equipment shall be used to handle oocytes and embryos from different batches of donor animals;

(r) ovaries and other tissues shall not be be allowed to enter the processing laboratory until completion of the post-mortem inspection of the batch. If relevant disease is found in the batch of donors, or in any animals slaughtered in that abattoir on that day, all tissues from that batch must be traced and discarded;

(s) the washing and examination procedure laid down in subparagraphs (i) and (j) shall be carried out after the culture procedure has been completed;

(t) any micromanipulation which involves penetration of the *zona pellucida* shall be carried out in accordance with the provisions of subparagraph (j), after the procedures laid down in subpargraph (s) have been completed;

(u) only embryos from the same batch of donors should be stored in the same ampoule/straw.**]**

2. Storage

Each embryo collection**[**F2or production**]** team shall ensure that the embryos are stored at suitable temperatures in premises approved for the purpose by the competent authority.

In order to be approved these premises must:

(i) comprise at least one lockable room intended exclusively for embryo storage;

(ii) be easy to cleanse and disinfect;

(iii) have permanent records of all incoming and outgoing movements of embryos. The final destination of the embryos in particular shall be specified in such records;

(iv) be subject to inspection by the official veterinarian.

The competent authority may authorize the storage of semen that fulfils the requirements of Directive 88/407/EEC in the approved storage premises.

3. Transport

Embryos for trade must be transported in satisfactory hygienic conditions in sealed containers from the approved storage premises until their arrival at their destination.

The containers must be marked in such a way that the number coincides with the number on the animal health certificate.