SCHEDULE 1

Regulation 5

CONDITIONS FOR THE APPROVAL OF BOVINE EMBRYO COLLECTION TEAMS FOR ALL PURPOSES INCLUDING INTRA-AREA TRADE

1. In order to be given approval under regulation 5, each bovine embryo collection team must fulfil the requirements of this Schedule.

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

3. The team must have at its disposal either a permanently sited laboratory or a mobile laboratory where bovine embryos can be examined, processed and packed, and which complies with paragraph 4 below in the case of a permanently sited laboratory or paragraph 5 below in the case of a mobile laboratory.

4. In the case of a permanently sited laboratory, the team must have at its disposal—

(a) a work surface, a microscope and cryogenic equipment;

(b) a room where bovine embryos can be manipulated which is adjacent to but physically separate from the area used to handle the donor animals during collection;

(c) a room or area equipped for cleansing and sterilising instruments and equipment used in bovine embryo collection and manipulation; and

(d) where micromanipulation of the bovine embryo which involves penetration of the *zona pellucida* is to be carried out, suitable laminar-flow facilities.

5.—(1) In the case of a mobile laboratory the team must have at its disposal—

(a) a work surface, a microscope and cryogenic equipment; and

(b) a specially equipped part of the vehicle consisting of two separate sections, one for the examination and manipulation of bovine embryos which shall be a clean section, and the other for accommodating equipment and materials used in contact with the donor animals.

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

SCHEDULE 2

Regulation 6

CONDITIONS FOR THE APPROVAL OF BOVINE EMBRYO COLLECTION TEAMS FOR PURPOSES OTHER THAN FOR INTRA-AREA TRADE

1. In order to be given approval under regulation 6 each bovine embryo collection team must fulfil the requirements of this Schedule.

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

3. The team must have at its disposal either—

(a) a permanent laboratory facility as specified in Schedule 1; or

(b) a mobile laboratory facility as specified in Schedule 1; or

(c) a mobile laboratory which:

(i) has separate parts so that there is no contact between used and unused equipment and materials, and

(ii) carries sufficient equipment to enable the examination and manipulation of bovine embryos to be carried out without contaminating them, and

(iii) has contact with a permanently sited laboratory to ensure the sterilisation of its equipment and the provision of fluids and other products necessary for the collection and manipulation of bovine embryos.

SCHEDULE 3

Regulation 7

CONDITIONS FOR THE APPROVAL OF BOVINE EMBRYO PRODUCTION TEAMS

1. To be approved as a team for the production and processing of bovine embryos derived by in vitro fertilisation and/or in vitro culture, a bovine embryo production team must fulfil the following requirements.

2. The production, processing and storage of bovine 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.

3. The production team personnel must be trained in appropriate disease control and laboratory techniques, particularly in procedures for working in sterile conditions.

4. The team must have at its disposal a permanently-sited processing laboratory which must—

(a) have adequate equipment and facilities, including a separate room for recovering oocytes fro ovaries, and separate rooms or areas for processing oocytes and bovine embryos, and storing bovine embryos; and

(b) have laminar-flow facilities.

5. Where oocytes and other tissues are to be collected in an abattoir, the production team 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.

SCHEDULE 4

Regulations 10 and 11

CONDITIONS RELATING TO THE COLLECTION, PROCESSING STORAGE AND TRANSPORT OF BOVINE EMBRYOS FOR THE PURPOSES OF INTRA-AREA TRADE

1. Bovine embryos shall be collected and processed by an approved collection team, without coming into contact with any other consignment of bovine embryos not meeting the requirements of these Regulations relating to bovine embryos intended for intra-Area trade.

2. Bovine 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.

3. Bovine embryos shall be processed (that is, examined, washed, treated and placed in identifiable 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.

4. All implements which come into contact with the bovine embryos or the donor animal during collection and processing shall be disposable or shall be properly disinfected or sterilised prior to use.

5. Products of animal origin used during collection of the bovine embryos and in the transport medium shall be obtained from sources which present no animal health risk or are treated prior to use in such a way that such risk is prevented. All media and solutions shall be sterilised by approved methods according to the recommendations of the manual of the International Embryo Transfer Society(**1**). Antibiotics may be added to the media in accordance with that manual.

6. Storage flasks and transport flasks shall be properly disinfected or sterilised before the commencement of each filling operation.

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

8. Each bovine 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 bovine 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.

9. Each bovine embryo shall be washed at least 10 times in a special fluid for bovine embryos which shall be changed each time and which shall contain trypsin, in accordance with internationally recognised procedures. Each wash shall be a 100-fold dilution of the previous wash and a sterile micropipette shall be used to transfer the bovine embryo on each occasion.

10. After the last wash each bovine embryo shall be subjected to microscopic examination at 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. Any micromanipulation which involves penetration of the *zona pellucida* must be carried out after the last wash and examination in the facilities approved for the purpose. Such micromanipulation may only be carried out on a bovine embryo having an intact *zona pellucida*.

11. Each consignment of bovine embryos that has successfully undergone the examination provided for in the preceding paragraph shall be placed in a sterile container marked in accordance with paragraph 8 of this Schedule and sealed immediately.

12. Each bovine 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.

13. Each collection team must submit routine samples of flushing fluids, washing fluids, disintegrated bovine embryos, non-fertilised ova etc., resulting from its activities to the appropriate Minister for official examination for bacterial and viral contamination.

SCHEDULE 5

Regulations 10 and 12

CONDITIONS RELATING TO DONOR ANIMALS FOR THE PURPOSES OF REGULATION 10 or 12

PART I

COLLECTION FROM LIVE ANIMALS

1. For the purposes of collection from live animals of bovine embryos or oocytes for the purposes of regulation 10 or 12, donor animals must meet the requirements of this Part of this Schedule.

(**1**) Edited by D A Stringfellow and S M Siedel and published in November 1990 by the International Embryo Transfer Society. It is obtainable from their headquarters at 309 West Clark Street, Champaign, Illinois, U.S.A.

2. The donor animals must have spent at least the previous six months within the territory of the European Economic Area or, in the case of a country outside that Area, in the country of collection.

3. The donor animals must have been present in the herd of origin for at least 30 days prior to collection.

4. The donor animals must come from herds which are—

(a) officially tuberculosis free,

(b) officially brucellosis free or brucellosis free, and

(c) either enzootic bovine leucosis free or for which certification has been obtained that there has not been any clinical case of enzootic bovine leucosis during the past three years.

5. During the previous year, the donor animals must not have been present in a herd (or herds) which have shown any clinical sign of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis.

6. On the day of embryo collection the donor cow—

(a) shall be kept in a holding which is not subject to veterinary prohibition or quarantine measures, and

(b) shall show no clinical signs of disease.

PART II

COLLECTION AFTER SLAUGHTER

7. For the purposes of collection of ovaries, oocytes and other tissues to be collected after slaughter in an abattoir, donor animals must comply with the requirements of this Part of this Schedule.

8. The donor animals should not have been designated for slaughter as part of a national disease eradication programme, nor should they have come from a holding subject to restrictions because of animal disease.

9. The abattoir where the ovaries and other tissues are collected must not be situated in a zone subject to prohibition or quarantine measures.

SCHEDULE 6

Regulation 11

CONDITIONS RELATING TO THE COLLECTION, PROCESSING AND STOR AGE OF BOVINE EMBRYOS NOT INTENDED FOR INTRA-AREA TRADE

1. Bovine embryos shall be processed (that is, examined, washed, treated and placed in identifiable 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.

2. All implements which come into contact with the bovine embryos or the donor animal during collection and processing shall either be disposable and unused, or shall be properly disinfected or sterilised before use.

3. Products of animal origin used during collection of the bovine embryos and in the transport medium shall be obtained from sources which present no animal health risk or are treated before use in such a way that such risk is prevented.

4. Storage flasks and transport flasks shall be properly disinfected or sterilised before the commencement of the initial filling operation.

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

6. Each bovine embryo container and the containers in which they are stored and transported shall be clearly marked with—

(a) the registration number of the collection team;

(b) the date of the collection of the bovine embryo; and

(c) either—

(i) the breed and identification of the donor sire and donor cow, or

(ii) a code from which this information can be readily established.

7. Each bovine embryo shall be washed at least 10 times in a special fluid for bovine embryos which shall be changed each time. Each wash shall be a 100-fold dilution of the previous wash and a sterile micropipette shall be used to transfer the bovine embryo on each occasion.

8. After the last wash each bovine embryo shall be subjected to microscopic examination over its entire surface to determine that the *zona pellucida* is intact and is free from any adherent material.

9. Each consignment of bovine embryos that has successfully undergone the examination provided for in paragraph 8 above shall be placed in sterile containers marked in accordance with paragraph 6 above and which shall be sealed immediately; and in this paragraph "consignment of bovine embryos" means a quantity of bovine embryos removed in one operation from a single donor.

SCHEDULE 7

Regulation 12

CONDITIONS RELATING TO THE PRODUCTION OF BOVINE EMBRYOS

1. The production of bovine embryos shall be in accordance with this Schedule.

2. Bovine embryos shall be produced by an approved production team, and, in the case of bovine embryos intended for intra-Area trade, without coming into contact with any other consignment of bovine embryos not meeting the requirements of these Regulations relating to bovine embryos intended for intra-Area trade.

3. Ovaries, oocytes and other tissues intended to be used in bovine embryo production 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.

4. Each production team must submit routine samples of flushing fluids, washing fluids, disintegrated bovine embryos, non-fertilised ova etc., resulting from its activities to the appropriate Minister for official examination for bacterial and viral contamination.

5. 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.

6. Materials and equipment coming into direct contact with ovaries, oocytes and other tissues shall be sterilised before use and, after sterilisation, used exclusively for those purposes. Separate equipment shall be used to handle oocytes and bovine embryos from different batches of donor animals. All laminar-flow facilities shall be properly cleaned and disinfected between batches.

7. Bovine embryos shall be produced, processed, and placed in identifiable and sterile containers in a permanent laboratory facility which is not situated in a zone subject to prohibition or quarantine measures.

8. Products of animal origin used during production of the bovine 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. All media and solutions shall be sterilised by approved methods according to the recommendations of the manual of the International Embryo Transfer Society. Antibiotics may be added to the media in accordance with that manual.

9. Storage flasks and transport flasks shall be properly disinfected or sterilised before the commencement of each filling operation.

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

11. Oocytes, semen and embryos shall be processed using the laminar-flow facility. However, the centrifugation of semen may be carried out outside the laminar-flow facility, as long as full hygienic precautions are taken.

12. Once the bovine embryo has been produced, each bovine 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 bovine embryos and the breed and identification of the donor sire and donor dam or batch, as well as the registration number of the team can be readily established.

13. After the culture procedure has been completed, each bovine embryo shall be washed at least 10 times in a special fluid for bovine embryos which shall be changed each time (and which shall, for bovine embryos intended for intra-Area trade, contain trypsin, in accordance with internationally recognised procedures). Each wash shall be a 100-fold dilution of the previous wash and a sterile micropipette shall be used to transfer the bovine embryo on each occasion.

14. After the last wash each bovine embryo shall be subjected to microscopic examination at 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. Any 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 bovine embryo having an intact *zona pellucida*.

15. Each consignment of bovine embryos that has successfully undergone the examination provided for in the preceding paragraph shall be placed in a sterile container marked in accordance with this Schedule and which shall be sealed immediately.

16. Each bovine 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.

17. Ovaries, oocytes and other tissues shall not be allowed to enter the processing laboratory until completion of the post-mortem inspection of the batch of donors. If any disease that might be transmitted in the material and make it unsuitable for producing bovine embryos 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.

18. Only bovine embryos from the same batch of donors should be stored in the same ampoule or straw.

SCHEDULE 8

Regulation 21

FORM OF CERTIFICATE BOVINE EMBRYO TRANSFER

Breed and type of bovine embryo(s).....

Registration number of collection team.....

1. I hereby certify that the animal(s) identified in the schedule overleaf was/were clinically examined by me on (date)..... at (address of premises) .....
.....
.....

2. I found the animal(s) to be in good health.

3. I was unable to detect any significant abnormalities of the reproductive tract(s) or birth canal(s).

4. I found the animal(s) to be in appropriate bodily condition and of a suitable size and conformation to receive the intended embryo(s).

5. On the basis of the above examination, I am of the opinion that the animal(s) is/are suitable to receive the embryo(s). I know of no reason existing at the time of my examination which would cause me to believe that the animal(s) would not be able to carry to term a normal calf of the breed and type specified and to calve naturally.

Signed ..... RCVS

Name (Block Capitals) .....

Date .....

Name of Practice .....

Address of Practice .....

Recipient Identification (Ear Tag No.)	*Recipient Breed and Type*	*Breed and Type of Intended Embryo(s)*
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		

The examining Veterinary Surgeon is required to sign immediately beneath the last entry on the above schedule.