SCHEDULE 7

Regulation 8(c)

Conditions relating to the production of embryos

- 1. Embryos shall be produced by a licensed bovine embryo production team, and, in the case of embryos intended for intra-Area trade, without coming into contact with any other consignment of embryos not meeting the requirements of these Regulations relating to embryos intended for intra-Area trade.
- 2. Ovaries, oocytes and other tissues intended to be used in 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.
- 3. Each bovine embryo production team must submit routine samples of flushing fluids, washing fluids, disintegrated embryos, non-fertilised ova etc, resulting from its activities to the Department for official examination for bacterial and viral contamination.
- 4. When ovaries, oocytes, and other tissues are to be collected at an abattoir, the abattoir should be officially approved and under the control of a veterinary officer of the Department who shall carry out ante- and post-morten inspection of donors.
- 5. 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 embryos from different batches of donor animals. All laminar-flow facilities shall be properly cleansed and disinfected between batches.
- 6. 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.
- 7. Products of animal origin used during production 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. 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.
- 8. Storage flasks and transport flasks shall be properly disinfected or sterilised before the commencement of each filling operation.
- 9. The cryogenic agent used shall not have been previously used for other products of animal origin.
- 10. 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.
- 11. Once the embryo has been produced, 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 or batch, as well as the registration number of the team can be readily established.
- 12. After the culture procedure has been completed, each embryo shall be washed at least 10 times in a special fluid for embryos which shall be changed each time (and which shall, for embryos intended for intra-Area trade, contain tryspin, 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 embryo on each occasion.
- 13. After the last wash each embryo shall be subjected to microscopic examination at a magnification of at least 50x over its entire surface to determine that the zona pellucida is intact

and is free from any adherent material. Any micro-manipulation 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 micro-manipulation may only be carried out on an embryo having an intact *zona pellucida*.

- 14. Each consignment of 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.
- 15. 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 a veterinary officer of the Department.
- 16. 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 embryos is found in the batch of donors, or in any animals slaughtered in that abattoir on that day, all tissues from the batch must be traced and discarded.
 - 17. Only embryos from the same batch of donors should be stored in the same ampoule or straw.