Terrestrial Animal Health Standards Commission Report

October 2008

CHAPTER 4.8.

COLLECTION AND PROCESSING OF *IN VITRO* FERTILISED BOVINE EMBRYOS / *IN VITRO* MATURING BOVINE OOCYTES

Article 4.8.1.

Aims of control

The purpose of official sanitary control of *in vitro* fertilised bovine 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 4.8.1.bis

Conditions applicable to the embryo ~~collection~~ production team

The embryo production team is a group of competent technicians, including at least one veterinarian, to perform the collection and processing of bovine ovaries/oocytes and the production and storage of *in vitro* fertilised (IVF) bovine embryos. The following conditions should apply:

1. The team should be supervised by a team veterinarian.

2. The team veterinarian is responsible for all team operations which include hygienic collection of ovaries and oocytes and all other procedures involved in the production of embryos intended for international movement.

3. The team veterinarian should be specifically approved for this purpose by an *official Veterinarian*.

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

5. The production team ~~must~~ should have adequate facilities and equipment for:

   a) collecting oocytes;

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

   c) storing embryos.

   These facilities need not necessarily be at the same location.

6. The collection team ~~must~~ should keep a record of its activities, which ~~must~~ should be maintained for inspection by the approving authority for a period of at least 2 years after the embryos have been exported.

7. The production team should be subjected to regular inspection by an *Official Veterinarian* to ensure compliance with procedures for the sanitary collection and processing of oocytes, and the production and storage of embryos.

8. The production team ~~must~~ should not operate in an *infected zone* for foot and mouth disease, contagious bovine pleuropneumonia, bluetongue or ~~and~~ rinderpest.

Article 4.8.2.

Conditions applicable to the processing laboratories

The processing laboratory ~~is a premises~~ may be mobile or permanent. It is a facility in which oocytes which have been recovered from ovaries are then matured and fertilised, and embryos are further cultured *in vitro*. It may be contiguous with the oocyte recovery area or may be at a separate location.

Embryos so produced may also be subjected to any required treatments such as washing before freezing, storage and quarantine in this laboratory.

Additionally:

1. The laboratory should be under the direct supervision of the team veterinarian and regularly inspected by an *Official Veterinarian*.

2. While embryos for export are being produced prior to their storage in ampoules, vials or straws, no oocyte/embryo of a lesser health status should be recovered or processed in the laboratory.

3. The 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 following each occasion on which embryos are processed.

5. The laboratory ~~must~~ should not be situated in an *infected zone* for foot and mouth disease, contagious bovine pleuropneumonia, bluetongue or rinderpest.

Article 4.8.3.

Conditions applicable to ~~the introduction of~~ donor animals

Oocytes for the production of IVF embryos are obtained from donors in one of two ways: individual collection or batch collection. The recommended sanitary conditions for these differ.

Individual collection usually involves the aspiration of oocytes from the ovaries of live animals on the farm where the donor animal resides or at the laboratory. Occasionally oocytes may also be recovered from individual live donors by aspiration from surgically excised ovaries. ~~When oocytes are recovered from individual live animals, the procedures for these donors should follow the recommendations set out in Article 4.7.4.~~

Cleaning and sterilisation of equipment is especially important and must be carried out between each donor in accordance with the requirements of the Procedures Manual of the International Embryo Transfer Society (IETS) [2].

Batch collection usually involves the removal of ovaries from slaughtered animals at an *abattoir* but may alternatively involve the surgical removal of ovaries from live donors; these ovaries are then transported to the laboratory where oocytes are removed by aspiration. Batch collection involving *abattoir* derived ovaries has the disadvantage that it is usually impractical to relate ovaries which are transported to the laboratory to the donors which were slaughtered at the *abattoir*. Nevertheless, it is critical to ensure that only healthy tissues are obtained and that they are removed from the donors in a hygienic manner.

Additionally:

1. The *Veterinary Authority* should have knowledge of, and authority over, the *herd(s)* ~~of origin of~~ from which the donor animals have been sourced.

2. The donor females should not originate from an *infected zone* for foot and mouth disease, contagious bovine pleuropneumonia, bluetongue or rinderpest and the removal of any tissue should not take place in an *infected zone* for foot and mouth disease, contagious bovine pleuropneumonia, bluetongue or rinderpest.

3. In the case of oocyte recovery from individual animals or batch collection from live donors, post-collection surveillance of the donors and donor *herds* based on the recognized *incubation periods* of the *diseases* of concern to determine retrospectively the health status of donors should be conducted.

~~3~~4. The *abattoir* should be officially approved 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 clinical signs of ~~contagious~~ *diseases* ~~of concern transmissible to cattle~~ that may be transmissible by bovine semen or embryos.

~~4~~5. The donor females should not have been designated for compulsory *slaughter* for a *notifiable disease* and other animals of a lesser health status ~~must~~ should not be slaughtered at the same time as donors from which ovaries and other tissues will be removed.

~~5~~6. Records of the identities and origins of all donors ~~must be kept~~ should be maintained for inspection by the approving authority for a period of at least 2 years after the embryos have been exported.

~~6~~7. Batches of ovaries should not be transported to the processing laboratory before confirmation has been obtained that ante and post-mortem inspection of donors has been satisfactorily completed.

~~7~~8. Equipment for removal and transport of ovaries and other tissues should be cleaned and sterilized before use and exclusively used for these purposes.

Article 4.8.4.

Testing of oocytes, embryos, semen and culture media

The main approach for ensuring IVF embryos are free of pathogenic organisms is the testing of non-viable oocytes/embryos and associated co-culture cells, fluid and media.

Tests may also be used to assess whether quality control procedures being applied in the processing laboratory are acceptable.

Tests may be carried out on the following materials to confirm the absence of pathogenic organisms that may be transmissible by bovine semen or embryos and that ~~which~~ are of concern to the *importing country*:

a) non-viable oocytes/embryos: all non-viable oocytes/embryos at any stage of the production line from batches intended for export should be pooled for testing;

b) *in vitro* maturation medium prior to mixing the oocytes with semen for the fertilisation process;

c) embryo culture medium taken immediately prior to embryo storage.

These samples should be stored at 4°C and tested within 24 hours. If this is not possible, then the samples should be stored frozen at -70°C or lower.

~~In the case of oocyte recovery from individual animals or batch collection from live donors, monitoring of clinical health status and post-collection testing of donors for *diseases* of concern may be considered.~~

Additionally:

1. Semen used to fertilise oocytes *in vitro* should meet the health requirements and standards set out in Chapter 4.5 as appropriate to the species.

   When the donor of the semen used to fertilise the oocytes is no longer living, 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 on the spare IVF embryos may be required to verify that these infectious *diseases* were not transmitted. An alternative may be to subject an aliquot of semen from the same collection date to testing.

2. Any biological product of animal origin, including co-culture cells and media constituents, used in oocyte recovery, maturation, fertilisation, culture, washing and storage should be free of living pathogen~~ic micro-organism~~s. Media 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 all fluids and media as recommended in the IETS Manual[2].

3. All equipment used to recover, handle, culture, wash, freeze and store oocytes/embryos should be cleaned and sterilised prior to use as recommended in the IETS Manual[2].

Article 4.8.5.

Conditions applicable to the processing, storage~~, quarantine~~ and transport of embryos/ova

1. After the culture period is finished but prior to freezing, storage and transport, the embryos should be subjected to washing and other treatments similar to those specified for *in vivo* derived embryos in accordance with the IETS Manual[2].

2. Only embryos from the same donor, in the case of individual animal recovery, or from the same batch collection, should be washed together.

3. The zona pellucida of each embryo ~~must~~ should be examined over its entire surface area at not less than 50X magnification and certified to be intact.

4. The IVF embryos should be stored in sealed sterile ampoules, vials or straws and then frozen in fresh liquid nitrogen or other cryoprotectant in cleaned and sterilised containers under strict hygienic conditions at a storage place, approved by the *Veterinary Authority* of the *exporting country*, ~~where no risk of~~ to avoid contamination of the embryos ~~can occur~~.

5. Only embryos from the same individual donor or batch collection should be stored together in the same ampoule, vial or straw.

6. Ampoules, vials or straws must be sealed at the time of freezing and should be labelled according to the IETS Manual[2].

7. Liquid nitrogen containers should be sealed prior to shipment from the *exporting country*.

8. Embryos must not be exported until the appropriate veterinary ~~certification documents~~ certificates are completed.

Article 4.8.6.

Procedure for micromanipulation

When micromanipulation of the embryos is to be carried out, this should be done after completion of the treatments described in Article 4.8.5. and conducted in accordance with Chapter 4.7.