

September 2009

CHAPTER 4.8.

COLLECTION AND PROCESSING OF
IN VITRO PRODUCED EMBRYOS / OOCYTES FROM
LIVESTOCK AND HORSES

Article 4.8.1.

Aims of control

Production of embryos *in vitro* involves the collection of oocytes from the ovaries of donors, *in vitro* maturation and fertilization of the oocytes, then *in vitro* culture to the morula/blastocyst stage at which they are ready for transfer into recipients. The purpose of official sanitary control of *in vitro* produced embryos intended for movement internationally is to ensure that specific pathogenic organisms, which could be associated with such embryos, are controlled and transmission of *infection* to recipient animals and progeny is avoided. The conditions outlined in this chapter are also applicable where the movement of *in vitro* maturing (IVM) oocytes is intended.

Article 4.8.2.

Conditions applicable to the embryo production team

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

1. The team should be approved by the *Competent Authority*.
2. The team should be supervised by a team *veterinarian*.
3. The team *veterinarian* is responsible for all team operations which include the hygienic collection of ovaries and oocytes and all other procedures involved in the production of embryos intended for international movement.
4. The team *veterinarian* should be specifically approved for this purpose.
5. 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*.
6. The production team should have adequate facilities and equipment for:
 - a. collecting ovaries and/or oocytes;
 - b. processing of oocytes and production of embryos at a permanent ~~site~~ or mobile laboratory;

- c. storing oocytes and/or embryos.

These facilities need not necessarily be at the same location.

- 67. The embryo production team should keep a record of its activities, which should be maintained for inspection by the *Veterinary Authority* for a period of at least 2 years after the embryos have been exported.
- 78. The embryo production team should be subjected to regular inspection at least once a year by an *Official Veterinarian* to ensure compliance with procedures for the sanitary collection and processing of oocytes and the production and storage of embryos.

Article 4.8.3.

Conditions applicable to the processing laboratories

A processing laboratory used by the embryo production team may be mobile or permanent. It may be contiguous with the oocyte recovery area or at a separate location. It is a facility in which oocytes which have been recovered from ovaries are then matured and fertilised, and where the resulting embryos are further cultured *in vitro*.

Embryos may also be subjected to any required treatments such as washing and 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 same 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 frequently and always before and after each occasion when embryos for export are processed.

Article 4.8.4.

Conditions applicable to donor animals

Oocytes for the *in vitro* production of embryos are obtained from donors basically in two different ways: individual collection or batch collection. The recommended conditions for these differ.

Individual collection usually involves the aspiration of oocytes from the ovaries of individual live *animals* on the farm where the *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 conditions for these donors should resemble those set out in Article 4.7.4.

In these cases the cleaning and sterilisation of equipment (e.g. ultrasound guided probes) is especially important and must be carried out between each donor in accordance with the recommendations in the Manual of the International Embryo Transfer Society (IETS)¹.

Batch collection involves the removal of ovaries from batches of donors slaughtered at a slaughterhouse/*abattoir* (hereafter '*abattoir*'); these ovaries are then transported to the processing laboratory where the oocytes are recovered from the ovarian follicles by aspiration. Batch collection has the disadvantage that it is usually impractical to relate the 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 and transported to the laboratory in a hygienic manner.

Additionally:

1. The *Veterinary Authority* should have knowledge of, ~~and authority over,~~ the *herd(s)/flock(s)* from which the donor animals have been sourced.
2. The donor animals should not originate from *herds / flocks* ~~which that~~ are subject to veterinary restrictions for *listed diseases of concern* (under study), and neither should the removal of any tissue or aspiration of oocytes take place in an *infected zone*, or one that is subject to veterinary restrictions for *listed diseases of concern* (under study)
3. In the case of oocyte recovery from live donors, post-collection surveillance of the donors and donor *herd(s) / flock(s)* should be conducted based on the recognized *incubation periods* of the *diseases* of concern to determine retrospectively the health status of donors.
4. In the case of oocyte recovery from batches of ovaries collected from an *abattoir*, the *abattoir* should be officially approved and under the supervision of a *veterinarian* whose responsibility 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 or pathological signs of infectious diseases (under study).
5. Donor animals slaughtered at an *abattoir* should not have been designated for compulsory *slaughter* for a *notifiable disease* and should not be slaughtered at the same time as donors from which ovaries and other tissues will be removed.
6. Batches of ovaries and other tissues collected from an *abattoir* 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. Equipment for the removal and transport of ovaries and other tissues should be cleaned and sterilised before use and exclusively used for these purposes.
8. Records of the identities and origins of all donors should be maintained for inspection by the *Veterinary Authority* for a period of at least 2 years after the embryos have been exported. While this may be difficult to achieve in the case of batch collection, it is to be expected that the identities of the *herds/flocks* from which the donors originated will be maintained.

Article 4.8.5.

Optional tests and treatments

~~The main~~ A supplementary approach for ensuring that *in vitro* produced embryos do not transmit *disease* is by testing various materials to confirm the absence of pathogenic organisms ~~which that~~ are of concern to the *importing country*.

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

Tests may be carried out on the following materials:

- a. non-viable oocytes/embryos from any stage of the *in vitro* production line from batches intended for export;
- b. samples of *in vitro* maturation medium taken prior to mixing the oocytes with semen for the fertilisation process;
- c. samples of 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.

Additionally:

1. Semen used to fertilise oocytes *in vitro* should meet the health requirements and standards set out in Chapter 4.56, as appropriate to the species.

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

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 pathogens. Media should be sterilised prior to use by approved methods according to the IETS Manual¹ 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 Manual¹.
3. All equipment used to recover, handle, culture, wash, freeze and store oocytes/embryos should be new or cleaned and sterilised prior to use as recommended in the IETS Manual¹.

Article 4.8.6.

Risk management

With regard to disease transmission, transfer of *in vitro* produced embryos is a low risk method for moving animal genetic material although the risk is not quite as low as for *in vivo* derived embryos. It should be noted that categorisation of *diseases*/disease agents by the IETS, as described for *in vivo* derived embryos in Article 4.7.14., does not apply in the case of *in vitro* produced embryos. Irrespective of the animal species, there are three phases in the embryo production and transfer process that determine the final level of risk. These are as follows:

1. the first phase comprises the risk potential for ovary/oocyte/embryo contamination and depends on:
 - a. the disease situation in the *exporting country* and/or *zone*;
 - b. the health status of the *herds/flocks* and the donors from which the ovaries/oocytes/ embryos are collected;

- c. the pathogenic characteristics of the specified disease agents (under study) that are of concern to the *Veterinary Authority* of the *importing country*;
2. the second phase covers risk mitigation by the use of internationally accepted procedures for the processing of embryos which are set out in the IETS Manual¹. These include the following:
 - a. after the *in vitro* culture period is finished the embryos should be washed at least ten times with at least 100-fold dilutions between each wash, and a fresh pipette should be used for transferring the embryos through each wash;
 - b. only embryos from the same donor (in the case of individual collection) or from the same batch (in the case of batch collection) should be washed together, and no more than ten embryos should be washed at any one time;
 - c. sometimes, for example when inactivation or removal of certain viruses (e.g. bovine herpesvirus-1, or Aujeszky's disease virus) is required, the standard washing procedure should be modified to include additional washes with the enzyme trypsin, as described in the IETS Manual¹;
 - d. the zona pellucida of each embryo, after washing, should be examined over its entire surface area at not less than 50X magnification to ensure that it is intact and free of adherent material;
 3. the third phase, which is applicable to *diseases* (under study) which are of concern to the *Veterinary Authority* of the *importing country*, encompasses the risk reductions resulting from:
 - a. post-collection surveillance of the donors and donor *herds/flocks* based on the recognised *incubation periods* of the *diseases* of concern to determine retrospectively the health status of the donors whilst the embryos are stored (in species where effective storage by cryopreservation is possible) in the *exporting country*. Post-collection surveillance of donors is not, of course, possible in the case of batch collection from an *abattoir*, although surveillance of the *herds/flocks* of origin may be possible;
 - b. testing of oocytes/embryos, co-culture cells, media and other samples (e.g. blood) (as referred to in Article 4.8.4.) in a laboratory for presence of disease agents.

Article 4.8.7.

Conditions applicable to the storage and transport of embryos

1. Only embryos from the same individual donor or from the same batch collection should be stored together in the same ampoule, vial or straw.
2. The embryos should if possible, depending on the species, be frozen in fresh liquid nitrogen or other cryoprotectant and then stored in fresh cryoprotectant in cleaned and sterilised tanks or containers under strict hygienic conditions at a storage place.
3. Ampoules, vials or straws must be sealed at the time of freezing and should be labelled according to the IETS Manual¹.
4. Liquid nitrogen containers should be sealed prior to shipment from the *exporting country*.
5. Embryos must not be exported until the appropriate veterinary certificates are completed.

Article 4.8.8.

Procedure for micromanipulation

When micromanipulation of the embryos is to be carried out, this should be done after completion of the treatments described in point 2 of Article 4.8.6. and conducted in accordance with Chapter 4.9.