Terrestrial Animal Health Standards Commission Report

October 2008

CHAPTER 4.9.

COLLECTION AND PROCESSING OF MICROMANIPULATED BOVINE EMBRYOS

Article 4.9.1.

Introduction

Chapter 4.7. recommends official sanitary control measures for the international movement of intact, *in vivo* derived ~~bovine~~ embryos, and ~~likewise~~ Chapter 4.8. recommends measures for *in vitro* fertilized bovine embryos/*in vitro* maturing bovine oocytes. Neither of those Chapters covers embryos which have been subjected to biopsy, splitting, transgene injection, intracytoplasmic sperm injection (ICSI), nuclear transplantation or other micromanipulations which breach the integrity of the zona pellucida. Such embryos are subsequently referred to here as 'micromanipulated embryos'.

It should be noted that complete removal of granulosa cells prior to micromanipulation of oocytes, zygotes and embryos is necessary to avoid lowering their health status.

To bring micromanipulated embryos within the scope of the above mentioned Chapters, the following conditions ~~shall~~ should apply:

Article 4.9.2.

1. Prior to any micromanipulation which involves breaching the zona pellucida, all embryos/oocytes ~~must~~ should be collected and processed according to the sanitary conditions laid down in Chapter 4.7. *in vivo* derived embryos) or produced according to the sanitary conditions laid down in Chapter 4.8. (*in vitro* fertilised bovine embryos/*in vivo* maturing bovine oocytes).

2. Responsibility for the embryos/oocytes ~~must~~ remains with the embryo collection team (*in vivo* derived embryos) or with the embryo production team (*in vitro* fertilised bovine embryos), and all processing involving micromanipulation should be carried out in an approved processing laboratory under supervision of an approved team veterinarian (see Articles 4.7.2. and 4.7.3., and Articles 4.8.1. and 4.8.2., as relevant).

3. Donor animals ~~must~~ should comply with the conditions laid down in Article 4.7.4. (*in vivo* derived embryos) or Article 4.8.3. (*in vitro* fertilised bovine embryos/*in vivo* maturing bovine oocytes), whichever is appropriate. The criteria for testing samples to ensure that embryos are free of pathogenic organisms are laid down in Article 4.7.5. and Article 4.8.4. respectively, and these should be followed.

4. All embryos to be micromanipulated ~~must~~ should be washed according to the protocols laid down in the IETS Manual ~~(1998)~~[1] and they ~~must~~ should be observed to have an intact zona pellucida before and after washing. Only embryos from the same donor, or, in the case of some *in vitro* produced embryos (see Chapter 4.8.) from the same batch collection, should be washed together at the same time. After washing, but before micromanipulation, the zona pellucida of each embryo should be examined over its entire surface area at not less than 50X magnification and certified to be intact and free of adherent material.

5. If surrogate zonae are used, they should be of bovine origin and the embryos/oocytes from which they are obtained should be treated in the same manner as if they were *in vivo* derived or *in vitro* produced embryos intended for international movement.

Article 4.9.3.

Procedures for micromanipulation

The term 'micromanipulation' covers several different procedures and a variety of specialized microsurgical instruments and other equipment may be used. However, from the standpoint of animal health, any cutting, penetrating or breaching of the integrity of the zona pellucida is an action that can alter the health status of an embryo. To maintain health status during and after micromanipulation, the following conditions should apply:

1. Media

   Any product of animal origin, including co-culture cells and media constituents, used in the collection of embryos, oocytes or other cells, and in their micromanipulation, culture, washing and storage should be free of pathogenic micro-organisms (including transmissible spongiform encephalopathy agents, sometimes called prions). All media and solutions should be sterilized by approved methods according to the IETS Manual[1] 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[1].

2. Equipment

   Equipment (e.g. microsurgical instruments which have direct contact with embryos) should either be of the single-use type (disposed of after each embryo) or should be effectively sterilised between embryos in accordance with recommendations in the IETS Manual[1].

3. Nuclei for transfer

   a) Where it is intended to transplant nuclei derived from pre-hatching stage (i.e. zona pellucida intact) embryos, the parent embryos from which those nuclei are derived should fulfil the conditions of this Chapter. Where nuclei derived from other types of donor cell (e.g. post-hatching stage embryos, embryonic, fetal and adult cells, including spermatozoa/spermatids for ICSI) are to be transplanted, the parent embryo, fetus or animal from which those donor cells originate, and the methods whereby they are derived, including cell culture, should comply with the relevant animal health standards recommended elsewhere in this *Terrestrial Code* and in the *Terrestrial Manual*.

   b) Where it is intended to transplant a nucleus into an oocyte (for ICSI), or into an enucleated oocyte (for nuclear transfer), those oocytes should be collected, cultured and manipulated according to the recommendations in this Chapter and/or in Chapter 4.7.

Article 4.9.4.

Optional tests and treatments

The *importing country* may request that tests[2] be carried out on certain samples or that embryos are treated to ensure that specified pathogenic organisms are absent.

1. Samples

   Samples to be tested may include those referred to in Article 4.7.7. and/or in Article 4.8.4. Where cells other that from zona pellucida-intact embryos (e.g. somatic or sperm cells) are used as donors of nuclei for transplantation, then samples or cultures of those donor cells may also be tested.

2. Treatments

   Treatments of embryos with the enzyme trypsin or other substances proven to inactivate or remove pathogenic organisms~~, and which are harmless to the embryo,~~ may be requested when pathogens that are not removed by ~~routine~~ washing may be present. ~~, but t~~These also should be applied prior to any micromanipulation, and according to the IETS Manual[1].

Article 4.9.5.

Conditions applicable to storage, quarantine and transport

Micromanipulated embryos should be stored, quarantined and transported according to the conditions laid down in Article 4.7.8. or in points 4, 5, 6, 7 and 8 of Article 4.8.5. Veterinary certification documents should identify all micromanipulations, where and when they were carried out.

1 Manual of the International Embryo Transfer Society ~~(1998)~~.

~~2 If the samples mentioned above in point 1. of Article 4.9.4. are to be tested for pathogenic agents, then the microbiological techniques in current use for those agents would be appropriate.~~