CHAPTER 4.9.

COLLECTION AND PROCESSING OF MICROMANIPULATED EMBRYOS/OOCYTES FROM LIVESTOCK AND HORSES

Article 4.9.1.

Introduction

Neither Chapter 4.7. which recommends official sanitary control measures for the international movement of *in vivo* derived embryos nor Chapter 4.8. which recommends measures for *in vitro* produced embryos/*in vitro* maturing oocytes covers embryos which have been subjected to biopsy, splitting, transgene injection, intracytoplasmic sperm injection (ICSI), nuclear transfer or other interventions which breach the integrity of the zona pellucida. Such embryos/oocytes are those referred to here as having been 'micromanipulated'.

It should be noted that complete removal of granulosa cells or other adherent material from the outer surface of the zona pellucida of oocytes, zygotes and embryos is necessary prior to micromanipulation to avoid lowering their health status.

Removal of such material from the zona pellucida of immature oocytes can be difficult. However, to bring micromanipulated embryos/oocytes within the scope of the above mentioned chapters, the following conditions should apply.

Article 4.9.2.

1) Prior to any micromanipulation which involves breaching the zona pellucida, all embryos/oocytes 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* produced embryos/oocytes).
2) Responsibility for the embryos/oocytes remains with the embryo collection team (*in vivo* derived embryos) or with the embryo production team (*in vitro* produced 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 4.8.2. and 4.8.3., as appropriate).
3) Donor *animals* should comply with the conditions laid down in Article 4.7.4. (*in vivo* derived embryos) or Article 4.8.4. (*in vitro* produced embryos), whichever is appropriate. Risk management and criteria for testing samples to ensure that embryos are free of pathogenic organisms are laid down in Articles 4.7.5. and Article 4.7.7. and in Articles 4.8.5. and 4.8.6. respectively, and these should be followed.
4) All embryos to be micromanipulated should be washed according to the protocols laid down in the IETS Manual[1] and they 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, embryos originating from the same batch of ovaries from an *abattoir* (see Chapter 4.8.), 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 from the same species 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 specialised 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 or production 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/oocytes batch) or should be effectively sterilised between embryos/oocytes batch in accordance with recommendations in the IETS Manual[1].

3. Nuclei for transplantation ('nuclear 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 intact oocyte (e.g. 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.

Article 4.9.4.

Optional tests and treatments

The *importing country* may request that tests be carried out on certain samples or that embryos be 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 4.8.5. 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 may be requested when pathogens that are not removed by washing may be present. If used, such treatments should also 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 Article 4.8.7. as appropriate. Veterinary certification documents should identify all micromanipulations, where and when they were carried out.

1 Manual of the International Embryo Transfer Society.