IMPORT RISK ANALYSIS REPORT ON THE IMPORTATION OF BOVINE SEMEN AND EMBRYOS FROM ARGENTINA AND BRAZIL INTO AUSTRALIA

PART 2: BOVINE EMBRYOS

November 1999

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EXECUTIVE SUMMARY

The animal health risks associated with importing bovine embryos from Argentina and Brazil were analysed in response to trade enquiries from Australian cattle breeders. Argentina and Brazil present quite different animal health risks to countries for which Australia has current bovine embryo import requirements, namely the USA, Canada, New Zealand, New Caledonia, Switzerland, Member States of the European Union (EU), Norway, South Africa and Zimbabwe.

The hazards identified in this import risk analysis (IRA) are causative agents of quarantinable diseases which could be imported with bovine embryos and which could adversely affect the Australian livestock industry if introduced.

The risks are qualitatively assessed. The assessment includes: consideration of the epidemiological features affecting the likelihood of disease agents infecting or contaminating bovine embryos; the likelihood of pathogens remaining after washing of embryos; and the likelihood of infected or contaminated embryos causing disease. The following pathogens were identified as requiring risk management measures:

- foot and mouth disease virus,
- vesicular stomatitis virus,
- bluetongue virus,
- Leptospira spp,
- rabies virus,
- Mycobacterium paratuberculosis,
- bovine spongiform encephalopathy infective agent,
- Brucella abortus,
- Mycobacterium bovis,
- bovine leukemia virus,
- Pasteurella multocida (serotypes B:2 and E:2),
- bovine herpesvirus-1,
- bovine pestivirus, and
- epizootic haemorrhagic disease of deer virus.

It is proposed that:

- collection and processing of embryos meet the minimal standards as recommended in the OIE International Animal Health Code (Code) Appendix 4.2.3.1.
- washing of embryos be considered as adequate risk management measures for:
  - bluetongue virus
  - Brucella abortus
  - bovine herpesvirus-1 (with trypsin treatment) and
  - epizootic haemorrhagic disease of deer virus.
- certification of country, zone, region or area freedom from disease be the sole quarantine measure for:
  - rabies virus
  - vesicular stomatitis virus and
  - Pasteurella multocida (serotypes B:2 and E:2).
- either certification of area/herd freedom from disease or donor cows meeting specified criteria such as negative blood tests for certain diseases be required for:
- foot and mouth disease virus
- bovine spongiform encephalopathy infective agent and
  - *Mycobacterium bovis*.

- a single disease test be required:
  - bovine pestivirus and
  - *Mycobacterium paratuberculosis*

- no risk management measures be necessary for
  - bovine leukemia virus.
ABBREVIATIONS AND ACRONYMS

AGID    agar gel immunodiffusion (test)
AI      artificial insemination
AQIS   Australian Quarantine and Inspection Service
AUSVETPLAN    Australian Veterinary Emergency Plan
BHV-1  bovine herpesvirus-1
BLV    bovine leukemia virus
Br     bovine brucellosis
BSE    bovine spongiform encephalopathy
BT     bluetongue
BTV    bluetongue virus
BVD    bovine viral diarrhoea
BVDV   bovine viral diarrhoea virus
CFT    complement fixation test
EBL    enzootic bovine leukemia
EE     equine encephalomyelitides
EHD    epizootic haemorrhagic disease
EHDV   epizootic haemorrhagic disease virus
EITB   enzyme-linked immunoelectrotransfer blot (assay)
ELISA  enzyme-linked immunosorbent assay
FMD    foot and mouth disease
FMDV   foot and mouth disease virus
HS     haemorrhagic septicaemia
IBR/IPV infectious bovine rhinotracheitis/infectious pustular vulvovaginitis
IETS   International Embryo Transfer Society
IRA    import risk analysis
JD     Johne’s disease
MAARA  Ministerio da Agricultura do Abastecimento e da Reforma Agraria (Brazil)
MD     mucosal disease
NAMP   National Arbovirus Monitoring Program
NCP    non-cytopathic
OIE    Office International des Epizooties
PCR    polymerase chain reaction
PI     persistently infected
SENASA Servicio Nacional de Sanidad y Calidad Agroalimentaria (Argentina)
Tb     bovine tuberculosis
USA    United States of America
VIAA   virus infection-associated antigen test
VNT    virus neutralisation test
VS     vesicular stomatitis
VSV    vesicular stomatitis virus
GLOSSARY

approved embryo collection team a group of competent technicians, including at least one veterinarian, approved or accredited by the national veterinary authority to assure compliance with recognised standards of ethical conduct and observance of established methods of handling donors and embryos.

embryo term used by convention to describe the conceptus from fertilised 1-cell to blastocyst stages.

establishment means an agricultural establishment in which animals for breeding, rearing or slaughter are raised or kept.


in-vitro refers to a process or procedure performed outside the body in a test tube or other laboratory apparatus.

in-vivo refers to a process occurring in a living organism or under natural circumstances.

IVF in-vitro fertilisation - a culture system in which matured oocytes and capacitated sperm are mixed to achieve conception outside the body.

trypsin treatment is a treatment additional to washing of embryos that may be necessary for the removal of certain pathogens adhering to the zona pellucida as described in Chapter 6 of the IETS Manual.

washed embryos that have been subjected to the washing procedure.

washing the washing of in-vivo derived embryos with intact zona pellucida as described in Chapter 6 of the IETS Manual where embryos are washed ten times to remove pathogens.
1. INTRODUCTION

1.1 Scope of risk analysis

This document analyses the risks associated with importing *in vivo* derived bovine embryos from Argentina and Brazil into Australia. As with bovine semen, there are two main concerns associated with the widespread use of embryos in the embryo transfer industry - the dissemination of undesirable genetic traits and the transmission of exotic and other significant diseases. The former is not a quarantine concern. Both Argentina and Brazil have a number of diseases that are exotic to Australia as well as a number of enzootic diseases that are present at very low levels or are enzootic only in certain parts of Australia. Embryo transfer may transmit some of these diseases to susceptible females or even to their offspring via infected embryos.

Importing *in vitro* derived bovine embryos is not considered because

- it is difficult to ascertain the health history of slaughtered commercial donor cows as ovaries and uterine tubes are usually collected randomly at slaughter houses and pooled in containers for transport and further processing at IVF laboratories;
- most media used in *in-vitro* fertilisation (IVF) processes contain sera, hormones and other additives which complicate risk assessment;
- there are differences in the morphology and physiology between *in vivo* and *in vitro* embryos, including differences in the structure of the zona pellucida;
- many pathogens which can be washed from the zona pellucida of *in vivo* fertilised embryos are not readily washed from *in vitro* fertilised embryos, and
- there is increased risk of true embryonic infection with the piercing of the zona pellucida with micropipettes before injecting the sperm for fertilisation of the oocyte.

This IRA

- identifies the disease hazards which may be found in washed *in vivo* embryos and which constitute a national quarantine risk;
- assesses the probability of these embryos being infected with these disease agents;
- assesses the probability of infected embryos transmitting the disease agents to other susceptible animals and causing disease;
- assesses the consequences if the diseases were introduced into Australia;
- identifies the risk management options for minimising the risks of introducing disease into Australia with bovine embryos, and
- lists the recommended risk management options which could be applied to each disease agent before importing *in vivo* derived bovine embryos from Argentina and Brazil.

Assessment of the consequences of the introduction of a number of diseases into Australia were discussed in Part 1: Bovine Semen of this IRA and are not repeated here.

Factors that influence risk assessment include:

- quality of veterinary services in both exporting and importing countries;
- animal health surveillance programmes, and
- disease zoning systems which can affect the probability of infection in the exporting country.
Effective quarantine relies on the partnership between the veterinary administrations of both importing and exporting countries and the embryo collection team veterinarian supervising the collection and processing of the embryos.

Both Argentina and Brazil have bovine gene pools that are of interest to Australian producers and there is a growing demand for importation of bovine embryos from Argentina and Brazil. The bovine embryo transfer industry is remarkably well developed in both Argentina and Brazil. The number of transfers of bovine embryos conducted in these two countries in 1997 is compared with several other areas in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Country</th>
<th>No. flushes</th>
<th>Transferred Fresh</th>
<th>Transferred Frozen</th>
<th>Total transferred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>1,855</td>
<td>4,142</td>
<td>5,135</td>
<td>9,277</td>
</tr>
<tr>
<td>Brazil</td>
<td>3,319</td>
<td>13,724</td>
<td>10,361</td>
<td>24,085</td>
</tr>
<tr>
<td>South Africa</td>
<td>3,011</td>
<td>5,213</td>
<td>3,407</td>
<td>8,620</td>
</tr>
<tr>
<td>Oceania (incl NZ)</td>
<td>3,074</td>
<td>7,610</td>
<td>6,827</td>
<td>14,437</td>
</tr>
<tr>
<td>(New Zealand)</td>
<td>1,567</td>
<td>3,930</td>
<td>3,830</td>
<td>7,760</td>
</tr>
<tr>
<td>North America</td>
<td>27,681</td>
<td>65,570</td>
<td>59,383</td>
<td>124,953</td>
</tr>
</tbody>
</table>

Argentina has a very active embryo transfer (ET) industry as close to 2,000 donors were flushed with close to 10,000 transferable embryos collected. Most of the embryos were from beef breeds with only 23.5% from dairy breeds. Approximately 20% of embryos transferred in Argentina were imported. Not many other countries transfer as many embryos as Brazil, which, according to 1997 data, ranked 5th in the world behind USA, Canada, Japan, and France. Of the Member States of the EU, only France, the Netherlands, the United Kingdom, and Germany transferred over 20,000 embryos in 1997.

International trade in bovine embryos is a rapidly growing industry. In 1997, USA exported approximately 11,000 embryos while Canada exported 8,351 embryos.

The unregulated movement of embryos involves considerably less disease transmission risk than does unregulated movement of live animals or semen. The risks of disease transmission as a result of embryo transfer can be further reduced by adopting processing methods designed to remove various disease agents from embryos.

Over 1,000 bovine embryos were exported from USA to France in 1983-87 without any prior testing of donors or washing of embryos. No evidence of disease transmission was reported. During 1997 nearly 170,000 fresh bovine embryos and over 190,000 frozen bovine embryos were transferred worldwide.

1.2 Current quarantine policy and practice

The Quarantine Act (1908) provides for the Governor-General to prohibit, by proclamation, the importation of goods, if the importation of those goods into Australia is likely to introduce any disease or pest. The Quarantine Proclamation 1998 Section 27 lists animal semen, embryos or ova as prohibited biological materials. Section 35 defines animal reproductive material as part of an animal from which
another animal can be reproduced, and includes semen, ova or an embryo. Section 28 (1) prohibits the introduction or importation of prohibited biological materials and Section 38 (1) prohibits the importation of animal parts into Australia, unless the Director of Quarantine has granted a person a permit to import as set out in Sections 28 (3) and 38 (4). Section 70 defines the factors the Director of Quarantine must consider when issuing a permit for the importation of semen, embryos or ova.

Australia permits the importation of in vivo derived bovine embryos from the USA, Canada, New Zealand, New Caledonia, Switzerland, Member States of the EU, Norway, South Africa and Zimbabwe. Licensed or accredited embryo collection teams and laboratories, managed according to the standards set by OIE (Code Appendix 4.2.3.1.) or equivalent national standards, are required for the preparation of embryos for export. To minimise the risk of importing diseases of concern donor animals at these centres are required to undergo disease testing before their embryos are exported.

As the animal health status of Argentina and Brazil differs markedly from countries that currently export bovine embryos to Australia, the development of import conditions requires an IRA.
2. HAZARD IDENTIFICATION

Hazard identification is defined in Part 1: Bovine Semen of this IRA.

2.1 General

It is not the purpose of this IRA to detail the interaction between the disease agent and embryos. However, for pathogens to be transmitted by embryo transfer, they must be present
- within the cells of the embryo (true embryonic infection);
- in association with the zona pellucida;
- in the embryo storage medium, or
- on contaminated personnel, instruments or equipment.

True embryonic infection may arise as a result pathogen
- being within the oocyte before fertilisation;
- being in the spermatozoon at fertilisation, or
- penetrating through the zona pellucida after fertilisation.

Such infections usually result in damaged or dead embryos. These embryos can be detected by microscopic examination, removed and discarded. However, current processing methods are unlikely to be effective in preventing the transmission of infection of healthy embryos with true embryonic infection.

The only known disease agents that appear to be capable of infecting bovine embryos in this way are
- bovine pestivirus
- enzootic bovine leukosis (EBL)
- bovine lentivirus (BLV) and
- bovine spongiform encephalopathy (BSE).

To date, there is no conclusive evidence of this type of infection occurring in bovine embryos. Embryos of different species differ in the glycoprotein composition of the zona pellucida. This can affect the way a pathogen may behave with embryos, eg, foot and mouth disease virus is more easily washed from bovine embryos than porcine embryos.

Although a number of different pathogenic agents have been reported in the semen of bulls, most were found in the seminal fluid or leucocytes rather than within or attached to the spermatozoon. Some pathogens suspected of being within the sperm cell include:
- bovine herpesvirus,
- bovine pestivirus, and
- bluetongue.

Again, there is no conclusive evidence of this happening. However, the possibility of this type of infection with these pathogens cannot be completely discounted.

The zona pellucida is not only a barrier to infection but may also act as a possible carrier of infections. There is no conclusive evidence of any disease agent being able to cross the intact zona pellucida into the oocyte. There is, on the other hand, significant cause for concern that pathogens may be carried on the
zona pellucida. Therefore the status of zona pellucida is critical in determining the health status of bovine embryos.

The zona pellucida, an extracellular shell composed of glycoproteins, protects the oocyte. It lasts for 8 to 9 days after fertilisation but when the embryo reaches hatching-blastocyst stage, the zona pellucida attenuates, and the embryo hatches. Sperm tracks apparently close quickly after fertilisation giving little opportunity for pathogens to follow.

Certain viruses and bacteria have been found to adhere so firmly to the zona pellucida that even 10 washes may fail to remove them. This appears to be true for:

- the enveloped viruses such as bovine herpesvirus-1
- Escherichia coli,
- Mycobacterium paratuberculosis,
- Mycoplasma spp, and
- Streptococci spp.

Once hatched from the zona pellucida, the embryo could become infected by these pathogens.

Special sanitary measures required for in-vivo production of embryos include the washing of embryos. This usually removes all traces of pathogens picked up by the embryo during uterine flushes.

The addition of trypsin appears to affect the “stickiness” of the zona pellucida and assist in the removal of pathogens such as certain enveloped viruses from the surface of the zona pellucida during the washing process. Trypsin should only be used judiciously and never as a general disinfectant for embryos.

Appropriate antibiotics can be used in the medium to reduce populations of some bacteria and mycoplasmas.

2.2 Biological agents identified

Table 2 lists the diseases that could be transmitted in bovine embryos. The diseases are grouped according to the Code List diseases.

Some disease agents (hazards) are not included in the risk assessment because they are endemic in Australia and are not the subject of official control programs or internal restrictions.

Australia, Argentina and Brazil are free from:

- Rinderpest,
- contagious bovine pleuropneumonia,
- lumpy skin disease, and
- Rift Valley fever.

This IRA is based on the continuing freedom of Argentina and Brazil from these four diseases.
<table>
<thead>
<tr>
<th>Hazard</th>
<th>Susceptible Species</th>
<th>Risk of being found in unwashed embryos derived from infected donors</th>
<th>Risk of being found in washed embryos derived from infected donors</th>
<th>Australia Health Status</th>
<th>Argentine Health Status</th>
<th>Brazil Health Status</th>
<th>Risk Assessment needed ?</th>
</tr>
</thead>
<tbody>
<tr>
<td>OIE List A diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot and mouth disease virus</td>
<td>cloven hoofed animals</td>
<td>Probable</td>
<td>Negligible</td>
<td>Not reported Officially free since 1871</td>
<td>Country free from FMD with vaccination</td>
<td>Enzootic with zone free from FMD with vaccination</td>
<td>Yes</td>
</tr>
<tr>
<td>Vesicular stomatitis virus</td>
<td>cattle, horses, pigs, and humans</td>
<td>Probable</td>
<td>Probable</td>
<td>Not reported</td>
<td>Last reported 1986</td>
<td>Sporadic</td>
<td>Yes</td>
</tr>
<tr>
<td>Rinderpest virus</td>
<td>cattle, pigs, sheep, goats</td>
<td>Probable</td>
<td>Negligible</td>
<td>Not reported Free since 1923</td>
<td>Not reported</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td>Mycoplasma mycoides subsp mycoides (cattle strain)</td>
<td>cattle</td>
<td>Probable</td>
<td>Probable</td>
<td>Not reported Free since 1967</td>
<td>Not reported</td>
<td>Not reported since 1921</td>
<td>No</td>
</tr>
<tr>
<td>Lumpy skin disease virus</td>
<td>cattle</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td>Rift Valley fever virus</td>
<td>multiple species include humans</td>
<td>Probable</td>
<td>Unknown</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td>Bluetongue virus</td>
<td>cattle (non-clinical), sheep (clinical)</td>
<td>Probable</td>
<td>Negligible</td>
<td>Enzootic region No virulent strains</td>
<td>Disease suspected but presence not confirmed</td>
<td>Serologic evidence only, no clinical disease</td>
<td>Yes</td>
</tr>
<tr>
<td>OIE List B diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptospira spp</td>
<td>all vertebrates except birds</td>
<td>Likely</td>
<td>Probable</td>
<td>Enzootic</td>
<td>Enzootic</td>
<td>Enzootic</td>
<td>Yes – public health risks</td>
</tr>
<tr>
<td>Coxiella burnettii</td>
<td>mammals, birds, arthropods (mainly ticks)</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Enzootic - no official control programs</td>
<td>Disease suspected but presence not confirmed</td>
<td>Not reported since 1983</td>
<td>No</td>
</tr>
<tr>
<td>Rabies virus</td>
<td>all warm blooded animals</td>
<td>Probable</td>
<td>Unknown</td>
<td>Not reported Lyssavirus in bats</td>
<td>Enzootic - outbreaks reported in cattle</td>
<td>Enzootic - outbreaks reported in cattle</td>
<td>Yes</td>
</tr>
<tr>
<td>Mycobacterium paratuberculosis</td>
<td>cattle, cattle strain may infect sheep</td>
<td>Probable</td>
<td>Probable</td>
<td>Enzootic in certain regions National control programs</td>
<td>Enzootic</td>
<td>Not reported since 1986 (cattle) and 1993 (sheep and goats)</td>
<td>Yes – all states have regulatory requirements</td>
</tr>
<tr>
<td>Brucella abortus</td>
<td>cattle, humans</td>
<td>Likely</td>
<td>Negligible</td>
<td>Not reported Free since 1989</td>
<td>Enzootic</td>
<td>Enzootic</td>
<td>Yes</td>
</tr>
<tr>
<td>Hazard</td>
<td>Susceptible Species</td>
<td>Risk of being found in unwashed embryos derived from infected donors</td>
<td>Risk of being found in washed embryos derived from infected donors</td>
<td>Australia Health Status</td>
<td>Argentine Health Status</td>
<td>Brazil Health Status</td>
<td>Risk Assessment needed?</td>
</tr>
<tr>
<td>-------------------------------------------------------------</td>
<td>--------------------------------------</td>
<td>---------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------</td>
<td>-------------------------</td>
<td>-----------------------</td>
<td>------------------------</td>
</tr>
</tbody>
</table>
| Campylobacter fetus subsp fetus                              | cattle                               | Probable                                                           | Negligible                                                         | Low sporadic occurrence  
No official control programs                                                              | Enzootic                | Enzootic              | No - no national control or regulatory program                                      |
<p>| Mycobacterium bovis                                          | cattle, deer, camels, humans, pigs   | Probable                                                           | Probable                                                          | Sporadic - OIE classified free since 12/1997.                                              | Enzootic                | Enzootic              | Yes                    |
| Bovine leukemia virus (BLV)                                  | cattle, sheep                        | Likely                                                             | Negligible                                                        | Enzootic - control programs only in dairy cattle                                          | Enzootic                | Enzootic              | Yes – dairy industry driven program in all states/territories to eradicate EBL. |
| Pasteurella multocida (Serotypes B:2 and E:2)                | cattle                               | Probable                                                           | Unknown - could be negligible                                     | Not reported                                                              | Not reported            | Reported sporadic but same expression for shipping fever                           | Yes                    |
| Bovine herpesvirus-1                                         | Cattle                               | Likely                                                             | Negligible if treated with trypsin                                | Low sporadic occurrence but pathogenic BHV-1.1 not reported                     | Enzootic                | Enzootic              | Yes                    |
| Tritrichomonas foetus                                        | Cattle                               | Probable                                                           | Negligible                                                        | Low sporadic occurrence especially in northern parts.                             | Enzootic                | Enzootic              | No - no national control or regulatory program                                      |
| Bovine malignant catarhal fever                              | cattle (clinical) sheep and wildebeest (nonclinical) | Not reported | Not likely | Exceptional occurrence | Not reported | Sporadic | No |
| Bovine spongiform encephalopathy                             | Cattle                               | Probable                                                           | Unknown but could be negligible                                     | Not reported - Classed free according to proposed OIE classification             | Not reported - Classed free according to proposed OIE classification | Not reported - Classed provisionally free according to proposed OIE classification | Yes |
| Other diseases                                               |                                      |                                                                    |                                                                     |                                                                                    |                         |                       |                         |
| Bovine pestivirus                                            | Cattle, sheep, pigs                  | Probable                                                           | Negligible                                                        | Enzootic - no pathogenic Type 2 recorded                                              | Enzootic                | Enzootic              | Yes                    |
| Epizootic haemorrhagic disease                               | Cattle, deer, sheep                  | Probable                                                           | Unknown but could be negligible                                     | Serologic evidence only                                                            | Disease suspected but presence not confirmed | Not reported            | Yes                    |</p>
<table>
<thead>
<tr>
<th>Hazard</th>
<th>Susceptible Species</th>
<th>Risk of being found in unwashed embryos derived from infected donors</th>
<th>Risk of being found in washed embryos derived from infected donors</th>
<th>Australia Health Status</th>
<th>Argentine Health Status</th>
<th>Brazil Health Status</th>
<th>Risk Assessment needed ?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine lentivirus</td>
<td>Cattle</td>
<td>Probable</td>
<td>Unknown</td>
<td>Low sporadic occurrence</td>
<td>Sporadic</td>
<td>Sporadic</td>
<td>No</td>
</tr>
</tbody>
</table>
3. RISK MANAGEMENT AND ASSESSMENT

FOOT AND MOUTH DISEASE VIRUS

It is possible to isolate foot and mouth disease virus (FMDV) from the embryo collection fluid from experimentally infected donor cows. The virus can be removed by washing the embryos. This was confirmed by the following reports:

1. All 111 susceptible cows which received intact washed embryos derived from viraemic cows and all steers which were injected intradermal lingually with washed and sonicated reject embryos derived from viraemic cows remained serologically antibody negative for FMDV. Only 15 calves were born, all were serologically negative at birth and at 30 days. The poor conception rate was probably due to the embryos being derived from febrile cows.²

2. No infectious FMDV could be found on any of the 169 zona pellucida intact bovine embryos which were exposed to FMDV and then washed but the virus could be detected on some of washed porcine embryos.³ This is most likely due to the different zona pellucida structure of the porcine embryos.

3. A total of 253 washed embryos collected from 48 cows which were positive to the VIAA test for FMD and which had experienced an outbreak of FMD were assessed for FMDV. No FMDV could be detected on 171 embryos. 42 susceptible cows were implanted and the VIAA tests were all negative in these cows and their calves.⁴

These trials demonstrated the efficacy of:
- the zona pellucida of intact embryos in protecting the germplasm from the virus, and
- the washing procedures in removing FMDV from bovine embryos.

Consequently the IETS ranks FMD as a Category 1 disease, that is, a disease for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer.

Risk management options and recommended measures

The introduction of FMD into Australia is most likely to have a major socio-economic impact. As proper handling of embryos is essential for FMD to meet IETS Category 1, an additional level of screening is proposed to overcome the very small risk of embryos from FMD infected areas not being properly handled.

The probability of importing FMDV in improperly handled embryos from FMD infected tries would be reduced if the donor cows were not
- incubating the virus,
- experiencing viraemia,
- persistently infected, or
- subclinically infected or seropositive to FMD while vaccinated against FMD.
Thus risk management options include:

- washing the embryos;
- assessing the donor for freedom from FMDV clinically and serologically with a test of high sensitivity and specificity;
- vaccination of donors for protection against FMD;
- requiring that the donor be kept in areas which have recorded no FMD outbreaks for a period long enough to eliminate the possibility of animals incubating FMDV being present in the area; or
- a combination of the above options.

The maximum acceptable level of risk of collecting infected embryos from a donor is negligible if

- the donor females
  - tested negative to a test of high sensitivity and specificity, eg EITB;
  - showed no clinical signs of FMD at the time of collection, and
  - were kept in an embryo collection centre where no animals had been added in the 30 days before collection, and
- FMD has not occurred within ten km for the 30 days before and after collection, and
- the embryos collected from the donor females were washed.

The Code gives options for the importation of frozen embryos of cattle from

- FMD free countries or zones where vaccination is or is not practised (Article 2.1.1.12.), and
- FMD infected countries or zones (Article 2.1.1.13.).

These options do not include serological testing of donor cows.

It is proposed to modify the Code (Article 2.1.1.13) by including EITB testing requirements for managing the risks of introducing FMDV with imported bovine embryos from FMD infected countries.

**VESICULAR STOMATITIS VIRUS**

Bovine embryos can become infected with vesicular stomatitis virus (VSV) through extrinsic contamination. However, VSV cannot be completely removed by washing as the virus adheres to the bovine zona pellucida. Pretreatment of embryos with trypsin did not remove VSV from washed embryos.

There is a risk of transmitting VS as a result of handling infected equipment. Although sunlight and disinfectants readily inactivate VSV, the procedures in embryo collection and processing are highly favourable for survival of the virus. Thus the virus is highly biohazardous and risk management measures are justifiable to ensure that the embryos, embryo straws, and the transport containers are not contaminated with VS when importing bovine embryos from VSV affected areas.

**Risk management options and recommended measures**

Risk management options and the recommended measures that may be considered are the same as those considered for bovine semen in Part 1 of this IRA. Thus it is proposed that the following risk management measure be adopted:

*VS had not been diagnosed within 15 km of the premises where the donors were kept during 30 days before the start of, and during, embryo collection.*
**BLUETONGUE VIRUS**

Bluetongue virus (BTV) is rapidly cytopathic to embryonic germplasm not protected by the zona pellucida. However cows susceptible to BTV can be implanted with zona pellucida intact embryos collected from BTV infected cows, both viraemic and non-viraemic, produce serologically antibody negative calves and remain seronegative throughout. This confirms that the zona pellucida of embryos provide good protection against BTV.

Bluetongue is an IETS Category 1 disease, that is, a disease for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer.

Australia has imported bovine embryos without any bluetongue testing requirements from USA for several years now without any reports of bluetongue transmission.

**Risk management options and recommended measures**

The washing of embryos has proven to be an effective risk management measure. As embryo washing is already a prerequisite for all importation of bovine embryos into Australia, no additional risk management measure is proposed.

**LEPTOSPIRA SPP**

Heifers experimentally infected with *Leptospira borgpetersenii sv hardjobovis* via uterine, cervical, supraconjunctival or intranasal routes can develop infection in the reproductive tract and Leptospira can be identified in the oviductal and uterine fluids by microscopy. The polymerase chain reaction (PCR) assay can detect leptospiral DNA on embryos. None of the recipients of embryos from infected heifers developed leptospirosis.

The washing procedure is ineffective for the removal of *L. borgpetersenii sv hardjobovis* from embryos.

**Risk management options and recommended measures**

The risk of infected embryos transmitting leptospirosis to recipients is low. However there are a number of serovars of leptospires isolated overseas and not reported in Australia. Risk management is justifiable to minimise the public health risk of introducing exotic serovars. Antibiotic cocktails mixed with media are used to reduce bacterial contamination and the consequent risk of disease transmission. It is proposed that antibiotics be added to the embryo media during processing as recommended in the *Code Article 4.2.3.1.5.a.*

**RABIES VIRUS**

Rabies virus antigen has been demonstrated in one of six embryos, the uterus and ovaries of a female skunk by immunofluorescence test and transplacental transmission of rabies has been reported. It is likely that
the rabies virus may be found in the uterine flush of infected cows. However it is not known whether the virus demonstrates adherence to the zona pellucida or whether it can be effectively removed by washing the collected embryos. It is presumed that there is a risk of transmission of rabies via bovine embryos from infected donor cows and risk management measures are justifiable where rabies in cattle are commonly reported.

**Risk management options and recommended measures**

Rabies from vampire bat bites is commonly reported in several South American countries. Because of the lack of knowledge on the risk of transmission of rabies with bovine embryos, risk management should aim at ensuring that the donor animals were in a rabies free environment long enough to give the rabies virus adequate opportunity to manifest clinically.

Risk management considerations for bovine embryos are similar to those considered for bovine semen in Part 1 of this IRA. The following certification would reduce the probability of importing rabies virus in bovine semen from Argentina and Brazil:

*The donor animal showed no clinical signs of rabies during, and for 15 days after, embryo collection.*

Risk management is not necessary for other forms of rabies.

**MYCOBACTERIUM PARATUBERCULOSIS**

*M paratuberculosis* can be cultured from the uterine flush of a cow with clinical tuberculosis and washing cannot remove *M paratuberculosis* from all infected embryos.\(^{11}\) Although short-term uterine infection can occur as a result of experimental inoculation of the uterus with the bacterium, there was no evidence of infection 4 weeks later.\(^{12}\) Foetal infection can occur in cows with or without clinical JD and is most likely the result of transplacental infection.\(^{13}\) Evidence indicates that small numbers of *M paratuberculosis* sometimes found with infected embryos would most likely be destroyed in utero rather than lead to systemic infection.

**Risk management options and recommended measures**

It is unlikely that *M paratuberculosis* can establish and spread if introduced with infected embryos. Furthermore, despite the official JD control programs now in place in Australia, there are no restrictions on embryo from infected donors. Risk management is not necessary.

**BRUCELLA ABORTUS**

As *Brucella abortus* (Br) can localise in uterine tissues, it is likely that the bacteria can be collected with the uterine flushes containing embryos. However, *washing* of embryos is effective in removing the bacteria from embryos with intact zona pellucida but this procedure was not very effective with embryos with damaged zona pellucida.\(^{14}\) Generally 6 washes is adequate to completely remove the bacteria from embryos.\(^{15}\)
Bovine brucellosis is an IETS Category 1 disease, that is, a disease for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer.

For several years there has been international trade in bovine embryos from countries with enzootic Br without any bovine brucellosis testing requirements and without any reports of transmission.

Risk management options and recommended measures
The washing of embryos has proven to be an acceptable risk management measure. As embryo washing is already a prerequisite for all importation of bovine embryos into Australia, no additional risk management measure is proposed.

MYCOBACTERIUM BOVIS

Genital tuberculosis (Tb) can occur in cows. Thus it is likely that M bovis can be found in uterine flushes containing embryos collected from infected donor cows. The Research Subcommittee of IETS Import/Export Committee has not yet determined the risks of transmission of M bovis. Washing of embryos did not always result in the removal of another Mycobacterium species, M paratuberculosis, following in vitro exposure. Hence it is presumed that there is a risk of transmission of Tb despite washing bovine embryos and risk management measures are justifiable when importing bovine embryos from Tb infected areas.

Risk management options and recommended measures
Risk management options are as discussed in Part 1: Bovine Semen in this IRA. However the management of donors differ from the management of bulls in that donor cows do not have to be kept at permanent collection centres and embryos may be collected on farm using mobile processing laboratories. Risk management options need to be adapted to the varying circumstances to which donor cows may be subject when being prepared for embryo collection.

Proposed risk management measures are:

Donors must

EITHER

• be kept in a country or part of the territory of a country officially free from bovine tuberculosis (Code Article 3.2.3.1);

OR

• be kept in a herd officially free from bovine tuberculosis (Code Article 3.2.3.1) and undergo a tuberculin test for Tb within 30 days after the end of embryo collection but prior to the export of the embryos;

OR

• be isolated from the herd at least three months prior to embryo collection and be held in isolation with no contact with other cattle until embryo collection is completed, and undergo a minimum of three tuberculin tests for Tb, each test being a minimum of 90 days apart, with negative results
  - at the start of isolation, at the end of isolation and
  - after the end of embryo collection but prior to the export of the embryos.
The purpose of the Tb test after the end of embryo collection is to give the cows a final check for Tb in case they were incubating the disease during isolation and embryo collection.
BOVINE LEUKEMIA VIRUS

Iatrogenic horizontal transmission, through procedures permitting the transfer of blood between cattle, has been shown to be the major route of transmission in most situations. Vertical transmission accounts for only a small proportion of total transmission.\(^{17}\) In utero or periparturient transmission were more likely in calves born to cows with high lymphocyte count during pregnancy or to cows with malignant lymphomas.\(^{18}\) BLV can occur in sediments of uterine flush fluid and this is most likely due to leucocytes being present in the lumen or from blood seeping from inapparent vessel damage during flushing. BLV could not be detected in eggs and embryos from infected donor cows.\(^{19}\) Prenatal transmission most likely occurs transplacentally after the third month of gestation, rather than through germinal cells.\(^{20}\) Studies have shown that BLV infection was not transmitted by embryos either to the recipients or to the calves.\(^{21}\)

Risk management options and recommended measures

As the risk of BLV transmission from infected donors to recipients or to the calves is negligible, risk management measures are not necessary.

PASTEURELLA MULTOCIDA (SEROTYPES B:2 and E:2)

The bacteria, *P. multocida* Serotypes B:2 and E:2, can be found in a range of tissues in infected animals. It is likely that it may be found in uterine flushes containing the embryos collected from donor cows. However it is not known whether the embryo washing procedure will effectively remove all bacteria. Risk management measures are justifiable to ensure that imported embryos are not infected with this bacterium.

Risk management options and recommended measures

Risk management options and measures are similar to those discussed in Part 1: Bovine Semen of this IRA. It is proposed that the importation of bovine semen be permitted only from countries free from HS (Code Article 3.2.12.2.).

BOVINE HERPESVIRUS-1

Bovine herpesvirus (BHV-1) can be recovered from ovarian oocytes, follicular fluid, granulosa cells, corpora lutea, and uterine tubal fluids of infected cows. The virus demonstrates strong adherence to the external layer of the zona pellucida of bovine embryos and cannot be removed by washing unless the trypsin treatment is performed. Susceptible cows, implanted with trypsin treated embryos from experimentally infected donors, and their calves, all remained seronegative for antibodies to BHV-1.\(^{22}\) Over 1000 untested and unwashed embryos were exported to France from USA during the early 1980’s, with the majority of embryos suspected to have originated from BHV-1 seropositive donors. No recipient cows seroconverted to BHV-1 as a result of the embryo transfer.

Risk management options and recommended measures

The trypsin treatment of embryos has proven to be an effective risk management measure for minimising the risk of importing exotic strains of BHV-1 with bovine embryos. It is proposed that
the embryos undergo trypsin treatment prior to freezing and export.

BOVINE SPONGIFORM ENCEPHALOPATHY

Embryos and uterine flush washes collected from cows with bovine spongiform encephalopathy (BSE) and bioassayed in susceptible mice have not been shown to be infective. Furthermore, BSE has not been detected in any of the calves derived from embryos collected from BSE confirmed cows and transferred to 347 heifers imported from New Zealand as calves. Because of the highly complex and unconventional nature of BSE, and the uncertain nature of the agent causing the disease, the probability of BSE infecting oocytes in the embryo cannot be dismissed as yet.

Risk management options and recommended measures

Risk management is justifiable in light of the uncertainty arising from the reported low level of maternal transmission of BSE and the reported transmission of scrapie in ovine embryos.

The Code Chapter on BSE is under review. AQIS has prepared a policy titled “Animal quarantine policy on bovine spongiform encephalopathy (BSE)” which includes risk management measures for importing bovine embryos.

The measures are:

Bovine embryos may be imported from:

1. **BSE free countries or zones** if it can be certified that the female donors have lived only in **BSE free countries or zones**.

2. **BSE provisionally free countries or zones** provided that:
   i) affected animals and, for females, their last progeny born within 2 years prior to or after the onset of clinical symptoms, were slaughtered and completely destroyed, and
   ii) the feeding of ruminant-derived *meat meal* to ruminants is banned, and
   iii) the embryos for export are derived from females which:
      • are permanently identified enabling them to be traced back to the dam and herd of origin;
      • are not the progeny of BSE suspect or confirmed females;
      • were not suspected of being affected with BSE at the time of embryo collection; and
   iv) the embryos were collected, processed and stored strictly in accordance with *Code* (Appendix 4.2.3.1.).

3. **Countries or zones** with a *low incidence of BSE* provided that:
   i) affected animals and, for females, their last progeny born within 2 years prior to or after the onset of clinical symptoms, were slaughtered and completely destroyed, and
   ii) the feeding of ruminant-derived *meat meal* to ruminants is banned, and
   iii) embryos for export were derived from females which:
      • are permanently identified enabling them to be traced back to the dam and herd of origin;
      • are not affected by BSE;
• are not the daughters of BSE affected females;
• were not suspected of being affected with BSE at the time of embryo collection; and either
  were born and remained in herds in which no case of BSE was confirmed during the preceding 7 years
  or
  were born after the ban on feeding ruminant-derived meat meal to ruminants.
iv) the embryos were collected, processed and stored in accordance Code (Appendix 4.2.3.1.).

4 Countries or zones with a high incidence of BSE provided that:
i) affected animals and, for females, their last progeny born within 2 years prior to or after the
  onset of clinical symptoms, were slaughtered and completely destroyed, and
ii) the feeding of ruminant-derived meat meal to ruminants is banned, and
iii) embryos for export were derived from females which:
  • are permanently identified enabling them to be traced back to the dam and herd of origin;
  • are not the progeny of BSE affected females;
  • are not affected with BSE;
  • were not suspected of being affected with BSE at the time of embryo collection; and either
    were born after the ban on feeding ruminant-derived meat meal to ruminants.
  or
  • have never been fed ruminant-derived meat meal and were born and remained in herds
    in which no case of BSE was confirmed during the preceding 7 years and which
    contains only cattle born on the farm or coming from a herd of equal status;
iv) the embryos were collected, processed and stored strictly in accordance with Code
  (Appendix 4.2.3.1.).

BOVINE PESTIVIRUS

Bovine pestivirus or bovine viral diarrhoea virus (BVDV), the cause of bovine viral diarrhoea and mucosal
disease, can be isolated from ovarian tissues, granulosa cells and uterine tubal epithelial cells of persistently
infected (PI) cows within a few days of infection. BVDV can be found at high levels in the reproductive
system in early infection yet be undetectable in the blood of some animals, and can occur in follicular fluid at
higher concentrations than in serum throughout the infection. BVDV can adversely affect the number of
embryos produced after superovulation and tends to interfere with fertilisation rather than cause
embryonic mortality. A small number of calves derived from embryo transfer have been found to be
persistently infected despite BVDV not being detected in any of the recipient cattle. Although there were
several theories on how this occurred, contamination during embryo transfer was considered to be a likely
factor. However normal calves free from BVDV were derived from embryos collected from PI heifers and
transferred to immune heifers. These calves later developed antibodies to BVDV.

The washing of embryos collected from PI cows effectively removed the virus and no virus could be
detected in the 10th wash after being detected in the flushing medium initially. The presence of BVDV2
in the uterine flush medium from one embryo can be enough for disease transmission if given by intravenous
inoculation to seronegative cows. It is not certain whether this can occur after normal embryo transfer.
In vitro studies suggest that BVDV2 behaves differently from BVDV1 and this may affect the efficacy of washing procedures.

**Risk management options and recommended measures**

BVDV is not an IETS Category 1 disease and risk management measures additional to the embryo washing procedure are justifiable.

Risk management options are limited to detecting donor cows, which are not persistently or transiently infected. Thus it is proposed that donor cows give a negative result to a virus isolation test (cell culture with immunoperoxidase test, antigen capture ELISA, or nucleic acid detection test) before embryo collection.

**ENZOOTIC HAEMORRHAGIC DISEASE**

There are similarities in the viral type and epidemiology between enzootic haemorrhagic disease (EHD) and bluetongue (BT). The epidemiological features of BT can be applied to EHD.

As bluetongue is an IETS Category 1 disease, that is, a disease for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer, it is proposed that EHD also be considered as a Category 1 disease.

The washing of embryos has proven to be an effective risk management measure, especially for the importation of embryos from USA where EHD occurs. As embryo washing is already a prerequisite for all importation of bovine embryos into Australia, no additional risk management measure is proposed.
ATTACHMENT 1

OIE International Animal Health Code - country freedom conditions.

Article 2.1.4.2  RINDERPEST

Rinderpest: free country

A country may be considered free from rinderpest when it has been shown that rinderpest has not been present for at least the past three years.
This period shall be six months after the occurrence of the last case for countries in which a stamping-out policy is practised, with or without vaccination against rinderpest.

Article 2.1.6.2  CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP)

CBPP: free country

A country may be considered free from CBPP when it has been shown that CBPP is not present and that one year has elapsed after the occurrence of the last case for countries in which a stamping-out policy is practised.

Article 2.1.7.2.  LUMPY SKIN DISEASE (LSD)

LSD: free country

A country may be considered free from LSD when:
1) LSD is notifiable in the country;
2) no case of LSD has been confirmed for at least the past three years.

Article 2.1.8.2  RIFT VALLEY FEVER (RVF)

RVF: free country

A country may be considered free from RVF when RVF is compulsorily notifiable, when no case, either clinical or serological, has been confirmed for the past three years and when the country has not imported any susceptible animals from a country considered infected with RVF during this period.

Article 3.2.13.2.  BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

BSE: free country

Countries may be considered free of BSE if:
1) there has been no clinical case of BSE, the disease is notifiable, and an effective and continuous surveillance and monitoring system is practised; or
2) all cases of BSE have been clearly demonstrated to originate directly from the importation of live cattle from countries where BSE has been reported, provided that the disease is made notifiable and suspect animals are slaughtered, investigated and, if disease is confirmed, completely destroyed and an effective and continuous surveillance and monitoring system is practised.

Article 3.2.12.2.  HAEMORRHAGIC SEPTICAEMIA

HS: free country

A country may be considered free from HS when:
1) the disease is compulsorily notifiable in the country;
2) no case of HS has occurred during the past three years.
This period shall be six months after the occurrence of the last case for countries in which a stamping-out policy is practised, with or without vaccination against HS.
Foot and mouth Disease

Article 2.1.1.12.

When importing from FMD free countries or zones (where vaccination either is or is not practised), Veterinary Administrations should require:

for frozen embryos/ova of cattle

the presentation of an international animal health certificate attesting that:

1) the donor females:
   a) showed no clinical signs of FMD at the time of collection and for the following 30 days;
   b) were kept in such a country or zone free from FMD since birth or for at least the past three months prior to collection;

2) the embryos/ova were collected, processed and stored strictly in accordance with Appendices 4.2.3.1., 4.2.3.4. or 4.2.3.5. as relevant.

Article 2.1.1.13.

When importing from FMD infected countries or zones, Veterinary Administrations should require:

for embryos/ova of cattle

the presentation of an international animal health certificate attesting that:

1) the donor females:
   a) showed no clinical signs of FMD at the time of collection;
   b) were kept in an establishment where no animals had been added in the 30 days before collection, and that FMD has not occurred within ten km for the 30 days before and after collection;

2) the embryos/ova were collected, processed and stored strictly in accordance with Appendix 4.2.3.1.

Bluetongue

Article 2.1.9.10.

Veterinary Administrations should require:

for bovine embryos/ova

the presentation of an international animal health certificate attesting that the embryos/ova were collected, processed and stored strictly in accordance with Appendices 4.2.3.1., 4.2.3.4. or 4.2.3.5. as relevant.

Brucella abortus

Article 3.2.1.5.(under study)

Veterinary Administrations of importing countries should require:

for embryos/ova

the presentation of an international animal health certificate attesting that:

1) when the embryos/ova come from a collection unit, the testing programme includes the buffered Brucella antigen and complement fixation tests;

2) when the embryos/ova do not come from a collection unit, the donor females:
   a) were kept in a country or zone free from bovine brucellosis; or
   b) were kept in a herd officially free from bovine brucellosis, showed no clinical sign of bovine brucellosis on the day of collection and were subjected to a buffered Brucella antigen test with negative results during the 30 days prior to collection; or
   c) were kept in a herd free from bovine brucellosis, showed no clinical sign of bovine brucellosis on the day of collection and were subjected to the buffered Brucella antigen and complement fixation tests with negative results during the 30 days prior to collection;

3) the embryos/ova were collected, processed and stored strictly in accordance with Appendices 4.2.3.1., 4.2.3.4. or 4.2.3.5. as relevant.

Bovine tuberculosis

Article 3.2.3.8.(under study)

Veterinary Administrations of importing countries should require:

for embryos/ova

the presentation of an international animal health certificate attesting that the donor females:

1) and all other susceptible animals in the herd of origin showed no clinical sign of bovine tuberculosis during the 24 hours prior to departure to the collection unit;

2) were kept in a herd officially free from bovine tuberculosis;
3) were isolated in the establishment of origin for the 30 days prior to departure to the collection unit and were subjected to a tuberculin test for bovine tuberculosis with negative results.

**Infectious bovine rhinotracheitis**

Article 3.2.5.8. **Veterinary Administrations** of importing countries should require: for embryos/ova the presentation of an international animal health certificate attesting that the embryos/ova were collected, processed and stored strictly in accordance with Appendices 4.2.3.1., 4.2.3.4. or 4.2.3.5. as relevant.

**Bovine Spongiform Encephalopathy**

Article 3.2.13.11. When importing from a BSE provisionally free country or zone, **Veterinary Administrations** should require: for bovine embryos/ova the presentation of an international animal health certificate attesting that:

1) the feeding of ruminants with meat-and-bone meal and greaves derived from ruminants has been banned and the ban has been effectively enforced;
2) if at least one indigenous BSE case has been reported in the country or zone:
   a) the affected cattle as well as, if these are females, their last progeny born within 2 years prior to, or after, clinical onset of the disease, if alive in the country or zone, are slaughtered and completely destroyed;
   b) embryos/ova destined for export are derived from females which:
      i) are identified by a permanent identification system enabling them to be traced back to the dam and herd of origin;
      ii) are not the progeny of BSE suspect or confirmed females; and
      iii) were not suspected of being affected by BSE at the time of embryo collection;
3) the embryos/ova were collected, processed and stored in conformity with the provisions of Appendix 4.2.3.1.

Article 3.2.13.12. When importing from a country or zone with a low incidence of BSE, **Veterinary Administrations** should require: for bovine embryos/ova the presentation of an international animal health certificate attesting that:

1) the feeding of ruminants with meat-and-bone meal and greaves derived from ruminants has been banned and the ban has been effectively enforced;
2) the affected cattle, as well as, if these are females, their last progeny born within 2 years prior to, or after, clinical onset of the disease, if alive in the country or zone, are slaughtered and completely destroyed;
3) embryos/ova destined for export are derived from females which:
   a) are identified by a permanent identification system enabling them to be traced back to the dam and herd of origin, and are not the progeny of BSE affected females;
   b) are not affected with BSE;
   c) were not suspected of being affected of BSE at the time of embryo collection; and
   d) either were born after the date from which the ban on the feeding of ruminants with meat-and-bone meal and greaves derived from ruminants was effectively enforced; or
   e) were born, raised and had remained in herds in which no case of BSE had been confirmed for at least 7 years;
4) the embryos/ova were collected, processed and stored in conformity with the provisions of Appendix 4.2.3.1.

Article 3.2.13.13. When importing from a country or zone with a high incidence of BSE, **Veterinary Administrations** should require: for bovine embryos/ova the presentation of an international animal health certificate attesting that:

1) the feeding of ruminants with meat-and-bone meal and greaves derived from ruminants has been banned and the ban has been effectively enforced;
2) the affected cattle, as well as, if these are females, their last progeny born within 2 years prior to, or after, clinical onset of the disease, if alive in the country or zone, are slaughtered and completely destroyed;
3) embryos/ova destined for export are derived from females which:
   a) are identified by a permanent identification system enabling them to be traced back to the dam and herd of origin, and are not the progeny of BSE affected females;
   b) are not affected with BSE;
   c) were not suspected of being affected by BSE at the time of embryo collection; and
d) either were born after the date from which the ban on the feeding of ruminants with *meat-and-bone meal* and greaves derived from ruminants was effectively enforced; or
e) have never been fed ruminant *meat-and-bone meal* or greaves and were born, raised and had remained in herds in which no case of BSE had been confirmed for at least 7 years, and which contain only cattle born on the farm or coming from a herd of equal status;
4) the embryos/ova were collected, processed and stored in conformity with the provisions of Appendix 4.2.3.1.
ATTACHMENT 2

4.2.3. COLLECTION AND PROCESSING

APPENDIX 4.2.3.1. BOVINE EMBRYOS/OVA

A. AIMS OF CONTROL

The purpose of official sanitary control of embryos/ova intended for movement internationally, is to ensure that specific pathogenic organisms which could be associated with embryos/ova are controlled, and the risk of infection being transmitted to recipient animals and progeny is reduced to an acceptable level.

The disease situation between exporting and importing countries may be similar or dissimilar, and national prophylactic programmes can vary widely, as can vaccination and testing requirements. Thus, exporting and importing countries may have different conditions for the approval of embryo collection teams and associated processing laboratories. For these and other reasons, the Appendix covers the main sanitary conditions under which embryos/ova may be collected, processed and transported.

B. GENERAL CONDITIONS

The Veterinary Administration should ensure that the general conditions relating to animal health set out in the following paragraphs are followed for the international movement of embryos/ova.

1. **Embryo collection team**

Embryo collection team means a group of competent technicians including at least one veterinarian to perform the collection, processing and storage of embryos. The following conditions should apply:

   a) This team should be supervised by a team veterinarian.
   b) The team veterinarian is responsible for all team operations which include sanitary handling and surgery of donors and disinfection and hygienic procedures.
   c) The team veterinarian should be specifically approved for this purpose by an Official Veterinarian.
   d) 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.
   e) The collection team must have adequate facilities and equipment for:
      . collecting embryos;
      . processing and treatment of embryos at a permanent site or mobile laboratory;
      . storing embryos.
   f) As these facilities are not necessarily at the same location, the collection team must keep a record of its activities which must be maintained for inspection by the approving authority for a period of two years after the embryos have been exported.
   g) The collection team should be subjected to regular inspection to ensure compliance with sanitary collection, processing and storage of embryos.
   h) The collection team must not operate in an infected zone unless the disease in question has been listed by the International Embryo Transfer Society (IETS) in category one*.

2. **Processing laboratories**

The processing laboratory may be mobile or permanent. It is a premises in which embryos/ova are recovered from collection media, examined, washed and subjected to any required treatments before freezing, storage and quarantine pending results of health control tests.

A permanent laboratory may be part of a specifically designed collection and processing unit, or a suitably adapted part of an existing building. It may be on the premises where the herd of donor animals is kept. In either case, the laboratory should be physically separated from animals. Both mobile and permanent laboratories should have a clear separation between dirty areas (animal handling) and the clean processing area.

   a) The laboratory should be under the direct supervision of the team veterinarian and regularly inspected by an Official Veterinarian.
   b) While embryos/ova for export are being handled prior to storage in ampoules/straws, no embryos/ova of a lesser health status should be processed.
   c) The laboratory should be protected against rodents and insects.
   d) 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.
e) The laboratory must not operate in an infected zone unless the disease in question has been listed by the IETS in category one.

3. **Donor animals**
   a) At the time of collection, donor animals should be clinically inspected by the team veterinarian and confirmed to be free of contagious and infectious disease transmissible to cattle.
   b) The herd of origin must be free of clinical signs of foot and mouth disease, rinderpest and contagious bovine pleuropneumonia and must not be situated in an infected zone for 30 days before and after collection, unless the disease in question has been listed by the IETS in category one.
   c) The Veterinary Administration should have knowledge of, and authority over, the herd of origin of the donor animals.
   d) The donor animals should not have been imported from another country during the previous 60 days and should have been in the herd of origin for at least 30 days.
   e) The owner of donor animals should sign a statement that to the best of his knowledge the donors are free of any known genetic defects and not associated with such defects in close relatives.

4. **Testing of donor animals and embryos/ova**
   There are two main approaches to ensuring embryos/ova are free of pathogenic organisms. The traditional method is based on the testing of donor animals over extended periods of time and is applied only to diseases not listed in category one and determined on the basis of their pathogenesis to pose more than a negligible risk of transmission by embryos. The checking of paired sera and the reapplication of other tests may be required after normal incubation periods to determine the health status of donors. The other method is based on recent well-documented work which identifies the high measure of freedom from specified bacteria and viruses of embryos/ova which have been processed in accordance with the IETS Manual**. It obviates long periods of isolation and repeated testing of donor animals and some of the inequities of testing of serum samples to determine disease status, provided they satisfy the basic criteria set out in paragraph 3 of this Appendix.

   a) **Traditional method**
      i) Testing of live donors by bilateral agreement:
         The holding of frozen embryos/ova in flasks of liquid nitrogen for periods which cover the normal incubation period of those diseases of concern to an importing country provides the opportunity to test sera at/or prior to and after collection from donor animals.
      ii) Semen used to inseminate donor animals artificially or fertilise ova should meet the health requirements and standards set out in Appendices 4.2.1.1. and 4.2.1.2.
         When frozen semen used to inseminate donor animals was collected from bulls no longer living and when the health status of the bulls concerning a particular infectious disease or diseases was not known at the time of collection, additional tests may be required of the inseminated donor female after ova/embryo collection to verify that these infectious diseases were not transmitted. An alternative may be to subject the semen to testing.
         Where natural service or fresh semen is used, sires should meet the same health requirements as donor females.

   b) **New method: processing of embryos/ova**
      The zona pellucida of each embryo/ovum must be examined over its entire surface area at not less than 50X magnification and certified to be intact and free of adherent material. The embryos/ova must be washed according to the IETS Manual and have intact zona pellucida before and after washing. Only embryos/ova from the same donor should be washed together. All shipments of embryos/ova must be accompanied by a statement signed by the team veterinarian in charge of the processing laboratory certifying that these examinations have been completed.

5. **Collection and storage of embryos/ova**
   a) **Media**
      Any biological product of animal origin used in the collection, processing, washing or storage should be free of living micro-organisms. Media and solutions used in the collection, freezing and storage of embryos/ova should be sterilised by approved methods according to the IETS Manual and handled in such a manner as to ensure that sterility is maintained. Antibiotics should be added to collection, washing and storage media according to the IETS Manual.
b) **Equipment**

All equipment used to collect, handle, wash, freeze and store embryos/ova should be sterilised prior to use according to the IETS Manual.

6. **Optional tests and treatment**

   a) The examination of the embryos/ova, collection or washing fluids can be requested by an importing country. Tests may be carried out on these fluids to confirm that the degree of quality control of the collection team is at an acceptable risk level:

   i) **Embryos/ova**

   Where the viable embryos are intended for export, all non-fertilised ova and degenerating embryos collected from a donor should be washed according to the IETS Manual and pooled for possible testing.

   ii) **Collection fluids**

   The collection fluid should be placed in a sterile container, such as a measuring cylinder, and allowed to stand undisturbed for one hour.

   The supernatant fluid should then be removed and the bottom 100 ml, along with accumulated debris, decanted into a sterile bottle. If a filter is used in the collection of embryos/ova, then any debris that is retained on the filter must be rinsed into the 100 ml of retained fluid.

   iii) **Washing fluids**

   The last four washes of the embryos/ova (washes 7, 8, 9 and 10) should be pooled (IETS Manual).

   iv) **Samples**

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

   b) Treatment of the embryos/ova with the enzyme trypsin may be required. This treatment should be carried out according to the IETS Manual.

   c) Only embryos/ova from one donor should be processed simultaneously.

7. **Storage, quarantine and transport**

   a) The embryos/ova should be stored in sterile ampoules/straws in sterilised liquid nitrogen containers under strict hygienic conditions at a storage place, approved by the Veterinary Administration of the exporting country, where no risk of contamination of the embryos/ova can occur.

   b) Only embryos/ova from the same donor should be stored together in the same ampoule/straw.

   c) Ampoules/straws must be sealed at the time of freezing and should be labelled according to the IETS Manual. The liquid nitrogen container should be sealed prior to shipment.

   d) Embryos/ova should be frozen in fresh alcohol or liquid nitrogen and stored in fresh liquid nitrogen in sterilised flasks.

   e) Stored embryos/ova must not be exported until health certification is completed.

* Based on available research and field information the IETS has placed the following diseases/disease agents of cattle in category one: enzootic bovine leukosis, foot and mouth disease, bluetongue, *Brucella abortus*, infectious bovine rhinotracheitis, (trypsin treatment required). This indicates that sufficient evidence has occurred to show that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer.

An up-to-date list of relevant scientific publications is available at OIE Headquarters.

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