



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

**PART 2: BOVINE EMBRYOS**



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# TABLE OF CONTENTS

<b>TABLE OF CONTENTS</b>	<b>ii</b>
<b>EXECUTIVE SUMMARY</b>	<b>1</b>
<b>ABBREVIATIONS AND ACRONYMS</b>	<b>3</b>
<b>GLOSSARY</b>	<b>4</b>
<b>1. INTRODUCTION</b>	<b>5</b>
1.1 Scope of risk analysis	5
1.2 Current quarantine policy and practice	6
<b>2. HAZARD IDENTIFICATION</b>	<b>8</b>
2.1 General	8
2.2 Biological agents identified	9
<b>3. RISK MANAGEMENT AND ASSESSMENT</b>	<b>13</b>
FOOT AND MOUTH DISEASE VIRUS	13
VESICULAR STOMATITIS VIRUS	14
BLUETONGUE VIRUS	15
<i>LEPTOSPIRA</i> SPP	15
RABIES VIRUS	15
<i>MYCOBACTERIUM PARATUBERCULOSIS</i>	16
BRUCELLA ABORTUS	16
MYCOBACTERIUM BOVIS	17
BOVINE LEUKEMIA VIRUS	19
<i>PASTEURELLA MULTOCIDA</i> (SEROTYPES B:2 and E:2)	19
BOVINE HERPESVIRUS-1	19
BOVINE SPONGIFORM ENCEPHALOPATHY	20
BOVINE PESTIVIRUS	21
ENZOOTIC HAEMORRHAGIC DISEASE	22
<b>ATTACHMENT 1</b>	<b>23</b>
OIE International Animal Health Code - country freedom conditions.	23
OIE Animal Health Code - recommended health conditions for bovine embryos.	24
<b>ATTACHMENT 2</b>	<b>27</b>
4.2.3. COLLECTION AND PROCESSING      APPENDIX 4.2.3.1. BOVINE EMBRYOS/OVA	27
<b>REFERENCES</b>	<b>30</b>

## 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

<i>approved embryo collection team</i>	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.
<i>embryo</i>	term used by convention to describe the conceptus from fertilised 1-cell to blastocyst stages.
<i>establishment</i>	means an agricultural establishment in which animals for breeding, rearing or slaughter are raised or kept.
<i>Code</i>	OIE International Animal Health Code 1999.
<i>IETS Manual</i>	Manual of the IETS, 3rd Edition, April 1998. Edited by Stringfellow DA and Seidel SM. Published by: IETS, 1111 North Dunlap Ave, Savoy, Illinois, 61874 USA.
<i>in-vitro</i>	refers to a process or procedure performed outside the body in a test tube or other laboratory apparatus.
<i>in-vivo</i>	refers to a process occurring in a living organism or under natural circumstances.
<i>IVF</i>	<i>in-vitro</i> fertilisation - a culture system in which matured oocytes and capacitated sperm are mixed to achieve conception outside the body.
<i>trypsin treatment</i>	is a treatment additional to <i>washing</i> of embryos that may be necessary for the removal of certain pathogens adhering to the zona pellucida as described in Chapter 6 of the <i>IETS Manual</i> .
<i>washed</i>	embryos that have been subjected to the <i>washing</i> procedure.
<i>washing</i>	the washing of <i>in-vivo</i> derived embryos with intact zona pellucida as described in Chapter 6 of the <i>IETS Manual</i> 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.<sup>1</sup>

**Table 1**

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

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 2**  
Those disease agents considered to be a hazard

Hazard	Susceptible Species	Risk of being found in unwashed embryos derived from infected donors	Risk of being found in washed embryos derived from infected donors	Australia Health Status	Argentine Health Status	Brazil Health Status	Risk Assessment needed ?
OIE List A diseases							
Foot and mouth disease virus	cloven hoofed animals	Probable	Negligible	Not reported Officially free since 1871	Country free from FMD with vaccination	Enzootic with zone free from FMD with vaccination	Yes
Vesicular stomatitis virus	cattle, horses, pigs, and humans	Probable	Probable	Not reported	Last reported 1986	Sporadic	Yes
Rinderpest virus	cattle, pigs, sheep, goats	Probable	Negligible	Not reported Free since 1923	Not reported	Not reported	No
Mycoplasma mycoides subsp mycoides (cattle strain)	cattle	Probable	Probable	Not reported Free since 1967	Not reported	Not reported since 1921	No
Lumpy skin disease virus	cattle	Unknown	Unknown	Not reported	Not reported	Not reported	No
Rift Valley fever virus	multiple species include humans	Probable	Unknown	Not reported	Not reported	Not reported	No
Bluetongue virus	cattle (non-clinical), sheep (clinical)	Probable	Negligible	Enzootic region No virulent strains	Disease suspected but presence not confirmed	Serologic evidence only, no clinical disease	Yes
OIE List B diseases							
Leptospira spp	all vertebrates except birds	Likely	Probable	Enzootic	Enzootic	Enzootic	Yes – public health risks
Coxiella burnettii	mammals, birds, arthropods (mainly ticks)	Unknown	Unknown	Enzootic - no official control programs	Disease suspected but presence not confirmed	Not reported since 1983	No
Rabies virus	all warm blooded animals	Probable	Unknown	Not reported Lyssavirus in bats	Enzootic - outbreaks reported in cattle	Enzootic - outbreaks reported in cattle	Yes
Mycobacterium paratuberculosis	cattle, cattle strain may infect sheep	Probable	Probable	Enzootic in certain regions National control programs	Enzootic	Not reported since 1986 (cattle) and 1993 (sheep and goats)	Yes – all states have regulatory requirements
Brucella abortus	cattle, humans	Likely	Negligible	Not reported Free since 1989	Enzootic	Enzootic	Yes

Hazard	Susceptible Species	Risk of being found in unwashed embryos derived from infected donors	Risk of being found in washed embryos derived from infected donors	Australia Health Status	Argentine Health Status	Brazil Health Status	Risk Assessment needed ?
Campylobacter fetus subsp fetus	cattle	Probable	Negligible	Low sporadic occurrence No official control programs	Enzootic	Enzootic	No - no national control or regulatory program
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 catarrhal fever	cattle (clinical) sheep and wildebeest (nonclinical)	Not reported Not likely	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

<b>Hazard</b>	<b>Susceptible Species</b>	<b>Risk of being found in unwashed embryos derived from infected donors</b>	<b>Risk of being found in washed embryos derived from infected donors</b>	<b>Australia Health Status</b>	<b>Argentine Health Status</b>	<b>Brazil Health Status</b>	<b>Risk Assessment needed ?</b>
Bovine lentivirus	Cattle	Probable	Unknown	Low sporadic occurrence	Sporadic	Sporadic	No

### 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.<sup>2</sup>
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.<sup>3</sup> 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.<sup>4</sup>

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.<sup>5</sup> Pretreatment of embryos with trypsin did not remove VSV from washed embryos.<sup>6</sup>

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.<sup>7</sup> 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.<sup>8</sup>

The *washing* procedure is ineffective for the removal of *L. borgpetersenii* sv *hardjobovis* from embryos.<sup>9</sup>

### ***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.<sup>10</sup> 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.<sup>11</sup> 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.<sup>12</sup> Foetal infection can occur in cows with or without clinical JD and is most likely the result of transplacental infection.<sup>13</sup> 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.<sup>14</sup> Generally 6 washes is adequate to completely remove the bacteria from embryos.<sup>15</sup>

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.<sup>16</sup> 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.<sup>17</sup> *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.<sup>18</sup> 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.<sup>19</sup> Prenatal transmission most likely occurs transplacentally after the third month of gestation, rather than through germinal cells.<sup>20</sup> Studies have shown that BLV infection was not transmitted by embryos either to the recipients or to the calves.<sup>21</sup>

### ***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.<sup>22</sup> 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.<sup>23</sup> 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.<sup>24</sup> 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.<sup>25</sup> 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<sup>26</sup> and tends to interfere with fertilisation rather than cause embryonic mortality.<sup>27</sup> 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.<sup>28</sup> 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.<sup>29</sup>

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.<sup>30</sup> 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.<sup>31</sup> 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.

## OIE Animal Health Code - recommended health conditions for bovine embryos.

### 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;
    - 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

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##### 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 the 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.

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\* 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.

\*\* Manual of the International Embryo Transfer Society, 1990.

## References

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- 1 IETS Embryo Transfer Newsletter, **16**: 4, December 1998.
- 2 Mebus CA; Singh EL (1988) Failure to transmit foot-and-mouth disease via bovine embryo transfer. *Proc. U.S. Animal Health Assoc.*, **92**: 183-185.
- 3 Singh EL; McVicar JW; Hare WCD; Mebus CA (1986) Embryo transfer as a means of controlling the transmission of viral infections. VII. The in vitro exposure of bovine and porcine embryos to foot-and-mouth disease virus. *Theriogenology.*, **26**: 5, 587-593.
- 4 Villar JA; Munar C; Salomone D; Caamano JN; Laporte O; Burry E; Vautier R; Sadir A; Singh EL; Acree JA; Carrillo B (1990) Transfer of bovine embryos free from foot and mouth disease virus. (Transferencia de embriones bovinos libres del virus de la fiebre aftosa.) *Revista de Medicina Veterinaria Buenos Aires.*, **71**: 6, 268-276.
- 5 Lauerma LH; Stringfellow DA; Sparling PH; Kaub LM (1986) In vitro exposure of preimplantation bovine embryos to vesicular stomatitis virus. *J Clinl Microb*, **24**: 3, 380-383.
- 6 Stringfellow DA; Lauerma LH; Thomson MS (1989) Trypsin treatment of bovine ova after in vitro exposure to vesicular stomatitis virus. *Am J Vet Res.*, **50**: 6, 990-992.
- 7 Singh EL; Thomas FC; Hare WCD; Mitchell D; Eaglesome MD; Randall GCB; Betteridge KJ; Dulac GC; Samagh BS; Papp-Vid G (1982) Embryo transfer in disease control. *Theriogenology.*, **17**: 1, 108.
- 8 Bielanski A; Surujballi O; Golsteyn Thomas E; Tanaka E (1998) Sanitary status of oocytes and embryos collected from heifers experimentally exposed to *Leptospira borgpetersenii* serovar hardjobovis. *Anim Reprod Sci* **54**: 2, 65-73.
- 9 Bielanski AB; Surujballi O (1998) *Leptospira borgpetersenii* serovar hardjo type hardjobovis in bovine embryos fertilized in vitro. *Can J Vet Res* **62**: 3, 234-236.
- 10 Howard DR (1980) Studies on the pathogenesis of rabies virus in the striped skunk (*Mephitis mephitis*). *Dissertation Abstracts International.*, **41B**: 5, 1673-1674.
- 11 Rohde RF, Shulaw WP (1990) Isolation of *Mycobacterium paratuberculosis* from the uterine flush fluids of cows with clinical paratuberculosis. *J Am Vet Med Ass* **197**: 11, 1482-1483.
- 12 Merkal RS, Miller JM, Hintz AM, Bryner JH (1982) Intrauterine inoculation of *Mycobacterium paratuberculosis* into guinea pigs and cattle. *Am J Vet Res* **43**: 4, 676-678.
- 13 Sweeney RW; Whitlock RH; Rosenberger AE (1992) *Mycobacterium paratuberculosis* isolated from fetuses of infected cows not manifesting signs of the disease. *Am J Vet Res* **53**: 4, 477-480.
- 14 Stringfellow DA; Wolfe DF; Lauerma LH; Sparling PH (1986) Resistance of preimplantation bovine embryos to infection with *Brucella abortus*. *Am J Vet Res.*, **47**: 9, 1924-1927.
- 15 Stringfellow DA; Scanlan CM; Brown RR; Meadows GB; Gray BW; Young White RR (1984) Culture of bovine embryos after in vitro exposure to *Brucella abortus*. *Theriogenology.*, **21**: 6, 1005-1012.
- 16 Rohde RF; Shulaw WP; Hueston WD; Bech Nielsen S; Haibel GK; Hoffsis GF (1990) Isolation of *Mycobacterium paratuberculosis* from washed bovine ova after in vitro exposure. *Am J Vet Res.*, **51**: 5, 708-710.

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- 17 Hopkins SG; DiGiacomo RF (1997) Natural transmission of bovine leukemia virus in dairy and beef cattle. *Vet Clin North Am Food Anim Pract* **13**: 1, 107-128.
  - 18 Lassauzet ML; Thurmond MC; Johnson WO; Holmberg CA (1991) Factors associated with in utero or periparturient transmission of bovine leukemia virus in calves on a California dairy. *Can J Vet Res* **55**: 3, 264-268.
  - 19 Bouillant AM; Ruckerbauer GM; Eaglesome MD; Samagh BS; Singh EL; Hare WC; Randall GC (1982) Attempts to isolate bovine leukemia and bovine syncytial viruses from blood, uterine flush fluid, unfertilized ova and embryos from infected donor cattle. *Ann Rech Vet*; **12**: 4, 385-395.
  - 20 Piper CE, Ferrer JF, Abt DA, Marshak RR (1979) Postnatal and prenatal transmission of the bovine leukemia virus under natural conditions. *J Natl Cancer Inst* **62**: 1, 165-168.
  - 21 : DiGiacomo RF; McGinnis LK; Studer E; Evermann JF (1990) Failure of embryo transfer to transmit BLV in a dairy herd. *Vet Rec* 127, 18, 456.
  - 22 Sing EL, Hare WCD, Thomas FC, Eaglesome MD, Bielanski A (1983) Embryo transfer as a means of controlling the transmission of virus infections. IV. Non-transmission of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis virus following trypsin treatment of exposed embryos. *Theriogenology*. **20**: 2, 169-176.
  - 23 Wrathall AE, Brown KFD, Fraser H, Chree A, Ferguson CA. (1997) Embryos and uterine flush fluids from cattle with bovine spongiform encephalopathy are not infective for mice. *Theriogenology* **47**: 384.
  - 24 Bradley R (1994) Embryo transfer and its potential role in control of scrapie and bovine spongiform encephalopathy. *Livestock Prod Sci* **38**: 1, 51-59.
  - 25 Tsuboi T, Imada T (1998) Bovine viral diarrhoea virus replication in bovine follicular epithelial cells derived from persistently infected heifers. *J Vet Med Sci* **60**: 5, 569-572.
  - 26 Kaji M, McGowan MR, Kirkland PD, Jillella D (1997) The effect of bovine pestivirus infection on the superovulatory response of Friesian heifers. *Theriogenology* **48**: 6, 985-996.
  - 27 Grahn TC, Fahning ML, Zemjanis R (1984) Nature of early reproductive failure caused by bovine viral diarrhoea virus. *J Am Vet Med Assoc* **185**: 4, 429-432.
  - 28 Liess B, Frey HR, Grambow H, Stahl C (1987) Embryo transfer and bovine diarrhoea virus infection in cattle. *Deutsche Tierärztliche Wochenschrift* **94**: 9, 506-508.
  - 29 Wentink GH, Aarts T, Mirck MH, van Exsel ACA (1991) Calf from a persistently infected heifer born after embryo transfer with normal immunity to (bovine diarrhoea virus) BVDV. *Vet Rec* **129**: 20, 449-450.
  - 30 Bak A, Callesen H, Meyling A, Greve T (1992) Calves born after embryo transfer from donors persistently infected with BVD virus. *Vet Rec* **131**: 2, 37.
  - 31 Bielanski A, Sapp T, Lutze-Wallace C, (1998) Association of bovine embryos produced by in vitro fertilization with a non-cytopathic strain of bovine viral diarrhoea virus type II. *Theriogenology* **49**: 6, 1231-1238.