

# Embryo transfer: a comparative biosecurity advantage in international movements of germplasm

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## Summary

This paper uses cattle as a model to provide an overview of the hazards involved in the transfer of *in vivo*-derived and *in vitro*-produced embryos. While scientific studies in recent decades have led to the identification of pathogens that may be associated with both *in vivo*- and *in vitro*-derived embryos, those studies have also been the basis of appropriate disease control measures to reduce the risks to a negligible level. These disease control measures have been identified and assessed by the International Embryo Transfer Society's (IETS) Health and Safety Advisory Committee, the expert body that advises the World Organisation for Animal Health (OIE) on matters related to the safety of embryo transfer. Through the OIE's processes for developing and adopting international standards, the disease control measures identified by the IETS have been incorporated into the *Terrestrial Animal Health Code*. The basic principles rely on the crucial ethical roles of the embryo collection team and embryo transfer team, under the leadership of approved veterinarians. Decades of experience, with nearly 10 million embryos transferred, have demonstrated the very significant biosecurity advantage that embryo transfer technology has when moving germplasm internationally, provided that the international standards developed by the IETS and adopted by the OIE are strictly followed.

## Keywords

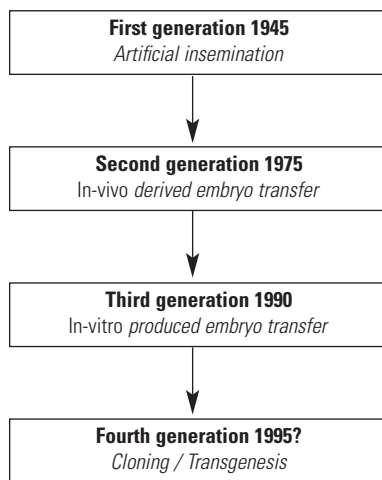
Animal reproductive biotechnology – Bovines – Cattle – Embryo transfer – *In vitro*-produced embryo – *In vivo*-derived embryo – Reproductive technology.

## Introduction

Historically, four generations of animal reproductive biotechnologies (ARBs) have been recognised, as shown in Figure 1 (25). These technologies developed gradually from the mid-1940s with the introduction of artificial insemination (AI). The next generation ARBs appeared 30 years later, when the production of *in vivo*-derived (IVD) embryos allowed the development of the embryo transfer industry. The next step, 15 years later, was the development of *in vitro*-produced (IVP) embryos. The fourth-generation ARBs, such as cloning, are still mostly restricted to experimental purposes or for specific applications, such as producing biopharmaceutical products.

The development of the first three generations of ARBs brought major benefits to the farming industry and enabled increased international exchanges of germplasm, at first through trade in doses of semen and then in straws of embryos. For various technical and economic reasons, these technologies have been used most widely in cattle. However, embryos are transferred, or oocytes collected, fertilised and transferred, in other livestock species, such as small ruminants, swine and horses.

The latest report of the International Embryo Transfer Society (IETS) Data Retrieval Committee (29) shows that, in 2008, 800,000 cattle embryos were transferred worldwide (two-thirds as IVD embryos and one-third as IVP embryos) onto almost all continents and into all regions. These figures have been more or less gradually



**Fig. 1**  
**The various generations of animal reproductive biotechnologies**  
(Thibier, 1990, 25)

increasing over recent years but they have remained in the same order of magnitude for the last ten years. This has meant that, in the first decade of the 21st Century, approximately ten million bovine embryos have been transferred worldwide.

Global statistics are difficult to retrieve. Nevertheless, an attempt was made recently to estimate the magnitude of the international movement of animal semen and embryos (28). It was reported that more than 23 million doses of bovine semen have been exported and approximately 70,000 cattle embryos. In terms of monetary value, Chillaud (Th. Chillaud, personal communication, 2007) estimated that the cattle embryo market is worth about €10 million (US\$14.5 million) or approximately 6% of the market value of semen. Almost half of these embryos originate from the United States (export value, in 2006, around €4.7 million or US\$6.8 million) and a third from the European Union (EU) (mainly France, Germany, Ireland, Italy and the Netherlands). Canada is also a significant exporter of embryos, particularly *in vitro* embryos. The major importing countries are, in decreasing order, the EU (€2 million or US\$2.9 million), East Asia (€1.5 million or US\$2.2 million), Oceania (€0.5 million or US\$0.72 million) and South America (€0.3 million or US\$0.43 million).

As has recently been pointed out (30), while such quantitatively important movements of embryos have occurred worldwide, no major contamination of pathogens associated with the embryos has ever been identified. While there have been significant epidemics of livestock disease in many parts of the world over the last two decades, none of these has been associated with embryo transfer.

Clearly, IVD and IVP embryo transfers have a significant value above their potential to transfer germplasm from one country to another. That is, they provide full biosecurity in such operations. This comparative advantage does not imply that such transfers are without risk. Rather, the safety of the trade depends on the rigorous implementation of appropriate validated disease control measures designed to manage any possible disease risks.

This discussion uses cattle as a model to review potential disease risks and outline the appropriate measures to mitigate such risks, thus giving these ARBs a comparative biosecurity advantage in the international trade in germplasm. In the first part, the author discusses IVD embryos and, in the second, IVP embryos.

## Transfer of *in vivo*-derived embryos

### Risk assessment

To implement appropriate disease control measures that effectively manage the potential risks posed by trade in IVD embryos, it is necessary clearly to identify those risks.

Pathogens may be shed into the genital tract and contaminate embryos, if those pathogens are present at the time of collection or between fertilisation and collection. This was recognised early in the development of embryo-transfer technology and so the veterinarians involved began to investigate the risks and to seek appropriate means to lessen them. For example, as early as 1979, Wrathall and Mengeling published a paper (39) showing, for the first time, the risk of infecting recipients with contaminated pig embryos. There have been many subsequent investigations into the interaction between pathogens and embryos. The IETS keeps a complete set of more than 400 references, which can be consulted on their website ([www.iets.org](http://www.iets.org)), and are known collectively as the IETS Health and Safety Advisory Committee Research Update.

For example, in bovine embryos, 89 pathogens have been investigated. Among them are some of the most serious livestock diseases, such as:

- foot and mouth disease (FMD)
- rinderpest
- bluetongue
- contagious bovine pleuropneumonia.

Diseases that are economically devastating in certain circumstances have also been investigated, such as infectious bovine rhinotracheitis/infectious pustular

vulvovaginitis (IBR/IPV), due to bovine herpesvirus 1 (BHV-1), or bovine viral diarrhoea (BVD). Table I summarises some of the data collated in the third edition of the IETS Manual (22). These data show that, for most diseases, and provided that defined disease control practices (see below) are followed during the process, the risk of transmission of a given disease from donor to recipient via an embryo is negligible. However, in some circumstances, and for a few diseases only, additional measures such as those recommended in the IETS Manual (treatment with trypsin, for example) should be implemented (see below).

**Table I**  
**Summarised results of studies on the interaction between pathogens and embryos where the zona pellucida is intact (22)**

Types of pathogens	Number of embryos exposed <sup>(a)</sup>	Assay of embryos
<i>In vitro</i> contamination and assay of bovine embryos <sup>(b)</sup>		
Viruses	12 to 169	0
Other viruses <sup>(c)</sup>	29 to 144	36% to 100% positive
Bacteria	38 to 96	0% to 26% positive
Mycoplasmas	20 to 111	30% to 100% positive
Assay of embryos from zona pellucida-intact bovine embryos from infected or seropositive donors		
Virus	2 to 372 <sup>(d)</sup>	Negative
<i>Brucella</i>	309	Negative
<i>Chlamydia</i>	5	Negative

a) Range of number of embryos per pathogen studied

b) High concentration exposure mimicking a 'worst-case scenario'

c) Bovine herpesvirus 1, bovine herpesvirus 4 and vesicular stomatitis virus

d) Foot and mouth disease virus-infected donors

In addition to the many viral diseases, bacterial and prion diseases have also been the subject of several major investigations. In the case of prion diseases, the detailed studies of Wrathall *et al.* (41) in cattle and Low *et al.* (13) in sheep in the United Kingdom clearly demonstrate that it is highly unlikely that these diseases will be transmitted by embryo transfer.

The mechanism for the safety of IVD embryo transfers has been reviewed recently by Van Soom *et al.* (34). That review demonstrated the key role of the *zona pellucida*. The same authors (34) also pointed out that, despite considerable research on embryo-pathogen interactions in farm livestock, there is little firm evidence for vertical transmission via the incorporation of the genome of endogenous retroviruses or, indeed, any other infectious agent.

The risks of a pathogen being associated with a given embryo come from a sequence of events that has been

summarised from an epidemiological standpoint by Stringfellow and Givens (21). This sequence includes:

- exposure to the pathogen
- the continued association of the pathogen with the embryo
- the maintenance of pathogen infectivity throughout embryo manipulation and processing
- delivery of an infective dose of the pathogen to a susceptible recipient.

Each step should be the target of disease control measures to ensure the biosecurity provided by this technology.

A consensus was reached on the epidemiological specificity of the embryo, as a proper entity in itself, during debate at a round table meeting organised by the OIE in 1985 (36). This consensus was based on a number of facts, e.g.:

- the embryo is present in the oviduct and uterus for only a very short time before collection, without any vascular link to the dam at these stages
- the embryo can be thoroughly examined under magnification.

Thus, the whole process of manipulating and storing embryos, which could potentially carry some hazards, can be placed under strict control.

Special attention was given recently by Wrathall *et al.* (43) to the risk of embryos being fertilised by pathogen-contaminated semen. They concluded that:

'... for *in vivo*-derived embryos, the risk of transmitting the disease when semen infected with enzootic bovine leukosis virus (EBLV) or bluetongue virus (BTV) is used for AI or natural service of the embryo donors, is negligible... the same is almost certainly true for semen infected with bovine herpesvirus 1 (BHV-1) if the embryos are also treated with trypsin.'

They did, however, express some reservations about BVD virus (BVDV): '...although field studies so far suggest that this is very unlikely'.

Special attention should also be paid to risks associated with materials of animal origin. Any biological product used in the recovery of gametes, sperm and oocytes or embryos, dilution, *in vitro* maturation of oocytes, washing or storage is potentially a source of contamination. This is particularly relevant to transmissible spongiform encephalopathies, as discussed in depth by Wrathall (38) and Wrathall *et al.* (42).

A putative additional source of contamination of semen or embryos which has also been examined is liquid nitrogen

in storage tanks. Bielanski *et al.* (7) demonstrated the presence of microflora, such as *Stenotrophomonas maltophilia*, in liquid nitrogen tanks and showed experimentally that this decreased the motility of semen with which the *S. maltophilia* came into contact. The same authors also suggested that embryos coming into direct contact with contaminated liquid nitrogen could lead to their contamination with viral agents. However, they also showed that all sealed samples of embryos stored in contaminated liquid nitrogen tanks tested negative for the presence of bacteria or viruses. Similarly, Bielanski (2) showed that the vapour phase of liquid nitrogen is a safe means for the short-term storage and transportation of embryos in so-called dry shipper dewars. A recent and comprehensive review on the risks of contaminating germplasm during cryopreservation and cryobanking (3) has been published in the latest edition of the IETS Manual.

In regard to IVD embryos, and with the proviso that the international standards developed by the IETS and adopted by the OIE are complied with (see below), the IETS has categorised diseases into four categories, by risk assessment. Category 1 is: 'that for which sufficient data are available to determine the risks to be negligible provided that the embryos are properly handled between collection and transfer'. As seen in Table II, only eight diseases are listed in this category. Unfortunately, it is not likely that this number will increase in the near future, due to insufficient research in this area.

**Table II**  
**Category 1 diseases, as listed by the International Embryo Transfer Society and the World Organisation for Animal Health (37)**

Disease/Pathogen	Species	Note
Foot and mouth disease	Cattle	
Enzootic bovine leukosis	Cattle	
Bluetongue	Cattle	
<i>Brucella abortus</i>	Cattle	
Infectious bovine rhinotracheitis	Cattle	Trypsin treatment required
Aujeszky's disease (pseudorabies)	Swine	Trypsin treatment required
Bovine spongiform encephalopathy	Cattle	
Scrapie	Sheep	

Scrapie in sheep has recently been added to Category 1, after an examination of research findings by the Research Sub-Committee of the IETS/Health and Safety Advisory Committee, and is now recognised as a Category 1 disease in the *Terrestrial Animal Health Code* of the OIE (the *Terrestrial Code*).

It is clear that, as long as all the procedures recommended by the IETS and adopted by the OIE are well implemented,

any risks posed by IVD embryos are extremely small – certainly much lower than those associated with the movement of live animals, and also lower than the risks associated with semen and most other animal products (34).

## Risk management

The risk management procedures that should be followed to ensure the safety of herds receiving IVD embryos are described in detail in the IETS Manual (20). This manual is a code of good practice that should be included, whenever possible, in any embryo-transfer quality assurance programme.

The first crucial step is the thorough clinical examination of the donor animal and its environment; in particular, ensuring the absence of infectious disease in the herd or surrounding area. However, such assurances are not required for diseases in Category 1 (see above), since the embryo-handling procedures recommended by the IETS have been shown to eliminate any risk posed by pathogens in this category. This may be particularly relevant for animals from endangered breeds, since these animals may not be available from a disease-free environment.

As pointed out by Wrathall *et al.* (43), the safety of the semen used should not be forgotten. It is always beneficial – and, in terms of international trade, compulsory – for semen to be processed in semen collection centres under the supervision of the national Veterinary Services (37).

Basic recommendations for handling embryos are described by Stringfellow (20) in the latest edition of the IETS Manual. These guidelines can be summarised as follows.

The first stage is to ensure appropriate washing, ten times consecutively with a new pipette each time, with immersion of the embryo(s) in each wash for a duration of one minute, with gentle agitation, and with a dilution factor of at least 1/100 between each wash. Convenient and time-efficient methods are now available for this step.

The embryo should be carefully inspected under magnification ( $\times 50$ ) and should only be processed if the embryo has an intact *zona pellucida*, with no adherent debris, because such debris could serve as a source of contamination and allow the pathogen to be 'carried over'. Treating embryos with the enzyme trypsin is often recommended when dealing with 'sticky' pathogens, such as BHV-1. This treatment is not always necessary (32) but is nevertheless a useful procedure and often required for exported embryos. The way in which trypsin is handled is also relevant since, as a protein enzyme, it is sensitive to its environment. Nonetheless, this treatment is by no means a

panacea. Even when used, it should not be considered to replace the need to take disease control precautions with the embryos' environment.

The media in which the embryos are handled should also be considered. Their nature and origin should be selected with great care. Adding antibiotics to the media may also be of value when used appropriately. Antibiotics are particularly useful for preventing the transmission of mycoplasmas, when these are a concern. Wrathall *et al.* (40) point out that prolonged exposure to high levels of antibiotics such as kanamycin or tylosin is needed to ensure the effective removal of mycoplasmas from IVD bovine embryos. Fortunately, such treatment does not seem to affect embryo viability.

Appropriate quality controls for the whole process are necessary and the standard procedure should be for used media to be collected and stored for pathogen testing, whether those pathogens may have originated from the donor or from some serum used in the media. This testing should also examine for saprophytic microflora and will contribute towards verifying the effectiveness of the quality assurance procedures.

These risk management procedures are incorporated into the OIE *Terrestrial Code* (37), which explicitly refers to the guidelines in the IETS Manual. These procedures are also usually included in national regulations for transferring embryos between herds.

Faithfully following these procedures ensures that embryo transfers contribute to improving the animal health status of a given population, by controlling the movement of germplasm between herds. The basic concept behind these regulations is official approval of embryo transfer teams. This approval is a very important component of the veterinary regulations, because such regulations usually focus more on the animals themselves, their confinement and their products. In this case, however, the safety of the embryo-transfer industry relies on the ethical and technical excellence of the person in charge of the embryo transfer team (27).

The criteria used by national Veterinary Services to approve embryo transfer teams are based on the *Terrestrial Code*. Chapter 4.7. states that: 'the embryo collection team is 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) the team should be approved by the Competent Authority;
- b) the team should be supervised by a team veterinarian;

c) the team veterinarian should be responsible for all team operations, including:

- verifying the health status of the donor
- implementing appropriate disease control measures when handling or operating on donors
- disinfection and hygiene procedures;

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 should have adequate facilities and equipment for:

- collecting embryos
- processing and treating embryos at a permanent site or mobile laboratory
- storing embryos;

These facilities need not necessarily be at the same location;

f) the embryo collection team must keep a record of its activities, which should be maintained for inspection by the Veterinary Authority for a period of at least two years after the embryos have been exported;

g) the embryo collection team should be subject to regular inspection at least once a year by an Official Veterinarian, to ensure compliance with the procedures for the appropriate collection, processing and storage of embryos.

Chapter 4.7. also deals with the recommendations for:

- processing laboratories
- the introduction of donor animals
- risk management
- the collection and storage of embryos
- optional tests and treatments
- the storage and transportation of embryos
- the procedure for micromanipulation.

While the OIE standards focus on international trade, it is interesting to note the wording in the *Terrestrial Code* on optional tests:

'The testing of samples can be requested by an importing country to confirm the absence of pathogenic organisms that may be transmitted via *in vivo*-derived embryos, or to help assess whether the degree of quality control of the collection team (with regard to adherence to procedures as described in the IETS Manual) is at an acceptable level. Samples may include:

- non-viable embryos/oocytes
- embryo collection (flushing) fluids



- washing fluids, the last four washes of the embryos/oocytes should be pooled (IETS Manual)
- the samples referred to above should be stored at 4°C and tested within 24 h. If this is not possible, then samples should be stored frozen at –70°C or lower.’

In conclusion, with respect to the biosecurity of IVD embryos, the worldwide system currently in place, founded on the international standards of the OIE, has proven to be effective. It is based on science and integrity in the collection and processing procedures and so provides an immense comparative advantage for this technique when moving germplasm from one herd to another and from one country to another.

## Transfer of *in vitro*-produced embryos

### Risk assessment

When dealing with the interaction between pathogen and embryo, we should never extrapolate from one species to another, or from one pathogen to another, even if they are generically very close (11, 26). Similarly, we should not extrapolate from one mode of production of embryos to another: whether *in vivo*-derived or *in vitro*-produced. Owing to apparent morphological differences in the *zonae pellucidae* of IVD and IVP embryos (35), some pathogens seem to adhere more readily to IVP embryos (23, 33). Such interactions might differ from one pathogen to another, not only between IVD and IVP embryos, but even within IVP embryos. One example of differences between IVD and IVP embryos is in their interaction with FMD virus, as shown in Table III.

**Table III**  
**Differing interactions of foot and mouth disease virus (type 0) with *in vivo*-derived embryos and *in vitro*-produced embryos (26)**

Embryos	<i>In vivo</i> -derived <sup>(a)</sup>	<i>In vitro</i> -produced <sup>(b)</sup>
Number	169	73
Type of virus	O <sub>1</sub>	O <sub>1</sub>
Viral concentration	10 <sup>6</sup> pfu	10 <sup>7</sup> TCID <sub>50</sub> /ml
Time of exposure	4-18 h	4 h
Test	Plaque and inoculation of epithelium	Plaque and polymerase chain reaction
Results	<b>Negative</b>	Virus isolation in all first fluids and cytopathogenically positive from the developed and degenerated embryos

(a) adapted from Singh *et al.* (19)

(b) adapted from Marquant-LeGuénié *et al.* (16)

pfu: plaque-forming units

TCID<sub>50</sub>: median tissue cell infective dose

The subtlety of the association between subtypes of viruses and IVP embryos was illustrated by an experiment performed by Bielanski *et al.* (4), involving two non-cytopathic BVDV biotypes: type 1 (NY-1) and type 2 (PA-131). These two viruses were experimentally added to bovine IVP embryos, which were then treated according to the IETS protocols and transferred to recipients. Some results of this experiment appear in Table IV. The authors concluded that:

‘... a large proportion of recipients that received embryos exposed to BVDV, especially those exposed to a high concentration of type 2 virus, became infected after [embryo transfer] and their pregnancies failed. However term pregnancies resulted in calves free of both virus and antibody.’

**Table IV**  
**Different interactions of subtypes of bovine viral diarrhoea virus with *in vitro*-produced embryos (3)**

Type of non-cytopathic BVDV	NY-1	PA-131
Number of pregnancies/number of transfers	20/33	25/61
Percentage of seroconversions in recipients	0%	51.4%
Number of seroconversions in offspring	0 (18 full-term calves)	0 (only 2 went to full-term and gave birth)
Virus isolation tests on non-transferred embryos (%)	25%	28%

BVDV: bovine viral diarrhoea virus

This study emphasises two points. First, considered as a whole, even in a ‘worst-case scenario’, this procedure is widely accepted as safe because it has a relatively low risk of producing contaminated embryos. Secondly, and in addition, there is indeed a low risk of infecting the recipients, which means that additional biosecurity measures should be taken when donors are infected with BVDV or ovaries are collected from abattoirs.

Although they have been investigated less thoroughly than viruses, bacteria have also been studied. Perry *et al.* (18) recently investigated *Mycobacterium avium* subsp. *paratuberculosis* (MAP). They demonstrated that all 109 IVP embryos obtained from oocytes collected from 12 cows with subclinical Johne’s disease were free of MAP, as was the freezing medium.

Thibier and Guérin (31) outline a sequence of risks involving infectious agents in the process of producing IVP embryos.

These risks are related to:

- the female donor and the mode of collection (abattoir collection or ovum pick-up)

- the maturation process
- fertilisation (the introduction of semen)
- co-culture *in vitro* development
- cryopreservation
- the last step, thawing and transfer.

The first point of interaction is the oocyte itself and its follicular environment (the surrounding cells of the oocytes and the follicular fluid). The amount of risk may be modified according to the source of the ovaries used, which may come either from ovum pick-up, in which case the donor animal's health status may be well defined, or from the abattoir, in which case a different approach to risk management should be taken (see below).

Since contamination of oocytes with two types of virus, BVDV and BHV-1 (IBR/IPV), is not uncommon (Table V), these viruses have been extensively investigated (23). They appear to adhere to the oocyte *zona pellucida* and thus are 'external' to the oocyte. This risk is complicated by the fact that these viruses often result in asymptomatic infection, which is why particular attention should be paid to these pathogens. Perry (17) conducted a quantitative risk analysis of the potential for transmitting BVDV through abattoir-derived IVP embryos. He concluded that, for each oocyte selected for IVP processing, there is a very low probability that the recipient cow might become infected with BVDV if the co-culture cells were derived from donor cows of unknown health status. The probability is even lower ( $1.2 \times 10^{-5}$ ) if the co-culture system used is tested and shown to be free from BVDV.

**Table V**  
**Level of contamination reported in materials of animal origin, sourced from abattoirs, used in multiple *in vitro* fertilisation laboratories**

According to Stringfellow *et al.* (23), and collated from the data of Avery *et al.* (1), Bielanski and Stewart (6), Bielanski *et al.* (8), Galik *et al.* (9) and Marquant-LeGuienne *et al.* (14)

Contaminant	Range of positive samples
Bovine herpesvirus-1	0% to 12%
Bovine viral diarrhoea virus	1% to 12%
Bacteria	13% to 68%

Risks also differ according to the oocyte collection methods used. In the case of abattoir collection, the first problem is to ensure that the females from which the ovaries are collected are free from infectious diseases. This implies that a good system for tracing the origin of the females is required. A second risk with abattoir collection is possible environmental contamination of the collected material.

One way to mitigate such risks is to ensure that, on the day the ovaries are collected, no herd that has been depopulated for disease-control reasons is being slaughtered. In the case of ovum pick-up, the risks are similar to those when collecting IVD embryos, as far as the donor female is concerned. Another source of contamination may come from the equipment used, especially when ova from a series of animals are collected at the same session. Transportation of this material to the laboratory is another potential source of external contamination. A further source of risk from individual animals is the risk from semen. As indicated above, this point has recently been revisited (40) and should not be overlooked.

During the handling and processing of embryos in the laboratory, from collection to transfer, there are many risks of environmental contamination that must be controlled (see below). As discussed in the section on IVD embryos, potential contamination may come from the media being used. Moreover, several types of media may be used during the week-long process, thus increasing the risk. A number of media contain products of animal origin and it is strongly recommended that such products should be replaced by others, such as amino acids of plant origin, wherever possible. Finally, the contamination of co-culture cells is also a potential risk, particularly when these cells are of primary origin. Several investigators have reported contamination of the cells by bacteria or viruses, such as BVDV or BHV-1 (12). The use of controlled cell lines, which have been determined as pathogen-free, is recommended whenever possible. Another risk-mitigation approach is to use a totally synthetic medium, i.e. synthetic oviductal fluid, which also reduces the risk of pathogen contamination.

Bielanski and Lalonde (5) studied the effects of cryopreservation, by conventional, slow, controlled cooling and by vitrification, on the presence of BVDV and BHV-1 infectivity associated with frozen-thawed, day-seven, bovine, IVP-produced embryos. They concluded that cryopreservation reduced the proportion of infected embryos but did not render all of them free from the pathogens.

## Risk management

The IETS has developed recommendations for controlling potential risks associated with IVP embryos (15). These recommendations should be considered by all practitioners as a mandatory code of good practice.

For oocytes being collected *in vivo* (ovum pick-up), the first risk management step is to survey the health status of the herd of origin and surrounding area, as well as the donor herself, to ensure that no infectious diseases are

present at the time of collection. However, when dealing with species or breeds threatened by extinction, it may be that these general conditions cannot be met because priority is given to the conservation of genetic diversity. That is, exceptions can be made, without unduly compromising biosecurity, because this technique is particularly valuable for quality control (see below), which is another comparative advantage of this method.

When ovaries are collected at a slaughterhouse, the establishment should be under official supervision. In addition, it is important to be able to trace the donors back to their herd of origin, to evaluate its health status, and to ensure that the animals do not come from a herd being depopulated for reasons of disease control.

The premises and working areas in which *in vitro* production of embryos takes place should be designed so that individual specialised units, with restricted access, are set aside for particular tasks. Wherever possible, a laminar flow chamber should be installed, with close attention being paid to cleaning and disinfecting (12).

The handling of embryos during the various steps should always be conducted with great care and under the strictest hygiene conditions. As stated above, the semen used should be specific-pathogen-free. Moreover, 'it is desirable to test each lot in IVF before it is used routinely, because some semen with low levels of bacterial contamination has been problematic' (23).

The quality of the media, and of the co-culture cells, when relevant, is one of the most critical points of the procedure. All biological products should be strictly controlled and guaranteed free from micro-organisms. Sera containing antibodies against agents of particular concern should be avoided. It is also strongly advised that researchers know which inactivation procedures have been used by the manufacturers of biological reagents to make them safe.

Adding antibiotics to the media is good practice (10). It contributes to the removal of pathogenic agents or saprophytic micro-organisms, which may have been inadvertently introduced at collection or at the time of fertilisation (12). Unfortunately, although they hold promise, approved antiviral compounds are not yet available for use in embryo production (M.D. Givens, personal communication, 2010).

Finally, the washing procedure recommended for IVD embryos, above, further reduces the likelihood of pathogens being associated with IVP embryos.

One of the major comparative advantages of IVP embryo technology is that the production system provides various control points and sufficient time to allow for the disease control status of each batch of embryos to be monitored. In

addition, the many different media used during the process provide an excellent sampling source, since it has been shown that these media, as an immediate environment of the embryos, serve as good indicators of any pathogens to which they could have been exposed during production (31).

Quality control is particularly important. It starts with the strict recording in the laboratory book of all the events in the process, from the identification of the ovaries to the release of the IVP embryos. In routine operations, and as mentioned above, quality control should include regular sampling of all media used as well as any degenerated embryos, as such testing gives an accurate indicator of the environment to which viable embryos may have been exposed. In some circumstances, Veterinary Services may require such testing before exports of IVP embryos.

As in the case of IVD embryos, these disease control considerations are reflected in the recommendations of the *Terrestrial Code*, which specifically refer to the guidelines published in the IETS Manual. The same issues are also usually included in national regulations. The basic concept of such regulations relies on official approval of embryo production teams. As for IVD embryos, the safety of the industry depends on the ethical and technical excellence of the person in charge of the embryo transfer team.

The criteria used by national Veterinary Services to approve embryo transfer teams are outlined in the *Terrestrial Code* (37). With respect to donor animals, the *Terrestrial Code* (Article 4.8.4.) distinguishes clearly between recovering oocytes from live donors and from batches of ovaries collected at an abattoir. In the latter case, the abattoir should be officially approved and the animals should not have been slaughtered for disease control purposes.

In conclusion, the procedures for IVP embryo production require particular care from the embryo production team because some pathogens have been shown to adhere more readily to the *zona pellucida* of such embryos. However, the whole process, carried out in a well-established laboratory with competent personnel, under the leadership of the team veterinarian, ensures that this third-generation ARB provides, as with IVD embryos, the highest level of biosecurity and thus has a definite comparative advantage when germplasm is traded internationally.

## Conclusion

Some 21 years ago, the author (24) stated that transfer of *in vivo*-derived embryos was the safest means of exchanging genes between herds, areas or continents. The passage of time has proven the accuracy of this



assertion, thanks, in large measure, to the high degree of professionalism and good practice of the embryo-transfer industry which, in turn, has been based on sound research readily adopted by the appropriate regulatory agencies. Recent research, particularly on BVDV, has shown that some viruses can be strongly associated with the *zona pellucida* of those embryos produced by *in vitro* fertilisation and culture. This finding places even greater responsibility on the embryo production team to meet its obligations

under the official approval granted by the veterinary authority. Strict adherence to the recommendations of the IETS, as adopted by the OIE, has shown that this technology too provides the most stringent biosecurity. It is this high degree of biosecurity which, at this time of globalisation and increasing international trade, provides animal reproductive biotechnologies with clear comparative advantages. ■

## Transfert d'embryons : un avantage comparatif en termes de biosécurité pour les échanges internationaux de matériel génétique

M. Thibier

### Résumé

Dans cet article, l'auteur fait le point sur les dangers liés au transfert d'embryons collectés *in vivo* et *in vitro*, en se basant sur l'exemple des transferts d'embryons de bovins. En même temps qu'elles ont identifié les agents pathogènes susceptibles d'affecter les embryons collectés *in vivo* et *in vitro*, les études scientifiques des dernières décennies ont également permis de mettre au point des mesures de lutte appropriées, qui ont pour objet d'atténuer ces risques jusqu'à un niveau de probabilité négligeable. Ces mesures de lutte ont été établies et évaluées par le Comité consultatif Santé et sécurité de la Société internationale de transfert d'embryons (IETS), qui est l'organisme compétent chargé de conseiller l'Organisation mondiale de la santé animale (OIE) en matière de sécurité sanitaire des transferts d'embryons. Dans le cadre de la procédure d'élaboration et d'adoption des normes internationales de l'OIE, les mesures de lutte préconisées par l'IETS ont été intégrées dans le *Code sanitaire pour les animaux terrestres* de l'OIE. Ces normes reposent sur les principes déontologiques encadrant le travail des équipes chargées de la collecte et du transfert des embryons, sous la responsabilité de vétérinaires certifiés. Comme l'a montré l'expérience acquise depuis des dizaines d'années, avec près de dix millions de transferts d'embryons réalisés, la technologie du transfert d'embryons offre un avantage comparatif important en termes de biosécurité des déplacements internationaux de matériel génétique, à condition que les normes internationales élaborées par l'IETS et approuvées par l'OIE soient strictement respectées.

### Mots-clés

Biotechnologie de la reproduction animale – Bovin – Embryon produit *in vitro* – Embryon collecté *in vivo* – Technologie de la reproduction – Transfert d'embryons. ■

# La transferencia de embriones y la ventaja comparativa que en materia de seguridad biológica presentan los movimientos internacionales de germoplasma

M. Thibier

## Resumen

El autor utiliza el ganado bovino como modelo para presentar a grandes rasgos los peligros ligados a la transferencia de embriones generados *in vivo* u obtenidos *in vitro*. Gracias a diversos estudios científicos, en los últimos decenios se han identificado una serie de patógenos que pueden acompañar a los embriones, tanto generados *in vivo* como obtenidos *in vitro*. Sin embargo, esos mismos estudios también han sentado las bases para establecer medidas de control sanitario que permiten reducir esos riesgos a un nivel insignificante. Tales medidas han sido definidas y evaluadas por el Comité Consultivo sobre Salud y Seguridad de la Sociedad Internacional para la Transferencia de Embriones (IETS), que es el órgano de expertos que asesora a la Organización Mundial de Sanidad Animal (OIE) sobre temas relacionados con la seguridad de la transferencia de embriones. Gracias a los procedimientos de la OIE para elaborar y adoptar normas internacionales, las medidas de control sanitario definidas por la IETS se han incorporado al *Código Sanitario para los Animales Terrestres*. Los principios básicos reposan en la importantísima función ética que cumplen los equipos responsables de la obtención y la transferencia de los embriones, dirigidos por veterinarios autorizados. Tras decenios de experiencia y cerca de 10 millones de embriones transferidos, está demostrado que la tecnología de transferencia de embriones presenta una importante ventaja desde el punto de vista de la seguridad biológica cuando lo que se desplaza a nivel internacional es germoplasma, siempre y cuando se cumplan estrictamente las normas internacionales definidas por la IETS y adoptadas por la OIE.

## Palabras clave

Bovinos – Ganado vacuno – Embriones generados *in vivo* – Embriones obtenidos *in vitro* – Tecnología de reproducción animal – Tecnología reproductiva – Transferencia de embriones.



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Embryo transfer shortens the generational interval, resulting in greater genetic progress. At the same time, artificial insemination and ET together constitute excellent tools for genetically improving flocks and herds isolated from suppliers of male improvers. International trade in frozen sheep and goat embryos has led to the world-wide diffusion of germplasm, with very low health risks. As a consequence, there have been rapid genetic improvements in different breeds and the establishment of alternate meat, milk, wool and hair production (e.g. mohair or cashmere) choices.

Table 3. Comparative embryo development in sheep and goats at different times after removal of intravaginal sponges (Adapted from Moore 1980). Days after removal of sponges. Sheep. Embryo washing is performed by transferring embryos into different, clean wells containing holding media. This procedure, recommended by the International Embryo Transfer Society, aids in removing cellular debris and potential pathogens adhered to the zona pellucida. Embryos are kept in holding media at room temperature until they are transferred to recipients or prepared for freezing, bisection (œsplittingœ), or determination of gender by embryo sexing. Alternatively, embryos can be refrigerated in transfer medium for up to 24 hr with no appreciable loss of viability.

Splitting embryos into i Thibier M. Embryo transfer: a comparative biosecurity advantage in international movements of germplasm. Rev Sci Tech. 2011;30(1):177–188.

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