Review Report

Embryo transfer and biosecurity: safeguarding healthy reproductive technologies in veterinary medicine

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Abstract

Embryo transfer (ET) is an essential reproductive biotechnology tool that supports genetic improvement, conservation programs and international germplasm trade across livestock species. Compared to the movement of live animals or semen, transport of properly collected and processed embryos presents a substantially lower biosecurity risk, owing to the protective role of intact zona pellucida, combined with standardized sanitary procedures established by the International Embryo Transfer Society (IETS) and the World Organization for Animal Health (WOAH). Rapid global expansion of in vitro produced (IVP) embryos, however, has introduced additional biosecurity considerations, as these embryos undergo multiple laboratory manipulations and are exposed to culture systems not encountered during in vivo development. Experimental evidence demonstrated that selected bacterial and intracellular pathogens may persist in IVP embryos following standard washing procedures under specific conditions; however, no confirmed outbreaks associated with commercially produced IVP embryos processed according to the standards have been reported. We summarize current evidence regarding pathogen transmission risks associated with ET; compare biosecurity characteristics of in vivo-derived (IVD) and IVP embryos; review current IETS and WOAH biosecurity guidelines; and discuss emerging challenges associated with continuing expansion of IVP technologies. Emphasis is placed on laboratory quality-control systems, donor health management and future research priorities needed to further strengthen international embryo biosecurity.

Keywords: Embryo handling procedures, international embryo trade, biosafety measures

Introduction: embryo transfer as a biosecure technology

Global use of embryo transfer in livestock

Embryo Transfer (ET) is an advanced assisted reproductive technique (ART) that is well established in dairy and beef cattle, horses, and increasingly used in small ruminants.[1] ET of either in vivo derived (IVD) or in vitro produced (IVP) embryos accelerates genetic progress, supports conservation programs for endangered breeds and species, and facilitates international trade in livestock species. Transport of properly collected and processed cryopreserved embryos presents a lower biosanitary risk compared to live animals and reduces logistic costs.[2-4]

Economic importance

Economic importance of ET in global livestock production is also considerable, with the most recent available data from the American Embryo Transfer Association recording substantial numbers of both IVD embryo collections and IVP embryos produced in domestic species in North America.[1] In cattle, the global ART industry is thriving, with ET enabling producers to maximize the reproductive potential of elite females that would otherwise be limited to as few as 1 offspring per year.[5] Furthermore, equine ET has become a routinely used biotechnology in several breeding programs, allowing mares to compete while simultaneously serving as embryo and/or oocyte donors.[6] In small ruminants, ET programs often underpin elite flock development and breed preservation.[7] Globally, the 34th annual report from the International Embryo Technology Society (IETS) reported bovine transferable embryo production numbers exceeding 2 million annual embryos worldwide, including over 2 million IVP embryos (8.1% increase from 2023) versus a little under 300 thousand IVD embryos (20.1% decrease from 2023).[8]

Biosecurity risks associated with germplasm movement

A key structure that contributes to the lower biosecurity risks of embryo versus live animal movement is the zona pellucida

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(ZP), a glycoprotein matrix that acts as a physical barrier to most microorganisms. Standardized procedures for embryo washing and grading developed by the IETS and the World Organization for Animal Health (WOAH) aim to further reduce pathogen load on embryos destined for transport, transfer or cryopreservation.[9–11]

However, rapid and ongoing growth of IVP, including in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) and somatic cell nuclear transfer (SCNT), has introduced additional biosecurity considerations.[12,13] Approximately 80% of all bovine ET worldwide are currently IVP embryos, representing a dramatic shift from the historical predominance of IVD embryos.[8] The most common technique for ruminant embryo production is IVF whereas most commercial equine IVP embryos are now produced by ICSI. In contrast, the commercial use of SCNT is more limited in all species.[8] Unlike IVD embryos, IVP embryos undergo multiple laboratory procedures, where gametes, culture media, serum supplements, and equipment can serve as potential sources of contamination. Nevertheless, commercial IVP laboratories operate under rigorous quality-control systems and standardized operating procedures designed specifically to minimize these risks, emphasizing that biosecurity depends not only on embryo washing procedures but also on strict donor selection, laboratory management and adherence to validated sanitary protocols.[9,10]

The Bovine Germplasm Movement Plan emerged as a framework to coordinate disease surveillance and certification requirements for germplasm trade, recognizing that even low-risk technologies require structured oversight. Thus, when donor and herd health, embryo handling, and certification all closely follow established biosecurity principles, ET can function as a disease-control process.[2,14,15]

We summarize the biological mechanisms underlying pathogen elimination associated with embryos and their surrounding media; compare biosecurity considerations associated with IVD and IVP embryo technologies; evaluate evidence for pathogen transmission in cattle, horses and small ruminants; and discuss current IETS and WOAH biosecurity guidelines, together with emerging challenges for ET programs in these species. Special emphasis is given to the expanding use of IVP embryos, recent updates in international sanitary recommendations and their implications for biosecurity, and international germplasm movement.

Biological barriers to pathogen transmission across in vivo and in vitro embryos

Physiological barriers

Zona pellucida: first biological protective barrier

Mammalian embryo is encased by ZP, a specialized extracellular matrix composed predominantly of cross-linked glycoproteins, named ZP1, ZP2, ZP3 (and in some species ZP4), secreted by the growing oocyte during folliculogenesis. ZP has roles in sperm binding, prevention of polyspermy, and protection of the blastomeres during transit in the oviducts and uterus.[16,17] From a biosecurity perspective, IVD ZP serves as a primary biological barrier to microorganisms, under physiological conditions, as its glycoprotein matrix physically prevents most viral and bacterial pathogens from reaching the trophectoderm and inner cell mass.[18,19]

Many viral and bacterial pathogens appear to remain adhered to the outer layer of ZP rather than penetrating into the perivitelline space and embryo cells, as long as ZP's structure remains fully intact.[18–21] Therefore, properly handled IVD embryos with an intact ZP are at minimal pathogen transmission risk, when combined with appropriate washing protocols. According to IETS guidelines, detailed microscopic evaluation to confirm ZP integrity and embryo quality is mandatory after washing, and only embryos with confirmed intact ZP should be cleared for export or cryopreservation for future transport.[9]

Equine embryonic capsule

Equine IVD embryos acquire an additional protective mucin-like glycoprotein capsule after entering the uterus (~ days 6-7), providing an extra layer between ZP and trophectoderm. This tough and elastic capsule, composed primarily of O-glycosylated proteins, sialic acid, galactose and sulfated sugars, is secreted by the uterine endometrium in response to tightly regulated embryo-uterine crosstalk, substantially thickening the protective envelope surrounding the equine embryo.[22–24] Capsule formation initiates as a delicate yet discontinuous matrix that progressively thickens and becomes more uniform, coinciding with blastocyst expansion and ZP attenuation, implying a compensatory protective mechanism for the loss of ZP integrity.[25,26]

The capsule provides mechanical stability and resilience during handling and in embryonic mobility, enabling embryos to withstand uterine contractions and repeated deformation during the intrauterine mobility phase that is essential for maternal recognition of pregnancy in horses.[25] Capsule may further reduce pathogen adherence and modulate interactions with uterine immune pathways, particularly as ZP is rapidly thinning as the embryo grows during this phase of development.[25,26] A similar mucin-like coating has been reported in IVD ruminant blastocysts; although less prominent than in horses, it is also absent or reduced on ruminant IVP embryos, representing a further vulnerability for these embryos.[16,17] In contrast, IVP equine embryos do not develop this capsule under laboratory culture conditions, since the embryo-uterine interactions are absent. Although a capsule forms postET, it is not present during handling and cryopreservation.[23,22,27]

Differences between in vivo and in vitro embryos

Morphological and developmental differences

IVD and IVP embryos differ in development and morphology. Direct comparisons between the 2 systems should be interpreted cautiously since they originate from biologically different oocyte populations. IVD embryos originate exclusively from ovulated follicles whereas IVP embryos are produced from a broader population of aspirated oocytes with variable developmental competence.[28,29] IVD embryos will develop within the physiological environment of the reproductive tract, where dynamic oviductal and uterine secretions (histotroph) provide optimal conditions for metabolism, cellular signaling, epigenetic programming, and embryonic development.[26,28–31]

In contrast, IVP embryos are cultured under laboratory conditions that, despite decades of research and optimization, cannot fully replicate the complexity of the maternal microenvironment.[28,29] Although IVP embryos often have lower blastocyst rates and more developmental arrest

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compared to IVD embryos of the same species and breed, significant advances in culture systems, embryo handling and laboratory quality-control procedures have markedly improved IVP embryo production and post thaw survival in recent years.[30]

Overall, IVP embryos generally exhibit slightly delayed development compared to same-day IVD embryos, although the extent and biological significance vary among species. In cattle, IVP embryos generally have increased lipid accumulation, reduced inner cell mass-to-trophectoderm ratios, lower pregnancy rates, and higher early embryonic loss, likely reflecting both suboptimal in vitro conditions and the absence of maternal reproductive tract factors.[28,29,31] In horses, IVP embryos contain fewer cells, have a less distinct inner cell mass (ICM), and typically reach the blastocyst stage ~ 1 day later than IVD embryos.[25,32,33] Despite this delay, pregnancy and live foal rates are generally comparable following embryo transfer, suggesting that uterine environment can compensate for slower in vitro development. Developmental kinetics remain an important predictor of embryo competence, with earlier blastocyst formation and cleavage events associated with improved foaling rates.[34–36]

Zona pellucida structural differences

Structural ZP differences between IVD and IVP embryos may affect biosecurity. Compared to IVD embryos, bovine IVP embryos often exhibit a thinner, less uniform and more porous ZP, likely reflecting the differences in oocyte maturation and in vitro culture conditions.[28,29,37] These alterations have been associated with increased pathogen adherence as demonstrated for *Mycoplasma bovis*, and support the concept that ZP functions as a biological barrier whose effectiveness depends on the pathogen, embryo species and developmental stage rather than an absolute barrier.[37,38]

Implications for pathogen transmission

Structural differences described above have important biosecurity implications, particularly when interpreting the applicability of sanitary protocols developed for IVD embryos to IVP systems. Standard IETS washing protocols were designed and validated using IVD embryos with intact, cell-free, structurally normal ZPs. Experimental studies have demonstrated that pathogens (e.g. *Chlamydia abortus*, *Coxiella burnetii*, *Mycoplasma bovis*) may remain associated with IVP embryos following standard washing procedures under specific experimental conditions.[37,39–41] These findings suggest that additional validation of IVD-derived sanitary recommendations for IVP embryos is warranted, rather than assuming direct equivalence among production systems.

As previously mentioned, IVP systems include several additional laboratory procedures that may serve as potential contamination points if appropriate sanitary practices are not maintained. These include ovum pick-up (OPU), in vitro maturation (IVM) and in vitro fertilization (IVF or ICSI), in vitro culture (IVC), embryo handling, cryopreservation, and micromanipulation procedures (e.g. embryo biopsy or SCNT).[15,29] Potential sources of contamination include donor tissues and gametes, culture media and supplements, laboratory equipment, and handling procedures. Culture media containing materials of animal origin, particularly fetal bovine serum (FBS), have historically been recognized as potential sources of pathogen introduction. Consequently, many commercial IVP laboratories have adopted more defined culture systems and minimize the use of animal-derived components whenever possible, whereas any serum used should be appropriately sourced, tested and quality controlled in accordance with current IETS recommendations.[9,15,29] The use of FBS for in vitro culture is very uncommon in the bovine industry due to known issues (especially increased risk for large calf syndrome) but is still common practice in the equine industry.

IVP embryos (produced by IVF, ICSI or SCNT) should be considered to have distinct biosecurity considerations compared to IVD embryos, due to additional laboratory procedures involved in their production. Conversely, it has also been proposed that IVP systems may reduce exposure to certain reproductive tract-associated pathogens by bypassing the oviduct and uterus during oocyte collection, while current commercial IVP laboratories further minimize contamination through sequential rinsing among IVM, IVF, and IVC stages, appropriate use of embryo-safe antibiotics, and rigorous quality-control procedures.[9,15,29,37,39–41] As a consequence, biosecurity assessments should consider the entire production system including donor health, laboratory quality management, embryo handling and sanitary procedures, rather than focusing exclusively on donor health status or maternal reproductive tract barriers.[9,15]

Semen versus embryo transmission risks

Semen contamination risks

Semen and embryos differ fundamentally as vehicles for pathogen transmission. Due to its intimate and prolonged contact with the male reproductive tract, semen may harbor cell-associated or free viral pathogens, and bacterial and protozoal organisms within seminal plasma or accessory sex gland secretions.[15,20,21] Representative semen-transmitted pathogens include *Tritrichomonas foetus*, equine arteritis virus (EAV) and bovine herpesvirus-1 (BHV-1).[20,42,43]

Semen processing techniques can reduce microbial load on sperm but do not completely eliminate pathogens, particularly those closely associated with sperm or seminal plasma.[15,20] Consequently, semen from chronically infected or latent carrier males, may remain infectious despite appearing macroscopically normal, reinforcing the importance of donor testing and biosecurity screening.[15,20,21] Additionally, antibiotics are often incorporated to semen extenders to control bacterial contamination during semen processing and storage. However, they are ineffective against viruses and some intracellular bacteria (e.g. *Mycoplasma* spp.) and their widespread use has raised concerns regarding antimicrobial resistance.[15,44–46]

Cryopreservation of semen does not fully eliminate pathogens, *Mycoplasma* spp. and several viruses (e.g. BHV-1 and bovine viral diarrhea virus [BVDV]) remain viable after storage in liquid nitrogen.[15,46] Appropriate donor testing, quarantine procedures and biosecure cryostorage therefore remain essential components of semen handling, particularly for venereal pathogens (EAV and contagious equine metritis).[43,47]

Comparative biosecurity: semen versus embryos

Embryo washing protocols effectively reduce the risk of transmission of several major pathogens via ET. Consequently,

there is broad scientific and regulatory consensus that, the transport and use of properly processed embryos present a lower biosecurity risk than the movement of live animals or semen, provided that current IETS sanitary recommendations are followed.[2-4,9,18,21,42] Although this principle also applies to IVP embryos, their additional laboratory manipulations require consideration of donor health, semen quality, embryo handling, validated culture systems and quality-control measures, with pathogen-specific validation of sanitary recommendations remaining necessary.[9,15,28,29]

Role of sperm washing and embryo washing

Sperm washing before IVF reduces seminal plasma and microbial contamination but does not provide the same level of biosecurity as embryo washing because sperm lack a protective ZP. Consequently, pathogens associated with sperm may still gain access to the oocyte during fertilization, particularly following ICSI, where the sperm is mechanically injected through ZP and oolemma.[15,16,29]

Washing protocols across IVD and IVP embryos

Embryo washing remains the cornerstone of embryo biosecurity. Current IETS recommendations prescribe a standardized washing procedure involving at least 10 sequential washes using fresh sterile medium and separate sterile pipettes for each transfer step, with supplementary trypsin treatment recommended for specific pathogens (e.g. BVDV in IVD bovine embryos).[9,10,48] Although these procedures have been extensively validated for IVD embryos, additional validation remains necessary for IVP embryos because of differences in embryo production systems and pathogen interactions.[9,29] Recent studies using *Brucella abortus* (*B. abortus*) strain RB51 as a model pathogen have further contributed to the evaluation of washing efficacy in IVP embryos, highlighting the need for continued pathogen-specific validation of sanitary protocols as IVP technologies continue to expand.[49,50]

Antibiotic treatments

Antibiotics are routinely incorporated into flushing and culture media, and cryoprotectant solutions to control bacterial contamination during embryo production and handling. Current IETS recommendations emphasize the use of validated antimicrobial protocols appropriate for the pathogen of concern and embryo production system.[9,10] Commonly used antibiotics include gentamicin, penicillin-streptomycin, tylosin, and lincomycin-spectinomycin combinations, while amphotericin B may be included for fungal control.[28,44,48,51–53] Despite their widespread use, antibiotics do not eliminate all pathogens and should be regarded as 1 component of a broader biosecurity strategy rather than a substitute for donor screening, laboratory quality management, and standardized embryo washing procedures. Some organisms, including *Mycoplasma* spp. and *Coxiella burnetii*, may persist despite conventional antimicrobial supplementation, whereas excessive antibiotic concentrations may adversely affect embryo development.[10,39,40,46,51] Overall, embryo biosecurity relies on multiple complementary measures, including donor health certification, appropriate laboratory practices, standardized embryo washing, and judicious use of antimicrobial agents. Together, these measures substantially reduce the risk of pathogen transmission associated with embryo transfer.[9,10,52,53]

IVP, ICSI and SCNT-specific risks

Risks unique to laboratory systems

Absence of maternal barriers and laboratory contamination sources

In vitro systems introduce a series of additional control points that have no parallel in natural embryo development or conventional IVD embryo collection and transfer procedures. During IVP, gametes and embryos undergo multiple laboratory procedures, including oocyte recovery, maturation, fertilization, culture, cryopreservation and micromanipulation, making biosecurity dependent not only on donor health but also in laboratory quality-management systems.[15,29] Culture media and supplements containing animal-derived (e.g. bovine fetal serum) components have historically been recognized as potential sources of pathogen introduction. Consequently, many commercial IVP laboratories have adopted more defined culture systems and minimize the use of animal-derived supplements whenever possible, while any biological products used should be appropriately sourced, tested, and quality controlled according to current IETS recommendations.[15,29,46] Cumulus cells surrounding the oocyte may also represent a potential contamination source. Intracellular pathogens (e.g. *Chlamydia abortus*, *Coxiella burnetii*) have been detected in cumulus-oocyte complexes, suggesting that cumulus cells could facilitate close contact between pathogens and the developing oocyte during IVM.[39,40] Whether this mechanism contributes directly to embryo infection remains incompletely understood and requires further investigation.

Micromanipulation and damage to zona pellucida

For ICSI procedures, 1 sperm is selected, immobilized, and mechanically injected using a micromanipulation pipette that first penetrates ZP and then oolemma. This process transiently disrupts ZP integrity and may give opportunity for potential pathogens present in the injection medium to gain direct access to oocyte interior through oolemma, representing a unique biosecurity concern in conventional IVF.[15,29,33]

Another procedure that disrupts ZP is preimplantation genetic testing (PGT), involving biopsy of trophectoderm cells, breaching ZP and trophoblast layer. Genetic material handling in PGT procedures introduces additional sample contamination risks, and cross-contamination among embryos harbored on the same plate and misidentification, all with implications for biosecurity management and genetic integrity of IVP embryo programs.[54–57] There are, however, noninvasive biopsy techniques, that preserve embryo integrity by collecting genetic material from extruded cells, presenting as safe and reliable PGT alternative.[55,58] Nonetheless, noninvasive techniques are not free from contamination risks, which are further compounded by concerns regarding genetic contamination during embryo biopsy, handling and molecular analysis, where even minimal DNA carryover or sample mix-ups may affect diagnostic accuracy and embryo traceability.[54–56]

SCNT procedures involve an even more extensive ZP manipulation, with zona drilling or partial zona dissection being required for introduction of the somatic donor cell into the perivitelline space of an enucleated oocyte. The reconstructed SCNT embryo therefore has a ZP that has been breached at

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multiple points and may not reestablish its full barrier integrity. Additionally, the somatic cell used as the nuclear donor may have cell-associated pathogens from the tissue of origin, representing potential contamination exclusive to this technique.[13,15,29]

Evaluating pathogen transmission

Evaluation of pathogen transmission through embryo transfer relies on a combination of complementary laboratory and in vivo approaches (Table 1). Experimental studies typically assess pathogen adherence or persistence following embryo exposure and standardized washing procedures using techniques (e.g. polymerase chain reaction [PCR], microbial culture, virus isolation, immunofluorescence or immunohistochemistry [IHC], electron microscopy and embryo transfer studies). Each method provides different information regarding pathogen detection, localization, viability, or infectivity, and should therefore be interpreted according to its respective strengths and limitations.[9,15,37–41]

PCR remains one of the most sensitive methods for detecting pathogen nucleic acids; however, it does not distinguish between viable and nonviable organisms. Consequently, PCR-positive findings should be interpreted cautiously, whenever possible, corroborated with culture, virus isolation, infectivity assays, or embryo transfer studies.[39,40] Although experimental inoculation models have been essential for validating embryo washing procedures and investigating pathogen interactions, many employ artificially contaminated gametes or embryos and pathogen concentrations that exceed those encountered under field conditions. Therefore, their findings may not fully reflect contemporary commercial IVP practices. Accordingly, embryo transfer studies remain the gold standard for assessing transmission because they directly evaluate recipient infection following transfer under biological conditions.[18,37–41,52,53,59,60]

Species-specific viral transmission risks

Viruses are among the most extensively investigated pathogens in embryo transfer biosecurity and had a central role in the development and validation of IETS sanitary procedures. However, available evidence varies among viruses and depends on factors including the production system (IVD versus IVP), experimental design, pathogen concentration, and detection method. Overall, properly collected and processed IVD embryos present a negligible risk for transmission of most viral pathogens when current IETS recommendations are followed. In contrast, evidence for IVP embryos remains more limited and is largely derived from experimental studies, highlighting the need for continued pathogen-specific validation, although available evidence supports a low risk of transmission following recommended embryo processing.[9.15,18,20,59,60] Viral pathogens studied that might be relevant to ET biosecurity in domestic species are summarized (Table 2).

Cattle

Among bovine viruses, BVDV is the best-characterized model for evaluating embryo-associated viral transmission and has been extensively used to validate IETS sanitary procedures. Several studies have demonstrated that properly washed IVD embryos present a negligible risk of BVDV transmission, supporting the effectiveness of current embryo washing protocols.[9,10,18,28,46,48] Other bovine viruses, including BHV-1, bluetongue virus (BTV) and foot and mouth disease, have also been investigated, with experimental studies generally supporting a low risk of transmission following appropriate embryo processing and current IETS recommendations.[10,18–21,46,61,62]

Small ruminants

In small ruminants, available evidence is comparatively limited. Border disease virus (BDV) and BTV have been investigated because of their biological similarities to bovine pestiviruses and arboviruses, respectively, and experimental studies generally support a low risk of transmission following appropriate embryo processing.[3,10,18,61,63–65] In contrast, scrapie remains largely a theoretical concern, as experimental transmission has only been reported under conditions with important methodological limitations, and there is no convincing evidence of disease transmission following commercial embryo transfer.[9,10,63,64] Consequently, continued pathogen-specific evaluation is appropriate, particularly for IVP embryos, where available data remain limited.

Horses

Among equine viruses, equine herpesvirus-1 (EHV-1), EAV and equine infectious anemia virus (EIAV) have received the greatest attention in embryo transfer biosecurity.[9,10] EHV-1 has been detected in embryo flush fluids from viremic mares, suggesting surface contamination; however, there is limited evidence that the virus penetrates an intact ZP and standard embryo washing appears effective in removing surface-associated virus.[66] EAV is considered a greater risk through semen than through embryos because persistently infected stallions are the primary reservoir. Experimental studies demonstrated that properly washed embryos produced using EAV-positive semen did not transmit infection, supporting current IETS and WOAH recommendations that properly processed equine embryos

Table 1. Summary of methods used to evaluate pathogen transmission in embryo transfer studies

Method	Detects	Main limitation
PCR	Pathogen nucleic acids	Does not determine viability or infectivity
Culture	Viable bacteria or fungi	May fail to recover fastidious organisms
Virus isolation	Infectious virus	Labor-intensive and time-consuming
Immunofluorescence/IHC	Pathogen localization	Does not confirm viability
Electron microscopy	Pathogen morphology/ attachment	Does not assess infectivity
Embryo transfer studies	Biological transmission to recipients	Gold standard but expensive and limited to few pathogens

Table 2. Summary of viral pathogens relevant to ET biosecurity in domestic species

Species	Pathogen	Main biosecurity concern	Evidence following recommended embryo processing	Evidence for IVP embryos
Cattle	Bovine viral diarrhea virus	Best-studied model for embryo-associated viral transmission	Properly washed IVD embryos present negligible transmission risk	Moderate; additional pathogen-specific validation recommended
	Bovine herpesvirus-1	Surface contamination of embryos	Washing effectively removes virus from intact IVD embryos	Limited
	Bluetongue virus	Experimental embryo contamination reported	Low risk for IVD embryos; persistence reported under some experimental conditions	Limited
	Foot and mouth disease virus	High-consequence transboundary disease	Experimental studies support low risk following recommended processing	Limited
Small ruminants	Border disease virus	Pestivirus with reproductive implications	Low risk following appropriate embryo processing	Limited
	Bluetongue virus	Experimental embryo contamination reported	Low risk after recommended processing; evidence remains limited	Limited
	Scrapie	Theoretical concern due to prion transmission	No convincing evidence of transmission following commercial embryo transfer	Very limited
Horse	Equine herpesvirus-1	Surface contamination from viremic mares	Washing appears effective; ZP penetration not demonstrated	Limited
	Equine arteritis virus	Carrier stallions and infected semen	Properly washed embryos have negligible transmission risk	Limited
	Equine infectious anemia virus	Theoretical contamination through blood	Donor testing and standard washing minimize risk; SCNT studies support negligible transmission	Very limited

represent a low transmission risk.[9,10,67–69] Likewise, although EIAV presents a theoretical concern because of cell-associated viremia, available evidence, including studies using somatic cell nuclear transfer (SCNT) suggests that the risk of transmission through properly processed embryos is negligible when standard embryo washing procedures and donor testing are implemented.[10,70] Overall, evidence supporting additional biosecurity risks specific to IVP equine embryos remains limited, emphasizing the need for continued pathogen-specific evaluation.

Species specific bacterial transmission risks

Bacterial pathogens represent one of the most extensively investigated groups in embryo transfer biosecurity because several bacterial species are associated with reproductive disease, abortion, or infertility and may interact with gametes and embryos during collection or in vitro production.[9,10,15,18] Similarly to viral pathogens, the level of evidence varies considerably among bacterial species and depends on the production system (IVD versus IVP), experimental design, and diagnostic method. Evidence for IVP embryos remains more limited and is derived primarily from experimental studies, emphasizing the need for continued pathogen-specific validation while recognizing that contemporary IVP laboratories employ multiple quality-control measures to minimize contamination risks. However, properly collected and processed embryos present a negligible risk for transmission of most bacterial pathogens when current IETS recommendations are followed.[9,15,18,29,60] Bacterial pathogens studied that might be relevant to ET biosecurity in domestic species are summarized (Table 3).

Cattle

Among bacterial pathogens, *B. abortus* remains one of the best-characterized models in embryo transfer biosecurity and has contributed substantially to the validation of IETS washing procedures. Historical embryo transfer studies demonstrated that properly collected and processed IVD embryos present a negligible risk of transmitting bovine brucellosis, while recent studies have further evaluated washing efficacy in

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Table 3. Summary of bacterial pathogens relevant to ET biosecurity in domestic species

Species	Pathogen	Main biosecurity concern	Evidence following recommended embryo processing	Evidence for IVP embryos
Cattle	*Brucella abortus*	Classical model for embryo transmission studies	Negligible risk following IETS washing	Moderate (RB51 validation studies)
	Mycoplasma bovis	Persistence on oocytes/IVP embryos after washing	Low risk for IVD; experimental persistence reported	Moderate
	Campylobacter fetus	Reduced embryonic development during IVP	Limited evidence of embryo transmission	Limited
	Chlamydia abortus	Persistence on IVP embryos after washing	Pathogen-specific risk assessment recommended	Moderate
	Coxiella burnetii	Persistence on IVP embryos after washing	Pathogen-specific risk assessment recommended	Moderate
	Leptospira spp.	Detection in reproductive tract and follicular fluid	Low risk following recommended procedures	Limited
	Mycobacterium avium subsp. *paratuberculosis*	Detection in reproductive tissues	No demonstrated embryo transmission	Very limited
Small ruminants	*Chlamydia abortus*	Major abortifacient; experimental persistence after washing	Pathogen-specific risk assessment recommended	Moderate
	Coxiella burnetii	Zoonotic abortifacient; persistence after washing	Pathogen-specific risk assessment recommended	Moderate
Horse	*Taylorella equigenitalis*	Venereal pathogen (CEM)	Washing considered effective; no documented ET transmission	Limited
	Mycoplasma spp.	Semen and laboratory contamination	Controlled through antibiotics and laboratory quality control	Very limited

IVP embryos using *B. abortus* strain RB51 as a representative pathogen.[9,10,14,52–54,71]

Experimental investigations have also evaluated *Mycoplasma bovis*, *Campylobacter fetus, Chlamydia abortus*, *Coxiella burnetii*, *Leptospira* spp. and *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Among these, *Mycoplasma bovis*, *Chlamydia abortus* and *Coxiella burnetii* have demonstrated persistence on oocytes or IVP embryos following standard washing procedures under experimental conditions, *Campylobacter fetus* has primarily been associated with reduced embryo development rather than persistence following embryo washing. Evidence for *Leptospira* spp. and MAP is primarily limited to detection within the reproductive tract or effects on *in vitro* embryo production. Still, much of the available evidence derives from artificial exposure models that may not fully reflect commercial IVP systems.[29,37,39,40,60,72–78] Contemporary IVP laboratories minimize these risks through donor selection, validated laboratory procedures, sequential washing, appropriate antibiotic use and quality-control systems, supporting the effectiveness of current sanitary recommendations while highlighting that biosecurity should be evaluated on a pathogen-specific basis.[9,15,29,60]

Small ruminants

Amongst bacterial pathogens affecting small ruminants, *Chlamydia abortus* and *Coxiella burnetii* are of greatest relevance since both are major causes of abortion and have been experimentally evaluated in embryo transfer systems. Studies using goat IVP embryos demonstrated that both pathogens may remain associated with embryos following standard washing procedures under experimental conditions, supporting their classification as pathogens requiring pathogen-specific risk assessment.[10,39–41,77,79] Nevertheless, available embryo transfer studies indicate a low transmission risk when embryos are collected, processed, and handled according to current sanitary recommendations. Overall, these findings reinforce the importance of standardized embryo handling procedures and appropriate donor health management to minimize bacterial transmission risks.[3,9,15,77–79]

Horses

Compared to cattle, fewer bacterial pathogens have been directly evaluated in equine embryo transfer systems. *Taylorella equigenitalis*, the causative agent of CEM, represents the principal bacterial pathogen of biosecurity concern because of its venereal transmission and potential economic impact on international horse movement.[10,47,80] Although contamination of embryo flush fluids has been reported, standard embryo washing procedures are considered effective for intact IVD embryos, and there is no evidence supporting transmission following properly processed embryo transfer.[9,10,47] *Mycoplasma* spp. and other opportunistic bacteria have also been recognized as potential contaminants of semen and laboratory

Table 4. Summary of protozoal and fungi pathogens relevant to embryo transfer biosecurity

Species	Pathogen	Main biosecurity concern	Evidence following recommended embryo processing	Evidence for IVP embryos
Cattle	*Neospora caninum*	Vertical transmission and abortion	Negligible risk following recommended embryo processing; ET historically used for disease control	Extensive
	Tritrichomonas foetus	Venereal infertility and embryonic loss	Limited evidence of embryo transmission	Limited
Small ruminants	*Toxoplasma gondii*	Major cause of abortion	No convincing evidence of transmission through properly processed embryos	Limited
	Neospora caninum	Abortifacient protozoan	Embryo-mediated transmission not demonstrated	Very limited
Horse	*Theileria equi*	Blood-borne protozoan (equine piroplasmosis)	No evidence of transmission through properly processed embryos	Limited
Fungi (all species)	Environmental fungi (*Aspergillus, Candida*, etc.)	Laboratory contamination	Controlled through aseptic laboratory procedures rather than embryo washing	Limited

environments, reinforcing the importance of donor health screening, appropriate antibiotic use, and strict laboratory quality-control procedures throughout embryo production.[44,45,52,53] **Collectively, these pathogens highlight the importance of controlling venereal transmission and maintaining stringent laboratory hygiene throughout equine embryo production.**[9,10]

Protozoa and other pathogens

Compared to viral and bacterial pathogens, fewer protozoal and other infectious agents have been investigated in relation to embryo transfer biosecurity. Available evidence is largely restricted to selected pathogens of major reproductive or trade importance, with most studies evaluating embryo adherence, persistence following washing, or transmission through experimental transfer. Broadly, current evidence indicates that transmission risk is pathogen-dependent and should be interpreted within the context of standardized embryo processing and established sanitary recommendations.[3,9,15,19] Fungal pathogens represent mostly environmental contamination concerns. Protozoa and fungal pathogens studied that might be relevant to ET biosecurity in domestic species are summarized (Table 4).

Cattle

Among protozoal pathogens, *Neospora caninum* is best characterized in cattle because of its major role in bovine abortion. Historically, embryo transfer has even been used as a management strategy to reduce vertical transmission in infected herds, and available evidence supports a negligible risk of transmission through properly collected and processed embryos.[7,9,10,18,79–84] In contrast, *Tritrichomonas foetus* is an important venereal pathogen associated with infertility and early embryonic loss.[81,82] Although contamination of reproductive tissues has been demonstrated, evidence supporting embryo-mediated transmission remains limited, and current sanitary procedures are considered effective in minimizing transmission risk.[3,9,18,81–84]

Small ruminants

As far as protozoal pathogens affecting small ruminants, *Toxoplasma gondii* is the most relevant since it is a major cause of abortion in sheep and goats.[85,86] Although the parasite can infect reproductive tissues, there is no convincing evidence that properly collected and processed IVD embryos represent an important route of transmission when recommended embryo handling procedures are followed.[9,10,85,86] *Neospora caninum* has also been investigated because of its role as an abortifacient in ruminants; however, evidence supporting embryo-mediated transmission in small ruminants remains limited. Collectively, available studies indicate that transmission risk should be considered individually for each protozoal pathogen while maintaining standardized embryo collection and processing procedures.[3,9,10,18,79–83]

Horses

Compared to cattle and small ruminants, few protozoal pathogens have been investigated in equine embryo transfer. *Theileria equi* and *Babesia caballi*, the causative agents of equine piroplasmosis, are primarily transmitted by ticks and through blood contamination.[87] Although donor mares with active parasitemia may theoretically contaminate IVD embryo flush fluids, there is no evidence that properly collected and processed embryos transmit infection following embryo transfer.[87–89] Consequently, routine donor screening and adherence to established sanitary procedures remain the principal biosecurity measures for equine embryo transfer.[3,9,10]

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Fungal

Fungal contamination has received considerably less attention than viral, bacterial, or protozoal pathogens in embryo transfer biosecurity. Most reports describe opportunistic environmental contaminants introduced during semen processing, embryo manipulation, or laboratory handling rather than true embryo-associated pathogens. Consequently, prevention relies primarily on good laboratory practices, validated culture systems, environmental monitoring and strict aseptic techniques rather than pathogen-specific embryo washing procedures.[9,10,90–92]

Current biosecurity guidelines

IETS guidelines

The IETS Manual, currently in its 5th edition,[9] provides the primary technical reference for procedures of embryo collection, handling and biosecurity in domestic species. Pathogens are classified into 4 categories according to available scientific evidence regarding the likelihood of disease transmission through embryo transfer. These classifications support international trade decisions and are periodically revised as new evidence becomes available. Importantly, current categorization system applies primarily to IVD embryos, while separate categorization of IVP embryos is under active development by the IETS Health and Safety Advisory Committee (HASAC).[9] Definition of each category and the classification of pathogens discussed in this review are presented (Table 5).

IETS Manual provides standardized recommendations for embryo collection, handling, processing and certification to minimize the risk of disease transmission. Key provisions of IETS guidelines for ET include: 1. mandatory use of at least 10 sequential washes in protein-supplemented medium; 2. use of separate sterile pipettes for each step; 3. microscopic evaluation and documentation of ZP integrity in each embryo to be transferred or cryopreserved; 4. specific additional steps (trypsin treatment when indicated, e.g. for exporting embryos) for certain high-risk pathogens (e.g. BVDV); 5. antibiotic supplementation of all media according to specific formulations; and 6. appropriate documentation and certification requirements for international germplasm movement.[9]

WOAH guidelines

WOAH Terrestrial Animal Health Code provides the international regulatory framework governing the movement of animals and animal products, including embryos, among member countries.[10] Code includes specific chapters on germplasm trade (cattle, small ruminants, horses) that establish health requirements for donor animals, certification procedures required for embryos intended for international trade, and pathogen-specific testing and treatment requirements. WOAH requirements for embryos traded internationally include: certification of the donor herd's freedom from, or low prevalence of specified diseases; pathogen-specific testing of donor animals at defined intervals around embryo collection; documentation confirming compliance with IETS-recommended embryo handling and washing procedures; and, when appropriate, additional testing of embryo batches before export. For selected pathogens, WOAH distinguishes between IVD and IVP embryos by applying additional sanitary requirements to IVP embryos. These distinctions reflect a precautionary regulatory approach based on current evidence base and ongoing evaluation by IETS Health and Safety Advisory Committee (HASAC), rather than documented evidence that properly produced and processed IVP embryos present a greater risk of pathogen transmission than IVD embryos.

Table 5. IETS (5th edition) disease categories for pathogens discussed in this review

IETS Category	Definition	Pathogens discussed in this review
Category 1	Sufficient evidence demonstrates negligible risk of disease transmission when embryos are collected and processed according to IETS recommendations	**Cattle:** *Brucella abortus*, bovine herpesvirus-1*, bluetongue virus - cattle, foot and mouth disease virus **Small ruminants:** Scrapie
Category 2	Available evidence suggests negligible risk, but additional embryo transfer studies are required to confirm existing data	**Small ruminants:** bluetongue virus - sheep
Category 3	Preliminary evidence indicates negligible risk, but additional in vitro and in vivo studies are needed before definitive conclusions can be drawn	**Cattle:** bovine viral diarrhea virus, *Mycobacterium avium* subsp. *paratuberculosis*, *Neospora caninum*. **Small ruminants:** *Campylobacter fetus*.
Category 4	Current evidence is insufficient to determine transmission risk or indicates that additional evidence is required	**Cattle:** *Chlamydia abortus*, *Leptospira* spp, *Mycoplasma* spp. **Horses:** Equine arteritis virus, equine herpesvirus-1, *Taylorella equigenitalis*. **Small ruminants:** Border disease virus, bluetongue virus - goats

*trypsin treatment is recommended according to IETS manual. Adapted from IETS manual (5th edition). Only pathogens discussed in the present review are described. Disease categorization applies to IVD embryos and may vary among host species for certain pathogens (e.g. bluetongue virus). Readers should consult IETS Manual (5th edition) for complete and current classification.

These recommendations are described in Section 4 (Disease prevention and control), and in Chapters 8 (IVD), 9 (IVP), 10 (Oocytes and micromanipulated embryos) and 14 (SCNT).[10]

Donor animal certification and pathogen testing

Comprehensive health certification of donor animals is a cornerstone of embryo biosecurity programs. Depending on the species, embryo production system and intended movement of germplasm, certification generally includes evaluation of herd health status, clinical examination of donor animals, and pathogen-specific testing in accordance with IETS and WOAH recommendations.[9,10] Commercial embryo transfer programs frequently implement additional surveillance measures, including serological and molecular testing, semen testing when applicable, and laboratory quality-control procedures that extend beyond minimum regulatory requirements.[9,10,61] Diagnostic approach and timing of testing should be selected according to pathogen biology. Serological assays primarily identify previous exposure and may fail to detect recently infected animals during the diagnostic window whereas PCR, culture, or virus isolation may identify active infection depending on the pathogen and sample type.[9,10] Consequently, surveillance programs should be tailored to individual pathogens and integrated with donor selection, embryo processing, and laboratory biosecurity measures rather than relying on a single testing strategy.[9,10,59]

Emerging concerns

Rapid expansion of IVP embryo technology

One of the most important emerging challenges in veterinary ET programs is the dramatic and ongoing shift from IVD to IVP embryos. Approximately 80% of all transferable bovine embryos worldwide are now IVP, a proportion that continues to increase annually.[8] This transition reflects the substantial commercial advantages of IVP, including the ability to produce large numbers of embryos from prepubertal donors, recover oocytes from abattoir-derived material, flexibility of OPU-IVP programs that do not require synchronization of donors, utilize genetically valuable animals with reproduction impairments, and accelerate genetic gain combining IVP with genomic selection. Recent work further highlighted that IVP systems enable continuous, scalable embryo production pipelines, particularly attractive for large-scale breeding programs while requiring rigorous quality-management systems to maintain biosecurity throughout in vitro handling steps.[60,71] As discussed throughout this review, the rapid expansion of IVP has prompted continued refinement of scientific evidence and regulatory recommendations. Although many IETS and WOAH sanitary guidelines were initially developed using evidence generated from IVD embryos, they are being progressively updated as new data become available.[9,10] Current evidence supports the effectiveness of contemporary commercial IVP laboratory practices when validated quality-control systems are implemented, while continued pathogen-specific research will further strengthen biosecurity recommendations for IVP embryo production.[9,10,60,71]

International trade of embryos

The volume of international embryo trade has increased substantially over the past 2 decades, with bovine IVP embryos now routinely shipped worldwide.[8] This continued expansion highlights the importance of maintaining harmonized international biosecurity standards to support the safe movement of germplasm. WOAH and IETS provide complementary frameworks for disease risk management through standardized donor certification, embryo processing, laboratory practices, and pathogen-specific health requirements.[9,10] As embryo production technologies continue to evolve, ongoing harmonization of scientific evidence and international regulations will remain essential to facilitate safe global embryo trade.

Antimicrobial resistance

Routine use of antibiotics in embryo flushing media and culture systems has raised concerns regarding antimicrobial stewardship and the potential contribution of assisted reproductive technologies to antimicrobial resistance.[93] Standard antibiotic combinations used during embryo production have remained largely unchanged for decades and include classes of antibiotics (aminoglycosides, penicillin, tetracyclines) that are important in both human and veterinary medicine.[15,92] Although antibiotics reduce microbial contamination during embryo production, they should be considered only 1 component of a broader biosecurity strategy rather than a substitute for appropriate donor screening, validated laboratory procedures, and strict hygienic practices.[18,92] Emerging approaches, including ultraviolet-C treatment of culture media, advanced filtration systems, bacteriophages, and antimicrobial peptides, have been proposed as potential alternatives or adjuncts to conventional decontamination strategies and warrant further investigation.[15,94]

Future research needs

Embryo-pathogen interactions

Despite substantial advances in embryo transfer biosecurity over recent decades, important knowledge gaps remain regarding the molecular mechanisms governing embryo-pathogen interactions. Most available studies have focused on pathogen adherence or persistence following embryo washing whereas the mechanisms regulating pathogen binding, penetration and interaction with embryonic structures remain incompletely understood. Improved understanding of these processes may support refinement of pathogen-specific biosecurity recommendations and embryo processing procedures.[11,40,95,96] Future research should continue to evaluate how embryo production systems, developmental stage and embryo-associated structures influence pathogen interactions. Although IVP embryos differ from IVD embryos in several biological characteristics, further investigation is also warranted to refine current understanding of IVD embryo biosecurity using contemporary molecular, microbiological and imaging techniques.[97,98] Likewise, naturally occurring protective mechanisms within the reproductive tract, including factors present in follicular and oviductal fluids, remain incompletely characterized and may contribute to embryo biosecurity.[29,33,60] Continued collaboration among researchers, regulatory agencies and international organizations will be essential to generate the evidence required to refine IETS and WOAH recommendations as embryo production technologies continue to evolve.

Improved embryo washing technologies

Continued refinement of embryo washing technologies remains an important area of research, particularly as IVP systems become increasingly widespread. Current IETS washing guidelines have been extensively validated for IVD embryos and have demonstrated excellent biosecurity performance when appropriately implemented.[9,97] As discussed throughout

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this review, however, embryo-pathogen interactions vary among pathogens and embryo production systems, supporting continued evaluation of washing procedures for specific applications and emerging technologies. For example, although standard washing procedures and pathogen-specific treatments effectively reduce the risk of BVDV and BHV-1 transmission in IVD embryos, additional studies have investigated their performance under experimental IVP conditions.[9,28,29] Future research should focus on optimizing washing procedures based on a better understanding of embryo-pathogen interactions. Potential approaches include refinement of washing parameters, evaluation of pathogen-specific treatments, and development of novel technologies that improve standardization and reproducibility. Microfluidic-based washing systems produced promising results in human and experimental veterinary assisted reproductive technologies by improving fluid dynamics and reducing operator-dependent variability, offering potential advantages over conventional manual washing procedures.[99,100] Any modifications to existing washing protocols should be validated by assessing both pathogen removal and embryo viability. Since excessive mechanical or chemical treatments may compromise embryo integrity and developmental competence, evaluation should include post transfer outcomes (e.g. pregnancy establishment, fetal development, offspring health) before widespread implementation.[98]

Pathogen screening in IVP systems

The complexity of pathogen exposure in IVP systems extends beyond the embryo to include oocyte donors, semen, culture media, supplements and laboratory environments.[9,10] Commercial IVP laboratories employ multiple quality-control measures to monitor these potential sources of contamination; however, continued refinement of pathogen screening strategies may further strengthen biosecurity and facilitate harmonization among laboratories and regulatory frameworks.[60] Reports of microbial contamination under experimental conditions and advances in molecular diagnostics have highlighted opportunities to develop more sensitive and comprehensive detection approaches.[92,93] Future research should prioritize development of multiplex diagnostic platforms capable of simultaneously detecting a panel of high-risk pathogens relevant to ET and international trade. These tools could be applied to diverse sample types, including follicular fluid, semen, embryo wash fluids and, potentially, culture media.[101] Real-time analysis of spent culture media represents a promising noninvasive approach that could be integrated with molecular diagnostic platforms, potentially enabling simultaneous embryo assessment and pathogen detection without direct embryo manipulation.[102] Next-generation sequencing approaches, particularly metagenomic analyses, also offer a powerful, unbiased strategy for detecting both known and emerging pathogens without prior assumptions regarding their presence.[103] Integration of these technologies into routine IVP workflows will require validation of analytical performance, practical feasibility, cost-effectiveness, turnaround time and standardized interpretation criteria before widespread implementation.[4,60]

Microbiome of reproductive tract

Mammalian reproductive tract harbors a distinct and dynamic microbial community. This reproductive tract microbiome may have important roles in modulating immune response, embryo-maternal interactions and developmental competence.[104,105] Although growing evidence supports reproductive microbiome importance in fertility, its role in embryo transfer biosecurity and IVP systems remains poorly characterized. Transition from in vivo to in vitro environments may alter microbial exposure through differences in oocyte collection, culture media, laboratory conditions and embryo handling.[29,93] However, the biological significance of these changes and their impact on embryo development and biosecurity remain largely unknown. Likewise, it is not yet clear whether structural or developmental differences between IVD and IVP embryos influence their interactions with commensal or pathogenic microorganisms.[32] Future research should characterize the baseline microbiome profiles of healthy reproductive tracts across species and investigate how reproductive tract microbiota, culture systems and embryo-associated microorganisms interact throughout embryo production. Attention should be given to identifying microbial signatures associated with dysbiosis and increased pathogen susceptibility.[105] Improved understanding of these relationships may contribute to the refinement of culture systems, laboratory biosecurity practices and antimicrobial stewardship strategies while supporting embryo health and developmental competence.[104]

Regulatory harmonization for IVP embryo systems

A final and important future research priority is the continued generation of pathogen-specific experimental evidence to support ongoing refinement of IETS and WOAH recommendations for IVP embryo systems.[9,10,59] A key research priority remains generating pathogen-specific experimental data to inform evidence-based updates. Particular attention should be given to pathogens for which experimental studies have identified unique embryo-pathogen interactions in IVP systems, including *Chlamydia abortus*, *Coxiella burnetii*, *Mycoplasma bovis* and BVDV, and economically important pathogens for which IVP-specific evidence remains limited (e.g. foot and mouth disease virus, BTV, EAV).[15,28,29] International collaboration will be essential to accelerate generation of this evidence base and its translation into updated recommendations. Coordinated research efforts supported by standardized experimental designs and data-sharing initiatives would facilitate accumulation of robust evidence necessary to refine certification standards. Ultimately, continued refinement of harmonized international guidelines will ensure that regulatory frameworks evolve alongside advances in embryo production technologies while maintaining a high level of biosecurity achieved by contemporary embryo transfer programs.

Conflict of interest

None to declare.

Authors' contribution and declaration

CR: literature review, preparation of the original draft and editing; **GC:** conceptualization and manuscript revision; and **RH:** conceptualization and manuscript revision. Authors have read and approved final submission.

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