Embryo transfer, a potential risk in disease transmission

Abstract

Several studies have been performed to determine the disease transmission potential of early bovine embryos. Most of them agree that the Zona Pellucida (ZP) is a barrier that generally protects the embryo from infection but can also be a potential route for the transmission of pathogens. This review will initially focus on the characteristics of the ZP and the potential ways in which an embryo can transmit disease and the possible alternatives to reduce this hazard.

Abbreviations: ZP, zona pellucid; BHV-1, bovine herpes virus type-1; BVDV, bovine viral diarrhea virus; IETS, international embryo technology society; CP, cytopathic; NCP, non-cytopathic; BLV, bovine leukemia virus; FMDV, foot and mouth disease virus

Introduction

The bovine ZP is a 12μm-thick acellular matrix that surrounds the oocyte and the early embryo. It is composed of three major sulfated glycoproteins generally designated as ZP1, ZP2 and ZP3.1 Its thickness is constant up to the blastocyst stage when it becomes progressively thinner until the embryo finally hatches approximately nine days after fertilization. This structure acts as a barrier to pathogens because of its thickness, its acellular nature and the lack of cellular receptors that may attract pathogens.2 Morphologically, it has a sponge-like appearance and is composed of a complex fibrous network containing numerous pores. The pores are channels that granulosa cell foot processes use to communicate with the oocyte prior to ovulation. They are largest at the outer surface and decrease in size towards the inside. After ovulation, the granulosa cells are shed and the structural changes of the ZP tend to obliterate these pores, although not completely.3

The importance of the ZP as a barrier is clearly shown when embryos with removed ZP are exposed to a pathogen. When embryos were exposed to Bovine Herpes Virus type-1 (BHV-1), no replication of BHV-1 in tubal cells was detected.4,5

Transmission of pathogens by the embryo

In spite of the characteristics of the ZP, the embryo can still be a potential carrier of pathogens by one of two ways: 1) a pathogen can penetrate the ZP and infect the ovum or the embryonic cells or, 2) a pathogen can attach to the ZP and infect the recipient female or the embryonic cells after hatching.

Pathogens penetrating through the zona pellucida

Small pathogens, mainly viruses, can potentially penetrate the ZP through its pores before, during, or after fertilization. Certain RNA viruses, especially oncornavirus, have been shown to penetrate the ZP, particularly before or during fertilization, and appear to be vertically transmitted.6 The information available suggests that there is species variation with regard to the ability of pathogens to cross the ZP. In the case of cattle embryos, this structure is quite resistant, since, of all the pathogens that have been studied, none have been able to pass this barrier. A study found Bovine Viral Diarrhea Virus (BVDV) inside the oocyte of Persistently Infected (PI) cattle, suggesting that this is a disease that could be vertically transmitted. On the other hand, Brock et al.,7 have shown that in vivo embryos from PI cows can be effectively washed free of BVDV and can be safely transferred to recipients without seroconversion.

The possibility of the embryo becoming infected after fertilization seems to be less likely due to the changes in the ZP after fertilization in vivo, additional proteins become associated with the ZP at that time. Interestingly, this does not hold true for some enteroviruses of 20-30nm in diameter that can readily penetrate the ZP of mouse morula. This finding suggests that factors, with regard to ZP-pathogen interaction, other than size, seem to be important, since not all similar size viruses can readily cross the ZP. A possible explanation for penetration of pathogens at more advanced stages of development arises from a publication of Gonzalez et al.,8 who showed that there are cytoplasmic extensions of the trophoblast cells through the ZP prior to hatching.

Penetration of pathogens into the embryo is more likely to be related to embryos that have hatched or have a broken ZP. If penetration does occur, degeneration of the embryo is the likely outcome with most pathogens. In these circumstances the risk of disease transmission is practically non-existent because the embryos will not be transferred into recipients. The importance of integrity of the ZP is highlighted by the International Embryo Technology Society (IETS)9 recommended protocol for successful removal of most bovine pathogens from the ZP of in vivo produced embryos. According to the Society, only ZP intact embryos should be washed and treated with trypsin and the ZP integrity should be confirmed before and after washing.10

Reliance on the cytopathic effect of a pathogen that has infected the embryonic cells is not applicable to non-cytopathic strains. Viruses such as BVDV can infect the embryo without affecting its development.11 Zona pellucida free oocytes, zygotes, 8-cell-stage embryos, morulae and hatched blastocysts were incubated for 1h with a cytopathic (CP) or a non-cytopathic (NCP) strain of BVDV. Only the
CP BVDV strain had a significant inhibitory effect on development of ZP-free morulae.\textsuperscript{12}

**Pathogens attaching to the zona pellucida**

Viruses and bacteria can become firmly attached to the ZP. In this case, penetration does not occur but the organism can still infect the recipient following transfer or the embryo after hatching. Stringfellow & Givens\textsuperscript{13} that have addressed this possibility have shown that pathogens show different affinity to the zona pellucida (Table 1) (Table 2). In one study, heifers persistently infected with BVDV were super ovulated and the embryos were recovered seven days after insemination. The embryos and unfertilized/degenerated ova were washed according to the IETS protocol. Bovine viral diarrhea virus was not detected in homogenates of embryos nor in the unfertilized/ degenerated ova, and a BVDV-negative calf was born.\textsuperscript{7} This study indicates that BVDV is not firmly attached or it is easily removed from in vivo produced embryos by the washing steps recommended by the IETS. By the IETS protocol. Bovine viral diarrhea virus was not detected in homogenates of embryos nor in the unfertilized/ degenerated ova, and a BVDV-negative calf was born.\textsuperscript{7} This study indicates that BVDV is not firmly attached or it is easily removed from in vivo produced embryos by the washing steps recommended by the IETS.

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<thead>
<tr>
<th>Pathogens</th>
<th>Association</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Parainfluenza-3 virus (PI-3)</td>
<td>None</td>
<td>Stringfellow &amp; Wright\textsuperscript{14}</td>
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<td>Bovine enterovirus</td>
<td>None</td>
<td>Thomson et al.\textsuperscript{15}</td>
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<td>Foot and mouth disease virus (FMDV)</td>
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<td>Akabane virus</td>
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<td>Bovine leukemia virus (BLV)</td>
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<tr>
<td>Bluetongue virus (BT)</td>
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<tr>
<td>Bovine viral diarrhea virus (BVDV)</td>
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<tr>
<td>Bovine herpes virus type 1 (BHV-1)</td>
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<tr>
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<tr>
<td>Vesicular stomatitis virus (VSV)</td>
<td>firmly attached</td>
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Table 1 Characteristics of the association between several viruses and the zona pellucida of bovine embryos\textsuperscript{11}

Due to their sizes, it is more likely for bacterial pathogens to become attached to the ZP than to penetrate it. *Brucella abortus* was not isolated from ZP-intact or ZP-free groups of bovine embryos after 10 sequential antibiotic-free washings but all groups containing ZP-defective embryos were positive.\textsuperscript{14}

The exact mechanism that underlies the interaction between pathogens and the ZP is poorly understood. The irregular surface of the ZP may entrap virions and bacteria or pathogens can enter the external pores and become entrapped further inside the ZP. Vanroose et al.,\textsuperscript{13} addressed this possibility by studying the passage through and the location in the zona pellucida of fluorescent microspheres with similar dimensions to BVDV (40-50 nm) and BHV-1 (180-200 nm). The smallest beads were detected halfway through the ZP. The conclusion was that the intact ZP of cattle oocytes and embryos is constructed in such a way that BVDV and BHV-1 should not be able to traverse the ZP and reach the embryonic cells but the risk exists that viral particles can be trapped in the outer layers of the ZP.

**Risk of disease transmission by embryos**

The information generated with regard to embryo-pathogen interaction has been compiled by the Research Subcommittee of the IETS Import/Export Committee. With the information available, the Society has categorized the diseases in four groups.\textsuperscript{21} Category 1 include disease agents for which there is sufficient evidence that the risk of disease transmission is negligible, provided that the embryos are properly handled. Bovine pathogens included in this group are Bovine Leukemia Virus (BLV), Foot and Mouth Disease Virus (FMDV), *Brucella abortus*, Infectious Bovine Rhinotracheitis IBR and Blue Tongue Virus (BT V). Category 2 includes those pathogens for which there is substantial evidence that the risk of disease transmission is negligible provided that the embryos are properly handled, but for which additional transfers are required to verify existing data. No cattle pathogens are included in this category. Category 3 includes agents for which there is initial evidence that the risk of disease transmission is negligible provided that the embryos are properly handled but for which additional information both in vitro and in vivo is required. *Rinderpest virus*, *Mycobacterium Paratuberculosis*, BSE, *Campylobacter fetus* foetus, *Neospora caninum*, *Haemophilus somnus*, and BVDV are included in this category. Category 4 includes the remainder of diseases in which there has been some preliminary work.

The four categories only consider in vivo produced embryos and not in vitro produced (IVP) embryos. Even though the Society has included in the IETS manual, recommendations for handling IVP embryos, there is preliminary evidence indicating that the recommendations for handling in vivo produced embryos are not as efficient in removing pathogens from the ZP of IVP embryos.\textsuperscript{15}

**Treatments to remove pathogens attached to the ZP**

As mentioned previously, it is more likely for pathogens to become attached to the ZP than to penetrate it. Therefore, it would be feasible to treat the ZP to eliminate or reduce the number of pathogens that are attached. One of the first approaches reported was to wash the embryos at least 10 times in a buffered solution such as Dulbecco’s phosphate buffered saline (dPBS). This treatment was effective in removing or reducing the number of pathogens carried by the embryo. For example, using this protocol, BVDV attached to the ZP of in vivo embryos from infected donors can easily be removed.\textsuperscript{22} Consequently,
wearing the embryo became a standard recommendation of the IETS for handling of in vivo embryos and was also adopted by the Office International des Epizooties (OIE) for the international movement of embryos. The protocol required a 100-fold dilution between each of the 10 washes and a sterile pipette had to be used each time.23

Washing the embryo is also important in eliminating or reducing the risk of transmitting intracellular viruses that can be present in flushing fluids of donor cows. This is particularly important in diseases such as bovine leukemia in which the virus is more likely carried intracellularly in somatic cells accompanying the embryo. In a study conducted by Bielanski et al.23 ET was successfully used to produce seronegative calves from seropositive BLV donor cows.

Certain pathogens such as BHV-1, BHV-4, VSV, H. somnus and U. diversum, which are firmly attached to the ZP, cannot be removed by washing alone. Hence, other treatments aiming for the inactivation or dislodging of the agent have been explored. Among them, the use of trypsin and antibiotics are the most widely recognized and are currently recommended by the IETS and the OIE. The treatment of embryos with trypsin is usually intercalated with the 10 washes with dPBS. It has been shown to be effective for inactivating or removing BHV-1, BHV-4 and VSV.24,25 On the other hand, pathogens such as H. somnus, U. diversum and Mycoplasma are not removed by trypsin treatment.23

Washing IVP embryos is beneficial in reducing the number of infectious particles that can be present in the fluids used for IVM, IVF or IVC but it is not totally effective. Pathogens such as BHV-1 become firmly attached to the ZP of IVP embryos and cannot effectively be removed by washing alone or with trypsin treatment.23 Therefore, the recommended IETS protocol for removing certain pathogens from in vivo produced embryos may not be adequate in the case of IVP embryos.

The use of antibiotics in the flushing and holding media, can effectively eliminate pathogens such as H. somnus from bovine embryos. Preimplantation bovine embryos were exposed in vitro to H. somnus to determine the effect of H. somnus on in vitro embryonic development. After exposure to H. somnus and before washing, some of the ZP intact embryos were held in antibiotic containing medium. H. somnus was not recovered from any of the 32 antibiotic-treated embryos. The coculture system was not compatible with normal embryonic development, and all embryos had begun to degenerate by the end of the 18-hour exposure period.23 This evidence supports the use of broad spectrum antibiotics not only to control bacterial contamination but also infectious bacterial diseases that can be transmitted from one animal to another. In any given situation, bacteria have to be sensitive to the antibiotics present in the media, which is not always the case.

The use of antisera to inactivate a particular pathogen has also been reported. Monoclonal antibodies were successfully used to inactivate BHV-1 from oocytes exposed during IVM. This procedure yielded 88% of the embryos free of the virus whereas all the controls were positive.26 Antibodies could also be present in animal products used during ET procedures as well as during the in vitro production of embryos. Although this may be an inconvenience in the detection of positively infected samples by serological status, it may be advantageous due to the potential in inactivating pathogens. In one study, BVDV antibodies were found in serum used in IVM and IVC but not in another media.

Unfortunately, the virus persisted in the system despite the presence of neutralizing antibody in the maturation and culture media, and both washing and trypsin treatment were ineffective for removal of the virus.27

Chen and Wrathal, reviewed other possible treatments that could remove a pathogen from its attachment to the ZP. The use of detergents or other enzymes such as glycosidase could potentially dislodge pathogens from the ZP without harming the embryo. Similarly, substances such as alpha-mannoside that can affect viral glycoproteins, or lectins that can block the binding sites of the glycosylated surfaces of the virions or the ZP, could be explored.

Conclusion

In vivo produced embryos can be effectively treated for the removal of pathogens if the IETS protocol is followed. This means that embryos can be used in embryo transfer programs both within a country and between countries with relatively low risk of disease transmission. On the other hand, there is no approved treatment that effectively eliminates pathogens from the ZP. In Vivo Produced embryos and further work is needed to assess the effectiveness of other alternative treatments as well as the effect that such treatments may have on embryo and fetal viability.

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Conflict of interest

The author declares no conflict of interest.

References


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