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PATHOGENS ASSOCIATED WITH MAMMALIAN EMBRYO (A Review)

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The advantages of transporting mammalian preimplantation embryos rather than postnatal living animals include reduced costs of transportation and rapid dissemination of genetic material between countries. However, the risk of transmission of diseases through the embryos must be considered. The disease control potential of embryos will depend on proper handling and washing, and the integrity of zona pellucida. Researches on embryo-pathogen interactions have shown that some pathogens are carried through the gametes and others could not infect the gametes. Some pathogens were found to adhere to the zona pellucida and others could penetrate the zona pellucida. To date, data presented appear to suggest no concrete model guidelines for embryo-pathogen interaction. The interaction seems to depend on the species and the pathogen involved.

Key words: embryo-pathogen, zona pellucida, gamete, bacteria, virus

INTRODUCTION

Recent technological advances for livestock improvement have created a demand for the use of embryo transfer (ET) to move genetic material between countries.⁵⁰⁾ The number of embryos from domestic animals, especially bovines moved internationally, continues to increase. Realizing the potential of ET for rapid, cheap and widespread dissemination of genetic material, the risk of introducing exotic disease must be considered.²⁾ Hence, a limiting factor that will hinder the efficient use of ET will be the uncertainty concerning the potential for the transmission of pathogens through the embryos.⁴⁾

Relatively little attention has been given to the potential role of ET in the control of infectious diseases.³⁷⁾ However, it has been recommended as a useful technique for the introduction of disease-free animals in established closed herds.¹⁷⁾ Similarly, there has been a limited amount of work regarding the mode of transmission of prenatal infections in domestic animals, but few viruses have been implicated in the

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infection of gametes.²⁰⁾ To date, data on diseases have been accumulated to indicate that the risk for disease transmission through the embryo is minimal or nonexistent provided that the embryos are handled properly. For other diseases, the data gathered would not support a conclusive statement. For some diseases, no work has been done to indicate transmissibility by the embryo. The purpose of this paper, therefore, is to investigate the extent of research activities that have been conducted about diseases that are associated with embryos and present a research proposal relevant to the topic discussed.

MODES OF TRANSMISSION

SINGH and HARE⁴¹⁾ reported that the potential of embryos to transmit infectious disease is considerably less than that of semen or live animals. Disease transmission through embryos would only occur when the pathogenic organism was carried in the gametes so that infection would be possible at fertilization. In addition, the developing embryo may be infected when the organism is present in the reproductive tract of its mother.

According to HARE,²⁷⁾ pathogens can be transmitted through ET if they are either within or on the embryo when it is transferred, or in the fluids transferred with the embryo. Direct embryo-pathogen association occurs under the following circumstances: (1) if the pathogen is in the ovum at the time of fertilization and capable of expressing itself in the embryo or fetus during its development, or in the animal after birth, (2) if the pathogen is carried into the ovum at fertilization either within or attached to the spermatozoa and is subsequently capable of infecting the embryo or fetus, (3) if the pathogen passes through, becomes embedded in, or adheres to the surface of the zona pellucida (ZP), and (4) if the pathogen reaches the embryo as a result of damage to the ZP due to handling or manipulation.

Evidence has shown the capability of integration of ectropic class of endogenous C-type viruses and the potential integration of exogenous C-type viruses into the genome of the mouse and their consequent expression at a later stage of embryonic or fetal development. It was also shown that the lymphocytic choriomeningitis virus can be present in mouse oocytes, and evidence for transmission of the disease via the ovum was demonstrated.³³⁾ Evidence has also been presented for the presence of bluetongue virus within the apical crests of spermatozoa from the bluetongue-infected bull, which would provide an opportunity for the virus to be carried into the ovum.²²⁾

Any pathogen passing into the embryo would be capable of infecting the embryo or, if it was in or on the surface of the ZP, it would be capable of infecting the recipient animal when the embryo is hatched. In studies conducted *in vitro*, certain viruses like bovine viral diarrhea virus¹⁾ and meningoencephalitis virus^{25,26)} are able to penetrate the ZP and infect the embryo. But, bovine parvovirus,¹⁰⁾ and Newcastle disease virus²⁴⁾ cannot penetrate the ZP and, therefore, these viruses cannot be

transmitted via embryos.

It has been shown that the ZP acts as a protective barrier for the embryo, and that the organisms attached to it can or cannot be removed through washings.⁸⁾ However, some viruses can be removed or inactivated by exposure of the embryo to trypsin or antiserum.⁴⁰⁾ Disease transmission through ET via fluids transferred with the embryo should not occur provided that the embryo is removed from the collecting fluid and washed several times in sterile medium prior to transfer. It has been shown that several embryos with viruses removed by washings can be transferred without transmitting infection.^{9,51)}

Researches on laboratory animals have shown that the pathogens vary as to whether or not they are capable of passing through the ZP or are transmissible by the gametes. Relating these to domestic animals, ET, therefore, could be used as a means of controlling or eliminating disease in a herd if the causal agent does not infect the early embryo via the gamete or by penetrating the ZP.

EMBRYO-PATHOGEN INTERACTION

Since the ZP has been shown to be an effective barrier against certain viruses, it is unlikely that the ZP would allow the passage of bacterial or fungal agents. In addition, there is an antibiotic added to uterine flushings and the culture media that would hinder the growth and development of bacteria and fungi. Thus, transmissible disease agents of concern in ET are likely to be viral rather than bacterial in nature.

Experiments on embryo-pathogen interactions were done with viruses and embryos of laboratory animals. Results from these studies indicated that there were no obvious guidelines as to what types of viruses can infect embryos and transmit disease and that each virus of concern in every species would have to be dealt with independently.

It was noted that only a few viruses have been implicated in the infection of the oocytes and that most viruses found in semen are in seminal fluid rather than within the sperm cell. It is likely that most infectious agents found in early embryos originate from the environment (uterus and oviduct) or through the ZP.

It was suggested by BOLIN et al.⁹⁾ that there are 3 possible mechanisms for a virus to interact with the ZP based on electron microscopic studies of porcine embryos exposed to several different viruses; namely: (1) virus interaction with receptors on the ZP, (2) virus interaction with cell debris associated with the ZP, and (3) virus entanglement in pores or sperm tracts of the ZP.

It is therefore, wise to investigate further the area of the ZP-pathogen interaction since it was mentioned previously that some viruses can be removed from the ZP by washing and some cannot. In which species viruses can be washed out and in which ones they remain in the ZP is a matter for careful investigation. In addition, since little work has been done on species difference in the composition and structure of the

ZP, it is necessary to elucidate further the factors that are involved (e.g., size of the ZP trabeculae or spaces, receptor sites, etc.) in determining whether or not a pathogen passes through, becomes enmeshed in, or becomes firmly adherent to the surface of the ZP.

PATHOGENS THAT CAN INFECT THE GAMETES

Through electron microscopic studies, immunocytochemical demonstration of antigen, autoradiography and the use of conventional techniques of cell culture, pathogens that are associated with oocytes, spermatozoa and early embryos were able to be investigated.

Some RNA viruses, particularly the endogenous onco-viruses, appear to be transmitted via the gametes from parent to offspring in mice.^{5,15)} Lymphocytic choriomeningitis virus has been demonstrated in mouse oocytes and embryos.³³⁾ Spermatozoa infected with simian virus (SV40 DNA) have been found to be transmitted to the zona-free, 2-cell and morula-stage mouse embryos.³⁾ After infection, the morula stage developed normally, but, there was degeneration of the 2-cell-stage embryos. It was found that the virus cannot penetrate the ZP.

Bluetongue virus (BTV) was isolated from semen of a bull with nonclinical persistent infection up to 300 days after exposure. However, in studies on preimplantation embryos from mice and cattle, BTV did not penetrate the intact ZP.¹²⁾ Results obtained in sheep confirmed that BTV can result in intrauterine infection and that embryo-recipient ewes can be infected by embryos exposed *in vitro* to the virus or those recovered from viremic donors.²³⁾

PATHOGENS THAT DO NOT INFECT THE GAMETES

Infectious diseases where the causative agent does not infect the gamete are potential candidates for control or eradication by embryo transfer, provided that these agents cannot infect the embryo through its environment.

Bovine leukemia virus (BLV) has been shown not to transmit disease via embryo transfer.¹⁸⁾ It was also demonstrated that semen and ova or embryos from BLV-infected cattle are not predisposed to infection by BLV.³⁰⁾ When spermatozoa were exposed to murine cytomegalovirus (MCMV) there was reduced capability for fertilization.³⁵⁾ However, it was shown that ova fertilized by these spermatozoa did not become productively infected. It has also been demonstrated that MCMV is not transmitted to the embryo by infected females bred with uninfected males.³⁴⁾ Sendai virus was also investigated and found to have no significant effect on the production of normal embryos or on the outcome of embryo transfer in mice.¹⁶⁾ However, this virus has been shown to be adsorbed to the sperm acrosome in the rabbit.²¹⁾ Since the outer membrane and acrosome contents of spermatozoa are lost prior to sperm penetration into the ZP, it is unlikely that the embryo would be infected. Likewise,

simian virus has been shown to be adsorbed on rabbit spermatozoa but not transmitted.³⁾

PATHOGENS THAT PENETRATE THE ZONA PELLUCIDA

In studies reported by ARCHBALD et al.,¹⁾ injection of bovine viral diarrhea (BVD) virus into the uterine horn of the bovine caused degeneration of the embryos. Electron microscopic examination of degenerated embryos revealed the presence of a structure which morphologically resembled the BVD virus. The results in this report indicate that the BVD virus within the uterine horns may interfere with the normal development of preimplantation bovine embryos. On the other hand, when BVD virus uptake by embryos was investigated in vitro, there was no significant inactivation of the virus during the incubation period and uptake by the embryos could not be detected by several assay systems.³⁶⁾ It is suggested that the lack of viral infectivity of the embryos may have been due to adverse effects of the experimental environmental conditions on the viral-embryo interaction.

Sendai virus was demonstrated in morulae of infected mouse colonies.¹⁴⁾ The virus replicated in mouse cleavage-stage embryos following exposure of zona-free embryos to virus. However, there was no viral replication when zona-intact embryos were exposed to the virus. In an in vivo study, CARTHEW et al.¹⁶⁾ reported that preimplantation embryos collected from mice in the acute phase of Sendai virus infection were not infected. Transfer of embryos from infected donors did not transmit the virus to the recipient foster mothers or to their fetuses.

PATHOGENS THAT CANNOT PENETRATE THE ZONA PELLUCIDA

Porcine parvovirus (PPV) has been found not to penetrate the ZP;⁵⁴⁾ however, it has been shown to adhere to the ZP and on transfer of the embryo to seronegative recipient animals, it subsequently infects the recipients and retards or kills a majority of the embryos.⁵⁵⁾ Bovine parvovirus (BPV) has been found not to replicate in zona-free bovine embryos.¹⁰⁾ Infection of unfertilized ova, 2-cell and morula-stage mouse embryos showed no alteration of their normal development and the virus could not penetrate the ZP.³⁾ In embryos exposed to MCMV, particles were seen beneath the ZP. However, no viral replication was noted. The embryos developed normally. The same results were reported when zona-free mouse embryos were exposed to MCMV.

In infection with Newcastle disease (NCD) virus, it was shown that in zona-intact mouse embryos the virus did not penetrate the ZP;²⁴⁾ however, when NCD virus was injected directly into blastocoele, only the trophoblast cells were infected. No infection in porcine zona-intact embryos was noted with pseudorabies virus (PrV) exposure,^{7,8)} but when porcine embryos were incubated on cell monolayers that had been previously inoculated with pseudorabies, it was found that PrV was adsorbed and

entered the sperm tracts in the ZP.⁹⁾

Infection of zona-free, 2-cell and morula-stage mouse embryos with simian virus (SV40) showed no deleterious results on their development.³⁾

EMBRYO-PATHOGEN INTERACTION IN FARM ANIMALS

CATTLE

Most of the experiments conducted in embryo-pathogen interaction in the bovine dealt with *in vitro* and *in vivo* methods using zona-intact embryos from infected donors that were properly washed before being transferred *in vivo*. The following is a brief review of the researches that have been carried out:

1. Bovine leukemia virus (BLV). So far, there have been 480 transfers carried out with embryos from BLV-positive donors to BLV-seronegative recipients.³⁸⁾ All of the recipients and all of the 143 calves produced from these transfers have remained BLV-seronegative. Zona-intact embryos from infected donors do not transmit the virus when properly washed.²⁸⁾

2. Bluetongue virus (BTV). Zona-intact embryos from viremic donors were washed and transferred to uninfected recipients. No recipient seroconversions were found.¹¹⁾ Assays and virus isolation from calves were negative. In addition, BTV did not infect zona-intact embryos exposed *in vitro* to BTV.¹²⁾

3. Infectious bovine rhinotracheitis virus (IBRV). Attempts to isolate IBRV from zona-intact embryos from IBRV-seropositive donors have proved unsuccessful. However, when embryos were exposed to tissue culture and then washed, approximately 65% of the embryos retained the virus though their development was not affected. Both trypsin and IBRV-antiserum were effective in removing the infectious IBRV from the embryos.³⁸⁾ Zona-intact embryos from IBRV shedders do not transmit the disease when washed and trypsin-treated before transfer.^{42,47)} On the other hand, when zona-micromanipulated embryos with zonae that were either damaged or removed were exposed to IBRV *in vitro*, results showed no significant difference in embryonic survival of the zona-cracked embryos exposed to IBRV and control embryos not exposed to IBRV.⁶⁾ However, there was a significant difference ($P < 0.001$) in the survival of zona-free embryos not exposed to IBRV (30% vs 80%).

4. Bovine viral diarrhea virus (BVDV). Preimplantation bovine embryos were exposed to BVDV to determine whether the virus had any effect on embryonic development and to allow viral replication to occur.⁴⁰⁾ No infectious virus was isolated from any of the embryos. *In vitro* development of virus-exposed embryos proceeded normally.³⁶⁾

5. Foot and mouth disease virus (FMDV). When zona-intact bovine embryos were exposed to FMDV and then washed, no infectious virus was detected on any of the embryos.⁴³⁾ FMD viral infectivity was found, however, in association with zona-free bovine embryos and a small number of zona-intact porcine embryos. FMDV

infectivity detectable in cell cultures or by animal inoculation was not found to be associated with any of the washed zona-intact embryos collected from cattle during the acute stage of the disease.³²⁾

6. *Brucella abortus*. Brucellae were not isolated from zona-intact embryos or from any washing beyond the sixth serial wash. Evidence tends to indicate that, provided embryos are washed, this agent is unlikely to be transmitted during embryo transfer procedures.^{48,49)}

7. Bovine parvovirus (BPV). Zona-free morula-stage embryos continued to develop normally after exposure to BPV and showed no evidence of embryonic infection when they were examined by electron microscopy.¹⁴⁾

8. Akabane virus (AV). Zona-intact bovine embryos exposed to AV for 1 to 24 hours were negative when assayed for infection. AV also had no effect on embryonic development compared with controls.⁴⁰⁾

9. *Hemophilus somnus*. Zona-intact embryos exposed to *H. somnus*, washed or exposed to antibiotic, washed and then assayed showed that this procedure removes *H. somnus* from embryos, but exposure in utero is detrimental to embryos.³¹⁾

10. *Chlamydia psittaci*. Zona-intact embryos exposed in utero to *C. psittaci* via contaminated semen showed no significant effect on embryo development. Chlamydial inclusions were not found in embryos.¹³⁾

SWINE

1. African swine fever virus (ASFV). The virus was detected in zona-intact porcine embryos that had been exposed, washed and cultured.³⁹⁾ Ninety-five percent of the embryos retained infectious virus after washing. Treating the embryos with papain, EDTA or ficin had no effect on the retained virus, whereas treating them with trypsin or pronase reduced the number of embryos carrying detectable virus to 30% instead of 95%, and lowered the amount of virus on the embryos. It has not yet been determined whether ASFV enters the embryonic cells but evidence suggests that most of the viruses, and possibly all of them, are bound to the ZP.

2. Porcine parvovirus (PPV). Porcine embryos were exposed to PPV in vitro for 4 days, washed and examined using immunofluorescence.⁵⁴⁾ All of the embryos had PPV adhering to the ZP. Likewise, when porcine embryos were exposed to PPV in vitro, washed and transferred to recipient pigs, disease transmission occurred.⁵⁵⁾

3. Hog cholera virus (HCV). Porcine embryos were exposed in vitro to HCV, washed and assayed. All of the embryos carried HCV and they were found to adhere to the ZP. When embryos were cultured and washed with trypsin, the virus could not be isolated from any of the embryos.¹⁹⁾

4. Foot and mouth disease virus (FMDV). When embryos were exposed in vitro to FMDV, washed and assayed, only 2~3% of the embryos carried FMDV. The virus adhered to the ZP when it was exposed to a high level of virus.⁴³⁾

5. Pseudorabies virus (PrV). Of the 257 embryos exposed to PrV in vitro, washed and assayed, some embryos (24%) carried PrV on the ZP.⁸⁾ When embryos (n=70) were collected from donors infected intrauterinely with PrV and transferred to uninfected recipients, disease transmission occurred in some recipients (40%).

6. Swine vesicular disease virus (SVDV). All of the embryos carried SVDV and the virus adhered to the ZP after it had been exposed to SVDV in vitro.⁴⁴⁾ No disease transmission occurred when embryos collected from SVDV-infected donors were transferred to uninfected recipients.⁴⁶⁾

7. Vesicular stomatitis virus (VSV). The virus was isolated from both porcine and bovine zona-intact embryos that had been exposed to VSV and then washed. When embryos were cultured for 24 hours after exposure and washing, the number of embryos carrying VSV and the amount of virus on each embryo was reduced. Trypsin (0.25%) was found to be effective in inactivating or removing the VSV from embryos, suggesting that most, if not all, of the viruses were bound to the ZP.⁴⁵⁾

SHEEP AND GOATS

There is a dearth of information regarding the embryo-pathogen interaction in these 2 species because the least amount of research has been done. Most of the pathogens reported involve unpublished data. The following are the published research works on bluetongue and caprine arthritis-encephalitis virus:

1. Bluetongue virus (BTV). Embryos recovered from seronegative superovulated donor ewes were incubated in vitro for 8 hours with BTV type 10. After incubation, the embryos were thoroughly rinsed by repeated transfer to sterile culture medium, and 12 of the embryos were then transferred to the uterus or oviduct of seronegative, synchronized recipients. Viremia and seroconversion were detected in 9 recipient ewes.²³⁾

2. Caprine arthritis-encephalitis virus (CAEV). Sixteen embryos from clinically affected, serologically positive donors were transferred to seronegative recipients in goats. This resulted in 2 pregnancies. One goat was stillborn and the other was alive. No virus was recovered from the stillborn kid and the live kid was seronegative for the virus beyond 4 months of age.⁵³⁾

SUMMARY

The pathogens that interact with the preimplantation embryos have been reviewed. The disease control potential of embryos will depend on proper handling and washing, and the integrity of the ZP. It should be pointed out that a pathogen, in order to be transmitted via an embryo, must be within the embryo, in the ZP or in the fluid in which the embryo is transferred. Embryonic infection occurs either via the gametes or via the environment.

Researches on embryo-pathogen interactions have shown that some pathogens are

carried through the gametes (Table 1), whereas others could not infect the gametes (Table 2). Some pathogens were found to adhere to the ZP pellucida but could be removed through proper washings. It was also reported that some penetrated the ZP (Table 3) and that others could not penetrate the ZP (Table 4). In this context, it is therefore suggested that the success of embryo transfer for disease control depends on the pathogenic organism and whether it can infect the gametes or the embryo prior to or during collection. Pathogens that are candidates for eradication using ET are those that cannot penetrate the ZP or, if these pathogens adhere to the ZP, that can be removed through proper washings.

Table 1. Pathogens that infect the gametes

Pathogen	Effect	Reference
Bluetongue virus	Infection of bull spermatozoa	Foster et al., 1980 ²²⁾
Lymphocytic chorio-meningitis virus	Demonstrated in mouse oocytes	Mims, 1966 ³³⁾
Oncovirus	Infection demonstrated in mouse oocytes and early embryo	Calarco and Szollosi, 1975 ¹⁵⁾
Simian vacuolating virus	Infected spermatozoa transmit the virus to mouse oocytes	Baranska et al., 1971 ³⁾

Table 2. Pathogens that do not infect the gametes

Pathogen	Effect	Reference
Bovine leukemia virus	Not transmitted via the gametes	Digiacomio et al., 1986 ¹⁸⁾
Murine cytomegalo-virus	Spermatozoa not infected when exposed; not transmitted to embryos when infected female mice were bred with uninfected males	Neighbour and Fraser, 1978 ³⁵⁾
Sendai virus	Virus adsorbed to acrosome of spermatozoa but transmission to egg is doubtful in mice	Carthew et al., 1983 ¹⁶⁾
Simian vacuolating virus	Adsorbed on rabbit spermatozoa but not transmitted	Baranska et al., 1971 ³⁾

Table 3. Pathogens that penetrate the zona pellucida

Pathogen	Classification (family)	Size (diameter)	Remarks	Reference
Bovine viral diarrhea virus	Togaviridae	50~65nm	Injection into the uterine horns caused degeneration of bovine embryos	Archbald et al., 1979 ¹⁾
Encephalomyocarditis virus (Mengo virus)	Picornaviridae	20~30nm	Penetrated the ZP and replica- ted in the 2-cell and morula- stage mouse embryos	Gwatkin, 1967 ²⁵⁾
Sendai virus	Paramyxoviridae	100~300nm	Demonstrated in morula of infected mouse embryo	Tuffrey et al., 1972 ⁵²⁾

Table 4. Pathogens that cannot penetrate the zona pellucida

Pathogen	Classification (family)	Size (diameter)	Remarks	Reference
Bovine parvovirus	Parvoviridae	20 nm	No replication in zona-free bovine embryos	Bowen, 1979 ¹⁰⁾
Moloney sarcoma virus	Retroviridae	100~120 nm	Virus did not penetrate the ZP of mouse embryo	Baranska et al., 1971 ³⁾
Murine cytomegalo virus	Herpesviridae	100 nm	Particles beneath the ZP but no replication in mouse embryos	Neighbour, 1978 ³⁴⁾
Newcastle disease virus	Paramyxoviridae	70~180 nm	No infection of the zona-intact mouse embryos	Glass et al., 1974 ²⁴⁾
Porcine parvo virus	Parvoviridae	20 nm	No infection of porcine embryos	Wrathall and Mengeling, 1979a ⁵⁴⁾
Pseudorabies virus	Herpesviridae	100~150 nm	No infection of porcine zona-intact embryos	Bolin et al., 1979 ⁷⁾
Simian vacuolating virus	Papovaviridae	45 nm	Inner cell mass remained free of the virus in mouse embryos	Baranska et al., 1971 ³⁾

Studies on embryo-pathogen interactions in farm animals have been continuously conducted. To date, results indicate that embryonic development is not affected by in vitro exposure to bluetongue virus, infectious bovine rhinotracheitis virus, bovine viral diarrhoea virus, foot and mouth disease virus, Akabane virus and bovine parvovirus. Embryos collected from bluetongue virus and bovine leukemia virus-infected donors when washed and transferred to uninfected recipients will not transmit these diseases. Bluetongue virus, bovine viral diarrhoea virus, foot and mouth disease virus and Akabane virus do not attach to the ZP under in vitro conditions and do not infect bovine embryos. Both trypsin and antiserum can remove the infectious bovine rhinotracheitis virus from the ZP. Bovine leukemia virus, infectious bovine rhinotracheitis virus, bovine viral diarrhoea virus and *B. abortus* have not been isolated from embryos (Table 5).

Table 5. Pathogens in farm animals (cattle)

Pathogen	Effect	Reference
Bovine leukemia virus	Zona-intact embryos from infected donors when properly washed do not transmit the virus	Hare et al., 1985 ²⁹⁾
Bluetongue virus	Assays and virus isolation were negative	Bowen et al., 1983 ¹¹⁾
Infectious bovine rhinotracheitis virus	Zona-intact embryos exposed to IBRV when washed and trypsin-treated do not transmit the disease	Singh et al., 1982b ⁴⁷⁾
Bovine viral diarrhoea virus	Infectious virus was not isolated from any of the embryos exposed to BVDV	Singh et al., 1982a ⁴⁴⁾
Foot and mouth disease virus	Zona-intact embryos from infected donors when washed do not transmit the virus	McVicar et al., 1986 ³²⁾
<i>Brucella abortus</i>	Zona-intact infected embryos that are washed do not transmit the disease	Stringfellow et al., 1984 ⁴⁹⁾
Akabane virus	Zona-intact embryos unlikely to transmit the disease	Singh et al., 1982a ⁴⁴⁾
<i>Hemophilus somnus</i>	Zona-intact infected embryos, antibiotic-treated and washed will not transmit the disease	Kaneene et al., 1986 ³¹⁾
<i>Chlamydia psittacci</i>	No effect on embryo development when agent exposed via semen	Bowen et al., 1978a ¹³⁾

Disease transmission occurred in pigs only when the pathogens were inoculated directly into the uterus of the donor just prior to embryo collection, and when the embryos had been exposed to large amounts of pathogens *in vitro*. African swine fever virus, hog cholera virus, foot and mouth disease virus, porcine parvovirus, vesicular stomatitis virus and swine vesicular disease virus were found to adhere to the ZP, but not necessarily to infect the embryo. No disease transmission was noted with hog cholera virus, pseudorabies virus and swine vesicular disease virus when infected embryos were transferred to uninfected recipients (Table 6).

Table 6. Pathogens in farm animals (swine, sheep and goats)

Pathogen	Effect	Reference
SWINE		
African swine fever	Virus adhered to the ZP	Singh et al., 1984 ³⁹⁾
Foot and mouth disease virus	Embryos exposed <i>in vitro</i> , 2~3% carried the virus	Singh et al., 1986 ⁴³⁾
Porcine parvovirus	Virus adhered to the ZP	Wrathall and Mengeling, 1979a ⁵⁴⁾
Hog cholera virus	No disease transmission	Dulac and Singh, 1988 ¹⁹⁾
Pseudorabies virus	Disease transmission occurred in some recipients	Bolin et al., 1982 ⁸⁾
Swine vesicular disease virus	Virus adhered to the ZP	Singh and Thomas, 1987a ⁴⁴⁾
Vesicular stomatitis virus	Virus adhered to the ZP	Singh and Thomas, 1987b ⁴⁵⁾
SHEEP and GOATS		
Bluetongue virus	Recipients became viremic	Gilbert et al., 1987 ²³⁾
Caprine arthritis-encephalitis virus	No virus recovery from offspring delivered from infected does	Wolfe et al., 1987 ⁵³⁾

The data presented appear to suggest no concrete model guidelines for embryo-pathogen association. The interaction seems to depend on the species and the pathogen involved. Hence, a good area for future research would be the study of the

interactions of specific pathogens that have not been investigated with specific species of animals via in vitro and in vivo methods. It would be worth investigating the role of the ZP in this interaction. In addition, another area to be studied is the mechanisms involved in the control, eradication and transmission of disease by embryo transfer. It should be determined whether or not particular pathogens are present in the reproductive tract in relation to the stage of the infection and the stage of reproductive cycle. The procedure for washing embryos should be elaborated and the factors that determine whether or not a pathogen passes through, is embedded in, or firmly adheres to the surface of the ZP, should be investigated in detail.

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REFERENCES

- 1) ARCHBALD, L. F., FULTON, R. W., SEGER, C. L., AL-BAGDADI, F. and GODKE, R. A. (1979): Effect of bovine viral diarrhoea virus on preimplantation bovine embryos. A preliminary study. *Theriogenology*, **11**, 81–89
- 2) ATWELL, J. K. (1987): The international movement of embryos and disease control: A regulatory perspective. *Theriogenology*, **27**, 5–20
- 3) BARANSKA, W., SAWICKI, W. and KOPROWSKI, H. (1971): Infection of mammalian unfertilized and fertilized ova with oncogenic viruses. *Nature*, **230**, 591–592
- 4) BETTERIDGE, K. J. and MITCHELL, D. (1977): Embryo transfer in farm animals: A review of techniques and applications. Monograph 16, Agriculture Canada, 66–68
- 5) BICZYSKO, W., SOLSTER, D., PIENKOWSKI, M. and KOPROWSKI, H. (1973): Virus particles in early mouse embryos. *J. Natl. Cancer Inst.*, **51**, 1041–1050
- 6) BIELANSKI, A., SINGH, E. L. and HARE, W. C. D. (1987): The in vitro exposure of bovine rhinotracheitis virus of zona pellucida micromanipulated bovine embryos with zona pellucida damaged or intact. *Theriogenology*, **28**, 495–501
- 7) BOLIN, S. R., RUNNELS, L. J., SAWYER, C. A., ATCHESON, K. J. and GUSTAFSON, D. P. (1979): Exposure of fertilized ova to pseudorabies virus. *Theriogenology*, **11**, 92 (Abstr.)
- 8) BOLIN, S. R., RUNNELS, L. J., SAWYER, C. A. and GUSTAFSON, D. P. (1982): Experimental transmission of pseudorabies virus in swine by embryo transfer. *Am. J. Vet. Res.*, **43**, 278–280
- 9) BOLIN, S. R., TUREK, J. J., RUNNELS, L. J. and GUSTAFSON, D. P. (1983): Pseudorabies virus, porcine parvovirus and porcine enterovirus interactions with zona pellucida of the porcine embryo. *Am. J. Vet. Res.*, **44**, 1036–1039
- 10) BOWEN, R. A. (1979): Viral infections of mammalian preimplantation embryos. *Theriogenology*, **11**, 5–15
- 11) BOWEN, R. A., HOWARD, T. H., ELSDEN, R. P. and SEIDEL, G. E. Jr. (1983):

- Embryo transfer from cattle infected with bluetongue virus. *Am. J. Vet. Res.*, **44**, 1625–1628
- 12) BOWEN, R. A., HOWARD, T. H. and PICKETT, B. W. (1982): Interaction of bluetongue virus with preimplantation embryos from mice and cattle. *Am. J. Vet. Res.*, **43**, 1907–1911
 - 13) BOWEN, R. A., SPEARS, P., STORZ, J. and SEIDEL, G. E. Jr. (1978a): Mechanism of infertility in genital tract infection due to *Chlamydia psittacci* transmitted through contaminated semen. *J. Infect. Dis.*, **138**, 95–98
 - 14) BOWEN, R. A., STORZ, J. and LEARY, J. (1978b): Interaction of viral pathogen with preimplantation mouse embryos. *Theriogenology*, **9**, 88 (Abstr.)
 - 15) CALARCO, P. and SZOLLOSI, D. (1975): Intercisternal A particle formation and inhibition in preimplantation mouse embryos. *Biol. Reprod.*, **12**, 448–454
 - 16) CARTHEW, P., WOOD, M. J. and KIRBY, C. (1983): Elimination of Sendai virus infection from mice by embryo transfer. *J. Reprod. Fert.*, **69**, 253–257
 - 17) CURNOCK, R. M., DAY, B. N. and DZIVK, P. J. (1976): Embryos in pigs: A method for introducing genetic material into primary specific-free herds. *Am. J. Vet. Res.*, **37**, 97–98
 - 18) DIGIACOMO, R. F., STUDER, E., EVERMANN, J. F. and EVERED, J. (1986): Embryo transfer and transmission of bovine leukosis virus in a dairy herd. *J. Am. Vet. Med. Assoc.*, **188**, 827–828
 - 19) DULAC, G. C. and SINGH, E. L. (1988): Embryo transfer as a means of controlling the transmission of viral infections. XII. The in vitro exposure of zona-intact porcine embryos to hog cholera virus. *Theriogenology*, **29**, 1335–1341
 - 20) EAGLESOME, M. D., HARE, W. C. D. and SINGH, E. L. (1980): Embryo transfer: A discussion on its potential for infectious disease control based on a review of studies on infection of gametes and early embryos by various agents. *Can. Vet. J.*, **21**, 106–112
 - 21) ERICSON, R. J., BUTHALA, D. A. and NORLAND, J. F. (1971): Fertilization of rabbit ova in vitro by sperm with adsorbed Sendai virus. *Science*, **173**, 54–55
 - 22) FOSTER, N. M., ALDERS, M. A., LUEDKE, A. J. and WALTON, T. E. (1980): Abnormalities and virus-like particles in spermatozoa from bulls latently infected with bluetongue virus. *Am. J. Vet. Res.*, **41**, 1045–1048
 - 23) GILBERT, R. O., COUBROUGH, R. I. and WEISS, K. E. (1987): The transmission of bluetongue virus by embryo transfer in sheep. *Theriogenology*, **27**, 527–541
 - 24) GLASS, R. H., CALARCO, P. G., LIN, T. P., FLORENCE, J. and OH., J. O. (1974): Development of mouse blastocyst following injection with Newcastle disease virus. *Biol. Reprod.*, **10**, 502–511
 - 25) GWATKIN, R. B. L. (1967): Passage of Mengovirus through zona pellucida of the mouse morula. *J. Reprod. Fert.*, **13**, 577–578
 - 26) GWATKIN, R. B. L. and AUERBACH, S. (1966): Synthesis of ribonucleic acid virus by mammalian ovum. *Nature*, **20**, 993–994
 - 27) HARE, W. C. D. (1984): Embryo transfer and disease transmission (an overview). *Proc. 10th Int. Cong. Anim. Reprod. & AI.*, Univ. of Illinois at Urbana-Champaign,

IX, 1-9

- 28) HARE, W. C. D. (1987): Cattle embryo-pathogen interaction. In: Int. Embryo Movement Symposium, IETS, XXXIII World Vet. Congress, Montreal, Canada, 117-122
- 29) HARE, W. C. D., MITCHELL, D., SINGH, E. L., BOUILLANT, A. M. P., EAGLESOME, M. D., RUCKERBAUER, G. M., BIELANSKI, A. and RANDALL, G. C. B. (1985): Embryo transfer in relation to bovine leukemia virus control and eradication. *Can. Vet. J.*, **26**, 231-234
- 30) KAJA, R. W. and OLSON, C. (1982): Non-infectivity of semen from bulls infected with bovine leukosis virus. *Theriogenology*, **18**, 107-112
- 31) KANEENE, J. B., COE, P. H., GIBSON, C. D., YAMINI, B., MARINEZ, R. O. and MORROW, D. A. (1986): The role of *Hemophilus somnus* in early embryonic death. I. The effect of the organism on embryos by day 8 postbreeding. *Theriogenology*, **26**, 189-198
- 32) McVICAR, J. W., SINGH, E. L., MEBUS, C. A. and HARE, W. C. D. (1986): Embryo transfer as a means of controlling the transmission of viral infections. VIII. Failure to detect foot and mouth disease viral infectivity associated with embryos collected from infected donor cattle. *Theriogenology*, **26**, 595-603
- 33) MIMS, C. A. (1966): Immunofluorescence study of the carrier state and mechanism of vertical transmission in lymphocytic choriomeningitis virus infection in mice. *J. Path. Bact.*, **91**, 395-402
- 34) NEIGHBOUR, P. A. (1978): Studies on the susceptibility of the mouse implantation embryo to infection with cytomegalovirus. *J. Reprod. Fert.*, **54**, 15-20
- 35) NEIGHBOUR, P. A. and FRASER, L. R. (1978): Murine cytomegalovirus and fertility potential in sexual transmission and the effect of the virus on the fertilization in vitro. *Fert. Steril.*, **30**, 216-222
- 36) POTTER, M. L., CORSTVET, R. E., LOONEY, C. R., FULTON, R. W., ARCHBALD, L. F. and GODKE, R. A. (1984): Evaluation of bovine diarrhea virus uptake by preimplantation embryos. *Am. J. Vet. Res.*, **45**, 1778-1780
- 37) ROWSON, L. (1971): The role of reproductive research in animal reproduction. *J. Reprod. Fert.*, **26**, 113-126
- 38) SINGH, E. L. (1984): Embryo-pathogen interactions in cattle. *Proc. 10th Int. Cong. Anim. Reprod. & AI., Univ. of Illinois at Urbana-Champaign*, **IX**, 17-23
- 39) SINGH, E. L., DULAC, G. C. and HARE, W. C. D. (1984): Embryo transfer as a means of controlling the transmission of viral infections. V. The in vitro exposure of zona-intact porcine embryos to African swine fever virus. *Theriogenology*, **22**, 693-700
- 40) SINGH, E. L., EAGLESOME, M. D., THOMAS, F. C., PAPP-VID, G. and HARE, W. C. D. (1982a): Embryo transfer as a means of controlling the transmission of viral infections. I. The in vitro exposure of preimplantation bovine embryos to Akabane, bluetongue and bovine viral diarrhea viruses. *Theriogenology*, **17**, 473-444
- 41) SINGH, E. L. and HARE, W. C. D. (1986): Embryo-pathogen interactions in relation to disease transmission. In: *Current Therapy in Theriogenology*, D. A. MORROW,

- ed., W. B. Saunders Co., 84–85
- 42) SINGH, E. L., HARE, W. C. D., THOMAS, F. C., EAGLESOME, M. D. and BIELANSKI, A. (1983): Embryo transfer as a means of controlling the transmission of viral infections. IV. Non-transmission of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis virus following trypsin treatment of the exposed embryo. *Theriogenology*, **20**, 169–178
 - 43) SINGH, E. L., McVICAR, J. W., HARE, W. C. D. and MEBUS, C. A. (1986): Embryo transfer as a means of controlling the transmission of viral infections. VII. The in vitro exposure of bovine and porcine embryos to foot and mouth disease virus. *Theriogenology*, **26**, 587–593
 - 44) SINGH, E. L. and THOMAS, F. C. (1987a): Embryo transfer as a means of controlling the transmission of viral infections. IX. The in vitro exposure of zona-intact porcine embryos to swine vesicular disease virus. *Theriogenology*, **27**, 443–449
 - 45) SINGH, E. L. and THOMAS, F. C. (1987b): Embryo transfer as a means of controlling the transmission of viral infections. XI. The in vitro exposure of bovine and porcine embryos to vesicular stomatitis virus. *Theriogenology*, **28**, 691–697
 - 46) SINGH, E. L., THOMAS, F. C., HARE, W. C. D. and EAGLESOME, M. D. (1987): Embryo transfer as a means of controlling the transmission of viral infections. X. The in vitro exposure of zona-intact porcine embryos to swine vesicular disease virus. *Theriogenology*, **27**, 451–457
 - 47) SINGH, E. L., THOMAS, F. C., PAPP-VID, G., EAGLESOME, M. D. and HARE, W. C. D. (1982b): Embryo transfer as a means of controlling the transmission of viral infections. II. The in vitro exposure of preimplantation bovine embryos to infectious bovine rhinotracheitis virus. *Theriogenology*, **18**, 133–139
 - 48) STRINGFELLOW, D. A., HOWELL, V. L. and SCHNURRENBERGER, P. A. (1982): Investigation into the potential for embryo transfer from *Brucella abortus*-infected cow without transmission of infection. *Theriogenology*, **18**, 733–743
 - 49) STRINGFELLOW, D. A., SCANLAN, C. M., BROWN, R. R., MEADOWS, G. B., GRAY, B. W. and YOUNG-WHITE, R. R. (1984): Culture of bovine embryos after in vitro culture to *Brucella abortus*. *Theriogenology*, **21**, 1005–1013
 - 50) STRINGFELLOW, D. A. and WRIGHT, J. C. (1987): Viewing the scientific evidence for the health status of embryos for international movement. In: *Int. Embryo Movement Symposium, IETS, XXXIII World Vet. Congress, Montreal, Canada*, 134–141
 - 51) THOMAS, F. C., SINGH, E. L. and HARE, W. C. D. (1983): Embryo transfer as a means of controlling the transmission of viral infections. III. Non-transmission of bluetongue virus from viremic cattle. *Theriogenology*, **19**, 425–431
 - 52) TUFFREY, M. M., SIZMAN, B. and BARNES, R. D. (1972): Sendai virus infection of mouse eggs. *Br. J. Exp. Path.*, **53**, 638–640
 - 53) WOLFE, D. F., NUSBAUM, K. E., LAUERMAN, L. H., MYSINGER, P. W., RIDDELL, M. G., PUTNAM, M. R., SHUMWAY, L. S. and POWE, T. A. (1987): Embryo transfer from goats seropositive for caprine arthritis-encephalitis virus. *Theriogenology*, **28**, 307–316
 - 54) WRATHALL, A. E. and MENGELING, W. L. (1979a): Effect of porcine parvovirus on

- the development of fertilized pig eggs in vitro. Br. Vet. J., 135, 249–254
- 55) WRATHALL, A. E. and MENGELING, W. L. (1979b): Effect of transferring parvovirus-infected fertilized pig eggs into seronegative gilts. Br. Vet. J., 135, 255–261

EXPLANATION OF PLATE

PLATE

- Fig. 1 A morula-stage bovine embryo showing its parts; zona pellucida (ZP), blastomeres (BM) and perivitteline cavity (PC), $\times 400$.
- Fig. 2 A blastocyst-stage bovine embryo showing its parts; zona pellucida (ZP), inner cell mass (ICM), blastocoele (BC) and trophoblast (TB), $\times 400$.
- Fig. 3 A diagram showing the important structures of an early embryo; zona pellucida (ZP), perivitteline cavity (PC) and blastomeres (BM). Surrounding the embryo is an illustration of the comparative sizes of bacteria; $4 \mu\text{m}$ (b); fungi, $30 \mu\text{m}$ (f); a small virus, 20 nm (sv) and a large virus, 300 nm (lv).

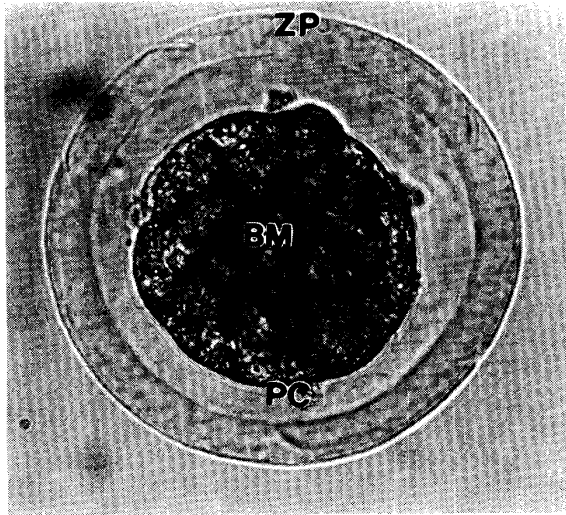


Fig. 1

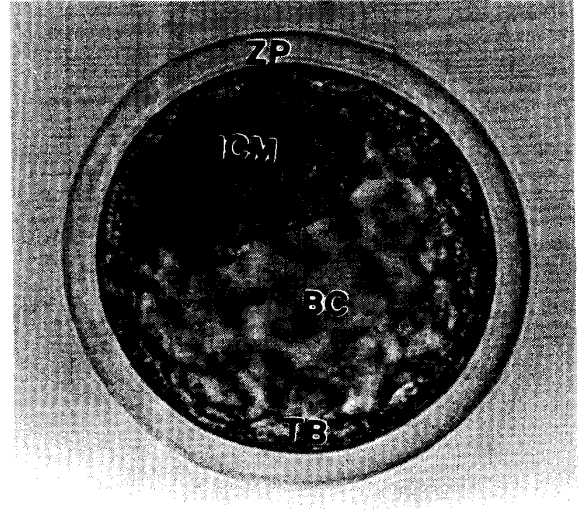


Fig. 2

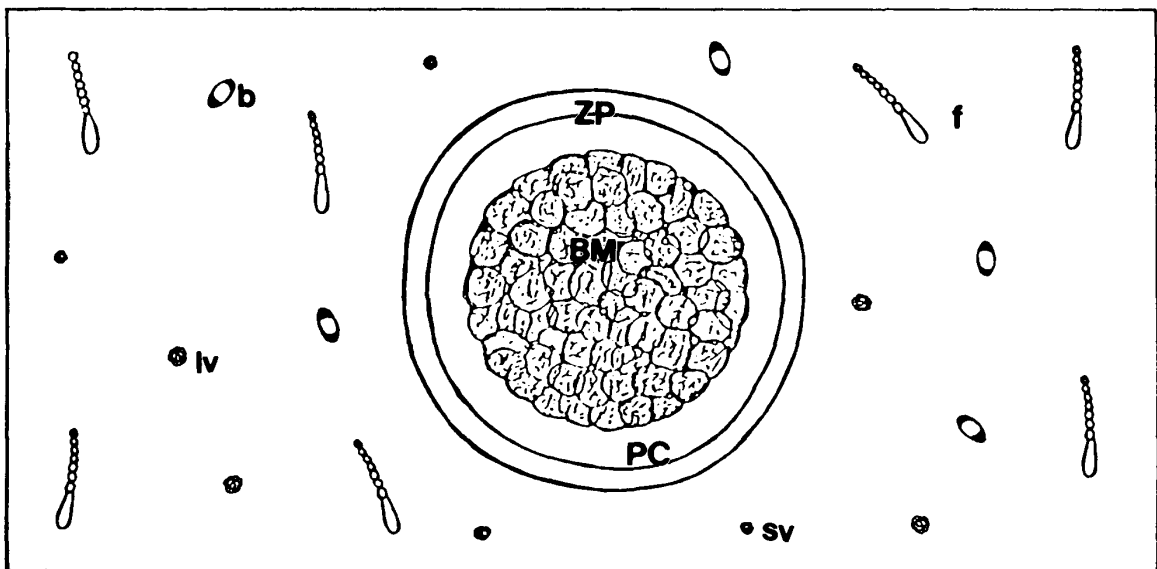


Fig. 3