Dairy Split Session III

Reproduction

Dr. Tom McDaniel, *Presiding*

Disease Transmission by Embryo Transfer in Cattle

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The number of calves born in North America from commercial embryo transfer has increased from zero in 1970 to approximately 100,000 in 1985. Improvements in basic embryo transfer procedures, as well as in ancillary techniques such as embryo freezing, have resulted in a significant demand for international transport of bovine embryos. Any technique for dispersing genetically valuable germplasm must also be shown to be relatively free of the risk of transmitting pathogenic microorganisms.

Today there are three methods that can be used for disseminating germplasm: transport of live animals, semen or embryos. The first and overriding priority of regulatory officials is to prevent ingress of disease; only secondarily are they charged with assisting individuals in genetic improvement programs. Although microbiologic and serologic screening can be used to aid in identifying and eliminating infected animals, most would agree that live animals can carry a variety of disease agents, often in a subclinical or latent form, and thus pose the most risk of these three techniques. A variety of viruses and bacteria have been isolated from bull semen, albeit often sporadically, and therefore, frozen semen is also viewed as a potential vector for disease spread.

There are a number of reasons to suspect that transfer of embryos can be conducted with minimal risk of disease transmission, but this attitude is currently supported by relatively little research and even less experience (7). Nevertheless, a strong case can be made for beginning to exploit the theoretical advantages of embryo transfer while maintaining classical methods of disease surveillance that are used for monitoring movement of live animals and semen.

Routes of Exposure and Transmission

The most direct route by which embryos can become infected with viruses is via contamination of either the oocyte or fertilizing spermatozoon. In mice, transmission of both endogenous and exogenous viruses via germ cell contamination has been demonstrated. Examples include the mouse leukemia viruses and lymphocytic choriomeningitis virus (12, 17, 22). In cattle, endogenous viruses have not been described. Structural abnormalities and virus-like particles observed in the spermatozoa of bulls from which bluetongue virus was isolated have been interpreted to represent the presence of virus in bull sperm (9), but unequivocal evidence for this phenomenon is lacking.

In contrast to actual carriage of viruses in bull spermatozoa, several agents have been isolated from semen. Embryos resulting from insemination with virus-contaminated semen could be infected either by adsorption of virus to the fertilizing sperm or, more likely, from establishment of a local infection in the maternal reproductive tract. Infection of the embryo by virus in the uterus or oviduct could also result from a systemic infection in the female.

A final route of exposure that is of unique concern in embryo transfer is the possibility that viruses would be present in the medium used to flush and manipulate embryos. The media used in embryo transfer are almost always supplemented with bovine serum. Bovine virus diarrhea virus has frequently been isolated from both fetal and adult semen, but the risk that this virus poses for the embryo is not known. Contamination of serum-supplemented media is of great concern when thinking of importing embryos into the United States from countries in which diseases such as foot and mouth disease are endemic; the best policy is such cases would be for the importing country to provide the serum or to utilize media supplemented with non-animal-derived macromolecules.

Aspects of Embryo Transfer Relevant to Disease Transmission

There is a profound lack of information about virus-

embryo interactions in vivo, particularly in cattle, primarily because this is a particularly difficult area to study. Consequently, in the absence of definitive evidence to the contrary, it is often necessarily assumed that embryos collected from cows that are infected with a certain disease agent are themselves potentially infected. There are several techniques that can be used to provide additional confidence that embryos will not be a vector of disease spread. Classical tools for prevention of disease transmission, including serology and virus isolation, are used to provide some assurance that the donor cow is free from infection. The availability of techniques for freezing embryos provides a means for holding the embryo while further post-collection testing the donor is completed, as is often done with with bulls and frozen semen to assure that the donor was not in the incubation stages of disease at the time semen or embryos were harvested. A final safeguard that can be applied with embryo transfer is to hold and test the recipient animals in quarantine for a period of time prior to their release into national herds.

An often-cited advantage of embryos over both live animals and semen is that the embryos can be "washed" to remove or at least greatly reduce the quantity of microorganisms that are potentially present in their environment prior to transfer. Washing embryos through several changes of fresh medium should indeed greatly limit the potential for transmission of cell-associated agents such as bluetongue virus and bovine leukemia virus. However, a caveat concerning washing is that some agents may stick to the zona pellucida and be resistant to removal; if this were to occur, virus on the zona pellucida could infect the recipient, but the significance of this problem in vivo is unknown.

The question of whether viruses can penetrate the intact zona pellucida and infect the embryo before hatching has been examined in a number of reports, with the tentative conclusion that the zona pellucida is a very effective barrier to viruses contacting the early embryo. One exception to this observation is that Mengo virus (a picornavirus) can apparently traverse the zona pellucida and infect early mouse embryos (11).

Research on bovine virus diseases in the context of embryo transfer

The viruses most adequately studied in the context of bovine embryo transfer are bluetonue virus, bovine leukemia virus and infectious bovine rhinotracheitis virus (IBRV). Other viruses that have been examined more superficially, include Akabane (19), bovine syncytial virus (2), BVD virus (1, 10, 19) and parainfluenza type 3 virus (6).

IBR virus has been relatively well studied with regard to its interaction with early embryos, both in vitro and in vivo. Hatched bovine blastocysts were readily infected in vitro with each of four strains of IBRV, as demonstrated by both EM and virus titration; embryos infected with IBRV underwent rapid degeneration (5). When younger bovine embryos with intact zonae pellucidae were exposed to IBRV in vitro, the embryos did not become infected and continued to develop normally (20). However, even extensive washing of such embryos failed to remove residual virus, indicating that IBRV would readily adhere to the zona pellucida; treatment with trypsin or antiserum to the virus was found to remove or inactivate such adsorbed virus (20). IBRV can also infect the hatched embryo in vivo, as shown by ultrastructural demonstration of replicating virus in a degenerating embryo collected from a heifer inoculated intravenously 7 days after breeding (16). Aside from direct infection of the embryo, IBRV may also cause early embryonic mortality by at least two other mechanisms. Intrauterine inoculation of virus at estrus can induce a necrotizing endometritis, which would adversely effect the embryo's environment, and the corpus luteum can become infected, with consequent luteal dysfunction (14, 15, 25).

One trial has been conducted to directly examine that risk of transmitting IBRV by embryo transfer (21). Embryos with intact zonae were recovered from cows inoculated with IBRV by intranasal, intravaginal or intrauterine routes, washed and treated with trypsin, and then transferred to seronegative recipients. Despite isolation of IBRV from the medium used to flush 10 of the 22 donors, none of the recipients or calves became infected, and virus was not isolated from any of the embryos or unfertilized oocytes that were not transferred.

Bovine leukemia virus is an exogenous retrovirus of cattle that infects a large number of cattle in this and other countries. There is no evidence that BLV is transmitted by germ-cell contamination, but there has been considerable concern that BLV could be transmitted by embryo transfer due to the near impossibility of recovering embryos free from some degree of contamination with blood. It is likely that BLV-infected lymphocytes are present in the recovered flushing medium from every infected cow, but standard techniques of washing embryos multiple times would almost certainly reduce the number of lymphocytes in the transfer volume dramatically. However, the possibility that free virus might be present in the uterine lumen cannot be totally eliminated at this time. Attempts to isolate BLV from early bovine embryos (zona-intact) and from unfertilized oocytes have failed (2). In those studies in which transmission of BLV by embryo transfer was investigated, several hundred bovine embryos collected from infected donor cows have been transferred to uninfected recipients without any evidence of transmission to the recipient and/or the resulting calves (8, 13, 18). It would thus appear that, using standard techniques for embryo transfer, the risk of transmitting BLV is minimal.

Bluetongue virus has been studied with regard to its ability to infect early bovine embryos and its potential for transmission by embryo transfer. Bovine morulae and early blastocysts exposed in vitro to BTV did not become infected if surrounded by an intact zona pellucida (3, 19), but were infected and destroyed if the zona pellucida was removed

(3). Two trials have been conducted in which donor cattle were infected with BTV (several serotypes represented) so that they were viremic when embryos were recovered. In one trial, virus was isolated from the flusing medium recovered from 11 of 20 donors, but none of the 39 recipients that received embryos (both zona-intact and hatched) seroconverted or became detectably viremic within 60 days after transfer (4). In the other experiment, BTV was isolated from one of 10 samples of flushing medium, but none of 28 recipients developed antibody to BTV following transfer, nor was antibody or virus detected in 14 calves after parturition (23). These studies indicate that embryos recovered from viremic donor cattle can be transferred without transmission of the virus. However, even with the relatively large number of embryos involved in these two trials, the upper limit of a 95% confidence interval for transmission is approximately 0.05 (5 transmissions per 100 transfers), which is not yet too comforting of a statistic. In interpreting this statistic however, it should be recognized that the donor cattle in these studies would have been easily detected as being infected by standard virologic procedures.

Conclusions

Any method of disseminating germplasm must be viewed in terms of risk versus benefit. Embryo transfer has some intrinsic advantages over transport of either live animals or semen, particularly for some disease agents, but these factors have been counterbalanced by our current ignorance concerning the possible interactions between disease agents and early embryos in natural situations. Recent studies dealing with transfer of embryos from cattle known to be infected with IBRV, BLV or BTV have failed to demonstrate disease transmission, and are thus very encouraging.

Additional research, particularly in the form of large field studies, is required to increase our confidence that transfer of genetically valuable embryos can be conducted with very minimal risk of transmitting infectious disease agents.

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