The potential of bovine embryo transfer for infectious disease control*

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Summary: The capability to collect preimplantation embryos, store them in the frozen state for extended periods of time, and subsequently thaw and successfully transfer them provides a new alternative to traditional means of improving blood lines. Research to date involving specific bovine pathogens has shown that this method of moving genetic material can be a valuable tool to those concerned with preventing the introduction of infectious disease into previously uninfected populations of cattle. In addition, an assessment of basic epidemiological factors involved in the process of embryo transfer leads to the conclusion that the movement of embryos is an innately safer process.

The embryo is an unlikely host for many pathogens because of primary resistance provided by the zona pellucida (ZP) and possibly the trophectoderm and secondary resistance factors such as young age, small size, and limited mobility.

The fact that many of the bacterial and viral pathogens have been shown to be too large to penetrate the ZP and that some do not survive well in embryo support media further reduces the possibility of an infectious agent being spread by embryo transfer.

Environmental factors involved in embryo transfer procedures are perhaps the most important to consider. The donor environment limits exposure to infectious agents and provides testing opportunities before, during, and after collection of embryos.

Dilution factors, mechanical washing, antimicrobial agents, and enzyme treatments may be provided to the embryo in its in vitro environment. Additionally, there is the possibility for visualizing the embryo in its entirety under the microscope and exerting control over the sterility of its surroundings.

The recipient animal provides the last safeguard since she represents a containment facility for the newly transferred embryo and can serve as a sentinel for the presence of any infectious agent.

KEY-WORDS: Bacteria - Cow - Disease control - Disease resistance - Embryo - Environment - Testing procedures - Transplantation - Viruses.

INTRODUCTION

Bovine embryos can now be collected nonsurgically from donor females, stored in the frozen state for extended periods, and transferred surgically or nonsurgically

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to recipients resulting in the birth of normal calves with a high degree of success (1). When this technology became available, it was suggested that the transport of embryos might be used as an alternative method to shipping livestock internationally (2). The economic and humane advantages of shipping flasks of frozen pre-implantation embryos as opposed to truck-, airplane-, or boat-loads of mature animals are obvious; however, early studies with laboratory animal species created concerns about the possibility of embryo transfer being a mode of transmission for livestock pathogens (3). With these concerns as the impetus, numerous studies have been conducted in recent years to investigate the potential for transmission of disease producing agents through embryo transfer. The results of these studies, involving specific agents have been summarized recently (4, 5) and lead to the conclusion that embryo transfer can be safer than traditional means of shipment of genetic material.

In addition to these research findings involving specific agent-embryo interactions there are sound epidemiological factors which indicate that the movement of embryos is innately safer than the movement of cattle. The purpose of this paper is to describe those agent, host and environmental factors which cumulatively support the use of embryo transfer as a means of controlling the spread of infectious diseases in national and international livestock populations.

HOST FACTORS

Host determinants of infectious diseases are categorized as intrinsic and extrinsic factors (6). In a consideration of the epidemiological aspects of bovine embryo transfer, the intrinsic and extrinsic factors for three potential hosts of disease, the donor, the embryo, and the recipient are of significance, but should be considered separately. For our purposes the embryo is considered the host of primary concern. The donor and recipient animals will be considered under environmental factors and testing possibilities. Determinants of disease of primary importance in the embryonic host are intrinsic and include primary resistance factors such as the zona pellucida (ZP) and trophoectoderm and secondary resistance factors such as age, mobility, and conformation. The size of the embryo also has epidemiologic significance.

The ZP is a glycoprotein shell which is secreted by the oocyte and the granulosa cells immediately surrounding it during the development of the secondary follicle (7, 8). Most of the functional and conformational information about this structure has been obtained through studies with murine embryos. It has been shown that the ZP is permeable to macromolecules (9) and even viruses (10) in mice. However, in the bovine embryo this protective shell resists adherence and penetration by many pathogens including Akabane virus, bovine leukemia virus (BLV), bluetongue virus (BTV), bovine viral diarrhea virus (BVDV), foot and mouth disease virus (FMDV) and Brucella abortus (11-15). In addition, it has been shown that the ZP resists penetration although not adherence by infectious bovine rhinotracheitis virus (IBRV) (16).

Attempts to demonstrate infection of zona pellucida-free (ZP-F) embryos after in vitro exposure with BLV, BVDV, bovine parvovirus (BPV) and B. abortus (12, 15, 17, 18) have failed, leading to the speculation that blastocysts possess the ability to resist infection by certain agents even without the protection of the intact ZP.
The demonstration of lysosome-like structures in ultrastructural studies of bovine blastocysts provides additional support for this speculation (19).

Young age, limited mobility, and proper conformation are important secondary resistance factors possessed by the bovine embryo. The majority of embryos for transfer are collected on day 7 (1). The embryo at this stage is approximately 160 microns in diameter and contains 80-120 cells surrounded by the ZP (20). In considering infectious diseases in which transovarian transmission does not occur, infection of the embryo would have to take place between ovulation and recovery of the embryo for transfer. This limits the opportunity for exposure to a 7-day time frame. The limited mobility of the embryo is also a consideration, since it remains relatively isolated in the oviduct and uterus until recovery. These factors serve to reduce the potential for exposure to a given etiologic agent. Conformation is the final important secondary resistance factor. Embryos that are better able to resist infection or adherence of pathogens will have perfectly symmetrical ZP of uniform thickness, will be free of adherent matter and will contain little or no extruded material.

The size of an embryo is not a resistance factor, but it is epidemiologically significant. At the stage in which it is recovered, the embryo can be visually examined in its entirety, enabling the integrity of its defence barrier (ZP) to be examined and the quality of its cells to be evaluated. Based on information available on many bovine pathogens, the presence of an intact ZP and viable healthy cells is indicative of noninfection.

AGENT FACTORS

The size and ability of living agents to survive in the environment are of epidemiological significance. Other properties of living agents related to their action in the host such as infectivity, pathogenicity, virulence, and immunogenicity are also pertinent in an assessment of the hazards of causing a diseased state in the host (6).

Penetration of the intact ZP by infectious agents which are not known to be present on or in the gametes must occur along channels left when follicular cell processes are withdrawn as the corona cells are shed shortly after ovulation. The penetration of mengovirus through the mouse ZP by this latter route has been demonstrated (10). Mengovirus is an extremely small RNA virus (picorna virus). Larger viruses and certainly bacteria will be prevented from passing through the intact ZP because of their physical size.

Survivability of aetiologic agents in the artificial environments of the embryo created by embryo collection and storage techniques is also important. For example, free BVD virus (i.e. not cell associated) has been shown to lose viability with time when held in embryo support media at room or incubator temperatures (15) and suspensions of B. abortus subjected to embryo freezing and thawing procedures have been shown to undergo a 64% reduction in viability (21).

Infectivity refers to the ability of an agent to lodge itself in the host, pathogenicity refers to an agent’s ability to produce disease, and virulence is the property of the agent which determines the severity of the disease produced (6). Published reports have only shown that one bovine agent, IBRV, is capable of attaching itself on the bovine ZP. Penetration of the intact bovine ZP has not been demonstrated...
for any agent. If future studies were to show that penetration of the ZP by an infectious agent is possible, then the pathogenicity and virulence of that agent for the embryo would have epidemiological significance. If penetration and infection occurred, and the embryo was rapidly destroyed, the hazards of transmitting the agent via embryo transfer techniques would be minimal since the degenerate embryo would not be transferred.

Immunogenicity is an agent factor which is not of importance in its direct relationship to the embryo since a day 7 embryo has not developed an immune system. However, the immune responses elicited in the donor and recipient animals are pertinent and will be discussed under environmental factors.

**ENVIRONMENTAL FACTORS**

Environmental determinants of the potential for disease transmission or non-transmission through embryo transfer are important factors to those concerned with preventing the spread of disease since they are most subject to inspection and control. Transferred embryos are exposed to three separate microenvironments, the donor environment, the *in vitro* environment and the recipient environment. Disease control provisions occur naturally or can be provided in each of these environments.

In the short time from ovulation until recovery for transfer the ova are afforded a degree of isolation and protection in the oviduct and uterus of the donor. The timing of this stay in the reproductive tract has epidemiological significance for it occurs during the first week of the oestrus cycle. At this time, the uterus has just experienced an immunological purging. The enhanced immune response in the uterus at the time of oestrus has long been recognized. The high blood levels of oestrogen achieved at oestrus are associated with a leukocytic infiltration and increased blood supply to the uterus with associated higher levels of immunoglobulins secreted and a relaxation of the cervix with retrograde flow of uterine content (22, 23). All of these activities serve to purge the uterus of any existing infections such as has been shown for *B. abortus* (24, 25) and to protect against introduction of infection at the time of breeding (22). Furthermore, there is some indication that hormones injected in superovulatory treatments may serve to enhance the humoral immune response in some donor animals (26). In addition to these natural provisions for a clean environment, freedom of the donor's reproductive tract from infectious agents can be ensured by traditional means of isolation, testing, and/or vaccination of the donor animal.

The *in vitro* environment of the embryo in normal transfer procedures provides multiple opportunities for elimination of pathogens which may be present. These include the dilution factor of recovery medium used, mechanical washing, antimicrobial agents in recovery and wash media, and enzyme treatments (27). There is also the opportunity to observe the embryo in its entirety under the microscope and, in addition, the embryo can be completely isolated and control exerted over the sterility of its environment.

The third microenvironment of the embryo is the uterus of the recipient. As with the donor, the recipient's uterus can be assured to be free of infectious agents by traditional means of isolation, testing, and/or vaccination and these precautions should be taken before recipients receive embryos.
TESTING POSSIBILITIES

The use of bovine embryo transfer techniques to replace traditional means of shipment of livestock permits unique testing opportunities which are associated with each of the microenvironments of the embryo. The donor female represents a quarantine facility for the embryo which is to be collected. For an agent-embryo encounter to occur, the donor must first be exposed to the agent. To detect the presence of agents for which a reliable serological test exists, paired sera collected at the time of embryo recovery and approximately two weeks after collection can be used to provide assurance that the embryo was free from exposure. Embryos can be held in the frozen state until serological evidence is provided. It should be noted that, while the absence of seroconversions could ensure that exposure did not occur, the presence of seroconversion does not automatically mean that exposure did occur.

Available techniques for testing embryos are destructive and they are not presently a viable option (5). Test procedures now include attempts to isolate agents from (i) flush fluids, (ii) embryo washes and/or (iii) selected embryos or nonfertilized or degenerate ova from a group recovered at a single collection.

The recipient can also be tested after receiving an embryo when a reliable serological test exists for detecting the presence of specific pathogens. However, good disease control practices dictate that the embryo’s health status should be known before transfer if possible. In selected situations the recipient could be held in isolation as a sentinel animal and tested for seroconversion following a certain period of time.

L’INTÉRÊT POTENTIEL DU TRANSFERT D’EMBRYONS BOVINS POUR LA PROPHYLAXIE DES MALADIES INFECTIEUSES. — D.A. Stringfellow.

Résumé : La possibilité de récolter des embryons transférables, de les conserver sous forme congelée pendant une période prolongée, puis de les décongeler pour les transplanter avec succès représente une alternative intéressante aux méthodes traditionnelles de l’amélioration génétique (importation d’animaux vivants, insémination artificielle).

Jusqu’à présent, les recherches sur les agents pathogènes spécifiques des bovins ont montré que ce mode de transport du matériel génétique est très utile pour éviter l’introduction de maladies infectieuses dans des populations bovines précédemment indemnes. En outre, l’évaluation des facteurs épidémiologiques fondamentaux impliqués dans le transfert d’embryons a permis de constater que ce procédé était par lui-même moins dangereux.

L’embryon est un hôte peu vraisemblable pour beaucoup d’agents pathogènes, à cause de la résistance primaire conférée par la zone pellucide et peut-être le trophéctodème, et de facteurs de résistance secondaires tels que son jeune âge, sa petite taille et sa mobilité limitée.

Beaucoup de virus et de bactéries sont trop volumineux pour traverser la zone pellucide et certains survivent mal dans le milieu de l’embryon, ce qui réduit encore le risque de propagation d’agents infectieux par le transfert d’embryons.
Les facteurs d’environnement impliqués dans cette méthode sont peut-être les plus importants à considérer. L’environnement de la donneuse limite les contacts avec les agents infectieux et fournit des possibilités de contrôle avant, pendant et après la collecte des embryons.

L’embryon peut être soumis à des dilutions successives, des lavages mécaniques, à l’action d’agents antimicrobiens et des traitements enzymatiques dans son milieu in vitro. De plus, on peut le visualiser entièrement sous le microscope et s’assurer qu’il est maintenu dans une ambiance stérile.

La vache receveuse représente la dernière garantie puisqu’elle est le réceptacle de l’embryon nouvellement transféré et peut être utilisée comme moyen d’alerte en cas de présence d’un agent infectieux.


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INTERÉS POTENCIAL DE LA TRANSFERENCIA DE EMBRIONES BOVIDOS EN EL CONTROL DE LAS ENFERMEDADES INFECCIOSAS. — D.A. Stringfellow.

Resumen : La posibilidad de recoger embiones transferibles, conservarlos en forma congelada durante un período prolongado, y descongelarlos después para transplantalos con éxito constituye una opción interesante frente a los métodos tradicionales de mejoramiento genético (importación de animales vivos, inseminación artificial).

Hasta ahora, las investigaciones de los agentes patógenos específicos de los bovinos han venido probando que esta forma de transporte del material genético es de suma utilidad para evitar que se introduzcan enfermedades infecciosas en las poblaciones bovinas que estaban libres de enfermedades. Además, la evaluación de los factores epidemiológicos fundamentales implicados en la transferencia de embriones permitió comprobar que este procedimiento era de por sí menos peligroso.

El embrión es un huésped poco probable para muchos agentes patógenos, a causa de la resistencia primaria que confiere la zona pelúcida y acaso la trofodermis, y de factores secundarios de resistencia tales como su joven edad, pequeño tamaño y movilidad limitada.

Muchos virus y bacterias son demasiado voluminosos para atravesar la zona pelúcida y algunos sobreviven mal en el medio del embrión, con lo que se aminoría aún más el riesgo de que se propaguen agentes infecciosos con la transferencia de embriones.

Acaso los factores ambientales implicados en este método sean los más importantes que se han de considerar. El medio ambiente de la donante limita los contactos con los agentes infecciosos y proporciona posibilidades de control antes, en y después de la recolección de embriones.

Se puede someter el embrión a diluciones sucesivas, lavados mecánicos, a la acción de agentes antimicrobianos y tratamientos enzimáticos en su medio in vitro. Se le puede visualizar completamente además en el microscopio, verificando que se mantiene en un ambiente estéril.

La vaca receptora representa la última garantía por cuanto es el receptáculo del embrión recién transferido, pudiéndose utilizar como medio de alerta en el supuesto de que existan agentes infecciosos.
REFERENCES


