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

M. D. EAGLESOME, W. C. D. HARE AND ELIZABETH L. SINGH*

SUMMARY

Studies on laboratory animals have shown that viruses vary as to whether or not they are transmissible by the gametes or are capable of passing through the zona pellucida and infecting the embryo.

Methods of studying early embryos for the presence of infectious agents include electron microscopy, immunocytochemistry and cell cultivation.

Determination that early bovine embryos do not become infected by certain agents might allow for easing of restrictions in the current import and export regulations for cattle embryos.

Embryo transfer could be used as a means of controlling or eliminating disease in a herd or flock if the causal agent does not infect the early embryo via the gametes or by penetrating the zona pellucida.

RÉSUMÉ

Transplantation embryonnaire: une discussion de ses possibilités comme moyen de contrôler les maladies infectieuses, d'après une revue des études sur l'infection des gamètes et des embryons par divers agents

Des études réalisées avec des animaux de laboratoire ont démontré que les virus varient selon qu'ils sont transmissibles ou non par les gamètes, ou qu'ils peuvent pénétrer la zone pellucide et infecter les embryons.

La microscopie électronique, l'immunocytochimie et la culture cellulaire constituent les principaux moyens de rechercher la présence d'agents infectieux dans les embryons.

La possibilité de déterminer que les embryons bovins ne subissent pas une infection par certains agents, pourrait permettre d'adoucir les restrictions de la réglementation actuelle sur l'importation et l'exportation de tels embryons.

On pourrait utiliser la transplantation embryonnaire comme moyen de contrôler ou d'éliminer certaines maladies, au sein d'un troupeau, si leurs agents étiologiques n'infectent pas les embryons par la voie des gamètes ou de la zone pellucide.

INTRODUCTION

Embryo transfer is now a recognized procedure in cattle, pigs, sheep, goats and horses (7), and more recently in dogs and cats (35). Generally used in combination with superovulation, embryo transfer has been promoted as a means of a) genetic improvement in cattle by i) the rapid expansion of limited gene pools and ii) the enhancement of selection intensity; b) producing more beef calves by twinning and shortening the generation interval; c) obtaining offspring from infertile female animals; d) introducing new blood lines into closed herds and e) conducting research in all species.

Relatively little attention has been given to the potential role of embryo transfer in the control of infectious diseases (8, 39, 45, 49). However, it has been recommended as a useful technique for the rapid establishment of minimal disease herds from a few animals with superior genetic background, or the introduction of disease-free animals with a different genetic background into established minimal disease or closed herds (22). Also, the import and export of animals can be facilitated by embryo transfer techniques provided that strict testing and quarantine procedures are combined with rigorous asepsis in the laboratory (50). These procedures form the basis on which several countries have developed regulations for the health certification of imported bovine embryos.

Import/export regulations for disease control in bovine embryos

At present, regulations with regard to the disease status of donor animals and herds of origin have been established by five countries (Argentina, Costa Rica, Mexico, Hungary and West Germany) to allow the importation of bovine embryos from Canada (36). Of the diseases specified in the regulations, five are bacterial, four are viral and one is protozoal (Table I). However, the health certification for each country differs in regard to the diseases included and the requirements to be met (Table I). For example, brucellosis and tuberculosis are the only diseases specified by all five countries and, for these diseases, the requirements vary. Health certifications for the export of bovine embryos from Canada are presently being negotiated with the U.S.A., Denmark, Italy, Switzerland and New Zealand (36).

Regulations regarding the import of embryos into Canada require that "the sire and the dam of the embryo and, if the embryo was transplanted, the animal in which it was transplanted a) be free from communicable disease, and b) proved negative to a test, or were treated for any disease specified by the Minister of Agriculture. Embryo collection and transfer in the country of origin must be done at an animal embryo transfer centre approved by that government" (2).

It is interesting that existing regulations relate only to the dam and sire of the embryo and to the herd of origin of the dam and not to the embryo or the flushing and transport media. Denmark, however, requires 20 mL of the flushing medium for diagnostic testing prior to the importation of embryos from other countries (44).

Possibilities for disease control by embryo transfer

The collection of uninfected

*Animal Pathology Division, Food Production and Inspection Branch, Agriculture Canada, Animal Diseases Research Institute, 801 Fallowfield Road, P.O. Box 11300, Station H, Nepean, Ontario, Canada K2H 8P9.

TABLE I

VETERINARY HEALTH CERTIFICATION FOR EXPORT OF BOVINE EMBRYOS FROM CANADA

Disease	Dam	Sire	Herd of Dam	Tests and Requirements	Country
Bacterial					
Brucellosis	x(6m)	x(6m)			Argentina
	x	x			Costa Rica
	x	x			Hungary
	x(45d)	x		AI and AET†	Mexico
	x(30d)		x,NC(6m)	Dam: standard agglutination test	W. Germany
Leptospirosis	x(6m)	x(6m)			Argentina
	x	x			Hungary
	x(45d)	x		AI and AET†	Mexico
	x(30d)		NC(12m)	Dam: agglutination lysis test, 9 serotypes	W. Germany
Paratuberculosis		x		AI†	Mexico
Tuberculosis	x(6m)	x(6m)			Argentina
	x	x			Costa Rica
	x	x			Hungary
	x(45d)	x		AI and AET†	Mexico
			x		W. Germany
Vibriosis	x(6m)	x(6m)			Argentina
	x	x			Costa Rica
		x		AI†	Mexico
			NS		W. Germany
Protozoal					
Trichomoniasis	x(6m)	x(6m)			Argentina
	x	x			Costa Rica
		x		AI†	Mexico
			NS		W. Germany
Contagious or infectious diseases	NS				Argentina
	NS				Costa Rica
	NS				Hungary
			NS		W. Germany
Viral					
Bluetongue	x(30d)			Dam: complement fixation test. Province of origin herd, negative 24m prior embryo recovery and shipment	W. Germany
Foot and Mouth			NC(3m)	Area within 10km of origin herd negative 30d prior to embryo recovery and shipment	W. Germany
IBR/IPV	x(45d)	x		AI and AET† Dam: negative test or inoculated intranasally with IBR vaccine	Mexico
	x(30d)		NC(12m)	Dam: serum neutralization test	W. Germany
Leucosis	x	x			Hungary
			NC(24m)	Animals in herd >24m, negative to agar gel immunodiffusion and to hematological tests within 3m prior to embryo recovery	W. Germany

x Tested with negative result within ( ) prior to embryo recovery and/or semen collection
NC No cases or suspicion of disease within ( ) prior to embryo recovery
NS No signs of disease at the time of embryo recovery
† Sire maintained on an approved artificial insemination (AI) centre which is under official veterinary control; embryos recovered from the donor cow by an approved animal embryo transfer (AET) centre registered by the Government of Canada
d Day
m Month

embryos from infected parents for transfer to uninfected recipients, thereby obtaining uninfected progeny, would be one approach to the control of infectious disease. For example, if a particular infectious disease were endemic on a farm and the farmer wished to eliminate the disease from his herd or flock, the usual recommendation would be to slaughter the animals, disinfect the premises and restock with disease-free animals. This might involve the loss of valuable blood lines and years of work. However, if it could be shown that the etiological agent is not transmitted from either parent to the embryo, it should be possible, using superovulation, embryo transfer and strict quarantine procedures, to rapidly establish a replacement herd or flock without loss of the gene pool. It should also be possible to develop health certification regulations for the import and export of embryos, based not only on the health status of the dam and sire, but also of the embryo itself.

Possible routes of transmission of a pathogenic organism from parent to embryo are twofold: a) via the gametes, in which case the organism has to be present either inside the ovum or spermatozoon, or attached to the surface of the spermatozoon and b) from the embryonic environment either through the zona pellucida or directly into the embryonic cells after the zona is lost. Organisms present in the embryonic environment could result from infections in the oviduct, uterus, or seminal fluid (14).

The purpose of this paper is to discuss studies on the infection of gametes and early embryos in relation to embryo transfer as a means of controlling or eliminating disease.

MATERIALS AND METHODS

Oocytes and spermatozoa have been studied for infection using electron microscopy (1, 11, 17, 24), immunocytochemical demonstration of antigen (38) and autoradiography (15).

Similarly, early embryos have been examined for productive infection by electron microscopy (3, 9, 13, 19, 25), immunocytochemical demonstration of antigen (12, 40, 48, 52) and the use of conventional techniques using cell culture (12, 27, 28, 32, 40, 42). Unlike electron microscopy and the immunochemical assays which allow direct demonstration of viral antigen at the

TABLE II
ORGANISMS THAT INFECT THE GAMETES

Pathogen	Effect	Reference
Bluetongue virus (BTV)	infection of bull spermatozoa	24
Lymphocytic choriomeningitis virus	has been demonstrated in mouse oocytes and embryos; evidence for transmission via the egg	38
Mouse mammary tumor virus (MTV)	evidence for oocyte, spermatozoa transmission	6
Oncornaviruses	infection demonstrated in oocytes, and early embryos (mouse, guinea pig, primates); breeding expts. implicate infection of the spermatozoa; in embryos, the virus particles are confined to the inner cell mass, none in tropho blast cells	1, 9, 11, 17 18,21,33,34
Simian virus (SV 40 DNA)	infected spermatozoa transmit the virus to the egg; infection of zona-free 2-cell and morula stage mouse embryos; after infection, morula stage developed normally but there was some degeneration of the 2-cell stage embryos; virus cannot penetrate the zona	4, 15, 46

cellular level, the demonstration of embryo infection using cell culture can be difficult. In general, either whole embryos have been cultured in the presence of permissive[1] cells (12, 32); or embryos have been disrupted and the resulting suspension has been incubated with permissive cells (27, 28, 40, 42). Unless it is quantitated by a plaque assay, this method of virus detection fails to distinguish between virus adsorbed onto the zona pellucida and virus replicating in the embryonic cells. Thus, infection of the embryonic cells is difficult to prove. Recent methodology involving micromanipulation with suction micropipettes allows the removal of all the embryonic cells from zona pellucida-intact embryos (47). The cells are then cultured and used for the demonstration of viral antigens. With this system, the demonstration of viral antigen proves infection of the embryonic cells.

RESULTS AND DISCUSSION

Organisms that infect the gametes

Some RNA viruses, particularly the endogenous oncorna viruses, appear to be transmitted via the gametes from parent to offspring (Table II). If this is so and if the embryonic cells are capable of supporting the virus, the diseases caused by them could not be controlled or eradicated using embryo transfer.

The virus of mouse lymphocytic choriomeningitis is an arenavirus, 50 to 300 nm in size, with the capability of causing two forms of the disease: mice infected *in utero* or at birth become infected without clinical signs or the development of antibodies, remain carriers for life and are completely resistant to reinfection. Mice infected as adults develop an acute disease with viremia. An antigen-antibody-complement complex results which causes variable pathological symptoms. None of the infectious diseases of domesticated animals is caused by arenaviruses.

The evidence that bluetongue is transmitted by the gametes is the apparently positive relationship between the bluetongue virus infectivity of semen samples obtained from bluetongue virus-infected bulls and virus-like particles and abnormalities observed by electron microscopy in the heads of spermatozoa. If, in fact, the virus particles in the spermatozoa are bluetongue and if these spermatozoa can infect the ovum at fertilization, the stage is set for the possible vertical transmission of the disease. However horizontal transmission by Culicoides is the generally recognized mode of transmission. Bluetongue is an orbivirus, 53-60 nm in diameter, which infects domestic ruminants; sheep show the most severe clinical signs of infection. Other orbivirus species are the viruses of African horse sickness, Colorado tick fever and epizootic hemorrhagic disease of deer.

The infection of rabbit spermatozoa with simian virus 40 (SV 40) DNA was done experimentally and is, therefore, unlikely to occur naturally. Entire SV 40 has been shown to be adsorbed onto but not to enter into rabbit spermatozoa (15).

Organisms that do not infect the gametes

Infectious diseases where the causal organism does not infect the gametes are potential candidates for control or eradication by embryo transfer provided that it can also be shown that the embryo does not become infected from its environment. Viruses which are known not to infect the gametes are listed in Table III.

Molecular hybridization studies on the DNA of cells from enzootic bovine leukosis (EBL) related cases of lymphosarcoma failed to show the bovine leukemia virus proviral sequence in tissues other than lymphoid tissue or tissue infiltrated by leukemic lymphocytes. This observation practically excludes transmission of the viral genome for EBL virus, an exogenous C-type virus, integrated as proviral sequences in either spermatozoon or ovum, unless transmission has resulted in embryonic or fetal death (16). Assuming these studies are correct and if it can be shown that BLV does not penetrate the intact zona pellucida, then EBL is a disease that would lend itself to this use of embryo transfer.

However, the infection of three day

TABLE III
ORGANISMS THAT DO NOT INFECT THE GAMETES

Pathogen	Effect	Reference
Bovine leukemia virus	not transmitted via the gametes	16
Murine cytomegalovirus (MCMV)	spermatozoa not infected when exposed; not transmitted to embryos when infected females bred with uninfected males	43, 54
Sendai virus	virus adsorbed to acrosome of spermatozoa but transmission to egg is doubtful	23
Simian virus (SV 40)	adsorbed on rabbit spermatozoa but not transmitted	4, 15, 46

[1]A permissive cell is one in which a given virus can enter and actively multiply.

old hamster embryos with feline leukemia virus (FelLv) may be of considerable significance (19). It is, like BLV, a C-type virus which suggests that BLV may also be able to penetrate the zona pellucida and infect the embryo. If so, then embryo transfer would be excluded as a tool in the control or eradication of EBL. On the other hand, Moloney murine leukemia virus, another C-type virus, does not infect zona pellucida-intact mouse embryos (30, 31).

Sendai virus has been shown to be adsorbed to the sperm acrosome in the rabbit (23). However, it is unlikely that these spermatozoa would infect an embryo at fertilization because most of the outer membrane and contents of the acrosome are lost from spermatozoa that penetrate the zona pellucida (5). It has also been shown that SV 40 is adsorbed to the surface of spermatozoa heads and that the spermatozoa do not carry the virus into the egg (15). Sendai virus is an RNA paramyxovirus, 100-300 nm in diameter, which was isolated originally from the mouse and later from the pig. It is closely related antigenically to human parainfluenza I (PI_1) virus. Other virus species in the same genus are PI_3 which affects cattle and horses and Newcastle disease virus which affects birds.

Simian virus 40 is a DNA polyomavirus with a diameter of 45 nm and is latent in Asiatic monkeys.

Spermatozoa exposed to murine cytomegalovirus (MCMV) have a reduced capability for fertilization. However, studies using indirect immunofluorescence, electron microscopy and cell cocultivation have shown that ova fertilized by these spermatozoa do not become productively infected (43). It has also been demonstrated that MCMV is not transmitted to the embryo by infected females bred by uninfected males (42). Murine cytomegalovirus is a DNA virus 120-150 nm in diameter, belonging to the family Herpetoviridae which localizes in the salivary glands producing a normally latent infection. Included in this family are the herpesviruses of horses, cattle, pigs and sheep.

The majority of organisms causing infectious disease are undoubtedly not transmitted by the gametes, but some of these agents have been shown to be capable of infecting the embryo *in vitro* and could, therefore, transmit the disease from parent to offspring if they were present in the seminal or uterine fluids. The age of the embryo may be a factor in infection here. Some organisms have been shown to be capable of penetrating the zona pellucida and infecting the embryo. Others appear not to infect the zona pellucida-intact embryo, but can infect the zona-free embryo. For example, it is impossible to infect zona pellucida-intact mouse embryos with SV 40 (46), Moloney sarcoma virus (MSV) (4), Moloney murine leukemia virus (30), vesicular stomatitis virus (31) or minute virus (40); but when the zona pellucida is removed, two-cell, morula and blastocyst stage mouse embryos can be infected with SV 40 (4, 10), two-cell and morula stage mouse embryos with Moloney sarcoma virus (4), two-cell mouse embryos with minute virus (40) and early mouse embryos with vesicular stomatitis virus (30). There is also evidence to indicate that zona pellucida-free, four to eight-cell stage, mouse embryos exposed to Moloney murine leukemia virus can transmit the virus to their offspring (30, 32), but it is stated that zona pellucida-intact embryos are protected (30).

That the age of the embryo can be a factor in infection is also demonstrated by work showing that zona pellucida-free mouse embryos at the two-cell stage are resistant to polyoma virus (10, 28), but the blastocyst and egg cylinder stages are susceptible to this virus (10).

Bacterium-like particles have been observed in day 10 rat blastocysts (53). This indicates that embryos that have lost the zona pellucida are vulnerable to infection by bacteria, but it provides no information with regard to zona pellucida-intact embryos.

Therefore, when considering embryo transfer as a means of preventing the spread of a disease from one generation to the next, it would be advisable to investigate the *in vitro* infectivity of embryos at different developmental stages by the causal agent, prior to proceeding with *in vivo* trials.

Organisms that penetrate the zona pellucida and infect the embryo

Studies have shown that some viruses are capable of penetrating the zona pellucida and infecting the embryo (Table IV). Mengo and Coxsackie B3 are RNA enteroviruses, 20-30 nm in diameter. Other species of virus within this genus include bovine and porcine enteroviruses.

Western equine encephalitis virus is an RNA alphavirus belonging to the family togaviridae. It is 40-70 nm in diameter and serologically related to the other equine encephalitis viruses. Bovine viral diarrhea (BVD) virus is also an RNA virus of the family togaviridae, and is 50-65 nm in diameter. The cows used in this experiment were superovulated and bred both naturally and by artificial insemination (3). Seven days after estrus, BVD virus was inoculated into one uterine horn. Three days later the embryos were

TABLE IV
ORGANISMS THAT PENETRATE THE ZONA PELLUCIDA AND INFECT THE EMBRYO

Pathogen	Effect	Reference
Bovine viral diarrhea virus (BVD)	injection into uterine horn caused degeneration of bovine embryos; BVD-like particles were found beneath the zona	3
Coxsackie B3 virus	infection of 2 and 4-cell stage mouse embryos; virus can penetrate the zona	[a]
Feline leukemia virus (FelLV)	replication of the virus in 3-day-old hamster embryos; virus can penetrate the zona	19
Human adenovirus	development of 8-cell mouse embryos arrested; viral replication	20
Mengo encephalitis virus	penetrated the zona and replicated in the 2-cell and morula stages of mouse embryos	27, 28, 29
Sendai virus	demonstrated in morulae of infected mouse colonies; conflicting evidence whether virus can penetrate the zona; replication does occur in zona-free mouse embryos.	13, 48
Western equine encephalomyelitis virus	degeneration of early mouse embryos	28

[a]P.A. Neighbour, personal communication 1979.

removed. Eight of 12 blastocysts recovered from the injected (infected) horn were degenerating and electron microscopy showed a normal zona pellucida with evidence of a structure that morphologically resembled BVD virus immediately below it. Embryos removed from the uninjected horn did not show degenerative changes or structures morphologically resembling BVD virus. This experiment is especially interesting because it is the only one reported in which embryos were exposed *in vivo* to a naturally occurring disease agent for the species. In most studies, mouse or hamster embryos have been exposed to agents that naturally infect other species (4, 10, 19, 20, 25, 28, 31, 46).

Most of the viruses that can penetrate the zona pellucida are small in size compared to other viruses and this may be a factor in their ability to penetrate. However, it cannot be the only factor since a number of viruses that do not penetrate the zona pellucida are no larger.

Organisms that cannot penetrate the zona pellucida or do not infect the embryo

Viruses that have been tested in this regard are listed in Table V. Diseases caused by these agents are strong candidates for control or eradication by embryo transfer. Of interest from the veterinary standpoint are the bovine and porcine parvoviruses, porcine pseudorabies virus and vesicular stomatitis virus. Porcine pseudorabies is a herpesvirus and other viruses within the same genus include the viruses of infectious bovine rhinotracheitis and equine rhinopneumonitis. Porcine parvovirus, although incapable of penetrating the zone pellucida and infecting the embryo, has been shown to adhere to the zona pellucida and, on transfer of the embryo to seronegative recipient animals, to subsequently (eight days) infect the recipients and retard or kill a majority of the embryos (52).

Therefore it is possible that the presence of virus on the zona pellucida might pose a threat to the embryo after hatching or to the recipient after transfer. This problem might be overcome by culturing the embryos, prior to transfer, in medium containing antiserum to the virus. Porcine parvovirus, for example, can be eliminated from infected cell cultures by inclusion of antiserum in the nutrient medium (37).

Maedi-visna, a disease mainly of sheep but also of goats, may also be appropriate for eradication by embryo transfer. Experiments to demonstrate transplacental transmission have given negative results (26), and lambs removed from infected ewes immediately after delivery and reared in isolation remained uninfected (41). The disease usually evolves slowly and is caused by an RNA virus having many of the characteristics of oncorna viruses.

There appear to be no guidelines indicating which agents can be transmitted from parent to offspring either through the gametes or from the environment. Therefore, before existing regulations for the control of disease in the international movement of embryos can be relaxed on the sire, dam and herd of origin and before embryo transfer can be used as a means for the control or elimination of a disease in a herd or flock, the etiological agent of each disease of concern will have to be investigated individually. For the certification of embryos, it will have to be determined whether the agent in question is capable of being transmitted via the gametes or by the zona pellucida-intact embryo. Embryo transfer can only be used as a means of controlling or eliminating disease if it can be shown that the embryo itself is not infected and will not infect the recipient animal.

TABLE V
ORGANISMS THAT CANNOT PENETRATE THE ZONA PELLUCIDA

Pathogen	Effect	Reference
Bovine parvovirus (BPV)	no replication in zona-free bovine embryos	14
Cytomegalic inclusion disease virus	no viral replication in 2-cell mouse embryos; developed normally after exposure	28
Herpes simplex virus	2-cell mouse embryos developed normally after exposure; no viral replication	28
Minute virus (MVM)	infection of 2-cell mouse embryos which continued to develop normally; virus cannot penetrate the zona	40
Moloney sarcoma virus (MSV)	infection of unfertilized ova 2-cell and morula stage mouse embryos; development proceeded normally; virus cannot penetrate zona	4, 46
Moloney leukemia virus (M-MuLV)	after exposure, 4 and 8-cell mouse embryos did not produce virus and developed normally; evidence that virus is integrated into germ line; virus cannot penetrate zona	30, 32
Murine cytomegalovirus (MCMV)	when embryos exposed, MCMV-like particles beneath zona, but no viral replication, embryos developed normally; same results with zona free mouse embryos	42
Newcastle disease virus (NDV)	infection of mouse embryos when virus injected into blastocoele; only trophoblast cells infected, inner cell mass cells were virus free; virus cannot penetrate the zona	25
Porcine parvovirus	no infection of porcine embryos; virus cannot penetrate the zona	51
Pseudorabies virus (PrV)	no infection of porcine zona-intact embryos; preliminary evidence shows zona-free embryos are also resistant	12
Rubella virus	no viral replication in 2-cell mouse embryos; developed normally after exposure	28
Simian virus (SV 40)	infection of zona-free 2-cell and morula stage mouse embryos; infection had no deleterious results on development; inner cell mass cells remained free of virus; virus cannot penetrate the zona	4, 10, 46
Vaccinia virus	normal development after exposure; no viral replication	28
Vesicular stomatitis virus	viral replication in early mouse embryos; virus cannot penetrate the zona	31
West Nile virus	after exposure, normal development of mouse embryos; no viral replication	28

ACKNOWLEDGMENTS

The authors are grateful to Dr. K.J. Betteridge for his constructive review of this manuscript.

REFERENCES

1. ANDERSEN, H.K. and T. JEPPESEN. Virus-like particles in guinea-pig oogonia and oocytes. J. natn. Cancer Inst. 49: 1403-1410. 1972.
2. ANIMAL DISEASE AND PROTECTION REGULATIONS. Animal Disease and Protection Act, Sect. 32, p. 46. Department of Supply and Services Canada. 1978.
3. ARCHBALD, L.F., R.W. FULTON, C.L. SEGER, F. AL-BAGDADI and R.A. GODKE. Effect of bovine viral diarrhea (BVD) virus on preimplantation bovine embryos: a preliminary study. Theriogenology 11: 81-89. 1979.
4. BARANSKA, W., W. SAWICKI and H. KOPROWSKI. Infection of mammalian unfertilized and fertilized ova with oncogenic viruses. Nature, Lond. 230: 591-592. 1971.
5. BARROS, C., J.M. BEDFORD, L.E. FRANKLIN and C.R. AUSTIN. Membrane vesiculation as a feature of mammalian acrosome reaction. J. cell Biol. 34: C1. 1967.
6. BENTVELZEN, P., J.H. DAAMS, P. HAGEMAN and J. CALAFAT. Genetic transmission of viruses that incite mammary tumor in mice. Proc. natn. Acad. Sci. (U.S.A.) 67: 377-384. 1970.
7. BETTERIDGE, K.J. Applications of embryo transfer in animal production. *In* Embryo Transfer in Farm Animals: A Review of Techniques and Application. K.J. Betteridge, Ed. pp. 57-66. Ottawa: Canada Dept. Agric. Monograph 16. 1977.
8. BETTERIDGE, K.J. Control of Reproduction in the Cow. J.M. Sreenan, Ed. pp. 645-649. The Hague/Boston/London: Martinus Nijhoff. 1978.
9. BICZYSKO, W., D. SOLTER, M. PIENKOWSKI and H. KOPROWSKI. Virus particles in early mouse embryos. J. natn. Cancer Inst. 51: 1041-1050. 1973.
10. BICZYSKO, W., D. SOLTER, M. PIENKOWSKI and H. KOPROWSKI. Interactions of early mouse embryos with oncogenic viruses — simian virus 40 and polyoma. I. Ultrastructural studies. J. natn. Cancer Inst. 51: 1945-1954. 1973.
11. BLACK, VIRGINIA H. Virus particles in primordial germ cells of fetal guinea pigs. J. natn. Cancer Inst. 52: 545-551. 1974.
12. BOLIN, S.R., L.J. RUNNELS, C.A. SAWYER, K.J. ATCHESON and D.P. GUSTAFSON. Exposure of fertilized ova to pseudorabies virus. Theriogenology 11: 92. 1979.
13. BOWEN, R.A., J. STORZ and J. LEARY. Interaction of viral pathogens with preimplantation embryos. Theriogenology 9: 88. 1978.
14. BOWEN, R.A. Viral infections of mammalian preimplantation embryos. Theriogenology 11: 5-15. 1979.
15. BRACKETT, B.G., W. BARANSKA, W. SAWICKI and H. KOPROWSKI. Uptake of heterologous genome by mammalian spermatozoa and its transfer to ova through fertilization. Proc. natn. Acad. Sci. (U.S.A.) 68: 353-357. 1971.
16. BURNY, A., F. BEX, H. CHANTRENNE, V. CLEUTER, D. DEKEGEL, J. GHYSDEAL, R. KELTMANN, M. LECLERQ, J. LEUNEN, M. MAMMERICKX and D. PORTETELLE. Bovine leukemia virus involvement in enzootic bovine leukosis. *In* Advances in Cancer Research. G. Klein and S. Weinhouse, Eds. Vol. 28. pp. 251-311. New York/San Francisco/London: Academic Press. 1978.
17. CALARCO, P. Intracisternal A particle formation and inhibition in preimplantation mouse embryos. Biol. Reprod. 12: 448-454. 1975.
18. CALARCO, P. and D. SZOLLOSI. Intracisternal A particles in ova and preimplantation stages of the mouse. Nature, New Biol. 243: 91-93. 1973.
19. CHAPMAN, A.L., H.M. WEITLAUF and W. BOPP. Effect of feline leukemia virus on transferred hamster fetuses. J. natn. Cancer Inst. 52: 583-585. 1974.
20. CHASE, D.G., W. WINTERS and L. PIKO. Replication of human adenovirus in cultured mouse embryos. J. Cell. Biol. 55: 39a. 1972.
21. CHASE, D.G. and L. PIKO. Expression of A- and C- type particles in early mouse embryos. J. natn. Cancer Inst. 51: 1971-1975. 1973.
22. CURNOCK, R.M., B.N. DAY and P.J. DZIVK. Embryo transfer in pigs: a method for introducing genetic material into primary specific pathogen-free herds. Am. J. vet. Res. 37: 97-98. 1976.
23. ERICSSON, R.J., D.A. BUTHALA and J.F. NORLAND. Fertilization of rabbit ova in vitro by sperm with adsorbed Sendai virus. Science 173: 54-55. 1971.
24. FOSTER, N.M., M. ALDERS and A.J. LEUDKE. Infectivity and electron microscopy of semen from bulls latently-infected with bluetongue virus. Proc. 58th Ann. Meet. Conf. Res. Workers Anim. Dis. 1977.
25. GLASS, R.H., P.G. CALARCO, T.P. LIN, J. FLORENCE and J.O. OH. Development of the mouse blastocyst following injection with Newcastle disease virus. Biol. Reprod. 10: 502-511. 1974.
26. GUDNADOTTIR, M. Host virus interaction in maedi infected sheep. Proc. 3rd Int. Conf. Cancer. Lung tumours in animals. pp. 381-391. Perugia, 1965.
27. GWATKIN, R.B.L. Passage of Mengovirus through the zona pellucida of the mouse morula. J. Reprod. Fert. 13: 577-578. 1967.
28. GWATKIN, R.B.L. Studying the effects of viruses on eggs. *In* Methods of Mammalian Embryology. J.C. Daniel, Jr., Ed. pp. 228-237. San Francisco: W.H. Freeman. 1971.
29. GWATKIN, R.B.L. and S. AUERBACH. Synthesis of a ribonucleic acid virus by the mammalian ovum. Nature, Lond. 209: 993-994. 1966.
30. JAENISCH, R. Germ line integration and mendelian transmission of the exogenous Moloney leukemia virus. Proc. natn. Acad. Sci. (U.S.A.) 73: 1260-1264. 1976.
31. JAENISCH, R. and A. BERNS. Tumor virus expression. *In* Concepts in Mammalian Embryogenesis. M.I. Sherman, Ed. pp. 287. Cambridge, Massachusetts/London, England: The MIT Press. 1977.
32. JAENISCH, R., H. FAM and B. CROCKER. Infection of preimplantation mouse embryos and of newborn mice with leukemia virus: tissue distribution of viral DNA and RNA and leukomogenesis in the adult animal. Proc. natn. Acad. Sci. (U.S.A.) 72: 4008-4012. 1975.
33. KALTER, S.S., R.L. HEBERLING, G.C. SMITH, M. PANIGEL, D.C. KRAEMER, R.J. HELMKE and A. HELLMAN. Vertical transmission of C-type viruses: their presence in baboon follicular oocytes and tubal ova. J. natn. Cancer Inst. 54: 1173-1176. 1974.
34. KALTER, S.S., M. PANIGEL, D.C. KRAEMER, R.L. HEBERLING, R.J. HELMKE, G.C. SMITH and A. HELLMAN. C-type particles in baboon (*Papio cynocephalus*) preimplantation embryos. J. natn. Cancer Inst. 52: 1927-1928. 1974.
35. KRAEMER, D.C., B.L. FLOW, M.D. SCHRIVER, G.M. KINNEY and J.W. PENNYCOCK. Embryo transfer in the nonhuman primate, feline and canine. Theriogenology 11: 51-61. 1979.
36. MANUAL OF PROCEDURES. Animal Health Directorate, Health of Animals Branch, Agriculture Canada. Sect. 26, p. 4. 1978.
37. MENGELING, W.L. Elimination of porcine parvovirus from infected cell cultures by inclusion of homologous antiserum in the nutrient medium. Am. J. vet. Res. 39: 323-324. 1978.
38. MIMS, C.A. Immunofluorescence study of the carrier state and mechanism of vertical transmission in lymphocytic chroriomeningitis virus infection in mice. J. Path. Bact. 91: 395-402. 1966.
39. MITCHELL, D. and K.J. BETTERIDGE. Embryo transfer for import and export and possible dangers of transmitting infectious disease. *In* Embryo Transfer in Farm Animals: A Review of Techniques and Applications. K.J. Betteridge, Ed. pp. 66-68. Ottawa: Canada Dept. of Agric. Monograph 16. 1977.
40. MOHANTY, S.B. and P.A. BACHMANN. Susceptibility of fertilized mouse eggs to minute virus of mice. Infection & Immunity 9: 762-763. 1974.
41. MOLITOR, T.W., M.R. LIGHT and I.A. SCHIPPER. Elevated concentrations in serum immunoglobulins due to infection by ovine progressive pneumonia virus. Am. J. vet. Res. 40: 69-72. 1979.
42. NEIGHBOUR, P.A. Studies on the susceptibility of the mouse implantation embryo to infection with cytomegalovirus. J. Reprod. Fert. 54: 15-20. 1978.
43. NEIGHBOUR, P.A. and L.R. FRASER. Murine cytomegalovirus and fertility potential sexual transmission and the effect of this virus on fertilization in vitro. Fert. Steril. 30: 216-222. 1978.
44. RASBECH, N. Control of Reproduction in the Cow. J.M. Sreenan, Ed. pp. 646-647. The

Hague/Boston/London: Martinus Nijhoff. 1978.
45. ROWSON, L. The role of reproductive research in animal production. J. Reprod. Fert. 26: 113-126. 1971.
46. SAWICKI, W., W. BARANSKA and H. KOPROWSKI. Susceptibility of unfertilized and fertilized mouse eggs to simian virus 40 and Molony sarcoma virus. J. natn. Cancer Inst. 47: 1045-1051. 1971.
47. SINGH, E.L. and W.C.D. HARE. Cytogenetics in relation to embryo transfer. 1st Int. Symp. Res. Cytogenet. Dis. Resist. Anim. Man. Vienna. 1979.
48. TUFFREY, M., B. ZISMAN and R.D. BARNES. Sendai (parainfluenza) infection of mouse eggs. Br. J. exp. Path. 53: 638-640. 1972.
49. WHITTINGHAM, D.G. Embryo banks in the future of developmental genetics. Genetics 78: 395-402. 1974.
50. WRATHALL, A.E., J.T. DONE, P. STUART, D. MITCHELL, K.J. BETTERIDGE and G.C. B. RANDALL. Successful intercontinental pig conceptus transfer. Vet. Rec. 87: 226-228. 1970.
51. WRATHALL, A.E. and W.L. MENGELING. Effect of porcine parvovirus on the development of fertilized pig eggs in vitro. Br. vet. J. 135: 249-254. 1979.
52. WRATHALL, A.E. and W.L. MENGELING. Effect of transferring parvovirus infected fertilized pig eggs into seronegative gilts. Br. vet. J. 135: 255-261. 1979.
53. WU, J.T. and R.K. MEYER. Bacterium-like particles in delayed implanting blastocysts. J. Reprod. Fert. 28: 105-107. 1972.
54. YOUNG, J.A., K.S. CHEUNG and D.J. LANG. Infection and fertilization of mice after artificial insemination with a mixture of sperm and murine cytomegalovirus. J. infect. Dis. 135: 837-840. 1977.

ABSTRACT

Factors affecting the availability of animal drugs. G.A. Mitchell (Ralston Purina, St. Louis, Missouri).

The miracle drugs of the 1940's and 1950's set the stage for the honeymoon between chemicals and consumers. This was to be a long and just relationship resulting in the birth of many new chemicals servicing many new consumers in many new ways. The honeymoon between chemicals and consumers ended when human safety concerns became public issues as a result of unfortunate mishaps, such as the thalidamide case. Since the demise of this chemical honeymoon, the human safety exceptions have been highlighted. Of greater significance to the public attitudes towards chemicals has been the publicity of numerous and continuous carcinogenicity concerns causing consumer alarm. Some concerns have been real, but far too many have been the result of conjecture and speculation. Perhaps the most serious potential harm resulting from the consumer alarm is the lack of consideration of the benefits for the chemical under attack. The consumer is only made aware of the problems. In short, a few unsatisfactory consumer experiences with a small number of chemicals coupled with conjecture and speculation has let to the funding of new and expanded federal agencies charged with the responsibility of regulating chemicals. The pendulum has swung too far. Today almost every aspect of the creation, research and development, manufacture, marketing and use of chemicals is regulated by one or more federal agencies. The bureaucratic apparatus that has been established by our Members of Parliament is presumably supported by a majority of voters who apparently agree that their tax dollars ought to be spent in this manner. Or do they? Our scientific community today is certainly divided as to what safety requirements should be established for regulating needed chemicals. Our regulatory agencies are even more confused as to how to evaluate the risks of a chemical in light of the chemical's potential benefits. Chemical regulation has become ever-increasingly and unnecessarily burdensome, costly and not generally applicable to product efficacy, and in some instances, not even directly to animal or human safety considerations. The agencies have overreacted to the safety panic stimuli and have become overly concerned with theoretical risks and absolute food safety requirements without regard for benefits to be derived from such products. More appropriately, the regulated industry, HPB and the scientific community should work together to establish testing requirements that produce meaningful results that can be equated to human health risk. Reduced animal drug approvals in both the treatment and mitigation of minor diseases in major species, and major diseases in minor species has been an obvious outcome of increased over-regulation. Historically, economic market principles have regulated basic industry R & D practices. Because of the increased costs of developing drugs now, minor diseases in major species and major diseases in minor species, no HPB approved claims are added, because no one affords the cost of generating the necessary safety and efficacy data. Environmentalists base their position on three myths: 1) Pre-industrial era of good crops, easy living and few insects, 2) A distinction between natural and unnatural chemicals and 3) Unnatural chemicals are causing a cancer epidemic when, in fact, all cancers except lung cancer are going down. In conclusion, I quote from William Tucker in *Harper's* "Of Mites and Man", "What keeps nagging in the back of my mind is this great Age of Environmentalism is what we are going to look like a few years from now. Somehow it seems we are going to appear as a generation that was so obsessed with misgivings, so afraid of what we didn't — and couldn't — know, so anxious to point hysterical accusing fingers at one another, that we neglected to pick up and use the simple tools we had at hand. I have no doubt that someone will eventually use these tools. I only wonder if we will ever calm down enough to do it ourselves."

Scientific Program of the Canadian Veterinary Medical Association and Ontario Veterinary Association Conference, Toronto 1979.