Export of animals as frozen embryos

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ABSTRACT

The basic technique for freezing cattle, sheep and goat embryos is briefly described. The number of offspring born from each transferred thawed embryo is usually satisfactory but extensive pre and post freezing culling of embryos means that the technique is usually inefficient compared with unfrozen embryos. Research to improve results continues and the 54 and 64% embryo survival/embryo frozen obtained from minimally selected sheep embryos at Ruakura is most encouraging. The technique is being used for the international export of cattle embryos. Its major advantage is that it enables the export of genetic material which could not otherwise be obtained by the importing country. Its use should increase as embryo survival rates increase and particularly if it is established as an effective means for infectious disease control.

Keywords Embryo; freezing; export; disease; pregnancy

INTRODUCTION

Since 1979, over 1000 frozen cattle embryos have been exported from New Zealand to Australia, Asia and South America. The use of the technique is not confined to New Zealand — there is a growing international trade in cattle embryos. The technique has not been used for the export/import of sheep, goat and pig embryos but, with the exception of the pig embryo which will not survive being cooled below 15 °C, live offspring have resulted from frozen embryos (Moore and Bilton, 1977) and the technique could be used for embryo transport.

This paper briefly outlines the technique used for embryo freezing, the results achieved, and examines economics and application.

TECHNIQUES FOR FREEZING EMBRYOS

The techniques used in all countries for freezing cattle, goat and sheep embryos are basically similar and involve: superovulation of donor animals and surgical or non-surgical recovery of embryos in modified Dulbecco's phosphate buffered saline (Tervit *et al.*, 1972) about 6 to 8 days after entire mating; examination of the embryos and rejection of unsuitable (retarded or degenerate) embryos; gradual and stepwise addition of a cryoprotectant at room temperature. The usual cryoprotectants are dimethyl sulphoxide (DMSO) and glycerol but ethylene glycol and propanediol have also been used; addition of the embryos to glass ampoules or mini-straws which are then sealed; placing the ampoules/straws into a programmable freezer which usually cools at -1 °C/min to -7 °C. At this temperature ice formation is induced in the freezing solution. Cooling then usually proceeds at -0.3 °C/min to around -35 °C, and finally at -0.1 °C/min to about -38 °C; plunging the containers into liquid nitrogen and storing for varying lengths of time.

Thawing usually involves placing the containers into a 37 °C water bath until all ice has melted. The embryos are then recovered and subjected to room temperature cryoprotectant removal either through gradually decreasing concentrations of cryoprotectant or a sucrose gradient (Niemann *et al.*, 1982).

EMBRYO VIABILITY AFTER FREEZING/THAWING

Typical results from frozen embryos are shown in Table 1. In most studies the number of offspring born (or recipients pregnant) from 100 embryos transferred is satisfactory. However, embryos found to be degenerate after thawing are usually not transferred (19% rejection rate; Elsden *et al.*, 1982) and when embryo survival is expressed relative to the number of embryos frozen, results around 30 to 40% appear the norm. Only the best embryos are usually frozen (15% rejection rate, Elsden *et al.*, 1982) and so the extensive culling of embryos pre and post freezing means that considerably fewer pregnancies are obtained per embryo collected compared to unfrozen embryos.

The results of trials conducted in 1982 at Ruakura to develop a successful technique for freezing sheep embryos are shown in Table 2. The results are again presented on the basis of embryos transferred and embryos frozen. The figures (152 and 166, respectively) differ because 7 embryos were not recovered from the ampoules post thaw and a further 7 embryos

TABLE 1 Survival of frozen embryos.

Species	No. offspring per no. embryos		Reference
	Transferred	Frozen	
Goat	3/6(50)[1]	3/48(6)	Bilton and Moore, 1976
Sheep	8/20(40)	8/62(13)	Willadsen *et al.*, 1976
	8/24(33), 2/8(25)	8/24(33), 2/8(25)	Willadsen *et al.*, 1977
Cow	5/16(31)	5/24(21)	Bilton and Moore, 1977
	22/34(65)[2]	22/40(55)[2]	Lehn-Jensen *et al.*, 1981
	23/34(68)[2]	23/68(34)[2]	Schneider *et al.*, 1980
	12/23(52), 6/9(67)[2]	12/39(31), 6/10(60)[2]	Willadsen *et al.*, 1977

[1] Percentage.
[2] Recipients diagnosed pregnant at about day 60.

were not transferred after cryoprotectant removal (only 1 of the latter was likely to have survived if it had been transferred). Embryos frozen in DMSO and ethylene glycol survived the freeze/thaw process very readily as did embryos subject to cryoprotectant removal via the sucrose gradient. The 152 embryos were transferred to 76 recipients and the survival of embryos (on the basis of embryos frozen) after the use of DMSO plus sucrose gradient and ethylene glycol plus gradient (54 and 64%, respectively) was similar to that obtained in recipients twinned with unfrozen embryos (56%; Moore *et al.*, 1960) and was far superior to that recorded previously (Table 1). The rejection rate of available late morulae and blastocysts pre freezing was low (1 and 2%, respectively) and so the efficiency of the technique is most encouraging.

TABLE 2 Survival of frozen sheep embryos.

Cryoprotectant	Method of cryoprotectant removal	No. lambs born per no. embryos	
		transferred	frozen
Dimethyl sulphoxide	dc[1]	11/26(42)[3]	11/26(42)
	sg[2]	15/26(58)	15/28(54)
Ethylene glycol	dc	12/24(50)	12/29(41)
	sg	16/25(64)	16/25(64)
Glycerol	dc	7/25(28)	7/31(23)
	sg	11/26(42)	11/27(41)
Overall		72/152(47)	72/166(43)

[1] Decreasing concentrations of cryoprotectant.
[2] Sucrose gradient.
[3] Percentage.

ECONOMICS AND APPLICATION

When live cattle are exported to Malaysia for example, the costs incurred (transport $500 to $1500 if many animals are shipped/flown at the same time; purchase cost of animal, say $300 to $1500) procure a live animal which, if it is a female, may already be pregnant and so production of offspring and other commodities begins shortly after arrival. When frozen embryos are exported, the costs involved are: transport, negligible; cost of embryo — a commercial grade embryo costs about 60 to 70% of the cost of the donor (embryo cost of say, $150) and the cost of a pedigree embryo is negotiable but is often about one-third the value of the donor (say, $500). The embryo costs obviously vary for each exportation but these figures can be used as an example and were generously supplied by Dr Sandy Ferguson of Gene Stock Ltd, Bombay, Auckland. The cost of the thaw/transfer in the importing country would be about $100. Table 1 shows that these costs usually procure about a 35% chance of a live calf. Thus the cost of producing a live calf in the importing country would average about $710 to $1700. The cost of producing a specific sex would average $1400 to $3400 and the offspring would need to be grazed for about 2 years before they produce offspring and other marketable commodities. These figures indicate that if embryo freezing is used simply for the transport of genetic material which could be sent as live animals, it is likely to have little or no economic advantage.

The major advantage of the technique, and currently its main use, is that it enables the export of genetic material which could not otherwise be obtained by the importing country. For example, embryos can be collected in the exporting country from proven dairy or rare dams which are not for sale. The technique could also be invaluable where quarantine restrictions prohibit the importation of live animals. Frozen embryos could be stored if a period of time is needed to establish whether parents were free from particular viral infections and the technique would be invaluable when it has been established that uninfected embryos can be collected from infected parents and then exported. Information on the potential of

embryo transfer for infectious disease control is slowly accumulating through studies on exposure of gametes to various organisms (e.g., Eaglesome *et al.*, 1980). The technique shows promise but before international regulations can be formulated the etiological agent of each disease of concern needs to be thoroughly investigated. It has been suggested that the technique may have advantages over the importation of live animals where the environments of the exporting and importing countries are very different. The transferred embryo would be born to an adapted native recipient and the resulting transfer of immunity through colostrum and transfer of behavioural characteristics may be beneficial. However, it is likely that in situations where live animal survival/performance is good, embryo transfer results will also be good and that in situations where live animal performance is poor, the skills needed to successfully conduct an embryo transfer programme and manage the exotic offspring will also be poor and the technique will be of little benefit. Finally, the technique can be attractive when used as an alternative to the export of a small number of live animals — under such circumstances the transport cost of importing each live animal is extremely high.

The export of animals as frozen embryos should increase as the survival rates from embryos increase and especially if it is established as an effective means for infectious disease control.

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