

EFFECTS OF EMBRYO FREEZING METHOD ON PREGNANCY RATE FOLLOWING BOVINE EMBRYO TRANSFER

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INTRODUCTION

Recent changes in the methods for bovine embryo freezing have the potential of affecting the efficiency of embryo transfer. Direct transfer of embryos frozen in ethylene glycol as a cryopreservative reduces the time required to prepare embryos for transfer. If pregnancy rates achieved following direct transfer are comparable to those following thawing, rehydration, and transfer of embryos frozen in glycerol, the direct transfer method could increase the efficiency of embryo transfer. As part of a large contract recipient bovine embryo transfer project, the effects of embryo freezing methods were examined.

METHODS AND PROCEDURES

Embryo freezing, transfer and pregnancy rate: A total of 3531 embryos were transferred in six episodes over a 3-year period. The embryos were frozen and thawed for direct transfer using ethylene glycol as a cryoprotectant (direct, n=3047) or were thawed and rehydrated after being frozen in 10% glycerol (glycerol, n=484). The frozen embryos were supplied by 25 different embryo transfer businesses.

Regardless of embryo type (direct or glycerol), the embryos were all transferred to synchronous recipients by a single practitioner. Thawing and rehydration of embryos frozen in 10% glycerol were conducted by a single practitioner, and all the embryos were loaded into side-delivery embryo transfer gun by one technician.

Pregnancy rates for this project are based on ultrasound detection of a viable fetus between 25 and 80 days of gestation.

Recipients and estrus detection: The contract recipient embryo transfer program was conducted at the Virginia Beef Corporation, Virginia's largest commercial beef producer. Embryos supplied by the embryo owner were transferred to recipient cows or heifers owned by Virginia Beef. Virginia Beef assumed all costs associated with embryo transfer and production of a weaned calf. The embryo owner received a weaned, pre-conditioned calf at a price negotiated prior to transfer.

Estrus was synchronized in crossbred cows or heifers using conventional methods. Estrus was detected exclusively using an electronic heat detection system manufactured by DDx Inc. (Boulder, CO). The HeatWatch™ system consisted of a battery-powered, reusable, pressure-sensing radio frequency transmitter contained in a pouch glued to the tailhead of the animal. The transmitter signal was emitted when the device was activated by pressure from mounting activity. A signal receiver (.4 mi. range) was hard-wired to a buffer which received and stored mounting activity data until it could be downloaded using HeatWatch™ software. The animal identification number, time of mounting and duration of each mount were recorded. Designation of a “heat” was defined by the operator as three mounts during a 4-hr period. A data file containing all mounting activity data was created and exported as a print file. The print file which can be imported to any spreadsheet program and manipulated with database functions was used to facilitate the assignment of embryos to recipients based on the stage of the embryo and synchrony of the embryo and recipient.

RESULTS

The overall pregnancy rate following the transfer of fresh and frozen bovine embryos was 59% (Table 1). There was no significant difference between the pregnancy rate following the direct transfer of embryos frozen in ethylene glycol and the embryos frozen in 10% glycerol.

Table 1. The Effects of Embryo Type and Embryo Quality on Pregnancy Rate Following Bovine Embryo Transfer

Embryo Type	Embryo Quality			Total
	Grade 1	Grade 2	Grade 3	
Direct	62.9% (1211/1953)	54.3% (584/1077)	47.1% (8/17)	59.2% (1803/3047)
Glycerol	59.8% (165/276)	55.0% (99/180)	39.3% (11/28)	56.8% (275/484)
Total	61.7% ^a (1376/2229)	54.3% ^b (683/1257)	42.2% ^b (19/45)	58.8% (2078/3531)

^{a,b} means in the same row with different superscripts are significantly different (p<.03).

While effects due to the source of the embryo could not be separated from the effects of freezing method in this data set, it is important to note that the pregnancy rate for embryo transfer companies which supplied >25 embryos was consistently at or above 50% (range 49.0% to 72.5%; Table 2).

Table 2. The Effects of Freeze/Thaw Method on Pregnancy Rate Following Transfer of Bovine Embryos from 15 Embryo Transfer Companies

ET Company ^a	Freeze/Thaw Method		Total
	Direct	Glycerol	
G	54.1% (13/24/1.25) ^b	44.4% (12/27/1.09)	49.0% (25/51/1.17)
C	N/A	49.2% (31/63/1.54)	49.2% (31/63/1.54)
D	49.5% (153/309/1.35)	62.1% (18/29/1.76)	50.6% (171/338/1.38)
O	50.9% (170/334/1.40)	0.0% (0/1/1.00)	50.7% (170/335/1.40)
M	52.6% (40/76/1.51)	N/A	52.6% (40/76/1.51)
E	53.1% (112/211/1.36)	63.9% (39/61/1.44)	55.5% (151/272/1.37)
J	61.2% (139/227/1.22)	22.9% (8/35/1.51)	56.1% (147/262/1.26)
I	N/A	60.4% (29/48/1.52)	60.4% (29/48/1.52)
P	53.8% (21/39/1.44)	78.6% (11/14/1.64)	60.4% (32/53/1.49)
F	58.8% (60/102/1.42)	64.1% (66/103/1.52)	61.5% (126/205/1.47)
L	N/A	61.8% (21/34/1.23)	61.8% (21/34/1.23)
B	62.0% (897/1446/1.43)	80.0% (12/15/2.07)	62.2% (909/1461/1.44)
N	69.4% (84/121/1.17)	N/A	69.4% (84/121/1.17)
K	85.7% (12/14/1.14)	53.8% (7/13/1.23)	70.3% (19/27/1.18)
H	72.5% (116/160/1.01)	N/A	72.5% (116/160/1.01)

^a companies with <25 embryos transferred were omitted from this table.

^b no. pregnant/no. transferred/average embryo quality.

Some practitioners have suggested that the stage of development of the embryo can affect the pregnancy rate following transfer of embryos frozen in ethylene glycol. The effect of stage of development on ethylene glycol frozen embryos was determined in this trial (Table 3). There were no significant differences among pregnancy rates for embryos frozen at different stages of development.

Table 3. The Effects of Stage of Development of Bovine Embryos Frozen in Ethylene Glycol on Pregnancy Rates Following Embryo Transfer

Stage	Pregnancy Rate	Average Quality
3	33.3% (1/3) ^a	1.33
4	58.1% (471/811)	1.50
5	56.8% (275/484)	1.29
6	52.3% (56/107)	1.04
7	25.0% (1/4)	1.00

^a no. pregnant/no. transferred.

CONCLUSION

The difference in pregnancy rates for bovine embryos frozen in ethylene glycol for direct transfer and those frozen in 10% glycerol was not significant. Furthermore, there were no apparent differences in the pregnancy rates following transfer of embryos frozen in ethylene glycol at different stages of development. The use of ethylene glycol as a cryoprotectant allows the direct transfer of embryos with less equipment, fewer supplies, less preparation and at a reduced cost to the client. These advantages of embryos frozen in ethylene glycol make it the method of choice for freezing of bovine embryos.