

## Freezing Embryos For Direct Transfer

In 1973 the first calf was produced resulting from a frozen embryo. Since that time embryo freezing has had a major impact on the livestock industry. Several of the advantages include: (1) embryos can be collected throughout the year from selected donors and implanted when appropriate; (2) embryos can be shipped over long distances for a low cost and (3) embryos surplus to a breeder's needs can be sold.

Embryo freezing was not discovered in a day. It took researchers years of hard work to find a method that allowed embryos to be frozen, thawed, and transferred resulting in an acceptable pregnancy rate (>50%). Many factors were found to be critical to maintaining a viable embryo, including (1) stage and quality of the embryo, (2) cooling rate and (3) cryoprotectant (antifreeze) used.

Best results are obtained with embryos recovered seven days after estrus (heat). Embryos at this age are at either the morula or blastocyst stage. The embryos are graded on the basis of the number of dead cells present at recovery. The highest quality of embryo, a #1, is an embryo that has no dead cells, A #1A has very few dead cells, and a #2A (the lowest quality frozen at AEG) has more dead cells. The high quality embryos yield more consistent results. At Alta Embryo Group we stress quality and our standards are recognized worldwide as being very high.

Cooling rate is another important factor in the success of freezing embryos. The freezing and thawing processes inevitably kill some cells within the embryo. However, by controlling the rate at which the embryos are frozen the number of cells dying can be minimized. To start ice crystals forming evenly throughout the straw, the straw is touched with a super-cooled instrument (process called seeding). Then the temperature is decreased by 0.6°C per minute down to -35°C. After reaching -35°C the embryos can be plunged directly into liquid nitrogen (-196°C) and stored indefinitely.

Embryos are frozen in antifreeze solutions containing cryoprotective agents. These agents displace some of the water in the embryonic cells and control ice forming within them. The first cryoprotective agents used successfully to produce pregnancies were dimethyl sulphoxide and glycerol. One drawback to these freezing solutions was that the embryos required extensive handling after thawing. The embryos were thawed in air for 10 second then plunged into a 35°C water bath for 10 seconds. They were then removed from the straw and placed in a sucrose solution for four minutes. The sucrose helped remove the cryoprotectant from within the embryo, allowing subsequent rehydration. Afterwards, the embryos were placed in holding medium, re-loaded into straws and transferred into recipients. Not only was time a factor in the processing of frozen embryos for transfer, but also the need for a trained technician and lab equipment.

In the early nineties, research focused on finding a reliable method of freezing embryos for direct transfer. Direct transfer means eliminating the handling of the embryo outside the straw between thaw and transfer. The cryoprotectant found to be successful was ethylene glycol. Within the straw, the embryo is contained in a column of ethylene glycol, but is surrounded by columns of holding medium. After thawing (in air and water as before) and placing the contents of the straw into the uterus, the columns will mix, diluting the cryoprotectant, thus exposing the embryo to the holding medium, allowing rehydration to take place. This procedure is much less time consuming and requires no lab equipment. Another advantage of direct transfer is that producers will be able to perform their own transfers with minimal training. Producers able to implant embryos into their own cattle will increase efficiency and flexibility in their recipient management. By implanting recipients on their natural heats, synchronization is not necessary, thus saving time, money, and labour. By eliminating these factors, producers will be saving up to \$150 per transfer.

Over the years Alta Embryo Group has frozen well in excess of 100,000 embryos. Since November 1994 all embryos frozen at AEG have been packaged for direct transfer. Over 4000 direct transfer embryos have already been thawed and transferred. The pregnancy rate, approximately 55%, is comparable to previous methods.

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