Update on Direct Transfer of Frozen Embryos

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The first calf to be born in the world after residing short term in the depths of a liquid nitrogen tank was named Frosty, reported in 1973.[1] This feat was the result of a colloquium of animal scientists, cryobiologists, and embryologists putting their ingenuity to test by freezing early whole life forms and bringing them back to life at a later date in a normal state of health. Since that time, the commercialism of routine cattle embryo transfer procedures includes freezing intact day 7 embryos. A rough estimate of the scope of the frozen embryo industry can be estimated from the American Embryo Transfer Association's most recent polling of its membership.[2] The data retrieved from commercial embryo transfer companies reveals that in 1994 more embryos were frozen than were transferred fresh, 88,969 vs. 68,066. These data indicate that the survival rates of frozen cattle embryos are certainly acceptable to cattle breeders.

Before a mammalian embryo can be frozen and subsequently thawed, it must first be cryopreserved. The physical properties of water molecules in a frozen state result in crystal formation. Therefore, intracellular water inside the embryo must be removed and replaced before freezing (cryopreservation) or the result would be lethal amounts of cell damage to the embryo. Early experiments with freezing mouse and cattle embryos utilized dimethyl sulfoxide (DMSO) as a cryoprotectant.[3,4] When an embryo is placed into a 10% solution of DMSO, water osmotically escapes the intracellular spaces and is replaced by DMSO. Water escapes faster than DMSO enters, thereby causing the embryo to visibly shrink like a prune for a few minutes until the DMSO has time to replace the water. The total time for DMSO to replace the water and ultimately equilibrate is about 10 minutes at room temperature. All this is well and fine for freezing, but the problem comes into play after thawing the frozen egg. If an embryo frozen with DMSO were to be transferred directly, a disastrous situation would occur. DSMO is less permeable than water (slower to leave the intracellular space than water moving in). Therefore, water from the recipient cow's uterus, in the case of direct transfer, would enter too rapidly into the intracellular space thereby causing the embryo to swell and burst. Consequently, the cryoprotectant must be removed slowly, in several steps, so that cellular overfilling with water does not occur. Unfortunately, this requires a trained technician and about 30 minutes to perform under a microscope, thus costing time and money. Glycerol ultimately replaced DMSO as the cryoprotectant of choice by the ET industry. Glycerol seemed to give more consistent results than DMSO so it was adopted by the ET industry as the recognized industry standard. However, even though glycerol enjoyed all the glamour for the last decade, it was not without drawbacks. It, like DMSO, has to go through a three-step removal to prevent cell bursting which requires time and skilled manpower.

Most recently, scientists have discovered a different cryoprotectant, ethylene glycol, which has a unique advantage over glycerol or DMSO relative to cryoprotecting cattle embryos.[5] Ethylene glycol is much more permeable than glycerol which allows it to cross the cell membranes almost as fast as water. This means that an embryo frozen in ethylene glycol can be transferred "directly" into the uterus of a recipient cow for rehydration without concern of overfilling with both water and cryoprotectant.

How successful are the pregnancy rates with direct transfer (DT) embryos compared with the old glycerol method? Table 1 represents accumulated pregnancy data from 3 commercial ET companies that have significant data on both glycerol and DT embryos.

Table 1. Pregnancy rates of frozen embryos using either glycerol or ethylene glycol (DT) as the cryoprotectant.

ET Company	Cryoprotectant	# of Embryos Transferred	Pregnancy Rates
A	Glycerol	325	67%
	Ethylene Glycol	117	58%
B*	Glycerol	56	70%
	Ethylene Glycol	56	50%
C*	Glycerol	883	50%
	Ethylene Glycol	550	52%

*embryos from a single flush were divided equally and frozen with either glycerol or ethylene glycol as the cryoprotectant.

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The advantages of direct transfer frozen embryos are far reaching for the veterinary and purebred cattle industries. Instead of just having ET practitioners transfer frozen embryos, veterinarians and experienced AI technicians are now candidates for transferring DT embryos frozen in ethylene glycol. Embryo owners with available recipients will be able to utilize their cow's natural heats instead of having to synchronize them and wait for their ET practitioner to make his monthly journey to the dairy or ranch. Instead of taking 30 minutes to thaw a glycerol embryo for transfer, the DT frozen embryo only takes a couple of minutes-not to mention the savings on petri dishes, thaw solutions, and a microscope. All factors considered, the DT process could open up new markets for the ET industry that never existed before. The bovine practitioner that has always shied away from ET because of its complexity and time consumption, can now directly involve himself with his clients that have ET performed by transferring his client's frozen embryos. There is no microscope and embryo manipulation, no special solutions to purchase, and frankly very little training to undergo so that one can begin serving the clientele.

Historically, every industry has several monumental events that in some way or other charts the course for its future. The ET industry is itself somewhat of a marvel. The most significant discoveries have been non-surgical embryo recovery, non-surgical transfer, freezing of embryos and now the direct transfer of frozen embryos. This DT process is relatively new to the industry and only time will tell if the results will hold up to older more proven methods of thawing. After all, our clients will ultimately determine if the process is effective enough to continue. **It is my opinion that a new standard has been established in the embryo transfer industry that is here to stay as long as cattle embryos are transferred.**

References

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Abstract

Acute recumbency and marginal phosphorus deficiency in dairy cattle

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Because of a mixing error at a local feed mill, a diet marginally deficient in phosphorus, compared with recommendations from the National Research Council, was fed to a high-producing dairy herd for 5 months. Two mature cows in early lactation became recumbent. Serum phosphorus concentration in 1 cow was low (1.8 mg/dl), but was not measured in the other cow. Ten other high-producing, first-lactation cows in the herd developed severe lameness.

Results of analysis of rib bone samples from the recumbent cows were consistent with changes associated with demineralization. Bone ash, calcium, phosphorus, and magnesium concentrations were lower than published ranges for healthy cattle. Serum calcium, phosphorus, and magnesium concentrations in 8 unaffected cows were normal. For 6 unaffected cows mean serum hydroxyproline concentration was higher during the period that the phosphorus deficient diet was fed than when an adequate diet was fed. Moderate (15%) restrictions in dietary phosphorus intake, compared with National Research Council recommendations, can possibly result in health problems in high-producing dairy cattle.