



## **FROZEN EMBRYOS**

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The first live foal following transfer of a frozen-thawed embryo was born in 1982. This milestone in horse reproduction followed previous successes in mice (1972) and cattle (1973).

Currently, only a relative few equine embryos are frozen or cryopreserved as compared to the number that are collected and transferred immediately into recipient mares. This is in contrast to the situation in cattle, in which cows are routinely superovulated and a large percentage of recovered embryos are frozen for future transfer into recipient heifers. A rule change in the AQHA allows for the use of frozen-thawed embryos beginning in January 2007.

There are several potential advantages that may be considered regarding freezing of equine embryos. Collection and cryopreservation of an embryo would conserve the genetic material from a specific mare-stallion cross. International transport of an embryo would be substantially less expensive than transport of a live horse. On a more local level, freezing embryos could decrease the number of recipient mares that are needed in an embryo transfer program. Embryos could be collected and frozen and subsequently transferred when a recipient became available. Finally, embryos collected during the middle or end of one breeding season could be frozen and then

transferred at the beginning of another breeding season. This may be advantageous in breeds or disciplines that desire early foals.

Several significant hurdles must be overcome before embryo freezing becomes a routine part of equine reproduction management. First, superovulation is not an efficient process in mares as compared to other species, such as cattle and sheep, and is therefore not used routinely. As a consequence, in a majority of embryo collection attempts, the mare has ovulated a single follicle and only one embryo is potentially available. This factor alone dramatically reduces the efficiency and usefulness of embryo cryopreservation.

A second obstacle to routine embryo freezing is that only small equine embryos have been successfully frozen to date. A series of research studies over the past two decades has confirmed that only morula or early blastocysts stage embryos that are less than 300  $\mu\text{m}$  (i.e. less than 0.3 mm) will survive the freezing and thawing procedures. Larger expanded blastocysts do not survive. The consequence is that in order to collect small embryos, the flush must be performed during a very narrow window of time relative to embryonic development. The equine embryo does not leave the oviduct and enter the uterus until late on day 5 or

early on day 6 after ovulation. To complicate matters further, equine embryos begin to expand rapidly on day 7 after ovulation. This means that the embryo collection attempt must be performed approximately 6.5 days after ovulation in order to have an opportunity to collect an embryo and have that embryo be small enough to freeze.

A third factor that has impeded progress in equine embryo cryopreservation is unique to the horse. The equine embryo begins to develop an acellular protein envelop, called the capsule, between days 6 and 7 that surrounds the embryo. This capsule may impede movement of the cryoprotectants into the embryo that are critical for cell survival.

The principles involved in freezing are similar between embryos of all species. Embryos are exposed to specific concentrations of cryoprotectants prior to freezing. The cryoprotectants help to remove water from within the cells of the embryo and consequently help the cell survive the freezing process. Mechanical damage that results from intracellular ice crystal formation is a major problem that can lead to a loss of embryo viability.

The embryo is loaded into a labeled 0.25-ml plastic straw after exposure to the cryoprotectants. The actual freezing process may involve a traditional or 'slow-freeze' process or may involve vitrification or an 'ultra-rapid freeze'. A vitrification procedure recently described for equine embryos has eliminated the need for expensive equipment and has reduced the time required to complete the cryopreservation procedure. Ultimately the straw containing the frozen embryo is stored in a tank of liquid nitrogen.

Transfer into a recipient mare involves thawing or 'warming' the straw containing the embryo. Depending on the cryopreservation procedure, the embryo may have to be moved through a series of dilution steps to remove the cryoprotectants or the dilution solutions may be contained within the original straw.

A day-6.5 frozen-thawed embryo is usually transferred into a recipient mare that has ovulated 5 days previously. It is anticipated that there will be a slight delay in embryonic development subsequent to the cryopreservation and the embryo-recipient synchrony noted above may be beneficial for maternal recognition of pregnancy.

Pregnancy rates following transfer of frozen-thawed embryos have been reported to be 50 to 70%. It is most likely that transfer success rate with frozen embryos will never equal that achieved after transfer of a 'fresh' embryo. Certainly, a trade-off exists between the potential advantages of a frozen embryo and the lower transfer success rates. It is anticipated that the success of frozen equine embryos will increase as the technology of cryopreservation advances.



**Photograph of a Grade 1 day 7 grade 1 blastocyst-stage equine embryo**