



## COOLED-TRANSPORTED EMBRYOS

Patrick M. McCue

DVM, PhD, Diplomate American College of Theriogenologists

Equine embryos may be transferred into a recipient mare immediately after collection, cooled for up to 24 hours prior to transfer, or frozen for transfer at a later date. The ability to cool and transport embryos long distances has opened up embryo transfer services to horse owners that do not want to ship their donor mare to a referral center.

The original medium used for shipment of equine embryos was called Ham's F-10. Unfortunately, the medium was somewhat cumbersome to use since a mixture of gasses (oxygen, carbon dioxide and nitrogen) had to be bubbled through the fluid to stabilize the pH. In addition, the solution required the addition of antibiotics and a protein source, such as calf serum prior to use. Once prepared, Ham's F-10 had a working shelf life of only about 72 hours. However, it served the industry well, as many thousands of equine embryos were successfully shipped in this medium since the late 1980's.

Several new holding media have been developed over the past few years for cooling transporting equine embryos. The media are complete, ready-to-use and have a shelf life of more than a year. Buffers, nutrients, growth factors, amino acids, antibiotics and surfactants have already been incorporated into the holding solutions. The composition of the media allows the equine

embryo to survive during short-term storage at 5°C. Many embryo transfer programs now use one of the new complete holding media instead of Ham's F-10.

The initial steps in the process of shipping cooled embryos are the same as for collection and transfer of fresh embryos. The donor mare is bred and the specific day of ovulation determined. An embryo flush attempt is performed 7 or 8 days after ovulation. The embryos is identified under a dissecting microscope and washed through several drops of holding medium. It is evaluated for morphology (i.e. morula or blastocyst), size and quality or grade.

A small (5-ml) plastic tube with a screw cap is filled with approximately 4.5 ml of pre-warmed holding solution. A small air gap is initially left at the top of the tube. The embryo is carefully transferred into the holding solution within the plastic tube. This is typically accomplished using a small syringe and 0.25-ml plastic straw. It is recommended that the straw be examined after use to ensure that the embryo was evacuated into the tube of holding solution.

Additional holding solution is then slowly added until the 5-ml tube is completely full. The screw cap is securely fixed into place and the junction of cap and tube is sealed with parafilm. A 50-ml

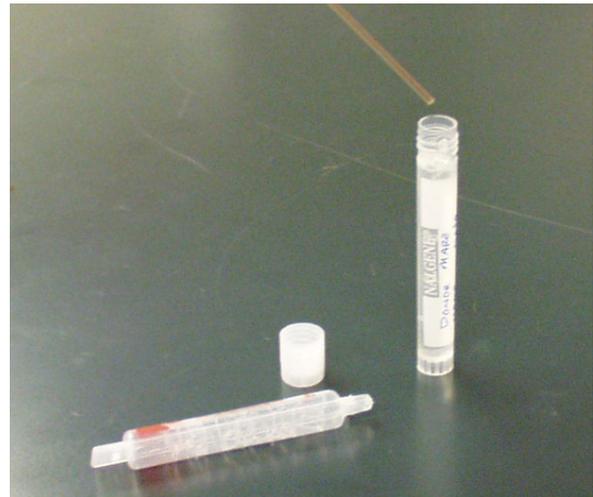
conical or centrifuge tube is then filled with flush medium that had been saved prior to the collection attempt. The 5-ml tube containing the embryo is placed within the 50-ml tube and the cap is attached, eliminating as much air as possible. This cap is also sealed with parafilm to prevent leakage.

The smaller tube is placed with the fluid-filled larger tube to provide physical protection during transport. Flush medium is used in the outer tube instead of water in case some leakage occurs into the smaller tube containing the embryo.

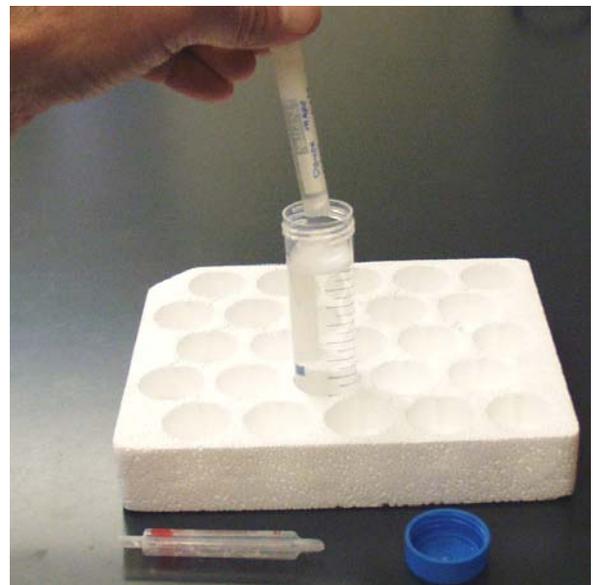
The tubes are placed into a passive cooling system, such as an Equitainer<sup>®</sup>, and shipped to a referral center for transfer into a synchronized recipient mare. Historically, a majority of embryos were cooled in Ham's F-10, shipped by overnight courier service, and transferred into a recipient within 18 to 24 hours after collection. Currently, most embryos are cooled in complete media, transported by counter-to-counter airline service, and transferred into a recipient within 8 to 16 hours after recovery.

Several research studies noted that there was no significant difference in pregnancy rates between embryos transferred immediately after collection and embryos cooled and transported in Ham's F-10. Although many equine embryos have been cooled and transported in the new complete holding media, additional research is need to determine how long embryos can be maintained in the new media. If embryos can be cooled for up to 24 hours, it would allow for transport by overnight courier service, which in many instances is more convenient and cost-effective than counter-to-counter airline service.

In summary, significant progress has been made in the area of embryo cooling, storage and transport over the past 20 years. This has resulted in dramatic changes in the practical utilization of embryo transfer in the equine breeding industry.



**Transferring embryo into small plastic vial**



**Placing small vial inside larger vial**



**Vial with embryo in shipping container**