



# Modern Techniques for Modern Horse Owners

## *Embryo Transfer*

by Dr Nichole Logan Belvoir Equine  
Clinic

***Technological advances in breeding research has brought the procedure of Embryo Transfer into the realm of all horse owners.***

**T**he options available to horse owners wishing to breed their mares in this technological advanced era are far greater than ever before. In 2010 prospective breeders can examine these procedures in the process of deciding which option is best suited for a particular mare, plus the owner's individual situation and financial commitment.

Horses have been bred for centuries using natural service, with either the mare and stallion running together or in-hand service where both animals are restrained and the stallion is bought to the mare to cover her.

Artificial insemination, where semen is collected from the stallion and inserted into the mare's uterus, has opened the door for access to top performance stallions from around the world, as well as increasing the genetic diversity of some breeds within Australia.

Embryo transfer involves removing a fertilized egg (an embryo) from a donor mare and transferring this embryo into the uterus of a recipient mare. The process was first used in horses in the 1970's and since then has become widespread across the United States and South America. The transfer of fresh and cooled embryos are now allowed by most equine breed registries, although the rules regarding how many foals can be registered each year out of a given mare differs among breeds. One breed that does not allow any

form of assisted reproductive techniques are the Thoroughbreds.

Other options for reproduction not widely used in Australia include Egg transfer, Gamete Intrafallopian Transfer (GIFT) and Intracytoplasmic Sperm Injection (ICSI). GIFT involves the mare's ovum and the stallion's sperm being deposited in the oviduct of a recipient mare. This process is very useful for stallions with a low sperm count, as fewer sperm are needed to achieve a successful pregnancy. ICSI is a newer, more successful technique than GIFT that involves collecting an ovum from a donor mare, injecting a single sperm into the egg with a micromanipulator and incubating the resulting gamete for a few days before transcervical transfer into the uterus of a recipient.

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Oocyte transfer procedure involves placement of a donor mare's oocyte (egg) into a recipient mare's oviduct (fallopian tube). The recipient mare is artificially inseminated with the desired semen prior to oocyte transfer; therefore, fertilization and embryo development occur within the

reproductive tract of the recipient mare. The recipient mare is either not cycling or her egg is removed, so it does not become fertilised. This process is best suited for donors with physical problems that may prevent them from carrying a foal to term, such as a torn cervix.

While these options briefly outline what is available around the world in the equine breeding arena, in this article Dr Nichole Logan discusses the intricacies of Embryo transfer and the process of preparing an embryo for long-term storage or vitrification.

### **Embryo Transfer**

There are numerous situations where one might choose embryo transfer in mares, with some of the most common being obtaining more than one foal per year out of top producing mares; foals from competition mares in training or showing; foals from late-foaling mares, leaving them open over the winter for an early start the following spring; foals from mares with reproductive problems that prevent pregnancy, pregnancy maintenance, or foaling, and foals from mares with other health problems that prevent pregnancy or carrying the established pregnancy to term.

#### **SELECTING A RECIPIENT MARE**

Recipient mares should be young and in good health. Maiden mares make

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## Embryo Transfers continued...

excellent recipients as they have the best quality uterus, however, there is a chance a maiden mare may deliver a small foal or be an unsuitable mother. Mares that have successfully carried and delivered a live, healthy foal are generally the best candidates. Generally speaking, the recipient mare would be between the ages of four and twelve and would not need a Caslick's procedure (stitches to the vulva to reduce the risk of infection in the genital tract) There should never be fluid in the uterus at any reproductive ultrasound examination as it can be an indicator of inflammation or infection, and ideally the mare would be monitored through at least one oestrous cycle to assess the quality of uterine and cervical tone at five days after ovulation, the time when she would most likely receive an embryo.

In order for an embryo transfer to be successful, the recipient should have the following characteristics five days after ovulating: 1) ovulation should occur from 1 day before (+1) to 3 days after (-3) the donor mare, with a -2 being the most desirable recipient, 2) the uterine tone should be good to excellent, 3) the cervical tone should be good to excellent, 4) there should not be any uterine oedema, 5) there should not be any intrauterine fluid, 6) finally, the mare should have a distinct corpus luteum (a hormone secreting structure that develops on the ovary after ovulation) on one or both of her ovaries, suggesting she is producing adequate progesterone to maintain a pregnancy.

### THE COLLECTION PROCEDURE

Mares can be bred with fresh, cooled or frozen semen. During the breeding process they are examined daily to accurately determine the day of ovulation. To aid in determining the age of the embryo when it is collected, an ovulation induction drug, is commonly used. Most mares reliably respond to the drug and ovulation occurs 36-38 hours after administration. Six and a half to eight days after ovulation a catheter is inserted into the donor mare's uterus and the uterus is sequentially flushed with two litres of embryo flush media two times. 'Y' shaped tubing allows fluid to move from the embryo flush media bag into the uterus via one tube and out of the uterus via a second tube. The solution is passed through a cup with an ultra-fine filter, which captures the embryo, along with some flush media for protection. The fluid in the cup is transferred to a petri dish and the embryo is searched for using a microscope.

If no embryo is recovered after the initial attempt, a final two litres of fluid is transferred into the uterus; oxytocin is administered and the fluid is allowed to set in the uterus for three minutes. The oxytocin causes the uterus to contract, and

*Right:*  
The recipient mares are carefully selected for their role in an embryo transfer operation and 'Blackie' shows the benefits of this selection process as she cares for 'her' new foal.

*Below:*  
When performance mares are at their competition peak, the option of continuing showing and possibly reproducing more than one offspring is attractive to owners.



Photographs supplied by Nichole Logan.



with fluid inside, acts as a sort of agitator to help loosen an embryo that may be trapped in intrauterine folds. After this time, the fluid is retrieved and again filtered through the cup and the search process is repeated with a second dish.

If an embryo is recovered, it is evaluated for quality, washed to remove any contaminants, placed in a nutrient media, and is transferred through the cervix and into the uterus of a suitable recipient mare. Alternatively, if no recipient mare is immediately available, an embryo can be placed in an embryo holding media and cooled in a semen-shipping container for transport to a facility where a recipient mare is available. Cooled embryos should be transferred within 12-24 hours of collection for best results.

### PREGNANCY CHECKS

The first pregnancy checks are usually five and seven days after transfer, which would be days 12 and 14 after ovulation. Success rates for embryo transfer with fresh embryos can be measured for two facets of the procedure: 1) Embryo recovery from

the donor mare and 2) Establishment of pregnancy in the recipient mare.

Embryo recovery is usually the rate-limiting step in equine embryo transfer. Recovery rates will vary with the individual mare's fertility; young mares can have very high rates, typically around 80% per cycle. Older mares with history of infertility, as a group, will have recovery rates around 30% per cycle. However, individuals within these groups will have higher or lower recovery rates. Once the embryo is recovered, a pregnancy success rate of approximately 80% at 14 days can be achieved, with about a 10% rate of pregnancy loss following that time. Older mares or chronically subfertile mares will have a decreased pregnancy rate and an increased rate of pregnancy loss. Cooling an embryo for shipment may also slightly decrease pregnancy rates.

If the embryo recovery attempt is unsuccessful, mares can be brought back to oestrus with an injection of prostaglandins and the process can be repeated, which means mares can have several embryo recovery attempts per season with no adverse affect on uterine health if strict hygienic technique is followed.

It is recommended that a donor mare be allowed to carry her own pregnancy every 3-4 years to promote a healthy uterus and aid in cervical relaxation.

### VITRIFICATION

Embryo vitrification - the process of storing the collected embryo in a sort of suspended animation in a glass-like state at sub-zero temperature - is a useful tool in the arsenal of a reproductive veterinarian, as it allows storage of embryos in the case that a suitable recipient is unavailable at the time of embryo collection. In 2007 vitrification was ruled to be an acceptable form of storage for an embryo by the American Quarter Horse Association, and many breed registries worldwide have since followed suit.

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**Embryo Transfere continued...**  
 This process can also be used to store an embryo for transfer so that the foal is born at an optimal time, or for storage of embryos from valuable individuals for use after that individual has died, as embryos can be held in this state indefinitely with proper storage conditions. The collection technique is identical to that for fresh or cooled transfer, however, the collection attempt must be made 6.5 days after ovulation as it is most successful when embryos are very small in size.



The embryo is flushed from the uterus of the donor mare and placed in increasing concentrations of ethylene glycol and glycerol, which are substances that prevent damage to cells that are exposed to freezing temperatures. This technique prevents the formation of ice crystals in the embryo, which destroys cells and helps to instead, make the water in the cells harden like glass, without the formation of crystals. The embryo is cooled in the vapour produced by liquid nitrogen for 60 seconds before being totally submerged in liquid nitrogen for long term storage.

Pregnancy rates from vitrified, warmed embryos can be as high as 80% when the procedure is precisely followed. The

best pregnancy results are obtained when young donor mares are bred with fresh or cooled semen.

#### **COST CONSIDERATIONS**

There are many factors to consider when calculating the cost of a foal produced by embryo transfer. Whether a breeder chooses to have their mare donate a single embryo, or multiple embryos and whether they provide their own recipient mare or lease a recipient, all add to the cost of the procedure. Vitrification adds a separate cost, but can be a convenient tool for owners with only one recipient mare whose ovulation time does not line up with the donor mare. Once an early pregnancy

is detected, it is recommended that the recipient mares be maintained on an oral progesterone supplement until 120 days of gestation. This too adds to the final cost and with the multiple factors available for the individual mare and the owner's situation, a total for the procedure will vary greatly. With fees included for a recipient mare and associated procedures the costs for Embryo Transfer could range anywhere between \$3000 to \$5000 - pending availability of recipient mare - however an enquiry to the local equine veterinarian with collection and breeding facilities, will establish an estimate that reflects each individual's situation.

### **About The Author**

#### **Dr Nichole Logan**

*A graduate from the Oklahoma State University College of Veterinary Medicine, Nichole completed a residency program and received a Master's degree in Equine Reproduction from Colorado State University. Her areas of research included superovulation in mares and embryo vitrification. Following her residency, she started an embryo transfer facility in South Dakota in the United States before spending two seasons in the Hunter Valley doing stud work. Currently she is working with Belvoir Equine Clinic in the Swan Valley, WA.*  
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