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EQUIFACTS

Embryo Transfer in Mares

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Embryo transfer (ET), an advanced reproductive technology, has several potential uses in equine reproduction. ET can be used to increase the annual reproductive rates in mares. For example, some breed associations will allow multiple foals to be registered annually per donor mare. Young mares (2 years old) can have offspring through ET where otherwise this might not be a good idea. Females in use for shows, training or exhibition can have offspring without being removed from their primary use. Last, less fertile mares — particularly those with uterine problems — can have foals.

Before opting to use ET, consider that it is a very technical, expensive and time consuming process with a fairly low success rate. Additionally, breed registries place limitations on the use of ET to produce registered offspring. For example, the Tennessee Walking Horse Breeders and Exhibitors Association Corporate Bylaws contain a rule that governs embryo transfer for Walking Horses. The rule is as follows: "Registration of foals resulting from embryo transfers will follow the same standard requirements for registration, except that multiple foal registrations from a donor mare during an eleven month period will be accepted. Blood typing of donor mare, stallion and foal is required. Blood typing will be the sole responsibility of the person(s) sending registration and entirely at his expense. Any foal that does not meet the blood type comparison will be forever barred from registration."

Each horse breed registry has different rules and requirements for ET registration. The Clydesdale Breeders of the USA requires genetic testing to verify parentage, and two foals per mare per

year may be registered. The American Quarter Horse Association will allow the registration of ET foals from a mare provided certain documentation has been provided to AQHA. Moreover, additional fees are often charged for registration of ET foals.

Donor mares should be carefully selected. These mares should have a successful reproductive history, be in moderate body condition and gaining weight. For best results, these mares should have a physical examination and should be experiencing normal heat cycles based on teasing, palpation and ultrasound. Uterine biopsy and cytology results and a vaginal speculum exam should be normal. They should have foaled at least 2 months before the ET procedure.

Once selected, the donor mare is synchronized to the recipients. For instance, the mare can be given 44 mg/kg of Altrenogest orally once a day for 10-15 days. On the last day of Altrenogest feeding, the mare is given prostaglandin. Heat should result in about three days. The mare is examined daily by ultrasound until a dominant follicle greater than 35 mm in diameter is identified. Human chorionic gonadotropin (HCG) is given intravenously (2500-3000 IU). The mare should ovulate in 24-40 hours. Natural breeding or insemination to a stallion of known high fertility should occur 36 hours or less before ovulation. Raw or extended semen can be used. Generally the mare is bred 12 hours after HCG administration. Ovulation is confirmed by ultrasonography.

Recipient mares should be 3 to 10 years old, in good physical health and gaining weight. Each potential recipient should be reproductively normal based on rectal palpation, ultrasound, uterine biopsy, culture and cytology. Mares should have at

least a 30 mm corpus luteum and no fluid in the uterus. The cervix should be tight and the uterus tubular. Teasing records should confirm the mare to be cycling and ovulating normally.

Recipient mares can be prepared in the following three ways:

1. Mares that have had their ovaries removed for less than one year can be given Altrenogest (66 mg daily) beginning six days before embryo collection and continued until 150 days of pregnancy.
2. Intact mares, present in sufficient numbers, can be teased to identify stage of heat cycle. Identify two mares that ovulated between one day before and 2 days after the donor mare.
3. Intact mares can have their estrous cycle manipulated by administration of hormones to synchronize their estrous cycle to the specifications listed under number 2 above. This has been the least successful method of supplying recipient mares.

The donor mare is collected seven days after ovulation (i.e., if the mare ovulates on Monday, she is collected the next Monday). A catheter with a 75 ml inflatable cuff is passed through the cervix. The cuff is inflated and pulled back against the internal opening of the cervix. One liter of Dulbecco's Phosphate Buffered Saline (DPBS) with 1 percent fetal calf serum and antibiotics added is allowed to flow into the uterus by gravity. After three minutes the fluid is drained from the uterus into a filter. A second liter of fluid is passed into the uterus and after rectal massage of the uterus, this fluid is also drained into the filter. The process is repeated with a third liter of fluid. Ninety percent of the fluid put in the uterus should be drained out.

The fluid in the filter is poured into a petri dish. Methodical embryo searching at 10 magnifications under a dissecting microscope completes the collection process.

Since mares cannot be superovulated like cows, only one or sometimes two embryos can be collected. In normal, healthy mares an embryo is found in 50 percent to 80 percent of attempts.

Following are three circumstances in which collection rates are lowered:

1. Collection rates for barren mares averaged 30 percent, while mares over 18 yielded an embryo in only 20 percent of cases.
2. Mares collected on the farm were less likely to be successful (27 percent) than mares collected at an ET center.
3. Foaling mares (53 percent) and older maiden mares (61 percent) were more likely to be successful than 2-year-old mares (36 percent).

Once the collected embryo is identified, it is assessed for quality. Most (69 percent) collected embryos are expanded blastocysts of good or better quality. Poorer quality embryos result a much lower pregnancy rate (16 percent). Indicators of poor embryo quality include collapsed blastocoeles, extruded blastomeres, and degenerate embryos. The size of collected horse embryos varies widely but averages .4 mm. Generally, the embryo is washed three times in DPBS with 10 percent fetal calf serum at room temperature before transfer. Most horse embryos are transferred at room temperature within one hour of collection.

For shipping, horse embryos have been stored in Hamm's F10 tissue culture fluid with 10 percent fetal calf serum added. A mixture of 5 percent carbon dioxide, 5 percent oxygen and 90 percent nitrogen is bubbled through the fluid before filtering. Embryos can be placed in this fluid and shipped in a controlled shipping container with no reduction in pregnancy rate if the embryo is transferred within 24 hours.

Embryos may be placed into the mare by surgical implantation or by a nonsurgical method through the vagina. Opinion is divided as to which method yields better pregnancy rates, but today most horse embryos are transferred by a nonsurgical method using a special embryo transfer gun. Pregnancy rates in recipient mares implanted with good quality embryos appear to range from 40 percent to 70 percent.

Equine embryo transfer can be complicated and expensive but does provide a useful method of assisting reproduction in more valuable mares.

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