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Embryo transfer challenges and perspectives

Transferência de embriões: desafios e perspectivas

E.L. Squires

Gluck Equine Research Center, University of Kentucky, Lexington, KY, USA.
Correspondence: edward.squires@uky.edu

Introduction

Embryo transfer has become a common technology in the horse industry in many countries (Squires and Seidel Jr, 1995). Initially, embryo transfer was used as a means of obtaining pregnancies from sub-fertile mares. These were generally older mares that had failed to produce a foal or had a history of becoming pregnant and then losing the pregnancy. More recently, embryo transfer has become a means of obtaining multiple foals from genetically valuable mares or obtaining foals from mares that are still competing in shows or races. Many breeders utilize embryo transfer as a means of obtaining several foals per year. This provides them with an opportunity of breeding the donor mare to several different stallions in one season. It is not unusual to obtain 2 to 3 foals per year out of the donor mare. Surprisingly, many breeders are opposed to obtaining multiple foals per year since they feel that this will flood the market and lower the price of the offspring. However, no one seems to oppose the use of the stallion that breeds several hundred mares are year.

Collection procedure

The techniques for embryo recovery were initially established in the late 70s and early 80s and very little has changed since that time. The major change has been the availability of commercial flush media for embryo recovery. Another trend is the use of fairly large volumes of flush media for recovery attempt. It is not unusual for the veterinarian to use 3 to 6 l of flush media per recovery attempt. Another change is the availability of several filter cups that allow fluid to move through the cup but trap the embryo above the level of the filter. This allows for easy identification of the embryo since only a small volume needs to be searched under the dissecting scope. We suggest that a quality dissecting microscope be used for identification and grading of the equine embryo .It is helpful to examine the flush media at a low magnification of .7 to 1.0X and then upon identification of embryo, the morphology and size of the embryo can be evaluated at a higher magnification (3.5X). Most technicians assign a developmental stage to the embryo of: morula, early blastocysts, blastocysts and expanded blastocyst. It is also important to use an eyepiece micrometer to determine the greatest diameter of the embryo. The embryo is also given a quality score of 1 to 3 based on size, shape, color and appearance of the trophoblast and inner cell mass (McKinnon et al., 1988).

Embryos can be recovered from the uterus on Day 6, 7, 8 or 9 post-ovulation. It is best to palpate and ultrasound the mare daily during late estrus in order to determine the exact time of ovulation. Day 0 is considered the day of ovulation. Most commercial operations collect equine embryos seven or eight days after ovulation. Collection of Day-9 embryos is usually not performed since these larger embryos are more fragile and result and lower pregnancy rates after transfer (Carnevale et al., 2000). For embryo freezing, the Day-6 embryo is much more viable than older embryos. Although pregnancies can be obtained with transfer of frozen Day-6 equine embryos the pregnancy rate is slightly less than that of fresh or cooled embryos (Seidel Jr, 1996). Therefore most embryos are transferred are either fresh or cooled embryos (Carney et al., 1991).

Management of donors

The donor mare is usually selected by the owner and not by the veterinarian or technician. However, the veterinarian and or technician should provide some advice to the owner regarding the criteria for selection of the donor. It is important to know that the success of embryo transfer decreases as the mare advances in age (Carnevale et al., 2000b). Studies have shown that as a mare ages her oocytes become less fertile (Ball et al., 1989; Carnevale and Ginther, 1995) Therefore, fertilization decreases and/or embryonic loss in the oviduct increases in the older mare and then embryo recovery from these donors is greatly reduced, particularly in mares older than 16 years of age. Having a good reproductive history on the donor mare can also allow one to predict the success of obtaining embryos. Older mares that have a history of becoming pregnant and then losing the pregnancy are better candidates for an embryo donor than mares with a history of no confirmed pregnancies.Mares that failed to become pregnant for multiple years may be more appropriate for an oocyte program (Carnevale et al., 2000a). Most commercial embryo transfer program report an embryo recovery rate of 50-60% recovery per cycle, though , embryo recovery rate for young fertile mares can be 70-80% per cycle and

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those of older subfertile mares 30-40% per cycle. The number of embryos recovered in a breeding season depends upon the number of cycles and the number of multiple ovulations that occur during the cycle. Mares that spontaneously double ovulate have a much higher embryo recovery rate than single ovulating mares. In addition, mares that are superovulated with exogenous hormones can average 2 to 3 embryos per cycle (Alvarenga et al., 2001; Scoggin et al., 2002).

Management of the embryo donor is similar to a mare bred to carry her own foal. One must be concerned about uterine fluid and infection after breeding and thus be prepared to manage the uterus in order to eliminate all extra uterine fluid and bacterial contamination after breeding. It is best to ultrasound the donor mare's uterus for 1 to 2 days after ovulation to it ensure that the uterus is devoid of excess uterine fluid. Generally donor mares are given an ovulatory agents once a pre-ovulatory follicle is diagnosed. Embryo recovery is generally attempted 7 to 8 days after ovulation. If embryos are to be frozen, then recovery attempts will be made 6 to 6 1/2 days after ovulation. Once embryos are measured and graded, they can be transferred immediately into recipients or placed into transport media and cooled to 5°C for shipments to a recipient station for subsequent transfer. Pregnancy rates are similar to that of fresh embryos for those stored up to about 24 h (Carney et al., 1991). This allows embryos to be collected on the farm by the veterinarian and then shipped to another facility that maintains large numbers of recipient mares. The embryonic loss between a 14 and 50 for embryo transfer pregnancies should be about 5 to 10%, which is similar to that of mares bred to carry their own foal.

Management of recipients

The recipient is the most important part of embryo transfer program. Thus, the selection and care of the recipient both before and after embryo transfer has a major effect on the success. Generally, the recipient should be between 3 and 12 years of age, normal cycling, with no reproductive problems and have a gentle disposition.

Depending upon the time of the year, recipients can either be anestrus/transitional, progestin-treated mares, cycling mares, or ovariectomized mares. Recipient mares should be placed under artificial lights beginning December 1 in order to hasten the first ovulation of the year. Ideally, recipient mares should have at least one normal cycle prior to being used in the breeding season. However, there are many occasions where early in the year an inadequate number of recipient mares are available for the number of embryos collected. Thus, the only option is to place the anestrus/transitional mare on progesterone for 5 to 7 days prior to being used as a embryo recipient (McKinnon et al., 1988). The transitional, progestin -treated mare tends to provide better pregnancy rates than the anestrus mare. In selecting transitional mares, they should have multiple 20 to 25 mm follicles and the presence of uterine edema prior to progestin treatment. The ideal recipient mare is a normal cycling recipients that has ovulated either 1 or 2 days before the donor, the same day as the donor, or one day to 3 days after the donor (Carnevale et al., 2000b).

It is important to do a final check on the recipient mare's uterus prior to embryo transfer. This is either done 1 or 2 days prior to the transfer (day 5) or on the day of the transfer (day 7-8). Studies have shown that recipient mares with good to excellent uterine tone and good to excellent cervical tone with the absence of uterine folds provide higher fertility after embryo transfer than mares that have a uterus that contains less tone or perhaps the presence of some endometrial edema (Carnevale et al., 2000b).

Challenges and perspectives

One of the major challenges is trying to obtain pregnancies from older mares, particularly older maiden mares. Performance mares are sometimes 12 or 16 years of age prior to being placed into an embryo transfer program. These mares generally have cervical problems that impact their ability to eliminate fluid after breeding. This also makes these mares more difficult to flush and recover embryos. Other challenges include clients that request 5 to 6 embryos out of each mare per season for several seasons. We know that continual flushing of the mare's uterus for multiple cycles year after year can result in endometrial changes. This shows up as an increase in cloudy flushes and a change in the endometrial biopsy score. We suggest that mares be bred to carry a foal to term at least once in their lifetime and hopefully, every few years in order to maintain the health of the uterus. By proper management of the uterus and allowing a mare to have a foal naturally on several occasion then it may be possible to increase the number of foals in their lifetime three to four-fold by using embryo transfer.

The cost of embryo transfer has remained high for many years. The major cost in an embryo transfer program is maintenance of recipient mares. When embryo recovery is low, recipient mares still have to be fed and managed. One way of decreasing the cost of embryo transfer is to increase the success of obtaining embryos .Experimentally, it has been shown that mares can be superovulated and that embryo recovery can be increased three to four-fold using superovulatory drugs (Alvarenga et.al., 2001; Logan et al., 2007). Unfortunately, none of these drugs are currently on the market and thus the veterinarian has no ability to superovulate mares. Another possible way of improving the efficiency of an embryo transfer program would be to incorporate embryo freezing into the program. Nearly 60% of bovine embryos that are recovered are frozen and transferred at a later

time when recipients are available. Unfortunately, only the small Day-6 6 1/2 embryos survive the freezing process. Transfer of these frozen thawed embryo can result in a 50 to 60% pregnancy rate per embryo (Stout, 2012). However, freezing of seven or eight embryos results in only a 30% pregnancy rate. Studies are needed to develop means of successfully freezing Day-7and 8 equine embryos.

Keywords: challanges, embryo transfer, perspectives.

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References

Alvarenga MA, McCue PM, Bruemmer J, Neves Neto JR, Squires EL. Ovarian superstimulatory response and embryo production in mares treated with equine pituitary extract twice daily. Theriogenology, v.56, p.879-887, 2001.

Ball BA, Little TV, Weber JA, Wood GL. Survival of Day-4 embryos from young, normal and aged, subfertile mares after transfer to normal recipient mares. J Reprod Fertil, v.85, p.187-209, 1989.

Carnevale EM, Ginther OJ. Defective oocytes as a cause of subfertility in old mares. Biol Reprod Monogr, n.1, p.209-214, 1995.

Carnevale EM, Maclellan LJ, Coutinho da Silva M, Scott TJ, Squires EL. Comparison of culture and insemination techniques for equine oocyte transfer. Theriogenology, v.54, p.981-987, 2000a.

Carnevale EM, Ramirez RJ, Squires EL, Alvarenga MA, McCue PM. Factors affecting pregnancy rates and early embryonic death after equine embryo transfer. Theriogenology, v.54, p.965-979, 2000b.

Carney NJ, Squires EL, Cook VM, Seidel GE Jr, Jasko DJ. Comparison of pregnancy rates from transfer of fresh versus cooled, transported equine embryos. Theriogenology, v.36, p.23-32, 1991.

Logan NL, McCue PM, Alonso MA, Squires EL. Evaluation of three equine FSH superovulation protocols in mares. Anim Reprod Sci, v.102, p.48-55, 2007.

McKinnon AO, Squires EL, Voss JL, Cook VM. Equine embryo transfer: a review. Comp Cont Educ Pract Vet, v.10, p.343-355, 1988.

Scoggin CF, Meira C, McCue PM, Carnevale EM, Nett T, Squires EL. Strategies to improve the ovarian response to equine pituitary extract in cyclic mares. Theriogenology, v.58, p.151-164, 2002.

Seidel GE Jr. Cryopreservation of equine embryos. Vet Clin North Am Equine Pract, v.12, p.85-99, 1996.

Squires EL, Seidel GE Jr. Collection and transfer of equine embryos. Fort Collins, CO: Colorado State University/Reproduction Biotechnology Laboratory Animal, 1995. (Bulletin, n.8)

Stout TA. Cryopreservation of equine embryos: current state of the art. Reprod Domest Anim, v.47, p.84-89, 2012.