EMBRYO TRANSFER IN HORSES

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Jonathan F Pycock and **James R Crabtree** discuss, in the second part of a series of articles on equine reproduction, the benefits that using the featured techniques can bring

Summary

The purpose of this article is to give the reader an overview of an embryo transfer programme and the separate phases involved. The factors that determine the success of such a programme are considered in detail, particularly the effect of semen quality, embryo quality and recipient mares. The practice of embryo shipping – where the donor mare is inseminated and flushed at home and the embryo shipped off to an embryo transfer centre with a large number of recipients – is growing in popularity. This transported embryo system is reviewed in the light of recent experiences. The transported embryo service is particularly useful where a recipient of suitable quality is not able to be provided by the owner of the donor mare. Sourcing, buying and maintaining recipient mares can be one of the most difficult and costly components of an embryo transfer programme. Success rates using transported, chilled embryos are comparable with those obtained where embryos are transferred immediately after collection. Embryo freezing opens up the possibility of preserving genetic material from mares. Until recently, pregnancy rates after freezing and subsequent thawing of the embryo were often poor. Studies in the past year have offered real advantages in the freezing of equine embryos.

Key words

embryo transfer, recipient mares, embryo shipping, embryo freezing

EQUINE embryo transfer (ET) was first described in the early 1970s and has been performed commercially since the 1980s. ET is now well established as a breeding technique.

Equine ET's progress has been slow in the UK due to the technique's non-acceptance by the thoroughbred breed registry, as well as the limitations imposed by many of the other breed registries on the number of foals born by ET that can be registered. Historically, ET was used in older donor mares that often conceived but failed to carry a mare to term.

More recently, ET has been used in performance mares that continue to compete. This development has seen ET combined with newer technologies, such as frozen semen.

Freezing stallion sperm to preserve valuable genetics is commonplace, and freezing sperm from the testicles of castrated, seriously ill or dead stallions is now available.

The opportunities for preserving mare genetics are, however, much more limited. Embryo transfer opens up the possibilities of preserving genetic material from mares through the process of embryo freezing. The freezing of mare oocytes, although not yet commercial, may be available in the future.

Why use embryo transfer?

The applications of embryo transfer include obtaining:

• foals from performance mares that continue to compete;

• multiple foals from individual mares each year;

• foals from young (two-yearold) mares;

• foals from reproductively unsound mares; and

• foals from mares with nonreproductive health problems.

In addition, freezing allows the long-term storage of embryos, thus preserving valuable genetics – which is especially important in rare breeds.

The process involves five separate phases.

• Daily monitoring and insemination of the donor mare.

• Daily monitoring of a minimum of two recipient mares (synchronised in oestrus with the donor mare) to determine when they ovulate. A recipient mare should have ovulated between one day

before and zero to three days after the donor mare (Jacob et al, 2010). These authors found that acceptable pregnancy rates could be obtained when the degree of synchrony ranged from the recipient ovulating one day before the donor to the recipient ovulating six days after the donor. The results of this study demonstrated that the degree of synchrony between donor and recipient mares does not need to be as restricted as previously reported in horses.

• Flushing the donor mare to retrieve the embryo. The donor mare is restrained in stocks and hygienically prepared (Figure 1), and then the uterus of the donor mare is flushed using a specially designed catheter introduced through the cervix. Up to six litres of flushing medium are introduced, one litre at a time. Fluid is recovered using gravity, and an in-line filter is used to reduce the amount of fluid that needs to be searched for the embryo. The use of 20iu oxytocin aids fluid recovery. The residual fluid in the filter, typically around 20ml, is poured into a searching dish and a stereo microscope, with 10× to 15× magnification, used to search for the embryo (Figure 2).

• Transfer of the embryo into a recipient mare. The embryo is aspirated into a 0.25ml or 0.5ml insemination straw (Figure 3), and transferred non-surgically using a specially designed ET pipette. The transfer pipette has a sterile, disposable outer sheath and a thin plastic wrap that the pipette is pushed through as it is passed via the cervix into the uterine body of the recipient mare.

• Pregnancy determination of the recipient mare.

Embryos are generally collected seven or eight days after ovulation, before it is possible to determine if the mare is actually pregnant by ultrasound examination. The embryo recovery rate from the donor mare is affected by the:

• type and quality of the semen used to inseminate the donor;

• age of the donor and her breeding history;

• number of days after ovulation when the flush is performed; and

• number of ovulations.

Semen type

The quality of semen used to inseminate the donor has a significant effect on the rate of embryo recovery.

Generally, mares inseminated with fresh or chilled semen are more likely to produce an embryo than those inseminated with frozen semen. Regardless of type, the semen’s quality is very important. The better the semen quality, the greater your chances of recovering an embryo.

Donor mare

The age of the donor and her reproductive history have a significant influence on embryo recovery. Older mares with poor reproductive histories produce fewer embryos. The causes of this include age-related degeneration of the oocyte and pathology of the uterus and/or oviducts. Young mares less than 12 years old are 10 per cent more likely to produce embryos than mares more than 18 years old (Uliani et al, 2010).

Many oocytes and embryos produced by aged, sub-fertile mares are inherently defective and have low survival rates. The rate of pregnancy loss (early embryonic death) following transfer from older donor mares is also higher than that obtained from younger mares.

The combined low embryo recovery rate from the older mare and the high rate of loss of transferred embryos from these mares greatly limit the number of foals that can be produced from this category of mare.

Day of flushing and number of ovulations

Embryos can be recovered from day six to day 10 post-ovulation. Recovery rates are, however, low on day six and day-10 embryos are large (Figure 4) and easily visible with the naked eye, but are difficult to handle and transfer.

Days seven and eight offer the best balance between recovery and ease of transfer. A study by Jacob et al (2010) found that embryo recovery rates between days seven and 10 were similar, but day seven embryos produced higher pregnancy rates when compared with embryos from days eight and nine.

Mares that double-ovulate have higher embryo recovery rates per cycle than single ovulating mares, but it does not double the rate (Riera, Roldan and Hinrichs, 2006). The same study compared embryo recovery rates when multiple ovulations occurred, from one ovary (unilateral double ovulation) or from both ovaries (bilateral double ovulation).

Interestingly, it also found that the embryo recovery rate per follicle ovulated was less in unilateral than bilateral ovulating mares. It was concluded that the lesser embryo recovery in unilaterally ovulating mares indicated that some mechanism at the level of the ovary or oviduct may interfere with ovulation or oocyte pick-up in multiple ovulating mares, and may explain the reduction in embryo recovery per follicle ovulated in superovulated mares.

The average recovery rate in a commercial embryo transfer programme is typically 50 per cent, but when one selects young, reproductively healthy donor mares, and uses fresh or chilled semen, the rate can be more than 70 per cent.

The success of the transfer phase of the process depends on the quality of the embryo recovered and the choice of recipient.

Embryo quality

The equine embryo's quality has a major effect on pregnancy rates and it is important to evaluate all embryos thoroughly.

An extremely rapid growth occurs in the equine embryo, from a morula (day five) to an expanding blastocyst (day seven to eight; Figure 5). Embryos with poor quality scores result in very poor pregnancy rates. Embryos smaller than normal for their age, or those that have morphological abnormalities, also result in lower pregnancy rates.

Recipient mares

Proper selection and management of recipient mares is the most important factor affecting the success of an equine embryo transfer programme. Recipient mares should meet all of the following requirements.

• Good health and body condition.

• Easy to handle and halter-broken.

• Body size around 500kg and 16 hands.

• Between four and 10 years of age.

• Sound breeding condition (free from uterine or ovarian abnormalities) and a history of regular cycling.

• Free from any disease.

Recipients can come in all shapes and sizes (Figure 6). They should have a body size that is equal to, or bigger than, that of the mare producing the embryo. The temperament of the recipient is very important and she will need to be a good mother.

The most important factors in selecting the recipient immediately prior to transfer of the embryo are the tone of the uterus and tone of the cervix. The recipient mares are examined by ultrasound for the presence of a corpus luteum and the absence of any uterine fluid or oedema.

In addition, the reproductive tract is assessed per rectum for uterine and cervical tone. Mares that have excellent uterine and cervical tone have higher pregnancy rates than recipients with marginal

uterine and cervical tone.

When selecting recipient mares, it should be remembered that the size, age and previous foals produced will govern the size and health of the foal at birth.

Ideally, recipient mares should be between five and nine years of age and should have already produced at least one healthy foal. If the owner wishes to provide their own recipients, the authors will accept mares from three to 12 years of age. However, they must be examined for suitability prior to inclusion in the programme.

This examination for breeding soundness should include taking an endometrial biopsy sample. When accepted into a programme, if a recipient is rejected on one occasion, then depending on the reason for rejection, she may well be suitable for use on another day.

It would be reasonable to achieve a pregnancy rate of 80 per cent with transfer of grade one embryos at the initial pregnancy examination (day 14), and a 70 per cent pregnancy rate at day 45.

Embryo shipping

When adequate recipients are not available, it is possible to ship embryos to another facility where recipients can be made available.

In controlled studies, there are no significant differences in pregnancy rates between embryos transferred fresh and those chilled and transported before transfer (Moussa et al, 2002). Embryos can be shipped in commercial holding media at 5°C in a semen shipper.

This transported embryo service is popular, as you avoid the need for your client or yourselves to source recipient mares and go through the process involved in selecting suitable candidates. However, you remain integrally involved in the process by inseminating and flushing the donor mare before loading the embryo into a semen shipper and sending it to the ET centre.

Success rates

The international average for embryo recovery is 50 per cent.

This, combined with a transfer/ pregnancy rate of 80 per cent (to 14 days), provides an overall success rate of 40 per cent (50 per cent × 80 per cent = 40 per cent) per embryo flush.

To maximise the chances of a live foal in an embryo transfer programme, one should aim to breed young mares to either fresh or chilled semen.

This does not mean, however, that one cannot attempt to breed an older mare with frozen semen,

but one should be aware that many of the factors noted above are beyond our control or management.

It should always be remembered that a recipient can lose a pregnancy just like any other mare, so their standard of care needs to be high to ensure the health of the foal carried within.

Costs of an embryo transfer programme

The procedures described above are very labour-intensive and, therefore, the cost of ET is considerable – this must be borne in mind from the outset.

Stocks for the mare, temperature-controlled laboratory facilities and appropriate equipment are essential. Phases one to three will be performed regardless of whether an embryo is recovered or not, so it is necessary to budget for at least two cycles and flushes to produce one pregnant recipient mare. The cost for two cycles/flushes and a transfer will be in the region of £1,500 to £2,000, not including livery, stud fees and semen collection or shipping costs.

Embryo freezing

Freezing stallion semen is now commonplace, and good pregnancy rates can be achieved using frozen semen.

Until recently, pregnancy rates after freezing and the subsequent thawing of the embryo were often poor. Two basic methods have been used: conventional, slow cooling – as described by Maclellan and associates (2002) – and vitrification (fast freezing). Before freezing, the embryo is exposed to cryoprotectants, the most common of which is glycerol (Figure 7).

Vitrification has gained popularity over traditional slow freezing procedures because of its speed and the fact that it does not require expensive computerised freezing units. The two methods only allow the freezing of small embryos (diameter less than 300µm) and vitrification of embryos greater than 300µm, which has resulted in few, if any, pregnancies (Eldridge-Panuska et al, 2005). Therefore, for successful freezing, small embryos need to be collected by flushing donors at six-and-a-half days after ovulation. This is more technically demanding, considering the equine embryo does not enter the uterus until approximately five-and-a-half to six days after ovulation. Recovery rates are generally lower.

One study (Araujo et al, 2010) has offered real advantages in the successful freezing of equine embryos. Being able to freeze them for future use provides the opportunity to wait until a suitable recipient mare is available. In addition, certain breed associations restrict the number of foals that can be born in any one breeding season. Workers from Brazil discovered that equine embryos could be frozen and thawed successfully under field conditions using a commercially available freezing kit. They reported freezing 64 embryos to achieve a 59 per cent pregnancy rate at 15 days

of pregnancy, compared with 82 per cent for their fresh embryos. This suggests that the vitrification of embryos is a commercially viable procedure.

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References

- Araujo G H M, Rocha Filho A N, Burns S D, Burns C M, Moya-Araujo C F and Meira C (2010). Pregnancy rates after vitrification, warming and transfer of equine embryos, *Animal Reproduction Science* **121S**: 299-300.
- Eldridge-Panuska W D, di Brienza V C, Seidel G E Jr, Squires E L and Carnevale E M (2005). Establishment of pregnancies after serial dilution or direct transfer by vitrified equine embryos, *Theriogenology* **63**: 1,308-1,319.
- Jacob J C F, Santos G O, Oliveira J P, Gastal M O and Gastal E L (2010). Evaluation of reproductive parameters in a commercial equine embryo transfer program, *Animal Reproduction Science* **121S**: 305-306.
- Maclellan L J, Carnevale E M, Coutinho da Silva M A et al (2002). Cryopreservation of small and large equine embryos pretreated with cytochalasin-B and/or trypsin, *Theriogenology* **58**: 717-720.
- Moussa M, Duchamp G, Mahla R, Bruyas J-F and Daels P F (2002). Comparison of pregnancy rates for equine embryos cooled for 24h in Ham's F-10 and EmCare holding solutions, *Theriogenology* **58**: 755-757.
- Riera F L, Roldan J E and Hinrichs K (2006). Patterns of embryo recovery in mares with unilateral and bilateral double ovulations, *Animal Reproduction Science* **94**: 398-399.
- Uliani R C, Ramires Neto C, Dell'Aqua J R, Pessoa M A, Camargo, A L, Alvarenga R and Alverenga M A (2010). Effect of mare breed and age on embryo transfer efficiency, *Animal Reproduction Science* **121S**: 303-304.