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Selection and management of the embryo transfer donor mare

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Summary

Embryo transfer (ET) is a useful tool for hastening genetic improvement and for procuring foals from mares incapable of carrying a pregnancy to term. However, ET is not cheap, primarily because of the costs of buying and managing recipient mares but also because of the failure, to date, to develop reliable techniques for superovulating mares or for cryopreserving horse embryos. In addition, since a properly synchronized recipient is vital to the success of transfer, and ovulation is difficult to synchronize in mares, at least 2 or 3 recipients per donor or a large pool of recipients are needed at the outset. Alternatively, since chilling embryos at 5°C for up to 30 h appears to have little detrimental effect on pregnancy rates, it is possible to flush embryos in one location and transport them to a large centre where suitable recipients are available; this system is now used on a large scale in the USA. To avoid predictable disappointment, it is important that the client is made aware that the intrinsic fertility of mare and stallion are critical to the success of ET. Embryo recovery and transfer success rates are much lower and pregnancy losses are higher when aged (>15 years) or sub-fertile mares are used as donors. Similarly, the use of chilled or frozen semen results in significantly lower embryo recovery rates. Nevertheless, where the potential (financial or sentimental) value of the resulting foal(s) is high, ET can be an extremely rewarding and technically straightforward way of increasing or salvaging the reproductive potential of mares.

Keywords: Embryo recovery, donor mare, selection, management, chilled transport

Auswahl und Management von Spenderstuten im Embryotransfer

Der Embryotransfer (ET) ist ein nützliches Verfahren um sowohl genetische Programme zu beschleunigen als auch um Fohlen von den Stuten zu erhalten, bei denen die Aufrechterhaltung der Gravidität nicht gewährleistet werden kann. Durch die Kosten für die Anschaffung und den Unterhalt der Empfängerstuten und aufgrund der bis heute ungenügenden Entwicklung von Techniken der Superovulation bei Stuten und der Kryokonservierung von Pferdeembryonen, ist der ET kostspielig. Desweiteren sind mindestens 2 bis 3 Empfängerstuten beziehungsweise initial eine große Gruppe an Empfängerstuten pro Spenderstute nötig, da die genaue Synchronisation der Empfängertiere die Voraussetzung für einen erfolgreichen Transfer darstellt. Die Ovulationssynchronisation bei der Stute ist jedoch schwierig. Seitdem bekannt ist, dass das Tiefkühlen von Embryonen bei 5°C bis zu 30 Stunden nur wenig negativen Einfluss auf die Trächtigkeitsrate hat, stellt das Ausspülen von Embryonen an einem Ort und der Transport zu einem großen Zentrum mit passenden Empfängerstuten eine Alternative dar und findet in der USA große Anwendung. Um vorhersehbare Differenzen mit den Patientenbesitzern zu vermeiden, ist es wichtig, diese über die Bedeutung der individuellen Fertilität der Stute und des Hengstes für einen erfolgreichen Embryotransfer aufzuklären. Werden ältere (> 15 Jahre) oder subfertile Stuten als Spender genutzt, ist die Erfolgsrate bei der Embryogewinnung und dem Transfers deutlich vermindert und es treten vermehrte Trächtigkeitsverluste auf. Trotzdem stellt der Embryotransfer in Fällen von hohem finanziellem oder ideellem Wert der Fohlen, einen erfolgsversprechenden und technisch einfachen Weg dar, das reproduktive Potential von Stuten zu erhöhen oder zu bewahren.

Schlüsselwörter: Embryogewinnung, Spenderstute, Selektion, Management, Kühltransport

Introduction

During the last two decades, the number of embryos flushed and transferred has increased dramatically in the Australian, South and North American sport-horse industries, primarily as a means of increasing the number of offspring from phenotypically desirable mares but also as a means of treating subfertility of uterine origin. European horse breeders have been slower to adopt ET partly because many Breed Societies were initially loathe to register more than one foal per mare per year but also because of the relatively high costs of buying and managing potential recipients, and misplaced fears among owners that the foals would behave or perform more like their surrogate than their genetic mothers. The major obstacles to the cost-effectiveness of equine ET are the failures to develop user-friendly and reliable means for inducing superovulation in mares or for cryopreserving embryos (see *Squires* et al. 1999 for review). In addition, owners often fail to appreciate the critical importance of both mare and stallion fertility to the success of ET. Most notably, embryo recovery rates fall significantly when aged or sub-fertile donor mares (*Vogelsang* and *Vogelsang* 1989) or frozen-thawed semen (*Meadows* et al. 2000) are used. In the absence of a user-friendly way of cryopreserving horse embryos, ensuring suitable recipients are available for every embryo recovery attempt makes ET time-consuming and expensive, particularly given that the long and variable duration of oestrus in mares complicates synchronization. Furthermore, since inadequate recipient quality or synchronization are major

causes of failed ET, if a large recipient herd is not available, at least 2 or 3 recipients per donor are needed at the onset of a synchronization attempt. One recent development that has helped to ease this problem is the success of chilled embryo storage and transportation; transport at 5°C for up to 30 hours appears to have little detrimental effect on subsequent pregnancy rates and allows embryos to be flushed in one location and sent to a specialized ET centre where suitable recipients are available (*Squires* et al. 1999). The aim of this review is to summarize the important aspects of donor mare selection and management and to emphasize the factors that may compromise the success of embryo recovery.

Selecting the donor mare

In theory, ET is of great potential benefit to sport-horse breeding because many of the best potential genetic dams remain in competition until rather late in life when they have few reproductively useful years left. ET allows for the production of foals from young mares before they enter competition (with an accompanying shortening of the generation interval), in competing mares between competitions or during an "off-season", and can also be used simply to increase the number of foals per year from a particularly valuable mare. Other indications for ET include conditions that preclude the carriage of a pregnancy to term such as a previous pelvic fracture or ventral hernia or sub-fertility of uterine origin (e.g. severe endometrosis), although it should be borne in mind that the embryo recovery rate and the likelihood of pregnancy are considerably lower and the risk of pregnancy loss is higher when aged (>15 years) or sub-fertile mares are used as donors (Tab. 1: *Vogelsang* and *Vogelsang* 1989; *Meadows* et al. 2000). Making the client aware of these facts in advance will prepare them for the possible disappointments and spiraling costs associated with ET from aged, sub-fertile donors. For competing mares, the demands of synchronization, insemination and embryo recovery can lead to a fall in the level of performance, while the stress of heavy training or competition may also have a negative effect on cyclicity and fertility.

Tab 1 The effect of donor mare age and fertility on the rates of embryo recovery, pregnancy and pregnancy loss after transfer to recipient mares.
Der Einfluss des Alters und der Fertilität der Spenderstute auf die Erfolgsrate bei der Embryogewinnung, der Gravidität und auf die Trächtigkeitsverluste nach dem Transfer auf die Empfängerstuten

Parameter	Authors	Factor	Success rate
Embryo recovery	*Vogelsang and Vogelsang 1989*	Age 2-8	61% (80/132)
		9-17	51% (94/183)
		18-28	30% (93/309)
		Maiden/Foaling	54% (186/342)
		Subfertile	29% (81/282)
	Meadows et al. 2000	Age <14	67% (89/134)
		>14	43% (37/86)
Pregnancy in recipients	*Vogelsang and Vogelsang 1989*	Age 2-8	70% (59/84)
		9-17	52% (50/97)
		18-28	56% (50/90)
		Maiden/Foaling	63% (121/193)
		Subfertile	49% (38/78)
Pregnancy losses	*Vogelsang and Vogelsang 1989*	Age 2-8	14% (8/59)
		9-17	24% (12/50)
		18-28	30% (13/38)
		Maiden/Foaling	18% (22/121)
		Subfertile	34% (13/38)

The ideal is therefore to devote a period of time solely to embryo recovery, as is done during the non-competition season for polo ponies in Argentina.

The choice of stallion/semen

An often neglected, but critical, aspect of a successful ET programme is the fertility of the chosen stallion. The embryo recovery rate will vary just as dramatically between stallions as the per cycle pregnancy rate (37-90%: *Morris* and *Allen* 2002), and a stallion with poor fertility figures is unlikely to yield good embryo recovery rates. Furthermore, the way in which semen is preserved critically affects embryo recovery rates, and AI with fresh semen yields considerably more embryos than chilled-transported semen or, in particular, frozen-thawed semen (Table 2: *Meadows* et al. 2000).

Examination and management of the donor mare

As compared to routine breeding management, the most important differences when managing ET donor mares are the need to know more precisely when ovulation has occurred, and the added complication of ensuring synchrony of ovulation with that in a recipient mare, if the latter are in short supply. In short, the donor mare needs to be examined at least daily to plan the time of embryo recovery accurately. Indeed, if the plan is to freeze the embryo, mares should be checked at least twice daily with the aim of recovering the embryo after its expected arrival in the uterus early on day 6 after ovulation but before it has expanded beyond 225 μm in diameter approximately 12 hours later (*Boyle* et al. 1989). With regard to synchronisation, many texts describe the blind use of 10-day progestagen or progestagen-oestrogen combinations followed by PGF2a analogue administration as simple labour-unintensive oestrous synchronization protocols. However, in the author's experience repeated scanning and synchronous or staggered (depending on the relative sizes of the largest follicle in each mare) administration of a PGF2a analogue during mid-dioestrus (days 6-14) together with the judiciously timed use of ovulation induction agents allows good synchronization with no apparent negative effects on embryo recovery, quality or pregnancy in recipients and a lower risk of surprising failures. In fact, the margins for acceptable synchrony are fairly wide, with recipients ovulating between –24 and +72 hours after the donor offering similar pregnancy rates (see *Squires* et al. 1999 for review). Nevertheless, the ideal is a recipient that ovulated 0-48 hours after the donor since early pregnancy loss rates seem to be higher at the outer margins of acceptable synchrony (*Carnevale* et

Tab 2 The effect of stallion semen preservation method on embryo recovery rates.
Der Einfluss der Aufbewahrungmethode des Hengstspermas auf die Erfolgsrate bei der Embryogewinnung.

Authors	Semen preservation method	Embryo recovery rate
Meadows et al. 2000	Fresh	88% (78/89)
	Chilled, transported	47% (45/95)
	Frozen-thawed	47% (48/103)
Lisa, Knaap, Colenbrander and Stout (unpublished data)	Fresh	71% (17/21)
	Chilled or frozen	32% (21/66)

al., 2000). With this in mind, inducing ovulation in recipients by administering human chorionic gonadotrophin (hCG) or deslorelin (Ovuplant implants®) at the time of donor ovulation is a good way of ensuring a well-synchronized recipient.

Superovulation

In an ideal world, donor mares would be superovulated, as is routinely done in species such as cattle where equine chorionic gonadotrphin (eCG) or follicle stimulating hormone (FSH) regimes result in embryo yields averaging 5 per cow and peaking at 50 (*Kafi* and *McGowan* 1997). However, early attempts to superovulate mares with pituitary extracts, FSH preparations or immunization against inhibin were disappointing (roughly a doubling of the ovulation rate: see *Squires* et al. 1999 for review) and it was feared that the combination of a relative insensitivity to gonadotrophins and the unique anatomy of the equine ovary, with its enormous preovulatory follicles that must all ovulate through a single small ovulation fossa, posed insurmountable barriers to multiple follicle growth and ovulation. Recent studies have, however, been more promising and in particular *Alvarenga* et al. (2001) reported an average of 7 ovulations yielding 3.5 embryos for mares treated twice daily with crude equine pituitary extracts beginning simultaneously with the induction of luteolysis on day 5 after ovulation. And while attempts to repeat these results have been less impressive (*Scoggin* et al. 2002; *W.R. Allen* pers. comm.), it is at least clear that superovulation is possible. Future studies will no doubt focus on identifying the most suitable agent and administration protocol, bearing in mind concerns over the use of pituitary extracts since the discovery of the spongiform encephalopathies, and the prohibitive costs of recombinant FSH preparations. Of course mares can also double or triple ovulate spontaneously, and some ET centers have reported a high incidence of spontaneous multiple ovulation (30%: *Losinno* et al. 2000) with corresponding increases in the embryo recovery and pregnancy rates.

Embryo recovery

Timing

Embryo recovery is usually performed on day 7 or 8 after ovulation, unless the embryos are destined for freezing. Because embryos expand extremely rapidly during days 7-10, day 8 embryos are much easier to find. However, if ovulation detection is performed only once daily there is a risk that a recovered embryo may be nearer to day 9 and too big to load into the transfer straw/pipette; larger embryos are also more easily damaged during recovery and transfer. For these reasons, flushing at 7-7.5 days after ovulation is usually preferred. On the other hand, it appears that embryo development and descent is slower in older mares (*Squires* et al. 1999; *Meadows* et al. 2000) and, if the first flushes from aged donors do not yield embryos, it may be prudent to wait until day 8.
When embryos are to be frozen, the best time to flush appears to be around 6.5 days after ovulation (*Lascombes* and *Pashen* 2001). Early on day 6 the embryo may still be in the oviduct while later on the same day the embryo may have expanded beyond the 225 μm likely to survive freezing and thawing (*Slade* et al. 1985). Therefore, detection of ovulation should be performed at least twice daily and, if no embryo is recovered, flushing may be repeated 12-24 h later, although this second flush may be contaminated with neutrophils as a result of the initial attempt.

Flushing technique

The technique for embryo recovery by transcervical uterine lavage with a cuffed catheter is straightforward and described in a number of texts (e.g. *Allen* 1982). Of course, operators differ with regard to their preferences for types of catheter, filter etc. and also over whether to use an open (faster fluid flow) or a closed (better sterility) flushing system. The most important considerations are to use a suitably buffered flushing fluid (e.g. Dulbecco's phosphate buffered saline) and to add a macromolecule/protein (e.g. 0.4% bovine serum albumin or 1% fetal calf serum) to prevent the embryo sticking to silicone or plastics in the collection system. Most operators perform 2-3 consecutive flushes of the uterus with 0.5-1.5 litres of fluid and massage the uterus per rectum to aid fluid distribution and recovery. After flushing, it is usual to administer a PGF2a analogue to bring the mare back into heat and reduce the likelihood of a resulting endometritis. However, induction of luteolysis may be delayed for 1-2 days to aid synchronization of donors and recipients for the next cycle.

Embryo searching and handling

Day 6 embryos are generally around 150-180 μm in diameter whereas day 8 embryos are usually between 0.3 and 0.7mm and visible with the naked eye. Nevertheless, a dissecting microscope should be used to search for embryos because of the possibility of developmental retardation or a second, smaller embryo from a later ovulation. Unfertilized oocytes (UFOs) may also be found on occasions and can be distinguished from viable embryos by their flat, granular and acellular appearance. Although UFOs are classically retained in the oviduct (*Betteridge* and *Mitchell* 1972), they do sometimes accompany an embryo on its passage into the uterus. Importantly, if only a UFO is found, the filter and search dish should be rechecked carefully for the expected embryo. Recovered embryos are usually scored on the basis of their developmental status (late morula, early or expanded blastocyst), and quality (1=good, 4= degenerate). While, in theory, only embryos of grades 1-2 are good candidates for transfer, because horse embryos are difficult to obtain even those of grade 4 are often transferred if there are sufficient recipients.

Conclusions

The mechanics of embryo recovery and non-surgical transfer are relatively straightforward, although experience is required to achieve good transfer results. However, intensive monitoring is essential to ensure recovery of the embryo and transfer to the recipient at the appropriate times. Where recipients are at a premium, cryopreservation of embryos can yield acceptable pregnancy results (54%: *Lascombes* and *Pashen* 2001), while still some 20% lower than those with fresh

embryos. However, collection of embryos for freezing requires more precise determination of the time of ovulation and, if ET is to prosper in Europe, it may be preferable to establish a limited number of large specialized recipient farms to which embryos can be transported at 5°C. Finally, if sub-fertile donor mares or frozen semen are used, the success of both embryo recovery and pregnancy may drop alarmingly.

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