

MODERN BREEDING TECHNIQUES

by

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This foal was by Cathargo by frozen semen AI from Zangersheide in Belgium

Some semen can be frozen and kept in flasks indefinitely until needed

A vet examining and scanning a mare to see how the embryo is developing

The practical application of artificial insemination and embryo transfer have forced the modernisation of techniques in horse breeding.

Artificial Insemination (AI) has become firmly established in the UK particularly with Sports Horse and Arab breeding. Embryo Transfer (ET), while not so well known, has the potential, particularly with frozen embryo technology, to once again add a new dimension to British horse breeding.

AI in horses, although recorded in Arabic texts as far back as the thirteenth century, has only relatively recently become accepted in the UK. Semen is collected from a stallion, usually by a technician, utilising an artificial vagina and the services of either a 'jump mare' in oestrus or increasingly a dummy mare, upon which the stallion is trained to jump. A third technique where a stallion's semen is collected on the ground applies to only a few stallions.

The semen is assessed, counted and tested as appropriate. The raw ejaculate is filtered and is then extended utilising a mixture of nutrients and antibiotics, to prolong its viability. This latter process will vary according to how the semen is to be used.

Three main categories of AI are recognised, specifically utilising fresh or fresh-extended semen, chilled semen or frozen semen.

Fresh or fresh-extended semen is used most commonly within the confines of the stud itself. Indeed, many studs operate very much in the manner of a traditional breeding establishment, i.e. teasing mares and inseminating those that are in season. The advantage of utilising AI, is that for most stallions only a daily, or even every other day, collection is necessary with the ejaculate being appropriately split. This means that insemination can be carried out efficiently without the need for multiple coverings at odd times of the day and night.

When combined with appropriate veterinary examinations of mares in season, the use of the stallion can be further reduced, leading to improved fertility rates.

Fresh semen lasts for a variable time once collected and for practical purposes is usually used immediately. If it is extended, however, and protected from extremes of heat and cold, it will last for several hours depending on the individual concerned, and therefore may be locally transported. Fresh semen or fresh-extended semen lasts for a similar time within the mare's reproductive tract to

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that derived from natural covering, i.e. in an average stallion two to three days.

The process of insemination is carried out as cleanly and hygienically as possibly. The mare in oestrus ('in season' or 'heat') is restrained appropriately, a tail bandage applied and her perineum cleaned. Utilising a gloved hand, a sterile disposable catheter is introduced via the vagina through the cervix, and the semen deposited into the uterus.

AI has advantages in individual mares that are otherwise unable to be mated naturally for reasons of aggression, injury, infection or contagious disease. AI techniques are relatively non-traumatic and being inherently clean, given the proper testing has been carried out on semen, allow effective and efficient disease control.

AI is also useful in those mares with an increased susceptibility to uterine infections. When combined with minimal contamination and vigorous veterinary procedures post-insemination, these can lead to significantly increased fertility rates in such mares.

Chilled semen is prepared as for fresh-extended, but then gradually cooled to about 4°c by use of a special container. Chilled semen has an extended life, usually in the region of 48 hours. This enables semen to be shipped, sometimes considerable distances. The majority of chilled semen utilised in our AI Centre comes from Western Europe where most Sports Horses have been bred by AI for many years.

Once chilled semen is introduced into the mare, its life span is approximately twelve hours. For this reason careful examination of the mare is required to ensure that she is at the appropriate time of her oestral ('heat') cycle when the semen arrives. Usually this involves several examinations both before the mare comes into season and during the heat period itself, together with the use of appropriate hormones to induce ovulation at precisely the right time. Semen is ordered in the early part of the heat period and delivered by courier.

Frozen semen differs from chilled by being stored in liquid nitrogen. Semen stored in this way may have an indefinite life span and it is therefore a good way of storing genetic material for the future. The process of freezing is more complicated than the chilling process and therefore frozen semen is usually collected only at specific centres where the necessary equipment is available. Unfortunately not all stallion semen will freeze and an important part of the procedure is to assess semen viability for this process.

Once semen is frozen successfully, it may be transported all over the world and stored indefinitely in specially designed flasks until it is ready to be used. It has many practical advantages in that stocks of semen can be retained at the most convenient location, always ready for use.

Frozen semen, once thawed and in the mare, has a relatively short life of about six hours, which means that insemination must be carried out very close to the time the mare ovulates, at the end of the heat period. For this reason such mares are resident to allow scanning during the latter part of oestrus to ensure that insemination is carried out at the most optimum time.

The administration of frozen semen involves one of two novel techniques which have increased our success rates for this method. Deep uterine insemination involves the passage of a flexible catheter through the vagina, cervix body and horn of the uterus by rectal and ultrasonographic control to allow the position of semen at the entrance of the oviduct on the same side as an impending or very recent ovulation.

The alternative technique is to carry out the same procedure utilising a flexible endoscope by which means the actual oviductal papilla in the horn can be identified and the semen actually placed on this via a long catheter. This latter technique is relatively labour intensive, requiring sophisticated equipment but it does permit the use of very low volumes of semen from, for example, a limited resource (perhaps of a dead stallion) or where the semen has been sex selected. About 5% of frozen inseminations are carried out in this way with 95% utilising conventional deep uterine technique.

Embryo transfer is the technique of removing early embryos from the donor mare and placing them in a surrogate. The donor mare is inseminated or mated in the usual way with the time of ovulation determined as exactly as possible. Six and a half to eight days later, embryos are flushed from the mare's uterus under sterile conditions, utilising a semi-sealed system with filtration, and any embryos identified. Embryos at this stage are slightly larger than the size of a pin head. Embryos may then be introduced via a special catheter into a synchronised surrogate mare, or alternatively placed in straws and transported under chilled conditions, utilising similar equipment to that used for chilled semen. Such embryos have a life span of about twelve hours.

If one is contemplating embryo transfer, one has to consider the practicalities of having surrogate mares. Currently there are only two commercially available herds in the UK where one can place surrogate pregnancies. Such mares are usually available to lease for a period of time while the pregnancy is being carried and the mare is kept on your own premises. An alternative is to have one's own surrogate mares; if one has large premises and adequate resources, this is quite practical. It does, however, require two to three surrogate mares per donor mare and of course if early programs in the year are successful, the number of surrogate mares required will increase for subsequent transfers!

An exciting new development in technology has appeared over the last two years with the increasing availability of frozen embryos. These are taken at approximately the six and a half day stage and deep frozen in liquid nitrogen. Such embryos may be transported all over the world. It is therefore technically possible to purchase stock of a better genetic make-up and place them in one's own mare as a surrogate. Surrogate mares, under these circumstances, should be of a suitable size compared to the genetic make-up of the embryo, in good general health and with no adverse breeding history, roughly aged between three and ten.

Embryo transfer in general can be used to obtain pregnancies from mares still undergoing an active riding career without seriously interfering with their competitive program. Embryos may also be derived from mares that for some reason cannot carry a foal to term, for example those that have pelvic damage. In theory, pregnancies may also be derived from older mares and placed in the younger surrogate, although here it has to be acknowledged that embryos derived from older mares are regarded as having a lower viability than those from young stock.

Artificial Insemination and Embryo Transfer are not the only techniques available for assisted reproduction, but currently these are the only ones commercially available. Most Breed Societies now allow the registration of stock produced by these means, with a notable exception of the thoroughbred breeding industry, which does not allow animals to race that have been produced either by AI or ET. This restriction, however, does not apply to those animals used for other sports such as eventing (there is a special Wetherbys' register for this purpose).

Concerns over security with such breeding techniques have now largely evaporated; the whole process is carefully documented and, if there is any question of parentage, DNA techniques now can give very clear answers.

These processes are, however, labour intensive from a technical perspective and require more input than conventional breeding practice. The key to success is particular attention to detail and hygiene issues and careful examination of mares as frequently as is necessary.