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**Embryo Transfer Tips and Tricks**

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**Introduction**

In recent years, the use of embryo transfer has significantly increased in the northern part of Europe. Several veterinary practices in The Netherlands, Belgium, Germany and France have integrated this technique in their reproductive services. The main reasons for this new development are a better understanding of the techniques of embryo transfer and transportation of cooled embryos as well as the availability of large, centralized recipient herds for the transplantation of transported, cooled embryos. It is especially the development of a practical method of short-term (24 hr) storage and transportation of equine embryos and the development of practical storage media that has allowed a breakthrough of this technique in Europe. This allows embryos to be collected in the “field” and then shipped to a centralized facility for transfer to suitable recipient mares. The ability to transport cooled embryos provides veterinarians with the opportunity to offer embryo transfer service without the onerous task of maintaining recipient mares, and eliminated the need to ship donor mares to a centralized facility. This article will review current equine embryo transfer techniques.

**ET applications**

The applications of embryo transfer include: 1) obtaining foals from (young) performance mares that continue to compete or that are for sale, 2) obtaining multiple foals from individual mares each year, 3) obtaining foals from two-year-old mares, 4) obtaining foals from reproductively unsound mares, and 5) obtaining foals from mares with non-reproductive health problems. Although embryo transfer was initially proposed as a promising method for obtaining foals from aged, subfertile mares, experiments utilizing oocyte transfer and embryo transfer have documented that many oocytes/embryos produced by aged, subfertile mares are inherently defective and have low survival rates after transfer to recipient mares; therefore, aged, subfertile mares are not optimal candidates for embryo transfer. Especially veterinary practices that start to offer this technique should carefully select fertile donor mares in order to give their ET program the best chances for success.

**ET timing**

Embryo collection is typically done on Day 7 or 8 after ovulation (Day 0 = day of detection of ovulation). Since the embryo only arrives in the uterus 6.5 days after ovulation, collection on Day 6 may be too early i.e. embryo has not arrived in uterus. Similarly, it has been described that in older mares (>18 years) embryos oviductal transport and migration into the uterus may occur later. Therefore embryo collection in old mares is typically performed on Day 8. Once present in the uterus, the embryo grows very fast and embryos collected on Day 9 are too large for transfer.

**Techniques**

The basic technique used by practitioners to recover embryos from donor mares has remained the same for many years. The standard method of embryo collection in the mare is a nonsurgical transcervical uterine lavage. A sterile catheter with an inflatable cuff (Bivona catheter) is inserted through the cervix. After the cuff has been placed in the uterine body, it is inflated with 60 - 90 ml of air and pulled back firmly against the internal os of the cervix. Embryo collection is performed using one-litre bottles of either lactated ringer solution (Hartmann’s Solution, Laboratoire Ageatan, France) or a complete equine embryo flushing medium that is provided in one-litre bottles (IMV Technologies). The uterus is lavaged a minimum of three times with 1 to 2 litres of prewarmed (30 - 35°C) embryo flush medium each time. The flush medium is allowed to flow back out the catheter by gravity flow and is collected in the original bottles. Recovery of the flush medium is aided by massage of the uterus per rectum. The flow rate and massage of the uterus are aimed at obtaining a maximum turbulence in the uterus and thus increase the chances that the embryo will be carried out with the out flowing flushing medium. After completion of the uterine lavage, the bottles are carried back to a clean area where the contents of the bottles containing the recovered flushing medium are poured through an embryo filter (Emcare Embryo Filter). The filter membrane is then carefully rinsed and the bottom of the filter is searched for the presence of an embryo. Once identified the embryo is washed by passing it through a series of cups containing each 1-2 ml embryo holding medium. This washing is preferably performed using a 12-well sterile culture plate and the embryo surrounded by a very small volume is passed from one cup to the next at least 10 times. This procedure will eliminate any contamination that will be present in the flushing medium. At this point, the embryo is ready to be transferred into a recipient mare or to be conditioned for cooled transportation.
Transfer of embryos

Transfer of equine embryos is generally performed using a nonsurgical transcervical approach. The embryo is loaded in a 1/4 ml straw (smaller, Day-7 embryos) or a 1/4 straw (larger, Day-8 embryos). The straw containing the embryo is then mounted in an embryo transfer gun or Cassou gun (IMV Technologies). The loaded gun and straw are protected by 3 layers. The first (most inner) layer is a sterile pipette that fits immediately around the metal transfer gun. This pipette has a rounded metal tip at the end with two side ports/holes. The rounded tip assures a smooth,atraumatic transition through the cervix. The side holes help with the unhampered deposition of the embryo in the uterus. The second layer of protection is a narrow plastic sheet (referred to as “schemise”) that protects the pipette from contamination in the vagina. This plastic sheet is carried through the vagina into the external portion of the cervix. At this place, the plastic sheet is punctured and the pipette is allowed to go forward through the cervix into the uterus. The third layer is a sterile palpation sleeve that is placed around the entire unit (Cassou gun plus sterile protection pipette plus plastic sheet) and serves as protection while going through the vulva and vestibular-vaginal junction. Practically, the veterinarian wearing a long sterile palpation sleeve places the Cassou gun with its protection metal-tipped pipette and plastic sheet in the palm of his/her hand. A second sterile palpation sleeve is placed over the hand holding the Cassou gun. The hand of this second sleeve is cut off and the end is held inside the fist of the gloved hand. This unit is now introduced through the vulva into the vestibulum. At the vestibular-vaginal junction, the fist is opened and the outer sterile sleeve remains in place. The Cassou gun still protected by the plastic sheet is placed in the external os of the cervix. The sheet is then punctured and the pipette with Cassou gun is then gently carried into the uterus. The proper placement in the body of the uterus is verified by rectal palpation.

Recent advances

In recent years the flushing technique has undergone several small modifications. The method with the one-litre bottles is still the preferred method because it provides the fastest flow rate (important to recover the embryo), it is a closed system (no open filter), it is easy to measure the recovered volume. The number of lavages which traditionally was 3 lavages has been questioned and more veterinarians use multiple lavages (3 to 6). McCue and colleagues have reported that a fourth flush with a 3-min incubation time and administration of 20 IU oxytocin results in an additional 7% embryo recovery. The increase in embryo recovery rate provided by the modified recovery procedure is economically very significant in a clinical embryo transfer program. The cost of the additional media, oxytocin, and clinician time is usually minimal compared with the potential value of a recovered embryo. There are differences in technique during the “extra” flush that were not routinely used in the initial flush procedure. Specifically, during the “extra” flush, the medium is allowed to remain in the uterus for several minutes before being recovered, and oxytocin is used before the “extra” flush to stimulate uterine contractions, even if fluid recovery during the initial flush attempts is adequate. Additional studies are needed to determine if the increased embryo recovery rate is because of the additional flush, the 3-min waiting period, or the oxytocin. We have tested this method at our ET station and have come up with similar results. From these experiments, we have concluded that additional lavages increases the embryo recovery. It is unclear if the 3-min incubation time and/or the oxytocin administration bring significant improvement to the method. With the low-low price of lactated ringer solution it is easily justifiable to perform additional lavages when in doubt. In our practice, we now routinely use a minimum of 4 lavages with oxytocin and 3-min incubation in the last lavage. The embryo filter (Emcare Embryo Filter) that we currently use has several advantages: a large filter membrane allowing a rapid flow and a bottom with grid that allows immediate and convenient searching of the embryo. The embryo is manipulated using the short straws provided by IMV Technologies (crystal clear short 1/4 ml straws). When using lactated ringer solution for lavage it may be prudent to moisten the straws with protein-rich embryo holding medium before aspirating the embryo from the filter cup. This extra step will ensure that the embryo will not adhere to the inside of the straw.

Previous reports suggest that some equine embryos may fail to leave the tip of the Cassou gun during nonsurgical transfer. Verifications in our ET Center confirm the statements by Pat McCue (CSU) that in about 3% of embryo transfer attempts the embryo can be found in the tip of the Cassou gun after completion of the transfer attempt. After embryo transfer, the tip of the Cassou gun should be gently rinsed with a fine needle and syringe containing embryo holding medium. Especially the inside of the tip needs to be carefully flushed with about 2-3 ml of fluid. Embryos recovered in this way can be transferred again with success. As a precaution, we routinely place a large volume of fluid in that part of the transfer straw that will be evacuated the last in order to maximize the “flush” effect. In summary, embryo recovery can be enhanced by slight modifications of the standard flush technique, and transfer success can be improved by verifying that the embryo was not retained in the tip of the ET sheath.

NOTE: The slide presentation used for this lecture can be viewed on-line at www.ivis.org/lectures/daels/.