The Art of Transferring Equine Embryos

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Equine embryos are transferred into a recipient mare using a non-surgical, transcervical procedure. Transfer rates are best if the recipient mare is synchronized with the donor and ovulates between 1 day before (+1) to 3 or even 4 days after (–3 to -4) the donor.

If a cycling synchronized recipient is not available, a non-cycling hormone treated mare may be utilized. Anestrus mares may be administered 5 to 10 mg of estradiol 17β once daily in the muscle for 2 days beginning the day the donor mare ovulates. Subsequently, the mare is administered 200 mg of short-acting progesterone in the muscle (or 0.044 mg/kg altrenogest orally) once daily for 5 or 6 days until the donor mare is flushed. If an embryo is collected and transferred, the recipient mare is maintained on progesterone or altrenogest until the pregnancy examination at 14 to 16 days of embryo age. Pregnant non-cycling hormone-treated recipient mares should be maintained on progesterone/progestin support until day 120 of gestation.

Equipment and Supplies

Handling Embryos - Option 1 (small embryos; < 1,000 µm)

- Cassou gun (0.25 ml, 53 cm (21 inches)), disposable sterile sheath, 0.25 ml straw.

Handling Embryos - Option 2 (large embryos; > 1,000 µm)

- Insemination pipette, syringe (1.0 ml), sterile chemise.

Additional Supplies for Manual Embryo Transfer Technique

- Sterile obstetrical sleeve
- Sterile obstetrical lube

Additional Supplies for Cervical Forceps Embryo Transfer Technique

- Equine vaginal speculum (3-pronged) (sterile)
- Equine Cervical Forceps (i.e. Wilsher Cervical Forceps)
- Penlight(s)
- Sterile obstetrical lube

Preparation of the Recipient Mare

- Recipient mares are often sedated 5–10 minutes prior to the transfer procedure with either acepromazine maleate (0.02–0.04 mg/kg IV), xylazine hydrochloride (0.3–0.4mg/kg IV) or detomidine hydrochloride (0.01–0.015mg/kg IV).
- It is common practice to administer a single dose of a non-steroidal anti-inflammatory drug such as flunixin meglumine (1.0 mg/kg IV) prior to transfer.
- Other medications that may be administered include corticosteroids, antibiotics, and tocolytic agents.

Manual Embryo Transfer Procedure

- The tail of the recipient is wrapped and held away from the perineal area.
- Wearing examination gloves, the perineum is washed with a non-residual soap, rinsed with fresh water, and dried with paper towels.
- Embryos less than 1,000 µm in diameter may be transferred using a 0.25 ml straw, disposable sterile sheath, and stainless steel Cassou gun. The embryo is placed in the center of the straw within alternating columns of medium and air (Figure 1). The straw is loaded into the disposable sheath, which is then attached to the Cassou gun.

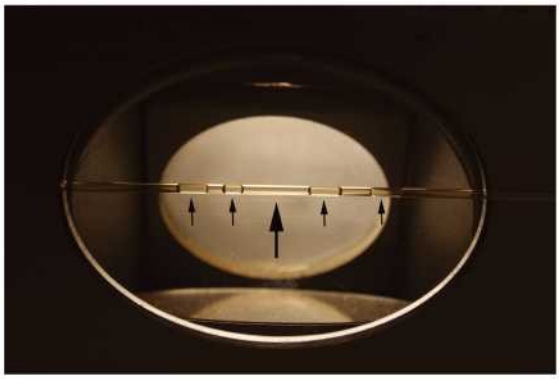

Figure 1. Embryo in a column of holding medium (large arrow) inside a 0.25 ml straw with two air gaps (small arrows) on either side of the column of fluid containing the embryo.

- Embryos greater than 1,000 µm in diameter are commonly transferred using a standard artificial insemination pipette, which is attached to a 1.0 or 3.0 ml syringe. The embryo is drawn up into the pipette in a small volume of medium between columns of medium and air. An outer sterile chemise should be used to protect the transfer gun or pipette from contamination with bacteria from the vulva, vestibule, or vagina. The transfer device should penetrate through the protective chemise within the external cervical os.

- Alternatively, a large embryo (i.e. >1,000 µm) may be transferred using a 0.5 ml straw and either a 0.5 ml sterile sheath and Cassou gun system or a 0.5 ml disposable embryo transfer gun.
- It is important to minimize manipulation of the cervix during the transfer procedure. The transfer instrument is gently maneuvered from the outside to place the tip of the instrument within the cervical lumen, and with a gentle, steady forward motion to facilitate passage of the instrument through the closed cervix into the uterine body. The embryo may be deposited into the uterine body or guided into a uterine horn via manipulation per rectum. The embryo should be gently deposited within the center of the uterine lumen as the transfer instrument is slowly withdrawn to lay the embryo in the space previously occupied by the transfer instrument. Consistent success is aided by attention to detail and a delicate transfer technique.

Cervical Forceps Embryo Transfer Procedure

- This procedure often involves multiple (at least 2) people.
- If available, one person should be at the head of the horse for light restraint.
- A small amount of sterile obstetrical lubricant is applied directly onto the vulva.
- The vaginal speculum, with the pen light(s) already fixed onto the device, is inserted through the vulva and vestibule and into the vagina.
- The cervix is visualized and the Wilshire forceps is passed through the speculum and used to grasp the lower aspect of the external os.
- Once the cervix is firmly grasped, the forceps is gently maneuvered caudally to straighten out the cervix and cervical lumen.
- The transfer device containing the embryo is subsequently passed into the vaginal vault, through the cervix and into the uterine body.
- Once fully into the uterus, the embryo is gently deposited into the uterine lumen.
- The transfer device is subsequently withdrawn, the forceps are disengaged from the cervix and removed and then the speculum is removed.
- It is common practice at some transfer centers to palpate the mare per rectum to remove residual air from the vaginal vault.

If another transfer is to be performed immediately, the speculum and Wilshire forceps are rinsed with tap water, then cleansed with at least three alternating applications of 4x4 gauze containing chlorhexidine scrub or alcohol. The devices are then rinsed with sterile water or sterile saline and placed onto a sterile field awaiting the next transfer procedure. If no other transfers are scheduled, the speculum and Wilshire forceps are cleaned and autoclaved using standard procedures.

Examination of the Transfer Instrument after Transfer

- Embryos are occasionally caught within the side ejecting stainless steel tip of the disposable sheath. Consequently, it is recommended that the tip of the sheath be rinsed after transfer and the rinse fluid examined under a dissecting microscope.

Failure to examine the sheath after transfer may result in the inadvertent loss of an embryo.

Management of the Recipient Mare After Transfer

- Minimizing stress in the recipient mare after transfer is important to optimize pregnancy rates. Consequently, it may be beneficial to keep the recipient mare in her original herd after transfer as opposed to moving her immediately to a different herd of post-transfer mares.
- It is common practice at some embryo transfer centers to administer progesterone or progestins following embryo transfer. If progesterone supplementation is provided, recipient mares may receive either altrenogest (0.044 mg/kg PO, once daily), progesterone-in-oil (200 mg IM, once daily), or a long-acting progesterone preparation (1,500 mg IM, once every 7 days).
- Progesterone supplementation may be discontinued at any time provided that endogenous levels are measured and determined to be sufficient to maintain pregnancy (i.e. >4.0 ng/ml). Progesterone therapy in a recipient mare may be discontinued between 45 and 70 days of pregnancy if an ultrasound examination confirms the presence of secondary corpora lutea. Alternatively, progesterone or progestin therapy may be discontinued at approximately 100–120 days of gestation without testing to determine concentrations of endogenous progesterone. The placenta produces sufficient progesterone and other progestins by day 90 to maintain the pregnancy.

Additional Comments

Transfer of an equine embryo is both a clinical technique and an art. Attention to detail and a gentle consistent approach are important for success. Pregnancy rates at day 14 following non-surgical transfer in mares range from <70% to above 90% (Table 1). Initial pregnancy examination should be performed 4 to 7 days post-transfer.

Table 1. Pregnancy rates following transfer of equine embryos.

Pregnancy Rate	Evaluation	Comments
≥ 90 %	Outstanding	Difficult to consistently achieve with large numbers of transfers
80 – 90 %	Excellent	Achievable with effort
75-80 %	Very Good	A solid goal
70-75 %	Good	Work on details
60-70 %	Fair	Need to improve
< 60 %	Marginal	Need remedial help

Further Reading

Hartman DL. 2011. Embryo transfer. In: McKinnon AO, Squires EL, Vaala WE, Varner DD (eds). Equine Reproduction, 2nd edn. Ames, IA: Wiley Blackwell, pp. 2871–9.

Jasko DJ. 2002. Comparison of pregnancy rates following nonsurgical transfer of day 8 equine embryos using various transfer devices. Theriogenology 58: 713–15.

Wilsher S, Allen WR. 2004. An improved method for nonsurgical embryo transfer in the mare. Eq Vet Educ 16: 39–44.