

EQUINE EMBRYO TRANSFER

by

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The first time a foal was born as a result of an embryo transfer procedure between mares was in 1974.

The technique of embryo transfer (ET) in the mare is relatively simple and offers considerable potential advantages for the breeding of horses. All too often mares spend their best breeding years being ridden or used in competition and consequently have little opportunity to become pregnant. By using ET, a mare can be mated and flushed with little interruption to her busy work schedule. In a similar way, by the use of E.T. the number of offspring that can be produced from a rare or highly prized mare can be increased by mating and flushing her on each estrous cycle of the breeding season, so that there is the possibility of producing several foals per mare versus the one obtained by natural methods. Older and multiparous mares are prone to endometrial degeneration which may render them incapable of sustaining a pregnancy to term. The breeding life of such mares can only be extended by the use of embryo transfer; recovering their eggs after fertilization and transferring the eggs to recipient mares with healthy uteri. The United States has been quick to realize the commercial potential of this procedure and has a number of commercial equine breeding Stations using embryo transfer. However the Jockey Club does not permit the use of embryo transfer, nor artificial insemination, in Thoroughbreds under any circumstances.

There are two physiological peculiarities of the mare which place limitations on equine embryo transfer, which are not present in other domestic species. The major drawback is the fact that mares cannot be readily superovulated as of this time. In other species Pregnant Mare Serum Gonadotrophin (PMSG, now called equine Chorionic Gonadotrophin eCG) and Follicle Stimulating Hormone (FSH) are used for superovulation. As eCG is naturally present in mare's blood in high concentrations during early pregnancy, the mare's ovary is not so readily stimulated by eCG and it is possible that the gonadal gonadotrophin receptors are capable of differentiating between pituitary origin gonadotrophin and eCG, because the mare's ovaries will respond to pituitary origin FSH and LH, but they are unresponsive to eCG. Attempts have been made to superovulate mares using equine pituitary extract, but the results have been poor and unpredictable. Until a reliable method of superovulation is found for the mare, embryo transfer in horses will continue to be more expensive and less reliable than in cattle.

The second physiological peculiarity is the variation in length of the mare's estrous cycle. Neither the onset of estrus, nor its end, provide a reliable indication of time of ovulation; this can only be accurately determined by daily rectal palpation. Furthermore, variability can exist both between cycles of the same animal as well as between individuals. This makes it difficult to achieve accurate synchronization between donor and recipient

In mares the embryo does not enter the uterus until day 5-6 (day 0 = day of ovulation) at the late morula stage. Good results have been obtained by flushing mares using a non-surgical technique on days 6-8 (late morula expanded blastocyst) with a 75% recovery rate. Transcervical Nonsurgical flushing of the mare's uterus is very easy and is the normal method for embryo collection. A body flush is used as the equine embryo is very mobile during the first 10-14 days it is in the uterus. Implantation occurs around day 35.

Transfer of an embryo should take place as soon as possible after collection, as it has been shown that pregnancy rates drop dramatically if the embryo is out of the mare for more than 1-2 hours. The recipient mare should be synchronized to have ovulated approximately 24 hours after the donor, with successful transfers reported when the recipient is as much as 24 hours ahead to 60 hours behind the donor. The transfer can be made either surgically or nonsurgically. In the surgical approach it can either be a midline approach under general anesthesia, or my preference is a standing flank approach under mild sedation and local anesthesia. Pregnancy rates of 75-80% can be achieved. Non-surgical transfer is very easy to do and virtually stress free, however pregnancy rates are not as high (35-40%), and success is probably due more to the skill of the operator than the technique involved.

Freezing of equine embryos is not as simple as in the bovine, probably due to the fairly high lipid content of equine embryos and their relatively large size. Best results for freezing have been achieved using early morulas obtained by surgical oviduct flushes.

There are relatively few commercial operations where equine embryo transfer are performed, as it is an expensive procedure and bearing in mind that the various breed societies have formulated rules and regulations that are fairly restrictive. Before undertaking any embryo transfer in horses it is wise to check with the appropriate breed society as to exactly what are their requirements, rather than run the risk of being unable to register the foal.

Non-surgical Equine Embryo Transfer

1. Collection

Day 6 to 8 after ovulation

2. Flushing Medium

This consists of Dulbecco's Modified Phosphate Buffered Saline (PBS) with 1% of heat inactivated bovine serum albumin or heat inactivated gelding serum added. One percent of Penicillin or Gentamycin is also added to the solution.

3. Technique

3.1. Preparation of donor mare

3.1.1. Secure In stocks

It is essential that the donor mare be restrained in a stock which prevents her from moving too far in any direction during the collection procedure

3.1.2. Bandage tail

3.1.3. Rectal palpation

Check to see if the donor mare has any follicular development. Where possible, palpate the ovary on which the follicle was found some 7 days before, to feel if there is a corpus luteum in its place.

3.1.3.1. Backrake to remove all feces

3.1.4. Wash perineal area

The entire perineal area is first cleaned of all fecal material. Then a surgical scrub is performed. This entails washing the perineum three times with a surgical scrub solution and rinsing well with clean water.

The area should then be dried with sterile cotton.

3.2. Embryo collection

3.2.1. Introducing the catheter

Wearing a well lubricated sterile palpation sleeve, an arm is introduced into the vagina. (In a small mare it may be preferable to use a sterile speculum) Care must be taken to choose a lubricant which is not embryo-cidal. An extra long sterile disposable Foley catheter (Size 28 or 30 French Gauge with an 80ml. balloon) is introduced manually through the cervix, until the tip is approximately 5cms. into the uterine body. The balloon of the catheter is then filled with air or flushing medium (80ml. of air or 60ml. of PBS) and seated into the internal os of the cervix. The inlet and outlet tubing is now attached to the Foley catheter, and both are clamped.

3.2.2. Flushing the uterus

The uterus is flushed by allowing about one half a liter of the flushing medium to run into the uterus under gravity, or until the mare shows signs of discomfort due to the stretching of the uterus. Once the uterus is full, the inlet tube is clamped off; and the outlet clamp released, and fluid allowed to flow back into an in-line filter. This procedure is repeated several times, until a total of 3 liters of flushing medium has been used. Normally 95% of the fluid placed in the uterus is recovered, and if this does not occur, the uterus can be massaged very gently per rectum.

3.2.3. Following the flush

After the flush, the uterus is irrigated with 50ml. of Furacin solution, to prevent any possible infection and to ensure that no pregnancy results if the embryo were to remain in the uterus.

3.3. Embryo handling

3.3.1. Identification

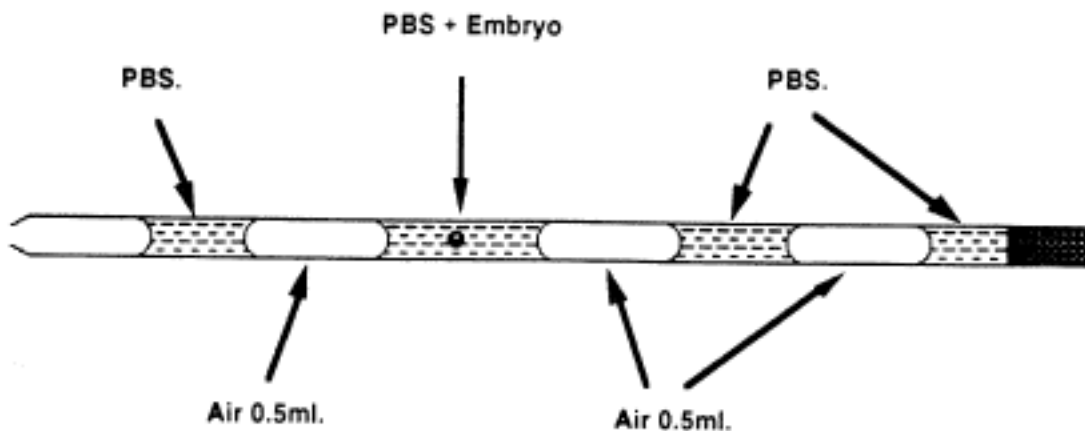
Many times it is possible to visually identify the embryo in the fluid in the filter. If this is not the case, the filter contents are poured into a "Quebec" Petrie dish and the filter flushed several times with PBS. A stereo microscope is used to identify and locate the embryo. The overall embryo recovery rate is 75-80%.

3.3.2. Preparation for transfer

The embryo is "washed" at least twice in PBS supplemented with 10% serum and held at 37°C before transfer.

3.3.3. Loading the transfer pipette

A sterile 55cms. insemination pipette is used for transfer. The embryo is positioned between two air spaces, in order to minimize movement within the pipette, as shown in the drawing below:



3.4. Recipient preparation

The recipient is prepared in the same way as the donor.

3.5. Transferring the embryo

The technician, wearing two sterile palpation sleeves, with the A.I. rod containing the embryo in place between the two gloves. After introducing the hand into the vagina, the cervix is "feathered" and the rod pushed through the tip of the glove and on into the cervix, where it is guided gently into the body of the uterus. After deposition of the embryo, the pipette is gently withdrawn while holding the lips of the vulva closed to prevent air entering the vagina. The pipette is rinsed carefully with PBS into a petri dish, which is then examined under the microscope to make sure that the embryo was transferred.

Timetable for Embryo Transfer in the Mare

compiled by J.M. Bowen, F.R.C.V.S., Dipl. A.C.T.

DAY 0	Prostaglandin (8-10mg.) to Donor and Recipients
DAY 13	Prostaglandin (8-10mg.) to Donor and Recipients
DAY 16	Beginning of Estrus (?)
DAY 17	Palpation, Donor and Recipients. Monitor follicle size and ovulation. BREED DONOR* (if using artificial insemination)
DAY 18	Palpation, Donor and Recipients. (Day 3 of estrus?) 3000IU of HCG given I/V to donor and recipients.
DAY 19	Palpation, Donor and Recipients. BREED DONOR*
DAY 20	Palpation, Donor and Recipients.
DAY 21	Palpation, Donor and Recipients. BREED DONOR*
OVULATION	Noted in Donor and Recipients
DAY OV. +7	COLLECT EMBRYO - Prostaglandin to Donor and New Recipients (Donor returns to Day 13 of schedule)
DAY OV. +16	PREGNANCY EXAM - Ultrasound
DAY 0/V. +28	PREGNANCY CHECK - Rectal palpation + Ultrasound
DAY OV. +45	Final PREGNANCY CHECK - Rectal palpation + Ultrasound

*Where Natural Service is utilized, breed to follicular size and fluctuance