

Management of Embryo Recipient Mares

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One of the most important components of any embryo transfer program is the recipient mare. The number of recipients needed for an embryo transfer program is dependent upon the number of donors in the program. If only a single embryo donor is involved a total of two or three recipients should be sufficient to ensure that one recipient mare is synchronized with the donor. Larger embryo transfer facilities will require a greater number of recipient mares, but a lower ratio of recipients to donors.

Acquisition of Recipients

Ideally, recipient mares should be identified and acquired in the fall or early winter prior to the onset of the next breeding season. It would be best to acquire all recipient mares needed for the next season at the same time. This will allow mares to acclimate to their new environment, provide a quarantine period to identify mares with medical issues and prevent exposure of the recipient herd to infectious disease from new mares added to the herd in mid-season.

Selection and Evaluation of Recipients

Recipient mares in large embryo transfer programs are usually maiden or open non-lactating mares between 3 and 12 years of age and weighing between 900 and 1,400 pounds. A recipient mare should be in good physical condition, easy and safe to handle, and have a gentle disposition. Recipient mares should be approximately the same size as the average donor mares. Research has shown that transfer of embryos from a large donor mare into a significantly smaller recipient mare will result in growth restriction of the fetus and birth of a foal that is small for its genetic potential. The size disparity was reported to be still evident at 3 years of age and may persist for life.

A mare may be used as a recipient every-other-year in most ET programs. The recipient mare receives an embryo, becomes pregnant and is sent to the home of the donor mare owner to foal out. The mare foals at home, nurses her foal for 4 to 6 months and is returned to the recipient station in the late summer or fall of the year. She will be put back into the recipient herd and may receive another embryo the following spring. Returned recipients that have done well with previous pregnancies can be used multiple times and may remain in the program well into their teenage years.

Foaling mares can be used as a recipient. It is advisable to avoid transfer of an embryo after the first postpartum (foal heat) ovulation. Pregnancy rates in subsequent heat cycles should be acceptable.

Barren mares, defined as mares with a history of breeding without a pregnancy, may or may not be good recipient candidates. If a mare cannot become pregnant herself, it is possible that she cannot become pregnant after receiving an embryo.

Initial Evaluation of Potential Recipients

Potential recipient mares should be critically evaluated on arrival and only mares that meet strict criteria retained as recipients. Maintenance of marginal recipients that are unlikely to receive a client embryo adds to the work load and the cost to maintain the overall program.

A general physical examination should be performed to identify abnormalities of the musculoskeletal system, eyes, oral cavity, mammary gland and other areas. The age of each mare should be estimated during the oral examination. Young mares (< 3 years of age) and aged mares should be identified and not selected as potential recipients. The behavior of each mare should be noted; fractious, dangerous or exceptionally nervous horses are a liability and should not be retained.

A thorough reproductive evaluation should subsequently be performed. The external genitalia should be examined for angle and muscular tone of the vulva. Mares that require a Caslick procedure to prevent aspiration of air may not be ideal recipients. The reproductive tract should be examined by palpation and ultrasound *per rectum*. The ovaries are examined to determine if both are present and to detect potential abnormalities. Size of follicles, presence of luteal tissue and other significant findings are recorded. The uterus is examined for muscular tone, edema pattern, presence or absence of fluid or air within the uterine lumen, endometrial cysts and other issues. The cervix is also evaluated for tone and morphology.

Uterine culture, cytology and biopsy samples are routinely collected for recipient mares at the initial examination in some embryo transfer programs. In other programs, uterine samples are only collected from mares with uterine fluid identified by transrectal ultrasonography. The goal is to evaluate uterine health and determine if the mare has an infectious or inflammatory condition in the uterus that may adversely affect her ability to become pregnant after transfer or prevent her from carrying a foal to term. Mares with active endometritis should not be used as recipients and it is questionable as to whether or not to retain a mare with endometritis as a potential future recipient mare.

The most common reasons for rejection of a potential recipient mare include musculoskeletal issues (i.e. laminitis, lameness), age, disposition or behavior, uterine abnormalities (i.e. fluid in the uterus, endometritis, endometrial cysts), pregnancy, and ovarian abnormalities (i.e. absence of ovaries, ovarian tumor).

Initial Management of Selected Recipients

Each recipient mare should be identified with a permanent system, such as freeze brands, neck bands, halter tags or microchips. In addition, the physical description of each mare is placed in a permanent medical record.

In most circumstances, the previous deworming and vaccination history of the recipient mare will not be known. Consequently, new recipient mares should be administered an anthelmintic agent and vaccinated against infectious diseases appropriate for specific geographic regions. A blood sample should be collected and tested for equine infectious anemia (Coggins test).

Housing of Recipients

Herds of recipient mares are typically housed in groups of 15 to 25 mares in paddocks or pastures. Mares housed in paddocks are often fed large bales of hay with free access to fresh water and shelter. The paddock system should be designed to allow the herd to be moved as a group to the examination area.

Stress should be minimized in recipient mares. Management procedures to minimize stress would be to avoid overcrowding, avoid moving mares between groups, provide adequate quality, quantity and access to hay or forage, and provide safe shelter.

Seasonal Management

In order to have cycling mares available to receive embryos at the onset of the breeding season, recipients should be maintained under a stimulatory artificial photoperiod beginning on December 1 (in the Northern Hemisphere). Approximately 60 to 70 days of an artificial photoperiod are required to stimulate follicular development and induce ovulation. The most common technique used in recipient mares is outdoor paddock lighting. Timers are used to turn lights on at dusk and turn lights off at 11:00 pm. The goal is to provide a total of 16 hours of light (natural and artificial combined) and allow 8 hours of darkness. The lighting regimen should provide approximately 10 ft-candles of light throughout the paddock.

Gonadotropin releasing hormone (GnRH) or GnRH agonists such as deslorelin acetate have also been used to stimulate follicular development in anestrus or transitional mares. In addition progesterone therapy may be effective at synchronization of ovulation when administered to mares late in the transition period.

Ultrasound examinations are performed occasionally during deep anestrus (follicles < 20 mm in diameter) and early in the transition period (follicles \geq 20 mm in diameter). Once the dominant follicle of a transitional mare becomes \geq 30 mm in diameter, mares are examined every other day. When the dominant follicle is \geq 35 mm in diameter, the mare may be administered hCG or deslorelin acetate to induce ovulation. Ultrasound examinations are subsequently performed daily to determine the day of ovulation.

Synchronization of Recipients and Donors

Synchronization of an individual donor and a single recipient mare may be requested by an owner. If the owner is providing the recipient(s), it is recommended that 2 to 3 recipients be synchronized along with the donor in order that at least one recipient mare ovulates during the critical time window. Ideally, the recipient mare would have ovulated 1-2 days after the donor mare.

Synchronization of estrus and ovulation can be accomplished by one of three techniques: 1) administration of two doses of prostaglandins 14 days apart, 2) administration of progesterone or a synthetic progestin once daily for 10 days plus a single dose of prostaglandins on the last day of progestin therapy, and 3) administration of progesterone or progestin for 18 days. The easiest technique is administration of two doses of prostaglandins 14 days apart. The tightest degree of synchrony is achieved by daily administration progesterone plus estradiol for 10 days followed by prostaglandin administration. Exogenous progesterone and estradiol will suppress both pituitary LH and FSH secretion, resulting in an overall suppression of ovarian follicular development. Follicular growth will resume when 'P+E' therapy is discontinued. It may be beneficial to stagger the onset of therapy in donors and recipients to increase the probability that the recipient mare(s) will ovulate slightly after the donor mare.

Once the donor and recipient(s) are in estrus, appropriate synchronization of ovulation can be obtained by administration of either hCG or deslorelin. The recipient mare is usually administered hCG or deslorelin the day ovulation has been confirmed in the donor mare. Consequently, ovulation in the recipient should occur 2 days after the donor mare.

Estrous synchronization is not typically required in large embryo transfer programs as there are usually enough mares that ovulate spontaneously on any given day to accommodate the embryos collected. However, it may be necessary to modulate the herd ovulation pattern by strategic use of prostaglandins to avoid large time periods without ovulations. Recipient mares that do not receive an embryo are usually administered prostaglandins 9 or 10 days after ovulation to cause luteolysis and an early return to estrus.

On a rare occasion an embryo has been transferred back into the original donor mare successfully when two embryos were collected and only one recipient was available.

Routine Examination of Recipient Mares

Recipient mares are examined on the day of prostaglandin administration. The reasons for an ultrasound examination prior to prostaglandin administration are to 1) confirm the identity of the mare, 2) confirm that the mare is not pregnant, 3) confirm the presence of a corpus luteum, and 4) determine the size of the largest follicle, which will help predict the interval to subsequent ovulation. In general, the interval to ovulation is longer in mares with small follicles at the time of prostaglandin administration (Table 1).

Table 1. Interval from prostaglandin administration to spontaneous ovulation based on follicle diameter at the time of treatment.

| Follicle Diameter at PGF | Interval to Ovulation (days) |
|---------------------------------|-------------------------------------|
| 10 mm | 9 to 12 days |
| 20 mm | 8 to 11 days |
| 25 mm | 6 to 10 days |
| 30 mm | 5 to 9 days |

| | |
|---------|--|
| ≥ 35 mm | <i>Possible outcomes</i> <ul style="list-style-type: none"> ▪ May ovulate dominant follicle within 1-2 days ▪ May ovulate dominant follicle 3 or more days after PGF ▪ May regress the dominant follicle and develop another follicle that ovulates 10 to 12 days after PGF |
|---------|--|

Timing of subsequent examinations is dependent on size of the largest follicle at the time of prostaglandin administration. In most instances, a mare will be examined 4 days after prostaglandin administration. Mares are examined every 2-3 days until a dominant follicle is ≥ 35 mm in diameter, after which the mare is examined once daily to determine the day of ovulation.

All recipients are examined 5 days after ovulation to determine if they qualify to receive an embryo on that cycle. Mares that are graded as 'acceptable' on this examination are available for use as recipients for the next 3 to 4 days. Pregnancy rates are generally higher for recipients that are graded as 'acceptable' on the '5-day Check' versus mares that are graded as 'marginally acceptable' or 'unacceptable'.

A recipient mare may be selected to receive a specific embryo based on the following criteria on the '5-day Check':

- Day of ovulation relative to the donor mare
- Day of ovulation relative to size and developmental stage of the embryo
- Quality of estrous cycle
- Presence and quality of the corpus luteum
- Progesterone level (optional)
- Tone of the uterus
- Tone of the cervix
- Absence of uterine edema
- Size of the recipient relative to size of the donor mare
- General physical health
- Behavioral characteristics
- Absence of reproductive abnormalities, medical issues or behavioral concerns

Many factors can be used to disqualify or decrease the likelihood of using a certain recipient mare as a candidate for receiving an embryo. These factors include:

- Poor quality cycle
- Ovulation within 2 days after receiving prostaglandins
- Ovulation of an abnormally small follicle
- Failure of ovulation or development of a hemorrhagic anovulatory follicle
- Absence of uterine edema during the cycle
- Presence of echogenic fluid within the uterine lumen during estrus
- Presence of fluid within the uterine lumen during diestrus
- Presence of a significant medical condition or behavioral issue

Equine embryos are usually collected on either day 7, or 8 (and rarely 9 days) after ovulation of the donor mare. Embryos are usually transferred into a recipient mare that ovulated during a window that may range from one day prior to the donor mare (+1 synchrony) to 3 or 4 days after ovulation of the donor mare (-3 or -4 synchrony). It is a common opinion that pregnancy rates are highest if the embryo is transferred into a recipient that ovulated 1 or 2 days after the donor (Table 1). Pregnancy rates are significantly lower if an embryo is transferred into a recipient mare that ovulated 2 or more days prior to the donor mare. Pregnancies can be obtained from embryos transferred into a recipient that ovulated 4 or 5 days after the donor mare. However, in commercial programs it may be necessary to utilize recipients that ovulated the same day as the donor (0 synchrony) or one day prior to the donor (+1) and save recipients that have recently ovulated for embryos to be collected in the near future. It is recommended that one always looks ahead at upcoming flushes in the next 2 to 3 days before making a final decision on recipients.

Table 1. Synchrony between donor mare that ovulated 7 days prior to embryo collection and potential recipient mares that ovulated between 2 days before to 3 days after the donor mare. Comments regarding order of selection of the recipient are also presented.

| Donor Flush Day | Recipient Ovulation Day | Synchrony | Selection Comments |
|-----------------|-------------------------|-----------|------------------------|
| | d 9 | +1 | Only use if necessary |
| Day 9 | d 8 | 0 | Use if needed |
| | d 7 | -1 | 1 st Choice |
| | d 6 | -2 | 2 nd Choice |
| | d 5 | -3 | 3 rd Choice |
| | d 4 | -4 | 4 th Choice |

Ideally several recipient mares would be available to choose from for each embryo recovered. Consequently, the best recipient mare could be selected to match the donor mare, the ovulation day and the developmental stage of the embryo.

Recipient mares may receive 2 or maximally 3 embryos in a given year. If they do not become pregnant or if they lose a pregnancy after 2 or 3 opportunities, they are generally culled from the recipient herd.

Hormone Therapy for Anestrous or Transitional Embryo Recipient Mares

If cycling recipients are not available early in the breeding season, non-cycling mares can be used as recipients. Deep anestrus is characterized by a prolonged period of minimal ovarian follicular development, with the largest ovarian follicles less than 20 mm in diameter. The transition period begins with the onset of the first follicular wave. From a practical standpoint, transition in the mare begins when a follicle first reaches 20 to 25 mm in diameter. The hormone therapy protocol for deep anestrous mares consists of intramuscular administration of estradiol-17 β (6.66 mg, IM) for 2 days beginning when the donor mare ovulates, followed by administration of a compounded short-acting progesterone preparation (200 mg, IM) once daily for 7 days. Embryo collection on the

donor mare is usually performed 8 days after ovulation. If an embryo is recovered and transferred, progesterone therapy is continued.

The hormone therapy protocol for transitional mares is similar, but estradiol-17 β is optional and may not be administered if ultrasound examination reveals the presence of moderate endometrial edema. In this situation, short-acting progesterone therapy would be initiated two days after the donor mare ovulates. Transitional mares without significant endometrial edema would receive the combination of estradiol-17 β for 2 days followed by progesterone therapy as described above. If a recipient mare is in late transition with a large dominant follicle, an ovulation induction agent, such as histrelin (500 μ g, IM), deslorelin (500 μ g, IM), or human chorionic gonadotropin (hCG, 2,500 IU, IV) may be given to induce ovulation followed by initiation of progesterone therapy. Initiation of progesterone therapy is especially important if ovulation did not occur following administration of an ovulation induction agent.

Emergency Hormone Therapy for Cycling Recipient Mares in Diestrus

In the event that a donor mare is about to ovulate or has just ovulated and no qualified anestrus, transitional or cycling mares in estrus are available, one can utilize the following 'emergency' protocol on a cycling recipient mare in mid-diestrus. A dose of cloprostenol (250 μ g, IM) would be administered to lyse the corpus luteum along with a dose of estradiol-17 β (6.66 mg, IM). A second round of cloprostenol and estradiol would be administered the following day. The intended recipient mare would then be administered a short-acting progesterone preparation (200 mg, IM) once daily for 7 days. Ultrasound examinations would be performed on the potential recipient prior to the onset of hormone therapy to confirm her reproductive status, again after the second day of estrogen therapy to evaluate endometrial edema, and again after 6 days of progesterone therapy to evaluate uterine status prior to receiving an embryo.

Overall, pregnancy rates after transfer and pregnancy loss rates of hormone treated non-cycling mares and short-cycled diestrus mares are not different from those of normal cycling mares.