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Equine Reproduction Embryo Recovery Embryo Transfer

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Protocol - On Farm Embryo Transfer Program

Embryo transfer is not rocket science. It is a relatively simple process, provided you pay attention to details. There are some short cuts that can be taken within a breeding program. However, if you follow the same practice with embryo transfer, it will not work for you. Embryo transfer can become a valuable tool in your breeding program if you "are willing" to work at it ... just a little bit.

It is understood that the owner of the ranch / farm (or his agents) have a working understanding of reproduction in the mare and its related terminology. It is also understood that this protocol's purpose is to help you ready your mares for embryo transfer. It is not intended as a home study course in embryo transfer or to give you credit towards your PhD in theriogenology. Therefore, it is important that your veterinarian review this paper with you.

I realize that most, if not all, embryo transfers undertaken by clients of Equine Reproduction Service (ERS) will be performed on a breeding farm (whether large or small). Although much of the contain information on reproduction in the mare may be repetitious to breeding farm personnel, I recommend that these guidelines be read and studied in their entirety. It is imperative that the services and advice of a technician experienced in broodmare practice be obtained to realize the benefit of the following undertaking.

BASIC NORMAL REPRODUCTIVE PHYSIOLOGY OF THE MARE

The mare exhibits normal estrus cycles starting in late spring continuing through summer and into early fall. In early spring (especially) and late fall the majority of mares will become transitional with erratic reproductive behavior, long heats and lowered fertility. Through the winter months most mares become anestrus (absence of reproductive function).

We will be concerned with the OVULATORY SEASON or the time of year when the mare experiences normal cyclic activity, "breeding season".

The estrus cycle in mares averages 22 days in length from the day of ovulation (rupture of a follicle within the ovary and the release of the ovum (egg) to the following ovulation in the next cycle. The time the mare is in estrus (heat or sexually receptive to a stallion) averages 4-7 days, longer early and late in the breeding season and often two or more days shorter during the mid-summer months. Diestrus (period of time the mare is "out" or sexually non-receptive to the stallion) averages 15-17 days. This cyclic activity continues throughout the breeding season until late fall with the mare then becoming anestrus or until pregnancy ensues.

When ovulation occurs, there is hemorrhage into the space once occupied by the follicle, a fluid filled cavity within the ovary containing the ovum or egg. The fluid portion containing [as a part of its make-up] estrogen a hormone which, among other functions, is responsible for the mare's showing of heat and preparing her reproductive tract for breeding.

The new organ produced by the ovulating follicle and the resultant hemorrhage once it organizes is called a "corpus luteum" (CL). The CL's function is to produce progesterone. Within a day or two after ovulation the mare will go out of estrus and will no longer accept the stallion. The Diestrus phase of the cycle now begins. If the mare is not pregnant or has not been bred, about the fifteenth day of Diestrus the uterus releases a natural substance called prostaglandin, resulting in autolysis or destruction of the CL. This allows the development of the next ovulatory follicle. If the mare is pregnant, substances (steroids) from the developing embryo and the fact, in early pregnancy, that the embryo is highly mobile block the natural prostaglandin release and the mare does not return to estrus.

ACQUISITION & QUALIFICATION OF RECIPIENT MARES

Although one recipient mare will work for one donor you might have to lose a cycle or two to get them synchronized together. Two recipients per donor is good. More better.

Recipient mares may be acquired through many methods. They may be purchased or borrowed from friends or neighbors. Recipient mares may be purchased through local sales. Mares not destined for the broodmare band and of limited value would also make excellent candidates.

Recipient mare candidates in a perfect world would be 5 to 7 years old and of sufficient physical size to be able to carry a pregnancy without cramping the normal development of the foal. Yes, older and younger mares will work just fine. I find the 5,6,7 year olds are just like teenagers, they are now pretty mature physically and reproductively and all they know how to do is get pregnant. They just haven't had time for too many bad things to happen to them yet reproductively. Four to ten is a good age group to shoot for. Recipients should be physically healthy with no apparent deformities or disease that would compromise the maintenance of a pregnancy. Take a look under the mare's tail for the presence of a discharge that could indicate infection of the uterus or vagina. The mare's vulva should be more vertical in orientation than horizontal. A vulva that is lying along a more horizontal plane with a sunken anus indicated a possible pneumovagina (wind sucking condition), which would promote irritation of the entire reproductive tract and infection. Pass her up.

Now that you have your potential recipient mares, further examination will be necessary. The services of that experienced individual mentioned earlier will now be required. The following criteria should be evaluated along with any additions considered complimentary.

- The condition and anatomy of the external genitalia,
- The condition and anatomy of the vestibule and vagina,
- The condition and normalcy of the cervix,
- The size, tone and consistency of the uterus,
- The presence, size, consistency and the activity level of both ovaries,
- Any other condition that would adversely affect a pregnancy the birth process or lactation.

After the above criteria have been judged to be within normal limits, taking into account the time of year when the examination is performed, I like to take a uterine culture and more importantly a uterine biopsy. If your going to do just one of these diagnostic techniques do the biopsy. Please, remember that it is preferable that the mare be in estrus when the culture and/or biopsy are taken. Uterine biopsy samples must be sent to a veterinary pathologist that is thoroughly experienced in the reading of uterine biopsy samples from mares. The reading of these biopsy samples by a human pathologist should be considered as unacceptable. I recommend the Diagnostic Laboratory at Colorado State University, Ft. Collins, CO.

Please remember that these recipient mares are not customer mares presented for breeding. Customer mares you are expected to do the utmost to fulfill your contract. If you see or "feel" (sometimes just a gut instinct) something you don't like, you do have the luxury of sending them to a better place than your recipient mare band. Most young, healthy mares should not have too much trouble passing these qualifications. Mares not passing the above criteria should be rejected. The expense of extended therapy to correct a "so called minor problem" unless it is truly minor, can quickly become a major expense. Spend that money on a better project.

METHODS OF MANIPULATING ESTRUS CYCLES

Mare owners wishing to breed their mares and transfer embryos early in the year, January, February, March, and many times April, will need to consider an artificial lighting program for the donor and her recipients. Sixteen (16) hours of light per day will hasten the onset of normal cyclic activity. This lighting program should start the end of November.

Mares should be teased on a regular basis to determine when they come into estrus. Teasing will also save you money on palpation and ultrasound fees. What fun is it having the expense of palpating mares that are not in heat? Unless you have some of those that will not tease, then you are going to have to check them. I personally don't like to spend a lot of time teasing wet mares (or at all for that matter), too many opportunities for foals to get injured.

Prostaglandin's (there are more than one available) can be given to the donor and recipient mares to encourage autolysis with resulting return to estrus and luteinizing hormone (HGC) or Deslorelin given, if necessary, to synchronize ovulation. Prostaglandin's used as a drug, mimic the natural prostaglandin released by the mare's uterus, resulting in autolysis of the CL and allowing the mare to come back into estrus. NOTE: Prostaglandin's will not be effective except on a mature CL. A CL is not mature until at least five (5) days after ovulation. Most broodmare practitioners wait until Day 7 after ovulation to administer prostaglandin products. Prostaglandin also becomes a little more unpredictable after 10 days post ovulation.

Knowing when the mares ovulate determines how and when we can attempt to manipulate their cycles. We want to manipulate the recipients after the donors as will become apparent as you read further. Also be aware that if you are working with a limited number of recipients that you may have to lose a cycle on your donor in order to synchronize them together. It all depends where the mares are in their cycles.

DETECTION OF OVULATION AND ITS IMPORTANCE

Knowing the day ovulation takes place is the KEY for timing of recipient mares with donor mares. It is also the KEY to the success or failure of your embryo transfer program. It is imperative to know the day that your mares ovulate. If you check mares every other day (both recipients and donors) your margin of error is 96 hours or four days. You might guess correctly at times, but you could be costing yourself successful pregnancies and adding additional expense to your program. I also end up with mud on my face from performing unsuccessful transfers. I don't need any advertisement from a failed ET program, thank you.

The day ovulation occurs is "DAY 0". My system is to consider any day discussed regarding heat cycles in mares as to originate from the day of ovulation. This will apply to both the Donor and Recipient mares. It is unimportant and confusing to start talking about this mare being + or - so many days from this or that other mare. All that matters is the number of days post ovulation in your record keeping and conversation.

When donor and recipient mares are in estrus and a preovulatory follicle is present that has reached sufficient size and/or tone to indicate that ovulation may occur they should be checked daily to accurately determine day of ovulation. The thing we are interested in is; yesterday the follicle was present today it is gone. Don't worry about; I think it was between ten last night and one in the morning (unless you checked the mare at that time, twenty forty hours is a most suitable time frame. There yesterday gone today is all I will need to know. The availability of ultrasound is imperative to this whole process, especially the qualification aspect.

SYNCHRONY BETWEEN DONOR AND RECIPIENT MARES

When are we going to flush the donor mare? Depends. With my favorite days being 8 and 9, day 10 on some older mares, that is what I would like to try to do. I am going to make a judgment call more off what recipient mare is available and what they look and feel like than the exact day of the donor. This also leaves time in both our schedules. Remember we are counting days post ovulation.

Ideally I want the recipient mare to be behind the donor (ovulating after her). I personally like the recipient mare to be (in order of preference) 5,6,4,7,8 days post OV when we transfer an embryo to her. Recipients 2 and 3 days post OV will work for transfer if they meet certain requirement. So being able to flush the donor mare at 7,8,9 days and the above recipient mare schedule there is some scheduling leeway.

The Big Embryo Myth:
I cut my teeth on flushing larger embryos. First of all, embryo recovery rates are higher. And they are easier to see and work with. A lot of experts have a hard time getting these bigger embryos to work; it's not what they learned. They don't know how to get along with them. They either damage them in the recovery process (flush) or during the transfer. I spent 31 years developing the equipment, the methodology and learning how to get along with big embryos and just love them. The equipment, the mindset, and the knowledge makes the difference.

EMBRYO RECOVERY AND DAY POST OVULATION

Embryo recovery attempts (flush) before day 6 produces the least number of embryos recovered. Oviductal transport in the mare takes approximately 5.5 days, (time from ovulation until the embryo reaches the uterus). Flushes are customarily performed 6 to 9 days post OV. A higher percentage of embryos will be recovered after day 7. I personally prefer to flush at day 8 and 9 if possible. Day 7,8 or 9 is OK. Some older mares will do better if flushed at day 10. This is because there is the possibility of delayed embryo transport in some of the older mares.

PREPARATION OF DONOR & RECIPIENT MARES FOR EMBRYO TRANSFER

The donor mare has been prepared for the day of embryo recovery by breeding and the detection of ovulation. The recipient mare has been prepared for the day of transfer by passing the qualification process and her ovulation date being known. Nothing remains to be done to them until the appointed day. Don't forget to feed them.

One or two days before the embryo recovery attempt I like for the recipient mare to receive an ultrasound examination. The mare should no longer be teasing. Her cervix should have some tone and be closing nicely. The uterus should look Diestrus and homogeneous (overall even appearance) with no estral folding remaining, absolutely no fluid present, and (not have a great amount of tone).

TRANSFER AND POST TRANSFER CARE OF RECIPIENT MARES

At the time of transfer, recipient mares are prepared with a meticulous scrub of the external genitalia being careful not to introduce any soap or disinfectant internally. The embryo is then transfer to her non-surgically. The last surgical transfer I performed was in 1991.

For two or three days after the transfer observe the mare's vulva for the sign of an abnormal discharge. Don't make one up. If nothing is out of the ordinary leave them alone for at least a couple of days post transfer. If you see something that doesn't look right, perform an ultrasound exam. If frank intrauterine fluid is observed, there is a problem and not much can be done. The transfer will fail. There was either an organism present inside the recipient mare that was carried into the uterus during the transfer or a contaminant was introduced from the outside that caused this. Occasionally a recipient mare will have a reflective white look on ultrasound that may involve half the uterine body, most of the body, and/or infrequently a horn, or only a few centimeters anterior to the cervix. This mare can be placed on an anti-prostaglandin product, an anti-inflammatory and antibiotics for a few days and the response will be acceptable depending on the degree of involvement.

Depending on the capabilities of your ultrasound equipment and the skill of the operator, you should be able to perform your initial pregnancy exam at the time the embryo is 11 to 12 days old. The cervix should feel elongated and firm and the mare's uterus should have a pleasant, soft, live feel. Smile, you will more than likely be pleased with what you see on your screen. If the mare's cervix doesn't have much tone and/or the uterus is really toned up, there will probably be no pregnancy established.

Sometimes large embryos placed in early day recipient mares will take an extra one or two days to show up. Do not become discouraged; if all the signs look good so far recheck in a couple of days. With a tight cervix and normally developing embryonic vesicle this embryo transfer pregnancy is the same as any other pregnancy. It is subject to the same rewards and pitfalls.

A METHOD OF IMPROVING PREGNANCY RATES IN RECIPIENT MARES

Many physiological responses are in place in allowing a mare to become pregnant and in the maintenance of pregnancy. Most of these responses we have little or no direct control over. However good animal husbandry and the elimination of as many stressful conditions as possible, will go a long way in promoting the health of the individual and in the maintenance of pregnancy in the broodmare, any broodmare.

Regardless of how carefully an embryo is transferred there is a certain amount of irritation produced by the manipulation necessary to perform the embryo transfer. During the transfer process the cervix is dilated to a certain extent in order to place the embryo into the uterus. We use Banamine as a prep for it's "anti-prostaglandin" effect and sedation for relaxation and the relief of anxiety it provides. Plus a physician friend of mine told me many ears ago while observing us perform surgical embryo transfers that when he went to medical school he learned that the doctor moves around during surgery not the patient. So, I don't like mares moving around or tying to kick me while I flush them or transfer the embryo. I just believe this creates a more harmonious outcome.

Regarding wet mares as potential recipients; although wet mares make acceptable embryo transfer recipients, I have found that dry mares are significantly easier to handle and manage. At times, a wet mare can require an extended period of time and may require therapy to fully restore reproductive health after foaling.

In this protocol there have been several references to different drugs, hormones and their uses. This information is supplied for educational purposes only. These substances should not be used without the consultation and approval of an individual familiar with their actions and uses.

If any part of this protocol is unclear or you require additional information, PLEASE, I expect you to call. I want embryo transfer to be a valuable and useful asset for your breeding program.

GOOD LUCK WITH YOUR PROGRAM !