Objectives

1) To introduce the process of superovulation and embryo transfer (ET) in cattle and horses.
2) To provide an overview of the advantages and disadvantages of ET in cattle and horses.
3) To review the steps of ET including flushing, searching, handling, grading, freezing, thawing, and transferring embryos.

Advantages and disadvantages of an ET program in cattle and horses

The reasons that cattle and equine industries utilize ET are very similar from both a production and economic standpoint. The actual procedures of collecting and processing embryos are also quite similar between the species as are the advantages and disadvantages.

Advantages

- Increase the number of offspring sired from superior females.
- Results in faster genetic progress.
- Increase the frequency of desired matings, capitalizing on excellence of a mating.
- Obtain offspring from old or injured animals incapable of breeding or calving naturally.
- Increased farm income through embryo sales.
- Exportation and/or importation of embryos is easier than with live animals.

Disadvantages

- Can be cost prohibitive and success rates are less than AI.
- Cost and maintenance of recipient females.
- Requires a technician with the skills to flush embryos from the reproductive tract.
- Possible spread of disease through recipients.

Animal components of ET Program (With an emphasis on bovine)

**Donor:** animal that donates embryos

1) Selection criteria are used to select a donor.
   - Genetic merit, reproductive performance, progeny performance
2) Embryo from a donor cow does not guarantee a superior calf.
   - Each embryo has a different genotype and more than likely a different phenotypic expression due to its environment.
3) Reproductive soundness
   - Animals must be exhibiting regular estrous cycles.
   - At least 60 to 90 days post calving.
   - Eliminate cows with a history of reproductive problems.

**Recipient:** animal that receives embryo from donor

1) Recipients (also known as surrogate) are the greatest single cost of an ET program because they need to be at the same stage of the estrous cycle as the donor when she donated the embryos. Estrous synchronization is typically used to manipulate recipients so they are at the correct stage of the estrous cycle.
2) Females should be healthy, good body condition, and vaccinated for all the common reproductive diseases.
3) Females should have been through at least two normal estrous cycles before use.

4) Pregnancy rates are greatest when day of the estrous cycles of the donor and recipient are within 24 hours of each other. The recipient should exhibit estrus from 24 hours before to 12 hours after the donor was in estrus. Embryos are typically transferred on day 7 of the estrous cycle.

Management components of ET Program (with emphasis on the bovine)

Superovulation (Figure 1 and Table 1)

1) This is the process of super stimulating the ovaries with FSH to produce multiple oocytes and it is the least predictable step of embryo production. There is tremendous variation in the number of embryos recovered after superovulation, which is due to variables like animal age, breed, lactation status, nutritional status, season, and stage of the estrous cycle when FSH treatment is initiated.

2) The process of superovulation includes frequent (2x daily) treatment of females with FSH for a period of four days. Treating with FSH allows for the recruitment, growth, and development of multiple ovarian follicles. Horses are typically not super-stimulated since they can only ovulate one follicle per ovary due to the presence of ovulation fossa.

3) Females are administered prostaglandin during the third day of FSH injections to regress any CL present. Females usually exhibit estrus within two days after the last day of FSH at which time they are artificially inseminated two times 12 - 24 hour apart.

4) Expected response is 5 to 12 embryos that are transferable and/or freezable. However, 0 to 20 oocytes can be ovulated, with nearly all being fertilized and transferable.

Figure 1. Example of a superovulated ovary. Notice numerous CLs, the very dark blister-like structures. The unovulated follicles are larger than the CLs and are more rounded in appearance. Photo by John Curtis, AgTech, Inc.

Flushing: Non-Surgical Embryo Recovery

- Most embryos are collected by a non-surgical process between day 6 - 8 (estrus = day 0) of the estrous cycle with day 7 being the most common. Since embryos have not hatched from the zona pellucida yet, they are easier to locate with a microscope after being flushed from the uterus.

- A specially designed instrument called a Foley catheter is used for the flushing procedure. The Foley is a 2-way catheter that has one channel for inflation of a balloon at the end of the catheter plus an additional channel for the inflow and outflow of flushing medium.
• **Flushing Process**

1. The donor is administered an epidural anesthesia (lidocaine) to relax the rear leg muscles during the flushing process.

2. The Foley catheter is inserted through the vagina, cervix and into one of the uterine horns where the balloon is inflated. The inflated balloon will seal off the anterior portion of the uterine horn, which prevents fluid from leaking out of the uterus as flushing media is added to the uterine horn to recover the embryos. After a small amount (100-300 mL) of fluid is “flushed” into the uterus the technician recovers the fluid from the uterus. This process is repeated on the other horn in cattle. In horses, the entire uterus is flushed since uterine horns are minimal in size in the horse.

3. The flushing media recovered from the uterus is filtered (75 μm pore size) to assist in separating embryos from the flushing media. Once the embryos are recovered they are evaluated for *stage of development* and *graded for quality*.

**Evaluation**

- Embryos can be maintained at room temperature for 12 - 24 hours at a pH of 7.1 to 7.5 when holding media is changed every few hours. Excessive temperature harms embryos (39°C). It is recommended that embryos be frozen within two hours after flushing.

- Embryos are classified by stage of development and graded based on gross morphological appearance. It takes a great deal of experience to do this well.

**Stage of Development Classification** (Figures 2, 3 and 4)

- Unfertilized, 2 to 12 cell, early morula, morula, early blastocyst, blastocyst expanded blastocyst, hatched blastocyst, expanded hatched blastocyst

**Quality Grades or Scores**

- **Excellent** (1) - Embryos with few or no recognizable imperfections, such as poor compaction or variation in cell size.

- **Fair** (2) - Embryos that show disarrangement, like a small embryonic mass with irregular shape or a large number of extruded and/or dead cells. Usually 1 to 2 days retarded development.

- **Poor** (3) - Embryos with signs of cellular degeneration, like a small ICM and several extruded cells. Development has been retarded by more than 2 days.

- **Dead or Degenerating** (4) - Embryos contain mostly dead cells and very few live. Tiny cell mass that is disorganized in appearance. Not worth transferring.
• After classification, embryos are prepared for the next processing procedure, which may include sexing of embryonic cells and (or) splitting the embryo in half to make twins. Embryos are either transferred immediately into a recipient or frozen for storage.

**Figure 2.** Example embryos from a flush done on day 7 of gestation. Tight morula grade 2 embryos (1 and 2); loose morula grade 4 embryos (3 and 4). Embryos 3 and 4 would neither be transferred fresh nor frozen since the likelihood of a pregnancy is very small.

**Figure 3.** Example embryos from a flush done on day 7 of gestation. Unfertilized ova (1, 4, and 5); 8-cell embryos, grade 1 embryos (2 and 3); morula, grade 1 embryo (6). A morula is an embryo with greater than 32 cells or blastomeres.

**Figure 4.** Example embryos from a flush. The embryos are classified as blastocysts since they have a clear, fluid-filled inner cavity (blastocele). The inner cell mass (ICM) is the darkened area of cells that are clustered near the edge of the embryos. Expanded blastocyst, grade 1 embryo (1); blastocyst, grade 3 embryo (2); hatched blastocyst embryos (3 and 4).

Photos credits: John Curtis, AgTech, Inc.
**Freezing**

1) Cryoprotectants like glycerol and ethylene glycol are used to dehydrate cells and protect the cells of the embryo during the freezing and thawing process. Ethylene glycol is the cryoprotectant of choice in cattle since embryos can be frozen in a single step and eventually thawed in a single step and immediately transferred into a recipient. Embryos frozen in ethylene glycol are known as direct transfer (DT) embryos. Freezing embryos is a common practice in cattle but not in horses since their embryos do not freeze well.

2) Embryos are equilibrated for 5 to 10 minutes in cryoprotectant freezing medium.

3) Embryos are loaded and stored in a 0.25cc polyvinyl straw.

4) Embryos are frozen in a step-wise procedure in a special freezing machine:
   - Embryo is placed in a freezing unit at -6°C for 5 minutes.
   - Embryo is “seeded” with forceps cooled in liquid nitrogen to facilitate uniform freezing.
   - Held at -6 to -7°C for another 5 minutes.
   - Then cooled at 0.5°C/minute to -32°C.
   - Hold at -32°C for 15 minutes and straws are plunged into liquid nitrogen (-196°C).

**Transferring** Embryos can be transferred in two different manners

1) **Same day transfer** Embryos are transferred into recipients on the same day they are collected from the donor. Transferring fresh embryos results in greater pregnancy rates, but requires a great deal of coordination to get recipients and donors on the same day of the estrous cycle when flushing occurs. More common in horse compared to cattle.

2) **Transfer of Frozen Embryos** Embryos are frozen the day they are flushed and then transferred at a later date. Although pregnancy rates for frozen embryos are slightly less than fresh, the process has numerous advantages including:
   - Frozen embryos offer important logistical and economic advantages. More cows can be flushed in a day, which facilitates freezing of embryos.
   - Frozen embryos can be marketed and imported and/or exported more easily.

3) **Process of transferring embryos**
   a) The recipient is palpated to determine the presence and location of the CL (right vs. left). Recipient is administered an epidural (lidocane) to relax the muscles in the pelvic area.
   b) If the embryo is frozen it is thawed in a warm water bath (92°F) for < 30 sec and placed in a specially designed transfer gun and covered with a sterile sheath.
   c) The transfer gun is passed through the vagina, cervix, and into the uterine horn on the side as the CL. The embryo is deposited 1/3 the way up the uterine horn.
d) Pregnancy rates are greatest when the day of the estrous cycles of donor and recipient are within 24 hours. Recipients should be in heat 24 hours before to 12 hours after the donor was in estrus. The embryos are typically transferred on day 7 of the estrous cycle.

Pregnancy Rates Resulting from Embryo Transfer

- When done by experienced technician, the transfer of fresh embryos yields pregnancy rates of 70 to 80%, while transferring frozen embryos yields pregnancy rates of 50 to 60%.
- The average cost to produce an embryo derived calf is $300. This does not include the cost of the recipient, which could be between $600 to $900/animal.
Embryo Transfer in the Equine

Embryo transfer is gaining in popularity in the equine industry for many of the same reasons as described for cattle. The ET procedure is quite similar between the species with the exception of the superovulation process not being conducted in the mare since she can only ovulate at a single location on the ovary. Although, aspiration of multiple follicles followed by in vitro fertilization, culturing, and eventually a transfer can be done to circumvent this obstacle.

Most equine breed associations allow embryo transfer, except for the Thoroughbreds (The Jockey Club). Originally, only one foal per mare per year could be registered but that is changing or has changed for most organizations. Most, but not all associations require intent forms (Example below) and all associations require reports that a transfer was completed. Furthermore, DNA testing is required of all ET mares, which is not the case in cattle.

Comparing and contrasting embryo transfer in bovine and equine

<table>
<thead>
<tr>
<th>Process</th>
<th>Bovine</th>
<th>Equine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super ovulation</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Number of embryos recovered per flush</td>
<td>0 to 20 Ave 5 -12</td>
<td>2 (Multiple ovulations can be induced)</td>
</tr>
<tr>
<td></td>
<td>(Superovulated donor)</td>
<td></td>
</tr>
<tr>
<td>Method of recovery</td>
<td>Non-surgical (Foley Catheter)</td>
<td>Non-surgical (Foley Catheter)</td>
</tr>
<tr>
<td>Day of estrous cycle of embryo recovery</td>
<td>Day 6 or 7</td>
<td>Day 6 to 9</td>
</tr>
<tr>
<td>Type of flush</td>
<td>Body flush or flush each individual horn</td>
<td>Body flush</td>
</tr>
<tr>
<td>Handling of embryos after a flush</td>
<td>Can be cultured up to 24 hours if transferred fresh. Embryos to be frozen should be frozen within 2 hours of flushing</td>
<td>Cultured for 24 hours, passively cooled to 5°C and shipped in Equitaner</td>
</tr>
<tr>
<td>Cryopreservation of embryos</td>
<td>Yes (glycerol or ethylene glycol)</td>
<td>No (unacceptable pregnancy rates)</td>
</tr>
<tr>
<td>Type of transfer of embryo to recipient</td>
<td>Non-surgical</td>
<td>Surgical or non-surgical</td>
</tr>
<tr>
<td>Location in reproductive tract where embryo is placed during a transfer</td>
<td>Uterine horn adjacent to CL</td>
<td>Surgical – horn adjacent to CL Non-surgical - uterine body via cervix (Similar to AI)</td>
</tr>
<tr>
<td>Day of estrous cycle embryo is typically transferred to recipient</td>
<td>Day 7</td>
<td>Day 7</td>
</tr>
<tr>
<td>Acceptable variation between recipient and donor</td>
<td>Recipient can be -24 to +12 hours asynchronous form donor</td>
<td>-3 to +1 days asynchronous form donor</td>
</tr>
<tr>
<td>Success rates (pregnancy rate)</td>
<td>Fresh 70 to 80%</td>
<td>Fresh surgical 65%</td>
</tr>
<tr>
<td></td>
<td>Frozen 50 to 70%</td>
<td>Fresh non surgical 45 to 65%</td>
</tr>
</tbody>
</table>
Embryo Transfer Enrollment

This form and enrollment fee must be submitted to AQHA before the collection of an embryo. The enrollment, once complete, is not transferable or refundable. Only one enrollment per year is required, regardless of the number of embryos being transferred.

This is notification of the intent to transfer an embryo from the mare below:

MARE'S NAME

REGISTRATION NUMBER

BREEDING YEAR

Please provide the name, full address, and AQHA identification number of the clinic where the transfer will be performed.

AQHA ID NUMBER, if known

Before a resulting foal can be registered, its parentage must be verified by genetically testing the donor mare, sire and foal. (Occasionally, the donor mare and sire are already typed. Please check with AQHA.) You may request a kit for the donor mare or sire by sending us the registered name and number plus $50 for each DNA kit.

☐ Check here if you intend to transport the embryo in accordance with 212(c) of the AQHA Official Handbook.

☐ Check here if you wish to order a DNA kit for this mare. The fee is $50 plus the enrollment fee.

The person signing below agrees to pay all genetic testing expenses for the parentage case of the resulting foal. By submitting this document to AQHA, I hereby agree to be bound by all the terms and conditions of AQHA's Official Handbook of Rules and Regulations.

SIGNATURE OF PERSON AGREEING TO PAY EXPENSES

DATE

NAME AND COMPLETE ADDRESS FOR PERSON SIGNING

AQHA ID NUMBER

DAYTIME TELEPHONE NUMBER

EMAIL ADDRESS

Enrollment fee for the Embryo Transfer Program is $100 for members or $140 for nonmembers. For the member fee to apply, the recorded owner of the donor mare must be an AQHA member for the year that the enrollment is submitted. Membership can be purchased at the time of this transaction to receive the member fees. Refer to rule 212 for a complete listing of embryo transfer requirements.

If paying by credit card, your membership will automatically be enrolled in the convenient auto-renewal program. You can cancel at any time.

☐ I would not like to enroll my membership in the auto-renewal program.

If you wish to pay your fee with a Visa, MasterCard or American Express, please complete the following information:

Card Number: ___________________________ Expiration Date: __/___

Cardholder's Name: ___________________________ Amount to be charged: ______$

Address

Daytime Telephone: (____) ____________

Revised 2/25
212. EMBRYO/OOCYTE TRANSFER

(a) A horse foaled by a mare that is not its genetic dam but transferred to her by embryo/oocyte transfer technique shall be eligible for registration. In addition to other AQHA registration rules, the offspring shall not be eligible for registration unless:

(1) Prior to the intended collection of the fertilized egg, record owner or lessee has notified AQHA in writing of its intention to attempt an embryo/oocyte transfer and has paid the proper fee as per rule 222. For mare enrollments received after collection of the embryo/oocyte, but prior to foaling, a late fee of $25 will be assessed, in addition to fees required by rule 222. For mare enrollments received after foaling, a late fee of $50 will be assessed, in addition to fees required by rule 222. This enrollment must be made each year that a transfer is to be performed, and once made, the fee is not refundable, nor can any substitution be made.

(2) Its pedigree has been verified through genetic testing of foal, sire and donor mare; and by such other testing as AQHA reasonably deems necessary to verify the validity of the genetic testing, all expense of which shall be the registration applicant's.

(b) The enrollment notice must be sent by certified mail, return receipt requested, to preserve for the record owner or lessee of the donor mare, the only acceptable proof to AQHA of timely compliance, if such proof is requested.

(c) In accordance with AQHA-approved procedures, an embryo/oocyte transfer may be transported from the premises where the donor mare was located at the time of its removal from her for use in a recipient mare at another location. To be eligible for such transportation, notice of intention to transport the embryo/oocyte transfer shall be given AQHA in conjunction with the advanced notice of intended collection specified above.

(d) If a mare is designated with AQHA for embryo/oocyte transfer but the procedure is not attempted regarding the mare in the designated year to avoid necessity of genetic testing for parentage verification, AQHA must be notified in writing by December 31 of the designated year that the owner has elected not to attempt embryo/oocyte transfer. Without such notice, a foal produced the following year by designated mare is not eligible for registration without genetic testing for parentage verification.

(e) In the event a mare owner wishes to sell a mare, but retain rights to use frozen embryos, he may purchase frozen embryo permits for $50 each from AQHA. The application for purchase of a permit must be on a form provided by AQHA. Once a mare is sold, a former owner cannot purchase additional permits from AQHA.

(1) The application for a frozen embryo permit must be signed by both the mare owner and the stallion owner at time of breeding. This permit, when used to register a foal, will serve as both the stallion breeding report and the breeder's certificate.

(2) Each of the frozen embryo permits purchased may be used for the registration of only one foal. AQHA will record the number of outstanding certificates for each individual mare and that number will be a matter of public record. It is the ultimate responsibility of a prospective buyer to confirm with the seller, the number of outstanding permit applications not yet recorded on AQHA records as of the date of sale.

(3) The ownership of the permit may be transferred. Each transfer of ownership of the embryo permit shall be recorded by AQHA. The rules for transfer of ownership as listed in rule 224 for transfer of ownership of a horse shall apply, except the request for transfer of the permit shall be accompanied by the embryo permit instead of the certificate of registration.

(4) When a frozen embryo permit is used to register a foal, the owner of the permit shall sign the registration application as the owner at time foaling.

(f) When a foal is produced by an embryo/oocyte transfer, such fact will be listed on its registration certificate.

(g) AQHA may inspect the premises and practices of any party using or intending to use embryo/oocyte transfer procedures.

(h) The burden of verifying true parentage is the registration applicant's, and any question of parentage shall be resolved against the registration of a horse carried by a recipient mare through embryo/oocyte transfer.

Overnight Delivery
1600 Quarter Horse Drive
Amarillo, Texas 79104
www.aqha.com
(806)376-4811

Regular Delivery
Box 200
Amarillo, Texas 79168
registration@aqha.org

EMAIL QUESTIONS TO: registration@aqha.org

FAX YOUR COMPLETED ENROLLMENT WITH CREDIT CARD INFORMATION TO (806)349-6405
Additional Notes: