LECTURE 7

Advanced Breeding Technologies:

EMBRYO TRANSFER, cattle
Prior to AI with frozen semen, a bull could service up to 50 cows per year. When the techniques for freezing and thawing semen were perfected a top sire could potentially service a quarter million cows in his lifetime and even long after his death. This capability tremendously expanded these top sires' genetic influence on their breeds.

Now, with embryo transfer we are able to more heavily utilize the genetic material available to us from the top dams in our cowherd or from any other cowherd. Prior to embryo transfers we could expect a cow to produce 6 to 10 calves in a lifetime. With ET we may, on occasion, produce that many calves or more from one flushing and transfer procedure. A cow that is properly managed could conceivably produce up to 200 calves in her lifetime. On the average, a cow will produce 7 transferable embryos and 3 or 4 unfertilized or degenerate ova when superovulated.

DONOR SELECTION and EMBRYO FLUSHING SCHEDULE
The selection of donor cows is a process in which numerous factors will determine the ultimate success or failure of an embryo transfer program. The most important of those factors are listed below.

Phenotypic selection
In beef cattle, donor selection can be based on several type and production traits. The type traits can include general conformation, proportion, height (frame score), mature body weight, scrotal circumference, udder type, pelvic measurements and carcass traits such rib eye cross section area (muscle size), marbling, back fat thickness and yield grade or percent retail product. Dairy cattle, of coarse, are also selected based on milk production traits such as total milk production, butter fat content and protein content.

Many registered and commercial cattle breeders obtain individual animal data for production and body type. This data is used to calculate a group index. These indexes rank the cows or their calves, within the herd, or more specifically within a contemporary group, for many production traits. A group index for a particular trait (such as weaning weight) will rank an individual as a percentage above or below the average within its contemporary group. The trait average is assigned an index of 100. An animal that performs at 110% of average will be assigned an index 110. An inferior animal will have an index of 90, because it only performed at 90% of average. Individual animal measurements and the trait indexes calculated from them are used for breeding animal selection within a herd. Since a group index will rank all animals in a contemporary group from top to bottom, it is easy to select the top 10% of a male contemporary group as sire prospects, or the top 33% of a female contemporary group as replacement heifers.

Genetic selection
Cattle breeders can increase selection power by utilizing genetic statistics that incorporate production data not only for the individual in question, but also production data from its offspring, ancestors, siblings and other relatives. Breed associations, Dairy Herd Improvement Associations (DHIA), the USDA, and other organizations that have access to data from large numbers of cattle, can calculate genetic statistics for the individual animal based on its own performance and also the performance of all its recorded relatives. Genetic statistics are very helpful in making breeding decisions. Donor cows (and the bulls that are used for embryo production) are selected for calving ease traits, pre-weaning growth, yearling weight, carcass traits, maternal traits and milk production.

Individual animal data (phenotypic data) is submitted to breed associations, DHIA, etc. on thousands of animals from many different herds. This data, combined with data from related animals already submitted over time, is used in calculations that result in statistical predictors of an animals performance and more importantly the performance of those animals progeny. These statistics are called “Expected Progeny Difference” (EPD) for beef cattle and “Predicted Transmitting Ability” (PTA) for dairy cattle. EPD and PTA statistics are published in sire summaries for most popular sires. Breeders can obtain EPD or PTA statistics for every animal in their herd. EPD and PTA data tends to become more accurate as an animal gets older since the calculations are updated with production data from the individuals offspring. The accuracy is expressed as a decimal percentage. .98 is a very high accuracy score for an EPD.

Embryo donor and sire candidates can be selected based on group indexes and also EPD scores for birth weight, weaning weight and yearling weight, maternal ability (milking ability) and carcass traits, or in dairy PTA scores for total milk, total fat, total protein, percent fat and percent protein. In dairy cattle there is also a genetic statistic called a "Total Production Index" (TPI) which ranks a cow on overall genetic merit.

It is rare that an animal will excel in all production traits mentioned above. When an animal is evaluated as a embryo donor (or AI sire) prospect, a breeder will usually choose a few traits that are of import within his herd for genetic improvement or for cattle marketing purposes. Animals that excel in these traits (even if only average in other traits) are selected to effect genetic improvement for those traits.

Donor's reproductive status

Once genetically qualified cows have been selected as donor candidates, final selection of the animals should also include reproductive and general health exams, and health test results. Other criteria may become apparent on an individual animal basis. It is essential to have good restraint and adequate help when performing this physical exam. It is helpful to have help for sampling, control of the animal during examination and for thorough record keeping, etc. Each donor should pass a physical exam of all organ systems and the reproductive tract to assure general health and sound reproductive status. Some particular aspects of the reproductive system exam follow. Some of the conditions mentioned below do not effect the cows ability to conceive but do affect her usability and success as an embryo donor. Virtually none of the conditions listed below are not absolute reasons to exclude a cow as a donor but they should still be considered as donor selection is occurring. Some of the defects listed below, such as urine pooling, short cervix and excessively long vagina / deep uterus may affect fertility and can be considered genetic traits that should not be propagated.

A. Crooked cervix, if extreme. Seen frequently in Holsteins and Zebu breeds. Is merely a hindrance to flushing. .

B. Prolapsed cervical rings. Appears as a caudal enlargement of the cervix. May be reason to cull a cow as a donor if it is also associated with a shortening of the cervix. Can act as a source of chronic infection of the cervix, hindrance to flushing and can also compromise the cervical seal and result in chronic metritis and early embryonic loss if severe.

C. Short cervix. See above under prolapsed cervical rings

D. Cervicitis is detectable by irregularity in the size and consistency of the cervix and presence of vaginal discharge.

E. Vaginitis. From a myriad of specific and nonspecific causes such as IBR/IPV,

F. Ureoplasma, Mycoplasma and non-specific infections. If present it is reason to expel an animal from the donor program until resolved. The vulvar lips are always parted and the vulva and vaginal vestibule are examined during a reproductive exam in order to uncover these conditions. A speculum may be used to perform a vaginal and cervical exam

G. Metritis, Pyometra, Mucometra are all conditions which interfere with fertility. Cows that have fluid in the uterus, regardless of cause should be diagnosed and treated prior to use as a donor.

H. Pregnancy. If pregnancy has not advanced beyond 90 days the donor candidate may be aborted using prostaglandin F2 alpha. Time for at least one natural estrus cycle prior to synchronization of estrus for flushing should be allowed (30-40 days). Pregnant cows beyond the first trimester should be skipped over as donors and used the following year.

I. Urine Pooling. Frequently seen in cows with: a tipped forward pelvic conformation, restricted vulva, sacroiliac luxation, pelvic ligament damage resulting in elongation of the vestibule and vagina (usually caused by calving related injuries and dystocia). These cows will pass excess yellow stained mucous when the vagina is expressed caudally or when mounting another cow. It is a good idea to check for this condition if a cow with a very deep uterus is examined. This condition may require surgery before successful embryo recoveries can be performed. This may be adequate reason to expel a donor candidate.

J. Deep Uterus. Cows with deep uteri and long vaginas are sometimes difficult to flush embryos from. This is not to say that they have any fertility problems or are undesirable for routine breeding purposes. They are less than ideal donor but still may be used as long as the deep uterus is not caused by a pathological condition. Problem can results from trauma to the endometrium and cervix resulting from excess manipulation required to flush embryos from a cow with this type of uterine conformation.

Donor selection schedule

At least 4 to 6 weeks before the anticipated date of the first embryo transfers it is necessary to select the donor cows, whether embryos are to be transferred fresh or frozen. As soon as possible all candidates should be examined, receive vaccinations, parasite treatments and export health testing if the embryos are to be exported. Vaccinations should be given at least 30 days prior to the estrus period in which the donor is bred for embryo production.

Superovulation and recipient synchronization schedule

Superovulation is a procedure where a cow is administered **Follicle Stimulating Hormone** (FSH) to stimulate multiple follicles to develop and ovulate rather than the typical 1. Several other hormones may be used such as eCG (PMSG) but we generally use FSH. FSH is an extract of animal pituitary glands typically isolated from pigs (Folltropin-V) or sheep (Ovagen) pituitary glands.

Of course we must have a place to put all of these embryos when they are removed from the donor cow. If the embryos are to be transferred fresh we must synchronize the estrus cycle of several recipient or surrogate cows with the donor's estrus. Prostaglandins allow us to regulate the estrus cycle of cows and induce heat among a group of animals in a synchronized manner. This synchrony between the donor and recipient estrus cycle is essential for an embryo transfer to be successful. Superovulation and synchronization procedures are used simultaneously in order to accomplish fresh embryo transfer. A typical 25 day superovulation / recipient synchronization schedule would be as follows:

Day –3	Donors and recipients may be given prostaglandin to initiate synchronization.
Day 0	Donor is in heat (and many of the recipients are also in heat +/- a few days of the donor).
Day 6	Recipients may be given GnRH to synchronize a new follicular wave if an "Ov-Synch" or "Select-Synch" synchronization protocol is to be used. A CIDR may also be used at this time.
Day 6-7	Donor may be given GnRH or estradiol to synchronize a new follicular wave with the superovulation protocol.
Day 10	Donor is started on FSH injections twice a day for four to five days.
Day 12	Recipients receive a prostaglandin injection. If the recipients received GnRH on day 6, the prostaglandin injection is delayed until am of day 13.
Day 13	Donor receives a prostaglandin injection and FSH injections continue. Typically the donor will receive two prostaglandin injections with the 7th and 8th FSH injections.
Day 14	Donor receives her last FSH injection.
Day 15	Donor and recipients are in heat, only the donor is bred.
Day 16	Donor is bred a second time.
Day 22	At 7 days after the donor's heat, her uterus is catheterized and the embryos are removed by flushing with a tissue culture media. These embryos are then transferred to the recipients or frozen for future use.

RECIPIENT SELECTION

The selection of recipient cows is nearly as important to the success of an embryo transfer program as the selection of donor cows. The recipient will have a profound effects on several aspects including conception rate, calving success, calf performance and cost.

Occasionally the donor, embryos, facilities, drugs, equipment, expertise, etc. are all impeccable; yet failure of recipient heat synchronization, poor conception rate or poor calf performance occurs in spite of all other preparations. It is likely that the recipients have caused the results to be less than expected. If recipient selection and preparation is compromised poor result are possible. Of course excellent recipient procedures will not make up for deficiencies elsewhere. Following are important criteria for recipient selection.

Recipient size

Each recipient cow must be of sufficient size and conformation to reduce the chances of calving difficulty upon delivery of a term calf. Upon initial selection of a group of recipients one usually must choose the best prospect by eyeballing them. It is essential to develop a feel for recipient frame size, weight, age and general "quality" upon this initial inspection. Recipient size should approximate that of the donor animal if possible to reduce the chances of dystocia due to calf / recipient size mis-match. A person should select the best and healthiest recipient, even if a premium price is involved. It is silly to go to the expense to flush a donor, get a pregnancy and then on delivery loose the calf due to dystocia or inability of the recipient to otherwise rear her calf.

Some of the factors that should be considered are:

A. General size and condition

It is very difficult to say exactly what height and/or weight a recipient should be. We have not established a minimum height, frame score or weight for the recipients. We expect to select the best cows available, based on size and productivity for use as recipients for the embryos.

Body condition scoring is also important. Post partum cows that are to be used as recipients should calve with a body condition score of at least 5 and maintain a score of at least 4 at breeding time in order to achieve adequate success in estrus synchronization and conception.

B. Pelvic size

Pelvic measurements may be taken on recipients, especially if heifers are used, in order to eliminate those that will require excess traction or cesarean section to deliver a healthy calf. The cross sectional area and shape of the pelvis in a heifer (or cow) and the size and shape of the calf at birth are equally important factors relating to calving ease. Typically a 2 year old Angus heifer (20 to 24 months of age) will have a pelvic diameter of 275 sq. cm. at calving time. An Angus cow will usually have a pelvic area of at least 325 sq. cm. Typically a 2 year old Holstein heifer (24 to 28 months of age) will have a pelvic diameter of 300 sq. cm. at calving time. A Holstein cow will usually have a pelvic area of at least 350 sq. cm. If a projected minimum pelvic area of 270 sq. cm. at calving time is used as a selection criteria, it should be possible to limit severe calving difficulties for average size calves (71 to 90 pounds). Formulas are available to project the pelvic area at calving time when the actual measurements are obtained prior to breeding.

In a research study conducted at the University of Nebraska and at Colorado State University, published in 1971, it was found that heifers with a pelvic area of 231 to 240 sq. cm. experience 0% dystocia when the calf weighed 61 to 70 pounds. These heifers experienced 14% dystocia when delivering a 71 to 80 pound calf and a 40% rate of dystocia when the calves weighed 81 to 90 pounds. With a 251 to 270 sq. cm. pelvis the dystocia rate drops to 20% for calves that weigh 81 to 90 pounds. In all cases, calves weighing over 90 pounds resulted in 100% dystocia. Heifers with a pelvic area exceeding 271 sq. cm. had a dystocia rate of less than 10% for calves weighing 81 to 90 pounds and 20% dystocia for calves weighing 91 to 100 pounds. These studies concentrated on heifers, but should be applicable to the selection of small cows as recipient prospect as well. Our experience tells us that cows with pelvic openings over 300 sq. cm. do not experience dystocia until calf weight exceeds 100 pounds. It would be wise to not select virgin heifers as recipients for embryos that may exceed 90 pounds birth weight. If heifers are used as recipients only those projected to have pelvic dimensions in excess of 270 sq. cm. should be used.

Additionally some dystocia will occur that has no relation to calf size or pelvis size but rather is a result of malpresentation of the calf or other accident at birth. Careful attention at calving time is the only remedy for these problems.

C. Calving management:

It must be anticipated that some calving difficulties will occur when these cows deliver their embryo calves. The calving crew should not hesitate to assist the recipients in delivering their calves and be ready to perform a cesarean section if the need arises. Under no circumstances should extremely difficult vaginal extractions, using calf pullers, be allowed. After serious dystocia the recipient may or may not be able to raise the calf. If not, the calf should be raised on milk replacer as a dairy calf would be raised. The better the selection process is in selecting recipients of adequate size and health the less difficulty there will be in calving and raising of embryo calves.

Since it should be anticipated that some dystocia will occur, full-time monitoring of the recipients should be available at calving time. When embryos are transferred into a group of cows with estrus cycles that are synchronized, the period during which calving occurs is also synchronized. This enhances the herd manager and the crew's ability to closely watch the calving cows on a rotating 24 hour basis. In addition, any cows that exceed their calculated calving date by more than 2 days may be induced to calve using corticosteroid and prostaglandin. This will prevent the calf from growing any larger in-utero prior to delivery. With these techniques, most calving from a round of embryo transfers will occur within a 7 to 10 day period and can be highly supervised and attended. If in the opinion of the attending manager and veterinarian the average calf size is excessive for the recipients, induction of labor using the above mentioned compounds could be done prior to the expected calving due date.

Milking ability

The recipient must have adequate ability to produce milk for the ET calf. This is determined by assessing the udder of the lactating cow and the body condition and development of calves that are currently nursing a prospective recipient candidate.

If heifers are to be used as recipients it is more difficult to assess her future ability to produce milk. The heifers own performance at weaning time could be used as a measure of her genetic ability to produce milk.

If dairy cattle are to be used as recipients milking ability is assumed to be adequate. In the case of dairy recipients it may be decided to remove the calf at birth and raise it on milk replacer. This present additional management challenges.

Recipient's reproductive status

Once "good looking" young cows have been selected as candidates to be recipients the next step is to put them through a chute and make the final selection of the animals based on a physical exam. Further testing subsequent to the physical exam may disqualify the animal as a recipient, but usually these are laboratory tests and results will be pending for a week or more.

It is essential to have good restraint and adequate help when performing this physical exam. Help is required for sampling, control of the animals for exams and record keeping, etc. A proper chute and working facility is essential. A portable chute and corrals can be set up in situations where no other facility is available. When cows of marginal size are examined, a portable scale is useful.

Each recipient must pass a physical exam of all organ systems and the reproductive tract to assure general health and sound reproductive status. Not to be ignored is an examination of the udder for mastitis or evidence of poor milk producing capacity. Some particular aspects of the reproductive system exam follow.

It does absolutely no good to have a healthy recipient (including reproductive health) if it is impossible to easily transfer an embryo into her for reason of physical impedance or other hindrance. Some of the conditions mentioned below do not effect the cows ability to conceive but do affect her usability as a non-surgical embryo recipient.

A. Crooked cervix, if extreme. Seen frequently in Holsteins and Zebu crosses. Is merely a hindrance to transfer.

B. Prolapsed cervical rings. Appears as a caudal enlargement of the cervix. Is reason to cull a cow as a recipient if it is also associated with a shortening of the cervix. Can act as a source of chronic infection of the cervix, hindrance to transfer and can also compromise the cervical seal and result in abortion or embryonic loss.

C. Short cervix. See above.

D. Cervicitis. Detectable by irregularity in the size and consistency of the cervix

E. Vaginitis. From a myriad of causes. See reviews on IBR/IPV, Ureoplasma, Mycoplasma, non-specific infections, etc. If present in an occasional animal it is easy to expel that animal from the program. If several animals are afflicted or if a severe forms is present in epidemic proportions it may be best to locate recipients in another herd. Pregnancies can be established in spite of mild vaginitis but it is wise to use a vaginal sheath to accomplish the transfer. <u>Always spread the vulvar lips and examine the vaginal vestibule</u> when performing a reproductive exam. Always have plenty of paper towels handy to facilitate this examination. Over 90% of all non-palpable infectious or purulent vaginal or uterine infections will become obvious upon examining the vaginal vestibule, even without a speculum. Of course a vaginal speculum can be used in suspicious cases.

F. Metritis, Pyometra, Mucometra are all conditions which interfere with fertility. Cows that have fluid in the uterus or a purulent vaginal discharge should be excluded as recipients, regardless of the cause.

G. Pregnancy. If pregnancy has not advanced beyond 90 days the recipient may be aborted using prostaglandin F2 alpha. Time for at least one natural estrus cycle prior to synchronization of estrus for transfer should be allowed (30-40 days). Pregnant cows, beyond the first trimester, should be skipped over as recipient candidates until after they calve.

H. Urine Pooling. Frequently seen in cows with: a tipped forward pelvic conformation, restricted vulva, sacroiliac luxation, pelvic ligament damage resulting in elongation of the vestibule and vagina (usually caused by calving related injuries and dystocia). These cows will pass excess yellow stained mucous when the vagina is expressed caudally or when mounting another cow. It is a good idea to check for this condition if a cow with a very deep uterus is examined.

I. Deep Uterus. Cows with deep uteri and long vaginas are very difficult to transfer an embryo into non-surgically or surgically. This is not to say that they have any fertility problems or are undesirable for routine breeding purposes. They are poor recipient candidates though. Whenever the cervix of the non-gravid tract is fallen over the brim of the pelvis or if the uterus is not easily retracted and fully gathered by the palpator's hand in the rectum, the cow should be questioned as a recipient. Problem may results from excess manipulation required to transfer an embryo into a cow with this type of conformation, resulting in trauma to the endometrium and cervix.

Recipient processing

Prior to synchronization of estrus for embryo transfer the recipients should receive all vaccinations and parasite treatments. Thirty or more days is considered a safe interval for processing prior to the estrus that will be used for the embryo transfer.

Other recipient specifications

Before transferring embryos into recipient cows, the following criteria and specifications should be met by each recipient cow:

A. Each recipient cow must be of sufficient health, size and conformation to eliminate calving difficulties. Pelvic measurements will be taken on all heifer recipients to eliminate those that will not likely give birth without assistance.

B. Each recipient cow must pass a reproductive examination and have normal reproductive tract and ovaries.

C. Each recipient cow should have calved within 90 days of selection and be nursing a healthy calf, or be pregnant in the last trimester of pregnancy. Also, cows that are less than 60 days pregnant and nursing a healthy calf are acceptable as recipients, as these cows may be safely aborted. First calf heifers should not be selected as recipients as they generally result in a lower pregnancy rate. Open virgin heifers are acceptable as recipients if they meet the size criteria. Open cows without a healthy calf nursing should not be selected unless a record of calving within 90 days of selection can be produced and the reason the calf is missing is not related to the ability of the potential recipient to produce a live calf and raise the calf to weaning.

D. Each recipient cow should have calved at least 65 days prior to receiving an embryo and had at least one normal heat cycle prior to synchronization.

E. Each recipient cow should be identified with a legible tattoo or freeze brand and have a legible ear tag that is correlated with the tattoo.

F. Each recipient cow may be tested for Brucellosis and other diseases if it is necessary for health verification or sale/export certification.

G. All recipients must be vaccinated with those products deemed necessary by the veterinarian in charge at the time of recipient selection.

H. All recipients should be treated for internal and external parasites with products deemed appropriate by the veterinarian in charge at the time of recipient selection. Re-treatment of the recipients for parasites will occur at intervals deemed appropriate by the veterinarian in charge.

I. At any time during the selection process or at a later date, during the embryo transfer process, the veterinarian or transfer technician may exercise the right to remove any recipient cow from the recipient herd. Two common reasons for removing a recipient are lack of a heat at the time of estrus synchronization or lack of a corpus luteum at the time of embryo transfer, indicating that the recipient did not ovulate and will be incapable of establishing a pregnancy.

EMBRYOLOGICAL PROCEDURES

Embryo Recovery

On the day of the flush the donor cows vulva and adjacent area is prepped and a sterile Foley catheter is introduced through the cervix and into the uterus. The catheter has a balloon type retention apparatus that is inflated in order to maintain the catheter position in the uterus. This catheter is similar to those used for urinary tract drainage. After the catheter is in place, tubing is connected and the tissue culture media is infused into the uterus. The embryos are thus suspended in the media and then the media is drained from the uterus. Usually, this process is repeated for 3 or 4 cycles.

Embryo isolation

We can use two different techniques to locate the embryos in a flush. The fastest is to run the flushing media directly out of the cow through a filter apparatus that retains the embryos in a small volume of media. The media remaining in the filter apparatus is searched with a dissecting microscope. In cold weather we use the second method, which is a little more time consuming. With this method the entire flush is collected in a graduated cylinder. The embryos are denser than the media and therefore sink to the bottom. After allowing time for settling (30 minutes), the top of the media is siphoned off, leaving 100 to 150ml, which is then searched under a dissecting microscope. The second method prevents rapid changes in temperature because of the larger volume of media. Indeed the cylinder can be placed in a warm water bath to even further protect the embryos. The remaining media to be searched is rinsed into a 100 x 20mm dish, with a grid, for searching and isolation of the embryos.

Embryo washing
After the embryos are located they are moved to fresh sterilized media using 5 to 20 microliter pipettes to pick up and move the embryo between petri dishes. The embryos are washed up to 10 times through successive tissue culture dishes containing fresh sterile media. 35mm x 10mm tissue culture dishes are used to contain the culture media and embryo. Multi-well culture dishes, with 6 to 24 wells, are convenient for washing and sorting embryos.

Embryo Grading
At the time that embryos are washed, they are graded for quality and stage of development. These factors dictate the usability of the individual embryo and provide parameters that can be used to assess the prospects for pregnancy from the embryo.

Embryo Grade (Quality)

Grade 1	Very few imperfections over 85 % of cell mass intact, suitable for transfer or freezing
Grade 2	A few imperfections in shape, size or density, over 50% of cell mass intact, suitable for freezing
Grade 3	Poor quality embryo with under 50% of the cell mass intact, suitable for transfer but not freezing
Grade 4	Degenerate or dead, less than 25% intact cell mass, unsuitable for use.

Embryo Stages

Stage 1	One cell zygote or Unfertilized oocyte
Stage 2	2 to 16 cell
Stage 3	Early (loose) Morula

(stage 1 through 3 are not generally used, sometimes stage 3 embryos may be transferred fresh, stage 4 through 8 embryos are suitable for transfer, Stage 4 through 6 are best suited for freezing

Stage 4	Compact Morula
Stage 5	Early Blastocyst
Stage 6	Blastocyst
Stage 7	Expanding Blastocyst
Stage 8	Hatched(ing) Blastocyst
Stage 9	Expanding Hatched Blastocyst

Embryo handling pipettes
10 to 20 microliter (µl) glass or clear plastic pipettes are used for picking up and transferring the embryos between washes. A variety of pipettes can be used. The main requirements for any material that comes in contact with embryos are: biocompatibility, chemical cleanliness and sterility.

It cannot be assumed that all biomedical supplies are compatible with embryos or semen. For instance, syringes with rubber plungers (Monoject, B-D, etc.) all have a coating on the plunger that is toxic to semen and embryos. Some infusion pipettes are manufactured with plastics that are toxic. When possible always use tissue culture grade plasticware, syringes without rubber plungers (Air-Tite Syringe Company) and other materials that are proven to be compatible with embryos.

Embryo Transfer
After washing the embryos are ready for splitting, biopsy, transfer or freezing. When transferring embryos, they are loaded individually into plastic 1/4cc straws, similar to those used for frozen semen. These straws are then loaded into a specialized transfer gun and using clean technique the gun is passed through the cervix into the uterus of the recipient cow and the embryo is expelled. Alternatively, the embryo can be transferred into the recipient's uterus through a surgical incision. Although surgical transfers yield slightly better pregnancy rates, they are not very practical for routine embryo transfer because of the requirement for additional facilities and personnel.

Embryo splitting
Splitting of the embryo is usually done when there is an excess of available recipients for a group of fresh embryos. If a high quality morula or blastocyst embryo is evenly divided in half, each half embryo has only a slightly lower chance of conception as the whole embryo would have. If the conception rate per demi-embryo is 50%, that conception rate is equivalent to 100% conception rate per original whole embryo.

Embryos are split by using a very small microsurgery scalpel blade attached to a micromanipulator. The blade is lowered onto the embryo taking care to orient the embryo so an equal amount of inner cell mass and trophoblast cells are included in each demi-embryo. The zona pellucida is bisected with the embryonic mass. The zona is not required for viability during the subsequent embryo transfer.

The media used to split embryos is usually a surfactant free embryo culture solution. The lack of serum, BSA or other surfactants allows the embryo to adhere to the bottom of the 35mm or 60mm tissue culture dish. The electrostatic adhesion of the embryo to the dish facilitates the bisection technique.

In addition, an osmotic agent, such as .1M sucrose, is added to the solution to cause mild dehydration and shrinkage of the embryo cells. The shrinkage of the embryonic cells reduces damage caused by the scalpel.

Upon completion of the bisection, serum or some other surfactant is added to the splitting media to free demi-embryos from the bottom of the tissue culture dish. The half embryos are then washed in fresh media to remove the .1M sucrose.

Once the embryo is divided, it is loaded into a ¼ ml straw and transferred as usual. Bisected embryos contain too few cells to be frozen efficiently. Generally embryos are split and transferred fresh. Grade 1 and 2 embryos are only

Embryo biopsy, genetic testing and sexing

Using techniques that are similar to the embryo splitting technique, a small biopsy can be removed from the embryo. The microsurgery scalpel is used to slice a small section of trophoblast cells off the edge of an embryo. Only 2 to 6 cells are required for the biopsy to be usable. Biopsied embryos are freezable with very little decrease in viability as long as 90% of the embryonic mass is still intact. The presence of the zona pellucida is not required for viability during freezing or transfer.

The most common genetic test that is performed on embryos is a polymerase chain reaction (PCR) test for the presence of a repeated DNA sequence present only on the Y chromosome. A positive test for the Y chromosome DNA assures that the embryo is a male.

Care must be taken not to get stray cellular debris into the PCR reaction tube as male cell contamination of the test will yield a false positive. The most common cause for a false negative would be loss of the biopsy prior to placing it in the reaction tube or improper use of reagents.

The PCR test is available as a field ready test kit available from AB Technologies of Pullman, WA.

Embryo freezing

When freezing of the embryos is to be done, they are placed in a special media containing a cryoprotectant such as glycerol or ethylene glycol. Cryoprotectants act to protect the embryo by replacing water inside the embryo, dehydrating the cytoplasm, decreasing the freezing temperature, reduce the amount of injurious ice crystals formation and probably change the character of the crystals that do form. Cryoprotectants may also afford some protection to cells by acting as a non-frozen solvent for intracellular macromolecules after the intracellular water has frozen. While the embryos are equilibrating in the freezing media they are loaded into ¼ ml straws. The end of the straw is plugged or heat-sealed. The straws are then placed in a programmable freezer and the temperature is lowered at a very specific rate. At –6º C., ice crystal formation is induced with a cooled forceps or cotton swab dipped in liquid nitrogen. Induction of ice crystals is called "seeding". At 10 minutes after seeding the freezing machine is set to decrease the temperature at .3 to .6º C. per minute to an end point of -30 to -35º C. The embryos are then plunged into liquid nitrogen at a temperature of -196º C. The embryos are stored at -196º C in liquid nitrogen until they are thawed for use. Embryos will remain viable for years at this temperature. Studies are still being done to determine how long embryos can be stored in liquid nitrogen but we expect viability to be maintained for many decades or even hundreds of years. Background ionizing radiation would be responsible for the majority, if not all, of the damage that could occur to an embryo properly frozen and maintained in liquid nitrogen.

Embryology media

Embryo flushing and embryo holding media is typically Modified Dulbecco's Phosphate Buffered Saline solution (MDPBS) with several additives. The additives that are typically used are: glucose, sodium pyruvate, a protein source such as bovine serum albumin or fetal calf serum and an antibiotic such as kanamycin or penicillin and streptomycin.

Modified Dulbecco's phosphate buffered saline

	MM	mg./L	ml/L (stock)	mOsmol.
NaCl	136.90	8000.815		273.80
KCl	2.70	201.288	2.700	5.40
NaHCO3				
Na H2PO4 . H2O				
Na2HPO4	8.10	1149.866	8.100	32.40
KH2PO4	1.40	190.519	14.000	5.60
HEPES				
CaCl2 . 2H2O	0.91	133.785	0.910	2.73
MgCl2 . 6H2O	0.49	99.618	4.900	1.47
	Additives			
Na Lactate (60% Syrup)				
Ca Lactate Pentahydrate				
Na Pyruvate	0.33	36.315	3.300	0.66
Glutamine				
Glucose	5.55	999.872		5.55
Penicillin		5 U/ml		
Streptomycin		5 microgram/ml		
Bovine Serum Albumin		.4%		
Fetal Calf Serum		10%		

Modified Dulbecco's PBS	Molecular composition			
Sodium :	3527.320	mg/L	mOsmolar.:	327.61
Potassium	160.302	mg/L		
Calcium	36.473	mg/L		
Magnesium	11.90945	mg/L		
Chloride	5048.507	mg/L		
Bicarbonate	0.000	mg/L		
Phosphate	902.228	mg/L		
Glucose	999.8725	mg/L		

Some of the newer commercially available media for embryo transfer are called Synthetic Oviductal Fluid (SOF) and replace the phosphate buffers with organic buffers such as HEPES, MOPS or PIPES.

Freezing media is usually MDPBS or SOF with 1.4 molar glycerol or 1.5 molar ethylene glycol added as a cryoprotectant.

When ethylene glycol is used as a cryoprotectant, the embryo can be thawed and directly transferred to a recipient. With Glycerol the embryo must be rehydrated (the glycerol removed) prior to transfer of the embryo. Glycerol removal involves rinsing the embryo through 2 to 6 washes that contain decreasing concentrations of glycerol. Sucrose (.3 to 1 molar) in PBS can be used as a single step process to remove glycerol from the embryos. Almost all embryos at ARC have been frozen in ethylene glycol in recent years due to the simplicity of the direct transfer procedure that E.G. allows.

Splitting media contains .1 molar sucrose and no protein source. The sucrose causes shrinkage of the cells, which decreases the damage done by the blade. The lack of protein allows electrostatic forces to develop between the embryo and the bottom of the petri dish. The electrostatic force holds the embryo tightly against the bottom of the dish and limits rolling of the embryo while the bisecting incision is made.

A few statistics and facts:
1st successful embryo transfer: Walter Heape, 27 April 1890, using a rabbit donor and recipient.
Superovulation of cattle was first described in 1950.
1st commercial application of Embryo Transfer in cattle in 1971 using surgical techniques.
1st commercial use of non-surgical embryo collection and embryo transfer, 1976.
In 1982, it was estimated that 50,000 bovine embryos were transferred in North America. In 1992, it was estimated that 126,000 bovine embryos were transferred in North America. There were 282,000 embryos transferred worldwide in 1992.
With ideal circumstances, a pregnancy rate of over 70% should be achieved with fresh embryos. Frozen embryos will yield a pregnancy rate over 60% if only top quality embryos are frozen. Recipient quality is the other main determinant of success in an embryo transfer program.
Embryo recovery rates, from donor cattle superovulated with FSH, are 5-7 transferable embryos per flush when all breeds are considered.
At ARC we have been averaging about 9 embryos per flush from Angus, Fresian and Hereford donors over the last several years.
A bovine embryo is approximately 135 microns in diameter. This size is at the limit of resolution for the unaided human eye. These embryos contain anywhere from 16 to 100 cells at the time we collect them from the cow at 6 to 8 days after conception.

EMBRYOLOGY SUPPLIES

Mfr/supplier	Category	Size	Unit	Item
	An/Trq	50	ml	Acepromazine, 10mg/ml, 50ml
Phoenix	An/Trq	100	ml	Lidocaine 2mg/ml
Alconox	Cleaning	1	ea	Alconox, Detergent
Flow	Cleaning	128	oz	Linbro or Orvus, soap
Sherwood	Dosing	100	ea	Needle 16ga x 1", Alum hub
Sherwood	Dosing	100	ea	Needle 18ga x 1 1/2", Plas. hub
Sherwood	Dosing	100	ea	Needle 20ga x 1 1/2", Plas. hub
Sherwood	Dosing	50	ea	Syringe, 12cc
Sherwood	Dosing	100	ea	Syringe, 1cc
Sherwood	Dosing	25	ea	Syringe, 20cc
Sherwood	Dosing	25	ea	Syringe, 35cc
Sherwood	Dosing	100	ea	Syringe, 3cc
Sherwood	Dosing	20	ea	Syringe, 60cc
Sherwood	Dosing	20	ea	Syringe, 60cc, catheter tip
Air Tite	Dosing	100	ea	Syringe, all plastic, 10cc
Air Tite	Dosing	200	ea	Syringe, all plastic, 2.5cc
Air Tite	Dosing	30	ea	Syringe, All plastic, 50cc
Air Tite	Dosing	100	ea	Syringe, all plastic, 5cc
Sovereign	Emb-Cu	1	ea	Catheter, tom cat, 3.5 Fr.
Falcon	Emb-Cu	20	ea	Dish, tissue culture, 35x10mm
Falcon	Emb-Cu	20	ea	Dish, tissue culture, 65x15mm
Falcon	Emb-Cu	10	ea	Dish, w/ grid, 100x15, square
Gelman	Emb-Cu	50	ea	Filter, Acrodisc, .2um
	Emb-Cu	100	ea	Pipettes, disposable, 5ml
	Emb-Cu	100	ea	Pipettes, disposable, l0ml
	Emb-Cu	250	ea	Pipettes, disposable, lml
Kimble	Emb-Cu	250	ea	Pipettes, micro, 20ul
Kimble	Emb-Cu	250	ea	Pipettes, micro, 50ul

Kimble	Emb-Cu	250	ea	Pipettes, micro, 5ul
B-D	Emb-Cu	1	ea	Pipettes, Unopette, sterile, 10ul
Falcon	Emb-Cu	50	ea	Plate, 24 well tissue culture
ESP	Emb-Cu	1	ea	Plate, 6 well tissue culture
Falcon	Emb-Cu	500	ea	Tube, Centrifuge, 15ml
Falcon	Emb-Cu	500	ea	Tube, Centrifuge, 50ml
ASP	Emb-Cu	1	ft	Tubing, Latex, 1/4"x3/8" (good)
ASP	Emb-Cu	50	ft	Tubing, Latex, 3/32"x7/32" (Good)
Bard	Emb-Fl	1	ea	Catheter, foley, 16 Fr. x 18"
Bard	Emb-Fl	1	ea	Catheter, foley, 18 Fr. x 18"
A.B.Tech	Emb-Fl	1	ea	Catheter, foley, 18 Fr. x 22"
A.B.Tech	Emb-Fl	1	ea	Catheter, foley, 20 Fr. x 26"
EmCon	Emb-Fl	1	ea	Filter, Embryo, EmCon
IMV	Emb-Fl	1	ea	Stylette, for Foley catheters, 18"
IMV	Emb-Fl	1	ea	Stylette, for Foley catheters, 22"
IMV	Emb-Fl	1	ea	Stylette, for Foley catheters, 26"
PETS	Emb-Fl	1	ea	Y Junction,Tubing, foley connector
PETS	Emb-Fz	1	ea	Cane, large, 13mm
PETS	Emb-Fz	1	ea	Cane, small, 10mm
PETS	Emb-Fz	1	ea	Goblets, cryogenic, 10mm, clear
PETS	Emb-Fz	1	ea	Goblets, cryogenic, 10mm, yellow
PETS	Emb-Fz	1	ea	Goblets, cryogenic, 13mm, clear
PETS	Emb-Fz	1	ea	Goblets, cryogenic, 13mm, yellow
PETS	Emb-Fz	1	lb	Straw sealant, PVC
PETS	Emb-Fz	1	lb	Straw sealing plugs 1/4" to 1/2"
IMV	Emb-Fz	100	ea	Straw, labeling, 1/2cc non sterile
IMV	Emb-Fz	100	ea	Straw, labeling, 1/4cc non sterile
	Emb-Re	1	ea	Record form, donor record
	Emb-Re	1	ea	Record form, embryo transfer fresh
	Emb-Re	1	ea	Record form, embryo transfer frozen
	Emb-Re	1	ea	Record form, frozen embryo report
	Emb-Re	1	ea	Record form, frozen semen inventory
	Emb-Re	1	ea	Record form, pregnancy check report
	Emb-Re	1	ea	Record form, recipient record
	Emb-Re	1	ea	Record form, semen collection
	Emb-Re	1	ea	Record form, superovulation/flush
IMV	Emb-Tr	80	ea	Sheath, sanitary
IMV	Emb-Tr	5	ea	Sheath, transfer, mini
IMV	Emb-Tr	5	ea	Straw, .25cc, sterile
Sanofi	Hormone	1	ea	Cystorelin, 2ml
Phoenix	Hormone	30	ml	Epinephrine, 1:1000
	Hormone	10	ml	Estradiol Valerate
Haver	Hormone	20	ml	Estrumate, 250mcg cloprostenol
AUSA	Hormone	1	ea	FSH, Folltropin-V 400 Unit
Schering	Hormone	1	ea	FSH-P, Sioux Biochem. 50mg.
Upjohn	Hormone	30	ml	Lutalyse, 5mg/ml, 30ml
Sanofi	Hormone	25	ds	Syncromate-B, 25 ds.
	Lab-Su	1	ea	Microscope, bulb, spare
	Lab-Su	1	ea	Microscope, lens cleaner, 50ml
	Lab-Su	1	ea	Microscope, lens paper
ASP	Lab-Su	1	ea	Tape, Label
ASP	Lab-Su	1	ea	Tissue, Kim-Wipe
ICP	Media	20	ml	Albumin, Bovine serum, 20%
Gibco	Media	1	ea	Antibiotic Antimycotic,100x,LY,20ml
ICP	Media	1	ea	DPBS, Mod., Flushing Media, l000ml

ICP	Media	1	ea	DPBS, Mod., Holding Media, l00ml
ICP	Media	1	ea	DPBS, Mod., W/ Ethylene glycol 20ml
Gibco	Media	100	ml	DPBS, modified,+ BSA+ Kanamycin, ETFM
Sigma	Media	100	ml	Glycerol, Cell culture
Gibco	Media	100	ml	Serum, fetal calf, heat inactivated
Gibco	Media	100	ml	Sodium Pyruvate Sol.
Gibco	Media	500	gm	Sucrose
Sigma	Media	50	ml	Trypsin .25%, HBSS w/o Ca, Mg + shipping
	Off-Su	1	ea	Label tape
	Off-Su	1	ea	Label, Avery 3.5"x5/8"
	Off-Su	1	ea	Pen, black fine tip
	Off-Su	1	ea	Pen, Sharpie, x-tra fine point
ASP	Sterile	60	ft	Autoclave tape, 3/4"
ASP	Sterile	100	ft	Dualpeel, tubing, 4" roll
ASP	Sterile	100	ft	Dualpeel, tubing, 9" roll
ASP	Surgical	128	oz	Alcohol 99.5% ethanol
ASP	Surgical	100	ea	Applicator, cotton tipped, 100/pk.
Aveco	Surgical	128	oz	Disinfectant solution, Nolvasan
Sherwood	Surgical	100	ea	Glove, Exam, Lrg. Latex
Kane	Surgical	100	ea	Glove, O.B. Maxi-Sleeve M125
	Surgical	128	oz	Lubricant, O.B.
Carter	Surgical	1	ea	Lubricating Jelly, HR

EMBRYOLOGY LABORATORY

Isolation, Washing and Freezing of Embryos

Preparations

- Place flushing media in incubator 2-3 hours prior to flushing @ 25 to 30ºC.
- Place holding media, freezing media and embryo filter rinsing media on lab bench @ room temp 1 hour prior to flush.
- Assemble the flush tubing and embryo filter assembly.
- Add BSA, antibiotics and antimycotic to the flushing media if necessary.
- Add BSA or serum, antibiotics and antimycotic to the embryo culture media if necessary.
- Place all materials necessary for flushing near the collection chute.

Flush donor

Remove flushing solution and embryos from the embryo filter

- Use a 50cc all plastic syringe with a 22 or 20 ga. needle to rinse the embryo filter into a gridded search dish.
- Use flushing media without BSA to rinse the filter.
- If BSA is used in the rinsing solution too many bubbles are formed.

Searching for embryos

- Gentle swirling of the search dish will usually bring most of the embryos to the center of the dish, expediting the search.
- Use a pipette to move bubbles to one side of the dish.
- Locate the embryos.

Prepare an embryo holding dish, an embryo washing plate, the freezing media dishes and embryo loading dishes.

- Use a 10 ml all plastic syringe to fill the embryo holding dish and a 6 well embryo washing dish with embryo culture media.
- The initial holding dish is a 35mm x 10mm tissue culture dish (petri dish). Use 4 ml of media.
- Embryo washing is done in a 6 well or 24 well tissue culture plate. Use 1 to 2 ml per well.
    - 6 well dishes are used for a single flush.
    - 24 well plates can be used to wash embryos from two or 3 flushes in one plate.
    - 24 well plates are also used for washing embryos from a single flush when special handling is required, such as when embryos for international export require exposure to trypsin to inactivate certain viruses (IBR).
- Embryo freezing media (1.5 Molar ethylene glycol in embryo culture media) is placed into 35mm x 10mm dishes, if embryos are to be frozen. Wells in the 24 well plate may also be used for embryo freezing media.
- The 35mm dishes to be used to load embryos into straws may also be prepared now.

Place embryo into 1st wash (holding media)

- Use a 5 µl to 20 µl micropipette to pick up embryos from the search dish and place them into the holding media.
- Continue to locate embryos in the search dish and transfer them to the holding dish.

Wash embryos 10X

- Once all embryos have been isolated in the holding dish they must be washed 10 times.
- Pick up the embryos with a micropipette and move them into the first wash (really the second wash if you consider the holding dish as the first).
- Transfer the embryos serially through each wash.
- Each wash should represent at least a 1:100 dilution of the media containing the embryos, i.e. 10 µl of media with the embryos is placed into 1000 µl (1 ml) of wash media.
- Only embryos from a single donor are washed together.
- Care is used to leave any debris in the previous well when picking up the embryos.
- Count the embryos at each wash to assure that none are left behind or lost.

The following are requirements for embryo that are to be exported:

- A new sterile pipette is used for each dilution.
- A maximum of 10 embryos are washed together at one time.
- Only zona pellucida intact embryos are washed. (No naked embryos or embryos with a cracked zona.) Embryos should be zona intact both before and after washing.
- Only embryos free of any adherent material are washed. (The usual adherent materials are attached granulosa cells, which can be difficult to remove, or tenacious mucous.)

- Trypsin treatment: (to inactivate IBR virus and pseudorabies virus
    - If trypsin washing is required for export embryos it is incorporated into the washing procedure after the 5th regular wash.
    - The first 5 washed must be supplemented with BSA not serum. Residual serum will inactivate the trypsin activity.
    - Embryos are passed through 2 washes of Hank's balanced salt solution (with or without Ca^{++} or Mg^{++}), containing .25% 1:250 trypsin. (1:250 means 1 gram of trypsin will hydrolyze 250 grams of casein)
    - Total exposure to trypsin is 60 to 90 seconds.
    - The embryos are then washed 5 times in embryo culture media that contains 2% serum to inactivate and dilute the trypsin.
    - It is suggested in the IETS manual 3rd Edition, that serum is not required and that simple dilution of the trypsin in media containing BSA is adequate for post-trypsin washing of the embryos.

Embryo Freezing

- Make labels for the embryo straws (see IETS manual)

- Start the freezing machine
    - Turn on machines control unit
    - Fill the liquid nitrogen reservoir 2/3 full of LN_2
    - Place cryo-chamber into the LN_2 reservoir
    - Allow the cryo-chamber to equilibrate at –6°C. (The freeze program start temperature)
- Select embryos to be frozen. Only grade 1 or grade 2 embryos should be selected for freezing. Frozen grade 3 embryos yield disappointing pregnancy rates. (Refer to IETS manual for grading of embryos)
- Place embryos into cryoprotectant, 1.5 Molar ethylene glycol in embryo culture media.
- Load embryos into straws, 1 embryo per straw. The embryo lies in a column of media between 2 air bubbles.
- Seal the straws containing the embryos
    - Place a "¼ to ½ cc straw adapter" with an attached labeling straw into the open end of the ¼ ml straw containing the embryo.
    - Examine the embryo straw under the microscope to verify proper position of the embryo in the cryoprotectant media column.
- Place the loaded straws into the freezing machines cryo-chamber. The embryos require at least 5 minutes in ethylene glycol before being loaded into the freezing machine at –6.0°C.
- Seed ice crystals in the straw at 2 to 5 minutes after loading the machine (at –6.0°C).
    - Seeding involves touching the side of the straw with an instrument or a cotton swab chilled in liquid nitrogen (LN_2) to minus 196°C.
    - This causes local chilling inside of the straw and induced ice crystallization.
    - The normal freezing point of the embryo freezing media with the cryoprotectant is minus 12°C. By seeding ice crystals at minus 6°C it is possible to eliminate the rapid rise in temperature to nearly 0°C that occurs when spontaneous ice crystallization occurs. This rise in temperature is caused by the "latent heat of fusion" that is released from the solution at the time of freezing. Any rapid change in temperature is detrimental to embryos but at the freezing point it is critical to eliminate fluctuations. Also, the ice crystals that form at higher temperatures when the embryo straw is seeded are smaller and probably less injurious to the embryonic cell membranes and organelles. Always seed at a point away from the embryo itself.
- After seeding the embryos are left to equilibrate for ten minutes while ice crystals form throughout the straw.
- Run the freeze program.
    - Turn the appropriate switch, on the freezing machines control unit, to the "run" position.
    - The current program decreases the temperature .5°C per minute from –6°C to –31°C. Other freezing programs are also effective.
    - At –31°C the embryos are left to equilibrate for an additional 10 minutes.
    - The complete freezing program, including seeding and the equilibration periods at the beginning and end of the program, is about 1 hour and 12 or 13 minutes.
- Plunge embryo into liquid nitrogen (LN_2)
- Load straws into labeled canes and goblets.
- Store embryos in the LN_2 tank

Embryo Splitting & Biopsy

- Embryo splitting is used to increase the number of fresh embryos available for transfer to available recipients. Pregnancy rate of whole fresh embryos should be near 70%. The pregnancy rate with split embryos is about 50% to 60%. In spite of the lower pregnancy rate, more pregnancies should be obtained than when whole embryos are transferred since a 50% pregnancy rate with a demi-embryo is equivalent to a 100% pregnancy rate based on the original whole embryos. Another possible advantage is the acquisition of an occasional set of identical twins in about 25% of the sets of split embryos transferred.
- Procedure for splitting follows:
    - Splitting media is embryo culture media that contains no serum, BSA or other surfactants. The lack of proteins allows electrostatic attraction between the bottom of the tissue culture dish and the embryo to develop. This attraction holds the embryo in place while it is divided in half with a micro-scalpel.
    - Aliquot either 50 µl drops or a full 4ml of splitting media into a 35x10mm dish or a 65x15mm dish to be used as the splitting dish
    - Aliquot 4 ml of splitting media into a 35x10mm dish to be used as a pre-wash.

- Select embryo to be split. Only grade 1 or high grade 2 embryos with large cell masses should be selected for splitting. (refer to IETS manual for grading of embryos)
  - Rinse embryo in the pre-wash and before it settles to the bottom of the dish pick it back up and place it into the splitting media in the splitting dish.
  - Place splitting dish under scope that is fitted with the micromanipulator and microscalpel.
  - Locate embryo and lower the microscalpel onto the embryo and make a 50:50 cut through the zona pellucida through the embryo cell mass and into the bottom of the dish to assure complete division of the demi-embryos. Care must be taken to orient the blastocyst so that equal amounts of inner cell mass and trophoblast cells are shared between the two halves.
  - After the embryo is split 5 to 10 µl of serum or 20 to30 µl of .4% BSA containing media is added to micro-drops or .5 to 1 ml of BSA containing media is added to full splitting dishes. The added protein will release the demi-embryos from the bottom of the dish
  - Wash embryo through embryo culture media, load embryo into a straw and transfer immediately to a waiting recipient
- Embryo biopsy is very similar to splitting.
  - With biopsy, the embryo is not bisected but rather the only a small portion of trophoblast is removed. The biopsy should contain 3 to 10 cells.
  - If desired an embryo may be biopsied instead of split.
  - The biopsy specimen should be placed in a 1ml ultracentrifuge tube.
  - The remaining embryo may be frozen or transferred immediately.

Embryo Transfer

- Prepare straws, sheaths and ET guns for loading (near embryology bench)
- Load embryo into a ¼ ml straw place embryo
- Place the ¼ ml straw containing the embryo into an ET gun and apply the sheath to the gun
- Provide thermal protection for the embryos before taking them into adverse weather conditions for transfer