

# Bovine Embryo Transfer



## Introduction

In this lab you are going to learn how to synchronize ovulations and cause superovulation in cows for the purpose of embryo transfer. By the end of this lab you should be familiar with the processes involved in synchronizing donors, using several different and not equally effective systems. You should be able to set up and administer an effective superovulation program utilizing FSH to cause a hyper response. You should be familiar with and able to assist in embryo recovery, embryo handling and embryo transfer into a synchronized recipient.

## History and Applications

Embryo transfer developed right along with the advances in artificial insemination. In the early 1930's as artificial insemination gained a stronghold in this country, it became a standard practice among America's cattlemen. In the 1950's a company named American Breeder's Service out of Wisconsin, developed a way to freeze and transport frozen semen. All of this laid the groundwork for the development of embryo transfer in cattle. In the 1970's surgical embryo transfer( no longer used), began to see commercial application on America's farms and ranches. At this point, the process was rather rudimentary as PGF2  $\alpha$  had yet to become commercially available and the processes of refining FSH were not as exact as they are today. Results varied extremely widely and the process was complicated and expensive, yet it began to gain popularity fairly rapidly; especially in dairy cattle. Embryo transfer was a way to get multiple calves out of a cow in a year as opposed to one or two every nine months. Soon cows were having up to thirty daughters and as many sons. From genetically elite cattle whose offspring were worth up to several hundred thousand dollars in the early 1980's, it was profitable to have many offspring. In the middle eighties the procedure began to be refined. Soon it was possible to flush cows without surgically entering the cow and the recipient. It also became possible to freeze embryos (possibly indefinitely) instead of throwing away the ones that there wasn't a recipient set up for. This allowed for international trade in genetics in a much easier way than ever before thought possible. Soon you could send female and male genetics worldwide in a cryopreservation tank without the hassle and complications of exporting live animals and the associated risk (disease and animal loss concerns). As the procedure became non-surgical the price began to drop (thought still expensive), and it became even more widespread. This along with advance in embryo splitting in the later 1980's to achieve even higher pregnancy rates, and in the early 1990's advances into PCR and embryo sexing , along with the freezing of embryos in ethylene glycol as opposed to the traditional glycerol in the quick thaw technology as allowed it to become a vital process in cattle genetic improvement. Embryo transfer is still a costly procedure and takes time and good management, and should only be used on the genetically elite because of it. The future for cattle embryo transfer looks bright and the advances and possibilities are limitless.

## Recipient Synchronization

Recipient synchronization is one of the most crucial elements of embryo transfer. Recipients at this point have hopefully been fed a balanced diet, with particular emphasis paid to macro and micro mineral levels in their feed. Hopefully accurate heat records have been kept for the last 30-60 days, so we know approximately where every animal is in their cycle. We want to have our recipients in excellent health condition, and we hope to have recipients at as close to identical uterine conditions as the donor cow to help increase our success rates from this expensive procedure. Embryos collected from the donor cow are pulled or collected at day 7 of her cycle. Due to this we hope to have recipients that are as close to day 7 as possible. Day 7 is ideal but days 6-8 are also acceptable. Cattle cycle on a 21-day cycle. If we take this day and count it as 0, and want to implant five embryos from a day 7 donor, we need a couple of recipients that are 6-8 days into their cycle. It is estimated that 5% of a naturally cycling herd will be in heat on any one day, the odds of having five recipients on days 6-8 of their cycle is pretty slim. To combat this we have several options.

### Option # 1 Natural Heat

One way to achieve our five recipients is to maintain a large inventory of open recipient candidates. With this you would maintain accurate heat records, and would need a herd of at least 40 head to achieve your five recipients. Figuring that five percent came in a day, that is two the day before (day 6), two the day of (day 7) and two the day after (day 8). This will work, but requires a large recipient pool and under farmer-breeder conditions, delays breeding long enough as to be impractical. There are too many expenses involved with keeping the heifers or cows open.

### Option # 2 Progesterone Implant and Induced Heat

With this system an animal can be made to synchronize with any other animal, because of the fact it doesn't matter where she is in her cycle to start it. First the animal is injected with 2 ml of Norgestomet/estradiol to lyse any existing CL's that may be present and producing progesterone on the ovaries. At the same time the shot is administered an ear implant containing progesterone is injected. Approximately ten days (implant day is day 1) later the implant must be pulled, and within 36 hours about 90% of animals implanted will show signs of heat.

### Option # 3 Prostaglandin F2 alpha

This is by far the most common way of synchronizing recipients. This hormone prostaglandin F2  $\alpha$  is responsible for lysing any corpora lutea present to remove progesterone from the system and allow the cow to come into heat. There are several synthetic prostaglandins available with the most common being known as Lutalyse. Cows that are presently between days 6 to 16 of their cycle will respond to a Lutalyse injection and come into heat about 60 hours later.

There are 3 commonly administered ways of doing this.

a.) Prostaglandin (1 injection) with palpation

Take all available recipients and palpate their ovaries. Cattle with a palpable CL, are given prostaglandin (about 5 mL depending on size), and about 90% of those will show heat within 60 hours.

b.) Prostaglandin (1 injection) with no palpation

In this protocol you take the entire available group and shoot them all, with no rectal palpation. It's cheap, its easy and only 45% come into heat 60 hours after injection, so success rate is lower.

c.) Prostaglandin double injection with no palpation.

Shoot all available recipients, with total disregard to place in cycle. Eleven days later shoot them all again. 60 hours after the second injection, 90% of the cattle will be in heat. Success rate is higher, but the amount invested increases also, but it may outweigh the hassle and expense of palpation.

The important key to remember is, there is no measurable difference in pregnancy rates between natural and induced heats. There are many factors to consider, including amount of recipients available, when needed, time available, drugs and labor available; in determining your synchronization program.

## Superovulation with FSH

Cows and heifers normally cycle on a 21-day cycle. About 12-16 hours after estrus a single egg, yet to be fertilized is sent down from one of two ovaries. If inseminated there is a good chance fertilization would occur and the ova would develop to be an embryo. The whole idea of superovulation is to cause this to happen but on a multiple number of ova as opposed to just one. In the United States this is performed using a drug known as FSH-P. Using this drug cattle can be repeatedly superovulated on a 30-day or more cycle. There are two common regimens available to superovulate cattle. Both of these are used with the understanding that the donor is healthy and cycling regularly.

### Regimen # 1 Corpus Luteum Present

After rectal palpation it has been determined that the cow (donor) has a well-formed CL. Any time during the cycle between days 8-13 the donor can start treatment, once it is determined that the CL is present. The way that this is done is a three to four-day series of FSH shots. They are give twice daily and at least twelve hours and no more than twelve hours apart. Each day the does decreases and prostaglandin is given with FSH on day 3 of the treatments series. Within a range of 36-60 hours after this the onset of estrus begins. It is important to keep a close watch on heat to determine optimum time for artificial insemination. It is important to note here that FSH should be administered with a clean needle each time, including drawing it from the bottle, as bacteria quickly break down and destroy FSH.

## Regimen # 2 Corpus Luteum Absent, Cystic Ovaries or Acyclic ovaries

The first key here is good palpation to determine exactly what is going on. You don't like to superovulate cows with these conditions, but it sometimes is necessary with extremely valuable donors to do so. When one of these conditions is present the donor is attempted to be brought into heat with the progesterone implant program. The exception is the cystic donors. With these, using rectal palpation you attempt to rupture the cyst manually and give them the implant but not the injection of Norgestomet/estradiol valerate. Now that your donor has her implant, seven days after it's insertion you start her on her FSH treatment. On day 3 or 4 ( depending on practioner) you pull the implant and 24-36 hours later, the donor shows heat and 12 hours after standing heat you inseminate her. This regimen works well for scheduling large groups of donors together.

Here is a sample Donor Injection Schedule for a cow due to be in heat on January 23.

Date	Time	Dose	Drug
Jan. 19	7 am	1.3 cc	FSH ( Schering FSH-P)
Jan. 19	7 pm	1.3 cc	FSH
Jan. 20	7 am	1.0 cc	FSH
Jan. 20	7 pm	1.0 cc	FSH
Jan. 21	7 am	4.0 cc	Lutalyse
Jan. 21	7am	.8 cc	FSH
Jan. 21	7 pm	.8 cc	FSH (remove implants)
Jan. 22	7am	.6 cc	FSH
Jan. 22	7pm	.6 cc	FSH

\*\*\*Good heat detection is vital to both recipient synchronization and donor superovulation...\*\*\*

Heat detection should be done twice daily (at the bare minimum ) for at least 60 days to use on both recipients and donors. Effort should be made to do it as much as possible. The best times to do it are early morning and later evening particularly in summer. Try to get the cattle off of concrete and your heat detection results will improve, as cattle are more likely to show heat on a more comfortable and better gripping surface. Suspicious behavior in the way of heats should be recorded, but the only definite sign of heat, especially for people new to heat detection is the telltale stand to be mounted by another cow or the teaser bull. Discharge, chalked tail heads, chin ball markers, k-mar are all secondary to this...

## Breeding

Cattle will be bred four hours after first estrus detection and then 12, and 24 hours after that. Breeding technique is critical, but will not be focused on for this lab. If you are interested in breeding practices, take Les Ferriera's AI class in the dairy science department.

# Embryo Recovery

## Materials:

Foley Catheter and tubing (with diverters and Clamps )

Insertion Rod

Fluid Container (either bag or bottle ) and Media ( PBS due to open air conditions)

Em -Con Filter with lid

## Procedure:

On Day 7, the donor is brought in and restrained in a chute or head gate. Preferably with her front end elevated.



**Restraining Chute (above)**

The next step is to administer an epidural using up to ten cc's of lidocaine administered in the spinal junction.



**Lidocaine Block ( above )**

You insert the needle and establish that you have suction and administer the dosage. Within two minutes the tail should be completely limp and you should be able to palpate without restriction. The first order of the day is to palpate all recipients and the donors, and figure out where they all are in cycles and pinpoint which ovary they ovulated from of in the case of the recipients.



**Palpating and Cleaning the vulva and anus ( above )**

Next the donor's vulva and anus is washed with a beta iodine solution (tail tied out of the way first), and then depending on practitioner it is rinsed off, due to the fact that the solutions can be lethal to embryos. Next the Foley catheter (20 gauge for cows and 16 gauge for heifers ) is inserted into the uterus. This is accomplished by using a metal rod, which slides into a slot on the catheter. This rod helps you navigate through the cervix, which is tight and constricted due to the fact that the cow is not in heat.



**Inserting metal rod to make cervical navigation possible (above)**

This helps to maintain rigidity and make cervical navigation possible. The catheter is slid through to just inside the uterine body and slowly inflated (balloon is slowly inflated) to avoid tearing the uterus. This inflated balloon or cuff serves to hold the catheter in place.



**Inflating cuff on Foley Catheter (above)**

From here the uterus or individual horn is filled with fluid depending on whether you are doing a body or a horn flush. In a horn flush, the tip of the catheter is navigated into each horn and each (must do both) horn filled and drained of the fluid.



**Filling the uterus with fluid and draining it out through filter (above)**

In a body flush, the uterine body is filled with fluid (PBS because we are working in open air) and then each horn is milked or manipulated to flush out the embryos. Only a skilled practitioner should do this to avoid damage to the cow and/or embryos.



**Complete Tubing Apparatus (above)**

The fluid is then drained out through a series of tubes down to a filter that is 17  $\mu\text{m}$  across (embryos are 100-120  $\mu\text{m}$  across), so there is a minimal amount of fluid to be searched

through at the end. The procedure itself is not overly complicated but takes extreme amounts of experience to be done successfully.

## Embryo Handling

### Materials:

Microscope

Search dish with grids

Pipette (any type will do depending on individual preference)

Programmed Freezer (see cryopreservation lab)

Freezing media (either Ethylene glycol or glycerol derivatives )

Dish with four wells

### Procedure:

Take the filter and extract all liquid into the search dish with grids. Then take a syringe filled with media and carefully hose down the sides and bottom of filter to make sure and knock loose any embryos that may be sticking to the sides or filter screen itself.



**Rinsing the Filter (above)**

Once this is done, filter may be discarded. The next step is searching through the grid dish to locate all embryos underneath the microscope.



**Searching on the grid plate for embryos (above)**

Upon location the embryos are sorted into a dish, using our pipetting skills acquired earlier on , with four wells into viable and nonviable groups of embryos.



**Grading embryos in four well dish (above)**

Once the search dish has been thoroughly searched and care has been taken to search through the bubbles and the uterine debris that obviously didn't pass through the filter, you can grade the embryos. Once the embryos are graded we can determine which ones

we want to transfer today (generally the poorer quality ones) fresh and which ones we want to freeze (see Cryopreservation lab ).

**Seeding Straws in Freezer**



**Embryos as viewed through the microscope, these are viable. ( above )**

# Embryo Transfer

## Materials:

Horn Gun

Straw for transfer

Transfer media (PBS with pyruvate, glucose and BSA )

Sheath to go over horn gun

## Procedure:

The recipient is brought into the headlock or chute and restrained. Then a lidocaine block or epidural is performed on their tail head to numb it. They are washed in the same fashion as the donor above. Since we palpated them earlier and marked their ovulation side with chalk and what day they felt to be in their cycle (and graded them 1,2 or 3 as recipients), we can match up younger or older embryos and increase our odds for success. We determine what embryos are to go in what recipient on grade or embryo and recipient. Then we load up our straw in the same fashion as we do for cryopreservation, but we don't seal the end. The embryo is loaded in a transfer solution of PBS supplemented with pyruvate, glucose and BSA. The straw is then inserted into the extra long horn gun with a round end and a hole about ½ inch from the end of it so embryo can go out, but don't damage lining of horn.



**Inserting Horn Gun into horn to implant embryo. (above )**

A sheath is then placed over the gun for sanitation reasons. We are now ready to enter the recipient rectally. We go in and locate the left or right horn depending on ovulation sight and using the broad ligament (avoiding touching uterus as

much as possible to avoid prostaglandin releases that reduce pregnancy rates) we straighten out the horn and pass the rod through the cervix and into the horn as far as we can go without encountering resistance, careful not to force it and damage delicate uterine lining. Once a slight bit of resistance is encountered, the gun is discharged by pushing the plunger, the embryo is sent to it's happy home in the uterine horn and hopefully nine months later a beautiful live calf results.



**Pushing Plunger to insert embryo. ( above )**

## Conclusion

After completion of this lab you should be able to synchronize recipients, set up superovulation schedules using FSH and be familiar with the processes involved in embryo recovery (collection procedures and materials) and embryo handling and finally transfer into the recipient cattle that you have synchronized and determined useable to this process. You should have combined knowledge from your prior labs on embryo handling, grading, freezing etc. with the new found knowledge of bovine hormone cycles and how to manipulate them to what we want and desire to achieve in our successful embryo transfer program.