►Producers may select a donor on the basis of performance data, showring appeal or a combination of personal preferences.

PHOTO BY SHAUNA ROSE HERMEL

ET Basics

Learn the details of an embryo transfer program, from prepping the donor to freezing embryos.

by ***Maggie Malson***

Because the average cow will have one calf per year, only a small amount of the reproductive potential of an outstanding female can be utilized with a natural breeding program. However, through embryo transfer (ET), a superior female can have a broader influence — and much more quickly.

Becoming acquainted with what an ET program involves and learning techniques to help ensure a successful experience will safeguard the time and investment ET requires.

"A sound herd health program is imperative to a successful embryo transfer program," says veterinarian Gregory Garcia, who owns and operates Precision Embryonics Inc., Glide, Ore. External and internal factors may affect whether a cow will produce viable embryos. "Having a good herd health program is the first step in eliminating some of those problems."

Garcia encourages producers to have a good rapport with their herd health clinicians. "Producers should meet with their local health professionals who are well aware of the diseases present in that area," he says. "Together, they can develop vaccination and deworming programs to meet the needs of animals in their particular locale."

Garcia warns that certain areas have vaccine requirements that differ from others.

"As an ET practitioner, I can't overemphasize the need for adhering to what the local health professionals feel are the best protocols for that particular area and vaccinate for them," he says. "Be very aware of vaccination protocols and how these vaccines react in relation to their function in reproduction."

Veterinarian Robert Derby, Nyssa, Ore., further explains the importance of herd health management. "Every herd is different in terms of nutrition and genetics, but the focus of any herd health program is on reproduction," he says. "Producers are in the business to make money, and if a cow isn't producing a calf because of poor body condition or a poor reproductive system, she's not doing her job."

If cows are too thin or too fat, they will have trouble conceiving. Minerals are also important in nutrition. Some animals can have phosphorous (P) deficiencies if fed mostly alfalfa, since it is high in calcium (Ca). Certain areas of the country deal with selenium (Se) and copper (Cu) deficiencies. Producers have to be aware of these and supplement accordingly.

"It is essential to have nutritional programs tailor-made and designed around the nutritional requirements and/or deficiencies that are unique to a specific geographic area," Garcia says.

Superovulating the donor

Once selection of the donor cow has been made, she must be prepped for superovulation using various hormones to cause the release of multiple eggs during a single estrus. Garcia says a superovulated cow releases eight to 12 ova, on average, during one stimulation period. Approximately 85% of cows will respond to treatment, with an average of six transferable embryos.

Fig. 1 offers a superovulation protocol recognized by industry standards. To understand the physiology of the estrous cycle (see Fig. 2) and how synchronization occurs, review "Synchronizing with GnRH" in the October 2000 *Angus Journal* and "Synchronized Breeding Made Simpler" in the October 2002 *Angus Journal.* Both articles are available by a back-issue search at *www.angusjournal.com.*

As Colorado State University's (CSU's) Jack Whittier and Tom Geary explain in "Synchronizing with GnRH," ovarian follicles grow and regress in waves during the 21-day estrous cycle. At the beginning of each wave, a group of similar-size follicles begin to increase in size. As the wave develops, most follicles die one by one until a dominant follicle remains. In the presence of high levels of progesterone, even it will regress; however, if the right hormonal signals are sent, the dominant follicle matures and ultimately ovulates. In a synchronization program, the producer manipulates the timing of the hormonal surges by administering synchronization products within an approved protocol. To superovulate the donor, follicle-stimulating hormone (FSH) is administered to prevent follicles from dying off, so most of the follicles in the wave will ovulate.

Choosing a synchronization strategy

If recipient cows are available for the immediate transfer of viable embryos, they should be prepped using estrus

synchronization at the same time donor cows are being readied.

"Synchronization of recipient cows is essential in relationship to embryo transfer because the timing of embryo recovery and age of embryo should be as close to the recipient cow as possible," Garcia says. "With today's new technology, we are more able to control the synchronization times to have tighter, more efficient synchronies."

He explains: "In the ideal world, a seven-day embryo would receive a cow with a seven-day heat. In the practical world, that's not always the case because cows don't always read the book. You try to have a six-, seven- or eight-day recipient receive a 7-day-old embryo."

There are numerous synchronization programs available to producers (a variety of articles are available via a back-issue search for the key word "synchronization" at *www.angusjournal.com*). "The bottom line is people should develop a synchronization program that works for them because tighter synchrony lends itself to higher success rates for embryo transfer," Garcia says.

Inseminating the donor cow

If a producer feels his herd sire will produce marketable offspring, he may opt to allow the herd sire to breed the donor naturally, Garcia says. "But most producers choose to AI (artificially inseminate) their donor cows."

Females used for ET are usually bred 12-24 hours after the onset of standing heat.

Because many eggs are released from several follicles during a period of time, the need to have viable sperm reach the oviducts is greater than normal. Therefore, many ET technicians will choose to inseminate the cow several times during and after estrus.

"In our program, we call for an early breeding in case a donor cow begins ovulation earlier," Garcia adds. "It may be redundant, but it provides good security."

Using high-quality semen with a high percentage of motile cells is important also, he advises. Semen is placed in either the body of the uterus or at the entrance into each uterine horn.

Flush, evaluate, transfer

Embryos are recovered nonsurgically using a small, synthetic rubber catheter, which is inserted through the cervix of the donor cow (see illustration). First, the technician administers an epidural block, allowing for a pain-free, more comfortable experience for the cow and more efficient work for the technician.

The technician then evacuates manure from the rectum, cleanses the rectal/vulval

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Fig. 1: Industry protocol for superovulating donor cows

Day		
−10	Insert CIDR implant and administer 2cc of GnRH	
−3	Remove CIDR and administer shot of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)	
0	Onset of estrus	
	a.m.	**p.m.**
10	FSH	FSH
11	FSH	FSH
12	FSH + $PGF_{2\alpha}$	FSH + $PGF_{2\alpha}$
13	No FSH	Begin estrus detection
14	Expected heat	Breed
15	Breed	
22	Nonsurgical embryo recovery	

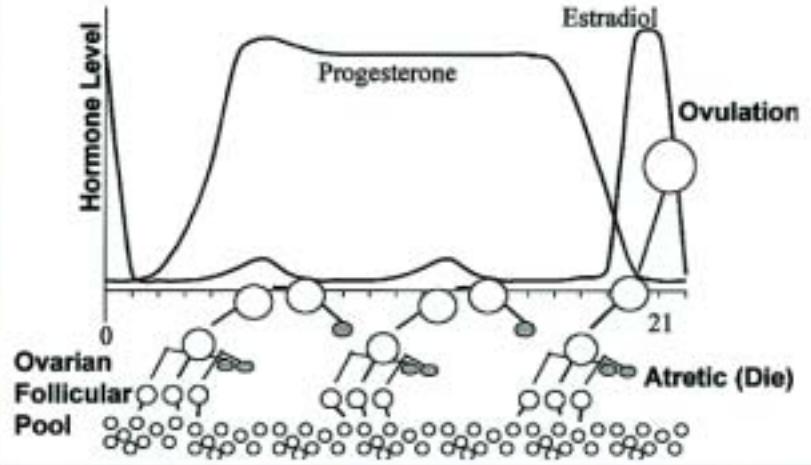
CIDR=Eazi-Breed™ CIDR® Cattle Insert, a controlled internal drug release insert containing progesterone.

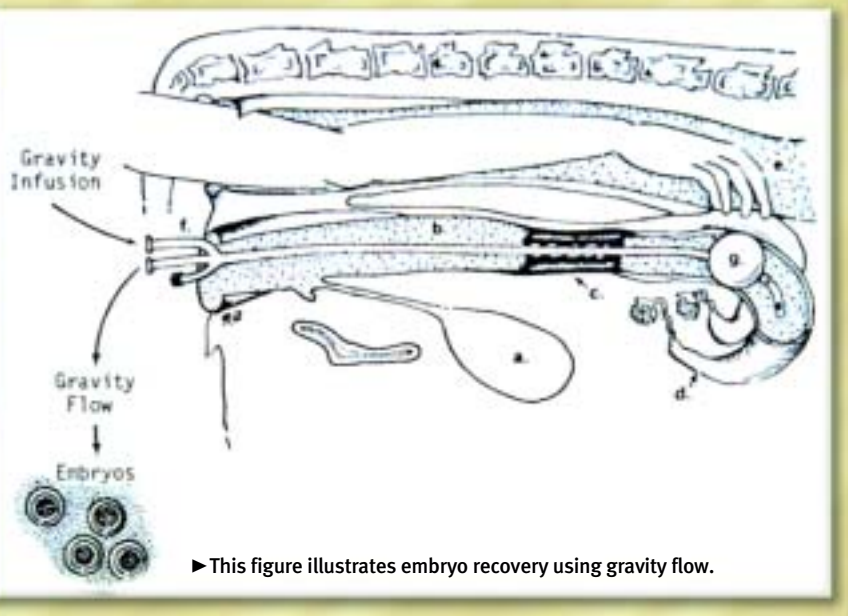
GnRH=Gonadotropin-releasing hormone, causes the release of luteinizing hormone (LH), which causes two events — (1) ovulation of the egg from a follicle on the ovary and (2) conversion of the follicle to a corpus luteum (CL).

$PGF_{2\alpha}$=Prostaglandin $F_{2\alpha}$, triggers a drop in the female's progesterone level that results in estrus and ovulation.

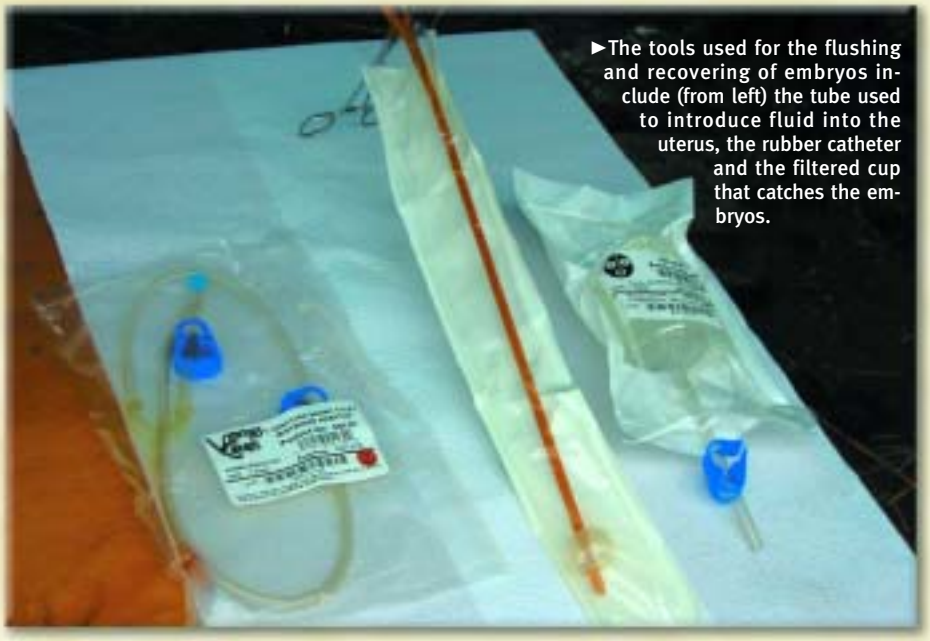
FSH=Follicle-stimulating hormone, prevents follicles from dying off, so most of them will ovulate.

Fig. 2: Development of follicular waves during the 21-day estrous cycle of cattle. Progesterone levels are elevated from Day 5 to Day 17, and estrogen levels peak at approximately 21 days.





►This figure illustrates embryo recovery using gravity flow.



►The tools used for the flushing and recovering of embryos include (from left) the tube used to introduce fluid into the uterus, the rubber catheter and the filtered cup that catches the embryos.

PHOTOS COURTESY OF CALIFORNIA STATE UNIVERSITY AT PAMONA

►After inserting the catheter and inflating the cuff, Gregory Garcia, Precision Embryonics Inc., Glide, Ore., introduces saline with a syringe in order to control the volume of liquid entering the uterus. He then massages the uterine horns, allowing the free-floating embryos to flow out into a filtered cup. The embryos are then transferred to a petri dish and are examined under a microscope.

area and places the catheter for inflation of the balloon or cuff.

"This step is crucial as it seals off the cervix to prevent the liquid from flowing back out," Garcia says.

Once the catheter is in place, the uterus is flushed with a phosphate-buffered saline solution. It has the same acidity and osmolarity (Osm) as body fluid.

There are two ways to flush a cow, the horn method or the body method, Garcia says. The horn method may be more efficient since it uses a lower volume of fluid to recover the embryos. Each uterine horn is flushed separately. The disadvantage is it usually requires two entries into the uterus. With the body method, both uterine horns are flushed simultaneously.

"Some advanced ET technicians can clamp off one uterine horn with their hand, while flushing the other, eliminating the need to reenter the uterus," Garcia says, describing a modified body flush. "This helps prevent any foreign material from entering the sensitive area."

There also are several methods used to introduce fluid into and remove it from the uterus. With the gravity-flow method, a bag of saline is held 1 meter (m) above the back of the cow, and the liquid is allowed to flow in via gravity. The saline, along with the embryos, is also allowed to flow back out via gravity.

Some technicians use a syringe to introduce the fluid. This method is usually used with low-volume flushes.

The modified version used by Garcia is a closed system, like the gravity-flow method; however, it is coupled with a one-way valve on a syringe to introduce the fluid while using gravity flow to recover the embryos. He says this allows him more control of the amount of fluid going in.

As the uterus is flushed with fluid, the technician massages it to move fluid throughout and loosen any embryos that may be trapped in the villi of the endometrial folds.

Embryos are sent through the saline into a cup with a filter attached. The size of the embryo is 100-120 microns, while the filter is approximately 70 microns. The fluid is then put into a small petri dish displaying a grid. There are other types of filters, some of which double as petri dishes. The technician looks through a microscope to find the embryos and gives them a quality grade of 1, 2 or 3.

"The best ones can be frozen, while the lower grades need to be transferred immediately," Garcia explains. "The embryos

are transferred to a holding container before freezing or transferring them."

Before the transfer is made, the ET technician palpates the recipients to determine the presence of a CL.

Embryos are transferred using a 0.25-mL French ET gun, which is similar to an AI gun. Embryos are placed into the uterine horn on the same side as the ovary containing the CL. The CL produces progesterone to maintain the pregnancy. Transfers can be made as high as comfortably possible without causing damage to the horn. Conception rates among recipients average 60%.

"ET is a delicate process, which should be done with as little stress to the recipient cow as possible," Garcia says. "Stress in handling the cows can result in the release of the hormone cortisol, which can result in the subsequent release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterine cells, which can cause loss of the pregnancy. Excessive or rough handling of the uterus may also lead to the release of this hormone."

►**Left:** High-quality embryos are frozen using the ethylene glycol method, indicated by the yellow canes. Freezing embryos allows producers more options by enabling them to transfer them at a later date or market the genetics to other producers.

►**Below:** Garcia transfers the embryos using a 0.25-mL French ET gun to the uterine horn containing the CL of the recipient cow. He tries not to damage the horn, as stress and excessive handling can cause the pregnancy to be lost.

Embryo freezing

Freezing high-quality embryos allows producers more opportunities in marketing superior genetics or making herd decisions, because frozen embryos can be shipped to other producers or saved to be placed in recipient cows at a later date.

Two methods are used in the industry today. One uses glycerol and the other ethylene glycol as a freezing medium. The method using 10% glycerol has been used since the 1980s. It requires a microscope to thaw embryos because glycerol is removed from the embryo via a dilution process, either with a decreasing concentration of glycerol or by using sucrose preparation to draw the glycerol from the embryo cells.

International rules set by the International Embryo Transfer Society (IETS) require embryos frozen in glycerol to have white cane tabs with clear or opaque goblets, straws and labels.

The other method uses 1.5 M (molarity) of ethylene glycol to free embryos. This technique is gaining favor with ET practitioners and producers alike because it does not require a microscope for thawing. The thawing of embryos is much like thawing semen, with transfer immediately to the recipient animal. Rehydration of the embryo occurs within the cow's uterus using her own fluids. This is referred to as direct transfer.

The international designation for embryos frozen with ethylene glycol is yellow goblets, straws and cane tabs.

"Thaw times and temperature in air and water baths are practitioner-dependent and should be adhered to," Garcia says. "There are as many different techniques as there are technicians performing them. The important thing is success. Whichever method achieves a higher conception rate should be used."

Technological advances

Further research into embryo technology includes finding a new method to freeze embryos without a freezer. Seidel says research conducted at CSU has found a method called vitrification, in which the water becomes glass-like instead of crystal-like. "It's the same water, but it has a different structure that doesn't damage the cells as much," he says.

CSU has also done research with XY Inc. on sexed semen.

"It isn't readily available commercially right now, but could be in the future," Seidel says. "Fertilization rates in superovulated cows are lower with sexed semen, but are nearly normal with synchronized cows. Sexed sperm are damaged slightly, and the environment in the superovulated cow's reproductive tract is more stressful to sperm than normal, so some eggs do not get fertilized with sexed sperm.

"Commercialization of sexed semen has been a slow process because companies are charging high fees for the technology," he says. "Sexing semen, as well as sexing embryos is about 90% accurate."

While research into ET continues, purebred producers can be assured the use of this technology will help them add genetically superior genes to their cow herds, as well as give them another means of marketing those genetics through the sale of embryos. Just as AI helped producers incorporate the genetically superior genes of a bull into their herds, ET allows for a genetically important cow to influence the herd as well.

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