

Taking non-surgical ET to the farm

BY TRISH WILLIAMS

QUARRYVILLE — Embryo transfer (ET) certainly is not a new concept. The first successful embryo transfer was recorded in 1890 at the University of Cambridge when two fertilized eggs were obtained from an Angora doe rabbit and transferred into a Belgian hare doe. But it wasn't until the mid-1960's that surgical embryo transfer became practical in cattle.

Research findings over the last twenty years have rapidly advanced the applications of ET. This rapid progress in research is due in part to interest in exotic beef breeds during the '60's, and by purebred dairy breeders, but just as important in obtaining funding for research was the interest in genetic engineering for application in human medicine.

In recent years we have been hearing about laboratory research being conducted on sexing, freezing and splitting embryos. These techniques have become refined enough that they are now being done in the field by a few of the more progressive veterinarians.

Carl G. Troop, V.M.D. is one such veterinarian. Dr. Troop who has been performing embryo transfers for four years, has

refined his technique for non-surgical ET, and has been using only the non-surgical method for ET for the past two years.

Troop works hard at keeping abreast with the latest advances in ET. As a member of the Embryo Transfer Society he attended its annual meeting and a workshop this winter in Denver, Colorado. Troop learned how to freeze embryos at the workshop. He also works closely with researchers at the University of Pennsylvania's New Bolton Center in Kennett Square.

Troop owns his own dairy herd, this gives him the ability to practice and master new techniques on his own animals before offering the service commercially. Troop's herd is also used by researchers at Penn, currently Troop has two recipient heifers who were implanted at Penn and are pregnant with test tube calves. When these two calves are born later this summer they will be second and third calves in the world to be born from an in vitro fertilization (conceived in a test tube). The first calf born from an in vitro fertilization is at the New Bolton Center.

Troop credits progressive dairymen with much of the recent success of application of new

techniques in the field. The dairymen are responsible for synchronizing recipients with the donor, and maintaining accurate records.

The estrous cycle of donors must be accurately detected and characterized to get the best results from superovulation treatments. It is best to observe two normal heats postpartum before starting to treat a cow with prostoglandins for superovulation.

Heat detection is also important in recipient heifers. Recipients must be in estrus within one day, plus or minus, of the donor for a high success rate. To synchronize recipients with the donor, Troop gives the dairymen the necessary drugs and a 'Donor and recipient programming schedule' to follow. The schedule gives a time table for the drug treatments for both donors and recipients and indicates when they should show signs of estrus.

Troop recommends breeding the donor early in standing heat and again in about 18 hours. This is because when the donor is superovulated the ova are released from the ovary over a period of time.

The synchronization of the donors and the recipients is the same for this non-surgical transfer technique as is used for surgically performed ET.

Troop uses the non-surgical procedure for recovering the fertilized embryos six to eight days after the donor is bred. Doreen Lowe, lab technician and transfer assistant, helps with the procedure.

After the flushing procedure has been performed the nutrient solution containing the embryos is taken back to the lab at Pennstar. The nutrient solution is allowed to sit for about a half-hour, to allow the embryos to settle to the bottom of the flask. Doreen Lowe and Nancy Hoffer then start the search for the embryos by examining the solution under stereo-variable microscopes. This job takes a trained eye and a great deal of patience. Once the embryos are found under the microscope they must be isolated and classified, then with sterile technique the embryo is manipulated into another medium for transfer into the recipient.

After the number of fertilized embryos suitable for transfer has been established Troop notifies the dairymen how many recipient heifers are needed for the transfer. Now, with the facilities to freeze embryos it is not necessary to transfer all the embryos fresh. Like semen, the embryos can be frozen in a liquid nitrogen tank for later transfer.

The synchronized recipient heifers are brought to Pennstar the same day and implanted non-surgically with the embryos and sent back to the farm immediately.

Recipient heifers are prepared for the transfer the same way the donor was prepared for flushing. The tailhead is clipped and the vulva area is scrubbed. A spinal nerve block is given in the tailhead. Troop checks the heifer for presence of a corpus luteum to maintain the pregnancy.

The non-surgical method for embryo transfer is very similar to the procedure use in artificial insemination. The embryo is drawn into a French straw like those used in artificial insemination. The loaded straw is put into an inseminating gun, the lips of the vulva are parted by an assistant so that Troop does not carry any contamination into the uterus on the inseminating gun.

Troop manipulates the inseminating gun through the cervix into the horn of the uterus that is on the same side as the corpus luteum.



Stereo microscopes, Doreen Lowe, left, and Nancy Hoffer examine the fluid flushed from the donor cow for embryos.



Embryos are implanted in the recipient heifers by a method very similar to artificial insemination, using no surgery.



Donors are given a spinal nerve block before flushing to relax them. This donor, Galena, is so relaxed that she is eating hay while Dr. Troop performs the flush.



Fluid for flushing is gravity fed from 36 inches above the uterus, through the uterus and is collect by Ms. Lowe in a thermal container.

"Placement of the embryo in the uterine horn is very critical," said Troop. "It took me alot of practice on my own herd to be able to place the embryo accurately and consistently. Heifers often have a very tight cervix at this stage in the estrus cycle. That can make passing the inseminating gun through the cervix difficult."

As soon as resistance is met in the uterine horn the embryo is expelled and the gun is removed.

This method of transfer requires

a great deal of manual dexterity. The endometrial lining of the uterus is very fragile and ruptures easily.

It takes Troop about 15 to 20 minutes to do the transfer. Then the heifers are loaded back in the trailer and shipped back to the farm. Flushing the donor, isolating the embryos and transferring them into recipient heifers can all be done within a matter of a several hours, all without a single cut of the scalpel.



Ms. Lowe first locates the tiny embryo under the microscope, then draws it into a glass straw for implanting.