at Em Tran

Cutting a fine line - embryo splitting

BY TRISH WILLIAMS

ELIZABETHTOWN — Embryo transfer technology has advanced rapidly, taking the purebred cattle industry to new levels of sophistication. Ten years ago embryo transfers outside of the research labortories were done on a very limited basis in the cattle industry. Today embryo transfer is an industry in its own right.

Enough bovine veterinarians have mastered the embryo transfer technique and are offering it commercially that in some areas the competition within the industry is becoming very noticable.

To stay abreast with the new technology and the competition in the embryo transfer industry requires a commitment to continuing education and to experimentation with the developing ET techniques.

Em Tran Incorporated has made that commitment. It is a commitment that has its obvious rewards, but it also has its definite costs. ET is a high stakes business to cattlemen. Low pregnancy rates or reproductive failures are not taken lightly, financially or mentally.

To make the decision to take the new techniques from the laboratory to the farm requires a strong commitment. Investment in new equipment, in training and research, and just in practicing the technique enough to confidently offer it commercially, must be weighed against the financial rewards.

So when Em Tran made the decision to develop the technique for embryo splitting for commercial service it was after much serious deliberation.

Embryo splitting is a technique used to divide the six to seven day old embryo, called a morula, into two complete embryos.

Dr. R. Allen Rushmer, one of five veterinarians on Em Tran's staff has been working on refining the splitting technique for over a year. Rushmer was taught how to split an embryo in January 1982 at a workshop conducted at Colorado State University, by Dr. Tim Williams, the man responsible for developing the technique.

Since that time Em Tran has acquired the necessary equipment for micromanipulation of the tiny but very valuable embryos. Rushmer has also been working on developing his manipulative abilities when embryos were available to practice.

Having five veterinarians on staff allows Rushmer to specialize in splitting. On the day Rushmer demonstrated the splitting technique to me, he and his four associates performed five on the farm flushes for embryo recoveries from cows that had been superovulated. Each veterinarian has a technician to assist with the flush on the farm and to isolate the embryo back in the Em Tran lab. Transfers were also performed that same day, and details worked out for transport of embryos for export.

The embryos Rushmer split had been recovered earlier in the day from a cow owned by a John Hertzler, a nearby farmer. The cow is not of great genetic worth herself but is making a contribution toward the advancement of genetic engineering by providing embryos for developing this potentially profitable technique.

After the embryos had been identified, isolated, and classified by technician Susan Hallowell, Rushmer was able to start his work.

Rushmer uses a Leitz micromanipulator to perform the split. The micromanipulator is a microscope specially equipment with three microinstruments to manipulate the embryo.

Rushmer must manufacture these microinstruments himself because as yet they are not available commercially. To make the microinstruments also requires special equipment, a microneedle puller and a microforge. Rushmer makes the microinstruments from 1 mm glass capillary tubing on the microneedle puller. This bends and tapers the tubing to the desired shape. Then on the microforge he bevels and sharpens the microinstruments.

One of the microinstruments is used to hold the embryo in place by means of a suction exerted by a syringe mechanism. The second microinstrument is a blade used to pierce the embryo and divide it. The third instrument is connected to another suction line and is used to draw out half of the embryo.

It takes Rushmer about a half hour just to set-up the micromanipulator and prime all the tubing.

Research in splitting embryos has proven that the zona pelucida of the embryo is necessary for embryo development. The zona pelucida, or zona, is the protective, elastic outer coat of the embryo similar to the shell of an egg. So Rushmer must have an empty zona ready to put the half of the embryo in after the split. Unfertilized embryos and empty zonas recovered from flushings are saved and frozen in liquid nitrogen for this purpose.

The empty zona and embryo to be split are put into a glass dish under the microscope, and final adjustments are made on the equipment.

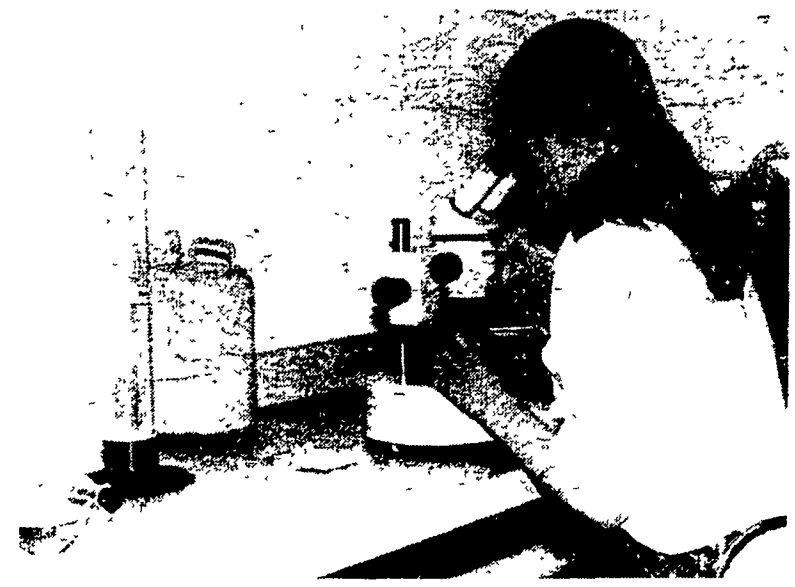
Rushmer locates the embryo and brings it into focus in the microscope field. With a delicate turn of the screw adjustment on the syringe he holds the embryo in place for the split. Then he brings the microblade into position for the cut. First he pierces the zona, then with great precision he divides the embryo in half. The microblade is removed and the third microinstrument, a suction line is used to draw out half of the nuclear material of the embryo. The elastic qualities of the zona allow it to quickly form around the remaining half to form a complete embryo. The complete embryo is released from the microholder and the empty zona is then held in place, pierced with the blade and the other half of the embryo's nuclear material is deposited into it. The finished product... two complete identical embryos from one.

The embryos are placed in culture medium in a test tube and placed in an incubator overnight. The next day they are examined to see if they have continued to develop as they should. If they are found to be normal they are either implanted into a recipient or frozen for later use.

Rushmer demonstrates his competency for the technique and says "We may be able to offer the service commercially in a couple of months if everything continues as well as it is now, but it requires alot of time, so time is an important factor."

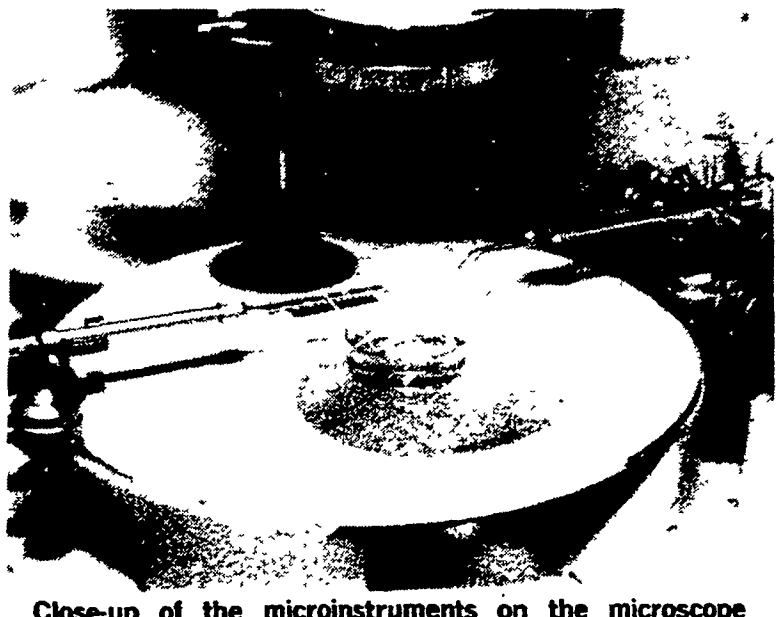
Considering how such a technique can be used to speed up genetic progress, a few months seems like a 'microsecond'.

Embryos are recovered by flushing this superovulated cow. Performing the flush are Dr. Halbach and technician Meg Turn.



Technician Susan Hallowell identifies, isolates, and classifies embryos from the flushing medium.

Dr. Rushmer assembles some of the "sophisticated plumbing" on the micromanipulator.



Close-up of the microinstruments on the microscope platform. On the left is the microholder, on the right are the

Dr. Rushmer watches the embryo and the microinstruments through the microscope as he manipulates them by controls.