

Frozen embryos new implement for industry

By JULIE SMILEY

The idea of freezing embryos used to be prime material for best-selling science fiction novels and movies. The idea is fiction no longer.

Just as the idea of freezing bull semen and breeding a cow later with that semen seemed unnatural and unrealistic 30 or 40 years ago, researchers predict that embryos can be frozen and stored in a similar manner. Artificial insemination, at first, was used only by innovative ranchers and dairy farmers, but now AI is common practice among producers.

Nitrogen tanks have been developed to freeze semen, and techniques improved so semen can be stored for an undetermined number of years without adverse effect to viability of the semen. Similarly, a biological freezer with a computer bank, developed by a company in England, is now being used to freeze mammalian embryos at Texas A&M University.

The idea, animals and equipment are available and the technique on its way, according to Dr. D.C. Kraemer, associate professor of veterinary physiology and pharmacology at Texas A&M.

"I don't want to suggest we're advanced compared to others," said Kraemer. "Our study is a basic comparative study of mammalian embryos to learn basic information to help us improve upon (freezing) methods currently being used."

Granada Embryo Transfer Services, Bryan, Texas, has loaned Texas A&M a biological freezer for research purposes. Kraemer said Granada's interest is to incorporate frozen embryos in its purebred Brangus breeding program and commercial embryo transfer company.

Kraemer said Granada has a working relationship with Texas A&M to provide cattle and equipment for the university's research and teaching program in return for training and research. He said this agreement is not exclusive to Granada, but because the Bryan-based operation is so close to Texas A&M it is convenient for both parties.

"As we obtain the capability to successfully freeze, thaw and implant viable embryos, we'll train them (Granada) as well as others," said Kraemer.

He said the freezer is one of six or eight foreign-made biological freezers in the United States. The University of Pennsylvania, Rio Vista Farms in San Antonio, Texas, and American Transplant in Michigan are others who have a freezer.

The freezer can be programmed to slowly cool embryos to minus 65 degrees Celsius by a two-stage process. Slow freezing reduces shock to the embryo. An anti-freeze, dimethyl sulfoxide, protects embryos during freezing and prevents them from becoming brittle, rupturing or crystallizing.

For freezing, each embryo is put into a vial with the anti-freeze. The vial is coded so complete records can be kept on each future calf. The embryos are stored in liquid nitrogen at minus 196 degrees Celsius.

Interest in preserving cells, other than sperm cells, has been steady and slow for 20 years, said Kraemer. Frozen-embryo research is more difficult because the larger the cell, the harder it is to freeze.

The diameter of an ovum is about 120 microns and the diameter of a sperm cell is about five microns. Once the ovum is fertilized, the embryo is a growing unit of many cells. The embryo could have as many as 160 cells. Successfully freezing and thawing this delicate unit makes research slow.

The attention-getter was in 1971 when the first mouse embryo was frozen in Cambridge, England. Mouse ova are successfully frozen, thawed and implanted all the time, said Kraemer.

In 1978, Carnation's Genetics Research in Hughson, Calif., claimed the first calf born from a frozen embryo in the United States. A frozen embryo, stored for 46 days at minus 160 degrees Celsius, became an 80-pound Holstein bull calf.

Kraemer said frozen embryos have about a 40 percent conception rate now, but he said he also hopes for a future rate of 50 percent or better. Fresh embryos, implanted non-surgically, have a conception rate of 60 to 70 percent. Conception rates depend on how the embryos are handled during collection and in the lab before freezing.

Joe Massey, director of Granada Embryo Transfer Company, said cattle producers are going to reap the benefits of research as the freezing technique is perfected. Massey performs all transfers, nonsurgically, at the Diamond G Ranch between Normangee and Marquez, Texas, and has about a 60 percent conception rate with fresh embryos.

He said one of the big advantages in freezing embryos will be cutting costs of maintaining "recipient" cows. Keeping a cow open and not producing and feeding her while waiting for a fresh embryo costs Granada time, money and investment.

When embryos are successfully frozen and thawed, these "recipients" will be implanted with a thawed embryo as they cycle naturally rather than waiting for a specific "donor" to give fresh embryos.

After a high-quality donor is enrolled in an embryo transfer program, she is superovulated with Follicle Stimulating Hormone (FSH) to make her produce many ova. FSH is much like a fertility pill for women.

If the donor produces five to 10 good embryos, then without the freezing technique, five to 10 recipients must be ready for implantation that very day. Keeping enough recipients on cycle with every donor is expensive, especially if the donor is flushed and no viable embryos are found.

The process of embryo transfer involves giving the donor 10 injections of FSH at 12-hour intervals. In conjunction with the fifth FSH injection, prostinglandin is also given to bring her back into estrus 48 hours later. She is given the final five FSH injections and then bred artificially.

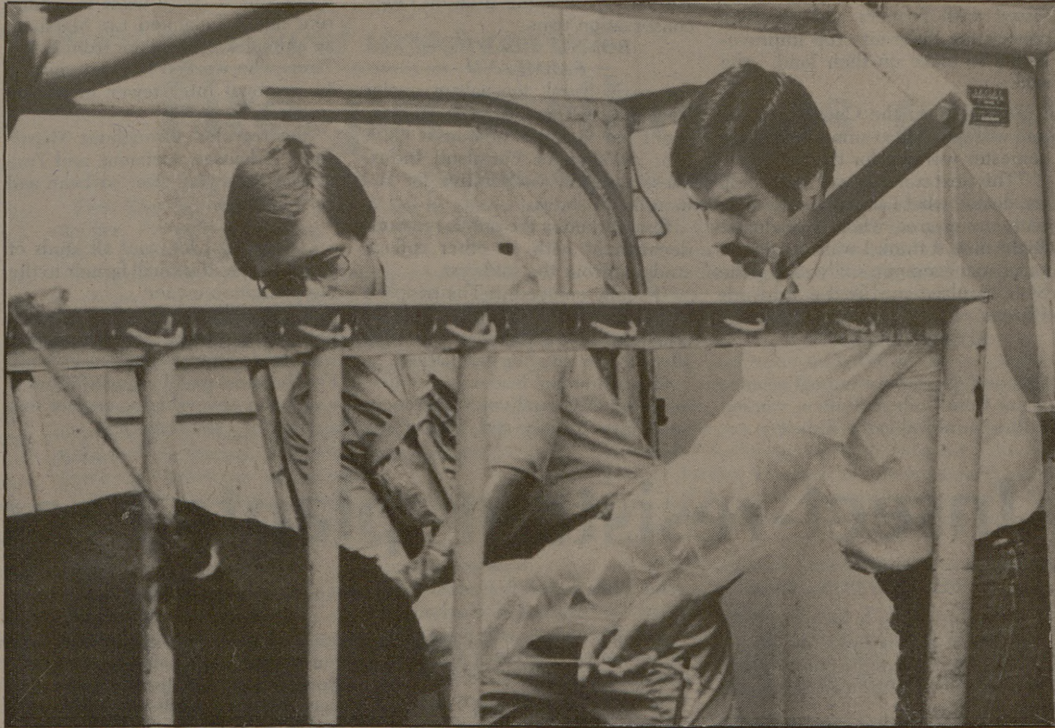
Seven days after she is bred, Massey collects fertilized embryos with the help of a catheter. Each horn of the uterus is separately flushed with a liquid medium. The medium is flushed through the uterus, and back out the catheter into a petri-dish. Embryos are then viewed under a microscope for quality and viability.

Massey said a cow can ovulate from one to as many as 20 to 30 eggs at one time when she is superovulated, but this inconsistent average is

about six to eight embryos. For every good embryo ready to be implanted, one recipient must be at the point in her estrus cycle to accept an embryo as her own.

Other advantages of freezing are easy shipment to other parts of the United States and less accessible areas, creation of embryo banks of superior genetic material, measurement of genetic drift, development of new breeds and studying of diseases by reproducing herds of "carrier" cows for genetic research.

Armed with a freezer, manpower and new technology, Granada plans to enlarge its commercial embryo transfer business with completion of a \$250,000 unit at the Diamond G Ranch.



Photos by Julie Smiley

ABOVE, Joe Massey (right), director of embryo transfer at Granada, helps Lanny Vinson (left), a graduate student from Texas A&M, pass a catheter and learn technique involved in embryo transfer. BELOW, once embryos are collected from the "donor" with the help of a catheter. A liquid medium is

flushed through the uterus, out the catheter and into glass petri-dishes. At left, after the embryos are flushed from the uterus and collected the search for viable embryos begins. Each dish is carefully examined under a microscope to locate embryos.

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