

One of the possibilities growing from embryo transfer research, and already finding commercial application is more offspring during the reproductive lifetime of genetically superior cows. These calves all had the same mother. U.C. researchers transferred them as embryos to "incubator" cows.



Using EMBRYO TRANSFERS to increase twinning

Gary A Beall

To determine the effects of twinning on beef production, researchers at University of California, Davis, are using embryo transfers to increase the percentage of twins produced by beef animals. The biggest cost in producing a beef calf is maintaining the cow, and economies should result if each cow produces two calves instead of one.

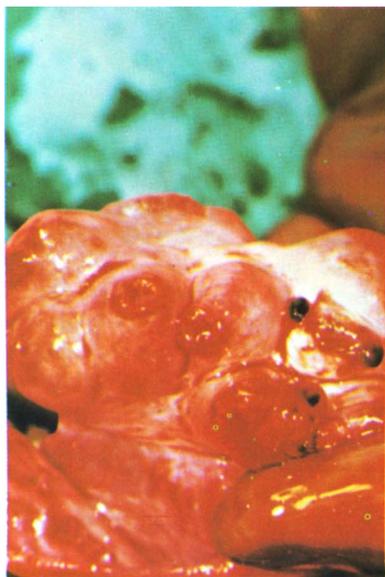
The natural twinning rate for cows is only 1 to 2 percent, however. With

management systems geared toward production of single calves, ranchers usually do not want cows to produce twins. Such cows often have calving difficulty and produce small calves.

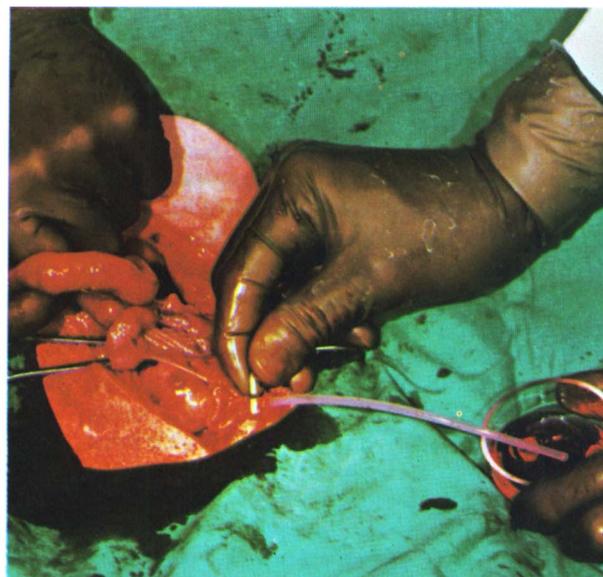
The researchers—Gary B. Anderson, assistant professor of animal science, Perry T. Cupps, professor of animal science, and Maarten Drost, associate professor of veterinary reproduction—hope to establish a herd that can be

managed for the production of twins. Since 1973 they have been surgically removing embryos from one cow and transferring them into the uteri of other cows. By transferring two such embryos or adding one embryo to a cow that already contains her own embryo, the percentage of twin births can be greatly increased.

An experiment using embryo transfer to increase the twinning percentage in



A superovulated ovary contains two or more corpora lutea, indicating multiple ovulations.



Embryos are flushed out of the uterus of the donor and collected in a small dish.



The 16-celled embryo is transferred through a small puncture made in the uterine horn.

a beef herd was started last fall when the researchers began transferring embryos to 80 Hereford cows and heifers. Part of the program will be to study dietary needs of animals pregnant with twins and to compare growth rates and meat production of twins and single calves.

The researchers will also attempt to advance embryo transfer techniques. They agree that several improvements are needed before embryo transfer becomes a practical way to increase the number of twins in commercial beef herds. Improvements include: greater and less variable numbers of embryos from donor cows; better estrus synchronization; increase in the time the microscopic embryos can be kept viable while in transit from donor to recipient animal; and nonsurgical transfer methods. Techniques to determine sex of embryos before transfer also would be helpful, because heifers born with a male twin are usually sterile.

All embryos in the twinning project are being transferred surgically. About four days before the desired time of estrus, donor animals are injected with pregnant mare's serum gonadotropin. This is a hormone that causes the ovary to produce more than the usual number of egg-containing follicles. Forty-eight hours later, the donors and recipients are given prostaglandin F₂-alpha, an experimental drug that synchronizes their estrous cycles. Forty-eight hours after this treatment, the animals come into estrus. The donors are then mated. Five days later, when the embryos are at the 16-cell stage, they are surgically collected and transferred.

The uterine horn of the donor animal is exposed, a small tube is inserted into the oviduct, and the embryos are flushed out of the oviduct into a dish. A microscope is used to locate the embryos in the dish while the recipient animal is being surgically prepared. In each uterine horn of the recipient, a small puncture is made, and the embryo is transferred through a fine pipette. In some cases in the current experiment, an embryo is transferred to only one uterine horn of a recipient that has already been bred.

In 1975, the first year the researchers used embryo transfer to increase twinning, they transferred embryos to 48 Hereford, Angus, and crossbred heifers. Thirty-six (75 percent) became pregnant, but 10 pregnancies terminated

prematurely. Of the remaining 26 going to term, 16 produced twins, and 10 had single calves.

This year, half of the 80 animals receiving embryos are older cows and half are first-calf heifers. The researchers feel that the older cows will have fewer abortions and more twins. To determine which technique produces the highest twinning percentage, half of each group is receiving two embryos, and the other half is being bred and receiving only one embryo. The animals receiving only one embryo are being bred to a Red Angus bull. The donor animals (Hereford heifers) are being bred to a Black Angus bull. This provides a color coding system for the researchers: the transferred embryos will become black calves, the others will become red calves.

Success with surgery under field conditions, and with nonsurgical transfer techniques, has been spotty so far. In 1974 and 1975, the researchers surgically collected embryos at U. C., Davis, flew them to a northern California ranch, and surgically transferred them to recipient animals in special facilities prepared by

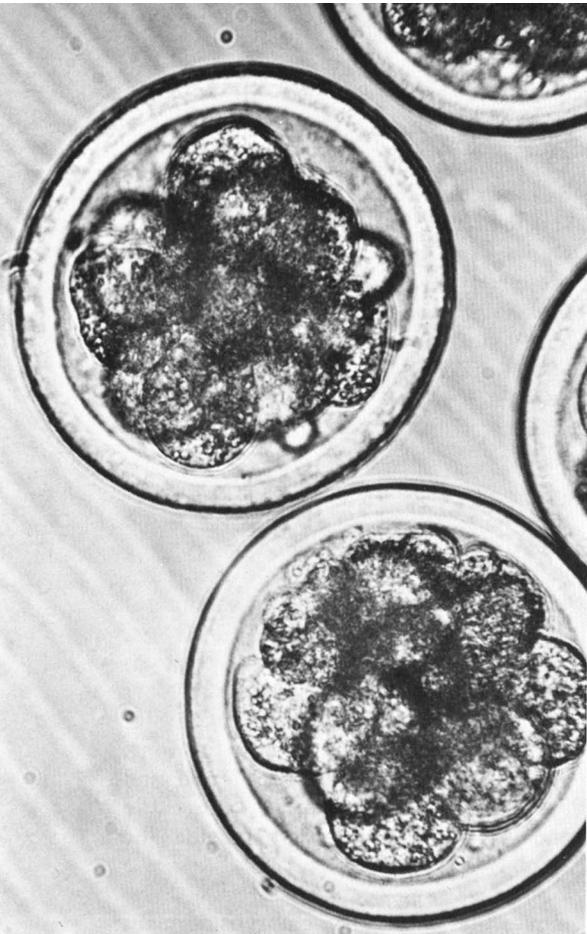
the producer. During the first year, 19 pregnancies were obtained among the 34 animals receiving embryos; one aborted, and 18 live calves were born. During the second year, 21 recipients produced seven calves. This year, embryos were collected both surgically and nonsurgically at Davis, flown to the ranch and transferred nonsurgically. Only one pregnancy (with one of the nonsurgically collected embryos) was obtained among the six recipients.

Improvements are being made, however. Transfer usually has to be completed within several hours to ensure a high degree of success, but this time has been gradually extended in the laboratory by perfecting the embryo culture system. The researchers have maintained embryos at body temperature for up to eight days but have not yet attempted to transfer embryos kept outside the cow this long. They have successfully transferred and produced healthy offspring from cattle and sheep embryos kept outside the animals for 24 hours.

Anderson is exploring freezing techniques that could keep embryos dormant



Maarten Drost (left) and Perry Cupps perform embryo transfer surgery.



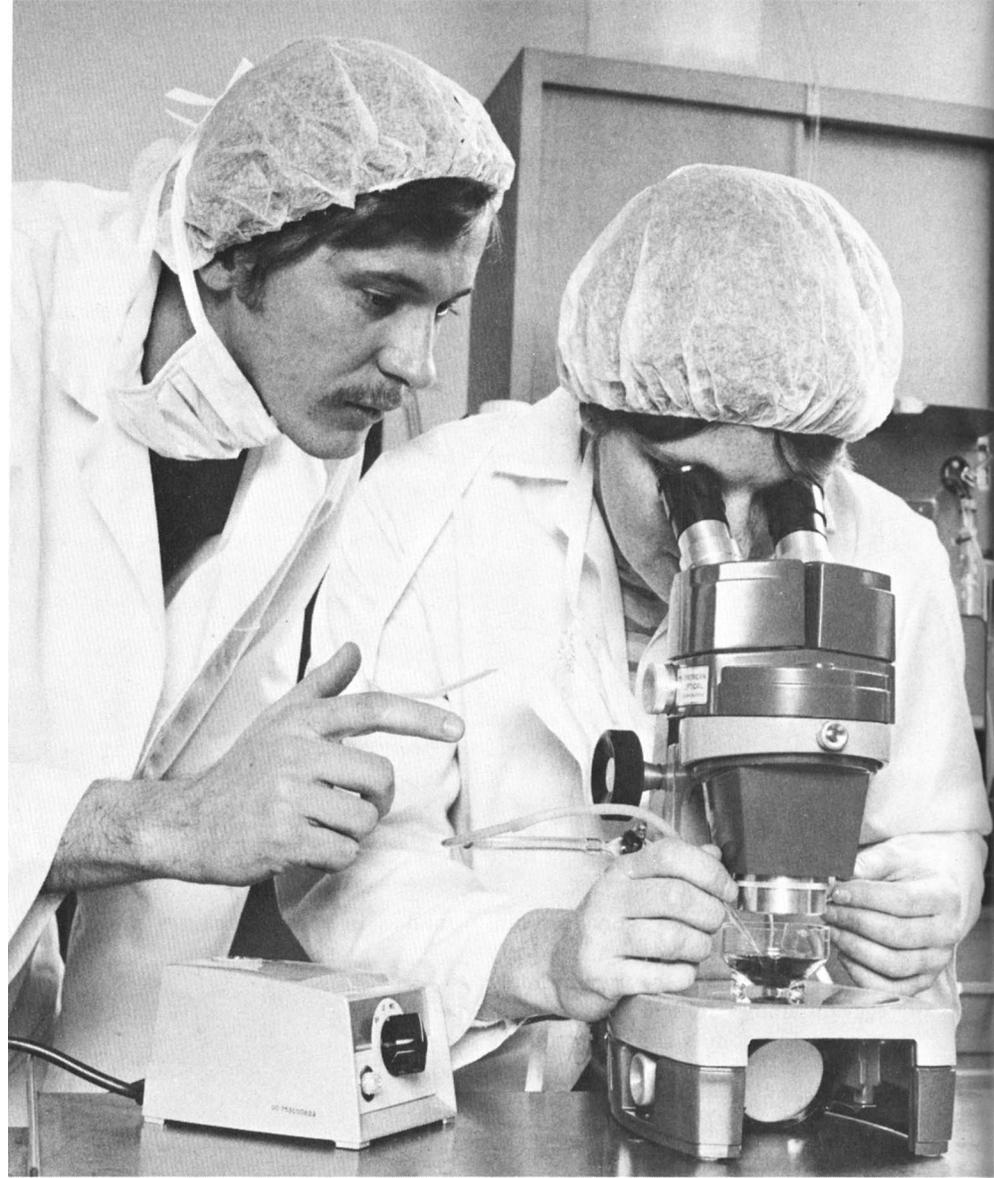
These 16-celled embryos, collected five days after estrus, are only one-tenth of a millimeter in diameter.

Using embryo transfers . . .

indefinitely. Duncan Peters, a graduate student working with him, has obtained live births from mice embryos frozen for as long as six days.

Development of nonsurgical transfer techniques has been slow. Considerable variation exists in the pregnancy rate after nonsurgical transfer, according to Drost, who leads this part of the research. Some possible causes of this variation are insufficient experience with the technique, and infection and damage to the uterus. More work with nonsurgical collection and transfer is planned.

If reliability of nonsurgical embryo transfer can be improved, it offers several advantages over surgical techniques. It can be performed on the farm, is quicker than surgery, does not require a recovery period for the cows, lacks the risks associated with surgery, and can be repeated



Gary Anderson and Susan Donahue, staff research associate, search for microscopic embryos flushed from the uterus of the donor animal. The embryos will be stored at body temperature in a special culture medium until transferred to a recipient animal.

more times on the same cows because, unlike surgical transfer, it does not carry the risk of scarring the uterus.

A third improvement being sought, and the one Cupps is most interested in, is getting donors and recipients into estrus at the same time. This is necessary for successful embryo transfer. The hormone prostaglandin has been very effective in helping synchronize estrus, but there still is more variation in estrus timing than is desirable. The goal is to have the donors and recipients come into estrus within 12 hours of each other. Approximately 50 percent of the animals being used in the research are falling within this range.

Cupps hopes that this percentage can be improved by manipulating timing, dosage, and methods of administering prostaglandin. Other factors, such as age,

breed, season, genetic makeup, lactational status, and physical condition of the animals, also may influence the success rate.

This research is the first large-scale attempt to use embryo transfer as a technique for increasing twinning in livestock, but the procedure is being used commercially to increase the number of offspring from genetically superior animals. However, cost of this process, which involves collecting embryos from outstanding animals and transferring them to lower quality "incubator" cows, limits its application to exceptional animals.

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