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Futuristic Application of New Reproductive Technologies

Charles R. Looney
Trans Ova Genetics, Sioux Center, Iowa

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Charles R. Looney, Ph.D.
Trans Ova Genetics
Sioux Center, Iowa

INTRODUCTION

Animal reproduction has enjoyed the most impact and progress of all the animal sciences in the development of new options for cattle producers. With the development of embryo transfer (ET) in the mid-1970's, animal reproduction has entered a new era of technical achievement. During this time a strong embryo transfer industry has enjoyed new advanced techniques of estrous cycle regulation, follicular growth dynamics and improved procedures in embryology. These and other advances in molecular biology will likely lead to changes in the traditional approaches to livestock breeding and further stimulate researcher's interest in areas of genetic engineering.

The objectives of this paper is to provide information to the cattle breeder of the history of reproduction and embryo transfer, an update of the embryo transfer industry and a review of some futuristic technologies that could soon impact the cattle breeder.

EMBRYO TRANSFER HISTORY

Experimentation in embryo transfer actually dated back to at least 1672 when Regnie de Groof first saw and recognized a blastocyst of a rabbit. Sheep ova were described in 1840 followed by the dog (1845) and deer (1854). Embryos were seen from pigs (1897), cats (1911) and man (1928). Cow embryos were not visualized until 1931.

The first embryo transfers were performed at the end of the last century by Walter Heape (1898). He transferred embryos between rabbits while studying uterine influence on an embryo phenotype.

In England in the late 1920's and early 1930's Marshall, Hammond and Asdel successfully transferred rabbit embryos. It was recognized at this time that pituitary gonadotropin was superior to PMSG for superovulation. The first successful embryo transfers reported in sheep and goats was in 1934, cows in 1949, pigs in 1951, horses in 1974 and humans in 1978.

Hammond (1927) was the first to synchronize heat in cows by manually expressing the corpus luteum by palpation. The first synchronization of a donor and recipient that produced a calf was accomplished at the University of Wisconsin in 1951. They used synthetic progestational compounds that were developed in 1950 and prostaglandins (tradenames Lutalyse, Estrumate) were approved in the early 1970's. Without prostaglandins embryo transfer would be impossible.

EMBRYO TRANSFER UPDATE

There is little doubt that the success the commercial embryo transfer industry enjoys is a direct result of the development of non-surgical recovery of embryos from donor cows and the non-surgical transfer of embryos to recipient cattle. The collection procedures are relatively simple and can be completed in less than thirty minutes. During this non-surgical technique less than one liter of fluid is flushed in then out of the uterus of a superovulated donor. Uterine washing fluids then are filtered through a patented Em-Con filter apparatus. Embryos are then evaluated for viability and individual embryos are placed in plastic straws for non-surgical transfer or freezing. The standard transfer technique is completed with a Cassou A.I. device. Recipients can be handled in standard squeeze chute. A total of twenty to twenty-five embryos can be transferred in one hour with the non-surgical techniques.

Today, the number of commercial embryo transfer companies have increased to serve farmers and ranchers throughout the United States. While the number of ET calves produced per year is only estimated between 100,000 and 150,000 the cost has steadily decreased along with the simplicity of the procedures. Throughout the United States the average number of total ova is twelve with five to six good embryos recovered. The fresh embryo pregnancy rate is 60 to 70 percent while the frozen-thawed embryos yield pregnancy rate of 50-60 percent. The embryo transfer industry is thought to be stabilized as far as numbers of recoveries and transfers done in a single years time.

TABLE 1 SURVEY OF ET WORK COMPLETED IN 1994

Donors Recovered	28,641
Embryos Recovered	157,736
Non-frozen Embryos Transferred	68,086
Embryos Frozen Commercially	88,969
Thawed Embryos Transferred	50,773
Embryos In Storage	87,596
Embryos Exported	8,981

98 of 267 ET Businesses completed survey by American Embryo Transfer Association.

One of the most exciting new developments in the ET industry has been the implementation of the direct transfer of frozen-thawed embryos. The procedure was first developed by Texas researchers and taken advantage of a highly permeable cryoprotectant named ethylene glycol. Prior to this new technique of thawing and direct transfer embryos were thawed and then a skilled embryologist using a microscope would rehydrate the embryos in a series of dilution then reloaded the embryo in another straw. This procedure takes twenty to thirty minutes and usually requires double handling of the recipients for pre-palpation. This new

technique makes it practical for technicians to thaw embryos and transfer into recipients. This user-friendly technique will decrease the cost of transfer and likely increase the number of frozen embryos that will be transferred in the future.

TABLE 2 PREGNANCY RATES OF "DIRECT TRANSFER" FROZEN EMBRYOS

PRACTITONER'S NO.	TRANSFERS	% PREGNANT
A	271	49.1
B	471	64.3
C	175	57.7
D	4	75.0
E	2167	52.1
F	131	57.3
G	1677	56.8
H	27	59.3
TOTAL	4,923	55.1

Source: Canadian Embryo Transfer Association 1995

Another development that has changed the embryo transfer business is the leasing arrangement of recipients between purebred seedstock breeders and commercial cattlemen.

Another development that has changed the embryo transfer business in the leasing arrangement of the commercial cattleman cow herd for recipients by the purebred seedstock producer. This arrangement has allowed commercial cattleman to obtain a premium for weaned purebred calves owned by the seedstock producer. The purebred breeder has the advantage in that he does not have to purchase recipients and enjoys utilizing the calving skills and the heavy weaning rates of the commercial cattleman. This program should gain popularity as the direct transfer method of utilizing frozen embryos become a standard program.

IN VITRO FERTILIZATION

The process of maturing, fertilizing and culturing the oocytes (eggs) and resulting embryos outside the body of the cow is called in vitro production (in vitro is Latin for in glass). The process has been commercialized since 1986 and has been utilized with ultrasound guided follicle aspiration to produce embryos and offspring from cows and heifers. To date, Trans Ova Genetics and four other labs are currently offering this service to cattlemen. The procedure has been very useful in lengthening the productive lives of donors with acquired infertility and from pregnant (up to 90-100 days) cows and heifers. In addition, studies have shown that prepuberal

heifers as young as six to eight weeks can become embryo producers using this technique.

The procedure of oocyte retrieval entails placing an ultrasound probe and needle guide into the vagina of the female and by rectal palpation the ovary is retracted back onto the probe end which holds the electronic crystals for visualization of the ovarian follicles. Follicles being fluid filled appear as dark spots on the ultrasound screen and are easily targeted by pushing a 60cm needle through the vaginal wall and into the follicle. An aspiration pump is used to vacuum out the contents of the follicle including the oocytes. The procedure requires an epidural injection similar to uterine flushing and takes approximately fifteen minutes to perform. Oocyte retrieval procedures are normally scheduled one time per week on problem cows but studies on healthy cows have shown benefits of twice per week oocyte retrieval sessions.

Typically six to eight oocytes are aspirated from each retrieval and of those 60 to 70 percent are of good quality for fertilization. In our hands, the embryo production of problem donors results in one embryo produced in vitro from each retrieval session and with a 50 percent pregnancy rate a producer should expect to obtain one to two pregnancies per month on an average. Because the technology is not fully developed to date, 40 percent of the retrieval results in no embryos produced. We have achieved several collections of 60 to 80 oocytes and it is not uncommon to produce 20 to 30 embryos from these collections. Because the embryos are grown in vitro culture the pregnancy rate of frozen-thawed IVF embryos are much lower than desired (30 to 40 percent).

MICROSURGERY OF EMBRYOS

Embryo microsurgery has created much interest both with researchers and commercial livestock producers because of the many options if offers in embryo bisection and biopsy also there are other special use procedures such as microinjection of spermatozoa.

In 1982, the embryo bisection procedure were developed by three independent labs, two in the USA (Colorado State University [CSU] and Louisiana State University [LSU]) and one in France. Each procedure requires specific microscopy and micromanipulators to conduct the extremely small delicate incisions needed in working with embryos. The procedures differed in the micro tools needed for the procedure. The LSU method required fine glass tools and probably caused less cell damage to the embryo but required more practice and patience than the CSU method which used a razor blade chip mounted on a glass pipette. A modification of this method is what is common today in commercial embryo transfer. Embryo bisection or splitting as it is commonly called requires good to excellent quality advanced morula or early blastocyst stage embryos and is very efficient in increasing pregnancy production when the embryos are transferred immediately after the procedure. Pregnancy rates usually range from 50 to 60 percent on each half embryo when transferred to a single recipient. This gives the producer a potential 120 percent pregnancy rate on a whole embryo basis. Many producers use this technique for donors who produce few good quality embryos from superovulated flushes.

Embryo biospy techniques also offer advantages to producers in knowing the sex of a calf prior to its implantation into a recipient. The procedure begins with a small sample of two to

three cells aspirated from each embryo and a three hour DNA analysis to determine the sex. Normally, embryos collected in the morning from superovulated donors can be "sexed" before noon the same day. The DNA analysis consist of a
DNA amplification process using a patented technique called PCR (poly-chain reaction). The presence (male) or absence (female) of a specific DNA sequence found on the bovine Y chromosome is determined with the procedure and is very accurate. Table 3 shows Trans Ova Genetics experience with these new techniques.

Table 3 PREGNANCY RATES WITH SPLIT AND BIOPSIED EMBRYOS[1]

	NO. TRANSFERRED	NO. PREGNANT	PERCENT
Split Embryos (1 Half Embryo)	57	35	61%
Sexed Embryos (Female)	35	19	54%

[1]Trans Ova Genetics November 1, 1994 - February 15, 1995

Microinjection of a single sperm cell into oocytes is another reproductive technology using microsurgery that has tremendous potential for livestock production. During normal in vitro fertilization, conditions of polyspermy (more than one sperm fertilizes) is common. Using a single sperm for microinjection this condition could be eliminated. Also, this technique could be utilized to conserve valuable and rare spermatozoa from bulls that are either dead or produce low sperm numbers. It is feasible to use this procedure on young bulls before they produce large numbers of viable sperm for A.I. The technique is become a standard procedure in human IVF laboratories and is expected to become a larger part of our cattle production scheme.

TRANSGENIC ANIMAL PRODUCTION

Of all these technologies the production of transgenic animals has the most to offer cattleman in the future but will require the longest time to develop. The process begins by microinjecting the one-cell embryos (sixteen to eighteen post-fertilization) with a specific fragment of DNA. This DNA is injected directly into the male or female pronucleus in very small volume. The DNA fragment has been genetically engineered to direct the DNA into specific tissues in the resulting calf. Therefore, if one wanted to produce a protein particularly specific for use a human drug it could be expressed in the mammary gland. This process today is very inefficient. Normally it takes 1000 fertilized and microinjected one-celled bovine embryos to produce one transgenic calf. This majority of the calves born do not have the transgene inserted into their genome. A large part of the embryos do not even produce a pregnancy. However, this technology is powerful when one puts a value on human pharmaceuticals such as tissue plasminogen activator (TPA) a heart-attack treatment drug that could be produced in gram quantities in the milk of cows. Because the huge opportunities for production of the valuable

proteins in this manner large amounts of capital can be raised with investors and funneled into this technology.

This is exciting for the cattle producer because of the potential opportunity to manage and produce the cattle for these companies. The potential to utilize this technology to alter the growth parameters of beef cattle and modify the milk constituents of dairy cattle is exciting. For example, it may be possible to alter by transgenic technology the marbling quality of a beef carcass and to produce milk that could be easily digested by human infants.

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