General Updating of Status of Embryo Transfer

The following article was a presentation at the 1983 Conference on Artificial Insemination and Embryo Transfer in Beef Cattle. The event was cosponsored by the National Association of Animal Breeders and the International Embryo Transfer Society.

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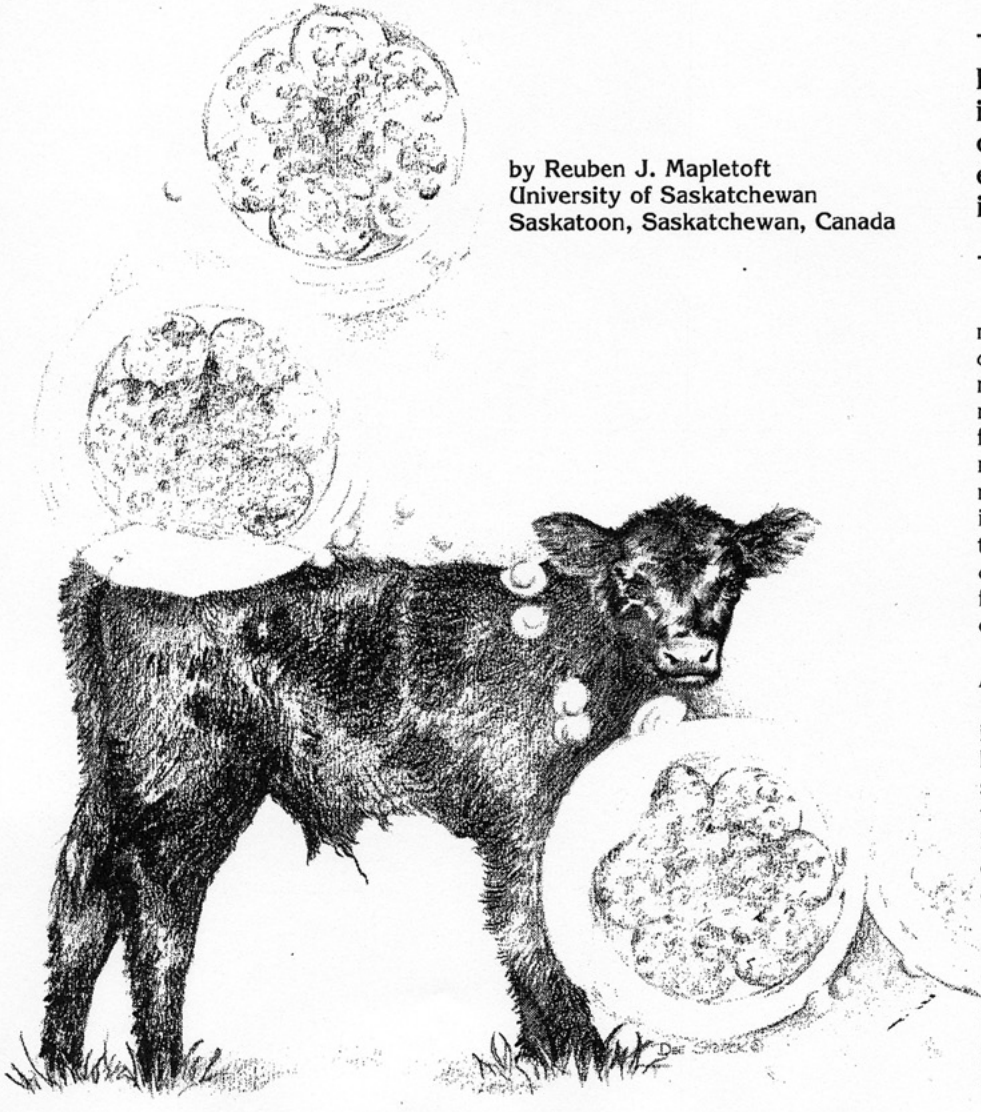

The technique of artificial insemination has made it possible to increase greatly the impact of a genetically superior bull on the cattle population. It is now possible to increase the influence of a genetically superior female on the cattle population through the use of embryo transfer. Briefly, embryo transfer involves the stimulation of a genetically valuable donor cow to produce more eggs than she would normally ovulate. This is called superovulation. At a selected time (usually 6 to 8 days) after breeding or A.I., the fertilized embryos are removed from the donor cow and are inserted singly into genetically inferior recipient, or nurse cows. The recipient cows must have their estrous cycles synchronized with that of the donor cow. The recipient then carries the embryo through pregnancy.

Although the first successful surgical transfer of a bovine embryo was reported in 1951, it was not until the early 1970s that the commercial utilization of bovine embryo transfer became a reality in North America. It was with the introduction of so-called "exotic" breeds of cattle from Europe that this research procedure became useful for the expansion of a limited gene pool. Interest and use increased to the extent that embryo transfer is now being used for many other reasons in all breeds, including the more traditional North American breeds. It is estimated that in 1981 more than 30,000 pregnancies resulted from bovine embryo transfer in North America. There are approximately 150 embryo transfer units and veterinary practitioners in the United States offering embryo transfer services at this time. Although embryo transfer centers were the traditional approach, the trend today is for "on-farm" services using the breeders help, facilities and recipients.

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Applications

Many of the benefits of embryo transfer in cattle breeding programs are obvious. It has been suggested that genetic progress in selection programs can be doubled through the use of embryo transfer with no increase in herd inbreeding. However, most geneticists agree that A.I. still constitutes a more effective method of obtaining genetic gain.

The most common use of embryo transfer in cattle breeding programs has been to increase the number of offspring from genetically superior females bred to superior A.I. bulls. In many cases, offspring are in turn subjected to superovulation and embryo transfer, resulting in a markedly reduced

generation interval and an increased selection intensity. This in itself should contribute to genetic gain, providing that genetic superiority can be identified in the young female. Certainly, embryo transfer is now being used to develop genetically superior herds from a few genetically superior parents. Furthermore, embryo transfer may realize its greatest domestic use for increased beef production through twinning. It has been suggested that an increase of 60% in unit beef production can be realized through twinning.

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Embryo transfer techniques are used extensively as a diagnostic and as a research tool. The benefits of embryo transfer have been realized in the areas of progeny testing, testing animals as carriers of genetic defects and in the study of genetic disease. The technique has also been widely used in the study of reproductive physiology, e.g. fertilization, implantation and the maternal recognition of pregnancy. Finally, embryo transfer has been successfully used in the diagnosis, treatment and salvage of repeat breeder cows. We have also used embryo transfer to salvage the genetics of terminally ill cows, producing a final one, two or three offspring.

One of the greatest potential uses of embryo transfer has not yet been fully realized, i.e. the importation and exportation of valuable breeding stock. Embryo transfer offers a number of advantages over traditional methods of transporting livestock internationally. Overseas transportation and quarantine costs add many thousands of dollars to the high cost of purchase. Obviously, the advantages of transporting embryos would be that initial costs could be reduced, the genetic material could be retained in the country of origin and transportation costs could be reduced to that of a single person's plane fare. Unfortunately, health requirements in most countries are a major obstacle. Although most research indicates that embryos are unlikely to transmit disease, the interaction between embryos and many specific pathogens still needs to be investigated. With this in mind, embryo transfer also offers great potential in the establishment of disease-free herds. Provided that it can be confirmed that the embryo does not carry disease organisms, embryo transfer could be used to retain genetics in the face of a disease outbreak, or to set up disease-free herds from infected populations. The value of embryo transfer in this regard should not be overlooked.

Selection of Donors

Although embryo transfer was initially used without paying much attention to the genetics involved, economics have finally dictated that it be directed toward the production of increased numbers of offspring from matings of genetically superior parents. Every breeder will have his own reason for using embryo transfer in a specific breeding situation. However, if it is not cost effective, it is unlikely to be done. Optimal results will no doubt reduce costs.

Therefore, it has been suggested that prospective donor cows in embryo transfer programs be selected on the following criteria:

1. Regular heat cycles commencing at a young age.
2. No more than two breeding services per conception.
3. First three calves born within two calendar years.
4. Superior individual performance for traits of economic importance.
5. Above average productive performance of offspring from previous matings of the same sire and dam.
6. No parturition difficulties or reproductive irregularities.
7. No conformational or detectable genetic defects.

These criteria have been suggested only as a guide. I want to emphasize that each breeder will have his own reasons for wanting to use a specific animal. Economics will eventually dictate the use of the most productive animals in embryo transfer programs. Generally it is felt that embryo transfer will be cost effective if the average value of offspring ranges from $2,000 to $3,000.

Techniques

Next, I would like briefly to review the various steps that must be followed in a successful embryo transfer program. There have not been any significant changes in the technology of embryo transfer in the last several years, although techniques have been refined and rates of success have increased. Success depends on constant attention to detail. Failure to do so in any one step of the operation can result in overall failure and disappointment. The decision to embark on an embryo transfer program involves a major commitment and, if you are unable to make that commitment, I would suggest that you do not start an embryo transfer program.

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Superovulation. In order to amplify the reproduction of high quality females, the donor must ovulate a greater number of eggs than normal. The ovary of a calf harbours between 75,000 and 300,000 ova at birth. A cow usually ovulates one egg in each estrous cycle. Therefore, there are a large number of ova, which go to waste. By superovulation more of these ova could be used in the production of live offspring.

Hormones that stimulate ovaries are used for superovulation. The hormones used are usually obtained from the serum of pregnant mares (pregnant mare's serum gonadotropin—PMSG) or from the pituitary of slaughtered animals (follicle stimulating hormone—FSH). Variability of response to hormone treatment continues to be a problem. Although imperfect, these preparations meet the needs for superovulation in most embryo transfer procedures.

Ovaries have been found to respond best to superovulation treatments beginning during the latter half of the cycle, i.e. days 8-14. Normally, twice daily injections of FSH are administered for 5 days. On the second or third day, donors are injected with prostaglandin (PG) F_2 alpha to bring them in heat 36 to 48 hours later. Donors are usually inseminated 12, 24 and 36 hours after the onset of heat. Natural service has also been successful. Regardless of the method used, it is absolutely critical that the semen be of a very high quality.

At this time, virtually all bovine embryos are collected non-surgically, except for some specialized applications.

Estrus Synchronization. The recipient animal must be in a suitable reproductive stage to receive and nourish the fertilized embryo. In other words, the donor and the recipient must be synchronized to ovulate within 24 hours of one another. Estrus synchronization may be achieved through the use of PGF_2 alpha or by the selection of animals with natural heats at the proper time, which requires the maintenance of a large recipient herd.

Recipients are usually injected with PGF_2 alpha 12 to 24 hours before the donor cow receives her PGF_2 alpha. The use of PGF_2 alpha to synchronize heats has greatly reduced the number of open animals that it is necessary to have on hand as potential recipients, making it possible for a breeder to supply his own recipients.

Collection of Embryos. At this time, virtually all bovine embryos are collected nonsurgically, except for some specialized applications. Basically, all nonsurgical collection techniques are variations of a single technique, which involves the passage of a rubber catheter through the cervix 6-8 days after breeding and washing out the embryos. The catheter has an inlet for allowing the flushing fluid to run into the uterus,

an outlet to collect the fluid, and an inflatable cuff to prevent both the catheter's slipping out of the cervix and loss of embryos into the cervix and vagina. The large amounts of fluid flushed through the uterus are examined under a microscope to locate the fertilized eggs.

Nonsurgical embryo collection is safe in the hands of experienced operators and may be repeated often with no damage to the reproductive tract. It is usually recommended that donors be superovulated not more than every 2-3 months. Between superovulations, single embryos may be collected after each normal breeding. In this way, the average number of offspring may be 20-25 per year. There are numerous cows that have been collected 3-4 times per year without any detectable loss in responsiveness and without any particular deleterous effects to the cow herself.

Embryo Storage. Embryos are flushed from the uterine lumen with a fluid, which closely resembles the natural fluid within the reproductive tract. As it is most practical to store embryos (for which requirements are more critical) in the same type of fluid, the quality and composition of this fluid must be very carefully controlled. Generally, embryos are maintained in the incubator only until transfer. However, it is occasionally necessary to culture embryos overnight, which is done with no particular problem. The long-term storage of fertilized embryos is possible using a computerized biological freezer and liquid nitrogen. Embryo freezing will be discussed more extensively later in the program.

Embryo Transfer. The term "inovulation" has been used to describe both surgical and nonsurgical methods of embryo transfer. To date, surgical transfers have resulted in slightly higher pregnancy rates than nonsurgical methods. However, nonsurgical techniques must be used in order to reduce the cost sufficiently to make embryo transfer more available to breeders.

Surgical transfer is generally performed on standing recipients through a flank incision. Nonsurgical embryo transfer is accomplished by inserting the embryo through the cervix using a Cassou A.I. straw gun. Certainly, the nonsurgical technique is more adaptable to "on-farm" work. However, both surgical and nonsurgical transfer is successfully used "on-farm."

Production of Live Offspring

The ultimate success of an embryo transfer program is judged by the production of live, healthy offspring. Obviously, husbandry of recipient animals must be excellent and calving must be supervised as with normal pregnancies. One of the most important considerations in an embryo transfer program is the use of recipients that are capable of calving normally and of producing adequate quantities of milk to enable calves to grow to their genetic potential.

As far as results are concerned, I can only refer to Dr. Elsden's paper last year at this meeting. In it, he stated that six to seven em-

bryos will be transferred from each donor animal and on the average, four pregnancies will result. He emphasized that the range may be from zero to 20 or more. We have averaged up to 20 transferable ova in certain herds and less than one in others.

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Studies of the results of embryo transfer do not suggest any greater incidence of congenital defects, nor does there appear to be any greater incidence of abortion or still births than in the normal population. The ability of the recipient to nourish the embryo can influence its growth and development, but its genetics are determined at the time of fertilization. In this regard, it is important to identify correctly the parentage of a calf resulting from an embryo transfer. Breeders must obtain the specific regulations for embryo transfer from each breed organization before initiating an embryo transfer program.

Future Research

Later in the program, you will hear about splitting embryos and freezing embryos. These are two experimental procedures, which have become commercially applied very recently. Another procedure that shows a great deal of promise is "in vitro" fertilization, or the fertilization of the egg in a test tube. Although not perfected, this is possible, having resulted in live offspring in at least one study. If you will remember the numbers of potential ova that a calf harbours at birth, you can imagine the great potential of this procedure. Once these ova are collected from an ovary (providing that they can be frozen successfully), the liquid nitrogen tank will become the donor with vast potential. Finally, a procedure that has received considerable study over the past few years is embryo sexing. Although this is still a laboratory procedure, it is successful and will be commercially available in the near future. Collectively, these procedures make the possibilities with embryo transfer almost limitless.

Breeding Programs with Embryo Transfer

Many researchers suggest that the greatest potential benefit of embryo transfer to long-term livestock improvement may be in the beef industry. This is because replacement animals can be selected for growth on their own performance before they reach reproductive maturity. Therefore, embryo transfer could double the response for growth rate by increasing selection among both heifers and bulls. In addition, fewer young females would have to be retained in the herd as replacements, due to the increased reproductive rate.

An elite breeder could select the top 15-20 percent of his breeding herd for transfer. As soon as cows resume cyclic activity after calving, they could be superovulated and the embryos collected nonsurgically and transferred. Cows in the bottom 25 percent of the herd could be synchronized with PGF_2 alpha and used as recipients. All procedures could be conducted on the breeders' premises. This program can be worked into a normal breeding program very easily. The normal breeding program can begin the day following the embryo transfer procedure so that breeding is not delayed.

There are other breeders that select the top two or three cows in their herd for transplant every spring. Prior to the breeding season, the cows are superovulated and recipients from within the herd are synchronized with PGF_2 alpha. On the day following embryo transfer, recipients are turned with a bull or the A.I. program is initiated. Recipients that were synchronized but not used can be given a second injection of PGF_2 alpha and bred 72-96 hours later.

Another approach is to increase the number of offspring from older, proven cows by superovulation and embryo transfer, interposed with single collections throughout the year. Certain offspring that show superior conformation, growth and beef character could also be superovulated, mated to similarly superior males and transferred. These procedures could be conducted "on-farm,"

at an embryo transfer center or a combination of both. It is difficult for a breeder to maintain recipients for a year-round embryo transfer program. However, the embryo transfer center is often able to supply recipients under these circumstances.

Finally, breeders who maintain a very narrow breeding season have also benefited from year-round collections. Embryos collected out of season are deep-frozen to be transferred during the desired breeding season. A stockpile of frozen embryos is also advantageous to transfer to extra recipients on any particular day. One is rarely able to synchronize the exact number of recipients required. Similarly, freezing capability provides a very good alternative when more embryos are collected than there are recipients available.

Embryo Transfer—1982

Beef breeders have the choice of participating in an embryo transfer program conducted either at a transfer center, or "on-farm." Both alternatives have advantages and disadvantages. Sometimes it is possible to minimize disadvantages by utilizing both alternatives at the same time, i.e. an owner may provide his own recipients, while donor cows are superovulated and collected at an embryo transfer center. Alternatively, donor cows may be superovulated and collected "on-farm" while embryos are transferred to recipients at an embryo transfer center. Most centers maintain a large recipient herd, which can be very convenient, especially for year-round embryo transfer.

The advantage of the embryo transfer center is that results will generally be better, providing embryo transfer center personnel conduct all procedures including heat detection and A.I. The major disadvantage at an embryo transfer center is that costs are generally higher and that the center is often a great distance from the breeder's herd.

There is a very definite trend today toward conducting all procedures "on-farm." This approach tends to be less expensive, and because the breeder does not have to pay for his own time and recipients, the initial capital outlay is very much less. However, results do tend to be somewhat lower "on-farm." In addition, a minimum quality of facility is necessary for "on-farm" work. It is imperative that management be optimal and that a great deal of attention be paid to detail. The responsibility of this lies with the producer, with supervision from the embryo transfer personnel. At the very least, a breeder should have had a successful A.I. program before considering an embryo transfer program. Furthermore, I recommend that the practicing veterinarian be included in the development of the program and that he be in close contact with the people conducting the embryo transfer service. Remember, he is left with the success or failure after the embryo transfer procedures have been completed.

Embryo transfer procedures consist of a series of critical steps, each of which must be accomplished successfully for the overall program to be successful.

The greatest advantage to "on-farm" embryo transfer is that the breeder can use his own recipients. It is seldom that the recipients you receive from an embryo transfer center are the same quality as those you may have at home. Furthermore, many breeders are concerned with diseases and accidents when their donor cows are transported great distances and with the periodic introduction of recipients into their herd. Finally, there are intangible benefits associated with conducting the program on your own premises. You are part of your own breeding program and can take a great deal of pride in the final results. As Dr. Seidel says, this is a most important human endeavour. Without it, we would all probably be raising grain crops and counting our money.

Summary

In summary, embryo transfer procedures consist of a series of critical steps, each of which must be accomplished successfully for the overall program to be successful. Breeders may use the services of an embryo transfer center or have these services conducted on his own premises. For "on-farm" embryo transfer, management must be optimal and very close attention must be paid to the various steps in the procedure. Greater potential exists for failure with "on-farm" embryo transfer. However, the use of one's own recipients and the sense of participation in one's own program makes this approach more appealing in many cases. Certainly it is possible to be very successful with an "on-farm" embryo transfer program. AJ

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