Applications of Embryo Transfer and Related Technologies to Cattle

G. E. SEIDEL, JR.
Animal Reproduction Laboratory
Colorado State University
Fort Collins, Colorado 80523

ABSTRACT

It is possible to recover embryos from superovulated cows nonsurgically, divide each embryo in half, and routinely obtain pregnancy rates of greater than 50% per half embryo after nonsurgical transfer, which is equivalent to greater than 100% per original embryo. It is also possible to freeze and sex embryos, although the cryopreservation process kills some embryos and the sexing process is so new that efficacy under field conditions is unknown. Embryo transfer techniques are applied to thousands of dairy cows, but in 1982 only about one dairy calf per thousand born in North America was from embryo transfer. Nevertheless, use of this technology is increasing, in part because of simplification of procedures, increased efficacy, and lower costs. It is difficult to predict when additional technologies will become available for commercial use, but it is likely that several additional exciting developments will occur in cattle breeding before the end of the century.

SCOPE OF THE EMBRYO TRANSFER INDUSTRY

A systematic survey of the volume of commercial embryo transfer in North America showed that about 10,000 pregnancies were produced in 1978 and 18,000 in 1979 (26). A recent survey by the International Embryo Transfer Society indicated that over 115,000 unfrozen and 25,000 frozen bovine embryos were transferred in the United States and Canada in 1983; this should result in 65,000 to 75,000 calves (born mostly in 1984). About one-third of these embryos were from Holstein donors, and nearly all of the rest were from beef cattle.

Received November 3, 1983.
TABLE 1. Number of Holstein calves registered in the United States resulting from embryo transfer.

<table>
<thead>
<tr>
<th>Year of birth a</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1974</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1975</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>1976</td>
<td>43</td>
<td>93</td>
</tr>
<tr>
<td>1977</td>
<td>119</td>
<td>161</td>
</tr>
<tr>
<td>1978</td>
<td>315</td>
<td>319</td>
</tr>
<tr>
<td>1979</td>
<td>730</td>
<td>774</td>
</tr>
<tr>
<td>1980</td>
<td>1632</td>
<td>1952</td>
</tr>
<tr>
<td>1981</td>
<td>2441</td>
<td>3270</td>
</tr>
<tr>
<td>1982</td>
<td>2854</td>
<td>4403</td>
</tr>
</tbody>
</table>

aData are incomplete, especially for 1982, as many breeders do not register calves for months or even years after birth.

sons for progeny testing. This increases the selection intensity, particularly because many cows contracted for this purpose never produce a son that can be sampled because of failure to conceive, death of the calf, birth of a female calf, or other factors. Having a litter of calves increases the probability that an appropriate male is produced. A survey was conducted early in 1984 that included responses from all major dairy bull studs (N=14) in the United States and Canada; 43% of the young Holstein bulls sampled in 1983 (455/1047) were from embryo transfer.

Most dairy farmers who use embryo transfer simply want more heifer calves from their best cows. In most cases the bull calves are more a nuisance to merchandise than an asset. The effect of this use of embryo transfer is to increase the selection intensity of dams to produce female herd replacements. Current costs of embryo transfer vastly exceed any genetic benefit from this selection path. However, many offspring produced in this way can be sold at a profit because factors other than genetic value for milk production influence the market value of top females. For example, 47 of the 128 Holsteins selling for more than $30,000 in 1982 were embryo transfer calves (15).

Moreover, replacement females can be produced by embryo transfer for less than the cost of purchasing registered cattle of similar quality. This is especially so when dairy producers are changing from grade to registered herds. Thus, the major reason for the profitability of embryo transfer is the demand for registered cattle of good quality. Registered Holsteins sold at auction in 1982 averaged $2800 each, whereas grade Holsteins averaged about 40% of this (15). This is partly due to a steady increase of the percentage of registered dairy cattle in the national herd. Should this trend reverse, it is doubtful that registered cattle would be worth even 1.5 x their grade counterparts instead of 2.5 x as is true today.

Selling embryos, cryopreserved or fresh, is merely a variation on these applications. It is an excellent way to export genetic material, as embryos are less likely to spread disease than semen or live animals (6, 33). Also, calves born to indigenous recipients obtain the appropriate passive immunity for that environment from the colostrum and presumably survive better than animals introduced into foreign environments at an older age. There are a number of other applications of embryo transfer technology, such as testing for recessive alleles and correcting some kinds of infertility (26), but only a small fraction of embryos are transferred commercially for such purposes.

STATE OF THE ART OF EMBRYO TRANSFER PROCEDURES

Superovulation

The single greatest problem in the embryo transfer industry is unreliability of superovulatory response. Virtually all large studies show that the average production per attempt to superovulate normal donor cows is in the range of 5 to 7 transferable embryos (9, 14). However, many donors yield no transferable embryos or only a few, whereas in rare cases 40 or more normal embryos are produced. This wide variability creates tremendous logistical problems that greatly increase costs. The mean response is much lower for donors with histories of infertility (5, 11). Moreover, a donor’s response sometimes decreases with repeated superovulation. As more fundamental knowledge of the process of follicular growth is gained, it may be possible to improve superovulatory response. However, prospects for great improvement in the next year or two seem remote.

Journal of Dairy Science Vol. 67, No. 11, 1984
Insemination

Standard practice is to inseminate donors two or three times at 12-h intervals, frequently with two standard doses of semen on each occasion (25). Such schemes are based more on copying a pragmatically successful procedure than on appropriate experimentation. It is likely that a standard procedure will evolve of inseminating one dose of semen half a day after estrus first was observed followed by a second dose half a day later. With expensive semen, one dose or even one-half dose or less sometimes is used. Well-designed experiments are critically needed to optimize insemination procedures.

Embryo Recovery

Except in rare cases of infertility, all commercial embryo recovery is by nonsurgical methods that, if done by properly trained personnel, damage the donor cow only in extremely rare instances. But there are nearly as many variations of the procedure as there are technicians who recover embryos. The basic procedure is to irrigate the uterus with a buffered NaCl solution in such a way that most of the fluid placed into the uterus is recovered. The procedure is not completely efficacious, as some donors remain pregnant, sometimes with multiple embryos. To prevent this, donors routinely are given a luteolytic dose of prostaglandin F2α after nonsurgical recovery. An occasional donor remains pregnant, even after this treatment.

Efficiency of embryo recovery is probably in the 70 to 90% range with most techniques. It improves as embryos migrate from oviduct to the tip of the uterine horn. Although some embryos can be recovered 4 days after the beginning of estrus, most workers wait at least until day 6, and there are hints that day 7 may be slightly better (14). There is no particular problem in recovering bovine embryos through day 14, and it sometimes can be successful until day 18 (22). However, embryo recovery attempts are rare after day 9, because embryos are more difficult to locate after hatching from the zona pellucida. Also, the superovulated uterus may be a suboptimal environment.

Because embryo recovery procedures are already about 80% efficient, do not damage the uterus, and usually take less than 30 min, dramatic improvements are unlikely. Nevertheless, simpler, faster, and more efficient procedures probably will evolve. Experimentation in this area is of relatively low priority.

Short-Term Storage of Embryos

Bovine embryos recovered 6 to 8 days after estrus normally have 30 to 200 cells. They are termed morulae if there is no cavity and blastocysts if a cavity is present. Such embryos are amazingly resilient when presented with physical and chemical insults over short periods. They can be kept for hours without measurable deterioration under the following conditions, temperature 0 to 38°C, osmolality 260 to 310 mOsM, pH 7 to 7.4, and freedom from noxious agents such as microorganisms, intense light, heavy metals, etc. The presence of serum albumin is beneficial as are energy sources and cations K⁺, Mg++, and Ca++. In standard culture media where these components are controlled precisely, embryos can be kept for 20 to 30 h with only slight deterioration of pregnancy rates. Storage for longer periods leads to progressive deterioration so that pregnancy rates approach zero after 2 to 3 days of storage unless embryos are cryopreserved.

Another approach to short-term storage is to cool embryos to about 5°C. Although this procedure seems to work well for rabbit embryos, results have been mixed for bovine embryos (4, 16). This approach works fairly well for storage up to 2 days, and with additional research it is likely that storage for 3 or 4 days would be feasible. At these temperatures embryos cease developing until warmed. When there are more embryos than synchronous recipients, this would provide the option of waiting a few days until more synchronous recipients are available.

Cryopreservation of Embryos

Embryos, like sperm and many other kinds of cells, can be preserved at the temperature of liquid nitrogen, certainly for decades and probably for centuries (38). Processes of adding and removing cryoprotectant and freezing and thawing frequently damage cells. However, even if half of the cells of an embryo are killed, the remaining cells often are sufficient to develop into a normal calf. With current technology, 20 to 40% of frozen-thawed embryos that were of high quality before freezing are damaged so
severely that they are not viable. Thus if all cryopreserved embryos are transferred, pregnancy rates are 60 to 80% of those with unfrozen embryos. If one culls embryos after thawing, pregnancy rates of the selected embryos approach those of unfrozen embryos (31). However, even with very experienced evaluators, some viable embryos will be culled.

About 10 to 15% of embryos recovered fall into a marginal category (10). Pregnancy rates for these embryos when transferred directly after recovery are about half of those for morphologically normal embryos. Cryopreservation procedures kill most marginal embryos.

Multiple insults to embryos can have devastating effects. For example, pregnancy rates remain high after transfer of embryos cultured for 24 h; however, pregnancy rates after cryopreservation of such embryos frequently are IOW.

Current methodology of cryopreservation of embryos is cumbersome and requires complex equipment. However, procedures continually are being simplified and may become similar to those for freezing and thawing semen (20). Success rates should continue to improve as well. Even though about one-third of potential pregnancies are lost from cryopreservation, the process is of enormous value in several circumstances. For example, the number of embryos recovered frequently exceeds the number of recipients available. Cryopreservation is a marked improvement over discarding embryos and also opens new domestic and export markets.

Embryo Transfer

Pregnancy rates are best if donors and recipients are in estrus within a day of each other. With embryos of good quality, asynchrony of 2 days results in a drop in pregnancy rates of only 10 to 20 percentage points, but asynchrony of 3 or more days is generally disastrous (25). Recipients can be in natural estrous cycle synchrony with donors, or estrous cycles can be synchronized with appropriate progestins or prostaglandins without lowering pregnancy rates.

Most embryos are transferred nonsurgically with equipment similar to that used for artificial insemination. A number of practitioners still use surgical methods of embryo transfer, usually by exposing the uterus through the flank and using a local anesthetic. The disadvantages of surgical procedures are that they must be done under veterinary supervision in most situations, with more time and slightly more sophisticated facilities required than for nonsurgical procedures.

The main disadvantage to nonsurgical procedures is that pregnancy rates are lower for most people (sometimes markedly lower) than with surgical methods. However, nonsurgical pregnancy rates for some technicians are similar to surgical ones (25). The reasons for the huge differences among technicians are not known, although experience is important. More research is required to determine how to train technicians to become skilled at nonsurgical embryo transfer. Personnel at embryo transfer firms that still use surgical methods predominantly feel that the slight extra investment in time is justified by the higher pregnancy rates under their circumstances. However, it is likely that surgical methods for commercial embryo transfer will disappear within several years. Pregnancy rates with good embryos transferred to properly synchronized recipients can exceed 70% under routine conditions and under some circumstances approach 80%. However, pregnancy rates are sometimes below 10% when cryopreserved embryos are transferred nonsurgically by inexperienced personnel.

Splitting Embryos

One of the most exciting recent developments has been practical microsurgical procedures to divide the bovine embryo in two (23, 40, 41). In our hands, pregnancy rates exceed 50% per half embryo transferred nonsurgically, and there are unpublished reports by others exceeding 60%. The net effect is pregnancy rates exceeding 100% per original embryo. However, when one demi-embryo is transferred per recipient, more recipients are required per calf born than with whole embryos.

There are a number of advantages to demi-embryos. The major commercial advantage is that more calves result per embryo. This is especially valuable when only one or a few embryos are recovered from a valuable donor. Such embryos can be twinned without obtaining freemartins, although this is not recommended.
generally. When both demi-embryos develop, even if they are in different recipients, identical twins result. These are extremely valuable for many kinds of research, because fewer animals are required to obtain statistically significant results.

As with cryopreservation, marginal embryos are not good candidates for bisection. One problem with the procedure is that embryos must be 6.5 to 8.0 days old for good results, which is a narrow time. Also, the procedure requires considerable training as well as expensive equipment. Several workers have combined splitting and freezing (19), but this double insult results in fairly low pregnancy rates. With more research, it may be possible to combine these techniques successfully. The success with demi-embryos clearly demonstrates the remarkable resilience of bovine embryos.

**Sexing Embryos**

There are two basic methods of sexing embryos. The first requires biopsy and killing a number of embryonic cells to examine the chromosomes. This procedure is called karyotyping. It is a tedious, time-consuming process that works 60 to 70% of the time under ideal circumstances (3). Although it is extremely accurate, a sex ratio of one-third male, one-third female, and one-third unknown is less than ideal. It is unlikely that this method will be used on a commercial scale, although it already has been used experimentally to sex hundreds of bovine embryos.

The second method of sexing involves making an antibody to molecules found only in male tissue. These antibodies bind to male but not female embryos or their secretory products. Next, a second antibody with attached fluorescent molecule or enzyme binds to the first antibody. The net result is that male embryos can be distinguished from female ones with a fluorescence microscope or by an enzymatic product. This procedure is about 80% accurate with mouse embryos and probably can be improved (37). A preliminary report suggests that similar accuracy can be achieved for bovine embryos (36). One firm already provides this service, but success rates on a commercial scale will not be known for some time. There are two practical considerations, the efficacy of the technique and whether it leads to lower pregnancy rates. Although much research remains to be done, it is likely that this technology will become widely available within a year or two. Initially costs will be high as companies seek to recoup their investments. However, the cost probably will decline to less than $20 per embryo, which will be about $100 per female returned to the milking herd if accuracy is 90%, male embryos are discarded, the pregnancy rate with female embryos is 60%, and there is normal attrition in terms of neonatal death, infertility, etc. This procedure, however, would revolutionize the embryo transfer industry, especially as applied to dairy cattle. Because male embryos generally will be discarded, splitting techniques will be especially appropriate for doubling the remaining female embryos.

**Future Techniques**

Dozens of additional technologies eventually will become available. One of the first is in vitro fertilization. One calf already has been born, and additional pregnancies have been established (7). Initially, in vitro fertilization will have the limited application of circumventing certain forms of infertility. It probably also will be used for evaluation of fertility of semen. The big disadvantage of bovine in vitro fertilization is the need to obtain oocytes surgically, by laparoscopy, or at slaughter. In the long run, in vitro fertilization will be used in conjunction with evolving biotechnology much as embryo transfer is used to exploit sexing, splitting, and freezing embryos.

A technology that is some years away is androgenesis. It involves fertilization of an oocyte with two sperm and removing the female genetic material microsurgically (27). Such an animal would have two genetic fathers and no genetic mother (except for certain cytoplasmically inherited characteristics, e.g., mitochondria). The sex ratio would be 1 female: 2 males: 1 lethal YY. One could cross two males or a male with himself. The technique would be especially useful experimentally, although problems such as molar pregnancies remain to be studied (1).

Another technology frequently mentioned is cloning. Bisecting embryos is a form of cloning. Several methods are already available for
making up to five identical multiplets (29, 39), but they are not practical for routine commercial use. Methods probably will become available within a decade to make hundreds of copies of embryos (30).

Recombinant DNA technology also will impinge on cattle breeding eventually. Unfortunately, little is known about the bovine genome or the molecular biology of how genetic information regulates growth or milk production. Much additional basic research is required before such techniques can be exploited except for a few special cases.

DELIVERY OF EMBRYO TRANSFER SERVICES

Embryo Transfer Centers

Embryo transfer can be done on the farm, but most procedures can be done more efficaciously at embryo transfer centers. Although success rates are frequently higher at embryo transfer centers, so are costs. Prior to 1976, nearly all embryos were recovered surgically, which necessitated that donors be at embryo transfer centers. After nonsurgical methods of embryo transfer came into widespread use in the early 1980's, the need for embryo transfer centers declined (32). However, most large embryo transfer companies still operate primarily through central laboratories established in the 1970's. Another factor influencing continued survival of the embryo transfer center is that it facilitates new technologies such as cryopreservation, splitting, and soon sexing, because of the nature of the equipment required.

The major characteristic of most embryo transfer centers is resident herds of donors and especially recipients. Under most conditions, it is possible to obtain more embryos from donors when they are under close supervision of personnel at embryo transfer centers than with on-the-farm procedures. This is especially true for infertility cases. One big drawback, however, is that most centers are not equipped to handle lactating dairy cows properly.

The most expensive aspect of most embryo transfer centers is maintenance of the recipient herd. The major expense is for feed for keeping nonpregnant cattle waiting for embryos. Maintaining appropriate herd health programs is also time-consuming. Holstein heifers frequently are used as recipients. Although heifers are intrinsically fertile, they have more calving problems than cows.

Fees at embryo transfer centers are generally a $500 entry fee per donor, $2 per day board for the donor, and around $1,700 per recipient pregnant at 90 days. As the value of fresh Holstein heifers is about $1,000, the net fees per dairy calf would be under $1,000.

On-Farm Embryo Transfer

All of the procedures in common use can be accomplished on the farm, frequently with equipment in a vehicle taken to the farm. Nonsurgical methods of transfer have made on-the-farm embryo transfer practical. The big difference in on-the-farm embryo transfer is that donors and recipients are under supervision of the farmer. In well-managed herds this works well, but in some herds it is disastrous. The big advantage of on-the-farm procedures is lowered costs. However, there are many hidden costs that make it less attractive than it seems on the surface (28). The “up front” costs are clearly lower. On-the-farm transfer is especially attractive in large herds and when nonpregnant but normally cycling cattle are available. Another attractive point is that herd health is not compromised if recipients are not introduced from other sources. Frequently, farmers make the mistake of buying outside cattle for use as recipients, which gives them all of the problems and expenses that embryo transfer recipient herds have, and frequently will lead to introduction of diseases as well.

Fees for on-the-farm work vary widely. Typically, there is a $300 fee to collect the embryos and a fee of $200 to $400 per pregnant recipient. Travel and lodging for technicians is an additional expense. All expenses of feeding recipients and synchronizing estrus, of course, are borne by the farmer. The economics of on-the-farm transfer are discussed in detail in (28).

Success Rates

Making cows pregnant by embryo transfer is accomplished by successful execution of a series of processes, each of which must be done well if the recipient is to remain pregnant. Under ideal circumstances an average of 3 to 4
pregnancies results per superovulatory attempt. Superovulation can be repeated at 7- to 8-wk intervals, but response declines after three or four superovulations of some cows (9, 14). With normal dairy cows, more than one superovulatory attempt per year would increase the calving interval well beyond the optimum; even one superovulation delays breeding back in most cases. Occasionally, normal dairy cows are superovulated twice in succession, but as a rule, those that are superovulated continually are infertility cases.

The variability of number of normal embryos obtained is truly striking. About 2% of donors produce more than 20 embryos. Obtaining more than 30 embryos is unusual, but on rare occasions more than 40 normal embryos have been produced. The mean number of pregnancies produced in a recent study was 3.4, the mode 0, and the median 3 (26).

In most cases when large numbers of embryos are produced, insufficient recipients are available. Thus, litters of more than 20 calves are exceedingly rare. This is even more true lately, as recipient herds have been reduced in size because of availability of cryopreservation to buffer demands for synchronous recipients. Cryopreservation leads to additional variability. In some cases, nearly all embryos from a donor survive the cryopreservation procedures intact, whereas all of those from other donors may be destroyed. Reasons for this are unknown but seem not entirely chance. Occasionally, disaster strikes; even without cryopreservation one sometimes obtains 0, 1, or 2 pregnancies from transferring 10 apparently normal embryos. The problem is exacerbated by splitting. In such a case one might transfer 20 demi-embryos and get little or nothing in return.

There is risk in purchasing a single cow with the expectation of an average response. The risk is reduced greatly if several donors are part of a program or if a certain number of embryo transfer pregnancies is guaranteed as part of the purchase agreement of the donor.

FRINGE COSTS AND BENEFITS

Pregnancy Delay in Donors and Nonpregnant Recipients

As pointed out earlier, two or more superovulations in succession will result in a longer calving interval than normal. In most cases this means fewer lactations per lifetime. For the kinds of elite cows being superovulated, the loss of milk to sell is trivial relative to the opportunity to add another lactation to the credentials of the cow. Long calving intervals also detract markedly from credentials. When embryo transfer techniques come into routine use for increasing milk production, it rarely will be profitable to superovulate donors more than once per lactation.

Currently costs of delaying pregnancy in recipients are even greater than for donors. Not all recipients that are synchronized come into estrus, frequently all recipients in estrus are not needed due to insufficient embryos, and many of the recipients receiving embryos do not become pregnant. When costs of maintaining all these unproductive animals are added up and divided by the number of resulting females entering the milking herd, costs are substantial. There are many other costs that are not apparent at first (28). In most circumstances, calves from embryo transfer must be worth approximately $2,000 (average of both sexes) for it to be profitable, even with on-the-farm procedures.

Semen Requirements

More calves are produced per unit of semen with embryo transfer than with conventional reproduction. If two units of semen are used per donor and an average of five calves results (if embryos are split), the number of calves per unit of semen is 2.5. This is about five times the normal .5 calf per breeding with dairy cattle. Use of one unit of semen instead of two for superovulation would be even more efficacious in terms of calves per unit of semen, but probably fewer ova would be fertilized. The fringe benefit of more calves per unit of semen is especially important with expensive semen.

Pregnancy Rates

Under ideal conditions, pregnancy rates with embryo transfer are similar to those with artificial insemination. Theoretically, they should exceed those with artificial insemination because all of the unfertilized and abnormal ova have been discarded. If only the best embryos are transferred to ideal recipients, pregnancy rates approach 80%. Perhaps they will reach 90% when we learn to evaluate embryos more
accurately and refine methods of embryo transfer.

High pregnancy rates with embryo transfer would be a distinct fringe benefit under some conditions. The problem is to have embryos available for all recipients, which is difficult because of variability in response to super-ovulation. One solution would be to have high pregnancy rates with cryopreserved embryos. Such a goal might be achieved within a few years.

Sex Selection

Of all the fringe benefits of embryo transfer, sex selection probably will be the most important, providing the cost is not excessive, it is reasonably accurate, and pregnancy rates are not lowered. The cost per female pregnancy will be lowered dramatically. It is clear that it will be an exciting time.

Cytoplasmic Inheritance

Several clear examples of maternal inheritance in mammals from factors other than nuclear DNA have been documented. The simplest example is the genome of mitochondria (18), which are organelles responsible for the bulk of energy production in most cells. An important point is that the mitochondrial genome only specifies a small proportion of the structural components of mitochondria; the bulk of genetic information for making mitochondria is specified by DNA in the cell's nucleus.

Embryo transfer will be important for propagating those beneficial things inherited through the cytoplasm of an ovum. A recent study suggests that over 1% of the variance for milk production may be due to cytoplasmically inherited factors (2), which is significant, as the component of variance for sires normally would account for only about 6% of the variance. Thus, embryo transfer may make this untapped source of genetic improvement available.

Tax Advantages

The tax laws of the United States and many other countries are structured to encourage certain kinds of investments. The three major aspects of tax regulations that result in fringe benefits from using embryo transfer with breeding stock in the United States are: 1) ability to convert ordinary income into long-term capital gains income, which is taxed at lower rates; 2) use of investment credit, whereby a percentage of the purchase price of breeding cattle can be deducted from taxes; and 3) most expenses from embryo transfer, including depreciation, can be deducted from gross income, which lowers taxable income. Optimizing these tax advantages is complicated. Of course, such advantages are most important when one has a large tax liability. This situation has led to infusion of some capital into the cattle industry. It is unclear whether this is beneficial to the dairy industry in the long term, as the capital is not necessarily productively invested. For example, certain cattle prices are inflated artificially and unrelated to improving efficiency of milk production.

Sense of Accomplishment

It is intrinsically appealing to many people to trick nature by the technologies described. For example, without embryo transfer, the average is one heifer calf from a given cow biennially. With embryo transfer, it is not unusual to have several heifers from a favorite cow born within a week of each other. There is much less reluctance to consign one heifer from a litter to a prominent sale than a single heifer from a cow who never may have another heifer calf. This phenomenon definitely has resulted in a qualitative change in the nature of promotional cattle actions. A number of sales are only for animals produced by embryo transfer; unborn embryo transfer fetuses are sold neatly packaged in their pregnant recipient (the recipient also is included as an incidental extra in the purchase price); and one recent sale required that for each bull consigned, a maternal sister also must be consigned. These are but reflections on the options that embryo transfer makes available to those who enjoy breeding cattle. Although the impact of these techniques on milk production is smaller than frequently imagined, they add to the pride of work of many dairy farmers, and that is far from trivial.
THE FUTURE OF EMBRYO TRANSFER AND RELATED TECHNIQUES

Goals for the Next 2 to 5 Years

The embryo transfer industry has matured considerably over the last decade (32). It is a diffuse industry that is influenced by a number of factors, including demands for services, new technologies that are provided from universities and other research institutions, new and improved technologies that evolve from within the industry, and technologies provided by mechanisms such as new biotechnology companies funded by venture capital. The first major product from the latter source probably will be a practical method for sexing embryos. Improvements in cryobiology are another area in which such contributions may be made. Mechanisms of funding research and development frequently involve patents, licenses, etc. It is unclear how the industry will react to this method of developing new techniques, especially when manipulation of biological processes is involved rather than a more defined product.

Major goals for the next 2 to 5 yr might be divided into three categories: development of new technologies, improving current technologies, and streamlining delivery systems to reduce costs greatly. Two new technologies, sexing embryos and in vitro fertilization, probably will soon become available commercially. The technology for splitting embryos is already available, although it may be simplified greatly in the next few years.

There are three current technologies that are ripe for improvement: superovulation, nonsurgical transfer, and cryopreservation of embryos. At times all three of these work exceedingly well, but they do not work well consistently. The extreme variability in response to superovulation and variability of fertilization rates are great sources of frustration. Fundamental work on follicular growth, timing of ovulation, and sperm transport probably will lead to improvement in this area. Standardization of gonadotropin preparations also might be of value.

The major problem with nonsurgical transfer is differences in pregnancy rates among technicians. A related problem is the great investment required for atechnican to become proficient. Reasons for differences among technicians need to be determined, much as was done for artificial insemination. Possibly location of embryos in the uterus will explain differences in much the same way that improper placement of semen was major source of variation among artificial insemination technicians (13). Likely the situation will be more complex. In any case, this is an area worthy of continued investigation.

Cryopreservation of embryos almost certainly will become greatly simplified over the next half decade. Expensive equipment will not be needed, and the process will not require as much time as current procedures. Probably great attention to detail will continue to be required, however. The real challenge will be to improve success rates. Some methods of cryopreservation of mouse embryos result in 90% survival (34). It is probable that pregnancy rates of cryopreserved bovine embryos will approach 90% of these of unfrozen embryos when optimal procedures have been developed. Marginal embryos will have to be discarded much as marginal ejaculates of semen are discarded rather than cryopreserved. When cryopreservation methods improve sufficiently, nearly all embryos will be frozen, analogous to what has happened with bovine semen.

Streamlining delivery systems may be the most challenging of the short-term goals. Under ideal circumstances, it appears that costs per pregnancy for embryo transfer services could be less than $100. In some parts of the United States, it may be possible to incorporate embryo transfer services with artificial insemination services. There seems to be no more need for a veterinarian to transfer embryos than to breed cows. Veterinary supervision of herd health programs is extremely important, however. Embryo transfer should be least expensive on large dairy farms. It will be difficult to provide inexpensive embryo transfer services when cattle are widely dispersed because of the same kinds of problems that limit artificial insemination of beef cattle. The real key to low costs is high pregnancy rates with cryopreserved embryos transferred nonsurgically.

Long-Term Goals

Ten or fifteen years ago few would have forecast accurately today's technological achievements with mammalian embryos. Conversely,
most would have predicted much wider application of artificial insemination (24). Development of technology is a complex process recently described with uncommon insight (12).

Possibilities for manipulation of the genome of animals are essentially unlimited. For example, methods of making an infinite number of genetic copies of a particular animal probably will become available (30), but making copies of the best animals available will be a trivial exercise relative to making animals better than the best. Of course, there is a best animal for each environment, which makes this complicated.

Mammalian genetic material is immensely complex. Understanding how all the pieces fit together will not be feasible until approaches evolve that are fundamentally different from those in use today. Much fundamental research on life processes in general and into the specific organisms of interest in particular will be needed before we can manipulate all aspects of the genome.

Our long-term goals, therefore, should be to understand how the organisms of interest works. That understanding then can be put to greater and greater use as it develops. We cannot expect too much too quickly. Research is expensive, and some of the obstacles are formidable. But there will be immense fringe benefits, like developing cures for diseases.

Conclusions

There is a large and growing bovine embryo transfer industry in North America. Although only approximately 1/1,000 dairy calves born is from embryo transfer, this represents a 10-fold increase in less than 5 yr. Improved techniques, such as nonsurgical methods of embryo recovery and transfer and cryopreservation, contributed greatly to this increase. Further improvements in existing technology plus new technologies like splitting and sexing embryos may lead to another 10-fold increase in the next 5 yr. Already more than 1% of Holstein calves registered annually are from embryo transfer. Most technologies are complimentary such that costs will continue to decline markedly. For embryo transfer to be used for more than 1% of dairy calves born, costs per pregnancy probably must decline to less than $100. Only then do these technologies start to make economic sense for producing the next generation of females to produce milk more efficiently. Even at $100 per pregnancy, it is the fringe benefits such as sex selection and more calves per unit of semen that really will make embryo transfer appropriate for more widespread use. It is unclear how soon directed chemical modification of bovine genetic material will find application. Because of costs, these modifications probably will be made primarily in bulls for inexpensive dissemination to the population through artificial insemination. Technologies of embryo transfer will be an integral part of the exciting genetic manipulations that will be applied in the decades ahead.

ACKNOWLEDGMENTS

Many of the ideas presented arose from discussions with colleagues and friends. R. E. Nelson provided the data in Table 1. I especially acknowledge the help of Sarah Seidel in improving the manuscript.

REFERENCES

2796 SEIDEL, JR.

hormone and pregnant mare's serum gonadotropin. Theriogenology 9:17.


Journal of Dairy Science Vol. 67, No. 11, 1984