Superovulation and Embryo Transfer in Cattle

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Summary. About 17,000 bovine pregnancies were produced by superovulation and embryo transfer in North America in 1979. The major use of these techniques is to increase the reproductive rate of valuable cows. Other applications include circumventing infertility, exporting embryos, and testing potential carriers for Mendelian recessive alleles. Cryopreservation of embryos is beginning to be used commercially, and sexing embryos before transfer may soon become routine.

A multimillion dollar industry centered on recovery, in vitro culture, and transfer of bovine embryos has evolved over the last decade. This article describes the techniques being used, examines commercial applications, and describes developing techniques and applications that are likely to be used in the near future.

Gestation in cattle lasts just over 9 months; usually a single offspring is born, but twins result from a few percent of pregnancies. Cattle are not considered to be seasonal breeders but are often managed to reproduce seasonally, calving before the time of the year when the cheapest forage is available. Genetic progress is limited by the low reproductive rate and long interval between generations, an average of 6 to 7 years (*1*). Thus, during a 40-year career, a cattle breeder works with an average of only six or seven generations. Moreover, it takes years to increase cattle numbers in response to economic incentives. This is responsible, in part, for the 10-year cycle, in the United States, of high and low beef cattle numbers and the consequent fluctuations in beef prices (*2*). Similarly, it takes decades for cattle numbers to recover after depletion due to war, famine, or social upheaval (*3*).

Ovum and egg are the general terms used to refer to the female gamete and early embryo. The ovulated ovum is technically termed an oocyte which, upon fertilization, becomes an embryo, and when the major organs form, a fetus (Fig. 1). Embryo transfer refers to placing an embryo into the lumen of the oviduct or uterus (*4*). In a broad sense, however, embryo transfer has come to mean the sequence of steps for moving embryos from one female to another, including superovulation, embryo recovery, and storage of embryos in vitro. The donor is the genetic mother from which embryos are recovered; the host or surrogate mother is called a recipient.

The first successful embryo transfer was performed in rabbits 90 years ago at Cambridge University (*5*). Since that time, embryo transfer has been used for research in more than a dozen species, although extensively only in the mouse, rabbit, sheep, and cow.

The first successful bovine embryo transfer was reported in 1951 (*6*). Over the next two decades a number of researchers repeated this feat, culminating in the high success rates achieved by Rowson and his colleagues (*7*). In the last decade, the development of new drugs and techniques coupled with demands for embryo transfer services by the cattle industry have led to significant advances (*8*). Two human infants, a girl and a boy, were born after in vitro fertilization and return of the embryos to the original donors by transfer (*9*).

Commercial embryo transfer has been centered in North America, but the size of this industry had not been documented until a systematic survey was undertaken of all companies known to offer such services in the United States and Canada (*10*). The survey, summarized in Table 1, is an underrepresentation of the volume of business both because two firms did not respond and because some were probably overlooked. However, it is likely that more than 90 percent of the commercial embryo transfer activity in North America is included. Data for 1979 are estimates based on 80 to 90 percent of the year's business. Although the volume of business appears to be very large, the most noticeable aspect is the 80 percent increase in pregnancies between 1978 and 1979. This may be explained in part by the increase in cattle prices after the liquidation of breeding herds in 1976 and 1977 when cattle prices were low (*2*). However, improved technology and acceptance of embryo transfer techniques have also contributed. Embryo transfer has become a $20-million-a-year industry (*11*); essentially no commercial activity existed before 1972.

Commercial activity outside North America has been less extensive. Over the past decade, several European firms have operated commercially for brief periods (*12*), and several centers have become more firmly established. Governments in Western Europe, particularly England, France, Ireland, and West Germany, have supported considerable research in embryo transfer. This has stimulated commercial application worldwide, especially in Canada and the United States.

Embryo transfer is a larger industry in Australia and New Zealand than in Europe (*13*), although it declined sharply a few years ago when cattle prices decreased, and it is only now recovering. Interest in embryo transfer is high in dozens of other countries, but the amount of commercial activity is probably small.

Current Technology

Treatment of cattle with gonadotropins leads to ovulation of numerous ova instead of the usual one. The basic mechanisms of superovulation are understood only superficially (*14*). Gonadotropin treatment is usually initiated between days 9 and 14 of the estrous cycle (estrus is day 0), causing ovarian follicles to grow (*8*, *15*). Two or three days after the start of treatment prostaglandin $F_{2\alpha}$ or an analog is injected to terminate the luteal phase of the estrous cycle prematurely by lysing the corpus luteum; about 2 days later estrus occurs. Estrus lasts

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about half a day; ovulation occurs about one-half day after the end of estrus; and fertilization probably occurs a few hours after ovulation (*8*).

Before prostaglandins became available, superovulation was initiated about 4 to 5 days before the end of the estrous cycle, a time that could not be estimated accurately. Availability of prostaglandin $F_{2\alpha}$ has improved the efficacy of superovulation and has also provided flexibility in scheduling donors. Few would have predicted that basic research with prostaglandins would eventually result in such an application (*16*).

Because the best bulls are usually propagated only with frozen semen, artificial insemination is used routinely for valuable cows. Sometimes mixtures of semen from two or three bulls are used with superovulation, and the progeny are sorted out after birth on the basis of blood type.

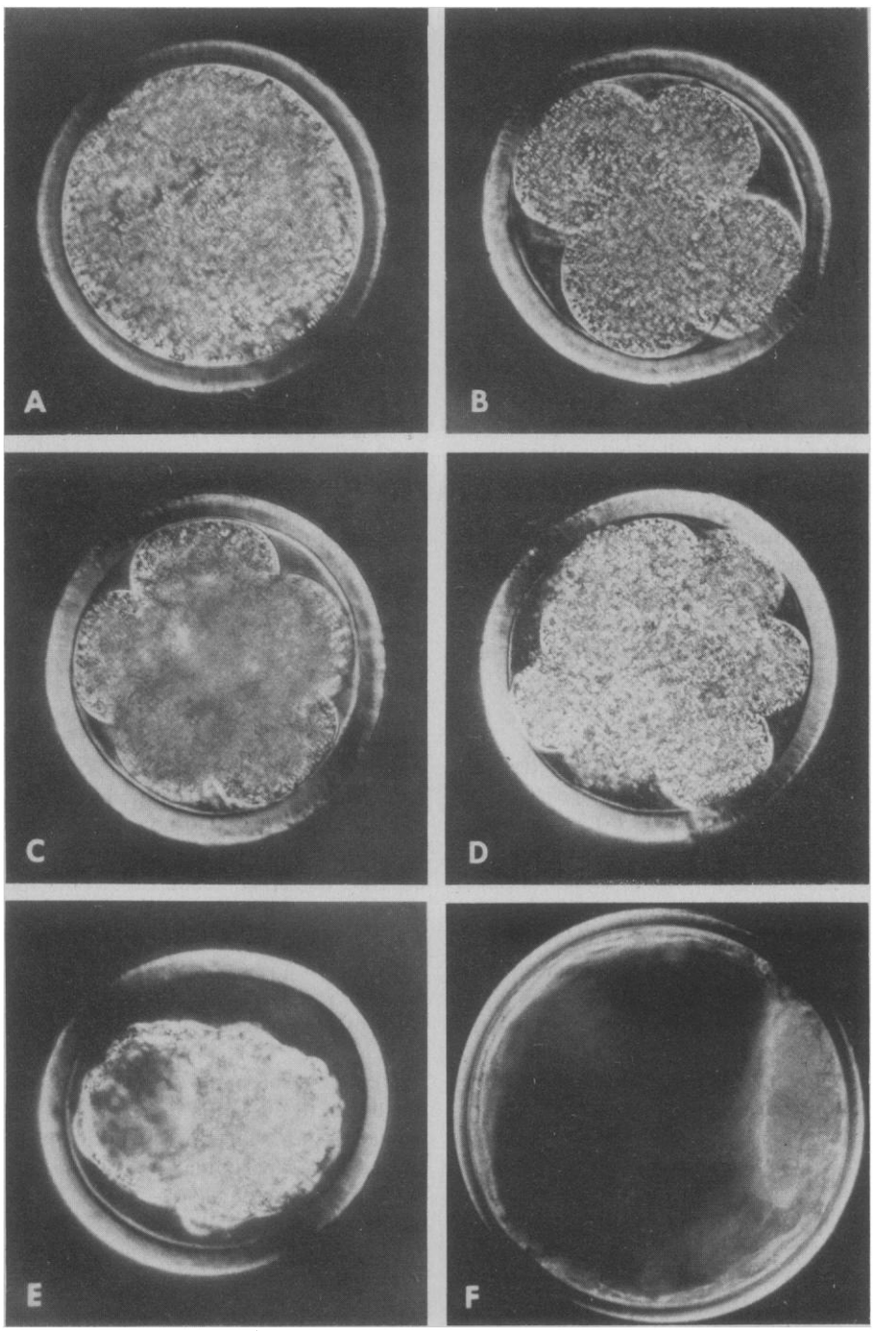

Fig. 1. Bovine ova. (A) Unfertilized oocyte recovered from the ovary. Follicle cells were removed mechanically. The outside layer is the zona pellucida. (B) Four-cell embryo recovered from the oviduct on day 2 of pregnancy (estrus = day 0). Note perivitelline space between the cells and the zona pellucida. (C) Eight-cell embryo recovered from the oviduct on day 3 of pregnancy. (D) An 8- to 16-cell embryo (morula) recovered from the oviduct on day 4 of pregnancy. (E) Very early blastocyst stage; approximately 60 cells recovered from the uterus on day 7 of pregnancy. The inner cell mass from which the fetus will develop will form to the right. (F) Expanded blastocyst with > 100 cells recovered from the uterus on day 8 of pregnancy. The inner cell mass is to the right. All photomicrographs were taken with differential interference contrast (Nomarski) optics. Magnification approximately ×300.

Bovine embryos move from the oviduct to the uterus 4 to 5 days after estrus (3 to 4 days after ovulation), although in superovulated cows a few remain in the oviduct through day 7 (*17*). A high percentage of embryos can usually be recovered nonsurgically from the uterus six or more days after the beginning of estrus (*18, 19*). Recovery of embryos from the oviduct requires surgery and, therefore, is recommended only in certain cases of infertility (*20, 21*).

Procedures for obtaining, storing, and transferring embryos are illustrated in Fig. 2. To recover embryos, an 18-gauge Foley catheter is inserted through the cervix into the uterus by palpating through the wall of the rectum with one hand as is done for artificial insemination. The latex catheter consists of three channels for inflow, outflow, and inflation of a balloon-like cuff that prevents the escape of fluid after insertion (Fig. 2). Each uterine horn is filled and emptied five to ten times with 30 to 200 milliliters of fluid each time, according to the size of the uterus. The embryos are flushed out with this fluid into large graduated cylinders. After 30 minutes, embryos settle and can be located under a stereomicroscope by searching through an aliquot from the bottom of the cylinder. They are then stored in small containers until transfer.

Embryos from the one-cell to the early blastocyst stage (7 to 8 days after estrus) are between 120 and 140 micrometers in diameter exclusive of the zona pellucida (Fig. 1) (*8, 22*). Between days 8 and 10, they double in diameter, hatch from the zona pellucida, and then grow to 20 centimeters or more in length by day 18 (*23*). Since bovine embryos form no intimate attachment to the uterus before day 18, they can be recovered nonsurgically until this time, although they are increasingly prone to damage after day 14. It appears that a larger number of normal embryos can be obtained nonsurgically 6 to 8 days after estrus than at other times.

In vitro culture and storage of bovine embryos. The ability to keep embryos in vitro provides considerable flexibility. For example, it is not unusual to recover embryos from a dairy cow and then fly hundreds of miles to a recipient herd. Bovine embryos, particularly those at the morula and early blastocyst stages, continue development in vitro at 37°C for 24 hours in nearly any properly buffered and balanced salt solution supplemented

with blood serum or bovine serum albumin; however, for periods greater than 24 hours, a complex medium, such as Ham's F-10 plus 10 percent fetal calf serum, appears best (*24*). Pregnancy rates for embryos cultured up to 24 hours are similar to those of embryos cultured for only a few hours, but there may be more subsequent embryonic death after transfer of embryos cultured for 24 hours or more (*25, 26*).

Although, for commercial purposes, embryos are usually kept in vitro for less than 10 hours, a pregnancy rate of 44 percent has been reported for embryos collected in Canada and transferred to recipients in Europe 18 to 33 hours later (*27*). Embryos also continue to develop normally when stored in the oviduct of a living rabbit for 2 to 4 days, and pregnancy rates are high when they are recovered and retransferred to the bovine (*28*).

Embryos are often kept at ambient temperature (15° to 25°C) between collection and transfer. Storage of embryos at 0° to 10°C halts embryonic development until they are again warmed to 37°C. Pregnancy rates with bovine embryos stored in this manner for a day or two are reduced somewhat (*29*); occasional pregnancies have resulted after transfer of rabbit and sheep embryos stored for up to 10 days at 10°C (*30*).

For long-term storage, embryos are frozen to −196°C in liquid nitrogen. About one-third of bovine embryos are killed or severely damaged after freezing and thawing, but pregnancy rates following transfer of the other two-thirds are not much lower than those with fresh embryos (*26, 31*). Despite the losses, freezing is sometimes used commercially when recipients are not available. Just as nearly all bovine semen used for artificial insemination in North America is frozen despite the fact that freezing and thawing kills half of the sperm (*32*), most bovine embryos probably will be frozen between collection and transfer if losses due to freezing and thawing can be reduced to less than 20 percent.

Embryo transfer. Embryo transfer technology is commercially viable because reproductive rates of valuable donor cows can be increased through less valuable recipient cows. A common misconception is that the recipients are undesirable cattle rejected for other purposes. In fact, though less valuable than donors, recipients usually are superior to the general cattle population. The successful recipient must be fertile, free from disease, and able to give birth to a calf without complications. With many systems of management, it is desirable that she also produce sufficient milk to ensure that the calf grows at an optimal rate. Easily the largest single cost of embryo transfer is procuring suitable recipients and maintaining them in a nonproductive (nonpregnant) state until embryos are available for transfer.

Table 1. Companies producing pregnancies by bovine embryo transfer in North America. Pregnancies were confirmed between days 60 and 90 of gestation. The 1979 data were estimated between October and November. In Canada, pregnancies totaled 2020 in 1978 and 3697 in 1979. In the United States, pregnancies totaled 7149 in 1978 and 13,008 in 1979.

Pregnancies (No.)	Companies (No.)	
	1978	1979
> 1000	1	4
500 to 999	5	7
250 to 499	10	11
100 to 249	6	7
< 100	13	16

Recipients must be at the same stage of the estrous cycle as the donor. Asynchrony between the donor's and the recipient's estrous cycles of 1 day results in pregnancy rates similar to those with exact synchrony; however, pregnancy rates plummet when the estrous cycles of donor and recipient are more than 2 days out of phase (*26, 33*). The requirement for estrous-cycle synchrony necessitates maintenance of herds of hundreds of cows to provide sufficient synchronous recipients for each superovulated donor. Manipulation of the estrous cycles of recipients with drugs such as prostaglandin $F_{2\alpha}$ is useful under some circumstances (*34*). Unfortunately, drugs for estrus synchronization must be given before actual recipient needs are known. The great advantage of frozen embryos is that embryos wait for recipients, instead of the converse.

Embryos are normally transferred to the uterus. Currently, nearly half of the commercial embryo transfers are performed nonsurgically, and this method is rapidly replacing surgical ones. It is accomplished with the same equipment that is routinely used for artificial insemination; however, the embryo is deposited further into the uterus than semen, and much more care is required to prevent introduction of microorganisms, since the progesterone-dominated endocrine environment makes the uterus especially prone to infection. In contrast, it is extremely difficult to infect the uterus when cows are routinely inseminated because of estrogen domination at that stage of the estrous cycle (*35*).

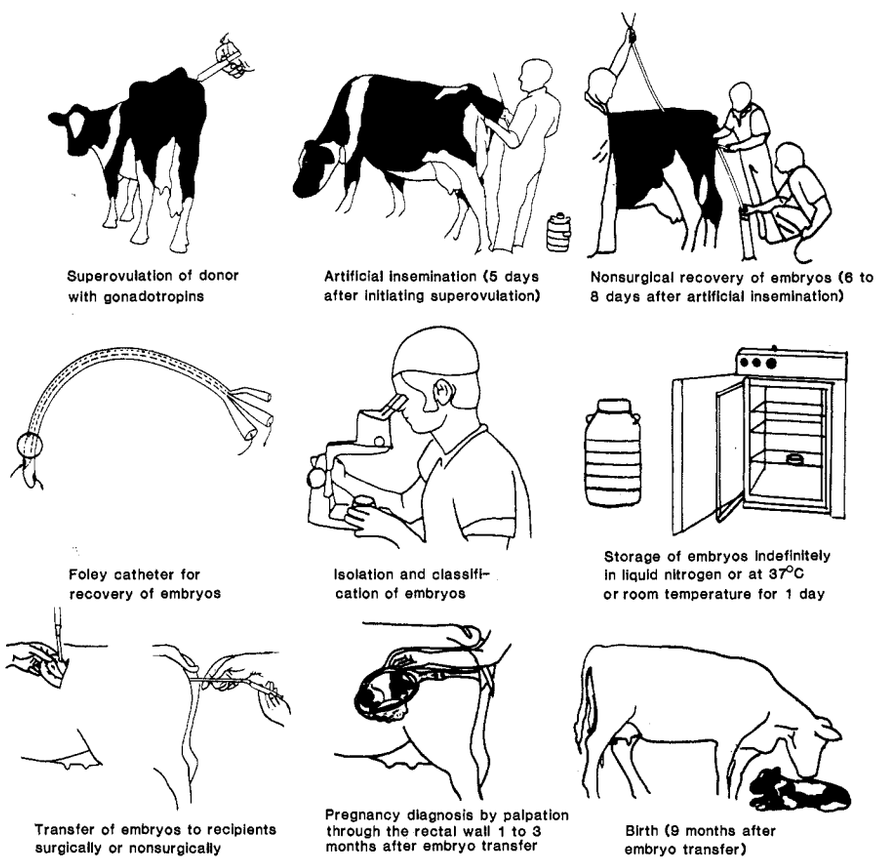

Fig. 2. Synopsis of bovine embryo transfer procedures.

Pregnancy rates with nonsurgical transfer are lower and more variable than with surgical transfer (*36*). For surgical transfer, the uterus is exposed either through an incision in the flank for which local anesthesia is used or through an incision between the umbilicus and udder for which general anesthesia is used. A puncture wound is made in the anterior portion of the exposed uterine horn ipsilateral to the ovary containing the developing corpus luteum (site of ovulation), and the embryo, in about 0.1 ml of medium, is transferred into the uterine lumen through a fine pipette, several centimeters long and about 1 millimeter in diameter at the tip. If the embryo is transferred to the uterine horn contralateral to the corpus luteum, pregnancy rates are reduced by half (*36, 37*).

Pregnancy rates with normal embryos from superovulated cows range from 30 to 60 percent with nonsurgical transfer and from 50 to 70 percent with surgical methods (*8, 15, 26, 36*). The latter rate is slightly higher than the pregnancy rates of 50 to 60 percent with routine artificial insemination (*38*), in part because unfertilized ova and abnormal embryos are discarded before transfer.

Tens of thousands of calves have been born as a result of embryo transfer, and there is no evidence of increased abnormalities. However, no systematic comparison has been made between calves produced by embryo transfer and those produced conventionally. Findings (*39*) from survey data of 178 pregnant recipients showed four abortions between 90 days of pregnancy and term, seven deaths of newborns (including one abnormal calf), and seven additional deaths before weaning. Thus 160 (90 percent) of the 178 pregnancies resulted in normal, weaned calves; this weaning rate is similar to that for routine cattle husbandry in well-managed herds (*40, 41*). The sex ratio was 51 percent male, which is normal. There were no identical twins, although there are anecdotal accounts of identical twin calves resulting from transfer of single embryos. Statistical documentation of normal offspring and sex ratios was not a prerequisite for accepting embryo transfer technology by the cattle industry.

Rates of success. When cows are inseminated without superovulation, it is possible for skilled personnel to recover the ovum nonsurgically in 60 to 70 percent of the cases (*19, 42*). About 10 percent of the ova recovered are unfertilized and another 10 percent are morphologically abnormal. Thus, a normal embryo is obtained about 50 percent of the time. Pregnancy rates after transfer of such embryos are close to 70 percent (*15*); therefore, the probability of getting a calf from each attempt is about 35 percent. A cow ovulates about 17 times a year, although estrus would probably only be detected about 15 times; thus, about five calves per year could be produced by embryo transfer without superovulation.

With commercial embryo transfer, donors are usually superovulated. The best treatments result in six to seven normal and two to three abnormal or unfertilized ova per normal cow treated; three to four pregnancies result on the average when embryos are transferred surgically (*15, 26*). To illustrate success rates, repeatability, and variability with current procedures, data from donors at the clinic at the College of Veterinary Medicine and Biomedical Science at Colorado State University are presented in Table 2.

From three courses of superovulation with follicle-stimulating and luteinizing hormone (*15*) of each of 32 apparently normal donors (*43*), 328 pregnancies resulted. Embryos were recovered nonsurgically and transferred surgically. The donors were beef cows from 1 to 14 years of age. They are fairly representative of the beef cattle in embryo transfer programs (*44*). Although superovulation and embryo transfer are frequently used with dairy cattle, similar data are not available since it is rare to keep normal, fertile dairy cattle nonpregnant for three courses of superovulation because pregnancy and parturition are required every 12 to 13 months for optimum milk production.

Table 2. Distribution of pregnancies from donor cows. Pregnancy was confirmed by palpation through the rectal wall between days 90 and 100 of gestation.

Donors		Pregnancies from superovulation (No.)		
Age (years)	Breed	First	Second	Third
1	Simmental	4	7	8
1	Simmental	5	0	9
4	Limousin	0	0	1
2	Hereford	5	1	4
2	Pinzgauer	6	1	0
2	Pinzgauer	4	8	20
13	Angus	4	2	8
10	Hereford	2	0	5
9	Hereford	9	3	7
1	Simmental	7	0	0
1	Simmental	2	1	0
4	Charolais	7	9	0
7	Brangus	2	0	1
2	Simmental	5	11	4
6	Hereford	1	11	5
8	Hereford	2	2	0
5	Hereford	2	0	1
14	Hereford	12	0	1
12	Hereford	0	3	2
2	Hereford	3	1	4
4	Angus	3	0	2
7	Hereford	4	7	3
5	Angus	4	2	0
5	Angus	4	3	0
5	Angus	4	9	0
4	Simmental	1	1	0
5	Angus	0	7	2
4	Hereford	0	4	8
10	Angus	0	6	0
1	Simmental	0	2	0
5	Angus	3	6	0
3	Simmental	3	4	14
			Average	
		3.4	3.5	3.4

Perhaps the most obvious characteristic of the data in Table 2 is variability. The data are not normally distributed and, therefore, many standard statistical descriptions are not appropriate. For example, calculated standard deviations for second and third superovulations are larger than the means, resulting in coefficients of variation greater than unity. A second characteristic of the data is the lack of predictability from one superovulation to the next; the repeatability, calculated from variance components was only 1 percent. The low repeatability of the number of pregnancies is partly due to accumulation of variability in superovulation, fertilization rate, efficiency of embryo recovery, availability of suitable recipients, and other factors.

Mean numbers of pregnancies were similar for all three courses of superovulation. However, half of the donors had fewer than two pregnant recipients from third superovulations, in contrast to one-fourth of the donors for first superovulations. Data from the third superovulation must be interpreted with caution because of the two high values of 14 and 20 pregnancies.

Although the number of animals is limited, the data are typical (*8, 26*). Under commercial conditions, one can reasonably expect an average of three to four pregnancies from one superovulation and about ten pregnancies from three, but results for an individual cow are unpredictable. Typically, one-half of the pregnancies are from one-fourth of the donors. This makes it extremely difficult for the farmer to plan breeding programs and for the embryo transfer unit to schedule workloads. The major determinant of the number of pregnancies is the number of normal embryos obtained, although pregnancy rates among donors also vary widely. Thus far, the variability has not been explained, although age, breed, nutrition, and season have been marginally implicated (*8, 45*).

Superovulation is not recommended until a donor exhibits a normal estrous cycle, characterized by two estrous peri-

ods 17 to 24 days apart. Because estrous cycles are often modified temporarily by superovulation, an interval of 2 months or more is usual between superovulations. For the donors in Table 2, the median interval between the first and second superovulation was 78.5 days and between second and third superovulations, 82 days. The median elapsed time between arrival of donors at our embryo transfer unit and the first superovulatory treatment was 46.5 days. Thus, nearly 7 months were required for three superovulations. On the average, however, reproductive capacity was amplified more than tenfold. The yield can be increased further by the transfer of unsuperovulated embryos recovered before and between superovulations while normal cycles are being established. Recovery of a single ovum is not usually attempted because of greater expense per pregnancy relative to superovulation; however, it was attempted from about 60 percent of the eligible estrous periods of the donors in Table 2, and 15 extra pregnancies resulted.

A number of cows have been superovulated four or more consecutive times without an intervening pregnancy (*46*). Some of these donors continued to respond, but data are insufficient to recommend superovulation more than three times in a row.

Cost of embryo transfer. Embryo transfer units currently charge about $2000 per pregnant recipient. When the various other costs are taken into account, and the value of the recipient is deducted, it turns out that calves must be worth about $2500 each at 6 months of age for embryo transfer to be profitable (*47*). About $1500 of the cost is attributable to embryo transfer; the remainder represents conventional costs.

Although the majority of bovine embryo transfer work is still done at embryo transfer centers, there is a definite trend toward doing both recovery and transfer on the farm, where, under certain conditions, embryo transfer costs may be much lower. As deep-freezing methodology for embryos improves, most embryos will be transferred on the farm and costs will plummet.

Commercial Applications

By far the major use of embryo transfer is to increase the reproductive rate of valuable cows. Although it is impressive for a cow to have ten calves per year, it is extraordinary that some cows have had more than 50 calves in a 12-month period. This must, of course, be weighed against those that have only a few, despite persistent effort.

Donors are usually chosen on the basis of the anticipated commercial value of the calves rather than on the basis of direct genetic considerations. At a recent auction, a bull calf produced by embryo transfer was the top selling animal at $131,000; the third highest priced animal at $27,000 was a maternal sister to the bull, also from embryo transfer. However, this technology can lead to such rapid multiplication of formerly scarce animals that they become commonplace and cannot be sold for a large premium. In the mid-1970's, propagation by embryo transfer of several breeds of cattle that were rare in some countries resulted in a "boom and bust" cycle, and many entrepreneurs suffered serious financial losses (*48*).

Embryo transfer technology is frequently used to obtain additional progeny from cows with certain kinds of infertility. In some cases, the infertility is corrected in the course of embryo recovery procedures (*21, 49*). This application could lead to propagation of genetically defective animals, but it is justified when valuable cows become infertile due to injury, disease, or senescence. Many infertile donors are in the latter category; their ovaries produce normal ova, but they are incapable of maintaining a pregnancy. Similar phenomena occur in a number of other aged mammals (*50*). Pregnancy rates are normal when morphologically normal embryos recovered from infertile donors are transferred to suitable recipients (*21, 49*). On the average, only a few normal embryos are recovered per infertile donor, however, which makes such reproduction very expensive. Nevertheless, on rare occasions results are spectacular. For example, we have obtained 30 pregnancies over a 15-month period from one infertile donor.

Hundreds of bovine embryos have been exported from Canada to Europe (*27*) and to other regions of the world too. Clearly, it costs much less to transport embryos than cattle. Although some exported embryos were frozen, most were not. Logistics are complicated when embryos are not frozen—for example, synchronizing estrous cycles of donors and recipients in widely different time zones can be confusing.

Theoretically, there is an important epigenetic consideration in the export and import of embryos. Frequently, animals die or are severely debilitated by infection with local pathogens when they are introduced into a different country. When embryos are transferred to indigenous recipients, however, the young receive appropriate passive immunity through the colostrum (first milk), and the developing immune system of the calf becomes specifically attuned to the pathogens in the environment. There may also be other physiological and behavioral adaptations that are facilitated when an animal is born in a particular environment rather than being placed there at an older age. Although artificial insemination usually is the method of choice for introducing new genetic material into indigenous cattle, it is slower than embryo transfer because three generations are required to produce an animal with seven-eighths of the genetic makeup of the new breed. On the other hand, embryo transfer is considerably slower than importing breeding adults, if they survive.

One limitation to the export of embryos is the potential for spreading disease. In all likelihood there is less potential than with movement of animals, but very little is known about this (*51*). Embryo transfer has been used successfully to introduce new genetic material into specific pathogen-free swine herds (*52*).

Testing for Mendelian recessive alleles. If a homozygous recessive individual is mated to a suspected carrier of a Mendelian recessive allele, and eight normal offspring result, the probability that the individual is not a carrier is 99.6 percent $[1 - (1/2)^8]$. Without superovulation and embryo transfer, it would take more than the average reproductive lifespan to prove that a cow was not a carrier of a particular undesirable trait. Furthermore, the eight normal progeny required for the proof would all be carriers and, therefore, undesirable for breeding purposes. With embryo transfer, comparable proof can often be obtained within a year. This is especially important when an outstanding cow is a daughter of a carrier.

Embryo transfer is also used to prove statistically that bulls are not carriers of undesirable traits (*53*). Without embryo transfer, proof can be very difficult to obtain because sexually mature females with certain homozygous recessive alleles are extremely rare. Examples are dwarfism and syndactyly (mule-foot). If a single homozygous female is available, however, she can be superovulated and her genes thus amplified for testing bulls. Furthermore, for many traits, diagnosis can be made at 60 days of gestation. The recipients can be slaughtered at that time to speed up the test without harming the rare, homozygous donor. Recently four of eight sons of a bull known to be a carrier of syndactyly were found to be carriers (*53*).

Future Technologies and Applications

In vitro fertilization. Several reports provide some evidence of in vitro fertilization of bovine ova (*54*), but no pregnancies have been reported from such procedures. In fact, production of young from oocytes fertilized in vitro has been reported in only four mammalian species: the rabbit, mouse, rat, and human (*55*). There probably have been hundreds of attempts at in vitro fertilization in human beings, and many were successful in terms of ovum penetration by sperm; however, continued normal embryonic development to term has rarely been documented (*9*, *56*).

Little work has been done with in vitro fertilization in cattle, partly because of the lack of economic incentive and research support and partly because of alternatives to in vitro fertilization. Oocytes can be taken from bovine donors, transferred to inseminated cows to be fertilized, and then recovered for evaluation. Normal embryos can then be transferred to recipients for gestation (*57*, *58*). This procedure is tedious, and success rates are low. For commercial application, it will be necessary to mature oocytes and capacitate sperm reliably, preferably in vitro. Few young have been produced from oocytes matured in vitro (*57*), possibly because such oocytes are usually abnormal. Capacitation, that is, modification of sperm in the female reproductive tract required for fertility, remains relatively unstudied in the bovine.

One application of in vitro fertilization will be to circumvent infertility. Probably a more important use, however, will be as a quality control test for semen, since current laboratory tests are not highly correlated with fertility (*59*). Another significant application may be to provide large numbers of embryos from oocytes recovered from ovaries at slaughterhouses. A final application would be to extend the use of rare, valuable semen. Currently, millions of sperm are required per insemination dose for acceptable conception rates, yet only one fertilizes the oocyte. With in vitro fertilization, this number could be reduced, possibly even to one, if sperm were injected directly into oocytes (*60*).

Sexing embryos. It is not yet possible to separate X- and Y-bearing bovine spermatozoa reliably, but the sex of embryos can be determined karyotypically. Cells can be obtained by biopsy of morulae or by cutting off the tip of an elongating blastocyst (*61*). Most embryos continue normal growth after biopsy, although pregnancy rates appear to be slightly lower than those with unsexed embryos. The bovine sex chromosomes are easily distinguished; thus, sex determination is highly accurate when suitable metaphase chromosomes are available for examination. Unfortunately, procedures are tedious and time-consuming and usable preparations are obtained from only about two-thirds of the embryos (*61*). Therefore, three categories of approximately equal numbers result: male, female, and unknown. Before this technology can be applied on a broad commercial scale, it must be made simple, fast, inexpensive, reliable, and nondamaging to embryos. A fringe benefit of karyotyping is detection of chromosomal abnormalities.

An alternative to karyotyping could be detection of the gene product of the Y chromosome, H-Y antigen (*62*). Sexing embryos will be replaced when it becomes possible to separate X- and Y-bearing sperm without damaging them, a feat that may, however, be many years away.

Commercial applications of sexing embryos are based on the following considerations. Because of artificial insemination, the number of bulls required for breeding is greatly reduced, and the value of the few very good bulls is greatly increased. Thus, in a very few instances male calves will be more valuable than female calves. In the great majority of cases, however, females are considerably more valuable than males. It is financially risky if one must count on at least five females from ten pregnancies for profitability; for example, the probability of seven or more offspring of one sex is 17 percent if the true sex ratio is 50:50 (*63*). In most cases, then, female embryos would be transferred and male embryos discarded, used for research purposes, or transferred to produce bulls for fattening (*64*). An advantage of sexing when inducing twinning is that one can ensure that a male and female fetus do not develop together, resulting in a sterile female (a freemartin).

Twinning. Nearly 80 percent of the cows in North America are beef cows whose sole function is to produce one calf each year. Approximately 70 percent of the nutrients consumed by each beef cow are for her own maintenance, while only about 30 percent are for growth and maintenance of the calf during pregnancy and lactation (*65*). The conversion of feed into meat would be more efficient if cows had twins even though there are more problems with twins, such as higher losses at birth (*40*, *66*). Currently, embryo transfer is the most effective method of inducing pregnancies of twins in cattle. Hormonally induced twinning often results in two embryos in one uterine horn, which leads to more abortion than when one fetus implants in each uterine horn (*67*).

Because of a shortage of calves to fatten for beef, twinning in cattle may become economically viable in Europe and Japan before it does in North America (*68*). Average prices for newborn dairy calves to be used for meat were less than $100 in North America in early 1980 (*69*); and embryo transfer costs per successful twinning would have to be considerably less than this to be profitable. However, lower costs of the technology and inexpensive embryos from unwanted frozen male embryos or from in vitro fertilization of oocytes from slaughterhouse ovaries may eventually lead to commercial twinning.

A special case of twinning is the production of identical twins by separating blastomeres at the two-cell stage or cutting morulae in half (*70*). This would lead theoretically to twice as many calves per embryo and eliminate the occurrence of freemartins from twinning without the need for sexing. When methods of producing identical twins (or triplets, or quadruplets) become more practical, the major use may be control of genetic variation in experiments.

Progeny from prepuberal calves. The ovary of the prepuberal calf can be stimulated by exogenous gonadotropins to produce viable ova long before the uterus is capable of maintaining pregnancy. Such ova can be transferred to postpuberal animals to produce calves, although with very low rates of success (*71*). This is extending reproductive life in the opposite direction from the cow with a senescent uterus. In general, genetic and phenotypic information about prepuberal calves is limited; therefore, using them as donors would only be profitable in special situations.

Progeny-testing females. It would seem that embryo transfer technology could be used routinely to progeny test females for traits like growth rate, carcass characteristics, and milk production. However, the time required to collect the information would increase the generation interval to such an extent that the added information would be of little value in a well-managed breeding program (*72*).

Benefits of Embryo Transfer Technology

Currently, 70 to 90 percent of the female calves must be kept as replacements in the national dairy herd for those removed by death, senescence, disease,

injury, infertility, and so forth. If all replacements could be obtained from the top 10 percent of the herd, then genetic progress from this source would be between three and four times as rapid as with the current 70 percent level of selection intensity (*73*), and even more rapid with sex selection. With a good artificial insemination program, however, nearly all genetic progress comes from selection of sires, not dams, so that even tripling progress from selecting dams leads only to a net 10 to 15 percent increase in overall genetic progress (*72*). Thus, the current cost of embryo transfer may be much higher than the return in terms of increased milk and meat production (*74*). Embryo transfer might be relatively more useful for beef cattle because selection of males is not as overwhelming a driving force in genetic improvement as it is with dairy cattle.

The industry should not rely too heavily on genetic and economic analyses of production traits. Farmers have used embryo transfer technology almost exclusively for purebred animals, and the relation between market value of purebred cattle and genetic value is low, although usually positive. Factors such as scarcity and economic health of the livestock industry have much greater impacts on market value of high-priced animals than does their genetic value in terms of production traits. Thus, cattle breeders use potential profit rather than some other measure of genetic value to determine if and when embryo transfer should be used.

The genetic value of dairy cows produced by embryo transfer was determined from data provided by the National Cooperative Dairy Herd Improvement Program in the United States. The 50 progeny with records in early 1979 (resulting from embryos transferred before 1976) were at the 18th percentile from the top for genetic value for milk production. Donors on file to date ($N = 275$) were about at the 25th percentile (*75*). Thus, dairy cattle donors selected for embryo transfer and the resulting progeny have been considerably above average in genetic value for milk production, even though there was also selection for traits such as body conformation, longevity, and popular blood lines. Of interest is that sires of embryo transfer progeny were approximately at the second percentile in genetic value for milk production of all bulls born.

It is too early to assess the long-term impact of bovine embryo transfer technology on the rate of genetic improvement nationally. Because of the relative ease of amplifying male reproduction, most of the genetic influence of embryo transfer in the near future will be through males produced by this process, or born to females as a result of embryo transfer. Although the cost and complexity of embryo transfer probably will limit its application to less than 1 percent of reproduction in cattle for the next decade, certain specialized uses will have far-reaching effects. For example, the genetic base of certain breeds rare in North America was quickly broadened by embryo transfer (*76*).

Analogously to putting eggs from whooping cranes into nests of sandhill cranes, embryo transfer can be used to rescue rare breeds or species from extinction. An example is the current effort to rescue the Angora breed of sheep from extinction in Australia. The breed is being reestablished from only a few animals by transferring resulting embryos into other breeds of sheep. The same technique might be used with endangered species such as the black-footed ferret and mountain bison. Females of closely related species must be available as recipients; if recipients of the rare or endangered species must be used, embryo transfer is not indicated because amplified reproduction of donors is usually at the expense of decreased reproduction of recipients.

It should be emphasized that embryo transfer is extremely useful experimentally, especially for distinguishing effects of the uterus from those of the embryo (*77*). An example is probing the causes of declining reproductive function as females age by transferring ova from young to young, young to old, old to old, and old to young uteri (*50*). Another illustration of the power of these techniques is that one could make identical twin female embryos, freeze one of them, and transfer it to the other when she becomes sexually mature so that a female gives birth to her identical twin sister.

Conclusions

Commerical bovine embryo transfer has developed into a $20-million-a-year industry in less than a decade. Currently reproduction of fewer than 5000 cows in North America (<1 in 15,000) is being increased in this manner, although use of embryo transfer technology is increasing rapidly. Probably 20,000 calves will be produced by embryo transfer in 1980, and their genes or those of their sons will be greatly amplified by artificial insemination.

Techniques will continue to be simplified and efficacy will increase, especially for freezing embryos; consequently, costs will decline drastically. It will probably be decades, however, before embryo transfer will be used on a scale comparable to artificial insemination. Techniques of genetic engineering, which require in vitro manipulation of ova, will require embryo transfer for application.

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44. Two potential sources of bias in the data in Table 2 probably cancel each other. (i) Owners become discouraged with donors that do very poorly and remove them from embryo transfer programs before three superovulations, and (ii) owners remove donors that do exceedingly well before three superovulations because no more calves are desired (usually because of the expense). Comparison of the mean number of pregnancies from the first superovulation of this group, 3.4, with the mean for first superovulations of all normal, nonexperimental donors treated similarly, 3.3 (N = 110), indicates that the net bias is negligible.
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78. The cooperation of the commercial embryo transfer industry in providing data on volume of business is greatly appreciated, as is the information that H. D. Norman provided from the files of the National Cooperative Dairy Herd Improvement Program. Most photomicrographs were taken by J. F. Hasler. I acknowledge the contributions of my colleagues at the Colorado State University Embryo Transfer Unit, especially R. A. Bowen, R. P. Elsden, L. D. Nelson, and S. M. Seidel. Our research has been supported by Select Sires, Inc., Plain City, Ohio; the Upjohn Company, Kalamazoo, Mich.; and the Colorado State University Experiment Station through regional project W-112.