GENETIC ASPECTS OF EMBRYO TRANSFER AND EMBRYO MICROMANIPULATION

C.R. YOUNGS, P.E. HUMES and R.A. GODKE
Department of Animal Science
Louisiana State University
Baton Rouge, LA 70803 USA

SUMMARY

Genetic progress from any selection regime is dependent upon four factors -- the heritability of the trait, the generation interval, the selection intensity, and the accuracy of selection. Embryo transfer is not expected to affect genetic change by reducing the additive genetic variation of a trait or by substantially reducing the generation interval. The greatest impact of embryo transfer will be on increasing selection intensity and accuracy. Embryo micromanipulation techniques will likely have their most immediate impact via embryo splitting. However, gene injection and other embryo engineering technologies may offer unique opportunities to alter the genetic constitution of an animal population.

INTRODUCTION

Perhaps the greatest contribution made to animal agriculture by reproductive physiologists has been the technology of freezing bovine semen. That scientific advancement provided a means of utilizing superior male genotypes and enabled quantitative geneticists to devise selection schemes which resulted in rapid genetic improvement, particularly in dairy cattle. Now other advancements in reproductive physiology, embryo transfer related technologies, are being promoted as an additional means by which to make genetic improvement. The objective of this paper is to review a number of these techniques, their applications to animal breeding, and their potential impact on animal improvement.

PRINCIPLES OF GENETIC IMPROVEMENT

Genetic progress from selection within a closed population is dependent upon four factors (Bradford and Kennedy, 1980):

(1) the additive genetic variation within the population for the trait(s) of interest (heritability)

(2) the length of time between the birth of the parents and the birth of the offspring which replace them in the breeding population (generation interval)

(3) the proportion of the population selected as the parents of the next generation (selection intensity)

(4) the ability of the breeder to accurately identify the genetically superior animals to be the parents of the next generation (accuracy of selection)

Theoretically, the only way to realize genetic improvement due to selection would be to alter one or more of these factors affecting genetic progress.

According to animal breeding theory, selection should lead to a reduction in genetic variability within a closed population as compared with a random mating population. Through selection, animals retained as replacements resemble each other more closely than the average of the population from which they were selected. Over time, the result should be a reduction in genetic variability due to these matings. However, this has not occurred in large animal populations, likely because of the large numbers of genes and gene complexes which control the expression of quantitative traits. Embryo transfer is not expected to have a measurable influence on reducing genetic variability, except perhaps in small populations. Care should then be taken to avoid inbreeding.

Reducing the generation interval would lead to increases in genetic improvement. However, the only means to accomplish this which is not also possible through conventional breeding programs involves the use of prepubertal females. At present, there are no consistent and reliable field techniques for oocyte maturation, in vitro fertilization, and long term embryo culture for all the major livestock species. An alternative approach, inducing estrus and ovulation in prepubertal females, also does not seem feasible on a routine basis. Furthermore, not all traits of economic importance can be assessed prior to puberty. Thus, the expected impact from a reduction in the generation interval from embryo transfer is neglible.

The increase in selection intensity which can be achieved is directly related to the number of offspring which can be obtained from each female during each breeding season. This is the area where embryo transfer will likely have its greatest genetic impact, especially in species which bear a low number of offspring per pregnancy. This can be demonstrated with the following example in beef cattle.

If a breeder's goal is to produce 100 calves annually, this would require approximately 111 cows in a conventional breeding program (0.9 calves/cow) but only 10 donor cows in an embryo transfer program (10 calves/donor). Based on a generation interval of five years, approximately 20% of the cow herd would be replaced each year. This would mean an annual replacement of 22 cows in a conventional breeding program, whereas only two donor cows would be replaced when embryo transfer is used. Thus, considerably more selection pressure can be applied in an embryo transfer program (retaining 5% of heifers) than in a conventional breeding program (retaining 40% of heifers). This principle can be shown numerically using yearling weight in beef cattle as an example.

As the proportion of animals saved to be parents of the next generation increases, the superiority of the female replacements over their contemporaries increases a proportionate amount. Table 1 lists this superiority at various selection intensities (assuming that yearling weight has a standard deviation of 40 kg).

If 5% of the heifers produced are retained as replacements in an embryo transfer program, the selection differential is 82.0 kg. This is more than twice as large as the 38.7 kg selection differential that would be present in a conventional breeding program where 40% of the heifers are saved for replacements.

Selection of male parents also contributes to genetic progress. If it is assumed that bulls selected to sire the next generation are in the top 1% of the males (which is likely with AI bulls), the selection differential for males would be 105.6 kg. Thus, the combined selection differential would be 93.8 kg with embryo transfer and 72.2 kg without embryo transfer. If it is further

TABLE 1. SELECTION DIFFERENTIALS FOR BEEF YEARLING WEIGHT AT VARIOUS SELECTION INTENSITIES

Proportion Saved (%)	Selection Differential (kg)
90	7.8
80	14.0
70	19.9
60	25.8
50	31.9
40	38.7
30	46.5
20	56.1
10	70.3
5	82.0
1	105.6

assumed that the heritability of yearling weight is 0.40, then the expected improvement in yearling weight after one generation would be 37.5 kg with embryo transfer compared with 28.9 kg without embryo transfer. Thus, an increase in yearling weight of 29.9% could be obtained by utilizing embryo transfer.

Genetic progress is also affected by the accuracy of the breeder's selection decisions. Accuracy may be defined as the correlation between the estimated breeding value of an individual and its true breeding value. If the breeding value of an animal is estimated from its own performance, then the accuracy is the square root of the heritability of the trait in question. So for a trait with low heritability, selection should not be based solely on the individual's own performance. This is especially true for cows to be used in an embryo transfer program, as the impact of individual donor cows on the next generation is several times greater than for individual cows in a conventional breeding program.

The best way to increase the probability that the estimated breeding value of the selected animal is highly correlated with its true breeding value is by using information on related individuals, since relatives have genes in common with the animal of interest. Table 2 gives the genetic relationships between an individual and its relatives along with the accuracy of selection for various levels of heritability and information from relatives.

Embryo transfer could greatly assist in identifying superior females through progeny testing. From one year's embryo collections, progeny tests could be conducted on young prospective donor females, thus enabling more accurate selection. In addition, embryo transfer has also been used to perform a progeny test to screen for genetic abnormalities (Johnson *et al.*, 1980).

Embryo transfer may also have an additional impact by producing offspring from females that are diseased (non-genetic causes), injured, or otherwise unable to normally maintain a pregnancy. For example, it has been reported that donor cows seropositive for brucellosis present minimal risks of disease transmission when their embryos are transferred to seronegative recipients (Voelkel *et al.*, 1983). Previously, brucellosis-positive animals were sacrificed and their potential genetic contribution was lost.

TABLE 2. GENETIC RELATIONSHIPS AND ACCURACY OF RECORDS FOR VARIOUS GROUPS OF RELATIVES FOR ESTIMATING BREEDING VALUES OF AN INDIVIDUAL

Relatives	Number	Genetic Relationship	Heritability		
			0.10	0.30	0.50
Individual	1	1.00	0.32	0.55	0.71
Parent	1	0.50	0.16	0.27	0.35
Half-sibs	4	0.25	0.15	0.25	0.30
	40	0.25	0.36	0.44	0.46
Progeny	10	0.50	0.45	0.67	0.77
	40	0.50	0.71	0.87	0.92

EMBRYO MICROMANIPULATION

One of the most widely utilized embryo engineering technologies is that of embryo splitting. Several simple and efficient methods for splitting bovine morulae and blastocysts have recently been reported (Ozil et al., 1982; Williams et al., 1982; Lambeth et al., 1982). These methods involve making a rent in the zona pellucida and bisecting the embryo into halves with either a metal blade or a fine glass needle attached to a micromanipulation unit. More recently, Rorie et al. (1985) described a simplified, less costly technique for splitting farm animal embryos using a hand-held razor blade. Genetically identical individuals produced by microsurgery techniques can be utilized in a wide variety of research areas, but their practical applications to animal improvement should not be overlooked.

The most obvious benefit of embryo splitting is to obtain an even greater number of offspring from a given female. Selection intensities for females could theoretically approach those of males with the halving or quartering of embryos, this even with a reduction in pregnancy rates following transfer of split-embryos. For example, 100 good quality embryos transferred to recipients may result in 65 embryo transplant calves, whereas 100 similar quality embryos divided into halves may yield 110 transplant calves. Splitting should not, however, be used to generate additional offspring for progeny testing, especially when screening for genetic defects. This is because split-embryo twins are merely copies of the same genotype.

Another use of embryo splitting could be in the assessment of animals' performance for traits where individuals are typically sacrificed (e.g., carcass traits). Few breeders would slaughter their fastest growing, most feed efficient animal to measure loin eye area or ham weight. However, splitting allows for one monozygotic twin to be sacrificed for the assessment of carcass characteristics while the remaining twin could be saved for breeding purposes. Such use of twins could provide for greater accuracy of selection (by using information on the individual in addition to that from relatives) and for reduced generation intervals (by circumventing progeny tests).

For species where artificial insemination is not widely practiced, an additional application of embryo splitting exists. If, for example, three or four genetically identical males were produced by embryo micromanipulation, each one could be placed in a different environment and be used as "reference sires", thus enabling among farm comparisons of sires.

Intra- or inter-species inner cell mass transfer (Butler et al., 1985) is another way to exploit rare or valuable genotypes. In this procedure, the inner cell mass of one specie could be placed within the trophectoderm of a second, with the second specie serving as the recipient female. The fetus originates from the inner cell mass, while the trophectoderm layers develop into the placental membranes. This might allow, for example, a Mouflon sheep to be born to a domestic goat. This technique would most likely be used to propagate endangered species or to increase the numbers from an animal with a valuable genotype.

The microinjection of DNA into the pronucleus of an activated ovum is one technique with great potential for making genetic improvement. Injection of the metallothionein-growth hormone gene into mice oocytes resulted in an 80% increase in body weight when the transgenic mice were fed a high-zinc diet (Palmiter et al., 1982). Similar major genes such as the Booroola gene (Piper and Bindon, 1982), the wool keratin gene complex (Ward, 1982) or the foot rot resistance gene (C.F. Parker, personal communication) could be microinjected into activated ova of sheep which do not possess the trait of interest. At present, however, gene injection attempts with farm animals have only met with limited success (Hammer et al., 1985). Depending upon the species, there can be problems with the visualization of the pronuclei, integration of the desired number of copies of the gene, and lack of expression of the integrated gene. In addition, microinjection may lead to mutations, and integrated genes are not always stably incorporated into the germ line.

Sperm cell microinjection methodology (Markert, 1983) offers the potential to obtain androgenetic offspring (Seidel, 1982). One example of this might be to microinject two sperm cells (from either the same or different males) into an activated ovum, followed by the removal of the endogenous pronuclei. This leaves only the male genetic material to be incorporated into the zygote. Such a technique should result in 2/3 male offspring and 1/3 female offspring, since the YY genotype is lethal. The possibility of producing offspring without a female genetic contribution could dramatically change the bull stud industry.

Another technique similar to the production of androgenetic offspring is gynogenesis, reproduction utilizing the female genome but without the contribution of a male genome (Seidel, 1982). No parthenogenetic large mammal has ever been scientifically verified, but parthenogenetic activation of mouse ova and subsequent birth of offspring have been reported (Hoppe and Illmensee, 1977). For example, an ovum could be activated by the penetration of a sperm cell, followed by the removal of the male pronucleus. The ovum could be cultured in the presence of cytochalasin B to cause the formation of a homozygous diploid ovum, and normal cell division would then be assumed to continue. If homozygous individuals were produced in this manner, they could then be utilized in crossbreeding schemes to exploit heterosis.

CONCLUSIONS

The use of embryo transfer offers the opportunity for more rapid genetic improvement than is possible with conventional breeding programs. Embryo engineering technologies may also afford additional methods by which to make genetic change. It appears that continued research will likely enable further applications of these new technologies to farm animal species. However, the number of breeders who will utilize these technologies remains to be seen.

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