Animal Biotechnology

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According to Webster's dictionary, biotechonology is, simply, "applied biological science" (Webster's, 1986). However, a more accurate definition of the emerging field of biotechnology would probably be similar to that given for NDSU's recently established undergraduate biotechnology program: "Biotechnology is an interdisciplinary field based on a combination of biology and technology." (NDSU Bulletin, 1990-92). It is appropriate, therefore, that biotechnology and related research are becoming a major thrust at many state agricultural experiment stations, which have emphasized applied research aimed at improving efficiency of agricultural production since their inception in the late 1800s (Kerr, 1987).

Examples of techniques currently being used in biotechnology research include cloning of specific genes, transfer of genes between or within species, and culture of cells and embryos. These technique have been successfully applied in several instances. For example, in the late 1970s, the human gene for insulin was succesfully transferred into bacteria, which then produced large quantities of the hormone (Watson et al., 1983). Because of its purity and ease of production, this "recombinant" insulin has become the standard insulin used to treat diabetes in humans (Gilman et al., 1985).

Another example of the potential of applied biotechnology is gene therapy. In gene therapy, a genetic disease caused by a defective gene producing a defective enzyme or hormone would be treated by transferring the correct gene into a patient's own cells, which then would provide the correct enzyme or hormone (Culliton, 1989; Friedmann, 1989). This approach to treatment of genetic diseases has recently been approved for clinical evaluation on a limited basis (Culliton, 1990). In addition to medical uses, application of biotechnology to agriculture was almost immediately recognized as having tremendous potential for economic benefit (Watson et al., 1983). For instance, one can envision transfer of disease-resistance or growth-promoting genes into crop plants or farm animals. Recognizing this potential, in 1985 Congress specified that a major portion of the funds in the U.S. Department of Agriculture's Competitive Grants Program would be used to support agricultural biotechnology research (Kerr, 1987).

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SPECIFIC EXAMPLES

Because of its potential for enhancing both the rate and efficiency of growth, transfer of the gene for growth hormone has been widely studied. The first successful transfer of a growth hormone gene into a mammalian embryo was reported by Palmiter and coworkers (1982, 1983). In these studies, early mouse embryos were injected with either the rat or human gene for growth hormone. Mice that were born with these genes, termed "transgenics," grew at four times the rate of normal mice during their maximal growth phase. Encouraged by this success in mice, researchers subsequently have transferred human or bovine growth hormone genes into early embryos to produce transgenic sheep, cattle and pigs (Hammer et al., 1985, 1986; Pursel et al., 1989; Roschlau et al., 1989).

These experiments have met with some success, especially in pigs. In pigs, transfer of the bovine growth hormone gene resulted, in some cases, in improvements in daily weight gain and feed efficiency for two successive generations (Pursel et al., 1989). These studies, however, also have had several drawbacks. For instance, the percentage of attempted gene transfers that have been successful in large domestic animals has ranged from 0.1 to 6.9 percent (Hammer et al., 1986; Pursel et al., 1989; Roschlau et al., 1986). In other words, for every 1,000 embryos injected with the gene, only 1 to 69 offspring which actually carried the new gene would be born.

In addition, the beneficial effects of gene transfer may be offset by serious side effects. For example, even though pigs with the bovine growth hormone gene grew faster with improved feed efficiency, they also showed increased incidence of arthritis, cardiac disease, dermatitis, gastric ulcers, renal disease and reproductive failure (Pursel et al., 1989). Thus, better understanding of regulation of gene expression, improved methods for gene transfer and perhaps even modified methods of husbandry will be required before the benefits of gene transfer will be realized in production of transgenic farm animals.

To date, the most successful method for gene transfer in large domestic animals has been microinjection of the gene directly into early embryos (Pursel et al., 1989; Roschlau et al., 1989; Sirard, 1989). Because of the need for large numbers of embryos, which must be manipulated in vitro (in a petri dish) and then successfully transferred to recipient animals, gene transfer has provided impetus for advances in technology of egg manipulation and embryo transfer. For example, it is now possible to stimulate growth of many ovarian follicles at the same time in a given cow, obtain the oocyte (egg) from each of these follicles either before or after ovulation, and fertilize these oocytes in vitro, to produce

many embryos which are synchronized at a specific stage of development (Greve et al., 1989; Sirard 1989). These techniques not only are useful for gene transfer but also are beginning to be applied in embryo transfer programs, with the goal of obtaining many offspring from outstanding dams. Development of these techniques is a good example of how the efforts of many dedicated researchers over many years will eventually benefit animal agriculture.

Another example of applied animal biotechnology comes from NDSU's laboratories. Induction of superovulation by using gonadotropic hormones is the most widely used technique for obtaining multiple eggs or embryos from cows (Lerner et al., 1986; Hasler et al., 1987). The usefulness of this technique, however, is limited by the variability of the superovulatory response among cows (Hasler et al., 1983, Lerner et al., 1986; Saumande et al., 1985). That is, during a given hormone treatment, some cows may respond well, whereas other cows do not respond at all. This lack of response in some cows has been estimated to cost the embryo transfer industry millions of dollars annually in the U.S. alone. Recently, NDSU researchers have developed a treatment regimen wherein all cows tested to date have responded well to treatment in terms of superinduction of follicular development, number of ovulations and number of embryos obtained (Tables 1 and 2). Because of the cost of superovulation regimens, this technique, which provides a means of obtaining a consistent superovulatory response in all cows, may be of tremendous value for use in gene transfer and embryo transfer programs. Development of this technique was only possible because a base of information built from many years of work by numerous investigators was available.

Table 1. Superinduction of follicular development in cows.[a]

Cow	Follicular size classes[b] 6 - <10	≥10[c]
06W7	12	11
896	17	7
14T1	9	5
06W1	19	20
437	15	11
454	6	6
02P1	14	5
42S1	9	14
02W7	10	19
09W1	3	9
12R1	2	3
453	11	9
342	12	8
22T1	3	8
05W1	10	6
09U7	2	9
Mean ± SE	9.8 ± 1.3	9.4 ± 1.2

[a]Redmer, Kirsch and Reynolds (unpublished, 1990). Cows used in this study were mixed beef breeds, including Hereford, Angus, Simmental, Shorthorn and crossbreds.

[b]Surface diameter (mm) of follicles for both ovaries from each cow. Ovaries were obtained at slaughter during the superovulatory treatment regimen.

[c]Most cows exhibit only one follicle greater than 10 mm at a time.

PROSPECTS FOR THE FUTURE

Better methods for transfer of genes into embryos have been developed for rodents and are beginning to be developed for domestic animals (Pursel et al., 1989; Cherfas, 1990). These improved methods not only are more efficient but also result in expression of the gene only by appropriate tissue or organs, which should minimize the side effects and also improve the ability to regulate function of these genes. In addition to transfer of genes into embryos, the techniques described for gene therapy could be used not only to treat genetic diseases but also to transfer beneficial genes, such as growth-promoting or disease-resistance genes, into young or even adult animals. These techniques have already been used to transfer "marker" genes into adult dogs and pigs (Wilson et al., 1989; Nabel et al., 1989).

The ability to transfer a beneficial gene into a growing or adult animal would allow for "designing" animals with specific traits suited to a given situation. Another gene transfer approach is targetting genes to the mammary glands so that the resulting transgenic animals would secrete a specific product in their milk. This approach already has been used successfully to obtain human proteins in milk from sheep (Cherfas, 1990). As with any gene transfer technology, these methodologies will not be practical until much more is learned about regulation of gene expression as well as husbandry of transgenic animals. In addition, we must always consider not only the potential benefits but also the consequences of gene transfer, since application of these techniques will greatly accelerate the rate at which characteristics of animals can be manipulated. To do so without much thought and careful investigation would be unwise and counterproductive.

An area closely related to gene transfer is gene mapping. Although quantitative geneticists and animal breeders have

Table 2. Induction of superovulatory response in cows.[a]

Cow	Laparotomy/Surgery Data Day after estrus[b]	No. of ovulations[c]
24x1	7	31
I17	8	19
M15	7	19
M6	7	20
28x1	7	23
H10	7	25
W12	7	34
P9	10	23
32	7	26
BT27	8	22
37	8	19
Mean ± SE	7.4 ± 0.3	23.7 ± 1.5

[a]Redmer, Kirsch and Reynolds (unpublished, 1990). All cows used in this study were Angus.

[b]Day on which laparotomy/surgery was performed after cows exhibited estrus (day 0) in response to superovulatory treatment regimen.

[c]Number of ovulations was determined by counting the number of corpora lutea at laparotomy/surgery.

been mapping genes at a relatively slow pace for many years, techniques termed restriction fragment length polymorphisms and *in situ* hybridization have greatly accelerated the rate at which specific genes can be mapped to specific chromosomes. Excitement over application of these techniques led to an international conference entitled "Mapping

Domestic Animal Genomes," which was held at the University of Illinois in April of this year. Mapping of genes will allow researchers to learn much about specific functions of genes, relationships among genes and specific traits, regulation of gene expression and causes of genetic diseases. In addition, mapping of domestic animal genomes will make more genes available for gene transfer. Mapping of all of the genes of domestic species could feasibly be accomplished within the next few decades.

Many other areas within animal biotechnology are advancing rapidly, such as cloning of embryos, development of recombinant hormones, and development of subunit vaccines and diagnostics. These techniques, developed through biotechnology, also are tremendously powerful tools for use in further research. Since the time between basic research discoveries and their practical application is shortening rapidly (National Academy of Sciences, 1989; Cherfas, 1990), it is important to continue support for the state agricultural experiment stations in their efforts in biotechnology. In fact, increased support for basic and applied agricultural research has been cited as being critical if the U.S. is to remain competitive in the world market (Holt, 1987; Reynolds, 1987; Redmer et al., 1988). It is likely that biotechnology and related research will play an ever increasing role in maintaining the economic vitality of animal agriculture in the future.

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