Chapter 4

Emerging Technologies in the Dairy Industry

Contents

Box

Figures

Tables

Chapter 4

Emerging Technologies in the Dairy Industry

Bovine somatotropin (bST) is the first major biotechnology product developed for the dairy industry. This product has been controversial and has raised many scientific and socioeconomic questions. BST, however, is not the only new technology that will affect the dairy industry. Advances in animal reproduction, animal health, and food processing are occurring, and many of the new technologies being developed use highly sophisticated and complex biotechnology methods. By comparison, the biotechnology methods used to produce bST are actually rather rudimentary; potentially some of these new technologies could make bST obsolete. These new technologies will increase the level of management skills needed to use them effectively; new information technologies will aid the decisionmaking process. Several conclusions can be drawn concerning the development of these new technologies:

- The dairy industry is on the verge of a technological revolution. Biotechnology methods that are more advanced than those used to produce bST are currently under development. The impact of these technologies, in conjunction with new information technologies in the not too distant future may rival that of bST. Assessment and analysis, similar to that of bST may be warranted for many of these technologies.
- The field of animal reproduction is advancing rapidly. Researchers 'have significantly improved their understanding of egg development in the ovary, how to stimulate the release of numerous eggs at once, and how to achieve fertilization and development of eggs outside of the cow. Embryos can be frozen for later use. Both embryos and sperm can be sexed. It *is also* possible to create multiple copies of an embryo, each of which can be transplanted into a cow whose reproductive cycle has been adjusted to be able to accept the embryo and carry it to term. These new technologies make it possible to improve herd quality more rapidly than can be achieved using traditional breeding *methods*.
- [t is now possible to create transgenic[1] cattle, however, the techniques currently used are inefficient and require the use of thousands of eggs to produce just one transgenic animal. These inefficiencies make it too expensive to commercially produce and market transgenic livestock. However, scientific breakthroughs are leading to the development of technologies that will improve the efficiency of transgenic animal production and substantially lower the cost of doing so. Transgenic livestock may become commercially available in small numbers by the end of the decade.
- BST potentially could be supplanted by the development of transgenic cattle. Dairy cows can be developed to produce higher levels of bST so that daily injections or timed release formulations are no longer needed. Alternatively, genes that code for chemicals that suppress bST production can be altered in the cow such that a cow's normal bST production will increase.
- New biotechnology products are also being developed to improve animal health. Products include new vaccines and diagnostic kits, as well as compounds that enhance an animal's ability to fight disease.
- Not only are new biotechnology products being developed for use in livestock production, but they are also being developed for use in food processing. New products will improve the production of milk products such as cheese and yogurt. They can also be used to detect milk contaminants.
- Effective use of these new technologies will place a premium on management skill. New information technologies are being developed to aid farm management. These new technologies can incorporate individual farm data, with pertinent information from national databases, into computer programs that will aid farmers in the decisionmaking process.

This chapter describes information systems, and biotechnology methods and products that have been developed, or are expected to be developed for use

[1]Animals whose hereditary DNA has been augmented by the addition of DNA from a source other than parental germplasm using recombinant DNA techniques.

in the dairy industry in the decade of the 1990s. Many of these technologies are highly sophisticated, cutting-edge technologies, and as such have not been extensively discussed in the lay literature. However, it is important that the potential of these technologies be understood. Given the nature of these new technologies, the following descriptions are, by necessity, somewhat technical.

BIOTECHNOLOGY AND THE DAIRY INDUSTRY

The term biotechnology refers to a wide array of techniques that use "living organisms (or parts of organisms) to make or modify products, to improve plants or animals, or to develop microorganisms for specific uses' (46). Under this broad definition, biotechnology includes many long-practiced dairy technologies such as animal breeding and cheese-making. New biotechnologies include recombinant DNA techniques, cell culture, and monoclinal antibody (hybridoma) methods (see box 4-A). The application of these new methods to the dairy industry has already generated a number of products for improving milk production, animal health, and food processing, and will continue to do so. Products now emerging range from rapid diagnostic tests for contaminants in dairy products to cows genetically engineered to produce high-value pharmaceuticals.

Reproductive Technologies and Transgenic Animals

Animal scientists generally agree that the most important cause of economic loss in the animal industries results from reproductive inefficiency

Box 4-A—Definitions of Commonly Used Terms

Antibody: Proteins produced by specific white blood cells (i.e., B lymphocytes) that bind specifically to foreign antigens in the body.

Antigen: Any substance that elicits a defensive (immune) response.

Cell **culture: The** growth and maintenance of cells derived from multicellular organisms under controlled laboratory conditions.

Chromosome: A thread-like structure composed primarily of DNA, that carries the genes which convey hereditary characteristics; in mammals chromosomes are contained in the nucleus and the X chromosome conveys female characteristics and the Y chromosome the male characteristics.

DNA (deoxyribonucleic acid): The molecule that is the repository of genetic information in all organisms (with the exception of a small number of viruses in which the hereditary material is ribonucleic acid-RNA).

Estrus: The period during which a female mammal is receptive to sexual activity.

Estrous cycle: The period of time needed for the reproductive cycle that includes egg maturation and ovulation in the ovary and preparation of the uterus to receive fertilized eggs. This cycle is under hormonal control and extends from the beginning of one period of estrus to the beginning of the next.

Hybridoma: A new cell resulting from the fusion of a particular type of immortal tumor cell line, a myeloma, with an antibody-producing B lymphocyte. Cultures of such cells are capable of continuous growth and specific antibody production

In vivo: Within the living organism.

In vitro: Outside the living organism and in an artificial environment, e.g., test tube.

Monoclinal antibodies: Identical antibodies that recognize a single, specific antigen and are produced by a clone of specialized cells.

Recombinant DNA: A broad range of techniques involving the manipulation of the genetic material of organisms; term is often used synonymously with genetic engineering; term also used to describe a DNA molecule constructed by genetic engineering techniques composed of DNA from different individuals or species.

Transgenic animals: Animals whose hereditary DNA has been augmented by the addition of DNA from a source other than parental germplasm using recombinant DNA techniques.

Uteri: The plural for uterus—the organ of the female mammal for containing and nourishing the young during development prior to birth.

SOURCE: Office of Technology Assessment, 1991.

(i.e., low conception rates and embryo mortality). The field of animal reproduction is currently undergoing a scientific revolution. In the 1986 report *Technology, Public Policy, and the Changing Structure of American Agriculture,* OTA predicted that, beginning about the year 2000, eggs matured and fertilized in vitro and transplanted to a recipient animal (artificial inembryonation) would in part, replace natural and artificial insemination in the animal breeding system, and embryos altered by recombinant DNA techniques (transgenics) would be commercially available. In fact, embryos produced by new reproductive methods are currently being marketed, although at present no transgenic embryos are commercially available. However, significant new advances are occurring, and the direction and timetable of developments will almost certainly be affected. This section focuses on recent advances in reproduction and recombinant DNA techniques and their application to the dairy industry during the decade of the 1990s.

It is now possible to select genetically superior females and induce them to shed large numbers of eggs (superovulation) that can be matured in vitro and fertilized with sperm from males selected for their desirable traits. The resulting embryos can be sexed, split to make duplicate copies, and stored frozen until needed. An embryo can then be transferred by nonsurgical techniques into the uterus of a recipient animal whose estrous cycle has been synchronized with the stage of development of the embryo. These new reproductive technologies can, and are being used to improve the quality of livestock herds more rapidly than could be achieved with traditional breeding, although currently many of these technologies are still relatively inefficient.

The ultimate goal of many workers in the field of animal biotechnology, however, is to produce animals whose hereditary DNA has been augmented by the addition of DNA from a source other than parental germplasm, using recombinant DNA techniques (transgenic animals) (47). Transgenic animals can be created that possess traits of economic importance including improved disease resistance, growth, lactation, or reproduction.

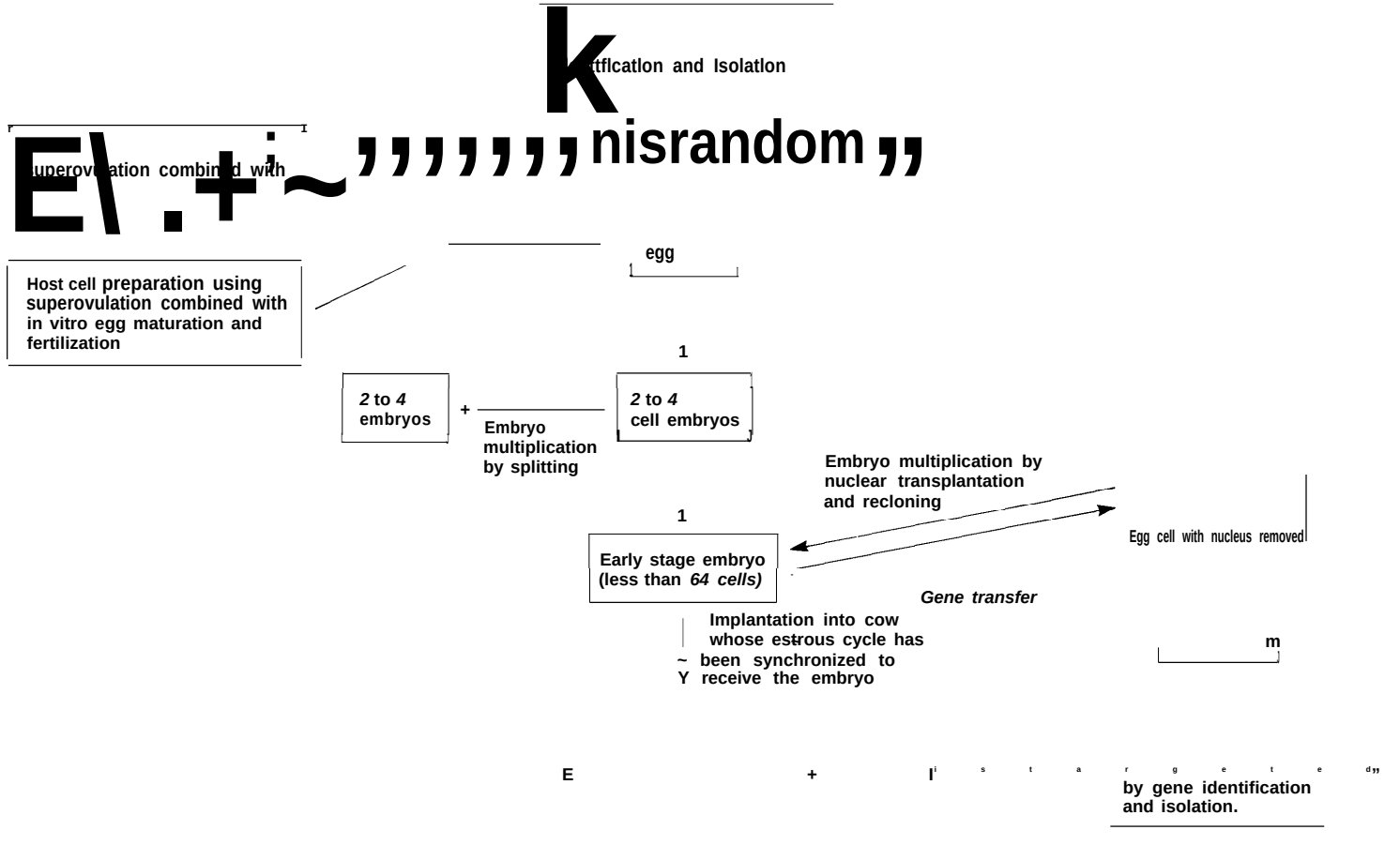
Transgenic livestock may also prove to be effective factories for the production of high-value pharmaceuticals, a development particularly pertinent for the dairy industry *(9).* Genes that code for animal proteins (e.g., tissue plasminogen activator-TPA) can be linked to regulatory sequences that direct high levels of gene expression in the mammary gland. This enhanced expression results in increased secretion of the desired animal protein into the milk of lactating females. This protein could then be extracted and purified from the milk. Transgenic cows producing pharmaceuticals have not yet been reported, but these animals are under development in a number of public and private laboratories. High levels of milk production coupled with the ease of milk collection may make this production method more cost effective than the cell culture systems currently used in the production of certain pharmaceutical proteins.

The production of transgenic animals is inextricably linked to development of new reproductive technologies. It is impossible to produce animals containing foreign DNA in their germlines without first manipulating the embryo and transferring it to a recipient animal. New reproductive technologies such as superovulation, in vitro egg maturation and fertilization, nuclear transplantation, and embryo sexing can be used to upgrade livestock herds. However, when they are combined with recombinant DNA technologies (the identification, isolation, and transfer of selected genes), they provide opportunities to efficiently and cost effectively produce transgenic animals, and to rapidly improve livestock quality (see figure 4-l). Therefore, advances in reproductive technologies will be discussed within the context of their applicability to the production of transgenic animals, recognizing that they are in their own right powerful tools for livestock improvements.

Steps in the Development of Transgenic Animals

The production of transgenic animals is a complex process, and involves four major steps. First, the desired gene must be identified, isolated, and prepared for insertion into a fertilized egg. Second, the host cell must be obtained and prepared for gene insertion. In livestock, the host cells used are generally fertilized eggs or early stage embryos. Third, the desired gene must be transferred to the host cell. And fourth, the resulting recombinant embryo is duplicated, and the resulting multiple embryos are transplanted into recipient cows that have had their estrous cycles synchronized to receive the embryo. This duplication process allows for the production of multiple offspring of genetically superior animals. Advances in each stage, discussed

Figure 4-1—Reproductive Technologies Used To Produce Transgenic Animals



SOURCE: Office of Technology Assessment, adapted from J.P. Simons and R.B. Land, "Transgenic Livestock," J. *Reprod Fert.* Suppl. 34:237-250, 1987.

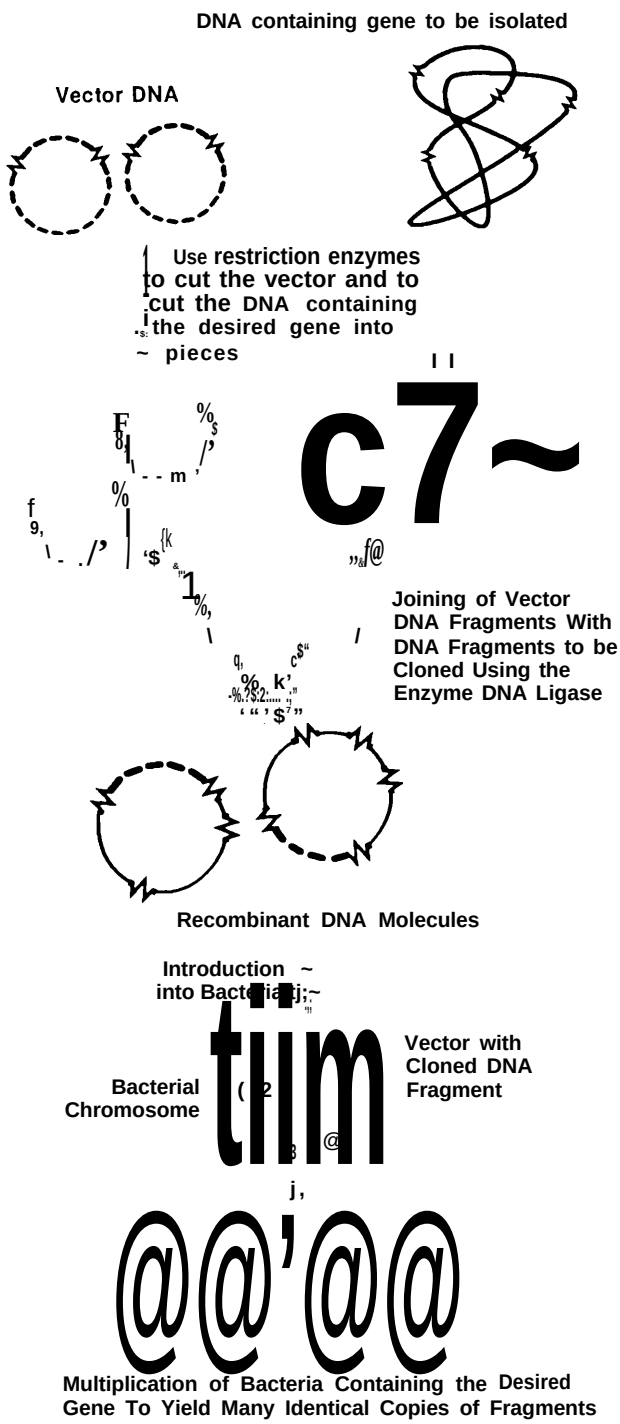
below, are improving the efficiency of transgenic animal production.

Step 1: Gene Identification and Isolation--Be-fore a foreign gene can be transferred into the genome of a host cell to create a transgenic organism, the gene must first be identified and purified. This is done by a process called cloning. The tools used to clone DNA include special enzymes that cut and paste DNA, nucleic acid fragments that can be used to identify specific DNA sequences (probes), vehicles used to carry a foreign gene into a host cell (vectors), and host cells that can be used to produce multiple copies of the gene. The host cells used to multiply gene numbers are usually bacteria. The vectors most commonly used are bacterial plasmids, circular pieces of DNA that are easily inserted into bacterial cells and are capable of independent replication inside the bacteria (see figure 4-2).

Isolating a single gene is complicated by the fact that a DNA sample may contain millions of genes. Researchers must be able to separate the one gene of interest from all of the other genes. To do this, the DNA sample containing the desired gene is cut into pieces with special enzymes (restriction endonucleases). The bacterial plasmid (vector) is also cut with the same enzyme. The pieces of sample DNA, including any pieces carrying the desired gene, are inserted into vectors and the loose ends of the sample DNA and bacterial plasmid are pasted together (using the enzyme DNA ligase). These recombinant DNA vectors are then inserted into bacterial cells, and the bacterial cells are grown. At this point the bacterial cells containing the plasmid carrying the desired gene must be identified and isolated from the rest. This is done by using a probe, a nucleic acid sequence that recognizes the desired gene. Once the bacterial cells containing the desired gene are found, those cells can be grown in isolation to produce millions of copies of the vector containing the desired gene. The vector can be removed from the bacterial cells, and the desired gene isolated. This procedure can yield millions of copies of the desired gene that are free from contamination by other genes (48).

The purified gene can then be combined with appropriate regulatory genes that control the circumstances under which the gene is turned on and off. The purified gene and its regulatory sequences can be inserted into a vector such as a virus, for example,

Figure 4-2—identification and Isolation of Desired Gene



SOURCE: Office of Technology Assessment, 1989.

that will carry these genes into an animal cell and incorporate them into the genome (see gene transfer technologies). The tools used to purify genes are well developed. The major challenge is determining which genes are to be purified.

Step 2: Preparation of the Host Cell—Because current technologies used to transfer a gene into a host cell are inefficient, to get just one transgenic animal requires the use of thousands of fertilized eggs. To attain such large numbers of embryos, cows must be induced to shed large numbers of eggs (superovulation) that can be matured and fertilized in vitro. Increased understanding of the control of ovarian functions is improving the efficiency of obtainin g sufficient numbers of fertilized eggs.

Control of Ovarian Functions-The **lack of** highly repeatable, efficient means for inducing superovulation is a major constraint. Induction of superovulation requires detailed knowledge of the hormonal factors that control the development of the egg in the ovary. The process of egg development has been subjected to intense investigation and a number of significant advances have been made during the past 5 years. Studies that have explored the basic mechanisms controlling egg growth and maturation, and corpus luteum[2] function, have paved the way for developing even more precise methods for regulating the estrous cycle, producing superovulation, and reducing the heavy losses due to early embryo deaths.

Perhaps the most important development in ovarian physiology in recent years is the discovery of the ovarian hormone inhibin, which decreases the ovulation rate.[3] Some breeds of animals with exceptionally high ovulation rates, such as the Booroola strain of Merino sheep in Australia, are known to have low levels of circulating inhibin (4). Cattle immunized against inhibin have lower circulating levels in their blood and show increased ovulation rates (21). The genes controlling inhibin production have been cloned and the potential exists for producing transgenic animals in which these genes are repressed or deleted.

Progress has also been made in understanding the control mechanisms that regulate corpus luteum function and its production of progesterone, a hormone that regulates the length of the estrous cycle and helps maintain pregnancy. This understanding paves the way for development of more precise methods for regulating the estrous cycle, which is needed to synchronize surrogate mothers, and for producing superovulation. Superovulation treatments are initiated when the ovaries are under the influence of progesterone. Currently, superovulation treatments use highly purified hormones produced by recombinant DNA technology and produce, on average, about 10 viable eggs per treatment (compared to the 1 egg a cow normally produces per ovulation) (21). As the new knowledge of the factors controlling egg development and corpus luteum function is applied, the number of viable embryos produced by each superovulation treatment is expected to increase.

In Vitro Maturation and Fertilization of Eggs—In vitro maturation and fertilization of eggs recovered by superovulation provides a means of overcoming the problem of livestock reproductive inefficiency. Normally, a bovine ovary contains about 50,000 immature eggs at puberty, however, on average only 3 to 4 of these eggs will result in the births of live calves during the animal's lifetime. Using present superovulation methods, about 10 viable eggs can be harvested from the ovaries of 1 treated cow and about half of these develop into embryos suitable for transfer. Improved superovulation technology may lead to the recovery of more eggs suitable for in vitro fertilization such that the number of live births resulting from a superior animal could be quite high.

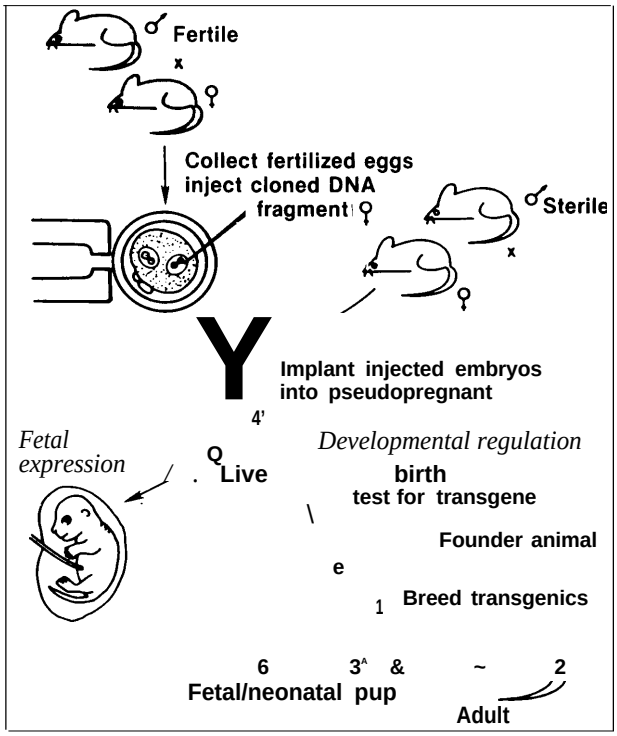
In vitro fertilization occurs only when a capacitated sperm (i.e., a sperm specially prepared to penetrate the egg cell membrane) encounters an egg at an optimal maturation state. Great progress has been made in understanding the factors involved in egg maturation and sperm capacitation in livestock. As a result, offspring have been produced in cattle, swine, sheep, and goats following in vitro fertilization (16) and attempts to market embryos produced with these techniques are already underway.

Step 3: Gene Transfer Technologies—Achieving the goal of transgenic animal production requires the development of efficient and cost effective ways to transfer the selected genes to an embryo. Mice were the first transgenic mammals created (see figure 4-3), and were produced by microinjecting

[2]The corpus luteum is a temporary endocrine organ that is produced at the site of ovulation during each estrous cycle.

[3]Inhibin decreases ovulation rates by suppressing the secretion of follicle stimulating hormone (FSH), a hormone produced by the pituitary gland.

Figure 4-3-Process of Producing a Transgenic Mouse



SOURCE: Sally A. Camper, Fox Chase Cancer Center.

cloned DNA into a fertilized egg[4] (32). Alternatively, viral vectors can be used to transfer cloned DNA into a host embryo (14). The embryo is then transferred to a recipient animal whose estrous cycle has been synchronized to accept and carry to term, the developing embryo.

A number of transgenic cattle, pigs, sheep, and chickens have been produced by these techniques, however, they are of limited use because of the high cost and low efficiency of microinjection techniques, and the absence of appropriate viral vectors for use in most livestock species. Additionally, DNA transferred by these methods is inserted randomly into the host genome, resulting in a lack of control of the gene transfer process (20).

Because of the deficiencies encountered with using viral vectors or microinjection methods to create transgenic livestock, alternative methods are being sought. A promising new method for generating transgenic animals has recently been developed in mice and may be applicable to other mammals. This new technique uses stem cells derived from an embryo. Stem cells are cells that are normally undifferentiated, that is, they do not become specialized tissue cells such as muscle, brain, liver cells, etc. However, stem cells retain their ability to become specialized cells when given the proper stimuli (i.e., they are pluripotent).[5] These stem cells can be used as vectors to introduce selected genes into a host embryo. This method has several significant advantages over microinjection methods, the most profound of which is that for the first time, it is possible to insert DNA at specific, predetermined sites within the genome of the stem cells (8). Targeted insertion is possible because stem cells have an intrinsic ability to recombine similar (homologous) DNA sequences, which results in the replacement of the endogenous gene with the desired gene.

Stem cells must first be isolated (see figure 4-4). An early stage embryo is cultured on a thin layer of specially prepared cells. The proliferating embryo cells are recultured until individual stems cells can be isolated. These individual stem cells can then be cultured indefinitely. At this stage, DNA sequences containing desired genes can be inserted into the stem cells.[6] A genetically transformed stem cell is then microinjected into an immature embryo to produce a chimera, an organism that contains cells from more than one source. If the stem cells are incorporated into the germ lines of these chimeric animals, then these animals can be interbred to obtain offspring that are homozygous for the desired trait (8).

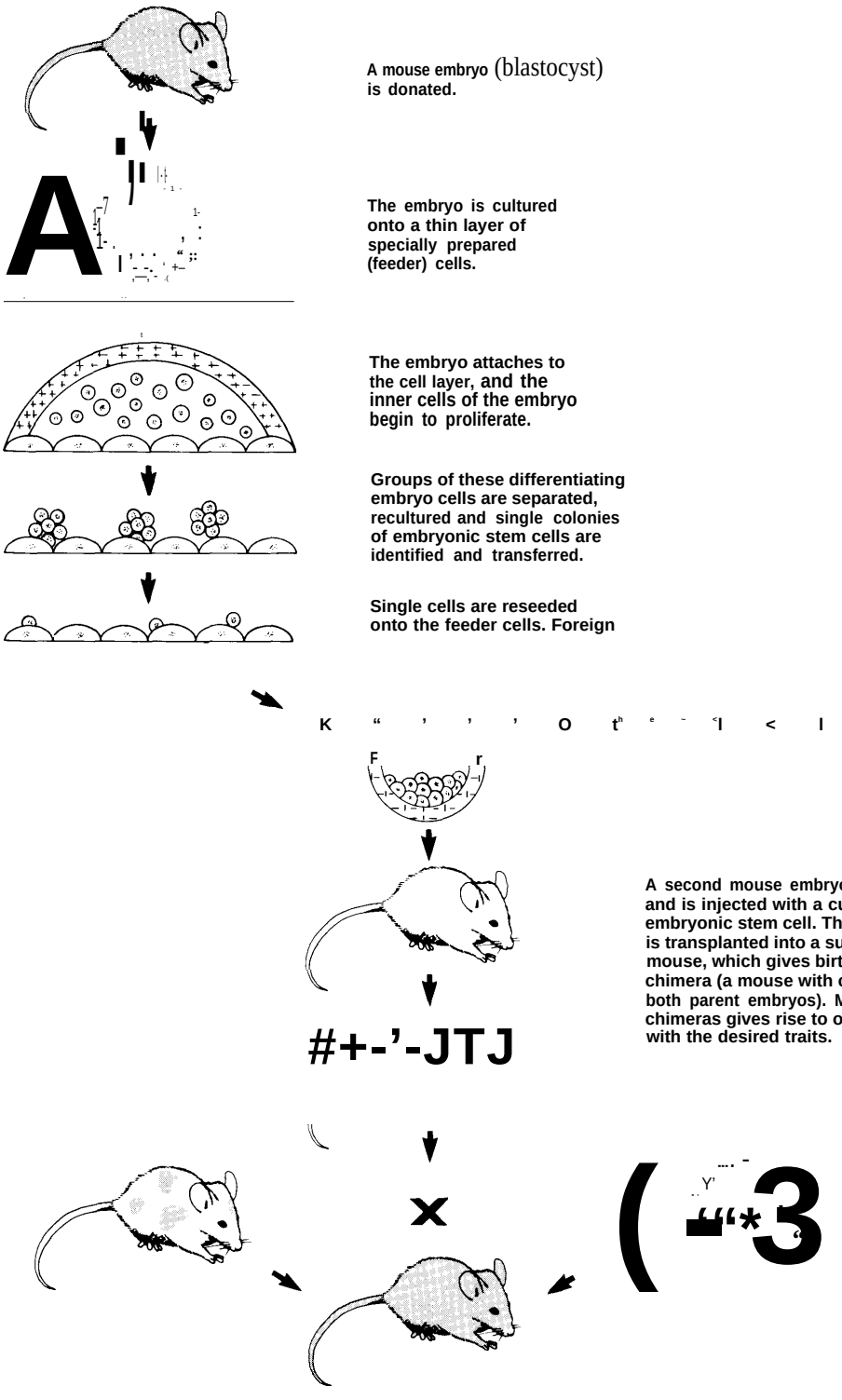
Application of the gene targeting method makes possible a broad range of phenotypes for transgenic animals that could not be produced economically using direct microinjection or viral vectors. Targeted gene insertion allows endogenous genes to be

[4]Specifically, the DNA is injected into the male pronucleus of the fertilized egg. The pronucleii are the egg and sperm nucleii Present after the sperm penetrates the egg membrane.

[5]Pluripotency help make stem cells attractive vectors of DNA transfer. While in tissue culture, DNA Cm easily be inserted into stem cells. When stem cells are injected into an early stage embryo, the conditions for tissue specialization are present, and stern cells undergo the normal tissue development that occurs as the embryo develops during pregnancy. Thus, using stem cells provides an efficient means to transfer DNA.

[6]Methods used include viral infection and use of an electric pulse to make cell membranes leaky (electroporation).

Figure 4-4—Gene Transfer Using Embryo Stem Cell Culture[a]



[a]This technique is currently developed only for mice and hamsters and possibly rabbits and swine. It is not yet developed for cattle.

SOURCE: M.R.Capecchi, "The New Mouse Genetics: Altering the Genome by Gene Targeting," *Trends in Genetics* 5:70-76, *1989.*

inactivated or replaced with modified forms of the gene, such as one that is expressed at a higher level, has anew pattern of tissue specific expression, or has a modified biological activity.

Perhaps the most significant advantage of the gene targeting approach is that it allows for endogenous genes to be effectively removed. Genes can be inactivated by targeting insertion into an essential region of the gene. This fact is of particular interest to the livestock industry, because inactivation of genes that have inhibitory physiological effects is likely to result in improvement in a number of productive traits. For example, bovine somatostatin is a hormone that inhibits bovine somatotropin production; inactivation of this gene would result in increased endogenous somatotropin secretion and, presumably increased milk production and more efficient growth. If successful, this technology would supplant the need to administer bST exogenously to increase milk production (see ch. 3). The genes controlling the production of inhibin, the ovarian hormone that reduces ovulation rate, is yet another example. The ability to inactivate genes also provides a powerful research tool that allows scientists to study the function of genes in vivo. The absence of a method to produce embryo stem cell lines from cattle is currently preventing the use of the gene targeting approach in the dairy industry,[7] however, a number of laboratories are trying to develop this technology.

Step* 4: *Embryo Multiplication-The production of multiple copies of a genetically engineered embryo is the final step in the development of transgenic animals. Efficient and inexpensive multiplication technologies are tremendously important for improving the efficient development of transgenic animals. Multiple copies of a mammalian embryo were first produced by physically splitting an early embryo into halves, giving rise to identical twins (21). If the embryo is divided more than twice, however, few offspring survive. Thus, no more than four identical animals can be produced by splitting and generally only two embryos are produced by this method. This procedure is already used in the cattle embryo transfer industry, nearly doubling the number of offspring produced.

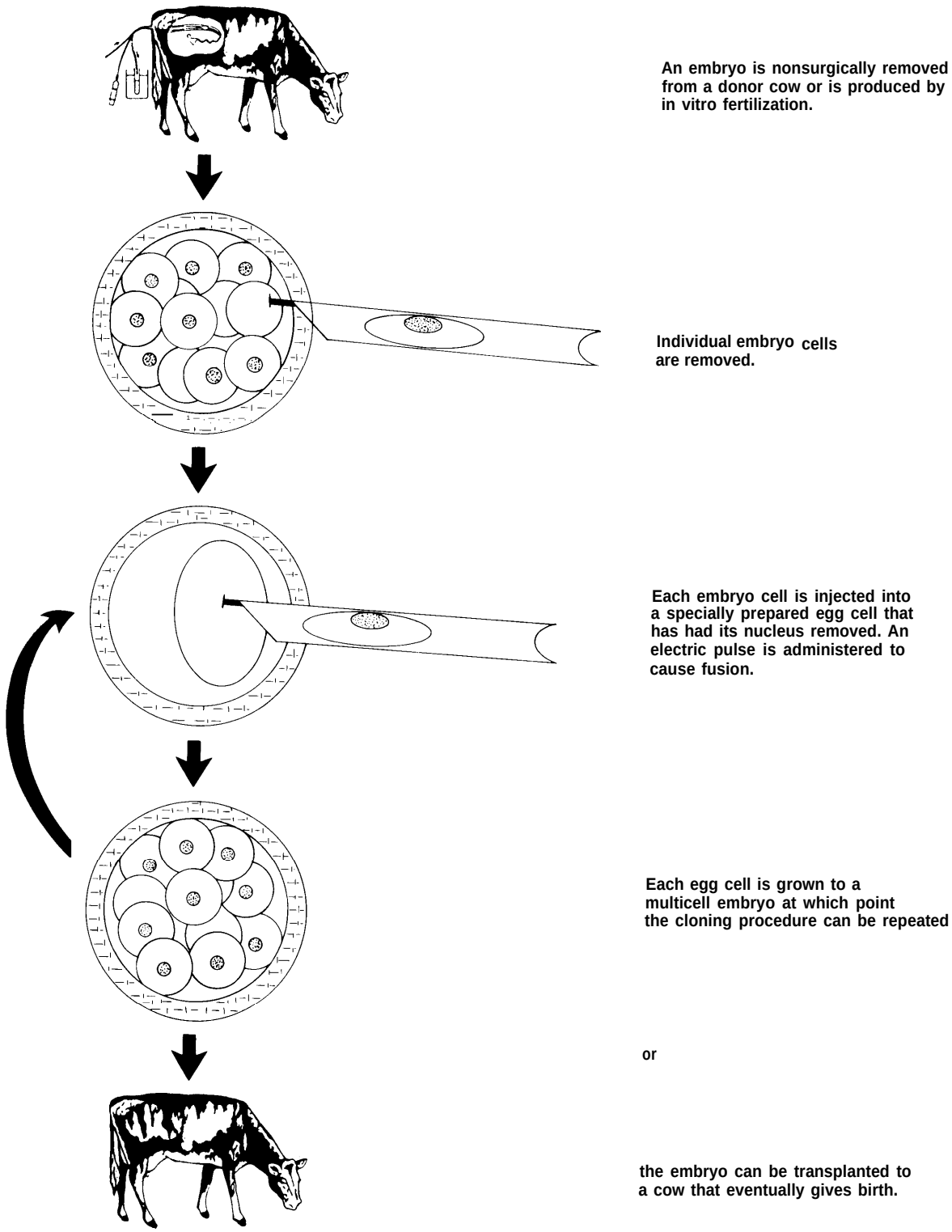
A more efficient and promising method of producing multiple copies of an embryo is by a technique called nuclear transplantation. Basically, the procedure involves the transfer of a nucleus from a donor embryo into an immature egg cell whose own nucleus has been removed. The recipient egg cell is activated by exposure to an electric pulse, allowed to develop into a multicelled embryo, and then used as a donor in subsequent nuclear transplantations to generate multiple clones. This procedure (outlined in figure 4-5) has been used successfully with cattle (7,35), sheep (42,49), and swine (36). Using this technique, hundreds of pregnancies have already been produced in cattle and recloning has been performed successfully resulting in as many as eight calves from one embryo (28).

The value of this technique to the dairy industry is enhanced by the ability to successfully transfer nuclei from frozen embryos into eggs whose nuclei have been removed. Conception rates obtained after transfer of embryos produced by nuclear transplantation are variable, but rates as high as 50 percent have been obtained. However, embryo losses after transfer are higher than normal, resulting in actual pregnancy rates ranging from 15 to 33 percent (7). Combining the techniques of in vitro fertilization, embryo cloning, and artificial estrous cycle regulation will likely result in major changes in dairy cattle breeding and in the rates of genetic improvement.

While significant advances in transgenic animal production have been made, it is unlikely that transgenic animals will be commercially available before the end of the 1990s at the earliest. The ability to produce transgenic cattle possessing traits of economic value is currently limited by the absence of embryo stem cell technology and the lack of knowledge about the relationship between the expression of a specific gene and the physiological consequences. While the techniques for isolating and sequencing bovine genes are now straightforward, understanding of the functions of the genes has lagged. Analysis of gene function is complicated by the fact that many traits are controlled by multiple genes. Thus, manipulation of such traits will require detailed understanding of these genes and their interactions. Ultimately identifying and understanding the physiology of the major genes controlling lactation, reproduction, and disease and stress resistance in dairy cattle is needed. An active genome mapping program could help enhance these developments.

[7]The stem cell technology has been developed for mice, titers, and swine.

Figure 4-5—Nuclear Transplantation



SOURCE: Office of Technology Assessment, adapted from R.S. Prather and N.L. First, "Cloning Embryos by Nuclear Transfer," *Genetic Engineering of Animals*, W. Hansel and B.J. Weir (eds.), *Journal of Reproduction and Fertility Ltd.*, Cambridge, UK, 1990, pp. 125-134.

Sexing of the Offspring

The availability of a technique to preselect the sex of the progeny is of great economic potential for the dairy industry where females are the major income producers and artificial insemination is already widely used. Until recently, none of the methods resulted in the degree of separation needed for commercial use. However, recent advances in the separation of the X and Y sperm, and sexing of the embryo have been made.

Separation of the X- and Y-Beating Sperm—It has long been a goal of mammalian physiologists to develop a method for effectively separating X and Y chromosome-bearing sperm to control the sex of the offspring. Most sperm separation techniques are based on potential differences in the size and density of the two sperm types.[8] These methods, however, have met with limited success (41).

Development of cell sorting techniques based on the differences in sperm size and fluorescence of sperm DNA (flow cytometric measurements) has provided the first effective method to sort the sperm cells. Johnson et al. (24) recently reported successful separation of intact viable X and Y chromosome-bearing sperm using this method (see table 4-l). Although the difference in DNA contents of the X and Y chromosome-bearing sperm in rabbits amounts to only about 3 percent, 94 percent of the rabbits (does) inseminated with X-bearing sorted sperm produced females and 81 percent of the does inseminated with Y-bearing sorted sperm produced males. Commercial use of this process is limited, at present, by the number of sperm that can be sorted per hour and by increased embryo mortality observed in the embryos produced after insemination with the sorted sperm. Neither of these factors is thought to represent an insurmountable difficulty.

Embryo Sexing—The most accurate method for sexing embryos is to create a picture of the number, size, and shape of the chromosomes contained in the embryonic cells (karyotyping). However, this method requires removal of about half of the cells of early-stage embryos, which decreases embryo viability and limits the number of embryos that can be transferred. Another method uses antibodies to detect proteins (antigens) unique to male embryos. This method is not damaging to the embryos and encouraging results have been obtained in one laboratory (2), however, the technique yields variable results and has not been widely adopted.

More recently, the sex of bovine embryos has been determined by using fragments of DNA that are contained only on Y chromosomes as a means of identifying the same DNA fragments in the embryo (6). Due to its chemical structure, a fragment of DNA will combine with a second DNA fragment that has a corresponding nucleic acid sequence. Therefore, a fragment of DNA that is specific to males can be used as a probe to identify male DNA fragments in the embryo. Combined with technologies that multiply the number of copies of the DNA fragments, this method determines the sex of the embryo using only a few cells. It is rapid (about 6 hrs) and extremely accurate, but may be overtaken by the rapidly developing technology, described above, for separating X and Y chromosome-bearing sperm.

Animal Health Technologies

Biotechnology is rapidly acquiring a prominent place in veterinary medical research. Initially applied to vaccine development, it most recently has contributed to efforts to develop diagnostic procedures and to improve detoxification systems.

Vaccines

Vaccines are agents that stimulate an effective immune response but do not cause disease. Traditional methods of vaccine development involved killing or modifying the pathogenic organism to reduce the potential for disease while preserving its ability to induce an immune response. Recombinant vaccine development involves either deletion or inactivation of genes necessary for disease, or insertion of immunizing genes into nonpathogenic vectors.

Gene deletion technology has been successfully used to develop both viral and bacterial vaccines. A naturally occurring mutant of E. coli, for example, has been shown to provide protection against gram-negative bacterial infections in cattle and swine (15,18). Live Salmonella modified to prevent

[8]Methods used are differential sedimentation techniques including differential velocity sedimentation free-flow electrophoresis, and Convection counter streaming galvanization.

[9]The antibodies are attached (labeled) to a fluorescent compound to allow for detection.

Table 4-1—Predicted and Actual Sex Ratios of Offspring After Intrauterine Insemination of Sorted X and Y Chromosome-Bearing Rabbit Sperm

Treatment of sperm	Number of does		Total of young born	Percentage and number of offspring					
				Predicted[a]		Actual[b]			
	Inseminated	Kindled		Males	Females	Males	(N)	Females	(N)
Sorted Y	16	5	21	81	19	81	(17)	19	(4)
Sorted X	14	3	16	14	86	6	(1)	94	(15)
Recombined X and Y	17	5	14	50	50	43	(6)	57	(8)
Total	47	13	51	—	—	47	(24)	53	(27)

[a]Represents the results of reanalysis for relative DNA content of aliquots of sorted X- and Y-bearing sperm populations.
[b]Represents actual births.

SOURCE: L.A. Johnson, J.P. Flook, and H.W. Hawk, "Sex Pre-Selection in Rabbits: Live Births From X and Y Sperm Separated by DNA and Cell Sorting," *Biol. Reprod.* 41:199-203, 1989.

reproduction in vivo have also proven to be effective vaccines for cattle (12).

The first gene deletion viral vaccine to be approved and released for commercial use was the pseudorabies virus vaccine for swine (26,30). Initially, a single gene deletion reduced the virulence of the virus. Since then, other genes have been deleted with a continuing reduction of virulence.

Vaccines can also be created by inserting into pathogens, genes that produce protective antigens that reduce the ability of the pathogen to cause disease.[10] Some of these vaccines, however, will have to be carefully tested because they have a slight potential to cause human infection (31). Others are in the early developmental stages (notably herpes virus vaccine and adenovirus vaccine).

Immunomodulators

Immunomodulators are chemical compounds that boost or accentuate immune response. Several such compounds (e.g., interleukins and interferon) have been identified in mammals, and the genes encoding some of these compounds have been isolated and cloned into bacteria (31). Mechanisms by which these regulatory proteins modulate immune response is now being investigated in domestic animals (l). Biotechnology is being used to identify and replicate these compounds so that their function can be investigated.[11]

Interleukin genes and genes for compounds that cause immune responses in animals (antigens) are being inserted together into viral or bacterial vaccines. This combination could possibly enhance the immune response of the animal and lead to increased protection against the antigen. The recombinant interleukins produced in bacteria or other expression vectors may also be used therapeutically to assist in overcoming certain infections.

Diagnostics

Safe, accurate, rapid, inexpensive, and easy-to-use diagnostic procedures are critical to the dairy industry at virtually all points in the production process. Examples of diagnostic tests include pregnancy tests and assays for pathogenic organisms. Many of the currently used diagnostic tests are costly, time consuming, and labor intensive, and some still require the use of animal assay systems. Monoclinal antibodies and nucleic acid hybridization probes can be used to produce simpler, easily automated, and highly sensitive and specific diagnostic procedures.

Antibodies are proteins produced by the body in response to foreign chemical substances. Monoclonal antibodies are produced by a cell line expressing only a single antibody type (see figure 4-6). They can be used to prevent disease,[12] and are the primary tools for biotechnology-based diagnostics. At least 15 different rapid diagnostic tests are on the market or will be soon. These tests are highly specific and most lend themselves to automation, potentially allowing their application in mass screening systems for disease surveillance and control. Some of the tests have been adapted to field use and can be used by veterinarians or producers. The rapid commercialization of these products is having a significant impact on animal health management and disease control.

Monoclinal antibodies are also being used in enzyme-linked-immunoabsorbent-assay (ELISA) systems to provide sensitive, quantitative blood assays (see table 4-2) of toxins, hormones, chemicals (e.g., pesticide and antibiotic residues), and a variety of antigens including microbial agents (19,29,34,38). Many of these tests are commercially available. In some instances monoclinal antibody diagnostics have been used to replace bioassays such as mouse inoculation tests.

"Nucleic acid hybridization' can also be used to diagnose the presence of microbes and parasites. Such assays rely on the bonding of a specific DNA or RNA segment to complementary RNA or DNA fragments in a test sample. Specific segments (probes) are available to detect viruses such as

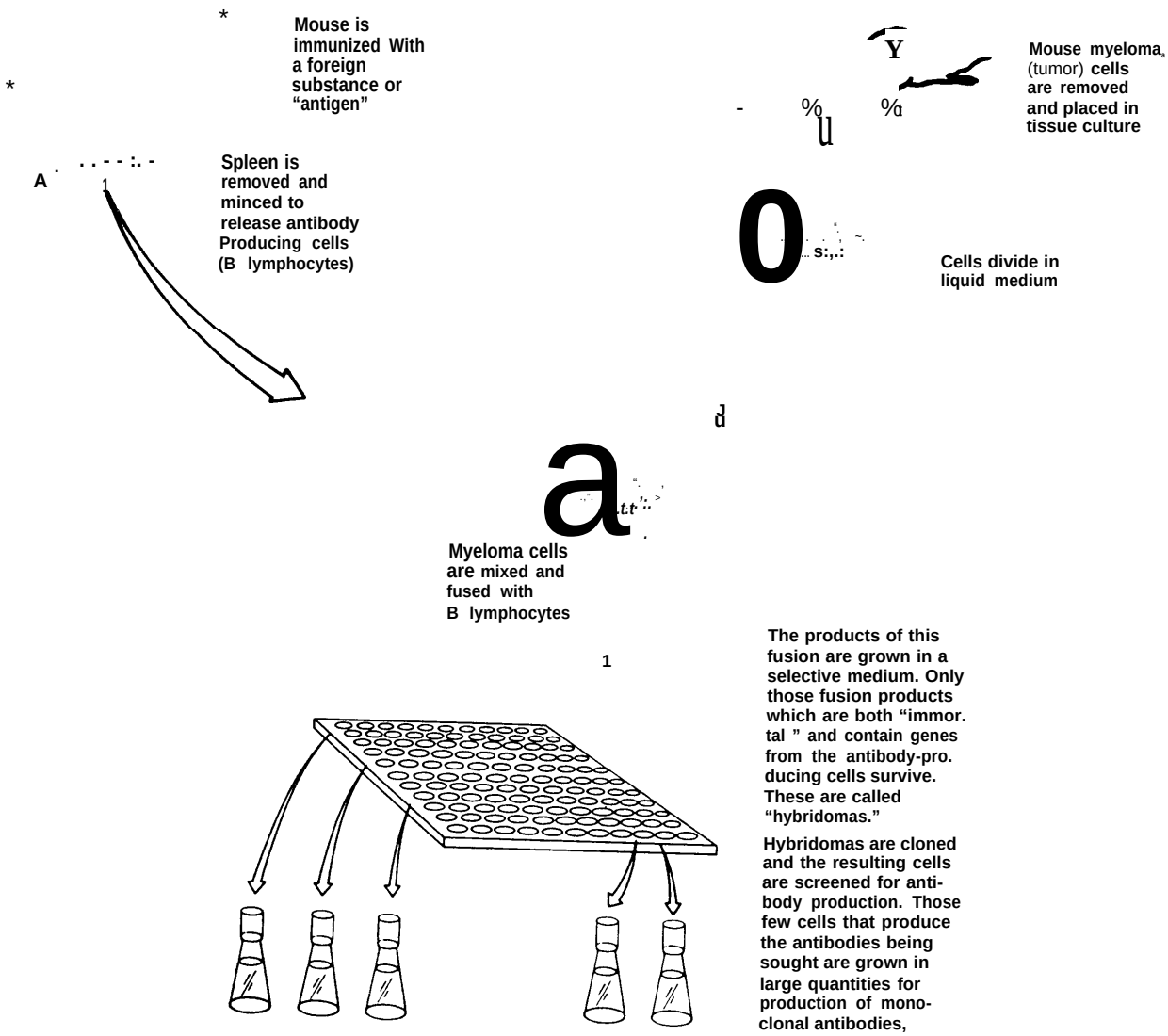
---

10The first veterinary recombinant viral vaccine was the vaccinia vectored vesicular stomatitis vaccine (27). This has been followed by the vaccinia vectored rabies and rinderpest vaccines (3,5,50).

11Cloned interleukin genes, such as bovine alpha, beta and gamma interferon, bovine interleukin-2 (IL-2) etc. have been studied both in vitro and in vivo.

12A commercial preparation of monoclonal antibodies directed to the K-99 pilus antigens of pathogenic E. coli, for example, Prevents diarrhea in newborn calves (31).

Figure 4-6—Preparation of Monoclinal Antibodies



SOURCE: Office of Technology Assessment. 1988.

bluetongue, bovine virus diarrhea, and foot and mouth disease as well as many parasites and bacterial diseases. The major limitation of this techniqu e is the small amount of target nucleic acid present in some samples. Also, the most reliable methods use radioactively labeled probes, and require expensive equipment and trained technicians, thus precluding their use in the field. Alternative calorimetric techniques currentl y in development will replace the radioactively labeled probes and make the use of this technology more commercially attractive.

Table 4-2—Monoclinal Antibodies for Diagnostic Tests

E. coli K99 antigen
Pseudorabies virus antibody
Avian leukosis virus antigen
Equine infectious anemia antibody
Avian reovirus antibody
Bluetongue virus antibody
Bluetongue virus antigen
Brucella abortus antibody
Avian encephalomyelitis antibody
Bovine progesterone
Sulfa methazine in milk
Crytosporidium
Bovine leukemia virus
Bovine herpes virus
Aflatoxin B
Sulfamethazine-swine

SOURCE: B.I. Osburn, "Animal Health Technologies," commissioned background paper prepared for the Office of Technology Assessment, Washington, DC, 1990.

Food Applications

Dairy products will be among the first food products to be impacted by biotechnology. For example, during the next decade, the genetically engineered version of the enzyme rennet, recently approved by FDA for use in cheese manufacturing systems, will replace the enzyme preparation normally extracted from the forestomach of calves. Other enzymes, which are added to the curd to accelerate ripening, or to produce dairy products acceptable for digestion by lactose-intolerant individuals, will also be produced more economically by engineered microorganisms (22).

Dairy starter cultures are living microorganisms used for the production of fermented dairy products including cheese, yogurt, butter, buttermilk, and sour cream. They have been safely consumed by humans for centuries and serve as ideal hosts for the production of these natural foods. The metabolic properties of these organisms directly affect the properties of the food product including flavor and nutritional content. In order to improve various properties of food products, food microbiologists attempt to manipulate the traits of the microorganisms, primarily through mutation and selection. The cloning and gene transfer systems developed in the 1980s are being used to construct strains with improved metabolic properties more rapidly and precisely than is possible with traditional methods. The development in this decade of new strains with precise biochemical traits will have an impact on several aspects of dairy fermentation, including production economics, shelf-life, safety, nutritional content, consumer acceptance, and waste management (22).

Although much of the current work in new strain development has focused on the use of E. coli and other nonfood microorganisms, there are distinct advantages to engineering starter cultures for producing high-value foods. For example, construction of cultures resistant to attack by viral infection will impact processing costs by eliminating waste. Cloning of the genes responsible for ripening of aged cheeses will decrease storage costs by accelerating ripening. Production of natural preservatives such as nisin, effective in inhibiting foodborne pathogens and spoilage organisms, will help ensure the safety and extend the shelf-life of fermented dairy products. Cloning of the gene(s) responsible for enzymatic reduction of cholesterol or modification of the degree of saturation of milk fat will improve the nutritional quality of fermented dairy products. The ability to engineer strains capable of producing enhanced flavors or natural stabilizers will influence consumer acceptance of fermented dairy foods.

Engineered yeast strains capable of fermenting the lactose in whey to value-added products, such as vitamin C, biofuels such as ethanol and methanol, or pharmaceuticals, will facilitate management of this waste product. Whey protein could potentially be used to produce specialty chemicals with biotechnology.

Nucleic acid probes and monoclinal antibodies can be used to analyze raw materials, ingredients, and finished products for pathogenic organisms, bacterial or fungal toxins, chemical contaminants (i.e., pesticides, heavy metals), and biological contaminants (i.e., hormones, enzymes). Animal cell cultures may partially replace whole animal systems to test for acute toxicity. Biosensors maybe used to monitor food processing, packaging, transportation, and storage (22).

KNOWLEDGE-BASED INFORMATION SYSTEMS

The economic vitality of an animal enterprise is dependent on expert managers who formulate, implement, and continually fine-tune relevant plans and goals in order to optimize resource use and output (10). However, producers may have difficulty evaluating the many interrelated factors that go into

such planning (17). Even a relatively simple animal operation requires that complex decisions be made, based on simultaneous consideration of dynamically changing factors related to risk, efficiency, disease, milk production, gestation status, and weather. With technology changing so rapidly, it has become almost impossible for agricultural managers to balance all of the facets of milk production now under their control (45).

Many producers rely on consultants and experts to sift through the data and information needed for informed management decisions. In addition, computers have come to play a significant role in dairying, an industry that historically has used records to make management decisions. Initially, data resided in mainframe computers. This allowed for professional maintenance of the database, but the ultimate user-producer had limited access to that database.

Examples of database access via telecommunication systems include the Direct Access to Records by Telephone (DART) system, run by the Dairy Records Processing Center at Raleigh, North Carolina; and the Remote Management System (RMS) available through Northeast Dairy Herd Improvement (DHI). Although dairy records processing centers (DRPCs) like the one at Raleigh have not developed software for onfarm data calculation and information storage, the private sector has. Pollock and Fredericks (33), for example, offer a microcomputer-based diagnostic program with which producers can avoid the time, recurring costs, and problems of phone access to a distant mainframe.

As computer languages have evolved and microcomputers decreased in price and gained computing capacity, database accessibility has increased. Microcomputers provide for direct, rapid delivery of management data, as well as more efficient data handling and user interfaces. They have, accordingly, revolutionized production record-keeping, and made possible onsite data manipulation and farm-level processing of information (45).

Expert Systems and Other Computer-Based Decision Aids

Management decisions rest on a knowledge base consisting of two kinds of information: that which is widely shared and generally publicly available (domain information); and rules-of-thumb judgments and sometimes educated guesses (heuristics), which typically characterize human decisionmaking. Both kinds of information are fundamental to computer-based expert systems (11), the objective of which is to raise the performance of the average producer to the expert level (39). Expert systems effectively and rationally integrate numeric, judgmental or preferential, and uncertain information, all of which come into play in the biologically based, weather-influenced production systems that typify animal agriculture (23). Another promising new information technology is the management-information system, with which managers can test the outcome of various management alternatives. Decision-support systems also hold high promise of enabling managers to balance production inputs in a way that maximizes response (output).

Expert systems, knowledge-based systems, or decision-support systems offer the potential of bringing the consultant to the farm through the microcomputer. An expert system provides a flexible yet structured approach to many problems that Extension specialists now solve relatively routinely (43). Interest in these systems is beginning to emerge as a field of research and development in agriculture, reflecting both industry awareness and appreciation of new information-management technologies (13). With the widespread introduction of specialized development tools, expert system construction has accelerated (25,37). For example, development of expert systems was, until recently, restricted to expensive LISP (a computer language) processing machines and mainframe computers. Recent advances in hardware and software have made possible the development of reasonably sized expert systems on microcomputers. Newly released expert system shells have removed the necessity to program in LISP (44). While conventional computer programs manipulate data (11), expert systems manipulate knowledge and help determine which data are useful to the decisionmaker. They are not competitors but extensions to conventional computer programs.

Application of New Information Technology

Pressure for the management expertise offered by these new information technologies will grow in the 1990s as farms increase in size, new technologies emerge, prices fluctuate, and consumer concern about food safety and diet increases. Problem solving and successful adoption of new agricultural technologies like bST will be facilitated if the knowledge acquired from research and the expertise

acquired in practice are combined and made readily available in easy-to-use forms.

Indeed, the possibility of fusing expert knowledge from different domains (extension, research, producer/managers) into a cohesive, accessible structure might be the most promising advantage of the new information technologies (11). This will allow management opportunities to be maximized, a wider group of individuals to be reached, and specialists to allocate more time to new areas of concern. Expert systems, for example, will provide farmers with online access to needed knowledge: the human expert farmers would otherwise rely on gains time for research and for expanding his or her expertise (40).

New information technologies will also revolutionize dairy record-keeping. For example, milk data are typically recorded once during a 30-day interval and extrapolated to predict total milk for that period. With new automatic metering devices, milk weights could be recorded from each milking, For a 305-day lactation, this would increase the data points from 12 to 710, if the cow is milked twice a day. With appropriate data-handling tools, this information could be tied to other information, such as measures of milk conductivity and temperature, and profiles developed to monitor cows for estrus. This would allow increased reproductive efficiency, while reducing labor requirements, and decrease the need for visual observation.

CONCLUSIONS

Advances in biotechnology and information technology will revolutionize the dairy industry. Attention by farm groups, consumers, and policymakers has focused on the first major biotechnology product from this new era—bST. In the future bST will be surpassed by more advanced biotechnology methods in animal reproduction, transgenic animal production, and animal health technologies. The more advanced technologies, for example, will increase a cow's endogenous bST production and milk synthesis by inactivating genes that inhibit bST production, eliminating the need to administer bST exogenously. Similar advanced technologies will produce higher quality cows, improve disease prevention and management, and allow for the production of high-value pharmaceuticals in milk.

These advanced biotechnologies will require sophisticated management capability to use them effectively. Knowledge-based information systems will assist in providing this management capability. Expert systems, for example, can help farmers integrate information for decisionmaking. To effectively use these systems farmers will need access to software that is specific to their individual situation and feasible for use in a variety of economic and policy situations.

The technologies from this new era are in various stages of development. Some of these technologies, such as embryo transfer, recombinant DNA vaccines, and information systems, are already commercially available or will be soon. Other technologies, such as transgenic cattle and advanced reproductive technologies, will not be available until the end of the decade. The next chapter examines the collective effect these emerging technologies, including bST, will have on the dairy industry in the economic and policy environment of the 1990s.

CHAPTER 4 REFERENCES

1. Altman, A. and Dixon, F.J., ''Immunomodifiers in Vaccines," *Adv. in Vet. Sci. and Comp. Med.* 33:301, *1989.*
2. Anderson, G. B., "Identification of Embryonic Sex by Detection of H-Y Antigen," *Theriogenology* 27:81-97, *1987.*
3. Belshaw, G.J. et al., ''Immune Response and Protection of Cattle and Pigs Generated by a Vaccinia Virus Recombinant Expressing the F Protein of Rinderpest Virus," *Vet. Rec.* 124:655, 1989.
4. Bidon, B.M. and Piper, L., "Reproductive Biology of Prolific Sheep Genotypes," *Oxford Review of Reproductive Biology,* vol. 8:414-457, J.R. Clark (cd.) (Oxford, England: Oxford University Press, 1986).
5. Blaneon, J. et al., "Safety and Efficacy of an Antirabies Vaccine Consisting of Recombinant Vaccina — Rabies Virus Administered to Fox, Dog and Cat," *Anal. Researches Veterin* 20:195, *1989.*
6. Bondioli, K.R. et al., "The Use of Male-Specific Chromosomal DNA Fragments to Determine the Sex of Bovine Preimplantation Embryos, " *Theriogenology* 31:95-104, *1989.*
7. Bondioli, K. R., Westhusin, M. E., and Looney, C. R., "Production of Identical Bovine Offspring by Nuclear Transfer," *Theriogenology* 33:165-174, *1990.*
8. Capecchi, M. R., "The New Mouse Genetics: Altering the Genome by Gene Targeting," *Trends in Genetics* 5:70-76, *1989.*
9. Clark, A.J. et al., ''Pharmaceuticals From Transgenic Livestock" *Trends in Biotechnology* 6:179-183, *1987.*

10. Conlin, J., "Emerging Technology for Dairy Management," *Proceedings of the American Farm Bureau Federation,* Orlando, FL, 1990.
11. Doluschitz, R. and Schmisseur, W. E., "Expert Systems: Applications to Agriculture and Farm Management," *Computers and Electronics in Agriculture* 2:173-182, *1988.*
12. Dougan, G., Smith, L., and Heffron, F., "Live Bacterial Vaccines and Their Application to Carriers for Foreign Antigens," *Advances in Veterinary Science and Comparative Medicine,* J.L. Bittle and F.A. Murphy (eds.), Academy Press, San Diego, CA, 33:271, 1989.
13. Eastwood, B., program leader, U.S. Department of Agriculture, Washington, DC, personal communication, 1989.
14. Egletis, M.A. et al., ''Gene Expression in Mice After High Efficiency Retroviral-Mediated Gene Transfer," *Science* 230:1395-1398, *1985.*
15. Fenwick, B.W. et al., "Mechanisms Involved in Protection Provided by Immunization Against Core Lipopolysaccharides of Eschevieria coli J5 from Lethal Hemophilus pleuropneumoniae Infections in Swine," *Inf. and Immune.* 53:298, *1986.*
16. First, N.L. and Parrish, J.J., "Sperm Maturation and In Vitro Fertilization, " *Proc. II Internat. Congr. Animal Reprod. and Artif. Insemin. 5: 160-168,1988.*
17, Gaultney, L. D., The potential for expert systems in agricultural systems management, American Society of Agricultural Engineers Summer Meeting, East Lansing, MI, 1985.
18, Gonzalez, R.N. et al., "Prevention of Clinical Coliform Mastitis in Dairy Cows by a Mutant Escherichia coli Vaccine, ' *Canad. J. Vet. Res.* 53:301, 1989.
19. Haines, D.M., Clark, E. G., and Chelak, B.J., "The Detection of Bovine Respiratory Syncytial Virus in Fornalin Fixed Bovine Lung with Commercially Available Monoclinal Antibodies and Avidin Biotin Complex Irnmunohistochernistry," *Canad. J. Vet. Res.* 53:366, *1989.*
20. Hansel, W., "Emerging Technology in Animal Reproduction,' U.S. Congress, Office of Technology Assessment, *Technology, Public Policy and the Changing Structure of American Agriculture,* OTA-F-285 (Springfield, VA: National Technical Information Service, March 1986).
21, Hansel, W., ''Reproduction and Embryo Transfer," OTA commissioned background paper (Washington, DC: 1990).
22. Harlander, S., "Biotechnology in Food Processing in the 1990s," OTA commissioned background paper (Washington, DC: 1990).
23. Higgins, L., Barrett, J. R., and Jones, D. D., "Expert Systems: Concepts and Opportunities," *Agri. Eng.,* Jan/Feb:21-23, 1986.
24. Johnson, L. A., Flook, J. P., and Hawk, H.W., "Sex Pre-Selection in Rabbits: Live Births From X and Y Sperm Separated by DNA and Cell Sorting," *Biol. Reprod.* 41:199-203, *1989.*
25. Kinnucan, P., ''Software Tools Speed Expert System Development," *High Technology,* Mar. 16-20,1985.
26. Kit, S., Kit, M., and Pirtte, E. C., "Attenuated Properties of Thymidine Kinase-Negative Deletion Mutant of Pseudorabies Virus," *Am. J. Vet. Res.* 46:1359, 1985.
27. Mackett, M. et al., "Vaccinia Virus Recombinant: Expression of VSV Genes and Protective Immunization of Mice and Cattle," *Science* 227:433, *1985.*
28. Massey, J. M., "Animal Production Industry in the Year 2000," *Genetic Engineering of Animals,* W. Hansel and B.J. Weir (eds.), *J. Reprod. Fert. Ltd., 1990,* pp. *199-208.*
29. Nantulya, V.M. et al., ''Monoclinal Antibodies That Distinguish Trypanosoma Congolese, T. Vivax, and T. Brucii," *Parasit. Immunol.* 9:421, *1987.*
30. Norby, E., "Modern Approaches to Live Virus Vaccines," *Advances in Vet. Sci. and Comp. Med.,* J.L. Bittle and F.A. Murphy (eds.), Academy Press, Inc., San Diego 33:249, 1989.
31. Osburn, B. I., ''Animal Health," OTA commissioned background paper (Washington, DC: 1990).
32. Palmiter, R.D. et al., ''Metallothionin-Human GH Fusion Genes Stimulate Growth in Mice," *Science 222,809-814, 1983.*
33. Pollock, R.V.H. and Fredericks, T.A., "Provides: A Complete Veterinary Medical Information System,' *Can. Vet. J.* 29:265-270, 1988.
34. Portelle, D., Marnmerick, M., Burny, A., "Use of Two Monoclinal Antibodies in an ELISA Test for the Detection of Antibodies to Bovine Leukaemia Virus Envelope Protein," *J. Virol. Methods* 23:211, *1989.*
35. Prather, R.S. and First, N.L., "Cloning Embryos by Nuclear Transfer,' *Genetic Engineering of Animals,* W. Hansel and B.J. Weir (eds.) *J. Reprod. Fert. Ltd., 1990,* pp. *125-134.*
36. Prather, R. S., Sims, M. M., and First, N. L., "Nuclear Transplantation in Early Pig Embryos." *Biol. Reprod.* 41:414-419, *1989.*
37. Richer, M. H., "An Evaluation of Expert System Development Tools," *Expert Systems* 3:166-183, *1986.*
38. Rodriguez, M. et al., "Detection of Bovine Herpesvirus Type 1 via an Irnmunoperoxidase Method, Using Monoclinal Antibodies," *Am. J. Vet. Res.* 50:619, *1989.*
39. Santarelli, M. B., "Artificial Intelligence: Impact on Business," *Software News 4(1)30-31, 36, 1984.*
40. Schreinemakers, J.F. et al., "The Introduction of Expert Systems in Animal Husbandry,' *The Veterinary Quarterly 10(4), 1988.*

41. Seidel, G.E., ''Sexing Mammalian Sperm and Embryos,'' *Proc. 11th Internat. Congr., Animal Reprod. and Artif. Insemin.* 5:136-145, *1988.*
42. Smith, L.C. and Wilmut, I., "Factors Affecting the Viability of Nuclear Transplanted Embryos," *Theriogenology* 33:153-164, *1990.*
43. Smith, T. R., "The Potential Applications of Expert Systems in Dairy Extension Education," *J. Dairy Sci.* 72:2760-2766, *1989.*
44. Spahr, S. L., Jones, L. R., and Dill, D. E., Symposium: Expert Systems —Their Use in Dairy Herd Management, *J Dairy Sci.* 71:3, *1988.*
45. Tomaszewski, M. A., "Use of Expert Systems in Animal Agriculture," OTA commissioned background paper (Washington, DC: 1990).
46. U.S. Congress, Office of Technology Assessment, *Commercial Biotechnology: An International Analysis,* OTA-BA-218 (Springfield, VA: National Technical Information Service, 1984).
47. U.S. Congress, Office of Technology Assessment, New *Developments in Biotechnology: Patenting Life — Special Report, OTA-BA-370* (Washington, DC: U.S. Government Printing Office, April 1989).
48. Wessells, N.K. and Hopson, J. L., *Biology, (New* York, NY: Random House, Inc., 1988), pp. 321-324.
49. Willadsen, S. M., "Nuclear Transplantation in Sheep Embryos, " *Nature* 320:63-65, *1986.*
50. Yilma, T., "Adjuvant Genes for Infectious Virus Recombinant Vaccines: Use of Lymphokines as Adjutants," *Biotechnology U.S.A.*, 1987:238.