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Animal Biotechnologies: Potential Impact on Animal Products and Their Production

Biotechnologies being developed for use in animal agriculture include the commonly practiced technologies of artificial insemination and embryo transfer, as well as the developing technologies associated with *in vitro* production of embryos, the splitting and cloning of embryos, marker-assisted selection including sexing of the embryo, and the transfer of new genes into an embryo. Each technology should be considered separately when assessing the benefits and risks of each to animals and humans because each is distinctly different and only one, gene transfer, involves recombinant DNA technology.

Genetic improvement of farm animals by traditional parent selection has been slow, especially for traits of low heritability. Nevertheless, the rate of increase in milk production has been greatly accelerated by artificial insemination of cows with semen from highly-selected, performance-tested bulls. In a limited way, the valuable genetics of a few very high production cows has been extended several-fold by the use of superovulation and embryo transfer. Conventional mating, artificial insemination and embryo transfer all have the disadvantage of propagating undesirable genes of a high-performance male or female along with the selected genes. Exciting new developments in animal biotechnology offer hope for modeling and designing animals to fit market and environmental needs and for rapidly propagating or identifying the animals of superior performance. This review will focus on each of these biotechnologies, their development status, use or potential use in animal agriculture, benefits and risks to the consumer of animal products and risks to animals.

ARTIFICIAL INSEMINATION

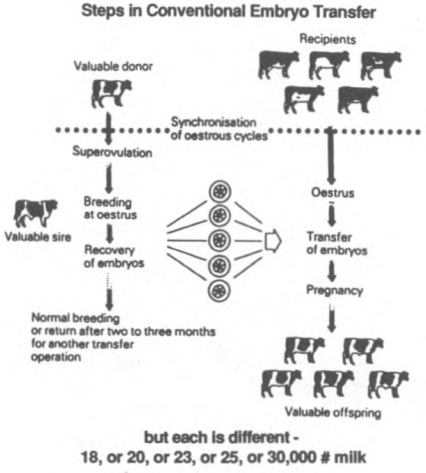
Use of artificial insemination developed rapidly in dairy cattle beginning in the late 1940s and 1950s when it was realized that bull sperm could be stored frozen and that 2-4 ejaculates per week from a bull could provide sufficient sperm after frozen storage to inseminate at least 2,000 cows. This allowed extreme selection of the sires, resulting in a more than two-fold increase in genetic ability for milk production of dairy cows. At present the pregnancy

rates are 60–65 percent and most dairy cows are mated by artificial insemination (70 percent U.S., greater than 90 percent Europe). While this technology has been developed for use in other domestic animals, the cost–benefit ratio has been favorable in the U.S. only for wide–scale use in dairy cattle or use by breeders at the top of the breeding stock pyramid for beef cattle, sheep and swine. It is a common practice in poultry breeding. The benefit to consumers has been low cost and availability of dairy products of high quality. The risks to the animals are essentially none. While it is sometimes argued that high milk production could reduce cow longevity, older cows declining in milk production are usually slaughtered for meat before becoming aged. For review of artificial insemination see Hafez, 1987.

EMBRYO TRANSFER

Embryo transfer (Figure 1) is being used primarily in dairy cattle and the top seedstock herds of beef cattle. It has the advantage of genetic improvement through both sire and dam rather than the sire alone, as with artificial insemination. There are approximately 250,000 calves born annually in the U.S. from embryo transfer. Its use has been limited because the technology of superovulation and embryo transfer has allowed only 20–30 calves per year from a cow and because it is more expensive than artificial insemination.

FIGURE 1



Blastocyst stage embryos are recovered nonsurgically by flushing the uterus of a superovulated, genetically superior cow that had been inseminated with sperm from a bull of high genetic value. The embryos numbering 3 to 10 or more are transferred each to a recipient cow synchronized in estrous cycle with the donor. The embryos can be stored frozen or sexed. Each calf born is no more alike than usual siblings and for traits such as milk production, where heritability of the trait is low, only a few reach the level of production of the parents.

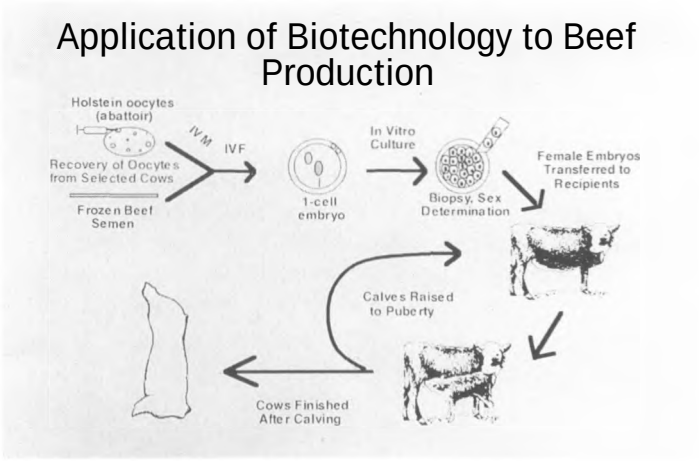


FIGURE 2

One commercial use for 'in vitro' production of embryos is illustrated above (Schaefer el al., unpublished). This breeding plan is used to eliminate maintenance of a beef brood cow. Other uses when oocytes are recovered by transvaginal laparoscopy from valuable cows include the production of large numbers of offspring from a valuable cow, or old or incapacitated cows.

Embryos can, and are, being frozen, and in a few cases sexed or split to double the number of embryos. Procedures for superovulation and embryo transfer are nonsurgical and present little risk to the cow donating the embryo or the recipient receiving it and cows can be successfully superovulated or bred at the second estrus cycle after superovulation. The animal risk is low and the benefit to consumers is manifest as more abundant, lower cost, higher quality milk and meat products. For review of embryo transfer see First, 1991; Seidel, 1991; and Wilmut et al., 1992. Artificial insemination and especially embryo transfer are the delivery mechanisms by which the new reproductive and genetic biotechnologies now under development will be delivered to animals for propagation.

IN VITRO PRODUCTION OF EMBRYOS

In vitro production of embryos depends on efficient systems for culture of oocytes and sperm, fertilization and embryo culture. This technology is being developed for all food-producing animals and is presently best developed for cattle where it is beginning to be applied. Parts of this technology are essential for cloning of embryos and for gene transfer. Several breeding companies are applying this technology commercially. Its application has been in two forms. In field trials in the U.S., Japan and Great Britain, embryos are produced *in vitro* from abattoir-recovered oocytes of selected breeds that are fertilized with semen of highly selected bulls. This is done with the objective

to replace the brood cow in beef production with *in vitro* produced embryos (Schaefer et al., unpublished) as shown in Figure 2. Each group has used this approach for a different genetic purpose. The application in Japan is to use, for both reproduction and valuable beef, the young Wagyu females that produce expensive Kobi beef. In Britain, the use is to produce valuable beef from dairy cows; while the use in the U.S. is to produce both embryo transfer recipient females and beef from young females derived from *in vitro* production of embryos, as shown in Figure 2 (Schaefer et al., unpublished). Cattle oocytes can also be recovered from follicles matured *in vivo* by recovery using ultrasound-guided vaginal laparoscopy. Recovery of these oocytes from genetically valuable cows provides a supply of oocytes of high genetic value for use in gene transfer and production of oocytes and embryos for cloning. Recent estimates indicate that one genetically valuable cow could produce 10 oocytes every 2-3 days throughout the year (Krimpenfort et al., 1991; Van der Schans et al., 1991) or approximately 100 calves per year, a big increase from that achieved by superovulation. Several embryo transfer companies are preparing to offer this *in vitro* embryo production service. The major challenge to researchers is to harvest and mature the thousands of growing oocytes and small follicles of domestic species. This would further increase the pool of oocytes available from genetically valuable animals. Application of this technique to fetal ovaries would allow rapid genetic progress through marker-assisted selection and velogenesis (Georges, 1991). The second part of producing embryos *in vitro* is the sperm capacitation and fertilization system. In general, any agent that causes Ca^{+}* entry into the sperm acrosome and a pH increase within the sperm causes capacitation. Numerous capacitation systems have been developed including high ionic strength media, glycosaminoglycans (such as heparin), aging, pH shift, calcium ionophores, caffeine and oviduct fluid. With appropriate sperm capacitation and incubation in serum-free medium at body temperature, *in vitro* fertilization rates have been reported as high as 70-80 percent in cattle, sheep, swine and goats (Parrish, 1990; First, 1991). Embryos of domestic animals can be successfully cultured in surrogate oocytes of rabbits and sheep or cultured with oviduct cells or oviduct cell conditioned media and recently successfully cultured in a defined media (Rosenkrans and First, 1990; First, 1991). While investigators are still searching for uterine factors and growth factors that may further increase the survival and development of cultured embryos, present methods are satisfactory for commercial use. The *in vitro* production of embryos as now practiced presents several benefits to society other than a lower-cost, more-abundant food product. This technology is economical to the environment and food supply as it eliminates beef brood cows. It permits use of gametes, cells and embryos in research rather than use of animals. To the livestock producer it could allow cost-effective genetic improvement. The risks are minimal when oocytes are recovered transvaginally and none when they are recovered as an abattoir by-product. Pregnancy rates are approximately

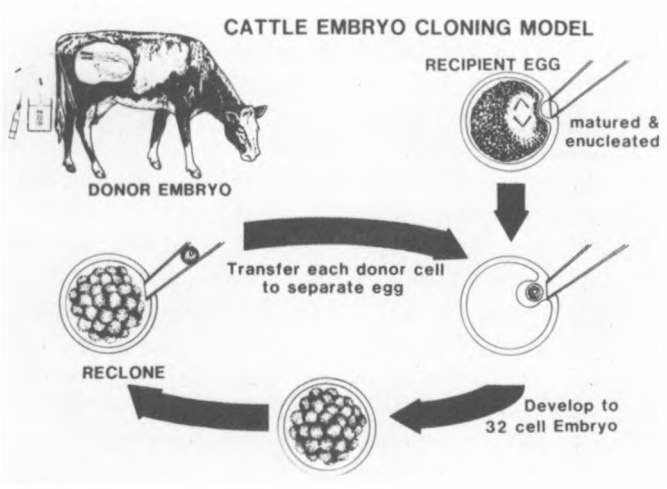


FIGURE 3

Donor embryos are obtained by flushing the uterus ofa genetically superior cow after superovulation and insemination with sperm from a genetically superior bull. Each cell of each 30 to 60 cell stage embryo is transferred into an enucleated oocyte. The embryos produced by this process can be used to supply donor cells to further increase the number of cloned embryos by recloning.

60 percent and offspring are normal (Monson et al., 1992). For a review of this technology, see Leibfried-Rutledge el al., 1989; Gordon and Lu, 1990; First, 1991; Flansel and Godke, 1992. These same methods for *in vitro* production of embryos are also beginning to be used to propagate zoo animals and to save endangered species (Wildt et al., 1992).

CLONING OF DOMESTIC ANIMALS

Twins are presently being produced with good efficiency by bisection of embryos and a few cells left on the bisection knife have been used to sex the bisected embryos. The most promising method for production of large numbers of offspring is nuclear transplantation. This procedure has successfully produced viable embryos and offspring in cattle, sheep, rabbits and swine. The procedure (Figure 3) involves transfer of a blastomere or nucleus from the valuable embryo at a multicellular stage (usually 20-120 or more cells) into an enucleated metaphase II oocyte. The oocyte then develops to a multicellular stage and is used as a donor in a serial recloning (First and Prather, 1991; Stice, 1992). Nuclear transplantation is being developed in private industry as well as by university research. Thus far, nuclear transplantation in cattle has been successfully performed using low-cost, *in vitro* matured oocytes from abattoir-recovered ovaries and with serial nuclear transfers. However, the efficiency is less than desired with approximately 20-25 percent of the nuclear transplantations resulting in transferable embryos and approximately

30 percent of the embryos transferred into the cows resulting in completed pregnancies. Throughout the U.S. and Canada, several hundred pregnancies have been produced in cattle by this procedure and recloning has been performed. To date, the largest number of calves cloned from one embryo has been 11 calves born at Granada Genetics in 1990 (First and Prather, 1991). The keys to a successful cloning system for a livestock industry are the ability to use donor embryos of larger cell numbers to produce many offspring and the capacity to use cells from cloned embryos as the donor nuclei for another generation of clones. In sheep embryos, the frequency of development to blastocysts after use of donor cells from the blastocyst inner cell mass was 57 percent and pregnancies resulted. In rabbits, blastocysts have been produced from inner cell mass cells but at a lower frequency than from the 32-cell morula stage blastomeres. In cattle, embryos at the stages of morula or the inner cell mass of blastocysts have produced good results as donors in cloning. This is approximately the stage where embryonic stem cells can be recovered and multiplied in culture in a mouse. If similar stem cell isolation and multiplication were done in domestic animals and if stem cells should prove useful in cloning by nuclear transplantation, the number of possible clones is unlimited (First, 1991). When developed to high efficiency, cloning provides a nearly phenotypic selection and propagation system for replicating valuable animals. For example, traits with heritabilities of approximately 30 percent are expected to increase to nearly 70 percent. It will also be used for rapid propagation of precious transgenic animals. The benefits of nuclear transfer include a nearly phenotypic selection, accelerated genetic improvement or environmental adaptation and characterized and predictable production performance, nutrient requirements, disease resistance and extensive screening of clonal lines for genetic defects, disease resistance and environmental adaptation before multiplication and release for use. The risks are low to none for the animals supplying donor embryo cells or the recipient oocytes, but the process at the present state of the art results in less than normal embryo survival before embryo transfer and less than normal pregnancy rates and calving rates. Also, some of the calves are born larger than normal and require assisted delivery. It is expected that with time and continued research these problems will be understood and corrected. For reviews of cloning of domestic animals, see First and Prather, 1991; Bondioli, 1992; Prather et al., 1992; and Seidel, 1992.

GENE TRANSFER

Successful production of transgenic food-producing animals requires the ability to efficiently achieve development of the transgenic embryo and genome integration of the transgene. Precise genetic modeling and appropriate promoter sequences to achieve expression at a high level in a tissue of choice and of the trait desired are necessary. Transgenic cattle, sheep, swine and rabbits have been made by microinjection of DNA into a pronucleus of a one-

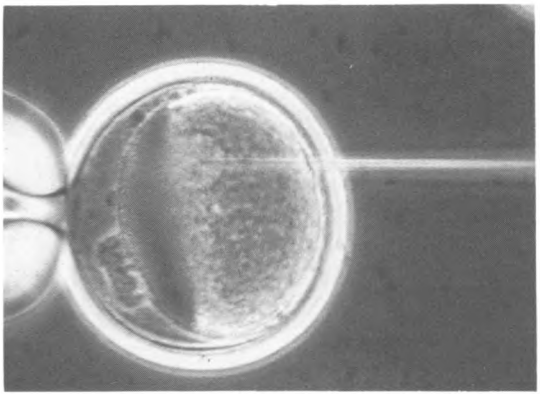


FIGURE 4

Microinjection of concentrated DNA (a gene) into a pronucleus of a bovine egg. Note the egg is first centrifuged to concentrate the cytoplasm against one side such that the pronuclei can be visualized in the cleared area.

cell zygote (Figure 4), transgenic fish by injection of DNA into oocytes and chickens by infection of genes into eggs (Hansel and Weir, 1990; First and Haseltine, 1991; Rexroad, 1992). Principle genes introduced into fish have been growth hormone genes resulting in production of fish that grow 2-3 times faster than normal. Genes imparting cold resistance to warm water species have also been expressed by transgenic fish. Transgenic chickens have been made which express increased growth from a growth hormone transgene and increased viral resistance from interference with cell receptors for avian viruses. Cattle, sheep and swine have been made transgenic for various growth hormones without significant increases in growth, but with decreased fat in the carcass. Use of tissue-specific promoter sequences and appropriate promoter control of the level of gene expression should improve growth responses. The SKI oncogene is an example of a gene that enhances muscle growth in mice and swine. Other genes may be identified that increase muscle tenderness. When appropriate disease resistance genes are identified, it should be possible to engineer high-producing animals for survival in high-disease environments. Genes for expanding the MHC complex and globins have been introduced into mice and sheep. Cells of animals have been genetically modified to resist the entire herpes family and defective viruses have been used in chickens to promote receptor resistance to pathogenic viruses (Hansel and Weir, 1990; First and Haseltine, 1991; Rexroad, 1992). The ability to target gene expression exclusively to the mammary gland will allow modification of milk composition to make novel cheeses, remove milk fat, lactose or allergenic proteins and increase protein content. Thus far, transgenic mice have been made which express new caseins or no milk fat. It is likely that some cows will be designed to produce milk for

specialty dairy products while most cows may be engineered to produce little or no fat in their milk. It is also expected that pharmaceutical products will be produced from milk of cows expressing pharmaceutical transgenes in their mammary glands. Already transgenic mice, sheep, goats and pigs have been produced which expressed either the pharmaceutical proteins of tissue plasminogen activator clotting factor 9, alpha-l-antitrypsin, lactoferrin, eurokynase, follicle-stimulating hormone, protein C, human growth hormone or interleukin 2 in their milk (First et al., 1991; Rexroad, 1992). Gene transfer usually results in one or two transgenic animals forming the beginning of a transgenic line. It, therefore, does not initially impact a large part of a population and requires artificial insemination, or *in vitro* production of embryos, or cloning or combinations of the above to produce animals or fish which are commercially useful. Gene transfer is most useful for introduction of genes not found in a population or deletion of genes not wanted, whereas marker-assisted selection is considered to be much more efficient in changing a population if the gene in question exists in the population. The benefits from gene transfer other than increasing the efficiency of animal production include the development of animals better fit for specific environments, including disease resistance; the production of new animal products and higher quality products, for example more digestible milk; removal of allergenic compounds from milk; etc. The technique of gene transfer imposes no direct animal risk. However, a greater than normal early loss of embryos occurs. The cost of gene transfer and use of animals can be considerably reduced by transfer of genes into *in vitro* produced embryos (First et al., 1991; Krimpenfort et al., 1991). Faulty modeling of the gene and promoter construct can result in insertion of the transgene at an inappropriate site with disturbance of expression of another gene or expression in other than the tissue or cell targeted in the modeling of the transgene, or expression at an inappropriate time in animal development. In the future, these risks will likely be reduced or eliminated by advances in several areas. These include the introduction of DNA into cultured cells that can be sampled and screened for appropriate expression before use in nuclear transfer to make embryos for transfer into cows. The use of cultured cells to make animals also allows site specific gene transfer or deletion through homologous recombination, thereby eliminating inappropriate sites of integration. Improved modeling of the desired outcome of the transfer gene will occur as gene mapping projects provide genome knowledge sufficient to allow accurate modeling of the genome and the gene construct. Perhaps the greatest challenge will be the development of consumer confidence that specific genetically engineered animals may not be at risk and that engineered products are safe whereas other transgenics may be rejected because animals are at risk. For review of gene transfer see Rexroad, 1992; First et al., 1991; First and Haseltine, 1991; Wilmut et al., 1990; and Hansel and Godke, 1992.

MARKER-ASSISTED SELECTION

Efforts to map the genomes of domestic animals and similarities with the mapped human genome have resulted in DNA markers that are beginning to be associated with desired or undesired productivity traits. For example, a restriction fragment linked polymorphism (RFLP) in at least one family of Holstein cattle has been associated with high milk production. Several artificial breeding companies now use this DNA marker to select for higher milk production. Markers for K-casein that relates to protein content of the milk and markers for selection against a neurological defect in Brown Swiss cattle are also in use (Georges, 1991). Rapid development of markers for use in phenotypic and genetic selection is expected as more of the genome and specific linkages to production traits are understood. Because the genomes of higher mammals are similar and gene mapping efforts across species are coordinated, we are rapidly increasing our knowledge of the genome. The applied value of gene mapping and genetic markers is primarily through association of markers with productivity, disease resistance and product quality: traits of interest. But marker-assisted selection has several other advantages. Markers can be used to perform early genetic selection on gametes, embryos or newborn animals. Markers can be used for DNA fingerprinting and accurate animal identification or association of product with animal, herd or processing plant. Markers are used to screen for genetic defects and when genes are introduced from other populations, markers can be used to track their segregation in the population. Because marker-assisted selection imposes little to no risk to the animals donating blood, sperm, or embryos for assay and imposes no risk to the consumer, marker-assisted selection is expected in the short term to be the most commonly used of the above animal biotechnologies. For review of marker-assisted selection see Dentine, 1992; Georges, 1991; Fries et al., 1989; and Massey and Georges, 1992. Several other biotechnologies impact animal agriculture, but also are expected to impose no risk to animal or consumer. These include the use of DNA fingerprinting for diagnosis of disease microorganisms, the development of new vaccines using recombinant technology and the use of engineered colostrums to protect animals and humans from disease.

SUMMARY

In summary, it is apparent that every biotechnology is different. Each gene construct is different and each must be examined for its individual benefits and risks. Some biotechnologies reduce the need for animals in research or reduce the numbers needed for food production. Some protect the health of the animal or make it more fit for a changed environment and some allow for the preservation and rapid repopulation of an endangered species. Most importantly, biotechnology is a series of tools to be used intelligently or carelessly by humans as a choice of humans. We must choose wisely, but avoid

condemnation and rejection of the tool. Like fire, clothing and the wheel, we may someday need it and be wise enough to use it.

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