

Application of Transgenic Technology in Animal Agriculture

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Abstract

Significant advancements have been made in bovine transgenic technology in the past 20 years. Currently, it is possible to target genetic sequences into predetermined sites in the host DNA, to transfer independent microchromosomes with the capacity to carry hundreds of genes into the bovine genome and to sequentially introduce multiple genetic modifications into a single genome. The most likely first genetically modified cattle to be commercialized will likely produce human therapeutic proteins.

Development of Transgenic Technology for Cattle

Over 20 years ago transgenic mice were produced carrying extra genes for growth hormone. The work was published in *Nature* (Palmiter et al., 1982) and the cover of the magazine showed a comparison of the transgenic mice and their non transgenic litter mates. The transgenic mice were huge; twice the size of their litter mates. This image stimulated the imaginations of both the public and scientists and created a tremendous amount of speculation about the potential impact of transgenic technologies for agricultural animals. It was surmised that by inserting a single growth regulating gene into an animal of agricultural value that growth rate and feed efficiency could be greatly increased and fat deposition reduced; transforming the entire meat animal industry. Furthermore, many other applications; including, enhanced milk production, production of milk with novel properties, enhanced disease and parasite resistance and increased wool production were imagined. Since then there has been a slow, but relatively steady, effort to apply transgenic technologies to agricultural species.

Initially, technical limitations, cost and a lack of understanding about genes and their regulation severely limited progress, particularly in species such as the cow. Up until 1998, transgenic animals were made by microinjection of a few thousand copies of a genetic sequence into one of the pronuclei in a newly fertilized zygote. And in the early 1980's, when transgenic technologies were first developed in the mouse, the only source of newly fertilized bovine zygotes was a superovulated cow. Zygotes had either to be recovered surgically or after slaughter from hormone treated animals. The donor cow could only be used once and yield of useable embryos was low (2 to 4 per cow) because of the precise timing required to obtain the optimal stage of

embryo for microinjection. Development of microinjected pronuclear embryos was generally low, so transfer directly back into recipient cows was considered impractical. Also, in vitro culture systems were not well refined; consequently, embryos were transferred into the oviduct of surrogate sheep for development to the blastocyst stage at day 7, then recovered and transferred, non surgically, into recipient cows. Production of a single transgenic calf required microinjection of over 1,000 embryos, supplied by 300 to 500 donor cows, and transfer of embryos into 150 recipients. Finally, when the offspring were born most would not be transgenic and those that did carry a copy of the exogenous gene often didn't express the gene or didn't pass it on to its offspring (reviewed by Pinkert and Murray, 1999). As one would expect, progress in making transgenic cows was minimal with these significant limitations.

By the late 1980's, oocyte in vitro maturation and fertilization systems were sufficiently well developed so that embryos could be obtained by fertilizing oocytes recovered from ovaries of random slaughtered cows. Furthermore, in vitro culture systems could finally be used to grow embryos for the 7 days necessary to produce blastocysts that could be transferred, non surgically, into recipient cows and develop at a reasonable rate into calves. In many laboratories around the world, in vitro produced embryos support calving rates well above 50%. These breakthroughs enabled researchers to produce, microinject and culture thousands of embryos at very low cost. Even with the damage caused by microinjection, transgenic calves can now be made with relative ease and at moderate expense. One study reports the microinjection of over 36,000 in vitro produced zygotes (Eyestone, 1999).

In spite of progress in technologies for making large numbers of inexpensive cow embryos the DNA microinjection system had several significant limitations. Integration of the transgene into the host DNA is random with microinjection and can result in detrimental mutations and variations in gene expression levels. Only a small percentage of calves born will actually be transgenic. Of those that are transgenic, the transgene may not be in the germ cells and, therefore, not transmitted to offspring. Finally, no two founder transgenic animals have the gene inserted into the same place, consequently, animals, homozygous for the transgene, can only be made by crossing offspring from a single founder animal. For the cow, production of a homozygous line from a single founder would require about 5 years.

The next breakthrough in bovine transgenic technology occurred with the discovery that somatic cell nuclei could support full term development of cloned calves (Cibelli et

al., 1998). The process of somatic cell cloning involves replacing the DNA in an unfertilized oocyte with DNA from a somatic (body) cell. The oocyte has the ability to reprogram the somatic cell DNA so that the unfertilized oocyte can develop as an embryo and, in some cases, give rise to healthy calves which have DNA that is entirely from the somatic cell. Because it is possible to obtain an unlimited number of genetically identical somatic cells from an animal, cloning is a technology that can be used for producing genetically identical calves. However, the somatic cell can also be genetically manipulated prior to being introduced into the oocyte, so cloning is also a convenient method of making transgenic cattle. Using cloning technologies, only about 10 to 15 recipients are needed to make transgenic calves, consequently, the cost of making transgenic cattle is substantially reduced.

A recent advancement in cattle transgenic technology is gene targeting. In all transgenic work with agricultural species that has been done up until the past couple of years, genes were inserted randomly into the host DNA by pronuclear microinjection. In the last couple of years a robust method for gene targeting in cattle, using somatic cell cloning technology, has been developed. Gene targeting is the insertion of a transgene, or any exogenous DNA sequence, into a specific, targeted site in the host DNA. The technique is more complex than random gene insertion but gene targeting is a much more powerful technology because it can be used to inactivate genes, insert new genes into predetermined sites or replace one variation of a gene with another variation. It overcomes many of the limitations of random gene insertion by microinjection. Because the insertion site is predetermined, a series of transgenic founder animals can be made, including both males and females, which can be mated to make homozygous offspring. An even simpler approach to making homozygous transgenic animals is to sequentially insert a copy of the transgene into one member of a pair of chromosomes and then insert a second copy into the other chromosome without germ line transmission of the transgene. To accomplish sequential gene targeting we have developed a rejuvenation system for bovine fibroblast cells. The system involves making a genetic modification in a fibroblast cell line established from a bovine fetus. Because the cells only grow for a limited number of cell divisions in culture only one genetic modification can be made before the cells become senescent and stop dividing. The cells are then used in a cloning procedure to produce cloned fetuses. Young healthy cell lines can then be made from the fetuses and used for a second round of genetic modification. When the genetic modifications are complete then the final fetal cell line can be used for making calves. Sequential gene targeting has been accomplished in our laboratory recently and homozygous transgenic calves have been produced (unpublished observations).

A second advancement in cattle transgenics, which has been accomplished recently, is microchromosome transfer

(Kuroiwa et al., 2002). A microchromosome is different from a typical transgene in a couple of characteristics. First, a typical transgene consists of a couple of gene sequences and may be up to 25,000 DNA bases long; whereas, a microchromosome typically consists of millions of DNA bases and can contain either very long genes or potentially hundreds of genes. Second, a typical transgene must integrate into the host DNA, either randomly or targeted to a specific sequence, to be carried along through cell division. Microchromosomes do not integrate but replicate on their own and are carried along during cell division as independent chromosomes. We have been successful in inserting a human-derived microchromosome into cattle. A microchromosome was needed because our objective was to transfer the human antibody genes into cows. Antibody genes are very complex and are up to several million DNA bases long; well beyond the capacity of a typical transgenic vector. The microchromosome is stable in cattle and appears to have no harmful effects on the animals.

In the 20 years since production of the first transgenic mice, work in cattle has focused primarily on technology development. At this time, many technical hurdles for application of transgenic technology to cattle have been overcome. In fact, transgenic technologies for the cow are now comparable to that of the mouse. The question now is, what are the challenges facing us in the next 20 years and will transgenic technologies be moved into commercial application? Two kinds of applications for transgenic technology in cattle are being pursued. One involves genetic modifications that are aimed at improving the efficiency of food (meat or milk) production. The second is for the production of novel products; such as pharmaceutical proteins for human health care.

Transgenic Cattle for Food Production

Of the few research reports describing the use of transgenic technologies in cattle only one is directed towards a food production application. Brophy et al., (2003) introduced additional copies of bovine beta or kappa casein into dairy cattle and evaluated the effect on milk production and composition. Transgenic offspring had an 8 to 20% increase in beta casein and a two-fold increase in kappa casein. In swine several attempts have been made at improving growth and composition by the addition of transgenes. In one study expression of an exogenous insulin-like growth factor gene in the muscle of pigs resulted in significant reduction in fat and an increase in lean muscle in gilts but not boars (Pursel et al., 1999). In another study, a widely expressed exogenous growth hormone gene tended to increase live weight gain, improve feed efficiency and reduce back fat thickness (Nottle et al. 1999). Although these studies demonstrate the feasibility of improving food production efficiency with transgenics, no attempts have been made to commercialize any transgenic food producing animals.

In addition to technology, there are several factors that will impact the use of transgenic cattle for food production. The first involves regulatory approval of meat or milk from genetically modified cattle. The federal agencies regulating genetically modified animals must address three factors; 1.) safety of the food product for human consumption, 2.) environmental impact of the genetically modified animals and 3.) welfare of the animals. Conceptually, many of the modifications that might be considered to enhance production efficiency would not have any impact on the safety or quality of the food product. Since there are no wild bovine species, the transmission of modified genes into wild species is not a concern with cattle as it is with genetically modified plants, therefore, it is unlikely that genetically modified cattle would have a significant impact on the environment. The welfare of the animal could be a concern with some genetic modifications but could be easily evaluated. Overall, the factors that are of concern to the federal regulatory agencies regarding genetically modified cattle could be scientifically addressed. However, obtaining approval for the first genetically modified animal food product is not likely to be straightforward due to the controversial nature of genetically modified food products.

The second factor to consider is the type of business model that would result in a financially successful commercialization effort for the modified genetics. A general lack of integration of the production chain in the beef industry would limit the kinds of genetic modifications that would be commercially viable. It is unlikely that a trait that might benefit the retailer would be adopted by the cow calf producer if the trait is not easily identified or if the financial benefit derived by the retailer is not shared with all components of the beef production chain. The most likely trait to be adopted would be one that produces an easily observed benefit for the cow calf producer since it is the cow calf producer that would make the decision about adopt the improved genetics.

The business model would also have to take into account whether the trait is dominant, additive or recessive. The value of a dominant trait would be observed in heterozygous offspring and therefore, could be passed on to all calves by a homozygous bull mated to non transgenic cows. A recessive trait, however, would require both parents to have homozygous genetic modifications for the trait to be observed in the offspring. The value of an additive trait would also depend on the zygosity of the parents.

The genetics of transgenes can be complex, particularly if the transgene is randomly integrated into the host DNA. To determine if the transgene disrupted any endogenous genes would require breeding a line to homozygosity and evaluating the animals in detail for a possible deleterious effect of the mutation. Breeding from a single animal is not ideal because of the inevitable inbreeding that would result. Furthermore, breeding a population from a single animal would reduce selection progress for other traits. A better

strategy would be to use gene targeting to ensure that the transgene does not cause a deleterious mutation. Gene targeting could be used to make homozygous animals without breeding and additional animals could be made with the same genetic modification at any time to add to the population.

Transgenic Cattle for Human Therapeutic Production

A second application for genetically modified cattle is the production of human therapeutic proteins. Human proteins that have been expressed in milk include human lactoferrin (van Berkel et al., 2002), human alpha lactalbumin (Eyestone, 1999), human serum albumin (Behoodi et al., 2001) and human bile salt stimulated lipase (Chen et al., 2002). The mammary gland in dairy cows is an excellent protein production factory. Large quantities of very complex proteins can be produced and collected at very low cost.

In our laboratory we are using microchromosome transfer and gene targeting technologies to develop a line of genetically modified cows that produce human polyclonal antibodies. A microchromosome transfer system is used to introduce the human antibody genes into cows. A microchromosome system is necessary because the human antibody genes are very large (millions of DNA bases) and very complex and two different gene products are needed to make an antibody molecule. To get rid of contaminating bovine antibody the bovine antibody genes are targeted with a knock out sequence to prevent expression.

Antibodies are currently used for many different human clinical applications; including treatment of infectious disease, cancer, transplanted organ rejection, autoimmune diseases and for use as antitoxins. To make a human antibody product the genetically modified cows are immunized with a vaccine containing the disease agent. For example, a product could be made for treatment of *Staphylococcus aureus* infections acquired following hospitalization by immunizing the genetically modified cow with the *Staphylococcus aureus* bacterium. Following immunization the cows build up an antibody response to the bacterium. To harvest the antibodies from the cows blood plasma is collected using a procedure that is similar to collecting plasma from human donors. The plasma is then processed to remove all contaminating bovine components so the final product is a human antibody that reacts to *Staphylococcus aureus* which can be injected into hospital patients to help them fight an infection.

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