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Animal Biotechnology: Scientific, Regulatory and Public Acceptance Issues Associated with Cloned and Genetically Engineered Animals

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What is animal biotechnology?

Biotechnology is defined as the application of science and engineering to living organisms. From this definition, it is obvious that animal breeders have been practicing biotechnology for many years. For example, traditional selection techniques involve using measurements on the physical attributes and biological characteristics of the animal (i.e., applying science) to select the parents of the next generation. One only needs to look at the amazing variety of dog breeds (see www.akc.org/breeds/breeds_a.cfm) to realize the influence that animal breeders can have on the appearance and characteristics of animals from a single species. Selection based on appearance is sometimes associated with unwanted deleterious effects on other traits such as fitness. In dogs it has been noted that each of the top 50 breeds has one aspect of breed type that predisposes the breed to a genetic disorder (Asher et al., 2009). For example Bulldogs are prone to airway obstruction syndrome, and Cavlier King Charles Spaniels are affected by a reduced-size malformation of the skull related to strong selection on snout shape and for skull conformations that are steep caudally, respectively.

Although the term biotechnology is often associated with the relatively modern biotechnologies of cloning and genetic engineering (**GE**), which are the foci of this chapter, it is important to realize that other technologies such as progeny recording schemes to objectively measure performance and the application of statistical methods to calculate the genetic merit of an animal have enabled rapid genetic progress in domestic livestock populations. Genetic improvement through selective breeding (i.e., carefully choosing which animals will become parents of the next generation based on their estimated breeding value or genetic superiority) has been an important contributor to the dramatic improvements in animal production that have been achieved over the past 50 years. Perhaps this is nowhere more evident than in poultry breeding.

The body weight of broiler (meat) chickens at 8 weeks of age increased from 0.81 to 3.14 kg between 1957 and 2001, and approximately 80% of this four-fold increase was due to genetic selection (Figure 28.1).

Animals that can be grown to market weight at a younger age use proportionally less of their total feed intake on maintenance energy. In 1960, the average time needed to produce a broiler chicken in the United States was 72 days. By 1995, this was reduced to 48 days, even as the average slaughter weight increased by 0.4 kg. Concurrently, the feed conversion ratio (kg feed/kg gain) was reduced by 15% (Table 28.1).

These remarkable improvements in production efficiency have resulted in a dramatic reduction in the inputs required to produce a kilogram of chicken. From an environmental perspective, this genetic improvement has also resulted in reductions in greenhouse gas emissions and global warming potential per unit of animal product (e.g., dozen eggs or kg of chicken). However, some have argued that productivity improvements were achieved without adequately considering the effects on associated animal well-being and the welfare implications of these genetic improvements.

The global number of livestock animals used in agricultural production has been estimated to be 1.8 billion large ruminants, 2.4 million small ruminants (sheep and goats), 20 billion poultry and nearly one billion pigs (Niemann et al., 2011). Since the early 1960s, livestock production has grown rapidly with a worldwide four-fold increase in the number of chickens, two-fold increase in the number of pigs, and 40–50% increases in the numbers of cattle, sheep, and goats. This so-called "livestock revolution" is being driven by the sharp rise in demand for animal food products in many developing countries, resulting in a pronounced reorientation of agricultural production systems (Delgado, 2003). The United Nations Food and Agriculture Organization predict the global population will rise to approximately 8 billion people by 2030, and will exceed 9 billion people by 2050. Accordingly the demand for animal protein is also expected to grow as consumers in developing countries become more affluent. Although some may yearn for low input, pastoral livestock production systems, the increasing demand for animal protein is likely to require a sustainable intensification of livestock production

Figure 28.1 Contemporary comparison of 1957 control and 2001 selected broiler carcasses fed the same diet and slaughtered at different ages (from left; 43, 57, 71, and 85 days). Modified from Hill and Kirkpatrick (2010), original photo by G.A. Havenstein. Reprinted with permission from the *Annual Reviews of Animal Biosciences*, Volume 1 © 2013 by Annual Reviews, www.annualreviews.org.

Table 28.1 Typical broiler performance in the USA from (a) Havenstein et al. (2003) and (b) Gordon (1974).

Year	Weeks of age when sold	Live weight (kg)	Feed efficiency (kg feed/kg gain)	Mortality (%)
1923[a]	16.0	1.00	4.7	18.0
1933[a]	14.0	1.23	4.4	14.0
1943[a]	12.0	1.36	4.0	10.0
1953[a]	10.5	1.45	3.0	7.3
1963[a]	9.5	1.59	2.4	5.7
1973[a]	8.5	1.77	2.0	2.7
1957[b]	12.0	1.43	3.84	4.7
2000[b]	6.0	2.67	1.63	3.6

systems. Visit the link, www.youtube.com/watch?v=6B-CH-NCdiY, to view a 5-minute music video contemplating the impact that genetic improvement has had on increasing the productivity of livestock over the past 50 years.

During the past century, several biotechnologies have been incorporated into programs aimed at accelerating the rate of the genetic improvement of livestock. One such technology is artificial insemination (**AI**), which is the deliberate introduction of semen into the reproductive tract of a female for the purpose of fertilization. AI allows the extensive use of well-proven, genetically superior sires and plays a major role in design of breeding programs and dissemination of advanced genetics. AI technology was introduced into the dairy industry and commercialized in the United States during the late 1930s to early 1940s. Today, approximately seventy per cent of all dairy cows in the US are bred using AI, as are virtually all turkeys and chickens. It provides an economical means for livestock breeders to improve their herds utilizing genetically superior males.

Although AI is now used routinely in animal breeding and human medicine, it was initially viewed with skepticism. There was a fear that AI would lead to abnormalities, and influential cattle breeders were originally opposed to the concept as they

Figure 28.2 The Holstein breeding bull, Elevation, lived in Plain City in the 1970s. Roughly half of the Holstein dairy cows in the United States today are believed to descend from Elevation.

believed it would destroy their bull market (Foote, 2002). When independent, university research demonstrated that the technology could be used to provide superior bulls, control venereal disease, and produce healthy calves, subsequent industry adoption was swift. To put the extensive use of AI in the US dairy industry in perspective, a single US bull named Elevation (Figure 28.2), born in 1965, had over 80,000 daughters, 2.3 million granddaughters, and 6.5 million great-granddaughters!

Such extensive use of this single exceptional bull clearly accelerated the rate of genetic gain, but also has the potential to reduce the genetic diversity of the dairy cattle population.

Cloning

Similar concerns regarding abnormal outcomes and reduced genetic diversity have been expressed about the use of animal cloning. A clone is an organism that is descended from, and has the same nuclear genomic DNA as, a single common ancestor. We routinely eat plant clones as many common fruits (e.g., bananas) and vegetables (e.g., potatoes) are clonally propagated. A variety of animals have also been intentionally cloned by animal breeders and researchers. There is a report of a cloned newt being produced as long ago as 1953! There are two basic methods that can be used to produce cloned animals: mechanical embryo splitting and nuclear transfer.

Embryo splitting involves bisecting a multi-cellular embryo at an early stage of development to generate clones or "twins." This type of cloning occurs naturally (e.g., human identical twins result from a spontaneous version of this process), and it can also be performed in a laboratory (Willadsen,1979) where it has been successfully used to produce clones from a number of different animal species. This technique was first used in agriculture to replicate valuable dairy breeding animals in the 1980s. The Holstein Association USA registered their first embryo split clone in 1982, and more than 2300 had been registered by October 2002 (Norman and Walsh, 2004). This method has a practical limitation in that only a small number clones, typically two, can be produced from each embryo and the genetic merit of the embryo is unknown (i.e., there are no individual or progeny performance

records available on an embryo to know whether it is a genetically-superior individual).

Cloning can also be performed using a technique called somatic cell nuclear transfer (**SCNT**). Nuclear transfer involves transferring the nucleus from a somatic cell (containing a full diploid set of paired chromosomes) to an unfertilized oocyte that has been "enucleated" by removal of its own haploid set of chromosomes. Oocytes at the metaphase II stage of meiosis are the most appropriate recipient for the production of viable cloned mammalian embryos. In order to begin the development process, the donor nucleus must be fused with the egg through the administration of a brief electrical pulse, and then the egg is activated though exposure to short electrical pulses or a chemical fusion process, after which the embryo starts to divide as if it had been fertilized. These "reconstructed" embryos are typically cultured in petri dishes for 5–7 days until they reach the blastocyst stage. In the case of mammals, the embryo is then placed into the oviducts or uterus of a surrogate or "recipient" dam where it will develop until birth.

The first mammals were cloned via somatic cell nuclear transfer in the early 1980s, almost 30 years after the initial successful experiments with frogs. Numerous mammalian clones followed, including mice, rats, rabbits, pigs, goats, sheep, cattle, and even two rhesus monkeys named Neti (Neti stands for "nuclear embryo transfer infant") and Detto in 1997. The Holstein Association USA registered their first embryo nuclear transfer clone in 1989, and approximately 1200–1500 cows and bulls were produced by embryonic cell nuclear transfer in North America in the 1980s and 1990s. However, all of these clones were produced from the transfer of nuclei derived from early (8–32 cell) embryos. This was based on the assumption that cells from mammalian embryos lose totipotency (ability of a single cell to divide and produce all the differentiated cells in an organism) after the fifth cleavage division, and therefore a theoretical maximum of only 32 clones could be produced from each individual embryo.

This assumption was shattered by the birth of Dolly the sheep on July 5, 1996 (Wilmut et al., 1997). She was the first animal to be cloned via SCNT from a differentiated somatic cell derived from an adult. This result opened up the possibility that clones could be produced from a potentially unlimited number of cells from an adult animal. From an animal breeding perspective, the importance of being able to clone from differentiated cells is that this opened up the possibility of cloning adult animals with known attributes and highly accurate estimated breeding values based on pedigree, progeny, and their own performance records.

Successful cloning from differentiated cells requires a remarkable epigenetic "nuclear reprogramming" to occur in the donor nucleus. This reprogramming involves a series of events where interactions between the donor nucleus and the oocyte cytoplasm induce change in the DNA structure towards a pluripotent (i.e., capable of giving rise to several different cell types) form that is more appropriate for embryonic development. To do this the nucleus must shut down the gene expression profile that was appropriate for its original somatic cell role (e.g., a skin fibroblast), and begin expression of the genes appropriate for embryogenesis. This requires down-regulating the expression of approximately 8000–10,000 somatic cell genes, and initiating expression of an equivalent number of embryonic genes. Currently this reprogramming process is not well understood, and several studies have shown that there appears to be an increased rate of pregnancy, early postnatal loss, and other abnormalities

in SCNT clones relative to offspring conceived in the traditional way. However, these problems are not seen in all SCNT clones, and many apparently healthy clones have been born, grown to maturity, and have gone on to conceive and have healthy offspring (Couldrey et al., 2011). Because the abnormalities seen in clones are largely epigenetic, meaning they are not based on changes in the underlying DNA sequence, they are corrected during gametogenesis and analogous problems have not been observed in the sexually-derived offspring of clones.

Significant improvements in the protocols for SCNT cloning have occurred over the past 15 years, and bovine cloning is now achieving efficiencies of 20–25% live cloned offspring per oocyte transferred (Panarace et al., 2007). Most embryonic loses occur in the first 2 weeks after transfer of the reconstructed embryo into the uterus of the recipient cow. This is the time when natural embryonic mortality in pigs and cattle is also high (35–50%). Porcine cloning can produce pregnancy rates as high as 80%, although the average litter size tends to be reduced compared to conventional breeding figures (~6 piglets as compared to 9–10 piglets).

The performance and behavior of cloned offspring that successfully survive the neonatal period are not different from age matched controls. An early study on Dolly suggested that clones might be susceptible to premature aging, due to shortened telomeres in their cells (Shiels et al., 1999). Telomeres are repetitive nucleotide sequences at each end of a chromosome, which protect the end of the chromosome from deterioration and prevent them from fusing with neighboring chromosomes. It was speculated that because the somatic cell nucleus that became Dolly was taken from a 6-year-old sheep, Dolly would have shortened telomeres in all her cells because she was genetically six years old at birth. This is because telomere length is reduced after each cell division and hence telomeres become shorter as an organism ages. Subsequent studies on other SCNT clones have not repeated this finding of shortened telomeres (Betts et al., 2001; Miyashita et al., 2011; Tian et al., 2000), and have shown that SCNT animals have telomeres of normal length. Dolly eventually died from a progressive lung disease in 2003. Roslin scientists stated that they did not consider that her death was the result of being a clone as other sheep on the farm had similar ailments. Such lung diseases are especially a danger for sheep kept indoors, as Dolly had to be for security reasons. Because longevity is a population statistic (i.e., it is the average age at death in a given population and thus cannot be determined based on a single observation), and SCNT cloning from adult cells has only been in general use since 1997, it is too early to assess the effects of cloning on lifespan and senescence (Niemann and Lucas-Hahn, 2012). Although some studies have reported that clones may experience a higher than normal annual mortality rate (Wells, 2005), others indicate no obvious problems with second generations of cloned cattle (Konishi et al., 2011) and mice that have been reiteratively cloned for six generations reveal no aberrant pathology (Wakayama et al., 2000).

A diverse range of 16 animal species have now been successfully cloned from adult tissues using SCNT including mice, rats, zebrafish, rabbits, ferrets, goats, horses, pigs, cattle, deer, camel, dogs, cats, and a range of endangered species including wild cats, muflon, gaur, wolf, and ibex. Although clones carry exactly the same genetic information in their DNA, they may still differ from each other, in much the same way as identical twins do not look or behave in exactly the same way. Clones do not share the same

Box 28.1

How animals are cloned and why problems sometimes occur

Cloning by nuclear transfer is a two-part process. First, scientists remove the nucleus from an egg, and then they fuse it with a somatic cell containing the nucleus and genetic material from another cell by the application of an electrical charge. The fused egg is then placed in a laboratory dish with the appropriate nutrients. Eventually the resulting embryo, which is a genetic copy of the animal that produced the somatic cell and not the egg, is transplanted into a surrogate mother.

The successful production of normal clones from differentiated somatic cells suggests that adult nuclear DNA retains the ability to direct the correct pattern of gene expression for embryogenesis. The process of resetting adult nuclear DNA to the embryonic pattern of gene expression is known as **reprogramming** and likely involves switching off certain genes and turning on others. Errors in reprogramming may lead to abnormalities in gene expression in cloned animals and affect the health and longevity of the animal.

Reprogramming involves changes at the epigenetic level. Epigenetic changes refer to alterations in gene expression resulting from modifications of the genome that do not include changes in the base sequence of DNA. Two key areas of epigenetic control are **chromatin remodeling** and **DNA methylation**. Epigenetic changes may also include the switching off of maternal or paternal copies of certain genes in a process called **imprinting**.

In the case of clones it appears that the reprogramming of somatic cell modifications is sometimes incomplete leading to inappropriate patterns of DNA methylation, chromatin modification, and X-chromosome inactivation in the developing clone. This can result in aberrant gene expression patterns and correspondingly high rates of pregnancy loss, congenital abnormalities, and postnatal mortality.

cytoplasmic inheritance of mitochondria from the donor egg, nor the same maternal environment as they are often calved and raised by different animals (see Box 28.1). It is also important to remember that most traits of economic importance are greatly influenced by environmental factors, and so even identical twins may perform differently under varying environmental conditions.

Applications of cloning

Cloned animals can provide a "genetic insurance" policy in the case of extremely valuable stud animals like Elevation, or produce several identical bulls in production environments where AI is not a feasible option. This so-called "reproductive cloning" could conceptually be used to reproduce a genotype that is particularly well-suited to a given environment. The advantage of this approach is that a genotype that is proven to do especially well in a particular location could be maintained indefinitely, without the genetic shuffle that normally occurs every generation with conventional reproduction and meiosis. However, the disadvantage of this approach is that it freezes genetic progress at one point in time. As there is no genetic variability in a population of clones, within-herd selection no longer offers an opportunity

for genetic improvement. Additionally, the lack of genetic variability could render the herd vulnerable to a catastrophic disease outbreak, or singularly ill-suited to changes that may occur in the environment.

Cloning offers an approach to reproduce otherwise sterile animals (e.g., mules or neutered animals). Cloning may also have some utility as one approach contributing towards the preservation of rare and endangered species. It should be noted in this regard, that oocytes can only reprogram and support the development to term where the donor nucleus species is closely related to the species of oocyte origin as was the case when a muflon was cloned in a sheep oocyte, and the gaur with a cow oocyte. Although embryonic development can begin in the case where species are not closely related, such as a cow and a pig, embryonic genome activation does not occur and development is arrested at the early cleavage stages of embryogenesis.

Although cloning does not alter the genetic makeup of the animal, there is a logical partnership between cloning and the process of using recombinant DNA technology to make transgenic or genetically engineered (**GE**) animals. As will be discussed later, cloning can be used to efficiently generate transgenic animals from cultured somatic cells that have undergone precise, characterized modifications of the genome. The first GE mammalian clones were sheep born in 1997 carrying the coding sequences for human clotting factor IX (Schnieke et al., 1997), which is an important therapeutic for hemophiliacs. Cloning has also been used to generate GE cows that produce human polyclonal antibodies (Kuroiwa et al., 2002). It is envisioned that these unique cows will make it possible to create an efficient, safe, and steady supply of human polyclonal antibodies for the treatment of a variety of infectious human diseases and other ailments including organ transplant rejection, cancer and various autoimmune diseases, such as rheumatoid arthritis. Genetically engineered proteins have been made and secreted in milk, blood, urine, and semen of livestock, although to date most commercial systems favor the mammary gland. Cloning also offers the unique opportunity to produce animals from cells that have undergone a targeted "knock out" (see http://learn.genetics.utah.edu/content/science/transgenic/) or deletion of an endogenous gene such as those that encode the allergenic proteins that cause the rejection of animal organs when used in human xenotransplantation surgeries (www.revivicor.com).

Studies examining the composition of food products derived from clones have found that they have the same composition as milk or meat from conventionally-produced animals (Yang et al., 2007). In 2001 the Center for Veterinary Medicine at the US Food and Drug Administration (FDA) undertook a comprehensive risk assessment to identify hazards and characterize food consumption risks that may result from the introduction of SCNT animal clones, their progeny, or their food products (e.g., milk or meat) into the human or animal food supply. As there is no fundamental reason to suspect that clones will produce novel toxins or allergens, the main underlying food safety concern was whether the SCNT cloning process results in subtle changes in the composition of animal food products.

In 2008 the FDA published its final 968-page risk assessment on animal cloning (available at www.fda.gov/AnimalVeterinary/SafetyHealth/AnimalCloning/UCM055489), which examined all existing data relevant to (1) the health of clones and their progeny, and (2) food consumption risks resulting from their edible products, and found that no unique food safety risks were

identified in cloned animals. This report, which summarized all available data on clones and their progeny, concluded that meat and milk products from cloned cattle, swine and goats, and the offspring of clones from any species traditionally consumed as food, are as safe to eat as food from conventionally bred animals. The FDA also has made available three public education fact sheets "Myths about Cloning," "Animal Cloning and Food Safety," and "A Primer on Cloning and Its Use in Livestock Operations," on their website (available from www.fda.gov/AnimalVeterinary/SafetyHealth/AnimalCloning/default.htm). Subsequent rodent feeding studies have revealed no obvious food safety concerns related to the consumption of cloned-cattle meat (Yang et al., 2011).

Genetic engineering

Genetic engineering (**GE**) is a process in which scientists use recombinant DNA (rDNA) technology to introduce desirable traits into an organism. DNA is the chemical inside the nucleus of a cell that carries the genetic instructions for making living organisms. Because the genetic code for all organisms is made up of the same four nucleotide building blocks, this means that a gene encodes the same protein whether it is made in an animal, a plant or a microbe. Recombinant DNA refers to DNA fragments from two or more different sources that have been joined together in a laboratory. The resultant rDNA "construct" is usually designed to express a protein(s) that is encoded by the gene(s) included in the construct. Genetic engineering involves producing and introducing the rDNA construct into an organism so new or changed traits can be given to that organism. A GE animal is an animal that carries a known sequence of rDNA in its cells, and which passes that DNA onto its offspring. Genetically engineered animals are sometimes referred to as genetically modified organism (GMO), living modified organism, transgenic, or bioengineered animals. Genetically engineered animals were first produced in the late 1970s. Forty years later GE animals have been produced in many different species, including those traditionally consumed as food although most have not moved from the laboratory to commercialization.

Techniques

The first method to produce GE animals was microinjection of rDNA into blastocysts to produce transgenic mice in 1974 (Jaenisch and Mintz, 1974). However, these mice were mosaic, meaning they did not carry the transgene in all of the cells of their body and most importantly their germ cells (egg and sperm), and so were not able to pass the transgene on to their offspring. Germline transmission (i.e., the rDNA construct is present in gametes produced by the GE animal) of the rDNA was achieved using a technique called pronuclear microinjection.

This technique involves injecting many copies of the recombinant gene into one of the two pronuclei of a newly fertilized single-cell embryo. Transgene integration happens randomly in the genome at sites of DNA double-strand breakage, and typically multiple copies of the transgene integrate into a single chromosomal locus in the embryo. If integration takes place prior to the first nuclear division, then all cells will carry the transgene. In many cases integration happens after the embryo has undergone cell division, which results in a mosaic animal in which some cells contain the gene construct while others do not. After micro-

injection, eggs are typically surgically transferred into the oviducts of synchronized surrogate females. The offspring resulting from injected eggs may or may not carry the transgene in their somatic and/or germ (sperm and egg) cells. Typically only 1–5% of the implanted embryos will test positive for the transgene. Animals that do have the GE construct integrated into their genome are called founders, and only those that are germline founders reliably transmit the transgene to their offspring.

Only a small fraction of GE founder animals produced using pronuclear microinjection show the expected phenotype. This is mainly due to the random nature of the integration site. The transgene can be affected by the surrounding DNA and result in animals that fail to express the transgene, a phenomenon called "gene silencing." Screening transgene expression levels in founder animals is currently the only way to identify animals with suitable expression patterns. Several alternatives to pronuclear microinjection have been developed to improve the efficiency and reduce the costs associated with generating transgenic animals. These include the use of targeted gene modifications in cell culture followed by SCNT of the modified cell, injection, or infection of oocytes and/or embryos by retro- and lentiviral vectors, cytoplasmic injection of circular plasmids (CPI), sperm mediated gene transfer (SMGT), and intracytoplasmic injection (ICSI) of sperm heads carrying foreign DNA (Figure 28.3).

As discussed previously SCNT offers an approach to clone cells that have been genetically modified in culture and thereby produce GE clones. Unfortunately the success rate of SCNT is also low, and although no mosaic animals are produced when a genetically engineered cell is cloned, reconstructed embryos have a low survival rate and typically only 1–10% of reconstructed embryos result in live births. Cattle seem to be an exception to this rule as levels of 15–20% can be reached (Kues and Niemann, 2004). Cloning also offers the possibility of producing animals from cultured cells that have had selected genes removed, a technique called gene targeting. The first "targeted gene knockout" technique that resulted in the selective inactivation of specific genes was developed in 1987 (Thomas and Capecchi, 1987) and gene targeting was the subject of the 2007 Nobel Prize in medicine (www.nobelprize.org/nobel_prizes/medicine/laureates/2007/advanced.html?print=1#.U3M-kvlSbiQ). This original gene targeting work was carried out in pluripotent embryonic stem cells (ESC) derived from mice. These cells have the ability to participate in organ and germ cell development following injection into the blastocysts. Despite extensive research, stem cells that are able to contribute to the germline are currently only available for rodents and not food animal species. However, gene targeting in somatic cells followed by SCNT offers an approach to allow additional species to employ high efficiency "targeted gene knockout" techniques. Somatic cell gene targeting directly recombines homologous genes in somatic cells and then GE animals can be produced through SCNT. This approach has been successfully used to produce cattle from cells lacking the gene for the prion protein responsible for mad cow disease (Richt et al., 2007), and pigs have been produced that lack the allergenic proteins that are responsible for the rejection of pig organs when used for transfer into human organ-transplantation patients (Whyte and Prather, 2011).

The disadvantage of this approach is that somatic cells have a limited lifespan *in vitro* and aged somatic cells result in a high number of abnormalities in cloned embryos. Recently, gene targeting technologies based on designer nucleases (e.g., zinc finger
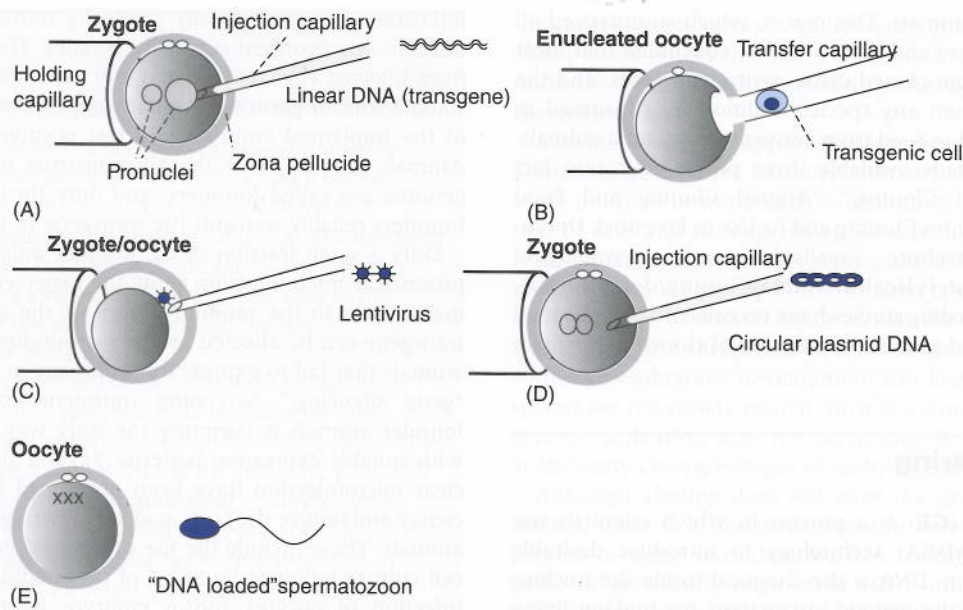
Figure 28.3 Methods for transgenesis in large mammals. Modified from Garrels et al. (2012). (**A**) Pronuclear injection (PNI): With a fine glass capillary linearized DNA molecules are injected into one pronucleus of a zygote. Requires highly skilled experimentalist. Random integration into the genome. High rates of transgene mosaic animals and unwanted concatemeric integrations. Approximately, 1–5% of treated zygotes develop to transgenic offspring. (**B**) Somatic cell nuclear transfer (SCNT): Requires highly skilled experimentalist for enucleation of oocytes and transfer of transgenic somatic cell. Integration into the genome of somatic cells is random in most cases, but can be targeted by homologous recombination. Genetic modification of donor cells with viruses, zinc finger nucleases and transposons, and subsequent use in SCNT has been shown. All offspring should be transgenic, but due to low developmental capacity only 1–5% of reconstructed embryos develop to vital offspring. (**C**) Lentivirus transfection: Requires advanced virus production facility and S2 safety laboratories. Replication-deficient lentiviruses are injected into the perivitelline space. Typically 50–90% of the offspring are transgenic, however, a high mosaicism rate and animals carrying multiple integrations are found. (**D**) Cytoplasmic plasmid injection (CPI): Circular expression plasmids are injected into the cytoplasm by employing transposon systems, active enzyme-catalyzed transgene integration of monomeric units can be achieved. Monomeric insertions into transcriptionally accessible regions are favored. Typically 40–60% of the offspring are transgenic, correlating to 6–10% of treated zygotes. (**E**) Sperm-mediated gene transfer (SMGT) and intracytoplasmic sperm injection (ICSI): For SMGT sperm cells are incubated with DNA, and are subsequently used for artificial insemination, thus avoiding any micromanipulation. However, the transgenesis rates are unpredictable and highly variable between laboratories. A more reliable extension of SMGT is the combination with intracytoplasmic sperm injection (ICSI). In this method sperm cell membranes are damaged (freezing, NaOH or drying) before incubation with DNA, then immobile (dead) spermatozoa are used for ICSI, followed by embryo transfer. However, the ICSI procedure is laborious and requires a highly skillful experimentalist, smoothing out the simplicity of SMGT. Reproduced with permission from Laible, G. Enhancing livestock through genetic engineering – Recent advances and future prospects. *Comparative Immunology, Microbiology and Infectious Diseases* 32, 123–137 (2009).

nucleases, transcription activator-like effector nucleases (TALENS), meganucleases) that target specific sequences in the genome have also been developed. These nucleases are like "molecular scissors" that introduce a double-strand break at a single predetermined location in the genome. They can be used for targeted gene modification including endogenous gene knockouts, targeted gene addition and/or replacement through homologous recombination, and chromosomal rearrangements. Gene knock-out plants, *Drosophila*, zebrafish, rats, pigs, and cattle have been successfully produced by zinc-finger nucleases (Miyashita et al., 2011). Recent progress in reprogramming somatic cells to become pluripotent stem cells that can divide indefinitely will likely further improve the efficiency of targeted gene modifications in the future.

Applications of genetically-engineered animals
Genetically engineered animals can be divided into six broad classes based on the intended purpose of the genetic modification: (1) to develop animal models for research purposes (e.g., pigs as models for cardiovascular diseases); (2) to produce products intended for human therapeutic use (e.g., pharmaceutical products); (3) to enrich or enhance the animals' interactions with humans (e.g., new color varieties of pet fish); (4) to produce

industrial or consumer products (e.g., fibers for multiple uses); (5) to enhance production attributes or food quality traits (e.g., faster growth); and (6) to improve animal health (e.g., disease resistance). Some of the most notable genetically engineered animals have been developed for a variety of reasons ranging from biomedical research to food production. All GE animals must receive regulatory approval before the products they produce can be commercialized for pharmaceutical or food purposes.

Regulation of genetically-engineered animals
The FDA is the lead agency responsible for the regulation of GE food animals in the United States. In 2009, the FDA outlined its science-based regulatory process (www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf) to assess GE animals and their edible products. To evaluate a GE animal the FDA requires the company interested in commercializing the GE animal to provide data to enable analyses of the following seven points:
1. Product definition: what does the GE animal do? For example, grow faster, disease resistant;
2. Molecular characterization of the construct: a description of the rDNA construct and how it was assembled;

3. **Molecular characterization of the GE animal lineage:** how was the rDNA construct introduced into the animal and whether it is stably maintained over time;

4. **Phenotypic characterization of the GE animal:** comprehensive data on the characteristics of the GE animal and its health;

5. **Durability plan:** plan to show that GE modification is stable over time, and will continue to have the same effect;

6. **Environmental and food/feed safety:** assessment of any environmental impacts, and for GE animals intended for food, that food from those GE animals is safe to eat for humans and/or animals;

7. **Claim validation:** does it do what it is meant to do?

In the United States, any animal containing an rDNA construct is subject to regulation by the FDA prior to commercialization. However, based on risk, there are some GE animals for which the FDA exercises something called "enforcement discretion," meaning they do not require an approval prior to commercialization. In general, this includes transgenic laboratory rodents such as mice and rats that have become increasingly important for biological and biomedical research and are sold to researchers around the world. GE livestock are also being developed specifically as biomedical research models. Several groups have created GE pigs with alterations in key genes in disease pathways to provide models for human disease (Whyte and Prather, 2011). Pigs are anatomically and physiologically similar to humans and these models will help to improve our understanding of the causes and potential therapies for human disease. The emerging technologies for gene targeting will likely mean more GE animals from a variety of species will be produced as valuable models to study human disease and therapies in the future. The FDA does not plan on exercising enforcement discretion for any GE animal of a species traditionally consumed as food. On a case-by-case basis, the FDA may consider exercising enforcement discretion for GE animals of very low risk, such as it did for Glofish (see www.youtube.com/watch?v=SA9PEBPnhWU), a GE aquarium fish that glows in the dark (www.glofish.com).

Pharming is a term used to describe the production of pharmaceutical proteins or drugs in GE animals following the introduction of a gene construct that directs the production of that drug. The mammary gland of dairy animals is a logical place to produce therapeutic proteins as it has the ability to produce large amounts of protein, and milk is easily harvested from the animal. In 2009, the first GE animal producing a pharmaceutical product, a GE goat (http://www.fda.gov/downloads/ForConsumers/ConsumerUpdates/UCM144055.pdf) synthesizing recombinant human antithrombin III in its milk (http://www.atryn.com), was approved by the FDA. This drug is an anticoagulant for the treatment of individuals with hereditary antithrombin deficiency, a blood-clotting disorder. Subsequently, a human recombinant C1 plasma protease inhibitor produced in transgenic rabbit milk was approved in Europe for treatment of patients with hereditary angioedema (www.pharming.com). Transchromosomal cattle carrying a human artificial chromosome harboring the entire sequence of the human major histocompatability complex have been made and these animals are able to make human polyclonal antibodies (Kuroiwa et al., 2002; www.hematech.com).

Agricultural applications of genetic engineering include making animals with improved food products, animal welfare (e.g., disease-resistant animals), and animals with a reduced environ-
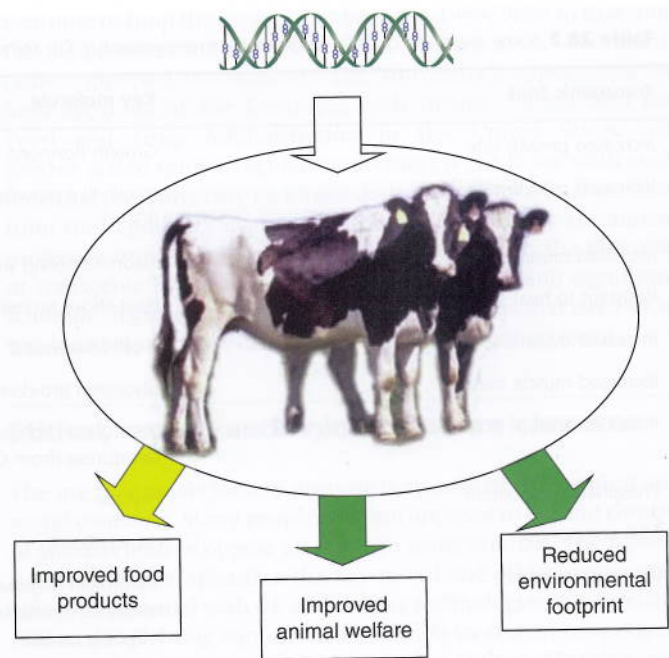
Figure 28.4 Main objectives of agricultural applications for transgenic livestock technology. Image from Laible (2009).

mental footprint per unit of food (e.g., egg or serving of milk and meat) (Figure 28.4). There is a much higher economic incentive associated with the production of GE animals for human medicine applications, than for agricultural applications.

The use of GE animals for agricultural applications tends to generate greater public scrutiny than the biomedical and pharmaceutical applications previously discussed. This may be partly due to the fact that GE animals for agricultural applications will enter the food supply. The advantage of GE for animal breeding is that unlike traditional selection approaches (Figure 28.4), the technology is not restricted by the species barrier and so entirely novel and unique characteristics can be introduced using genes derived from unrelated species. Traditional selection schemes make relatively slow genetic progress and are imprecise, meaning that selection for one characteristic is often accompanied by undesired changes in associated traits (e.g., production and fertility). Some of the GE animals that have been developed for agricultural applications are listed in Table 28.2, although none have yet received regulatory approval for commercialization and entry into the food supply.

A company called AquaBounty requested regulatory approval for a GE line of growth June 2014 enhanced Atlantic salmon intended for food. The AquAdvantage Atlantic salmon reaches market size twice as fast as wild-type salmon. Consisting of an "all fish" construct, the transgenic salmon contain an ocean pout antifreeze promoter driving a Chinook salmon growth hormone gene that allows the fish to grow up to six times larger than non-transgenic salmon of the same age (Du et al., 1992). The company has completed all of the major studies required to gain regulatory approval for the transgenic salmon to be consumed in the US. The data package included regulatory studies to address food safety, allergenicity, nutrient content, and genetic stability through inheritance, and evaluation was completed in September 2010. As of June 2014, the FDA had not made a decision as to whether this fish will be the first GE animal approved to enter the food supply.

Table 28.2 Some examples of traits targeted for improvement in GE animals for agricultural applications. Modified from Kues and Niemann (2004).

Transgenic trait	Key molecule	Gene transfer method	Species	Ref.
Increased growth rate	Growth hormone (GH)	Microinjection	Pig	Nottle et al., 1999
Increased growth rate	Insulin-like growth factor-1 (IGF-1)	Microinjection	Pig	Pursel, 1999
Increased muscle mass	Slaon–Kettering virus	Microinjection	Pig	Pursel et al., 1992
Resistant to heat stress	Heat-shock protein	Microinjection	Pig	Chen et al., 2005
Increased ovulation rate	B-cell Leukemia 2	Microinjection	Pig	Guthrie et al., 2005
Increased muscle mass	Myostatin pro-domain	Microinjection	Pig	Mitchell and Wall 2008
Increased level of polyunsaturated fatty acids in pork	Desaturase (from spinach) Desaturase (from *C. elegans*)	Microinjection Somatic cloning	Pig Pig	Saeki, 2004 Lai et al., 2006
Phosphate metabolism	Phytase	Microinjection	Pig	Golovan et al., 2001
Milk composition	α-Lactalbumin	Microinjection	Pig	Wheeler et al., 2001
Influenza resistance	Mx protein (Myxovirus resistance 1, interferon-inducible protein)	Microinjection	Pig	Muller et al., 1992
Enhanced disease resistance	Immunoglobulin (IgA)	Microinjection	Pig, sheep	Lo et al., 1991
Wool growth	Insulin-like growth factor-1 (IGF-1)	Microinjection	Sheep	Damak et al., 1996
Visna virus resistance	Visna virus envelope	Microinjection	Sheep	Clements et al., 1994
Bovine spongiform encephalopathy (BSE) resistance	Prion protein gene	Somatic cloning	Sheep	Denning et al., 2001
Milk fat composition	Stearoyl desaturase	Microinjection	Goat	Reh et al., 2004
Milk composition (increase of whey proteins)	β-Casein κ-Casein	Somatic cloning	Cattle	Brophy et al., 2003
Milk composition (increase of lactoferrin)	Human lactoferrin	Microinjection	Goat	Maga et al., 2006
Mastitis resistance	Lysostaphin	Somatic cloning	Cattle	Wall et al., 2005
Bovine spongiform encephalopathy (BSE) resistance	Prion protein gene	Somatic cloning	Cattle	Richt et al., 2007
Influenza resistance	Short hairpin RNA	Lentiviral transduction	Chicken	Lyall et al., 2011
Increased growth rate	Growth hormone (GH)	Microinjection	Salmon	Du et al., 1992

The extensive regulatory process to document the food and environmental safety of GE animals bred for agricultural applications is unique to GE technology. For example, genetic modifications that result from using traditional animal breeding approaches to select for faster growing salmon undergo no analogous regulatory scrutiny. While there may be some risks that are uniquely associated with some GE animals (e.g., potential introduction of an allergenic protein from a different species), there are other risks where there is no difference between those associated with GE animals and risks associated with conventionally-bred animals. For example, environmental risks associated with fast growing GE salmon would be similar to those associated with fast growing strains of farmed salmon developed using traditional selection for faster growth (Schiermeier, 2003). Subjecting conventionally-bred and GE animals to discordant regulatory requirements despite similar risks is inconsistent from a scientific perspective, and places a disproportionate regulatory burden on the development of GE technology. Commercialization of agricultural applications of GE animals in the US is currently being delayed by concerns about the cost and timelines associated with the regulatory process.

Yonathan Zohar, a professor at The University of Maryland, wrote an opinion piece entitled "Genetically modified salmon can feed the world" on the GE salmon. Read his opinion piece at http://edition.cnn.com/2010/OPINION/09/22/zohar.genetically.engineered.salmon/ and then consider the question in Box 28.2.

The University of Guelph in Canada was also interested in obtaining regulatory approval for its Enviropig – a GE pig that produces the enzyme phytase in its saliva (Golovan et al., 2001; www.uoguelph.ca/enviropig). This bacterial enzyme enables the Enviropig to process indigestible phosphorus in the form of phytate and better absorb the phosphate in its diet, thereby eliminating the need to supplement the diet with readily-available forms of phosphate supplement. As a consequence the phosphorus content of Enviropig's manure is reduced by as much as 60%. This pig is discussed on a CNN report entitled "Enviropig: the next transgenic food?" Watch the video at http://eatocracy.cnn.com/2010/09/25/enviropig-the-next-transgenic-food/ and then consider question in Box 28.3.

In May, 2012 the University of Guelph closed down its Enviropig project after failing to find an industry partner to

Box 28.2

The AquAdvantage™ salmon

Since the mid-1980s, the yield of food fish from wild capture fisheries has been static at about 60 mMT per year. The growth of the fish supply since that time has largely come from aquaculture. It has been calculated that an extra 52 mMT of aquaculture production will be needed by 2025 if the current rate of fish consumption is to be maintained. Atlantic salmon remain the most important farmed food fish in global trade. The AquAdvantage™ salmon is an Atlantic salmon carrying a Chinook salmon growth hormone gene controlled by an antifreeze protein promoter from a third species, the ocean pout. The mature weight of these fish remains the same as other farmed salmon, but their growth rate is increased, with a concomitant 25% decrease in feed input, decreased waste per unit of product, and decreased time to market. The application to market this fish for food purposes has been going through the FDA regulatory approval process for over a decade. Do you think this fish should be approved for commercialization? Give three reasons to support your answer.

Photo Courtesy of AquaBounty Technology.

Box 28.3

The "Enviropig"

Given the large increase that is expected in both pig and poultry production in the developing world over the next 20 years as a result of population growth and increased income, decreasing the phosphorus levels in the manure of these monogastric species would likely have a huge worldwide environmental benefit. However, using GE to reduce the levels of this important pollutant in swine manure has been subject to the criticism that this kind of approach encourages "non-sustainable, unecological approaches to livestock management." Critics argue that if farmers really want to be environmentally friendly, they should let pigs graze on pasture instead of feeding them grain. However, outdoor pig farming can itself exacerbate nutrient leaching into the soil and groundwater. What is your opinion of the Enviropig – can it help reduce phosphorous pollution in environment; or do you think this GE animal is a bad idea? Give three reasons to support your answer.

Photo by Cecil Forsberg.

continue to fund the project that began in 1999. Prior to that time the pig producer industry association "Ontario Pork" had financially supported the research. The University's applications for food approval of the Enviropig with Health Canada and the Food and Drug Administration in the United States will remain active until a regulatory decision is made, or until such time that the University no longer desires to obtain a final decision from the regulatory evaluators. The shelving of this GE animal project example emphasizes the fact that although the potential of transgenic livestock is tremendous, there are still significant scientific, regulatory and public acceptance issues that need to be resolved before this technology is widely adopted on farms.

Ethical, moral, and animal welfare concerns

The use of animals for any purpose is associated with ethical and moral concerns. Many people who are opposed to GE and cloning of animals tend to oppose all research using animals. The following discussion emphasizes the key moral and ethical issues specifically associated with GE and cloning technologies. An excellent resource explaining why animals and their treatment raise ethical and moral questions is a booklet entitled "Ethics, Morality, and Animal Biotechnology" prepared by the Biotechnology and Biological Sciences Research Council (BBSRC) in the United Kingdom (see www.bbsrc.ac.uk/web/files/policies/animal_biotechnology.pdf). That booklet discusses some of the key considerations and schools of thought when considering morality and ethics as it relates to animals. One important point that is stressed in that booklet is the difference between moral and ethical concerns. The distinction between morals and ethics is explained by the BBSRC as follows:

> Everybody (except perhaps the psychopath) can be said to have moral views, beliefs and concerns, to the effect that certain things are right or wrong and that certain actions ought or ought not to be performed. What issues arouse most moral concern will of course vary enormously between different individuals, cultures and periods of history…Such moral concerns may result from a lot of deliberation and reflection, or from very little; they may be firmly grounded in a consistent set of carefully considered principles, or they may not. We all probably hold some moral views almost unthinkingly, perhaps as a result of our upbringing. We may just "feel" that certain things are right or wrong; we have a "gut reaction" about them; and that may be the sum total of some people's "morality."
>
> Ethics is a narrower concept than morality, and it can be used in several different, though related, senses. The most general of these: " … suggests a set of standards by which a particular group or community decides to regulate its behavior – to distinguish what is legitimate or acceptable in pursuit of their aims from what is not." Hence we talk of "business ethics" or "medical ethics." More technically, ethics can also refer to a particular branch of philosophy which tries to analyze and clarify the arguments that are used when moral questions are discussed and to probe the justifications that are offered for moral claims. So ethics in this sense puts our moral beliefs under the spotlight for scrutiny.

Genetic engineering and cloning may be considered by some to be intrinsically wrong, meaning they are morally wrong under any circumstances, regardless of their consequences and intentions. If someone considers a practice to be intrinsically wrong, then no

further discussion can reverse their belief of that intrinsic wrongness. One of the intrinsic arguments that is often heard when scrutinizing animal biotechnology is that GE and cloned animals are "unnatural" and therefore they should not be allowed. However, there are a number of ethical questions that are raised when considering that moral argument. The first is that the techniques used to produce GE and cloned animals employ natural processes such as DNA repair mechanisms. And so this raises the question of what exactly is natural, and does the fact that something is natural make it right? Vaccinations are not natural and yet we routinely employ them to protect ourselves from disease. It might be more natural to let people die from exposure to naturally-occurring viruses like smallpox, but is that the ethically correct choice? The notion of "nature" and "natural" tends to be an interpretation drawn from the observer's perspective. In the context of ethical judgments the notion of nature is more a conclusion than an argument. There is a presupposition that because something is natural it is ethically correct. Asserting that GE animals are unnatural does not allow a conclusion about whether they should not be allowed. From a description of what is, there is no logical way to prescribe what ought to be. George Edward Moore first observed this fact in 1903 and called the conclusion of an "ought" from an "is" the "naturalistic fallacy."

Some may hold religious views that GE and cloning are intrinsically blasphemous and that humans are intruding into areas that are the realm of God. This argument obviously is one that will only be persuasive for people that believe in a Creator. However, not all religions share this perspective and there is no unanimous condemnation of cloning or GE among religious groups *per se*. Creation is defined as "bringing something out of nothing," and some may argue that GE animals and clones are produced from something (i.e., living cells) and hence this does not meet the definition of creation. There is also some support for the idea that God gave humans a position of "dominion" over Nature. Some may even see biotechnology as an opportunity for humans to work with God as "co-creators." Others may argue that GE is intrinsically wrong because it moves genes from one species to another. However, this occurs routinely in nature, although some GE animals like the phytase pig where a rDNA bacterial phytase gene driven by a porcine promoter was integrated into a pig chromosome could only exist as the result of human intervention. Many religions do not hold that the boundaries between species are sacred and immutable, nor indeed that they are so regarded by God. From an animal breeding perspective, it is exactly this ability to bring entirely new traits into an animal from a different species (e.g., disease resistance genes) that makes the potential benefits of animal GE so compelling.

Another intrinsic argument is that making GE animals interferes with the integrity or "telos" of the animal. Telos is defined as:

… the set of needs and interests which are genetically based, and environmentally expressed, and which collectively constitute or define the form of life or way of living exhibited by that animal, and whose fulfillment or thwarting matter to that animal.

(Holland and Johnson, 1998).

However, as discussed at the beginning of this chapter, all domesticated animals show characteristics that have been produced by selective breeding and that represent changes to their telos – for example, reduced aggression. Those who oppose GE because it alters an animal's telos must consider whether they would also raise ethical objections to the selective breeding methods that have produced all domestic pets (e.g., breeds of dogs) and farm animals.

Public opinion polls have repeatedly shown that the public acceptance is influenced by the utility or reason that a genetically engineered animal is being created. Medical applications are viewed more favorably than food applications and food applications with consumer benefits are viewed more favorably than those with producer benefits (e.g., increased growth rate). Here, we move into the realm of considering the extrinsic arguments, that is, evaluating the consequences and intentions associated with the production of GE and cloned animals and determining whether the benefits outweigh the risks. A patient awaiting an organ transplant from a GE pig may have a different view on the appropriateness of using GE to produce transplantation-friendly pigs than someone who is not facing a similar life-threatening situation.

Critics of GE contend that the risks involved are so great that any use of GE is irresponsible; that it is the particular and potentially dire risks associated with these techniques that make them ethically unjustifiable. Others, including the National Academy of Sciences, argue that there are no unique risks associated with GE and cloning that do not also arise from other genetic improvement techniques including conventional breeding. The risks that are associated with each unique rDNA/animal combination, will vary from case to case making generalizations about the "safety" of GE animals virtually impossible. However, excessive caution does not necessarily reduce risk. Abandoning research and development in all forms of GE animals might prevent the development of a technique or product that could allow animals to better adapt to climate change, help feed the world's growing population, or prove invaluable in the treatment of serious diseases in 50 years' time.

Methodologies to produce cloned and GE animals themselves sometimes create animal welfare concerns, not the least of which is the current inefficiencies of the techniques that result in the use of many more experimental animals than would be needed if success rates were higher. However, efficiencies have been increasing as researchers improve experimental protocols. Additionally the use of SCNT in conjunction with GE cells results in 100% GE clones thereby avoiding the inefficiencies associated with pronuclear microinjection where only a small fraction of microinjected eggs result in a GE animal, and even fewer of these turn out to be germline transgenic.

Some of the reproductive manipulations (e.g., embryo transfer, superovulation) that are required for the production of clones may cause pain or discomfort to the animal, but again these are not new or unique concerns to cloning as these techniques are commonly employed by commercial livestock breeders, and have been for many years. A problem that is often seen with bovine embryos cultured using *in vitro* embryo culture techniques (e.g., SCNT clones) is that the resultant calves tend to have high birth weights and long gestational periods. This phenomenon, known as large offspring syndrome, can result in calving difficulties and an increased rate of caesarian section for the dam. These abnormalities have predominantly been observed in ruminants (sheep and cattle), and mice (Niemann and Lucas-Hahn, 2012). Other naturally-occurring breeds of cattle have analogous calving difficulties. For example double-muscled cows of the Belgian Blue breed (see Box 28.4), routinely require a caesarean section to safely deliver their muscular calves.

An animal welfare concern that is more specifically associated with GE animals is poorly controlled expression of the introduced

Box 28.4

Myostatin GE cattle

Natural mutations in the myostatin gene result in the "double muscled" appearance of some beef breeds (e.g., Belgian Blue), and a 20% increase in muscle mass, the source of beef. This natural mutation is associated with major calving difficulties as the increased muscle mass in affected calves makes it difficult for the cow to deliver her calf. Additionally, cows who are themselves double-muscled have calving difficulties even when carrying an unaffected calf because of their narrower birth canal. In this example, there is a potential conflict between animal productivity and animal welfare arising from a naturally-occurring mutation. This mutation could be introduced into other breeds of cattle using traditional crossbreeding and marker-assisted selection to maintain the natural mutation in the new breed – a process called introgression. New gene editing approaches also offer an approach to introduce the same mutation into other breeds of cattle using GE. Are either of these approaches to introduce this mutation into new breeds ethically acceptable? Do concerns arise based on the process used to introduce the gene (i.e., selective breeding versus GE), the attributes of the animal (i.e., double muscling), or the combination of the two?

Reprinted by permission from Macmillan Publishers Ltd: *Nature Genetics*, vol. 17 issue 1. Luc Grobet, Luis José Royo Martin, Dominique Poncelet, Dimitri Pirottin, Benoit Brouwers, Juliette Riquet, Andreina Schoeberlein, Susana Dunner, François Ménissier, Julio Massabanda, Ruedi Fries, Roger Hanset, Michel Georges. A deletion in the bovine myostatin gene causes the double–muscled phenotype in cattle.

Copyright 1997.

gene. Various growth abnormalities have been noted in GE animals that are expressing a growth hormone transgene (Pursel et al., 1989). Many of the problems that were encountered in these early experiments have been minimized by the use of tissue-specific promoters that result in more targeted expression of the transgene. As technologies to make transgenic animals improve through the use of more sophisticated targeted gene modification approaches, it is likely these unintended effects will become increasingly rare. Of course GE animals that are produced as models for human disease are intentionally modified to have a disease phenotype, and they raise a distinct set of welfare issues. Reduction in animal welfare is intrinsic to the objective of this research and is therefore inevitable while for other applications animal suffering, when it occurs, might be seen as incidental. The decision as to whether the benefits derived from creating diseased animals outweigh the adverse animal welfare effects falls into the realm of ethics.

Public acceptance of agricultural applications of GE has generally been lower than that associated with medical applications of

this technology (e.g., recombinant insulin is used routinely by people with diabetes), and public acceptance may be even more of an issue when considering animal agricultural applications of this technology. In a 2012 survey commissioned by the International Food Information Council (see www.foodinsight.org/Content/5438/FINAL%20Executive%20Summary%205-8-12.pdf), about one-third (33%) of the US respondents were somewhat or very favorable towards animal biotechnology and slightly more than one-quarter (26%) were somewhat or very unfavorable. The primary reasons consumers give for being "not favorable" (i.e., somewhat or very unfavorable or neutral) toward animal biotechnology relate to lack of information and not understanding the benefits of animal biotechnology: More than half (55%) of not favorable consumers chose "I don't have enough information" about animal biotechnology as their primary reason, while 42% cited "I don't understand the benefits of using biotechnology with animals." Ironically, the development of GE animals with direct consumer benefits is unlikely to occur if developers are concerned about public acceptance – somewhat of a "Catch-22" situation.

Paradoxically it often seems that the arguments for and against GE animals overlap. Groups opposed to the technology argue that the risks GE animals pose to food safety, animal health, and the environment are too great to allow the technology to move forward. Proponents of the technology see the potential benefits for GE animals to produce safer food, improve animal health, and reduced environmental impact as too great to forgo the use of this technology in animal agriculture production systems. As with many complex issues there is no right or wrong answer. Polarizing the issue of GE and cloned animals into "all is permitted" or "nothing is permitted" prevents rational social progress on the issue. There are both benefits and risks associated with all technologies. Effective and responsible communication among scientific, community, industry and government stakeholders is essential to reach a societal consensus regarding the appropriate use of these technologies.

Summary

Animal biotechnology is a general term that encompasses older, well-accepted technologies for the genetic improvement of animals such as selective breeding and artificial insemination, and also the more recent "modern" biotechnologies of cloning and genetic engineering. Cloning entails making a genetically identical copy of an individual, whereas genetic engineering involves the use of rDNA to intentionally make changes in the genetic makeup of an individual. There are a number of different techniques that can be used to make cloned and genetically engineered animals, and the optimal approach will vary depending upon the desired outcome. The coordinated use of the two techniques simultaneously can greatly improve the efficiency of producing genetically engineered animals. The genetic modification of animals using any technique is associated with animal welfare, ethical, and moral concerns. Opinions about the appropriate use of cloning and genetic engineering vary greatly. Many countries are currently evaluating both the benefits and risks associated with these technologies, and wrestling to come to a societal consensus as to the appropriate use of these technologies when it comes to genetically modifying animals.

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Review questions

1. Breeds of dogs are all derived from a common wolf ancestor by selective breeding – are dogs genetically modified? If so, should their breeding be regulated by the FDA? Why or why not?

2. What is the difference between cloning and GE?

3. How can SCNT cloning be used to help improve the efficiency of GE?

4. Can you describe a use/application of GE animals that you consider to be ethically acceptable? How would you discuss your idea with someone who is morally opposed to GE engineering?

5. In 1918, an avian influenza epidemic killed more than 20 million people. If GE could be used develop influenza-resistant poultry, do you think chicken and eggs derived from these birds would be accepted by consumers?

6. The science-based regulatory review process undertaken by the FDA is designed to provide a predictable science-based framework that will ensure the safety and safe use of GE animals. Moral, ethical and broader social issues are not included in its review process. How should these issues be addressed in deciding which applications of animal biotechnology are acceptable?

7. How should society go about making decisions on technologies that are considered to be intrinsically or morally wrong by some members of society, and highly beneficial by other members? Can you think of other technologies that have faced this predicament, and how did society address this dilemma?