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Original Paper

A Review of the Use of Genetic Engineering Practices and the Impact of Gene Editing in Healthcare and Biotechnology

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Abstract

Genetic engineering is an integral approach to the development of new diagnostic techniques, drugs for human and animal diseases, foods for human health, development of tissues, and cells for xenotransplantation. The components of vaccine for disease control and nutraceuticals for human health provide proteins; peptides and other components may be an integral part of human life in the coming days. Genetically engineered animals also offer significant human health and environmental benefits; livestock becomes more efficient for converting feed to animal protein and reducing waste production, by imparting resistance to disease and good health. The techniques permit individuals or groups of genes to be isolated from large masses of DNA and produced in virtually unlimited quantities. Genetic engineering in animal production has a growing number of practical benefits, such as in the production of transgenic animal's resistant to disease, increasing the productivity of animals, in the treatment of genetic disorders, and the production of vaccines.

Keywords

in-vitro maturation of oocytes, in vitro fertilization, transgenesis, gene technology

1. Introduction

Genetic engineering is the name of a group of techniques used for direct genetic modification of organisms or populations of organisms using the recombination of DNA. These procedures are of use to identify, replicate, modify, and transfer the genetic material of cells, tissues, or complete organisms. Genetic (Bhatia, 2018; Blanco-Rojo & Vaquero, 2019) engineering involves the incorporation of DNA markers for selection (marker-assisted selection, MAS), to increase the efficiency of the so-called

"traditional" methods of breeding based on phenotypic information. The direct manipulation of DNA sequences These techniques involve the capacity to isolate cut and transfer specific DNA pieces, corresponding to specific genes (Raza, Faooqi, & Mubeen, 2016; Klug & Cummings, 2006). The mammalian genome has a larger size and has a more complex organization than in viruses, bacteria, and plants. Gene therapy to cure genetic diseases such as cystic fibrosis by replacing the damaged copies of the gene with normal ones in infants. In mammals, techniques for reproductive manipulation of gametes and embryos such as obtaining of a completely new organism from adult differentiated cells (cloning), and procedures for artificial reproduction such as in vitro fertilization, embryo transfer, and artificial insemination, are frequently an important part of these processes (Bhatia, 2018).

1.1 Transgenesis

Transgenesis is a procedure in which a gene or part of a gene from one individual is incorporated into the genome of another one (Bhatia, 2018). Transgenic animals have any of these genetic modifications with potential use in studying mechanisms of gene function (Mushenkova, Summerhill, Silaeva, Deykin, & Orekhov, 2019), changing attributes of the animal to synthesize proteins of high value, create models for human disease, or to improve productivity or disease resistance in animals (Montaldo, 2006; Bernabucci, Lacetera, Baumgard, Rhoads, Ronchi, & Nardone, 2010). They include animals that result from the molecular manipulation of endogenous genomic DNA, including all techniques from DNA microinjection to embryonic stem (ES) cell transfer and knockout' mouse production (Felmer, 2004), the production of transgenic mice by microinjection of DNA into the pro-nucleus of zygotes has been the most productive and widely used technique. Using transgenic technology in the mouse, such as antisense RNA encoding transgenesis, it is now possible to add a new gene to the genome, increase the level of expression or change the tissue specificity of expression of a gene, and decrease the level of synthesis of a specific protein (Gama Sosa, De Gasperi, & Elder, 2010).

1.2 Transgenic Methods

Microinjection of DNA and nuclear transfer are two methods used to produce transgenic livestock successfully (Gama Sosa, De Gasperi, & Elder, 2010). Once a specific fusion gene containing a promoter and the gene to be expressed has been cloned and characterized, sufficient quantities are isolated, purified, and tested in cell culture if possible and readied for preliminary mammalian gene transfer experiments (Bhatia, 2018; Blanco-Rojo & Vaquero, 2019). DNA microinjection experiments were first performed in the mouse (Bhatia, 2018; Rao, D., Nra, Iga, Itovic, 2000). While nuclear transfer might be considered inefficient in its current form, major advances in experimental protocols can be anticipated. The added possibility of gene targeting through nuclear transplantation opens up a host of applications, particularly with regard to the use of transgenic animals to produce human pharmaceuticals. The only major technological advance since the initial production of transgenic farm animals has been the development of methods for the in vitro maturation of oocytes (IVM), in vitro fertilization (IVF), and subsequent culture of injected embryos before transfer to recipient females (Gama Sosa, De Gasperi, & Elder, 2010; Murray & Maga, 2016).

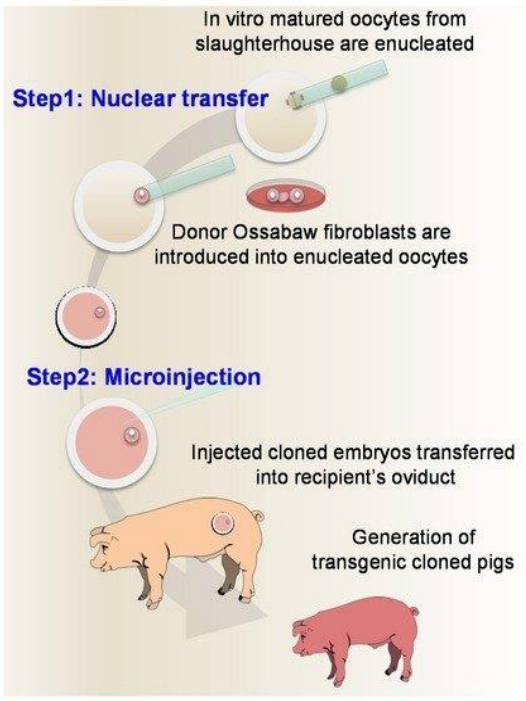

Figure 1. This Diagram Shows How Cloned Zygotes are Edited (ReSheets, Park, Park, Powell, Donovan, & Telugu, 2016)

1.3 DNA Microinjection

The direct DNA microinjection into the pro-nuclei of embryos was the first technique that led to regular and relatively easy success in mammals (Uh & Lee, 2022). In lower vertebrates and invertebrates, pro-nuclei are not a visible gene. In order to perform microinjections in the cytoplasm, much larger amounts of DNA must be used, and the approach varies from species to species (Meacham, Durvasula, Degertekin, & Fedorov, 2014). It proved efficient in several fish species and mainly in salmonids. It remains inefficient in the laboratory fish medaka, Xenopus and chicken. In these species, foreign DNA usually does not integrate into the genome of the animals (Houdebine, 2007). In insects (Drosophila) and worms (*Caenorhabditis elegans*), foreign DNA is injected into gonad syncytium (Lin & Yuen, 2020).

1.4 The Use of Transposons

Several transposons have been used successfully. Transposon has been used for years to generate transgenic Drosophila (Venken & Bellen, 2014). The mariner transposon originally found in Drosophila and adapted to different species is efficient to transfer genes in medaka (Hackett, Ekker, Largaespada, & Mc Ivor, 2005), chicken (Houdebine, 2002), and mouse (Dupuy et al., 2002).

1.5 DNA Transfer into Gametes

Introducing foreign DNA in gametes before fertilization. The yield of transgenic animals is usually low and largely unpredictable. Moreover, the integrated DNA is most of the time profoundly rearranged and no more functional. This phenomenon can seemingly be greatly attenuated by selecting the most

appropriate ejaculates and by removing DNAse by repeated washing and addition of DNAse inhibitors (Coward, Kubota, & Parrington, 2007). Experiments aiming at transferring foreign genes into sperm precursors either in vivo or in vitro are in the course (Coward, Kubota, & Parrington, 2007). The mechanism of gene transfer into epididymal spermatozoa by injection of a DNA- transfectant complex into testis is under study. This method permitted the generation of a limited number of transgenic animals so far (Sato, Ishikawa, & Kimura, 2002).

1.6 The Use of Retroviral Vectors

A family of vectors capable of infecting chicken primordial germ cells and of generating transgenic animals has been described (Tyack et al., 2013). Although laborious this method remains the only, which allowed repeatedly to transfer the foreign gene into the chicken. lentiviral vectors transfer foreign genes with quite a high (Lois, Hong, Pease, Brown, & Baltimore, 2002).

1.7 Gene Transfer Using Embryonic Cells

Appropriate it should be mentioned that in a work published recently, it was reported that appropriate vectors are capable of inducing targeted gene transfer into Drosophila by homologous recombination (Ellis, Hirsch, Porter, Samulski, & Porteus, 2013). Two recent studies indicate that chicken cultured primordial germ cells retransferred to embryos can participate in their development and transmit their genes to progeny (Macdonald, Glover, Taylor, Sang, & McGrew, 2010). ES-like cells from medaka embryo capable of generating chimeric animals have also been described and are potential tools for gene transfer and targeting in fish (Hong, Li, & Hong, 2011).

1.8 Gene transfer by Nuclear Transfer

The donor genome in bovine embryos generated by nuclear transfer from somatic cells is aberrantly methylated (Ogura, Matoba, & Inoue, 2021). This may have inactivated genes required for embryo development. Cloned sheep having the knocked-out gene did not survive after birth (Petersen, 2017; Houdebine, 2009). Gene addition by the cloning technique has been extended to goat (Kalds et al., 2019) and cow (Lonergan, 2007; Zakhartchenko et al., 2001) and pig (Whyte & Prather, 2011). Three groups obtained cloned pigs independently and by different methods. Gene knock-out was recently achieved in the pig. Moreover, cloning was successful in the rabbit (Chesne et al., 2002; Meng, Polgar, Tancos, Tian, & Dinnyes, 2014).

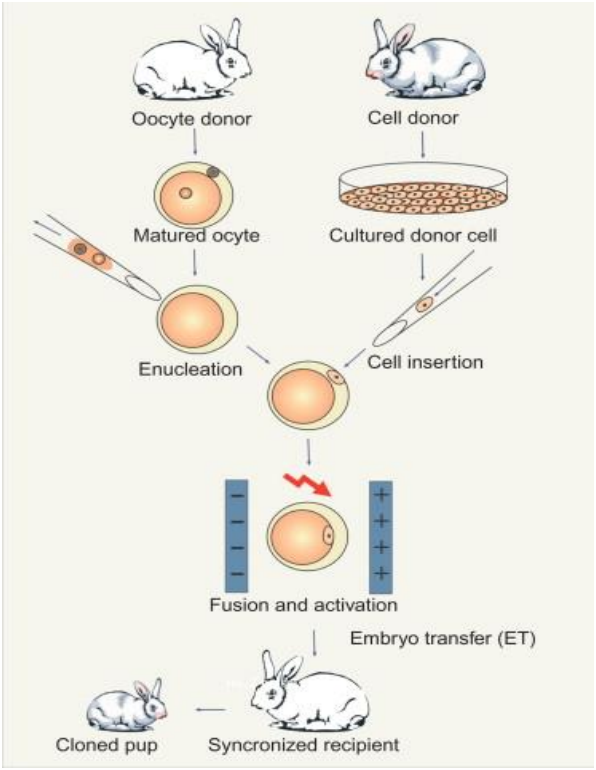

Figure 2. An Embryo Micromanipulation Technology was Developed Using Nuclear Transfer as a Rabbit Model (Meng, Polgar, Tancos, Tian, & Dinnyes, 2014)

1.9 Recombination of Genes Using Plasmids

Foreign DNA is inserted into host cells by combining the foreign DNA with the DNA of a vector. If the recombinant DNA gets inside a host cell, it can replicate along with the DNA of the host cell. This means that every time the host bacterium undergoes cell division, each new daughter cell receives a copy of the recombinant DNA, thus amplifying the recombinant DNA with each cell division (Thomason, Sawitzke, Li, Costantino, & Court, 2014). In order to use a plasmid to insert foreign DNA into a bacterial cell, two steps are required: First, the foreign DNA must be combined with a plasmid. In the second step, a bacterial cell must absorb the recombinant plasmid. For the first step, restriction enzymes and DNA ligase are used. Restriction enzymes are naturally occurring enzymes that cut DNA. Many restriction enzymes are valuable tools in molecular biology. Each restriction enzyme cuts DNA only where a specific sequence of base pairs occurs. The broken bonds between the deoxyribose and phosphate groups that form the "side rails" of the DNA double helix (the phosphodiester linkages) must also be repaired. DNA ligase is the enzyme that catalyzes this reaction (Thomason, Sawitzke, Li, Costantino, & Court, 2014).

1.10 Future Perspectives of Transgenesis

The techniques for obtaining transgenic animals in species of agricultural interest are still inefficient. Using these techniques, it is feasible to reduce to less than 50% the number of embryo receptor females, which is one of the most important economic limiting factors in domestic species. It would also

facilitate the further proliferation of transgenic animals (Edwards et al., 2003). High rates of perinatal mortality, and variable transgenic expression that requires to be evaluated before generalizing their application (Montaldo, 2006; Samiec & Skrzyszowska, 2005). Considerable effort and time are required to propagate the transgenic animal genetics into commercial dairy herds. Rapid dissemination of the genetics of parental animals by nuclear transfer could result in the generation of mini herds in two to three years. However, the existing inefficiencies in nuclear transfer make this a difficult undertaking. While continuous genetic improvements will be introduced in commercial herds by using artificial insemination breeding programs (Karatzas, 2003). In an alternative scenario of herd expansion, semen homozygous for the transgene may be available in four to five years. Extensive breeding programs will be critical in studying the interaction and co-adaptation of the transgene(s), with the background polygenes controlling milk production and composition. Controlling inbreeding and confirming the absence of deleterious traits so that the immediate genetic variability introduced by transgenesis is transformed into the greatest possible genetic progress is equally critical (Andorf et al., 2019).

2. Application of Genetic Engineering

2.1 Increases Milk Composition

Milk proteins are coded by unique copy genes that can be altered to modify milk composition and properties. Among the different applications of milk modification in transgenic animals. To introduce the human lactoferrin into the bovine milk, transgenic cows have been obtained. Lactoferrin is responsible for iron transport and inhibits bacterial growth. To express antibacterial substances in the milk, such as proteases to increase mastitis resistance. The objective is to alter the concentrations of antibacterial proteins such as lysozyme or transferrin in the milk (Bernabucci, Lacetera, Baumgard, Rhoads, Ronchi, & Nardone, 2010; Kerr & Wellnitz, 2003).

2.2 Improving Wool Production

The objectives are to improve the production of sheep wool and to modify the properties of the fiber. Because cysteine seems to be the limiting amino acid for wool synthesis, the first approach was to increase its production through the transfer of cysteine biosynthesis from bacterial genes to sheep genome. This approach did not achieve the efficient expression of these enzymes in the rumen of transgenic sheep. The control of the quality, color, yield, and ease of harvest of hair, wool, and fiber for fabric and yarn production has been an area of focus for genetic engineering in livestock. The manipulation of the quality, length, fineness, and crimp of the wool and hair fiber from sheep and goats has been examined using transgenic methods (Wheeler, 2007). Transgenic methods will also allow improvements to fiber elasticity and strength. In the future transgenic manipulation of wool will focus on the surface of the fibers. Decreasing the surface interactions between fibers could decrease the shrinkage of garments made from such fibers (Mahbubul Bashar & Khan, 2013).

2.3 Enhancing growth Rates and Carcass Composition

Using transgenic technology, it is possible to manipulate growth factors, growth factor receptors, and growth modulators. Transgenic sheep and pigs have been used to examine the postnatal growth of mammals. Growth hormone (GH) and IGF genes have been incorporated and expressed at various levels in genetically engineered animal. Frequently the used promoters have not allowed an efficient control of the expression of the transgene. It was assessed that it is necessary to develop more complex constructions that activate or repress the expression of the transgene more precisely. Adams et al. (2002) found inconsistent results regarding the effect of a growth hormone construct in sheep on growth and meat quality. Recently, a spectacular transformation was obtained by insertion of a plant gene in pigs generated transgenic pigs that carried the fatty acid desaturation 2 Gene for a 12 fatty acid desaturase from spinach (Sasaki et al., 2009).

2.4 Improving Reproductive Performance

Several genes that may profoundly affect reproductive performance were identified. These included the estrogen receptor (ESR) and the Boroola fecundity (FECB) genes. A specific form of the ESR gene is associated with 1.4 more pigs born per litter than is typical in lines of pigs that do not contain this specific ESR gene type (Asaye, Biyazen, & Girma, 2014). The introduction of a mutated or polymorphic ESR gene could increase litter size in pigs. A single major gene for fecundity, the FECB gene that allows for increased ovulation rate was identified in Merino sheep (Davis, 2004). Each copy of this gene increases ovulation rate by approximately 1.5 ova per cycle (Gottlieb & Wheeler, 2007).

2.5 Increasing Resistance to Disease

Animal biotechnology offers several approaches to fight diseases in animals. Firstly, through genetic selection, livestock producers can select for certain traits that are associated with disease resistance and populations of animals that are less vulnerable to diseases could be developed. Secondly, through genetic engineering, breeders can integrate disease resistance genes from new sources, allowing for improved animal disease resistance benefits not only livestock producers and their animals, but consumers also benefit as a result of safer animal products in the market and a reduction in the incidence of human transmissible diseases such as avian influenza (Pohlmeier & Van Eenennaam, 2009). Increased disease resistance can be achieved by introducing resistance-conferring gene constructs into animals or by depleting a susceptibility gene or locus from the animal Hence gain of function (additive), as well as loss of function (deletive, knockout) gene transfer experiments, can be used. Gene transfer experiments are often hampered by the lack of identified major genes or loci responsible for resistance traits (Savolainen, Lascoux, & Meriläˆ, 2013).

2.6 Agricultural Applications

Genetic engineering was originally envisioned to have a multitude of agricultural applications. Recombinant bovine somatotropin (BST) derived from genetically engineered bacteria is one product of genetic engineering that is currently being used in animal agriculture. This protein, which increases milk production in lactating cows, is widely used throughout the U.S. dairy industry. Administering the

protein rBST does not modify the DNA of the cow, and they do not become genetically engineered. BST was approved by the FDA in 1993 following extensive testing by numerous medical associations and scientific societies, which revealed no health or safety concerns for consumers (McGauran, Wieseler, Kreis, Schüler, Kölsch, & Kaiser, 2010).

3. Future of Genetic Engineering

A published report in California Agriculture entitled "Genetic engineering and cloning may improve milk, livestock production" (Bhatia, 2018). The detailed potential uses of these biotechnologies and optimistically concluded that "by midcentury most agricultural animals will be genetically engineered to be more efficient and healthier than the current stock, producing healthy products for human consumption in an environmentally friendly system." While the technologies undoubtedly have the potential to deliver such benefits, no genetically engineered food animals are currently on the market, and the U.S. Food and Drug Administration (FDA) continues to call for a voluntary prohibition on the marketing of milk or meat from clones and their offspring. This review examines the scientific, regulatory, ethical, and public acceptance issues faced by the animal biotechnology industry and discusses the implications of the current climate on the future of animal biotechnology. Biotechnology is defined as technology based on biology. From this definition, it is obvious that livestock breeders have been practicing animal biotechnology for many years. For example, traditional selection techniques involve using observations about the physical attributes and biological characteristics of an animal to select the parents of the next generation. Genetic improvement through selection, based on an increased understanding of population genetics and statistics, has been an important contributor to dramatic advances in agricultural productivity (Dekkers & Hospital, 2002). Many different biotechnologies have been incorporated into livestock breeding programs to accelerate the rate of genetic improvement. These include artificial insemination (AI), sire-testing programs using data collected from thousands of offspring, synchronization of estrus, embryo transfer, cryopreservation of gametes and embryos, and DNA-based marker assisted selection of genetically superior animals (2002).

3.1 Effect of Genetic Engineering

Effect on the Environment Livestock farming is thought to be the major source of steroid hormones found in regional groundwater (Itana & Duguma, 2021), several streams and rivers, and external surface water. Beef cattle wastes are strongly androgenic (Durhan et al., 2006). Significant amounts of synthetic and natural hormones and their metabolites are excreted in animal waste (Itana & Duguma, 2021; Bartelt-Hunt et al., 2012). Synthetic hormones excreted by animals are present in manure applied as fertilizer and in feedlot retention ponds, and from there they may be retained in soil or transported to the ground and surface water calculated the number of beef cattle implanted with estrogens and androgens or progesterone, and the percent of applied hormones that reach the environment via cattle excrement. Commonly used androgenic growth promoter trenbolone has been found in groundwater

near cattle feedlots, and that this growth promotor has androgenic effects. These numbers represent an increase in estrogens and androgens or progesterone over natural elimination rates (Khan, Lee, & Sassman, 2008; Khan et al., 2008; Fan, Wu, Chang, & Hu, 2011).

3.2 Effects on Human

Humans are potentially exposed to synthetic hormones by consumption of commercial meat products and from environmental exposures related to animal waste. Human exposure to both the synthetic and natural hormones causes cancer, reproductive effects, and other endocrine disruption outcomes. Estrogen is carcinogenic, anabolic steroids are reproductive toxicants and trenbolone is an anabolic steroid. TBA, zeranol, and MGA cross the placenta and are detectable in fetal tissues in rabbits (Itana & Duguma, 2021; Alemayehu, 2014) and are reflected in humans. Some evidence showed that xenobiotic growth promoters and their metabolites are thought to be genotoxic. Veterinary use of hormones causes postmenopausal women, and pre-pubertal children, leaving them more vulnerable to the effects of exogenous hormone exposure.

3.3 Effects on the Animal Health

The use of Recombinant Bovine Growth Hormone (rBGH) had problems like mastitis, lameness, loss of condition, and lowered immune system functions, which they attributed to rbST use (Raux, Bichon, Benedetto, Pezzolato, Bozzetta, Le Bizec, & Dervilly, 2022).

4. Limitations of Genetic Engineering

4.1 Environmental Impacts

Potential risks for the environment include unintended effects on non-target organisms, ecosystems, and biodiversity. Insect resistant GM crops have been developed by the expression of a variety of insecticidal toxins from the bacterium *B. thuringiensis* (Bt). Detrimental effects on beneficial insects, or a faster induction of resistant insects (depending on the specific characteristics of the *Bacillus thuringiensis* proteins, expression in pollen and areas of cultivation), have been considered in the area of several insectprotected GM crops (Hilbeck & Otto, 2015).

4.2 Impacts on Human and Animal Health

Healthcare research is the most well-known purpose for which animals are genetically engineered. Through animal genetic engineering, scientists have made breakthroughs in organ transplantation, cancer research, and other areas. Similarities between the genomes of humans and other animals also suggest that future genetic research on animals will yield additional benefits for humans. In the future, kidney, heart, and lung failure patients will likely benefit from animal organ transplants. Xenotransplantation is the procedure of transplanting organs from one species to another. Although xenotransplantation is not new, its use to solve immunological problems such as transplant rejection began recently. Some believed that animal organ transplantation may be able to solve the organ shortage problem (Hilbeck & Otto, 2015). The application of modern biotechnology to food production presents new opportunities and challenges for human health and development. Recombinant gene

technology, the most well-known modern biotechnology, enables plants, animals, and microorganisms to be genetically modified (GM) with novel traits beyond what is possible through traditional breeding and selection technologies.

4.3 Religious, Cultural, and Ethical Issues

The current and potential impact of rapid developments in biotechnology to effect innovations in medicine and drug development, as well as such diverse areas as crime detection, agriculture, pollution control, and industrial processes, brings into question how these techniques can be used constructively without damaging the cornerstones of religious ethics, namely respect for human life (Asaye, Biyazen, & Girma, 2014). Revolutionary innovations in genetic engineering, the decoding of the human genome now make it possible for vegetables in our food chain to bear animal transgenes (Asaye, Biyazen, & Girma, 2014; Akhmetova, 2016). Public opinion surveys have reported that some people are ethically uncomfortable with the idea of genetically engineering animals. There are two central ethical concerns associated with the genetic engineering of animals. The first has to do with breaching species barriers or playing God. Proponents of this view suggest that life should not be regarded solely as if it were a chemical product subject to genetic alteration and patentable for economic benefit (Brunk & Coward, 2009). The second major ethical concern is that the genetic engineering of 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 that animal" (Houdebine, 2007).

4.4 Economic Impact

Research and development budgets for biotechnology research are only beginning to pick up in developing countries. Financing early stages of business development could be achieved through seed funding, easier access to loans, and venture funds (Bruce & Bruce, 2014; Tonukari, 2004). The requirement of adequate infrastructure is a critical factor for the establishment of biotechnology companies.

5. Conclusion and Recommendation

Animal genetic engineering is here to stay. Genetic engineering plays an essential role in healthcare research and food production. It has resulted in huge profits for a variety of industries. Genetic engineering can also assist in sustaining species that are facing possible extinction. The current state and federal laws on animal rights suggest that all justifiable animal genetic engineering should be permitted. Free market environmentalism will test this hypothesis by allowing individuals, interest groups, and government agencies to use the market to indicate the optimal level for genetic engineering regulation. Genetic engineering if executed judiciously may provide practical benefits to mankind, as we have seen from other fundamental advances in life science. These benefits may be in terms of improvement in human health through the production of novel replacement proteins, drugs, vaccines,

research models and tissues for the treatment and prevention of human disease, genetically engineered animals for improvement of environment and human health, improved in food production traits enabling them to help meet the global demand for more efficient, higher quality and lower-cost sources of food. It may also be beneficial to animal health, well-being, and animal welfare and some beneficial high-value industrial products can also be produced such as spider silk used for medical and defense purposes. However, the practical benefits of this technology have not yet reached to the consumers due to the broader gap between myths and the reality of genetic engineering technology. Therefore, there is an urgent need to formulate the regulatory framework with predictable, rigorous, science-based monitoring, and authentication of the technology to deliver practical benefits through the science of genetic engineering.

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