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Transgenic Biotechnology in Animals and its Medical Application: Review

Enyew Mekuriaw* Asefa Asemare Animut Tagele
School of Veterinary Medicine, Wolaita Sodo University, P.O.Box 138, Wolaita Sodo, Ethiopia

Abstract
Genetically modified organisms are not really recent phenomena: thousands of years of selection have led to the domestication of many animal and plant species. However, with the modern methods of genetic engineering and biotechnology, it was possible to cross the interspecific barriers and create transgenic organisms with newly acquired properties. In this over view, we attempted to discuss different aspects of producing transgenic animals by microinjection procedure, use of transposons and use of intermediate cells like pluripotent cells, primordial germ cells and testis stem cells and somatic cell nuclear transfer. Typical medical application includes ;as a source of biologically active proteins, as donors in xenotransplantation**,** as disease models for the development of new treatments, antibody production in transgenic animals, blood replacement and for a research in cell and gene therapy. Despite having these applications, there are many pressing problems that need to be resolved for transgenic animal studies. Ethical considerations regarding animal biotechnology reveal that people are concerned about the purpose of the applications, the methods of research, long term impact on human health. Among the world's largest religions, there are very few clear-cut taboos prohibiting animal biotechnology, although ethical implications can be drawn from the general role of animals within the religious tradition and from beliefs and practices that address animal care and use, animal breeding, and human diet. In conclusion, transgenic animals can resolve serious problems of human health. However, continuous researches are required to break suspects on using transgenic animals with the oversight of respective governments.
Keywords: Biomedicine, DNA Microinjection, Ethical Consideration, Genetic Engineering, Xenotransplantation.

INTRODUCTION

Humans have altered the genomes of species for thousands of years through selective breeding, or artificial selection as contrasted with natural selection and more recently through mutagenesis. Genetic engineering as the direct manipulation of DNA by humans outside breeding and mutations has only existed since the 1970s**.**

In 1972 Paul Berg created the first recombinant DNA molecules by combining DNA from the monkey virus SV40 with that of the lambda virus [1].In 1973 Herbert and in 1974 Jaenisch created the first genetically modified (GM) animal. A year later was 1975 created a transgenic mouse by introducing foreign DNA into its embryo, making it the world's first transgenic animal [2]. These achievements led to concerns in the scientific community about potential risks from genetic engineering, which were first discussed in depth at the Asilomar Conference in 1975. One of the main recommendations from this meeting was that government oversight of recombinant DNA and research should be established until the technology was deemed safe [3]. In 1976 Genentech, the first genetic engineering company was founded by Boyer and Swanson and a year later the company produced a human protein (somatostatin) in *E. coli.* Genentech announced the production of genetically engineered human insulin in 1978 [4].

Over the last three decades biotechnology has advanced to a level where it is generally feasible to make particular changes to the genome, and therefore to the expressed characteristics of living organisms. The product of such a change is called a transgenic or a genetically modified organism (GMO). In the mid 1970s, several investigators infected mouse embryos with retroviruses and demonstrated that proviral DNA integrated into the genome and was passed to subsequent generations (Germ-line transmission of foreign DNA). By the early 1980s the technology gained momentum with various laboratories producing transgenic mice by microinjecting genes into the pronucleus of a fertilized egg, which has been hailed as a seminal event in the development of animal biotechnology [5].

Today, transgenic animals embody one of the most potent and exciting research tools in the biological sciences. Transgenic animals represent unique models that are custom tailored to address specific biological questions**.** Hence, the ability to introduce functional genes into animals provides a very powerful tool for dissecting complex biological processes and systems**.** This has made it possible to explore the regulation of gene expression as well as the regulation of cellular and physiological processes. Significant uses of live transgenic mammals are in the arenas of agricultural, biological, biotechnological and biomedical sciences including production of pharmaceuticals, human gene therapy, antibody production, as disease models for the development of new treatments, blood replacement and in the field of organ transfer from transgenic animals to humans [6]. These require the ability to target gene expression and to control the timing and level of expression of specific genes. Experimental designs have taken advantage of the ability to direct specific expression (including cell

types, tissue, organ type, and a multiplicity of internal targets) and ubiquitous, whole body expression in vivo [7].

The biotechnological production of new-generation drugs with the use of transgenic animals starts with gene-engineering. This must ensure the integration of a genetic construct into the animal genome, tissue specificity of synthesis of the corresponding human protein, biological functionality of the protein of interest, and it's economically significant expression inherited in successive generations of animals. There are several ways to introduce alien genetic information into the animal genome. The most common method, which has been employed in the construction of most currently known transgenic animals are the microinjection of recombinant DNA into a pronucleus of the zygote, use of transposons and use of intermediate cells. The integration frequency proved to depend substantially on several parameters such as the form (Linear or circular) of the DNA molecule, the DNA concentration, the DNA copy number and the injection site [8]. The microinjection procedure is now performed at a high technical level. Regardless of that, further improvement of this already classical method of constructing transgenic animals is possible only by elucidating the molecular mechanisms of the integration and function of foreign genes and by fulfilling the conditions ensuring their realization. As an alternative to microinjection, several protocols of sperm-mediated gene transfer have been developed. However, these approaches are still under investigation and are not routinely used by biotechnological companies specialized in the production of transgenic animals [8]. This paper also revised some key ethical, religious, and legal issues associated with animal biotechnology in traditional livestock species [56].

Therefore, the objectives of this review paper are;

- To discuss most commonly used method of producing transgenic animals.
- To summarize importance of application of transgenic animal in medicine.
- To elaborate the current possible use of transgenic biotechnology in agriculture.
- To indicate problems associated with transgenic animal biotechnology.

DEFINITIONS

Genetically modified animals is defined as an animal that carries a foreign gene which has been intentionally inserted into its genome in order to cause it to exhibit traits or characteristics which are not natural to that animal. Transgenesis is the process of introducing an exogenous gene called a transgene into a living organism so that the organism will exhibit a new property and transmits that property to its offspring. To describe animals carrying new gene (Integrating foreign DNA into their genome), this definition has since been extended to include animals that result from the molecular manipulation of endogenous genomic DNA, including all techniques from DNA microinjection to embryonic stem cell transfer and 'Knockout' mouse production [10].

The methods to generate transgenic animals

An animal is considered as transgenic if it has a copy of foreign DNA stably integrated into its genome. To reach this goal, the foreign gene can be transferred using different methods according to animal species**.** There are different methods of generating transgenic animals but the most commonly and recently used are the following.

DNA Microinjection

The direct DNA microinjection into the pronuclei of embryos was the first and commonly used technique which led to regular and relatively easy success in mammals. The most common method for producing transgenic animals is gene transfer by DNA microinjection, which involves the following steps:

- DNA containing the desired transgene is identified and cloned (Copied tens of thousands of times in bacteria) before insertion into the animal host.
- The host animals (Cows, pigs, or sheep) are induced to superovulate and their eggs are collected.
- The eggs are fertilized in a laboratory dish.
- Using a fine, hollow needle, a solution of DNA containing the transgene is injected into the male pronucleus of the fertilized egg (The nucleus of the sperm cell that entered the egg) before it fuses with the female pronucleus.
- The transgenic embryos are grown in cell culture and then implanted into the uterus of a surrogate mother, where they complete their development.
- Screening is performed to determine which of the offspring's have inherited the transgene.

The limitations of DNA microinjection are:

- It has a low success rate. Only between one and four percent of microinjected eggs result in the live birth of a sheep, goat, or cow containing the transgene, and about 80 to 90 percent of transgenic embryos die during early development.
- The number of embryos generated by super ovulation is low and the success of microinjection appeared accessible only if embryos were prepared in vitro after oocyte maturation and fertilization followed by *in vitro* development of the microinjected to the blastocyst stage.
- It remains laborious, cost and requires a large number of embryos obtained by superovulation (1000-5000

copies) of females followed by either mating with a male or by artificial insemination.

- In lower vertebrates and invertebrates, pronuclei are not visible gene. Microinjection must therefore be performed in cytoplasm, using much larger amounts of DNA.
- In non-mammalian species, pronuclei cannot be visualized, as the embryonic cell is embedded in the opaque vitellus. In these species, a large amount of DNA is microinjected into the cytoplasm of one-cell embryos.

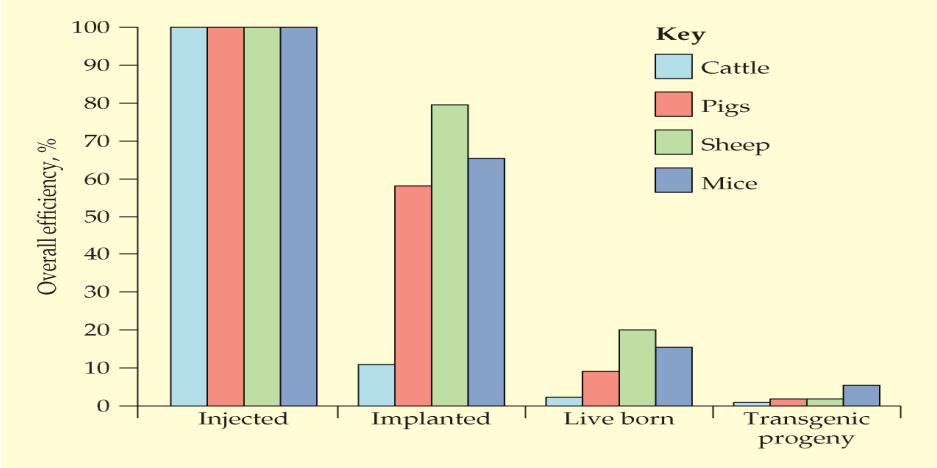

Fig 1.The overall efficiency of the microinjected fertilized eggs to become transgenic progeny.

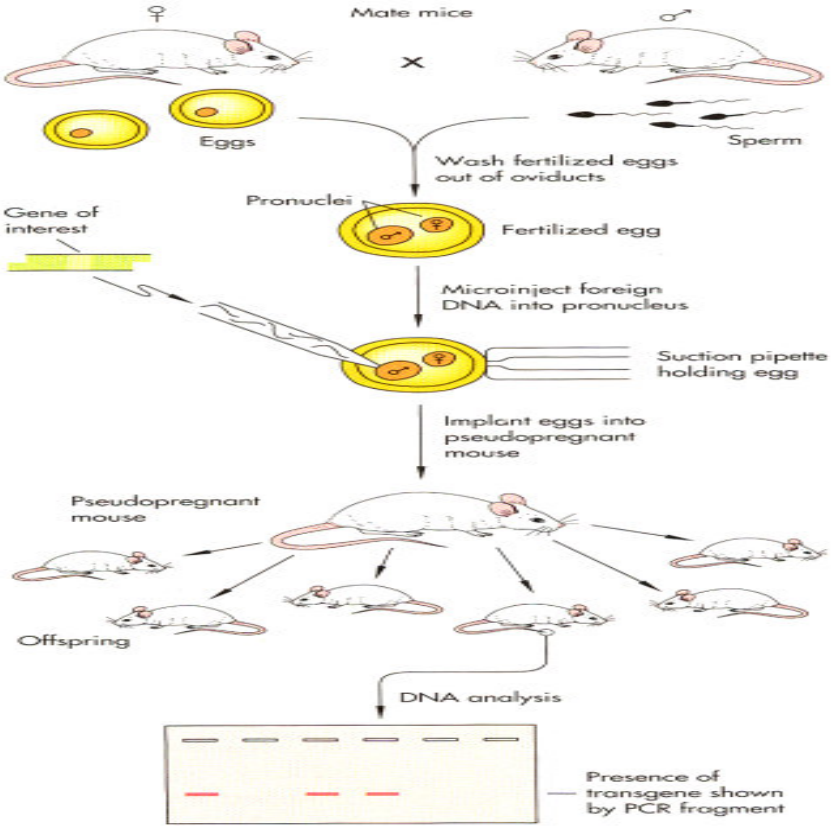

Fig 2. Establishment of transgenic mice by DNA microinjection

Use of Transposons

Transposons are short genomic DNA regions (About 1-3 kb) that autoreplicate and are randomly integrated as multiple copies within the same genome. Integration is achieved by the integrase enzyme encoded by the transposon itself. Foreign DNA can be introduced into the transposon to replace the integrase gene, and the recombinant transposon can be microinjected into one-cell embryos along with the transposon integrase enzyme. Using this method, the foreign gene can be integrated into the embryo genome insects, silk worms, chicken, fish and mammals [11, 12].

Use of intermediate cells

According to Adams and Van der Weyden [13], the gene transfer techniques described above are often too inefficient (*e.g.* gene targeting) to be used directly in embryos. Instead, gene modifications can be carried out in intermediate cells that can then participate in the development of an embryo and transmit the genetic trait to progeny.

Pluripotent cells

By definition, pluripotent cells have the capacity to participate in the development of all organs, including gametes. Pluripotent cells exist in early embryos (Morula and blastocysts), where they are known as embryonic stem (ES) cells. ES cells can be maintained in culture, genetically modified, selected and injected into recipient embryos at the morula or blastocyst stage, as well as in one-cell embryos, and then participate in embryonic development, leading to the production of chimeric transgenic animals . All cells of the chimeric animals, including the gametes, derive from either the pluripotent genetically modified cells or the recipient embryo. Therefore, a proportion of the gametes harbour the genetic modification, which can therefore be transmitted to progeny. Additional studies led to the identification of chemical compounds that can control the expression of these pluripotency genes. ES cells can now be obtained by adding these compounds to the culture medium of rat embryonic cells, thus enabling the development of rat *gene knock-out* and *knock-in* procedures [13].

Interestingly, the transfer of three or four genes normally expressed in pluripotent cells into somatic cells can induce their dedifferentiation into pluripotent cells. These induced pluripotent cells (iPS) have similar properties to ES cells [14, 15]. In addition, it was recently shown that the transfer of two microRNAs into somatic cells can induce their transformation into iPS cells [16].These experiments open important new avenues for cell and gene therapy. Induced potent cells have been obtained in different species, and are thus expected to be a new tool for generating transgenic animals in species for which ES cells are not available [17].

Primordial germ cells and testis stem cells

Primordial germ cells: Primordial germ cells (PGC) are embryonic multipotent cells involved in the development of gonads and the formation of gametes. Recent experiments have demonstrated that chicken PGC can be cultured under experimental conditions that maintain their multipotency, thus establishing stable embryonic gonad (EG) cell lines. To optimize the chance of EG cells colonizing an embryo, EG cells containing a gene of interest were injected into an early embryo in which the majority of the cells had been destroyed by irradiation. This approach proved very successful and has greatly simplified the generation of transgenic chickens [18].

Testis stem cells: In mice and a few other species, testicular sperm precursors can be isolated, cultured and genetically modified. They can also be induced to partially differentiate *in vitro* and then transplanted into recipient testis to give functional sperm capable of generating transgenic animals by fertilization. Sperm cell precursors can also be genetically modified *in situ* using either viral vectors or Transposons [19].

Somatic cells and somatic cell nuclear transfer

The birth of Dolly the sheep demonstrated that somatic cells can be dedifferentiated following their introduction into enucleated oocytes. This technique was developed to improve transgenesis efficiency in farm animals and to generate transgenic cloned animals. The somatic cell and somatic cell nuclear transfer (SCNT) technique starts with the process of enucleation in which the oocyte DNA is removed by aspiration with a tiny micropipette mounted on a micromanipulator. The donor cell is placed against the cytoplast cell membrane using a micropipette and micromanipulator. This couplet then is placed in an electrofusion chamber. Electrofusion is similar to electroporation in that a high voltage direct current electrical pulse is applied to the cell membrane to induce formation of small pores. But with electrofusion, the cells must be aligned so that the two membranes to be fused (The donor cell and cytoplast membranes) are perpendicular to the flow of current [20].

Application of the electrical pulse results in close apposition of the membranes resulting from the charge across the membranes. Eventually, the charge causes formation of tiny pores between the two cells, which eventually coalesce and effectively join the two cells into a single entity. Sperm-induced oocyte activation has been followed, after joining of the oocyte cytoplasm with the donor DNA, to elevate intracellular calcium and suppress activity of an intracellular enzyme that arrests cell division in the oocyte. After activation, the SCNT embryo either can be transferred immediately to a recipient female or allowed to grow for a few days in culture before [20].

Advantages of SCNT:

- It has been shown that SCNT does not induce mutations in the genome of the clone.
- SCNT is currently the most frequently used technique to generate transgenic ruminants and pigs.

Disadvantages of SCNT:

- Somatic cell nuclear transfer gives rise to abnormal fetus development in cow and sheep results from an

incomplete programming of the somatic cell genome. This leads to abnormal epigenetic modifications and to erroneous gene expression, is less frequent in sheep and goats [21].

Applications of transgenic animal biotechnology

A broad spectrum of genetically modified large animals has been generated for applications in agriculture and biomedicine.

Medical Applications

Genetically engineered livestock (Cows, goats, sheep, pigs or chickens) present a unique platform for producing a wide variety of medically-relevant products. The most popular uses of the transgenic animal are the following:

Xenotransplantation

Transplantation of solid organs: Approximately 250,000 people are currently only living because of transplantation of an appropriate human organ (*e.g*. all transplantation). In most cases no alternative therapeutic treatment was available and the recipients would have died without the organ transplantation. Today transplantation technology becomes the basis for a normal life of thousands of patients has led to an acute shortage of appropriate organs. However there is increased demand for appropriate organ, xenotransplantation (The transplantation of organs between discordant species *e.g.* from animals to human) [22]. According to Van Cott *et al*. [23] to solve these problems transgenic animal like pig seems to be the optimal donor animal because:

- The organs have a similar size as human organs,
- Porcine anatomy and physiology are not too different from those in humans,
- Pigs have short reproduction cycles and large litters.
- Pigs grow rapidly than other animals.
- Maintenance is possible at high hygienic standards at relatively low costs.
- Pigs are a domesticated species.

Essential prerequisites for a successful xenotransplantation are: prevention of transmission of zoonoses, compatibility of the donor organs in anatomy and physiology and overcoming the immunological rejection of the transplanted organ [24].

Transplantation of cells and tissues: Another promising area of application for transgenic animals will be the supply of xenogenic cells and tissues. Several intractable diseases, disorders and injuries are associated with irreversible cell death and/or aberrant cellular function. In the future, human embryonic stem cells may serve as a source for specific differentiated cell types that can be used in cell therapy. Xenogenic cells, in particular from the pig, hold great promise with regard to a successful cell therapy for human patients. These cells provide several significant advantages over other approaches, such as implantation at the optimal therapeutic location *(i.e.* immunoprivileged sites such as the brain), possibility for manipulation prior to transplantation to enhance cell function, banking and cryopreservation, combination with different cell types in the same graft [25]. According to Edge *et al*. [25] there are numerous examples for successful application of xenogenic cell therapy:

- Porcine islet cells have been transplanted to diabetic patients.
- Porcine fetal neural cells were transplanted into the brain of patients suffering from Parkinson's disease and Huntington's disease.
- The use of porcine neural cells for stroke and focal epilepsy.
- Cells from genetically modified pigs serve as therapeutic measure to restore electrophysiologically functional axons across the site of a spinal cord transaction.
- Bovine neuronal cells were collected from transgenic fetuses, transplanted into the brain of rats and resulted in significant improvements of symptoms of Parkinson's disease.
- Retinal pigment epithelial cells holds promise to treat retinal diseases such as macular degeneration which is associated with photoreceptor loss.
- Porcine or bovine fetal cardiomyocytes or myoblasts may provide a therapeutic approach for the testis stem cells and somatic cells and somatic cell nuclear transfer.
- Treatment of ischemic heart disease.
- Xenogenic porcine cells may be valuable for the repair of skin or cartilage damage.
- Xenogenic cell therapy using new powerful immunosuppressive drug from donor pig serve as an important therapeutic option for the treatment of human diseases.

The use of transgenic animal as biopharmaceutical production (Industrial use)

Gen pharming or known as biopharming implies any medicinal product manufactured in or extracted from biological sources or semisynthesized from them. Examples of biopharmaceuticals include vaccines, blood or blood components, allergenics, somatic cells, gene therapies, tissues, recombinant therapeutic protein and the living cells used in cell therapy. Perhaps one of the biggest incentives for the production of transgenic livestock

is their capacity to manufacture biopharmaceutical proteins. Transgenic proteins have been produced and secreted into the milk, blood, urine and semen of livestock [26].

According to Niemen *et al*. [26] mammary gland favor most commercial systems to produce for pharmaceutical proteins because it has significant advantages compared with other production methods in terms of:

- Safety, biological activity, and production costs for those biopharmaceuticals that are still harvested from human tissues
- Biopharming represents a safer procedure in regard to prevention of transmissible human diseases such as human immunodeficiency virus/acquired immune deficiency syndrome (*HIV/AIDS*) or Creutzfeldt-Jakob disease (*CJD*).
- Biopharming can be used to produce products (*e.g.,* human polyclonal antibodies).
- The proteins produced should be less likely to cause allergic response than the corresponding nonhuman products harvested from nonengineered animal tissues.

Many therapeutic proteins (Table, 1) that previously were harvested from animal tissues *(e.g.* insulin, growth hormone, hemophilic factors) now are being produced as recombinant human proteins in mammalian, yeast, or bacterial fermentation systems [27]. Acceptable levels of recombinant protein production have been demonstrated in the milk of goats, sheep, and cattle [27]. Cows are likely the most promising animals to be used as transpharmers because they produce large amounts of milk and they have a long lifespan compared to mice or goats. However working with larger animals, like cattle, is much more expensive than working with mice, pigs, sheep and goat, longer gestation period, which takes up large space than a cow and eats more food. So, recent work has focused on breed-early/lactate-early animals like sheep, goats' pigs *and e.t.c.* [28].

Table 1. Summary of therapeutic proteins that are currently in development

Animal	Drug/Protein	Use
Sheep	alpha1 anti trypsin	deficiency leads to emphysema
Sheep	CFTR	treatment of cystic fibrosis
Sheep	tissue plasminogen activator	treatment of thrombosis
Sheep	factor VIII, IX	treatment of hemophilia
Sheep	fibrinogen	treatment of wound healing
Pig	tissue plasminogen activator	treatment of thrombosis
Pig	factor VIII, IX	treatment of hemophilia
Goat	human protein C	treatment of thrombosis
Goat	antithrombin	treatment of thrombosis
Goat	glutamicacid decarboxylase	treatment of type 1 diabetes
Goat	Pro542	treatment of *HIV*
Cow	alpha-lactalbumin	anti-infection
Cow	factor VIII	treatment of hemophilia
Cow	fibrinogen	wound healing
Cow	collagen I, collagen	tissue repair, treatment of rheumatoid arthritis
Cow	Lactoferrin	treatment of GI tract infection, treatment of infectious , treatment of infectious arthritis

Antibody production in transgenic animals

Numerous monoclonal antibodies are being produced in the mammary gland of transgenic goats. Cloned transgenic cattle produce a recombinant bispecific antibody in their blood. Purified from serum, the antibody is stable and mediates target cell-restricted T cell stimulation and tumor cell killing [37]. An interesting new development is the generation of trans-chromosomal animals. A human artificial chromosome containing the complete sequences of the human immunoglobulin heavy and light chain loci was introduced into bovine fibroblasts, which were then used in nuclear transfer. Transchromosomal bovine offspring were obtained that expressed human immunoglobulin in their blood. This system could be a significant step forward in the production of human therapeutic polyclonal antibodies. Further studies will show whether the additional chromosome will be maintained over future generations and how stable expression will be Niemen *et al*. [26].

Blood replacement

The current production system for blood products is donated human blood and this is limiting because of disease concerns, lack of qualified donors, and regulatory issues. Genetically engineered animals, such as cattle carrying human antibody genes which are able to produce human polyclonal antibodies, have the potential to provide a steady supply of polyclonal antibodies for treatment of various infectious and medical conditions like organ transplant rejection, cancer and autoimmune diseases and other diseases. Functional human hemoglobin has been produced in transgenic swine. The transgenic protein could be purified from the porcine blood and showed

oxygen-binding characteristics similar to natural human hemoglobin [24].

Transgenic animals as disease models for the development of new treatments

An animal model is a living, non-human animal used for research and investigation of human disease, for the purpose of better understanding the disease without the added risk of causing harm to a human being during the entire drug discovery and development process. Transgenic animal models are created by the insertion of a particular human DNA into fertilized oocytes which are then allowed to develop to term by implantation into the different models of transgenic animals for various diseases oviducts of pseudo pregnant females. There are different models of transgenic animals for various diseases in Table 2 [29].

***Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/AIDS*):** Transgenic 26 human immunodeficiency virus associated nephropathy (Tg26 HIVAN) Mouse Model was the first transgenic model developed in 1991 for the study of HIV. These transgenic animals can express *HIV-1* proteins; develop symptoms and immune deficiencies similar to the manifestations of AIDS in humans. Other models are AIDS Mouse and Smart Mouse [24].

Alzheimer's disease: No animal models existed for the disease before transgenic technology was employed. Immunization of Amyloid precursor protein *(A42*) in transgenic mice showed that vaccination against Alzheimer's disease could have potential as a therapeutic approach. *E.g.* Alzheimer's Mouse [29].

Cardiovascular disease: Various transgenic animal models for gain and or loss of function of angiotensin, endothelim, natriuretic peptides, catechoalmines, calcium binding-signaling, sodium channel transporters, and nitric oxide synthesis involved in cardiovascular regulation are used to study cardiovascular diseases [30].

Diabetes mellitus: Transgenic models are developed for studying the genes, and their role in peripheral insulin action. Models of insulin secretion such as glucokinase, islet amyloid polypeptide, and hepatic glucose production in type two diabetes are developed. 18A transgenic mouse model that expressed Insulin Dependent Diabetes Mellitus by inserting a viral gene in the animal egg stage is also developed [31].

Angiogenesis: Mouse models of angiogenesis, arterial stenosis, atherosclerosis, thrombosis, thrombolysis and bleeding addresses techniques to evaluate vascular development. Inhibition of angiogenesis is currently one of the biggest opportunities for new cancer therapies. With the help of angiogenesis transgenic animal models inhibitors are identified which act on specific mechanisms of angiogenesis [32].

Cancer diseases: Oncomouse was first transgenic animal to be patented. Its germ cells and somatic cells contain an activated human oncogene sequence introduced into the animal at an early embryonic stage to ensure that the oncogene is present in all the animal cells. Mechanisms for tumor progression and metastasis via E-cadherin and other adhesion molecules are possible by various transgenic knockouts [24].

Pigs in particular, minipigs have been proposed to be better models than mice for studies relating to heart disease, organ transplantation, immunotherapy and obesity.

Transgenic models using livestock are ongoing on several topics: transgenic cattle lacking the *prion receptor* (Inhibits susceptibility to bovine spongy form encephalopathy (BSE), transgenic cattle with resistance to staphylococcus infection (Mastitis resistance) [33].

Disease models are needed in medicine so that one can discover the targets for drug development. Adding and deleting genes in these animals provide them new properties that make them useful for better understanding of disease or manufacturing a cure. It is not ethical or safe to perform the initial tests in humans, so transgenic animals are used. As the testing of new vaccines and drugs must first be performed on animals, these disease models are indispensable [29].

Table 2. Summary of use of transgenic animals as disease model and their proposed therapeutic use

S. No.	Disease model animals	Proposed therapeutic use
1	Sickle cell model	Sickle cell disease .*e.g.* sickle hemoglobin, sickle cell red blood cell (Hemophilia) and other genetic disease.
2	Alzheimer's mouse	Alzheimer's patients (Memory loss), Huntington's, cystic fibrosis, emphysema, diabetes, inflammatory arthritis and cancer *etc*. To create Alzheimer's vaccine
3	Oncomouse	To find better treatments and cures for cancer
4	Schizophrenia model	For schizophrenia
5	Pigs	For treatment of various human disease like cancer, diabetes, heart disease, organ transplantation, immunotherapy, retinitis pigmentosa and obesity.
6	Cattle	For treatment of cattle disease like scrapie, BSE(Animal lacking with prion receptor),mastitis resistance(*Staphylococcus*)
7	Tg26 HIVAN mouse model	To understand and test for vaccine human immunodeficiency vitus/acquired immunodeficiency syndrome (*HIV/AIDIS*).
8	ApoEgene model	To understand and find solution for cardiovascular disease *e.g*. heart failure and hypertrophy).
9	Tg mouse	To study path physiology of diabetes and their therapeutics.

Human gene therapy

Gene therapy uses genetically modified viruses to deliver genes that can cure disease in humans. Although gene therapy is still relatively new, it has had some successes. It has been used to treat genetic disorders such as severe combined immunodeficiency and Leber's congenital amaurosis. Treatments are also being developed for a range of other currently incurable diseases, such as cystic fibrosis, sickle cell anemia, Parkinson's disease, cancer, diabetes, heart disease and muscular dystrophy. Current gene therapy technology only targets the no reproductive cells meaning that any changes introduced by the treatment cannot be transmitted to the next generation. Gene therapy targeting the reproductive cells is called "Germ Line Gene Therapy" [34].

Problems associated with transgenic animal

Biotechnology

Transgenic animals have potentially broad application in biomedicine, agriculture and industry. However, there are many pressing problems that need to be resolved for transgenic animal studies:

Dietary and food safety concerns

According to FAO [35] food safety of bioengineered products is always a significant public topic. Homologous recombination or integration causes the formation of new virus. Foreign gene inserted in the chromosome of the recipient animals result in different genetic changes in different degrees, causing unintended effects. Transgenic animals also increase the risk of zoonotic disease and cause human allergic reactions.

Environmental impacts

If transgenic animals are in the external environment and mating with wildlife, foreign gene may spread, which results; changing the species composition of the original genes, causing confusion in species resources; leads to the loss of the wild allele, resulting in a decline in genetic diversity; disrupts the ecological balance of species, genetic diversity of threatened species. For example, once the transgenic fishes are into ponds or rivers and out of control, they may affect the balance of ecology [36].

Ethical and religious perspective

The ethical issues

According to CAST[37] the ethical issues associated with transgenic animals and mammalian cloning (as these techniques are applied to traditional food animals) fit into three broad categories:

- First are issues that pertain to the impact of this technology on the animals themselves
- Second are issues that relate to the institutions and procedures that govern the research and applications context within the agro-food system.
- Third the issues related to the relationship between humans and other animals; the way that humans think of or act in regard to nonhumans, irrespective of the effect that human conduct has on the animals.

Impacts on animals: Most cultural traditions have accepted the view that at least certain kinds of harm to non-human animals are morally significant. However animals do have subjectively felt needs and are capable of feeling pain. Three philosophical strategies have been proposed as a way to articulate the basis of ethical duties to animals. The animal welfare strategy usually is associated with the work of Peter Singer, a professor of

bioethics. Singer argues that people should attempt a rough estimate of the pain or suffering in dealings with animals, and then weigh this against the benefit derived. Practices in which benefits are offset by the suffering of animals are viewed as ethically unacceptable. This approach generally is understood as a version of ethical utilitarianism [38]. Animals possess a form of individual identity, coherence in their subjective experience that deserves ethical respect. This view would prohibit any use of animals that is contrary to the interest of the individual animal, including many common agricultural practices such as the slaughter of animals for food [39]. Bernard Rollin, a professor of both philosophy and animal science, has argued that those who manage livestock for a living have never doubted that animals have subjectively felt needs and are capable of feeling pain. Rollin also uses the term "Rights" to convey the fact that people do regard themselves as having duties to individual animals, but he regards the basis for these duties as residing in a social consensus on moral duty, noting that whereas this consensus forbids certain exploitative practices without regard to the benefits derived, it nonetheless continues to find the use of animals for food to be morally acceptable. This third strategy can be called the new social ethic for animals [40].

Of these three philosophers, only Rollin has written extensively on animal biotechnology. He has argued that transgenic and cloning technologies would be ethically unacceptable if they resulted in greater animal suffering or frustration than would be experienced by animals of the same species and breed under similar husbandry. If there are no adverse impacts on individual animals, however, there is no basis for an ethical objection to animal biotechnology. It seems likely that Singer's animal welfare approach would reach a similar conclusion [41].

Institutions and procedure

Animal research in the United States is subject to the provisions of the Animal Welfare Act (AWA) of 1966. Although agricultural animals technically are exempt from the Act, the majority of both for profit and nonprofit research organizations use the provisions of the Institutional Animal Care and Use Committee (IACUC) to oversee research. The IACUC committees are regarded widely as having an ethical as well as legal function. Applying a rough test commensurate at least with Rollin's new social ethic to projects involving animal biotechnology would be among these functions. One of the key ethical questions associated with an IACUC is: Has the committee been constituted so that animal interests will be taken into account when experimental protocols are reviewed? A final category of institutional issues addresses the need for consumers to retain the ability to lead lives consistent with the diverse values that exist throughout society. As other sections indicate, it is reasonable to expect that some individuals will resist animal products from genetic engineering or cloning, perhaps for religious or even arbitrary reasons [37].

Relationships between humans and animals

Some of the most strident ethically based opposition to animal biotechnology focuses on the ways modern technologies have caused the traditional relationship between humans and farm animals to change. Here, the ethical focus is on the moral character of the people or the society that undertakes these projects rather than on the ethical acceptability of what is done to the individual animal. Thus, whereas an animal rights view would object to any practice that sees the animal merely as the means to an end, the objection here is focused more on the venality or corruption of character either within the scientific and animal production community, or perhaps within society at large [37].

The fact that regulatory agencies are unable to intervene against specific technologies deemed to meet standards of animal, human and environmental health can be interpreted, in this regard, as part of a general societal failure to regulate human conduct in light of moral expectations. Here, too, the large scale and automation of husbandry associated with concentrated animal feeding operations (CAFOs) is undoubtedly a component of the concern. Although CAFOs currently in use do not in any way use biotechnology, they are the end result of scientifically based studies on animal nutrition, reproduction, and husbandry, combined with principles of agricultural engineering. As such, it is not unreasonable for someone not personally involved in science or animal agriculture to perceive a pattern of change in livestock production and to interpret developments in animal biotechnology as elements in this broader pattern. Thus, without regard to whether biotechnology will improve or materially affect the welfare of animals within a CAFO system, it is possible, particularly given no reason or evidence to draw a contrary conclusion, for a member of the public to associate ethical concerns with the general drift of science-based animal husbandry, and to see animal biotechnology as a particularly cogent example of this drift [37].

Religious views on animal biotechnology

Among the world's largest religions, there are actually very few clear-cut religious taboos prohibiting transgenic and other animal technologies. Ethical implications of religious traditions, however, can be drawn from the general role and status of animals within the religious tradition, as well as from traditions that address animal

care and use, animal breeding, and human diet. Western religions, those based on Christianity, Judaism, and Islam permits animal biotechnology because humans are the instruments through which God works toward bringing creation to final perfection. Whereas animals are God's creatures and have their own moral value, they are at the service of men and women, so that humans also can achieve their overall development through them. Humans cannot use animals indiscriminately, but if animals are used to provide a significant human benefit, that use is permissible. Thus, creating and using animals through biotechnology is permissible as long as the need is sufficient and animal welfare is respected [42].

Eastern religions such as Buddhism, Hinduism, and Confucianism do not use the concept of animals in the service of humans. Instead, these religions give animals a moral status that often is almost equal to that of humans. Humans have a higher status only to the extent that they are more capable of achieving the philosophical ideals of spiritual wisdom and liberation. For Hindus, incarnations of the Gods include animal forms. There are also specific religious concerns involving food use of animal biotechnology. For example, most Hindus attempt to be strict vegetarians, and there could be concerns about the extent that animal DNA is mixed in with GM plants. Both Jewish law and Islamic law have food restrictions that may be affected by biotechnology [43].

CONCLUSION

In the ongoing quest of transgenic animals, different methods have been used to create these transgenic animals. These techniques have developed rapidly and provided more and improved platforms for the preparation of transgenic animals since their emergence. These techniques provided an entirely new pathway for the accurate modulation of genes. All of these developments will provide new ideas and bring important changes in fields like medicine, health. Transgenic livestock have the potential to play a critical role in the production of new medications for the treatment of human disease. This role might consist of actual production of recombinant proteins (Including bio therapeutic proteins and antibodies) for treatment of human diseases. In particular, the economic and social benefits from the production of bioreactors, drug production, and gene-therapy and organ culture for human transplantation will be great. Scientists are also able to use animals as model systems for human diseases and thereby provide a system for testing new treatments and medications that is not possible with human subjects. Hopefully, new drug and gene therapies can be designed and tested in these animals and, therefore, provide new strategies for treating debilitating diseases. Some of the diseases for which transgenic animals have served as model systems include Alzheimer's disease, diabetes acute heart failure, hypertrophy, Sickle Cell Anemia (Hemophilia), *HIV/AIDS* and various forms of human cancers. Transgenic animals have numerous potential agricultural importances in modifying traits like breed, quality of agriculturally important livestock resulting in highly increased animal production in quantity and quality and improved reproduction performance. Also have importance on the development of environmental friendly and disease resistant animals. However, the use of transgenic animals is facing: dietary and food safety concern, worry of negative environmental impacts mainly by disrupting ecological balance, and ethical and religious concerns.

Based on the above conclusive statements the following recommendations are made:

- Continued support of research by both government and commercial entities to develop additional promising bio therapeutics and chromosomes
- Information concerning the genetic engineering, the production methods, products, and regulatory process should be available to the public
- Education regarding the advantages and challenges associated with this new technology is the key to public understanding
- Government oversights, stringent rules and regulations on the research of genetic engineering and use of transgenic animals

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