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RESEARCH PAPER

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Review on Transgenic Animal Technology Classifications, Applications and Implications

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ABSTRACT

Application of artificial insemination, embryo transfer and other biotechnological procedures has resulted in the well-known and remarkable increases in performances of animal production. Transgenesis is one of biotechnological procedure in which genes of one species can be transplanted to another species through the process known as Recombinant DNA technology. The first successful transgenic animal was a super mouse since then much has been accomplished to produce transgenic rabbits, pigs, sheep and cattle. Pronuclear injection, embryonic stem cell mediated gene transfer, retro viral mediated gene transfer, sperm mediated gene transfer and somatic cell nuclear transfer are the commonly used methods to transfer foreign gene from one species to another. Transgenic animals are created for many purposes and in many cases they have been useful, both in a positive light as well as in the negative, to science and society. There are five basic categories of transgenic animals, which include: disease models, transpharmers, xenotransplanters, food sources and biological models. Transgenic technology plays a great role in many fields including agriculture, medicine and industry. Even though it has enormous applications in different fields, still it has some limitations. It can be concluded that further efforts on the use, method of application and side effects of transgenic technology should be done.

Keywords: Recombinant DNA technology, super mouse, Transgenesis,Transgenic Animals, Transpharmers and Xenotransplanter.

INTRODUCTION

Breeding of animals have a long standing and very successful tradition. It began with domestication by which man habituated animals to live in his proximity. Scientifically based animal breeding has existed for approximately 50 years on the basis of the increasing knowledge in population genetics and statistics. From the early beginning, this approach incorporated biotechnological procedures for which artificial insemination is the preeminent example. In the 1980s, embryo transfer technology has been transferred from an experimental stage to commercial application (Thibier, 2001).

The efforts of animal breeders and the applications of artificial insemination, embryo transfer and further biotechnological procedures have resulted in the well-known and remarkable increases in performances of animal production. Gene transfer is one of the biotechnological procedures which are defined as the introduction of a protein coding DNA fragment into the host genome with the goal that the foreign DNA contributes to the protein synthesis of the host organism, e.g. the transgenic animal. Usually gene transfer involves gene constructs which are artificially combined DNA fragments consisting of regulatory and protein coding sequences (Niemannand Kues, 2003).

Transgenic animal technology is one of the fastest growing biotechnology areas. It is used to integrate exogenous genes into the animal genome by genetic engineering technology (Miao, 2012). In genetic engineering or genetic transformation the genetic material is modified by artificial means. It involves isolation of gene and cutting it at a precise location by using restriction enzymes', selected DNA fragments can be then transformed in to the cells of the target organism and that organism genetically modified (Johanson and Ives, 2001). Recent developments in animal gene transfer technique includes microinjection method, embryonic stem cell method, somatic cell nuclear transplantation method, retroviral vector method, germ line stem cell mediated method to improve efficiency andgene targeting to improve accuracy (Miao, 2012).But each of techniques has their own limitations and most of those techniques are uncertain and have long term effects on transgenic animal production. Transgenic farm animals are important in human medicine as sources of biologically active proteins, as donors in xenotransplantation (xenografts from transgenic pig) and for research in cell and gene therapy (adding the normal copies of human gene carrying defective copies of the gene). Typical agricultural applications include: improved carcass composition, lactational performance, wool production as well as enhanced disease resistance and reduced environmental impact (Niemann *et al*., 2005).

Conventional breeding methods much adapted by means of careful selection of particular animals but, the numbers of new gene combinations that can be achieved through transgenic animal technology are, however, limited since genes can only be transferred between members of the same or very closely related species. The transgenic animal technology reaches only 1–3% per year and is relatively low and this indicates that still this technology is in infant stage, its full potential is not been fully utilized and this technology is still experimental. Many proof-of-principle studies have been carried out, about transgenic livestock production but, the commercial application of this technology is still not-exist (Baguisi, 1999). Therefore, the objective of this paper is: to highlight transgenic animal technology, classifications, method of applications and its implications.

TRANSGENIC ANIMALS

A transgenic animal is an animal whose genome has been altered by the inclusion of foreign genetic material through transgenesis. Transgenesisis a process in which genes of one

species can be modified, or genes can be transplanted from one species to another. Nucleus of all cells in every living organism contains genes made up of DNA. These genes store information that regulates how our bodies form and function. Genes can be altered artificially, so that some characteristics of an animal are changed. For example, an embryo can have an extra, functioning gene from another source artificially introduced into it, or a gene introduced which can knock out the functioning of another particular gene in the embryo. Animals that have their DNA manipulated in this way are known as transgenic animals. The underlying principle in the production of transgenic animal is the introduction of a foreign genes into an animal (the inserted genes are called transgenes). The foreign genes "must be transmitted through the germ line, so that every cell, including germ cells, of the animal contains the same modified genetic material (AIBS, 2003).

History

In 1982, the world's first transgenic animal was created, an oversized mouse called super mouse containing a human growth hormone. Since then much has been accomplished in the generation of various types of first transgenic animals like, pig in 1985, goat in 1991, sheep in 1998 and cattle in 2003(Hafez, 2015).

Purpose

Interest in transgenic animals originally falls into three broad categories. Firstly to increase production efficiency of farm animals in a short period like to yield more meat or to perform a specific task better (AIBS, 2003). Secondly genetically modified organisms are created or modified in the laboratory to amplify desired characteristics which are beneficial to mankind (Niemann and Kues, 2003). Thirdly some transgenic animals are produced for specific economic traits. For example, transgenic cattle were created to produce milk containing particular human proteins, which may help in the treatment of human emphysema (AIBS, 2003).

Methods Used to Produce Transgenic Animals

Foreign gene to be used in creating the transgenic animal is constructed through a process known as recombinant DNA methodology. In this process, desired genes or a piece of DNA to be inserted from another species are cut with a restriction enzyme. Isolate plasmids from cloning vector and by using the same enzyme cut plasmids and then insert desired DNA into plasmid and then join the two DNA with DNA Ligase (Kinseyand Cooey, 2000). The transgeneis inserted into a vector (which allows it to be amplified to high copy numbers). The vector also contains a promoter which allows the inserted foreign DNA to be expressed by the cells of the host animals (Anderson and Dowdy, 2005).

Establishment of stable transgenic animals implies that the foreign DNA is present in gametes or one-cell embryos to allow its transmission to progeny (Houdebine, 2002).Five techniques existing by which cloned DNA could be engineered and transferred in to the host cell such as: pronuclear injection, retroviral-mediated gene transfer, embryonic stem cell-mediated gene transfer, sperm mediated gene transfer and somatic cell nuclear transfer (Primrose and Twyman, 2006).

Pronuclear injection mediated gene transfer

Injection of a DNA solution into the pronuclei of fertilized eggs is the most common method for making transgenic animals. Injection is done at the stage of development when the ova have two pronuclei, one from each gamete, which will later fuse to form the diploid nucleus. The egg donor females are super-ovulated to cause them to release more eggs than usual.

Then mated with fertile males and the eggs are harvested (UQ, 2017). For several hours following the entry of the sperm into the oocyte, the male and the female pronuclei can still be seen individually under a normal light microscope and they have not fused yet into a so called zygote. The foreign DNA is microinjected into either pronuclei with no difference in results; however, the DNA is typically injected into the male pronucleus because it is slightly larger and closer to the oocyte surface. Then with the transgenes are kept overnight in an incubator to develop to 2-cell stage. These oocytes are subsequently transferred into the uterus of pseudo pregnant recipient animals. The offspring are screened to confirm which of the offspring is expressing the gene of interest (Transgenic Mouse, 2005).

The introduced "foreign" DNA may lead to the over- or under-expression of certain genes or to the expression of genes entirely new to the animal's species. The integration of the transgene into the host nucleus is a random process, and there is no way of controlling where it integrates in the host's genome. The major advantage to this method is its applicability to a wide variety of species and it remains one of the most commonly used methods for creating transgenic animals (Corcoran *et al.*, 2004).

Embryonic stem cell mediated gene transfer

Embryonic stem (ES) cells are derived from the inner cell mass of blastocysts. This method involves isolation of totipotent stem cells, which are undifferentiated cells that have the potential to differentiate into any type of cells (somatic and germ cells) and therefore to give rise to a complete organism (Miao, 2012).

Pronuclear fertilized eggs are collected from the oviduct of a donor female. They are associated with sticky follicular cumulus cells that must be removed using the enzyme hyaluronidase. Once the cells are free from the follicular cumulus cells, the embryos are transferred into a pool of medium on a petridish and covered with a sterile, autoclaved mineral oil to avoid contamination by microorganisms and debris and to prevent evaporation. Preparation of the DNA transgene is also very important because any contamination will ruin the experiment. The DNA fragment that is to be used is purified to rid it of any contaminants or traces of agarose and to make sure that the DNA is intact and not ruined in any way. The transgene is cloned in a plasmid and then isolated from the plasmid by restriction enzymes (Blanchard and Kelly, 2005).

When the transgene is prepared, it ismicroinjectedinto the ES cells. The cells are then allowed to grow to the blastocyst stage and then transplanted into the uterus of the pseudo pregnant mother (Blanchard and Kelly, 2005). The offspring are screened to determine which of the offspring are heterozygous for the transgene and then two heterozygous animals are breed to create an animal that is homozygous for the transgene (Cunningham, 2011). Embryonic stem cells allow the use of homologous recombination to target where the transgene is inserted in the host's genome. In homologous recombination, regions of host DNA are engineered to flank the transgene. Once the rDNA is inserted into the ES cell, during normal DNA replication and cell division, the homologous DNA regions exchange between the rDNA and the host chromosome targeting the transgene to the site (PrimroseandTwyman, 2006).

Retrovirus mediated gene transfer

Retroviruses are viruses that have RNA as their genetic material instead of DNA. It also has an enzyme called reverse-transcriptase which can make DNA from RNA. These viruses are altered, so that they will not destroy the host cells that they are to invade (Blanchard and

Kelly, 2005). Retroviruses used as vectors to transfer genetic material into the host cell, resulting in a chimera, an organism consisting of tissues or parts of diverse genetic constitution (AIBS, 2003).

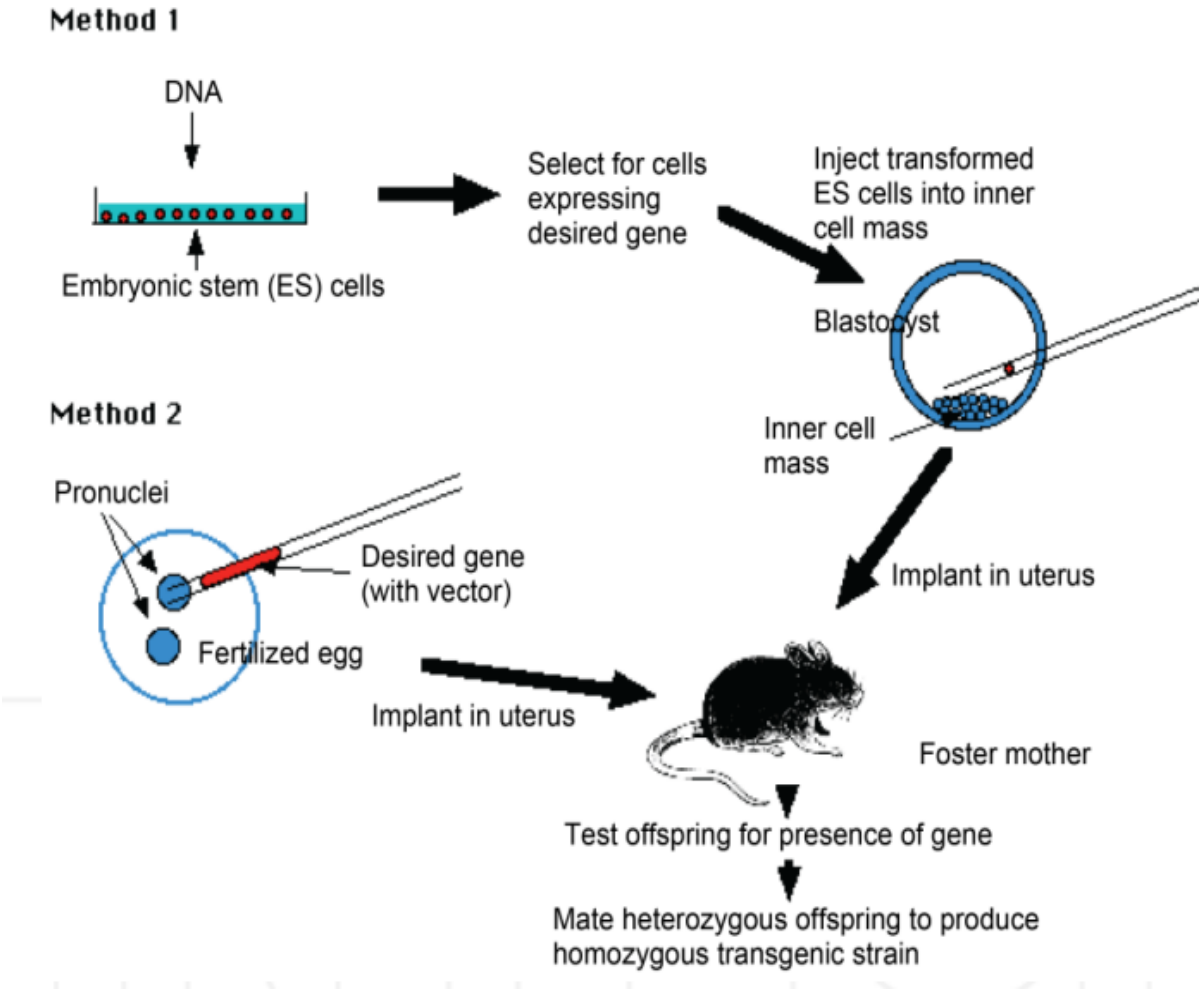

Figure 1. Diagram displays methods 1 and 2 of the embryonic stem cell method for making a transgenic animal.

Source: (Miao, 2012).

In this method, specific deleterious viral genes are removed from the viral DNA and then replaced by the transgene of interest (Anderson and Dowdy, 2005). Next they microinject the virus into the embryo and let it infect the embryo with the transgene. After that the embryo is allowed to grow to the blastocyst stage and is transplanted into the surrogate mother. This method can be problematic though because it creates animals that are mosaics (the virus does not infect all the cells) and the viral sequences necessary for the virus to do its job of infecting the cells may interfere with the expression of the transgene. Also the size of the transgene sequence that can be added to the virus is limited (Blanchard and Kelly, 2005).

Somatic cell nuclear transfer (SCNT)

In somatic cell nuclear transfer the nucleus, contains the organism's DNA, of a somatic cell (a body cell other than a sperm or egg cell) is removed and the rest of the cell is discarded(Das, 2003). At the same time, the nucleus of an egg cell is removed. The nucleus

of the somatic cell is then inserted into the enucleated egg cell. After being inserted into the egg, the somatic cell nucleus is reprogrammed by the host cell. The egg, now containing the nucleus of a somatic cell, is stimulated with a shock and will begin to divide. After many mitotic divisions in culture, this single cell forms a blastocyst (an early stage embryo with about 100 cells) with almost identical DNA to the original organism. Then Somatic cell nuclear transferderived blastocysts are implanting into the uterus of a surrogate mother, in which the embryo develops into a fetus (Beyhanet al., 2007).

Sperm mediated gene transfer

Sperm mediated gene transfer is successfully carried out in cattle. The sperm cells have the capacity to bind naked DNA or bound to vesicles like liposome's (Chang et al., 2002). Collect sperm cells from male animals and evaluate its quality, motility, survival rate, morphology and ability to take up exogenous DNA. Then Removal of seminal fluid and mix with transgene. Introducing exogenous DNA containing sperm cell into oocytes either through invitro fertilization or artificial insemination (Smith and Spadafora, 2005).

TRANSGENIC ANIMAL CLASSIFICATION

Mice are not the only animals being genetically altered; other animals including pigs, sheep, goats, fishes, flies and even a monkey have been transformed into transgenic animals. Transgenic animals are created for many purposes and in many cases they have been useful, both in a positive light as well as in the negative, to science and society. There are five basic categories that these animals fall under, which include disease models, transpharmers, xenotransplanters, food sources and scientific models (Corcoran et al., 2004).

Transgenic Disease Models

Disease models are among the most important transgenic animals. Animals have long been used in medical research to test medications, vaccines and other treatments (Anderson and Dowdy, 2005). Alzheimer's Mouse was created in 1995 which was models for Alzheimer's diseases. It was engineered with human amyloid genes to develop Alzheimer's symptoms (Games and Adams, 1995).Oncomouseare useful models for studying human cancers (Corcoran and Moore, 2004).

Farm animals, such as pigs, sheep, or even cattle, may be more appropriate models in which to study potential therapies for human diseases that require longer observation periods than those possible in mice, e.g. atherosclerosis, non-insulin-dependent diabetes, cystic fibrosis, cancer and neurodegenerative disorders (Hansen and Khanna, 2004).

Transpharmers

The second class of transgenic animals is called transpharmers. These animals are used to study the production of a pharmaceutical protein or antibiotics in their milk. Ultimately all transpharmers are female because males cannot produce milk (GMP, 2001).

Goats

Genetically altered goats expressing human antithrombin III (hAt) produced by injection of h Atgene directly into the oocyte by a procedure called somatic cell nuclear transfer(Baguisi, 1999). Human antithrombin III is a serum glycoproteinit controls blood clots by inactivating the clotting factor thrombin as well as inhibiting other clotting factors trypsin and chymotrypsin (Phadkeet al., 1998).

Sheep

The transgenic sheep expressing alpha-1-antitrypsin, which is also known as AAT. It is a protein produced in the liver, which protects the lungs, stimulates an enzyme that fights bacteria and gets rid of dead lung tissue to keep the lungs functioning properly (Corcoran and Moore, 2004).

Baby Herman

Baby Herman, the world's first transgenic bull and his daughters who carry a human gene for creating lactoferrin enhanced milk (Corcoran et al., 2004). It is hoped that his female offspring will produce the protein in their milk and thus alleviate the need for human babies to drink formula or mother's milk unnaturally low in this protein (Biotech Notes, 1994).

Xenotransplanters

Xenotransplanters are animals altered to grow human-compatible organs for animal to human transplantation (Butler, 2002).Transplantation of regular animal organs into a human will almost definitely result in rejection of the organ because the human immune system identifies the cells as foreign. The cells are destroyed by T-cells and therefore the transplanted organ will not function properly. These transgenic animals will no longer produce the animal specific proteins that will bring about the detrimental immune response and rejection (Kaiser, 2002).

Pig Xenotransplanters created by inactivating the gene for the enzyme 1, 3 galactosyltransferase which is the enzyme responsible for adding the sugar, 1, 3 galactosyl, to the surface of pig cells. These sugars are 32 specific to pigs, so in turn it triggers rejection in humans. By removing the sugar, the organ is more compatible to humans so rejection is less likely (Butler, 2002).

Food Sources

Food sources fall into the category of "super" animals, which are given this generic name to describe their size. These transgenic animals are genetically engineered to produce more meat, so fewer animals are killed for the same amount of meat. Often, the addition of growth hormones to the animal is used to increase size beyond that of the animal's natural stature (Corcoran andMoore, 2004).

Superpig was created by fusion of ovine growth hormone (oGH) into pig zygotes. It was larger in size after engineered with oGH and shows different amount of the growth hormone (Pursel *et al*., 1997).Super fish has been attempted using quite a few different types of fish. Rainbow-trout eggs obtained from a very slow growing wild strain were microinjected with a salmon growth hormone. The transgenic trout grew much faster than normal trout. Although these trout were considered super fish, their growth still could not match that of a fast-growing normal domesticated strain of trout (Devlin*et al.*, 2001).

Scientific Models

Working with transgenic animals has led to a great deal of scientific knowledge about the biological effects of over-expressing or under-expressing specific proteins. Animals producing more or less of a particular protein than usual this helps to study that protein'sin biological mechanisms or development and then applied to humans (Anderson and Dowdy, 2005). In 2001, Oregon Regional Primate Research Center, announced the creation of ANDi, the world's first transgenic primate (Chan *et al.*, 2001). ANDi, whose name represents "inserted DNA", backwards, is a rhesus monkey and carries the jellyfish gene encoding green fluorescent protein. The gene is inactive, but present and detectable in its cells. Successful creation of ANDi proves that the techniques used to insert foreign genes can work in primates (Vogel, 2001).

APPLICATIONS OF TRANSGENICANIMALS

Medical Applications

Nutritional supplements and pharmaceuticals

Application of transgenic animal in the production of recombinant and biologically active proteins in the mammary gland and this in turn could be used for the benefit of mankind. This is called as "Gene Pharming". Mammary gland is the preferred site for production of these proteins because large quantities can be extracted and purified (Meade et al., 1999). Moreover, milk is secreted body fluid that is normally produced in large quantities and which could be collected without causing any harm to the animals (Kumar et al., 2013).

Table 1. Lists of some recombinant proteins obtained from transgenic animals.

Protein	Source	Against
Antithrombin III	Goat	Thrombosis
Tissue plasminogen activator	Sheep, pig	Thrombosis
α-antitrypsin	Sheep	Emphysema
Factor VIII, IX	Sheep, pig, cow	Hemophilia
α Glucosidase	Rabbit	Pompe's disease
Fibrinogen	Cow, sheep	Wound healing
Glutarmic acid decarboxylase	Goat	Type 1 diabetes
Human serum albumin	Cow, sheep	Maintenance of blood volume
Human protein c	Goat	Thrombosis
Monoclonal antibodies	Chicken, cow, goat	Vaccine production
Pro 542	Goat	HIV
Lactoferrin	Cow	GI tract infection and infectious arthritis.

Source: (Rajoriya *et al.*, 2013)

Xenotransplantation

Xenotransplantation involves the transplantation of non-human tissues or organs into human recipients. Scientists use xenografts from domesticated pigs to close the growing gap between demand and availability of appropriate organs for humans. Prerequisites for successful xenotransplantation are: overcoming the immunological barriers, preventing the transmission of pathogens from the donor animal to the human recipient and adjusting the compatibility of donor organs with human (Yang and Sykes, 2007).

Human gene therapy

Human gene therapy involves in adding a normal copy of a gene (transgene) to the genome of a person carrying defective copies of the gene. The potential for treatments for 5,000 named genetic diseases is huge and transgenic animals could play a role. Transgenic calf carries a gene that responsible for making a substance which promotes the growth of red blood cells in human (Miao, 2012).

Agriculture

Breeding and quality

Farmers have always used selective breeding to produce animals that exhibit desired traits. Traditional breeding is a time-consuming and difficult task. When technology using molecular biology was developed, it became possible to develop traits in animals in a shorter time and with more precision. In addition, it offers the farmer an easy way to increase yields (Miao, 2013).Potential applications of transgenic technology in producing new varieties of livestock that has increased growth rate, reproductive performance, feed utilization, improved milk production, meat and eggs also could be modified by this technology (Kumar et al., 2013).

Transgenic animals have potentially broad applications in the improvement of animal production quality, for example: transgenic cows exist that produce more milk or milk with less lactose or cholesterol, pigs and cattle that have more meat with improved, quality and sheep that grow more wool. In the past, farmers used growth hormones to spur the development of animals but this technique was problematic, especially since residue of the hormones remained in the animal product (Miao, 2012).

Disease resistance

Scientists are attempting to produce disease-resistant animals, such as transgenic sheep have been developed that is resistance to visna virus infection (Clements et al., 1994). The transmission of bovine spongiform encephalopathy (Scrapie) is also prevented by the knock down of prion protein (Weissmann et al., 2002). Transgenic mice have been developed that secretes recombinant antibodies in milk to neutralize the coronavirus responsible for transmissible gastroenteritis virus (Castillaet al., 1998).

Industrial Applications

In 2001, scientists at Nexia biotechnologies in Canada spliced spider genes into the cells of lactating goats. The goats began to manufacture silk along with their milk and secrete tiny silk strands from their body by the bucketful. By extracting polymer strands from the milk and weaving them into thread, the scientists can create a light, tough, flexible material that could be used in such applications as military uniforms, medical micro sutures and tennis racket strings. Toxicity-sensitive transgenic animals have been produced for chemical safety testing. Microorganisms have been engineered to produce a wide variety of proteins, which in turn can produce enzymes that can speed up industrial chemical reactions (AIBS, 2003).

PROBLEMS ASSOCIATED WITH TRANSGENIC ANIMALS

Transgenic animals have potentially broad applications in the improvement of animal production quality, the enhancement of production capacity, the studies of human disease models and the production of biomedical materials. However, there are many pressing problems that need to be resolved for transgenic animal studies. Firstly, the transgenic technique is imperfect, resulting in low success rates and survival rates of transgenic animals. Secondly, inserted genes have multiple functions that the integration efficiency of extrinsic genes at the target site is low and unstable, and that affects the intrinsic gene, damages the host's genome, or activates the closed gene in normal conditions to express and subsequently produces abnormalities in animals (Miao, 2013).

Thirdly, Food safeties of bioengineered products are always a significant public topic. For the transgenic animals, some of the foreign gene and its promoter sequences from the virus may occur in the recipient animals. Homologous recombination or integration may cause the formation of new virus. Foreign gene inserted in the chromosome locus may also result in different genetic changes in different degrees, causing unintended effects. Transgenic

animals may also increase the risk of zoonotic disease and cause human allergic reactions (Miao, 2012)

Fourthly, Potential risks for the environment include unintended effects on non-target organisms, ecosystems and biodiversity (Sears *et al.,* 2001). If transgenic animals are in the external environment and mating with wildlife, foreign gene may spread, which results in changing the species composition of the original genes, causing confusion in species resources. Finally Ethical concern has also been discussed about the "unnaturalness" of genetic engineering and the ways it might devalue nature and commercialize life (Maio, 2013).

CONCLUSION AND RECOMMENDATIONS

Transgenic organism whose genetic characteristics have been altered using the techniques of genetic engineering. Disease models, transpharmers, xenotransplanters, food sources and scientific models are the major classification of transgenic animal. Transgenic animal technology holds great potential in many fields including agriculture, medicine and industry. Transgenic animals have broad applications in the improvement of animal production. However, there are many pressing problems; among them the transgenic technique is imperfect, resulting in low success rates and survival rates of transgenic animals, inserted genes have multiple functions, unregulated gene expression and also improper use of transgenic animals in biotechnological research causes great suffering to the animal and their welfare may be compromised.

Based on the above conclusion, the following recommendations are forwarded:

- ✓ Further studies are needed tominimize problems associated with transgenic animal technology and to extend its full potential.
- ✓ There should be consideration of welfare of transgenic animal especially reduction of improper suffering to the animals, while using these animalsin different fields.

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