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Review Article

FABRICATION TECHNIQUES AND UTILIZATION OF TRANSGENIC ANIMAL

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ABSTRACT

The contemporary methods of genetic engineering and biotechnology, it was potential to crossing inter specific barriers and fabricating transgenic organisms with newly acquired properties. In this article, we attempted to focus about different aspects of producing transgenic animals such as DNA micro-injection, embryonic stem cell-mediated gene and retrovirus gene transfer techniques. Typical agricultural application of genetic engineering and biotechnology includes improved growth and carcass composition, lactation performance, modification of reproductive prolificacy, wool production, enhanced disease resistance, food productions and reduced environmental impact. In addition, transgenic farm animals have applications in human medicine as a source of biologically active proteins, donors in xenotransplantation, disease models for the development of novel treatments, antibody fabrication in transgenic animals, blood replacement and for a research in cell culture as well as gene therapy. 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. The general role of experiment on animals within the religious convention were practices about animal care, use, breeding and diet. Finally, the transgenic animals could be used to resolve serious problems of human health after successful experiment on transgenic animal. However, continuous researches are required to break suspects on using transgenic animals with the supervision of respective governments.

1. Introduction

In the modern technology, there is much technical invention in the field of agriculture biochemistry and biotechnology developed very swiftly. In genetic engineering technology, the transgenic animal is one of the carries as foreign gene that has been deliberately inserted into its genome[1]. The numbers of new gene combinations that can be achieved by these approaches, however limited genes can only be shuffled between members of the same or very closely related species[2]. Many different genes have been characterized and annotated through several databases. This knowledge makes availability in possible methods in the field of genetic changing makeup in useful ways[3].

Transgenics are genetically modified organism with DNA from another source inserted into their genome, A large number of transgenic animals have been created such as mice, cows, pigs, sheep, goat, fish, and frogs[4]. Before sometimes ago, No transgenic animal and animal products were approved by the FDA, for human consumption. But now the USFDA just approved the first genetically modified animal for human food consumption, 19 November 2015 the FDA announced that it has approved the fish for consumption in the US. There historical backgrounds are prior to the development of molecular genetics, the only way of studying the regulation and function of the mammalian gene was through the observation of inherited characteristic or spontaneous mutation[5].

During the (1970), the First Chimeric mice were produced the cell of two different embryos of different strain were combined together at an early stage of development 8 cells to formed a single embryo that subsequently developed into a Chimeric adult, exhibiting the characteristic of each chain. Since (1981), when the term transgenic was first used by J.W Gorden and F.H Ruddle (1981) then there has been rapid development in the use of genetically engineered animals as investigations have found expend Number of application for the technology[6].

Transgenesis is a completely new technology for altering the characteristics of animals by directly changing the genetic material. Since DNA contains a universal genetic code, it can, in principle, be transferred between completely unrelated organisms to produce organisms with particular, useful characteristics that would not otherwise be available[7]. Moniruzzaman et al., 2014 Reported that, Over the centuries, animals with new combinations of genes have been produced using conventional breeding methods by means of careful selection of particular animals[8].

1.1 Fabrication of transgenic animals

There are three fundamental methods used for producing transgenic animals

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a. DNA Microinjection
b. Embryonic stem cell-mediated gene transfer
c. Retro-virus mediated gene transfer.

(a). The process of DNA microinjection

The DNA microinjection or pronuclear microinjection, a very fine glass pipette is used to manually inject DNA from one organism into the eggs of another [9]. Better time for injection is early after fertilization when the ova have two pronuclei. They fused to form a single nucleus, the injected DNA may or may not be taken up. Through the DNA microinjection, the ovum is transferred into the oviduct of recipient female or foster mother that has been induced by mating with a vasectomized male[10]. The University of California (Irvine) Transgenic Mouse Facility reports an estimated success rate of 10% to 15% based on experiments with mice testing positive for the transgene. If the DNA is assimilated into the genome, it is done so randomly. Because of this, there is always a chance the gene insert will not be expressed by the GMO, or may even interfere with the expression of another gene on the chromosome (Fig. 1)[11, 12].

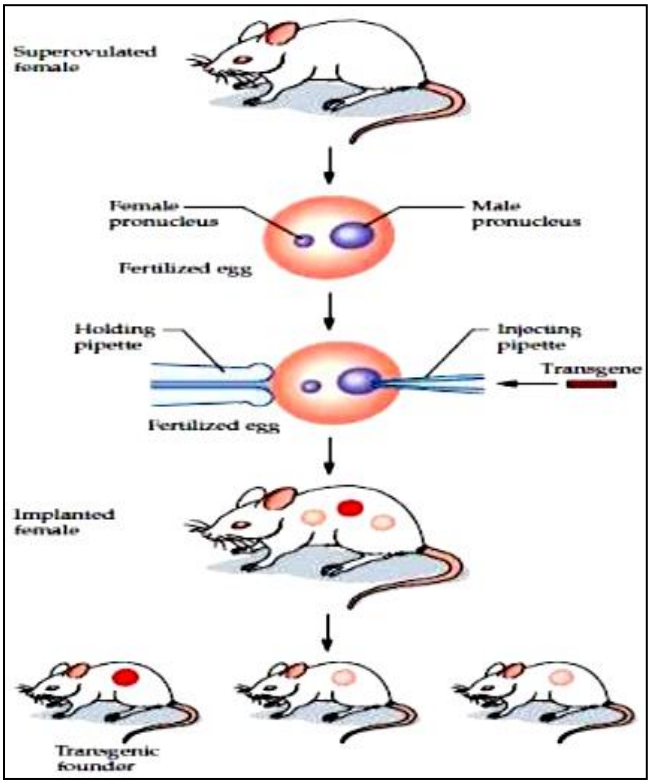

Figure No. 1: DNA microinjection process for producing transgenic mice.

(b). The Process of Embryonic Stem cell-mediated gene transfer

A second method of creating transgenic mice is embryonic stem cell-mediated gene transfer. It is the introduction of DNA into embryonic stem cells[13]. ES cells are from the very early mouse embryo and can differentiate into all types of cells when introduced to another embryo. DNA introduced into ES cells may integrate randomly, just like in pronuclear microinjection[14]. If the introduced DNA is like as in sequence to part of the mouse genome, it may undergo "homologous recombination" and integrate as a single copy at a specific site[15]. Embryonic stem cells will colonize a host embryo and often contribute to the germline. This results in the production of some sperm carrying the extra DNA. When these transgenic sperms fertilize a normal egg, a transgenic mouse will be produced with the same foreign DNA in every cell (Fig. 2)[16,17].

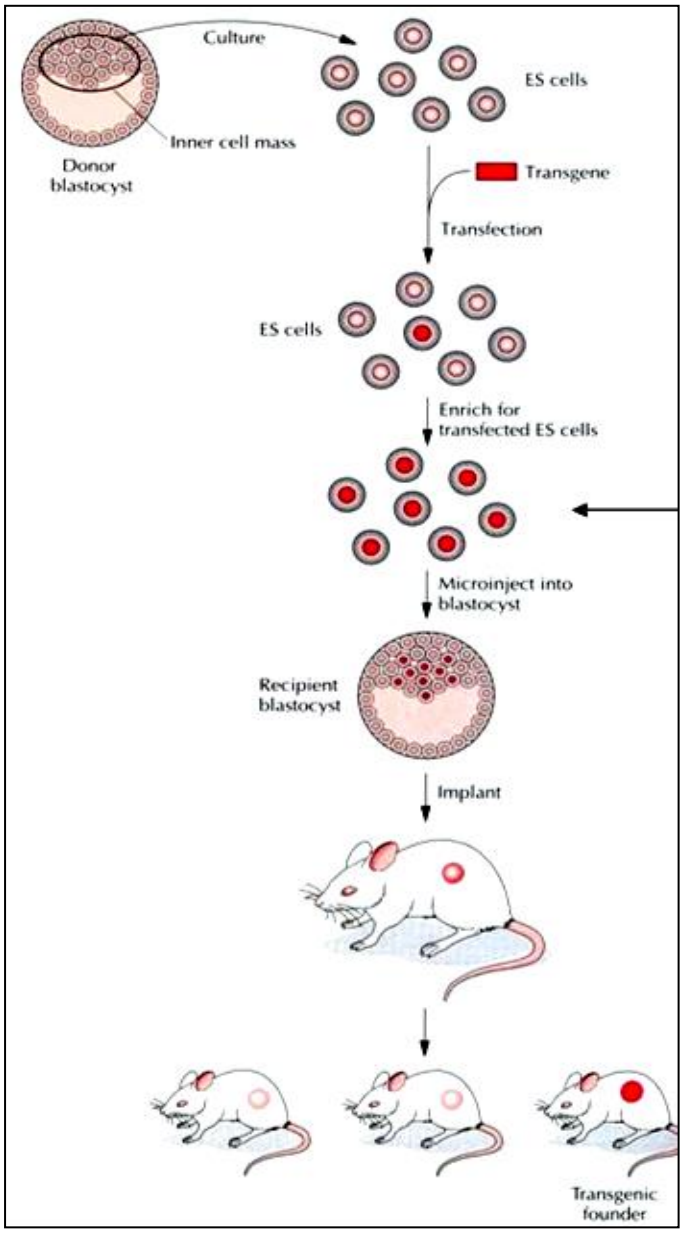

Figure No. 2: Embryonic stem cell method for producing transgenic mice.

(c). The Process of Retro-virus Mediated gene transfer

In this method gene transfer is mediated by a carrier or vector. Retroviruses are commonly used as a vector that carries its genetic material in the form of RNA rather than DNA[18]. Its transfer own genetic material into the cell, taking advantage of their ability to infect host cells. Offspring consequential from this method are chimeric, i.e., not all cells carry the retrovirus[19].

The killed virus is replication defective. The virus gene is replaced with trans-gene is inserted to the host cell by transfection. This can be used to transfect a wide range of cells such as embryonic cells. The outcome is chimera, an organism consisting of tissues or parts of diverse genetic constitution. Retro viral vectors that infects the cells of an

early stage embryo prior to implantation into a receptive female[20,21].

Technique: Immediately following infection, the retrovirus produces a DNA copy of its RNA genome using transcriptase. Completions of this process require that the host cell undergoes the S phase of the cell cycle. Therefore, retroviruses effectively transducer only mitotically active cell. The DNA copy of the viral genome, provirus, integrates randomly into the host cell genome, usually without deletions rearrangements because assimilation is not by way of homologous recombination[22,23].

Depending on the technique used, the Generation may result in chimeras. When the transgene has integrated into the germ cells, the so-called germline chimeras are then inbred for 10 to 20 generations until homozygous transgenic animals are obtained and the transgene is present in every cell[24]. Current research has shown that lentiviruses can overcome previous limitations of viral-mediated gene transfer, which included the silencing of the transgenic locus and low expression levels[25]. Injection of lentiviruses into the perivitelline space of porcine zygotes resulted in a very high proportion of piglets that carried and expressed the transgene. Stable transgenic lines have been established by this method (Hofmann et al', 2003). Lentiviral gene transfer in livestock promises unprecedented efficiency of transgenic animal production (Fig. 3)[26,27].

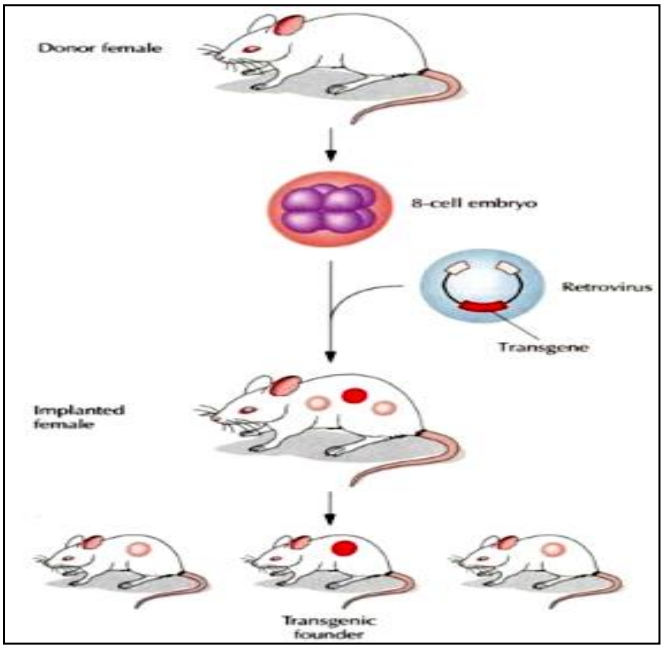

Figure No. 3: Retroviral vectors into germ line (8-cell embryo infected) transgenic animals

2. Utilization of transgenic animals

a. Human Health

The main potential application of transgenic animal is 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 "Gene Pharming"[27]. The mammary gland is the preferred site for the production of these proteins because large quantities can be extracted and purified Meade *et al*., 1999 and Rudolph, 1999). Additionally, milk is a secreted body fluid that is normally produced in large quantities and which could be collected without causing any harm to the animals[28].

b. Recombinant Therapeutic Proteins

Some novel therapeutic proteins have been derived from the mammary gland of transgenic animals. Many predictable methods were used for the production of therapeutic proteins through bacteria, plants, yeast etc, but most of them lack the machinery for post-translational modifications of eukaryotic genes. The transgenic livestock serves as potential bioreactors for the production of valuable proteins[29]. Proteins like antithrombin III (AT III), tissue plasminogen activator (TPA) and α-antitrypsin have been derived from the mammary gland of transgenic sheep and goats. The human AT III (for the treatment of heparin-resistant patients) is expected to be, in the market (Kues and Niemann, 2004).Glycosidase enzyme has been produced in the milk of transgenic rabbits, which is used in the treatment of Pompe disease (Vanden Hout *et al*., 2001).

A topical antibiotic against *Streptococcus mutants*, which is useful in the treatment of dental caries, is expected to complete clinical trials. Transgenic crops also play a vital role in the production of proteins coded by genes, which has complex regulation and fail to produce sufficient quantities in the milk of transgenic animals. A significant landmark has been achieved by the production of FMD vaccine in transgenic alfalfa crop (Wigdorovitz*et al*., 2004) (Table. 1[30].

Table1: 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	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	Collagen I	Tissue repair, treatment of rheumatoid arthritis
Cow	Lactoferrin	Treatment of infectious, treatment of infectious arthritis

A. Agricultural Applications

(a). Breeding

Farmers have always used discriminatory breeding to produce animals that exhibit desired traits (e.g. increased milk production, high growth rate). Traditional breeding is a time-consuming, difficult chore. When expertise using molecular [illegible] was developed, it became possible to develop traits in in a shorter time and with more precision. In sum, it offers the farmer an easy way to increase yields[31,32].

(b). Quality

Transgenic cows stay alive that produce more milk or milk with less lactose or cholesterol, pigs and cattle that have more meat on them, and sheep that grow more wool[33, 34]. In the past, farmers used growth hormones to stimulate the development of animals but this technique was problematic, especially since the residue of the hormones remained in the animal product[35].

(c). Disease resistance

Scientists are attempting to produce disease-resistant animals, such as influenza-resistant pigs, but inadequate number of genes is currently known to be responsible for resistance to diseases in farm animals[36].

(B). Medical Applications

(a). Xenotransplantation

Patients die every year to be deficient of replacement heart, liver, or kidney. For example, about 5000 organs are needed each year in the United Kingdom alone. Transgenic pigs may offer the transplant organs needed to improve the loss. Presently, xenotransplantation is vulnerable by a pig protein that can cause donor rejection but research is underway to remove the pig protein and replace it with a human protein[37]. One of the earliest genetic modifications of larger animals was the development of genetically modified pigs transport a human gene that could prevent the acute rejection of organs transplanted between pigs and humans[38]. The transplantation of tissues from one species to a different species is known as xenotransplantation. Whenever pig tissue is transplanted into another species, antibodies in the receiver attack the transplanted organ, and the resulting inflammatory response leads to graft rejection. By introducing a modification to some of the proteins on cells that cause the body to raise an immune response, called balance control proteins, rejection of the transplant can be disallowed[39].

(b). Nutritional supplement and pharmaceutical

Some Products such as insulin, growth hormone, and blood anti-clotting factors may soon be or have already been obtained from the milk of transgenic cows, sheep, or goats. Research is ongoing to manufacture milk through transgenesis for treatment of unbearable diseases such as phenylketonuria (PKU), hereditary emphysema, and cystic fibrosis. In 1997, foremost transgenic cow, Rosie, produced human protein-enriched milk at 2.4 grams per liter. This transgenic milk is an additional nutritionally balanced product than natural bovine milk and could be given to babies or the elderly with unique nutritional or digestive needs. Rosie's milk contains the human gene alpha-lactalbumin [40].

(c). Human gene therapy

Human gene therapy involves adding together a normal copy of a gene (transgene) to the genome of a person carrying defective copies of the gene. The prospective for treatments for the 5,000 named genetic diseases is huge and transgenic animals could play a role. For example, the A.I.Virtanen Institute in Finland produced a calf with a gene that makes the material that promotes the growth of red cells in humans[41,42].

(C). Transgenic Animals as Disease Models for theDevelopment of New Treatments

An animal model is a, the living, non-human animal used for study and investigation of human disease, for the purpose of better considerate the disease without the added risk of causing harm to a human being during the whole drug discovery and development process[43]. Transgenic animal models are created by the insertion of a particular human DNA into fertilized oocyte 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[44].

a. Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/AIDS)

S 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 to symptoms and immune deficiencies similar to the manifestations of AIDS in humans. Other models are AIDS Mouse and Smart Mous[45].

b. Alzheimer's Disease

There wasno animal models existed for the disease before transgenic technology was working. Immunization of Amyloid precursor protein *(A42*) in Pigs in transgenic mice showed that vaccination against Alzheimer's disease could have potential as a therapeutic approach. *E.g.* Alzheimer's Mouse[46,47].

c. Cardiovascular Disease

Various transgenic animal several 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 parameter are used to study cardiovascular diseases[48,49].

d. Diabetes Mellitus

Transgenic model are developed for studying the genes and their role in peripheral insulin accomplishment. Models of insulin secretion such as glucokinase, tislet amyloid polypeptide and hepatic glucose production in type II diabetes are developed. 18A model that expressed Insulin

Dependent Diabetes Mellitus by inserting a viral gene in the animal egg stage is also developed[50].

e. Cancer Diseases

"Oncomouse" was original transgenic animal to be patented. Its germ cells and somatic cells carry 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[51].

f. Industrial Applications

Two scientists discovered (Dr. Jeffrey Turner & Randy Lewis) a sliced spider gene into the cells of lactating goats in 2001 at Nexia Biotechnologies in Canada. The goats begin to produce 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 the strand, the scientists can generate a light, tough, flexible material that could be used in such applications as military uniforms, medical tiny caliber suture called (microsuture), and tennis racket strings. The toxicity-sensitive transgenic animals have been produced for chemical safety testing. Microorganisms have been engineered to produce spacious variety of proteins, which in turn can produce enzymes that can speed up industrial chemical reactions[52].

Conclusion

Interestingly, the fabrication of transgenic animals has resulted in a transfer in the use of laboratory animals from the use of higher-order species such as dogs to lower-order species like as mice and has reduced the number of animals used in such experimentation, especially in the development of disease models. This is doubtfully a good turn of procedures since transgenic technology holds great potential in many fields, including agriculture, medicine, and industry.

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Conflict of Interest

The authors declare no potential conflict of interest concerning the authorship, or publication of this review article**.**

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