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Transgenic Animals: A Review on its Various Dimensions and Applications in Animal Biotechnology

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***Abstract*-- Genetic modification of livestock proves beneficial to human health by economic and efficient production of important pharmaceutical proteins and to study human diseases. The creation of transgenic animals has resulted in the more use of laboratory animal such as mice instead of large size animals and has decreased the number of animals used in experiment related to the development of disease models. Since transgenic technology has great potential in many fields including livestock, medicine and industry. Several methods have been used for the production of transgenic animals like Microinjection of fertilized ovum, Embryonic stem (ES) cells mediated gene transfer and Retrovirus-mediated gene transfer.**

***Keywords*-- Transgenes, microinjection, transgenic animals**

I. INTRODUCTION

The animal whose genetic composition is altered by insertion of foreign genes is said to be transgenic animals transgenic animals. The Transgene is the foreign gene that is transferred to the recipient cell or recipient organism. This whole procedure is called Transgenesis. The first transgenic experiments in mammals were performed in mice (Gordon *et al.*, 1980; Gordon and Ruddle, 1981) afterward rabbits, pigs, sheep and cattle. (Hammer *et al.*, 1985, Pursel *et al.*, 1987, Rexroad *et al.*, 1989, Roschlau *et al.*, 1989)

Methods used for the production of transgenic animals

There are three main methods used for the production of transgenic animals. DNA microinjection, retrovirus-mediated gene transfer and embryonic stem (ES) cell-mediated gene transfer.

1) Microinjection of fertilized ovum

DNA microinjection is the first predominant method was developed. This technique was successfully used for the first time in 1980 (Gordon *et al.*, 1980).

The majority of transgenic animals are produced by the pronuclear microinjection method, which is accomplished by the transfer of a desired gene construct from another member of the same species or from a different species into the pronucleus of a fertilized ovum, which is subsequently implanted into the oviduct of recipient animals. This results in the recipient animal giving birth to genetically modified offspring.

Advantages

1. Foreign genes are expressed efficiently.
2. There is no clear limit to the size of the inserted DNA molecule.
3. Inexpensive process
4. Technique is simple
5. Application to a wide variety of species.

Disadvantages:

1. The technique cannot be used into the cell at later development stage.
2. Inserted gene may not insert itself into a site on the host DNA that will permit its expression.
3. The success rate of producing transgenic animals by these methods is very low.
4. The injected transgene is randomly integrated into the recipient genome and may cause a change in the normal physiological processes of the animal.
5. Manipulations of oocytes or embryos, or the disruption of parental DNA at the integration site of the gene construct can also influence the normal development of the transgenic animal.
6. The parental animals used for the production of the transgenic progeny can also experience discomfort from the experimental procedures to which they are exposed.
7. Sometimes resulted in multi-copies and multi-site integration.
8. Inefficient and results in random integration and variable expression patterns in the transgenic offspring (Wall, 1996)

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9. Time consuming and requires extensive intellectual, financial and material assets (Seidel, 1993)

2) Embryonic stem (ES) cells mediated gene transfer:

Embryonic stem (ES) cells mediated gene transfer method based on findings of Gossler *et al.*, (1986).

The presence of transgenes can be tested at the embryonic state in this method. ES cells are pluripotent cells, found in the inner cell mass (ICM) of embryos at the blastocyst stage of development. These cells have not yet differentiated and maintain the ability to develop into any type of tissue during the embryonic and foetal development. DNA can be introduced into the ES cells in vitro (Capecchi, 1994). embryonic stem cells grown at blastocyst stage, containing the desired DNA are incorporated into the host's embryo and then embryo inserted in the uterus of a surrogate mother, resulting in a chimeric animal. The method uses homologous recombination of DNA to permit precise targeting of DNA in embryonic stem cells. If the homologous sequence to be introduced into the cell carries a mutation or a gene from another species, the new sequence will replace the specific targeted gene. This is the method of choice for gene inactivation therefore called as "knock-out" method, particular important for the study of the genetic control of developmental processes.

Advantages:

1. Application of gene targeting thus enabling site-directed insertion of DNA (Müller, 1997).
2. Gene targeting involves inducing the embryonic stem cell to remove one of its own genes and replace it with a modified version of the same gene.
3. This method allows testing for transgenes at the early cell stage.
4. Embryonic stem (ES) cells are relatively efficient in homologous recombination in comparison to other animal cells.
5. To detect precisely mutations in the gene via homologous recombination

Disadvantages:

1. Production, characterization and maintenance of pluripotent Embryonic stem (ES) cells lines difficult.

3) Retrovirus-mediated gene transfer

Retrovirus-mediated gene transfer method based on findings of Jaenisch (1976). To increase the probability of expression, gene transfer is mediated by means of a carrier or vector; generally a virus or a plasmid. Retroviruses are commonly used as vectors to transfer genetic material into the cell because of their ability to infect host cells.

A retrovirus is a virus that carries its genetic material in the form of RNA rather than DNA. The code in the viral RNA is reverse transcribed to produce DNA, which is then incorporated into the host cell. The offspring derived from this method are chimeric, i.e., not all cells carry the retrovirus or an organism consisting of tissues or parts of diverse genetic constitution. Transmission of the transgene is possible only if the retrovirus integrates into some of the germ cells.

Advantages:

1. Readily integrate and pass through the germ lines allowing for their propagation into subsequent generations.
2. This system is technically simple.
3. Integration causes minimal disruption of host DNA and always involves integration of a single copy of the donor gene.
4. Infectious retroviruses are unable to infect human cells.

Disadvantages:

1. General limitation on the size of the foreign DNA insertion.
2. Some of the cells in the tissues of an organism receive the genetic change while the other cells without the desired addition.
3. The viral sequences may interfere with transgene expression.
4. Low copy number integration.

Applications of Transgenic animals

Improving livestock: Improve genetically size as well as production of livestock that are growing faster, efficient converter of food and resistance to various diseases. Transgenesis will allow larger herds with specific traits. Transgenic cows produce more milk or milk with less lactose or cholesterol and therapeutic proteins in their milk, which include hormones, antibodies, vaccines, growth factors and blood clotting factors. Therapeutic proteins are used to treat human diseases. Transgenic pigs and cattle produce more meat for human consumption and transgenic sheep that grow higher quality and quantity of wool. Transgenic fish shows increased growth rate, improved flesh color and increased disease resistance than the natural fish. Transgenic pigs used as an animal model of human diseases like cancer, Alzheimer's disease, cardiovascular diseases, cystic fibrosis and diabetes mellitus (Aigner *et al*., 2010). Transgenic pigs take care of the environmental issue of manure based phosphorous pollution and serve as donors in human organ transplantation (i.e. xenotransplantation).

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Xenotransplantation seems to be one option to close the growing gap between demand and availability of appropriate organs for human patients with acute or chronic organ failure (Yang and Sykes, 2007)

Research

OncoMouce or Harvard mouce carrying a oncogene significantly increases the mouse's susceptibility to cancer, and thus makes the mouse suitable for cancer research. Testing the animals for detection of toxicants, study of mammalian developmental genetics and molecular biology, analysis of the regulation of gene expression and evaluation of a specific genetic change occurring in entire animal.

Medical Applications

Milk-producing transgenic animals are especially useful for medicines. The milk of transgenic cows, sheep and goats contain nutritional supplements and pharmaceuticals products such as insulin, growth hormone and blood anti-clotting factors .Transgenic milk used for treatment of devastating diseases such as phenylketonuria and cystic fibrosis. Transgenic milk is a more nutritionally balanced product than natural milk and could be given to babies and the elder peoples with special nutritional and digestive requirements. A transgenic cow produces a substance to help human red cells grow. Human gene therapy involves adding a transgene to the genome of a person carrying defective copies of the gene. First recombinant protein of animal origin was human antithrombin produced by the transgenic goats was the used as a drug for the clinical use in humans (Moura *et al.*, 2011).

Industrial Applications

Uses in industry include creation of military uniforms, medical microsutures and tennis racket strings. Toxicity-sensitive transgenic animals have been produced for chemical safety testing and to produce a wide variety of proteins, which in turn can produce enzymes that can speed up industrial chemical reactions, production of pharmaceutical proteins, drug and products in the pharmaceutical industry.

Limitation of Transgenic animals

A creation of transgenic animal is a difficult, lengthy and expensive procedure. Generally leads to breeding problems, mutagenesis and functional disorders. Transgenic technique is not perfect with low success and survival rates of transgenic animals. The joining efficiency of external genes at the determined site is low and unstable and the effect of the intrinsic gene creating abnormalities in animals is unclear, this technique is still in immature stage which means it requires further studies.

Ethics of transgenic technology

There are safety concerns if new processes and products fail to gain consumer acceptance because of moral concerns. Animal suffering caused by the expression of transgenes inducing tumors or neurodegenerative diseases. Genetically altering the cells of an animal, thus side effects could result from modifying genes. Even though humans may benefit from transgenic animals, the animal itself may not benefit. Foreign genes affect the host and produce a lot of threats to ecological balance and species diversity (Miao, 2013).

II. Conclusion

Different methods used for generating transgenic animals, each method have its own advantages and disadvantages. There are wide range of applications of transgenesis in different species like cattle, sheep, goat, pig, chicken, fish, mice and humans. Transgenic technology helpful in human's health in organ transplantation and treating various disorders of humans such as diabetes mellitus, cardiovascular diseases and cystic fibrosis but there are also some limitations during production of transgenic animals, consideration should be given to ethical concern regarding animal welfare, human health and environmental issues.

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