Transgenic animal technology: Recent advances and applications: A Review

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ABSTRACT
Transgenic animal technologies are one of the fastest growing techniques in the biotechnology areas. It is used to incorporate exogenous genes into the animal genome by genetic engineering technology so that these genes can be inherited and expressed by offspring. Two fundamental determinants of the success of any transgenic exploitation of a livestock species are the transport of DNA across the plasma membrane of the recipient cell, and the transport of that DNA across the nuclear membrane to gain access to the chromosomes. A variety of transgenic technologies are available like microinjection method, chemical method mediated transgenesis, somatic cell nuclear transfer, restriction enzyme-mediated integration, retrovirus-mediated gene transfer, sperm mediated gene transfer and embryonic stem cell mediated method etc. Use of transgenic animals will provide solutions for drug research, xenotransplantation, disease resistance and tissue repair etc. This review focuses on various methods of transgenesis and its application.

Key words: Applications, Methods, Transgenesis.

The term transgenic animal refers to an animal in which there has been a deliberate modification of the genome, in contrast to spontaneous mutation. It is used to integrate exogenous genes into the animal genome by genetic engineering technology so that these genes can be expressed and inherited by offspring. Foreign DNA is introduced into the animal, using recombinant DNA technology, which gets transmitted through the germ line so that every cell, including germ cells, of the animal contains the same modified genetic material. If the germ cell line is altered, characters will be passed on to succeeding generations in normal reproduction. The transgenic efficiency and precise control of gene expression are the key limiting factors in the production of transgenic animals. Advance studies will allow transgenic technology to explore gene function, animal genetic improvement, bioreactors, animal disease models and organ transplantation. Transgenesis may involve whole organisms, rather than individual cells and there may be in vivo alteration of body function. Recent developments in animal gene transfer techniques are microinjection method, sperm mediated gene transfer method, embryonic stem cell mediated gene transfer, somatic cell nuclear transplantation method, nuclear transfer and retroviral vector method. These techniques can provide a better platform to develop transgenic animals for breeding new animal varieties and promote the development of medical sciences, livestock production and other fields.

Transgenesis allows improvement of nutrients in animal products, including their quantity, the quality of the whole food, and specific nutritional composition. Transgenic technology could provide a means of transferring or increasing nutritionally beneficial traits. For example, enhancing the omega-3 fatty acid in fish consumed by humans may contribute to a decreased occurrence of coronary heart disease. In fact, transgenic pigs that contain elevated levels of omega-3 fatty acids have been produced (Lai et al., 2006). Furthermore, transfer of a transgene that elevates the levels of omega-3 fatty acids into pigs may enhance the nutritional quality of pork (Lai et al., 2006). The production of lower fat, more nutritious animal products produced by transgenesis could enable improvements in public health. Another aspect of manipulating carcass composition is that of altering the fat or cholesterol composition of the carcass. By altering the metabolism or uptake of cholesterol and/or fatty acids, the content of fat and cholesterol of meats, eggs, and cheeses could be lowered. There is also the possibility of introducing beneficial fats such as the omega-3 fatty acids from fish or other animals into our livestock (Lai et al., 2006). In addition,
receptors such as the low-density lipoprotein (LDL) receptor gene and hormones like leptin are potential targets that would decrease fat and cholesterol in animal products. Recent progress has produced prion-free (Richt et al., 2007) and suppressed prion livestock (Golding et al., 2006). Prions are the causative agents in bovine spongiform encephalopathy (BSE) or ‘mad cow disease’ in cattle and in Creutzfeldt-Jacob disease (CJD) in humans. This is only a partial list of organisms or genetic diseases that decrease production efficiency and may also be targets for manipulation via transgenic methodologies. Small improvements in milk volume in Guzerat cows using genetic material from high producing Holsteins could have a significant impact on Brazilian beef production (Wheeler et al., 2010).

**What is transgenic animal?** A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. It is the one which has been genetically altered to have specific characteristics it otherwise would not have. In animals, transgenesis either means transferring DNA into the animal or altering DNA of the animal. Transgenic animal are genetically modified to contain a gene from a different species following gene transplantation or resulting from the molecular manipulations of endogenous genomic DNA. The new gene is inherited by offspring in the same way as the organism’s own genes. The earliest transgenic approaches involved transferring DNA, usually by injection into a fertilized mouse egg. However, since it is not possible to control the site of integration of the foreign DNA using this technique, it is a relatively imprecise tool. Mice resulting from this technique are generally called “overexpressors”.

Currently over 95% of transgenic animals used in biomedical research are mice. Over 80% of mouse genes function the same as those in humans. Mice also have a short reproduction cycle and their embryos are amenable to manipulation. Mice are therefore an ideal human surrogate in the study of most diseases. It is hoped that the refinement of transgenesis techniques in mice will ultimately allow for a corresponding reduction in the use of “higher” animals, such as dogs and non-human primates, in biomedical research. Other transgenic animals include rats, pigs and sheep.

**METHODS USED TO PRODUCE TRANSGENIC ANIMALS**

**Microinjection:** DNA microinjection has become the most commonly applied method for gene transfer in animals. Using DNA microinjection, mouse was the first animal to undergo successful gene transfer. Microinjection of embryos with DNA has been the traditional approach for generating transgenic livestock. For processes such as cellular or pronuclear injection the target cell is positioned under the microscope and two micromanipulators—one holding the pipette and other one, holding a micro capillary needle usually between 0.5 to 5 µm in diameter (larger if injecting stem cells into an embryo) are used to penetrate the cell membrane and/or the nuclear envelope (David and Matthew, 2013).

The method relies on random integration of the transgenic DNA via the recruitment of cellular DNA repair pathways and remains a highly inefficient process with success rates of only 1-4% (Niemann and kues 2000). Apart from all these drawbacks the method is still being used to generate transgenic animals (Baldassarre et al., 2003) and is being improved with co-injection of restriction enzymes with the DNA to mediate incorporation of the transgene into the chromosome (Thermes et al., 2002).

**Retrovirus-mediated gene transfer:** A retrovirus is a virus that carries its genetic material in the form of RNA rather than DNA. The retrovirus-mediated expression cloning method is efficient because the number of the provirus integrations in each cell is limited. This method was successfully used in 1974 when a simian virus was inserted into mice embryos, resulting in mice carrying this DNA. The most important features of retrovirus as vectors are the practically ease and effectiveness of gene transfer and target cells specificity. When cells are infected by retroviruses, the resultant viral DNA, after reverse transcription and integration, becomes a part of the host cell genome to be maintained for the life of the host cell (Ponder, 2002). Retroviruses are being explored widely for apply in human gene therapy and have been used in a precise condition to treat genetic diseases (Thermes et al., 2002). Recently lentivirus constructs have been made and used to infect embryonic tissue resulting in the generation of transgenic rats and mice (Rubinson et al., 2003). On the other hand, retroviral methods of modifying the chicken genome are progressing (Ivarie, 2003).

**Somatic cell nuclear transfer:** In genetics and developmental biology, somatic cell nuclear transfer (SCNT) is a laboratory technique for creating a viable embryo from a body cell and an egg cell. The technique consists of taking an enucleated oocyte (egg cell) and implanting a donor nucleus from a somatic (body) cell. It is used in both therapeutic and reproductive cloning. Dolly the Sheep, famous for being the first successfully cloned mammal was created using this process (Li et al., 2009). Somatic cell nuclear transplantation has become a focus of study in stem cell research. The aim of carrying out this procedure is to
obtain pluripotent cells from a cloned embryo. These cells genetically matched the donor organism from which they came. This gives them the ability to create patient specific pluripotent cells, which could then be used in therapies or disease research (Lomax and Dewitt, 2013). A potential use of stem cells genetically matched to a patient would be to create cell lines that have genes linked to a patient’s particular disease. By doing so, an in vitro model could be created, would be useful for studying that particular disease, potentially discovering its pathophysiology, and discovering therapies (Lo and Parham, 2009). For example, if a person with Parkinson’s disease donated his or her somatic cells, the stem cells resulting from SCNT would have genes that contribute to Parkinson’s disease. The disease specific stem cell lines could then be studied in order to better understand the condition. Another application of SCNT stem cell research is using the patient specific stem cell lines to generate tissues or even organs for transplant into the specific patient (Pera and Trounson, 2013). A number of animals with genetically identical appearance can be produced by somatic cell nuclear transfer (SCNT). From current advancement of SCNT and molecular techniques, production of a transgenic animal becomes easier. Although cloning efficiency in goat is low, the ability to propagate genetically identical animals, with a gene or genes of interest, would be important for increasing productivity and ultimately the economic livelihood. In this paper, the potential applications and uses of SCNT technology like production of transgenic goat for production of quality milk and meat are discussed (Abdullah et al., 2011).

Sperm-mediated gene transfer: In the year 1971, the first evidence of mammalian spermatozoa being able to take up and transfer exogenous DNA was demonstrated by Bracket et al. (1971). Sperm cells are exposed to foreign DNA, which binds to the surface of sperm through specific protein–protein interactions. There is currently a general agreement that only two steps in the processes are well-established and fully reproducible: (i) the spontaneous interaction between sperm cells and foreign DNA molecules, and (ii) delivery of sperm-bound DNA to oocyte at fertilization. At present research has been carried out to determine the appropriate conditions to use when incubating DNA with sperm (Lavitrano et al., 2006). This method is now being hopefully perceived as a valuable technique for transgenic animal production.

To increases the effectiveness of sperm uptake of DNA by various approaches are being taken. One is to attach the recombinant DNA to the sperm head via an antibody amalgamated to the DNA (Chang et al., 2002). The antibody used in this work recognises surface proteins common to sperm from cattle, pigs, sheep, chicken, goats, mice and humans. An additional approach likewise lipofection technique (Lai et al., 2006) or electroporation (Reith et al., 2000) method has been used to produce transgenic progeny by placing the DNA inside the sperm head. With the improvement in techniques for culturing and expanding spermatogonial stem cells there is now also the possibility of engineering these cells in vitro to generate transgenic sperm that could be used to fertilise oocytes and generate transgenic animals (Nagano et al., 2000).

Liposome’s mediated technology: Liposome is small bodies consisting of membrane-like lipid layers surrounding hydrous compartments. Cationic liposome was used to increase the transfection efficiency of sperm cells. Association of the cationic liposome/DNA complexes with sperm cells may allow DNA to be carried into oocytes at fertilization (Bachiller et al., 1991). However, sperm motility and fertilizing capability of spermatozoa was lower at the higher concentration of liposome as assessed by microscopic observation. Recent report that BSA, a major serum protein, could prevent the cellular uptake of liposome/DNA complexes in cells. The high transgenic rates were reported more recently in mouse F1 (41%) and F2 (37%) offspring via testis mediated gene transfer (TMGT) using liposome treated plasmid DNA (He et al., 2009). Furthermore, the existence of several different types of liposome makes it difficult to make general predictions as to the likelihood of success, in the absence of specific empirical studies.

Linker (receptor) based method: The process of linking the exogenous DNA to the head of the sperm was reported by using the monoclonal antibody mAbC (Chang et al., 2002). The antibody (mAbC) is a positively charged basic linker protein; it binds to negatively charged DNA via ionic interactions. These interactions specifically bind exogenous DNA to sperm in a precise way. DNA can bind to polycations in a strong but noncovalent manner forming soluble complexes. DNA coupled with antibodies or antibody-fragments offers the ability to internalize the complexes via receptor-mediated endocytosis (Varga et al., 2000).

Restriction enzyme-mediated integration (REMI): Restriction enzyme-mediated integration (REMI), involves the transformation of cells with a mixture of plasmid DNA, linearized with a restriction enzyme, along with a restriction enzyme that is capable of generating compatible cohesive ends in the genome. REMI has proven useful for genetic screens and for placing genetic and molecular markers at particular points in the genome. Plasmids were linearized with a restriction enzyme to generate single-stranded cohesive ends and then introduced in vitro into decondensed sperm nuclei using REMI. Shemesh et al. (2000) produced transgenic...
bovine sperms by combining REMI with liposome, and demonstrated that these transgenic sperms could be used to produce transgenic embryos and live offspring by IVF or AI.

Applications of transgenic animals

**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 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 and Rudolph, 1999). Moreover, 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.

**Recombinant therapeutic proteins:** Several novel therapeutic proteins have been derived from the mammary gland of transgenic animals. Many conventional 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 serve as potential bioreactors for the production of valuable proteins. Proteins like antithrombin III (AT III), tissue plasminogen activator (TPA) and á-antitrypsin have been derived from the mammary gland of transgenic sheeps and goats. The human AT III (for the treatment of heparin resistant patients) is expected to be in market (Kues and Niemann, 2004). Glycosidase has been produced in the milk of transgenic rabbits, which is used in the treatment of Pompes diseases (Vanden Hout _et al._, 2001). A topical antibiotic against *Streptococcus mutans*, which is useful in the treatment of dental caries, is expected to complete clinical trials.

**Blood substitutes:** Transgenic swine has been developed that produce functional hemoglobin which has the same oxygen binding capacity as that of normal human hemoglobin and that could be purified from porcine blood (D’Agnillo, and Chang 1988).

**Antibodies and transgenic animals:** Different varieties of monoclonal and recombinant antibodies were produced in transgenic goats and cattle (Meade _et al._, 1999; Grossse-Hovest _et al._, 2004). These antibodies are useful in targeting cancerous cell. Kuroiwa _et al._ (2002) reported that Transchromosomal animals could be used for the production of human therapeutic polyclonal antibodies.

**Human disease models:** Farm animals like cattle and pigs could be used as an appropriate model for the study of human diseases like cystic fibrosis, cancer and neuro-degenerative diseases and their therapies (Theuring _et al._, 1995; Palmarini and Fan, 2001 and Li and Engelhardt, 2003). Pigs could be used as an effective model for the study of growth hormone releasing hormone (GHRH) defects (Draghia – Akli _et al._, 1999).

**Carcass composition and growth enhancement:** Transgenic animals with exogenous gene constructs have been produced which has enhanced growth rate and improved quality of food. Growth hormone and insulin like growth factors genes have been expressed at different levels in transgenic animals. Transgenic cattle and salmon fish have been produced that contains foreign gene constructs. The introduction of chicken ski gene has caused muscular hypertrophy in case of pigs and cattle (Bowen _et al._, 1994). The acid meat gene or Rendement Napole gene has been involved in low processing yields of pork there by affecting the quality of meat in pig. Silencing the expression of this gene in case of pigs alter the post mortem pH and improve the quality of meat. Other genes like GH releasing factor, IGF binding proteins also play a major role in the modification of growth. Transgenic pig with human metallothionein promoter had a significant improvement in growth rate and feed conversion (Nottle _et al._, 2001).
**Milk production and lactation:** The advances in transgenic technology provide ample chances to improve both the quality and quantity of milk produced. The animals could be made to secrete nutraceuticals in milk that may have an impact over the growth of offspring. Casein variants are the main target for improving the milk composition, which in turn alters the physio-chemical properties of milk. Brophy et al. (2003) reported that cloned transgenic cattle have been developed that produce increased amounts of beta and kappa casein in milk that increase the value of milk in the production of milk based products like cheese, yoghurt and also increase the shelf life of milk products. Transgenic animals also could be developed to produce “infant milk” that has increased levels of human lactoferrin, to generate lactose free milk for lactose in tolerance populations by inhibiting the expression of lactalbumin locus and to produce hypoallergenic milk by knocking down the expression of B-lactoglobulin gene. Transgenic animals could also be made to secrete antibodies in their milk that give resistance against several diseases like mastitis or to secrete antimicrobial peptides like lysozyme. Grosvenor et al. (1993) reported that the milk composition could also be altered by making the transgenic animals to secrete growth factors in milk, which in turn affect the growth and maturation of newborn offspring.

**Disease resistance:** The most important application of transgenic technology is the manipulation of MHC (Major Histocompatibility Complex) genes, which influence the immune response and increase the disease resistance capacity of livestock. Clements et al. (1994) reported that transgenic sheep have been developed that is resistant to Visna virus infection. 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 corona virus responsible for transmissible gastro enteritis (TGEV), an economically important disease in case of pigs (Castilla et al., 1998). Transgenic dairy cows that secrete lysostaphin into their milk have higher resistance to mastitis due to the protection provided by lysostaphin, which kills the bacteria *Staphylococcus aureus*, in a dose-dependent manner (Donovan et al., 2005), that protects the mammary gland against this major mastitis-causing pathogen.

**Transgenics in the aquaculture industries:** Aquaculture species have been particularly amenable to the production of transgenics. Fish and shellfish tend to be highly fecund, producing a large quantity of gametes. Many species can be harvested for eggs and sperm and fertilisation in-vitro is often straightforward. Eggs are relatively large and fertilised eggs tend to develop outside the body, so no further manipulation, such as re-implantation is necessary. The first successful gene transfer experiment in fish occurred in 1985 in China. A DNA construct consisting of human growth hormone under control of the mouse metallothionein promoter was injected into the geriminal disc of an early-stage goldfish *Carassius auratus* embryo. Microinjection procedures were quickly perfected by other groups in Norway. Brem et al. (1988) were among the first to produce a commercially important fish (Nile tilapia, Oreochromis niloticus) bearing a human growth hormone transgene, again under the control of the mouse metallothionein promoter.

Recently, in Drosophila, zebra fish, and rats, direct embryo injection of engineered zinc-finger nuclease (ZFN) encoding mRNA or DNA has been used to generate heritable knockout mutations at specific loci (Carroll 2008; Geurts et al., 2009). Tsai et al. (2000) engineered a line of Japanese abalone *Haliotis diversicolor* supertexta which expresses Chinook salmon growth hormone.

**Production of pharmaceuticals in transgenic animals:** The production of therapeutic proteins from transgenic animals usually involves their expression from mammary-gland specific promoters to drive secretion of the transgene into milk (Martin et al., 2005). In January 2004, GTC Biotherapeutics submitted a Market Authorization Application to the European Medicines Agency for ATryn®, a recombinant form of human antithrombin produced in the milk of transgenic goats (www.gtc-bio.com/pressreleases/pr012604.html). This was the first product derived from a transgenic animal to be submitted for formal regulatory approval in Europe or the USA. Milk is presently the most mature system to produce recombinant proteins from transgenic organisms. Blood, egg white, seminal plasma and urine are other theoretically possible systems, but all have drawbacks. Blood, for instance, as of 2012 cannot store high levels of stable recombinant proteins and biologically active proteins in blood may alter the health of the animals (Houdebine and Louis-Marie, 2009).

**Xenotransplantation:** The extraordinary success of human-to-human (an example of intraspecies or allotransplantation) transplantation of vascularized organs (i.e. heart, kidney, liver, lung and pancreas) has saved many lives over the past 25 years, but it has also created a significant need for donor organs. It was recognized early on that for physiological, anatomical, ethical and supply reasons the pig was the best choice as a donor animal for vascularized organs. However, serious immunological issues had to be overcome before the pig-to-human transplantation model could become a reality (Platt et al., 1991).
The first published transgenic pig-to-primate xenograft used a novel transgenic delivery system for human complement regulatory proteins (McCurry et al., 1997). Transplant organs may soon come from transgenic animals. Currently, xenotransplantation is hampered 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.

**Tissue repair:** Using induced pluripotent stem (iPS) cells were directly injected into the vitreous of the damaged retina of mice, the stem cells engrafted into the retina, grow and repaired the vascular vessels (Mullin et al., 2014).

**Ethical issues related to transgenic animals:** The social opinion on transgenic animal research is divided almost in the middle. Opinion surveys in USA, Japan and New Zealand reveal that only 42, 54 and 58 percent, respectively, of the people participating in the survey favour such research. The main reasons for opposition of people are as follows.

1. Use of animals in biotechnological research causes great suffering to the animals. But most people seem to accept some animal suffering to serve the basic interest and welfare of mankind; this attitude has been termed as interest-sensitive speciesism.

2. It is felt that by using animals for the production of pharmaceutical proteins we reduce them to mere factories. This seems not to recognize that animals also are living beings which feel pleasure and pain just as we do.

3. Some people feel that animals should be regarded as equal to humans in that they have the same basic rights as human beings. However, in most societies animals are relegated to a position several steps below that of man.

4. An argument attempts to focus on integrity of species in that each biological species has a right to exist as a separate identifiable entity. But biologists do not regard a species as a fixed, water-tight entity; rather they are regarded as dynamic, constantly evolving groups.

5. Finally, the introduction of human genes into animals and vice-versa, may be seen by many as clouding the definition of “humaness”. But most of the known human genes are not unique and comparable genes do occur in animals. In addition, many retroviruses have integrated into the human genome without any recognizable devaluation of our humaness.

**Limitations of transgenics:** The transgenic technology even though has tremendous applications in livestock improvement programmes; still it has lots of limitations:

- Insertional mutations resulting in alteration of important biological processes.
- Unregulated gene expression resulting in improper expression of gene products.
- Possibility of side effects in transgenic animals like arthritis, dermatitis and cancer etc.

**CONCLUSION**

In the mid-1980s, the advent of transgenic technologies generated great excitement in the scientific community and the pharmaceutical industry. The acknowledged consequences and potential were equal to the societal implications and translation of the outlined basic research technologies toward fruitful applications. At the time, transgenesis were considered the next wave in the maturation of the developing field of biotechnology. Twenty-five years later, we now find ourselves positioned to reap the benefits of advances that are still deemed to be, if not in their infancy, in their adolescence. Although there are various products poised for launch, market, ethical and product concerns have matured considerably as we have advanced into the 21st century. The challenges are daunting, the implications thought provoking, with results still appearing to loom just around the corner. As in the case of related technologies, including stem cell-based genetic therapies, it will be interesting to observe the acceptance of newly engineered products that hold the promise of having a tremendous impact on societal needs and human health.

The emergence of transgenic technology has widened the scope of development in case of farm animals and the advent of new molecular biology techniques has paved way by giving a new dimension to animal breeding. The transgenic technology is one of an important tool to meet the future challenges for increased animals production. The biological products from animal source should be handled with safety as they are subject to contamination and could be damaged very easily. Thus, safety guidelines should be developed for the commercial exploitation of recombinant proteins and ensure that the transmission of pathogens from animals to human beings is prevented.

Therefore, the genetically engineered animals and biotechnology will play a vital role in the production of pharmaceutical proteins and bring about a complete refinement in agriculture production by increasing the quality and quantity of production, protection of environment, maintenance of genetic diversity and overall improvement in animals welfare.

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