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THE USE OF TRANSGENIC ANIMALS IN CLINICAL RESEARCH; THE PROS AND CONS (REVIEW)

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Abstract

Recent progress in the development of molecular genetic methods enables the manipulation of genes in intact mammalian organisms. The power of such techniques to elucidate complex biological systems was initially recognized and exploited by developmental biologists and immunologists . Before the advent of molecular genetics, the only practical way to study mammalian genetic regulation and function was to observe certain traits. The farmers used this technique for more production of milk. The first chimeric mouse was produced in 1970. The first transgenic animals were produced almost 20 years ago by using microinjection of foreign deoxyribonucleic acid into the pronuclei of zygotes. Production of transgenic livestock was demonstrated to be feasible over two decades ago. It became apparent almost immediately that the method used to produce the transgenic livestock had substantial limitations that would impede its use both for research and commercial applications. Substantial improvements have been made in transgenic technology over the past 20 years. Several DNA transfer systems are now available that greatly improve efficiency, thereby reducing the cost associated with transgenic animal production. Methods are now available for targeting foreign DNA to specific sites in the genome, alleviating concerns about harmful mutations and allowing the inactivation of endogenous genes.

Keywords: *Genetics; Transgenic; DNA; Mutations*

Introduction

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 one that carries a foreign gene that has been deliberately inserted into its genome [1]. 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 [2]. 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 [3]. 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 [4]. 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 [5].

The generation of transgenic animals is essential for the in vivo study of gene function during development, organogenesis and aging [6]. It also permits the evaluation of therapeutic strategies in models of human disease, as well as the investigation of disease progression in a manner not possible in human subjects [7]. Commercial applications include the preparation of recombinant proteins, protection of animals against disease, and introduction of new genetic traits into herds. Transgenic animals have been produced in a variety of species and transgenic vertebrates have been developed in species with both scientific and commercial value including fish, amphibians, birds, and mammals [8].

The foundation for the production of transgenic animals was started using sperm mediated gene transfer [9] and in 1980s, the transgenic mice was produced using the most popular microinjection technique. The creations of many transgenic animals were subsequently reported in1985s [10]. There are several methodologies employed in producing transgenic animals. Microinjection method used frequently but having some drawbacks like low efficiency, variable expression patterns [11]. So, alternative methodologies are used like sperm-mediated DNA transfer, intracytoplasmic injection of sperm heads carrying
foreign DNA [12], injection or infection of oocytes and/or embryos by different types of viral vectors ribonucleic acid (RNA) interference technology and the use of nuclear transfer [13].

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 [14].

Production of Transgenic Animals

The main principle in the production of transgenic animals is the introduction of a foreign gene or 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 [14].

- **Pronucleus microinjection**
  This method involves the direct microinjection of a chosen gene construct (a single gene or a

combination of genes) from another member of the same species or from a different species, into the pronucleus of a fertilized ovum. It is one of the first methods that proved to be effective in mammals [15]. The introduced DNA may lead to the over- or under-expression of certain genes or to the expression of genes entirely new to the animal species. The insertion of DNA is, however, a random process, and there is a high probability that the introduced gene will not insert itself into a site on the host DNA that will permit its expression. The manipulated fertilized ovum is transferred into the oviduct of a recipient female, or foster mother that has been induced to act as a recipient by mating with a vasectomized male [16].

Male and female pronuclei are microscopically visible several hours following the entry of the sperm into the oocyte. The transgene may be microinjected into either of these pronuclei [5]. All the transfection techniques are applicable to cultured animal cells, but microinjection is ordinarily not used due to the tediousness of the technique and the limited number of cells that can be handled. The method allows an early integration of the transgene into the host DNA, which is important to ensure that transgenic DNA is apparent in all cells of the host. A major advantage of this method is its applicability to a wide variety of species [17].

- **Embryonic stem cell-mediated gene transfer**

  This method involves prior insertion of the desired DNA sequence by homologous recombination into an in vitro culture of embryonic stem (ES) cells. Stem cells are undifferentiated cells that have the potential to differentiate into any type of cell (somatic and germ cells) and therefore to give rise to a complete organism. These cells are then incorporated into an embryo at the blastocyst stage of development. The result is a chimeric animal [18]. ES cell-mediated gene transfer is the method of choice for gene inactivation, the so-called knock-out method.

  This technique is of particular importance for the study of the genetic control of developmental processes. This technique works particularly well in mice. It has the advantage of allowing precise targeting of defined mutations in the gene via homologous recombination [19].

- **Retrovirus-mediated gene transfer.**

  Interestingly, the first successful foreign DNA transfer in a mammal was accomplished using viral DNA. In 1974, Jaenisch and Mintz [20] showed that seemingly healthy mice, carrying copies of foreign viral DNA, could be produced by microinjecting SV40 DNA into the blastocysts. Subsequently, it was shown that germline transmission of viral DNA into the animal genome could be achieved by directly infecting preimplantation stage embryos with Moloney leukemia viruses (MLV). Such pioneering work led to numerous successes of making transgenic animals using a retroviral genome as vector to deliver transgenes [3].

  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, taking advantage of their ability to infect host cells in this way. Offspring derived from this method

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are chimeric, i.e., not all cells carry the retrovirus. Transmission of the transgene is possible only if the retrovirus integrates into some of the germ cells [21].

For any of these techniques the success rate in terms of live birth of animals containing the transgene is extremely low. Providing that the genetic manipulation does not lead to abortion, the result is a first generation (F1) of animals that need to be tested for the expression of the transgene. Depending on the technique used, the F1 generation may result in chimeras. When the transgene has integrated into the germ cells, the so-called germ line chimeras are then inbred for 10 to 20 generations until homozygous transgenic animals are obtained and the transgene is present in every cell. At this stage, embryos carrying the transgene can be frozen and stored for subsequent implantation [3].

- **Sperm-mediated DNA transfer**

This is a transgenic technique that transfers genes based on the ability of sperm cells to spontaneously bind to and internalize exogenous DNA and transport it into an oocyte during fertilization to produce genetically modified animals [22]. Exogenous DNA refers to DNA that originates outside of the organism. Transgenic animals have been obtained using SMGT, but the efficiency of this technique is low. Low efficiency is mainly due to low uptake of exogenous DNA by the spermatozoa, reducing the chances of fertilizing the oocytes with transfected spermatozoa. In order to successfully produce transgenic animals by SMGT, the spermatozoa must attach the exogenous DNA into the head and these transfected spermatozoa must maintain their functionality to fertilize the oocyte [23].

Brackett *et al*. [9] first demonstrated that mammalian spermatozoa have the intrinsic ability to bind exogenous DNA. In 1989, Spadafora and co-workers [24], using mice as a model, reported that such DNA binding capacity of sperm could be used to introduce foreign DNA into eggs during fertilization for making transgenic animals. The report generated substantial interest because "sperm-mediated DNA transfer" was simple and low cost. However, difficulties in reproducibility and low efficiency in integrating transgenes into the animal genome, resulted in considerable controversy over the work for several years [25]. Nevertheless, numerous papers have been published in recent years confirming that sperm from many species, including livestock, poultry and fish, can be used as vectors to deliver transgenes into the animal genome. Genetically modified animals produced by SMGT are useful for research in biomedical, agricultural, and veterinary fields of study. SMGT could also be useful in generating animals as models for human diseases or lead to future discoveries relating to human gene therapy [26].

- **Somatic Cell Nuclear Transfer**

This is a laboratory strategy 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 [27]. "Therapeutic cloning" refers to the potential use of SCNT in regenerative medicine; this

approach has been championed as an answer to the many issues concerning embryonic stem cells (ESC) and the destruction of viable embryos for medical use, though questions remain on how homologous the two cell types truly are [28].

The process of somatic cell nuclear transplant involves two different cells. The first being a female gamete, known as the ovum (egg/oocyte). In human SCNT (Somatic Cell Nuclear Transfer) experiments, these eggs are obtained through consenting donors, utilizing ovarian stimulation. The second being a somatic cell, referring to the cells of the human body. Skin cells, fat cells, and liver cells are only a few examples. The genetic material of the donor egg cell is removed and discarded, leaving it 'deprogrammed.' What is left is a somatic cell and an enucleated egg cell. These are then fused by inserting the somatic cell into the 'empty' ovum [29]. After being inserted into the egg, the somatic cell nucleus is reprogrammed by its host egg cell. The ovum, now containing the somatic cell's nucleus, is stimulated with a shock and will begin to divide. The egg is now viable and capable of producing an adult organism containing all the necessary genetic information from just one parent. Development will ensue normally and after many mitotic divisions, this single cell forms a blastocyst (an early stage embryo with about 100 cells) with an identical genome to the original organism (i.e. a clone). Stem cells can then be obtained by the destruction of this clone embryo for use in therapeutic cloning or in the case of reproductive cloning the clone embryo is implanted into a host mother for further development and brought to term [30].

Modeling Diseases Using Transgenic Animals

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 oviducts of pseudo pregnant females [31]. There are different models of transgenic animals for various diseases as listed below

- Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/AIDS)
- Alzheimer's disease
- Cardiovascular disease
- Diabetes Mellitus
- Angiogenesis
- Cancer diseases

Clinical Research Application of Transgenic Animals

There has been greater research effort, progress, ethical justification, and economic incentive to generate transgenic livestock for various biomedical applications compared to those for agriculture and food production.

Specific human genes encoding medically important proteins, under the control of mammary-specific promoters, can be introduced into cultured cells and directed to be expressed in the lactating mammary gland, with secretion into the milk, in resulting cloned-transgenic females [32]. These therapeutic proteins are then extracted from the milk, purified, and used in clinical trials to evaluate their safety and effectiveness in treating particular human diseases and disorders before gaining final regulatory approval. Livestock are favoured where functional proteins are difficult to make in sufficient quantities, cost-effectively and

safely by other methods. In harnessing the potential of the mammary gland to synthesize heterologous proteins, the choice of species (rabbits through to cows) depends upon the quantities required. Indeed, other tissue systems may sometimes be preferable; for instance, human polyclonal antibodies produced in the blood of transgenic cattle [33], or proteins produced in the egg whites of laying hens, or the seminal fluid of pigs, which offer a more closed system for the production of highly bioactive molecules [34].

The shortage of human donor organs to treat chronic organ failure and various degenerative tissue diseases could be overcome by targeting specific genetic modifications to generate pathogen-free herds of pigs whose organs would be immunologically compatible with humans following xenotransplantation. Recently, pigs have been produced that completely lack the enzyme a-1,3-galactosyl-transferase [35] to counter hyperacute immune rejection. Subsequent immuno-suppressive drug therapy or additional genetic modifications could be used to manage the body's other rejection processes. It may be ethically acceptable to genetically modify farm animals to serve as models for various inherited human genetic diseases to aid research and preliminary evaluation of novel therapies. An ovine model of cystic fibrosis, for example, is considered superior to available mouse models because of the greater similarity in lung anatomy and physiology with humans [36].

Xenotransplantation

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) [37]. According to Van Cott *et al.* [38] 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.

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 [39]. There are currently at least 33 different drugs in clinical testing including several in pivotal trials that contain variable regions from transgenic mice encoded by human sequences. Also there are 17 therapeutic MAbs approved by the USFDA which are in different phases of drug development [40]. Functional human haemoglobin has been produced in transgenic swine. The transgenic protein purified from the porcine blood showed oxygen binding characteristics similar to natural human haemoglobin but only a small proportion of

porcine red blood cells contained human form of haemoglobin [41].

Production of Biopharmaceuticals

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 [41].

Many therapeutic proteins 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. Acceptable levels of recombinant protein production have been demonstrated in the milk of goats, sheep, and cattle [42]. 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. [43].

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 [44].

Antibody Production

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 [41]. 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. [41].

Ethical Concerns

One area of concern is the moral status of animals. This has sprung from the reflections of philosophers, the advocacy of animal rights and animal welfare organisations, and the incorporation of pets as part of the family circle. These factors have now made the status of animals a mainstream concern [45]. Studies of public views on animal experimentation have demonstrated that account is taken of the purpose of the experiment, whether there is unnecessary suffering of the animals, whether basic animal welfare is adhered to, and whether alternatives are available. The guidelines on the care of animals used in research, which are well established in many countries, are an indicator of the institutionalization of this care [46]. The United Nations World Charter for Nature declared: 'every form of life is unique, warranting respect regardless of its worth to man, and, to accord other organisms such

recognition, man must be guided by a moral code of action.' While not universally accepted, this resolution propounds a common scale of value that both human and non-human life have intrinsic merit and worth [47].

A second area of concern is the boundary constructed between what is considered 'natural' and 'unnatural'. To many people, the crossing of species boundaries is 'unnatural'. This becomes especially problematic when higher life forms are involved. This concern is not just limited to the general public; even among some scientists, this issue is problematic [48].

Another consideration for some revolves around offending human dignity, particularly in the case of human-animal chimeras. As argued by Johnston and Eliot [49], this notion suggests that our collective sense of humanity is compromised when we refuse to consider our obligations to the chimera or when we do not acknowledge how such activities reflect on the people or societies who create such creatures.

Conclusion

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. The application of transgenic animals showed that within the next five to eight years genetically modified animals will play a significant and important role in the biomedical field, in particular via the production of valuable pharmaceutical proteins and the supply of xenografts. New and exciting techniques being developed will continue to expand this important and useful area of experimentation.

References

1. Bachiller, D., Schellanderk, K., Peli, J. and Ruether, U. (1991). Liposome-mediated DNA uptake by sperm cells. *Mol. Reprod. Dev.*, **30**: 194-200
2. Baldassarre, H., Keefer, C.L., Wang, B., Lazaris, A., Karatzas, C.N. (2003). Nuclear transfer in goats using *in vitro* matured oocytes recovered by laparoscopic ovum pick-up. *Cloning and Stem Cells,* **5**: 270-285.
3. Jaenisch R: Germ line integration and Mendelian transmission of the exogenous Moloney leukemia virus. *Proc Natl Acad Sci.*1976; 73:1260-1264.
4. Transgenic Animal Mutagenicity Assays, World Health Organization Geneva, 2006, 10-11.
5. Kohler SW, Provost GS, Fieck A, Kretz PL, Bullock WO, Putman DL, Sorge JA, Short JM: Analysis of spontaneous and induced mutations in transgenic mice using a lambda ZAP/lacI shuttle vector. *Environ Mol Mutagen*1991; 18:316–321.
6. Abbott A (2004) Laboratory animals: the Renaissance rat. Nature 428:464–466
7. Adams DJ, van der Weyden L (2008) Contemporary approaches for modifying the mouse genome. Physiol Genomics 34:225–238
8. Agca C, Fritz JJ, Walker LC, Levey AI, Chan AW, Lah JJ, Agca Y (2008) Development of transgenic rats producing human betaamyloid precursor protein as a model for Alzheimer's disease: transgene and endogenous APP genes are regulated tissue specifically. BMC Neurosci 9:28
9. Brackett B G, Boranska W, Sawicki W, Koprowski: Uptake of heterologous genome by mammalian spermatozoa and its transfer to ova through

fertilization. *Proc Natl Acad Sci.*1971; 68:353-357.
10. Gordon J. W, Scangos G. A, Plotkin D. J, Barbosa J. A, Ruddle F. H: Genetic transformation of mouse embryos by microinjection of purified DNA. *Proc Natl Acad Sci.*1980; 77:179-184.
11. Hammer R.E, Pursel V.G, et al: Production of transgenic rabbits, sheep and pigs by microinjection. *Nature.*1985; 15(6021):680- 683.
12. Chang K, Qian J, et al: Effective generation of transgenic pigs and mice by linker based sperm-mediated gene transfer. *BMC Biotechnol.*2002; 2(1):5.
13. Shim H, Gutierrez-Adan A, Chen L.R, BonDurant R.H, Behboodi E, Anderson G.B: Isolation of pluripotent stem cells from cultured porcine primordial germ cells. *Biol. Reprod.*1997; 57(5):1089-1095
14. Vijg J. Douglas GR: Bacteriophage lambda and plasmid lacZ transgenic mice for studying mutations in vivo. In: Pfeifer GP ed. Technologies for detection of DNA damage and mutations. New York, Plenum Press, 1996; 391–410.
15. Gordon, J.W: Transgenic technology and its impact on laboratory animal science. *Scandinavian Journal of Laboratory Animal Science*1996; 23:235-249.
16. Brinster, R. (1974). The effect of cells transferred into mouse blastocyst on subsequent development. J. Exp. Med.:1049-1056.
17. Aine Bblanchard: Transgenic Animals, Project report of worcester polytechnic institute. 2005; 10-11.
18. Donnelly, S., McCarthy, C.R. and Singleton, R. Jr. (1994). The Brave new World of Animal Biotechnology, Special Supplement, Hastings Center Report.
19. Moore, C.J. and Mepham, T.B. (1995). Transgenesis and animal welfare. ATLA 23:380-397.
20. Jaenisch, R, B. Mintz: Simian virus 40 DNA sequences in DNA of healthy adult mice derived from preimplantation blastocysts injected with viral DNA. *Proceedings of National Academic Science* 1974; 71:1250-1254.
21. Makoto Nagano, Clayton J. Brinster, et al: Transgenic mice produced by retroviral transduction of male germ-line stem cells. *PNAS*2001; 98(23):13090–13095.
22. Lavitrano M, Giovannoni R, Cerrito MG. 2013. Methods for sperm-mediated gene transfer. Methods Molecular Biology. 927:519-529.
23. García-Vázquez FA, Ruiz S, Grullón LA, Ondiz AD, Gutiérrez-Adán A, Gadea J. 2011. Factors affecting porcine sperm mediated gene transfer. Research in Veterinary Science. 91(3):446-53.
24. Spadafora, C. (2017). *Sperm-Mediated Transgenerational Inheritance. Frontiers in Microbiology, 8.*doi:10.3389/fmicb.2017.02401
25. Brinster RL, Sandgren EP, Behringer RR, Palmiter RD. No simple solution for making transgenic mice. Cell 1989;59: 239–41.
26. Smith K, Spadafora C. Sperm-mediated gene transfer: applications and implications. Bioessays 2005;27:551–62.
27. Li, J; Liu, X; Wang, H; Zhang, S; Liu, F; Wang, X; Wang, Y (2009). "Human embryos derived by somatic cell nuclear transfer using an alternative enucleation approach". *Cloning and Stem Cells*. **11** (1): 39–50. doi:10.1089/clo.2008.0041. PMID 19196043
28. Liu, Zhen; et al. (24 January 2018). "Cloning of Macaque Monkeys by Somatic Cell Nuclear Transfer". *Cell*. **172** (4): 881–887.e7. doi:10.1016/j.cell.2018.01.020. PMID 29395327. Retrieved 24 January 2018.

29. Pattinson, Shaun D.; Kind, Vanessa (2017-09-12). "Using a moot to develop students' understanding of human cloning and statutory interpretation". *Medical Law International*. **17** (3): 111 133. doi:10.1177/0968533217726350. PM C 5598875. PMID 28943724.
30. Wilmut, I.; Schnieke, A. E.; McWhir, J.; Kind, A. J.; Campbell, K. H. S. (1997). "Viable offspring derived from fetal and adult mammalian cells". *Nature*. **385** (6619): 810–813. doi:10.1038/385810a0. PMID 9039 911.
31. Kandhare AD, Raygude KS, Ghosh P, Gosavi TP, Bodhankar SL. Patentability of Animal Models: India and the Globe. International Journal of Pharmaceutical and Biological Archives. 2011; 2(4):1024-32.
32. CLARK, A.J.(1998).The mammarygland as a bioreactor: expression. processing and production of recombinant proteins. Journal of Mammary Gland Biology and Neoplasia 3, 337-350.
33. ROBL, J.M., KASINATHAN, P., SULLIVAN, E., KUROIWA, Y., TOMIZUKA, K. AND ISHIDA, I. (2003). Artificial chromosome vectors and expression ofcomplex proteins in transgenic animals. Theriogenology59, 107-113.
34. DYCK, M.K., GAGNE, D., GUELLET, M. ETAL. (1999). Seminal vesicle production and secretion of growth hormone into seminal fluid. Nature Biotechnology 17, 1087-1090.
35. PHELPS, CJ., KO1KE. C.. VAUGHT. T.D. ET AL. (2003). Production of alpha 1.3-galactosyl transferase deficient pigs. Science 299.411-414.
36. HARRIS. A. (1997). Towards an ovine model of cystic fibrosis. Human Molecular Genetics 6, 2191-2194.
37. Heiner, N. and A.K. Wilfried, 2003. Application of transgenesis in livestock for agriculture and biomedicine. Anim.Reprod. Sci., 79:291-317.
38. Van Cott, K. E., P. E. Monahan, T. C. Nichols and W. H. Velander, 2004. Haemophilic factors produced by transgenic livestock: Abundance that can enable alternative therapies worldwide. Haemophilia 10 (Suppl) 4:70- 76.
39. Alison VE. Genetically Engineered Animals: An overview. [Serial on the Internet]. 2008. [Cited 2012 Nov 25]; Available from http://animalscience.ucdavis.edu/animalbiotech /Outreach/Genetically_engineered_animals_ov erview.pdf
40. Lonberg N. Human antibodies from transgenic animals. Nature Biotechnology. 2005;23(9):1117-1125
41. Niemen, H., K.W. Niemann, and J.W. Carnwath, 2005. Transgenic farm animals: Present and futureBiomedical applications of transgenic domestic animals. Rev. Sci. Tech. Off. Int. Epiz., 24:285-298.
42. Gulzar A. Niazi and S. Riaz-ud-Din, 2006. Biotechnology and Genomics in Medicine - A Review. World Journal of Medical Sciences 1: 72-81, 2006.
43. Clark, A.J., S. Ali, A.L. Archibald, H. Bessos, P. Brown, S. Harris, M. McClenaghan, C. Prowse, J.P. Simons and C.B. Whitelaw, 1989. The molecular manipulation of milk composition. Genome, 31: 950–955.
44. Mullin, E. (2014). Researchers repair retinas in mice with virus-free stem cell. *Fiercebiotech.com*.
45. Rollin B.E. (2003). – Ethics and species integrity. Am. J. Bioethics, 3 (3), 15-17
46. Agriculture and Environment Biotechnology Commission (AEBC) (2002). – Animals and biotechnology: a report by the AEBC, London. Website: www.aebc.gov.uk/aebc/pdf/ animals_and_biotechnology_report.p df (accessed on 6 March 2005).

47. United Nations (1982). – World charter for nature. Website: www.un.org/documents/ga/res/37/a37r007.htm (accessed on 6 March 2005).
48. DeWitt N. (2002). – Biologists divided over proposal to create human-mouse embryos. Nature, 420, 255.
49. Johnston J.M. & Eliot C. (2003). – Chimeras and "human dignity". Am. J. Bioethics, 3 (3), W6-W8.

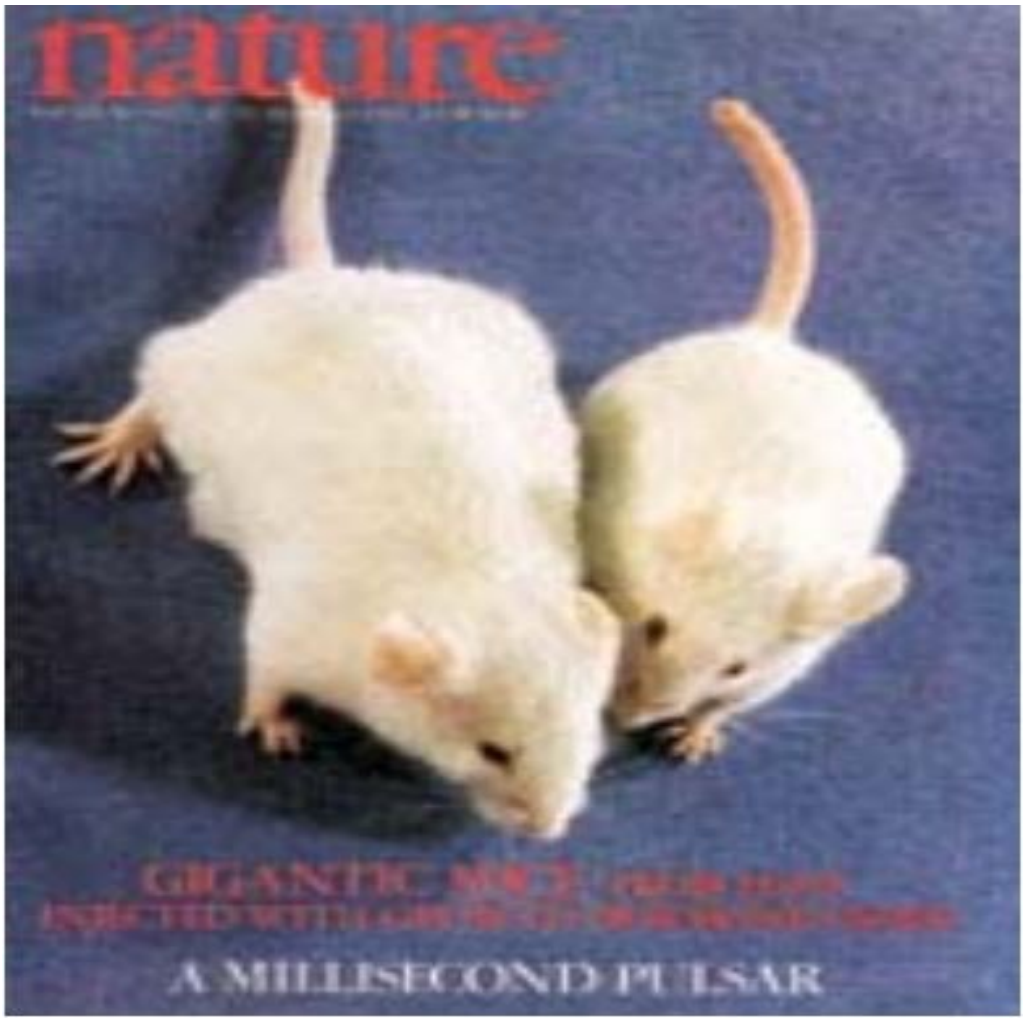

Figure 1: First transgenic mouse with a phenotype. This resulted in the expression of growth hormone gene (Source**:** https://nptel.ac.in/courses/104108056/module9/PNR%20lecture%2035.pdf)

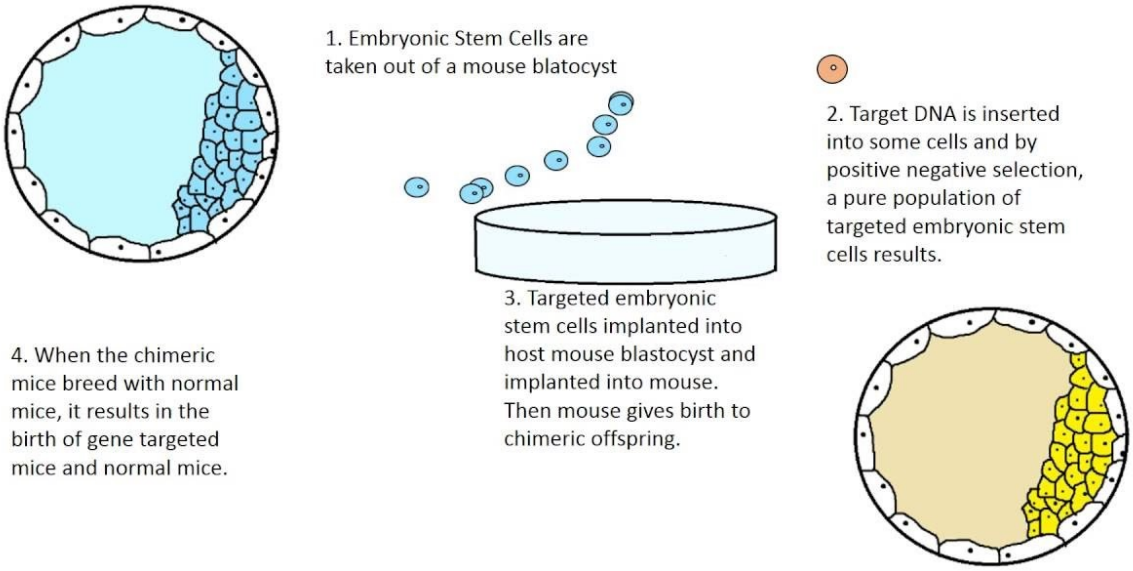

Figure 2: Embryonic stem cell-mediated gene transfer. (Source: https://stemcells.nih.gov/info/Regenerative_Medicine/2006Chapter1.htm)

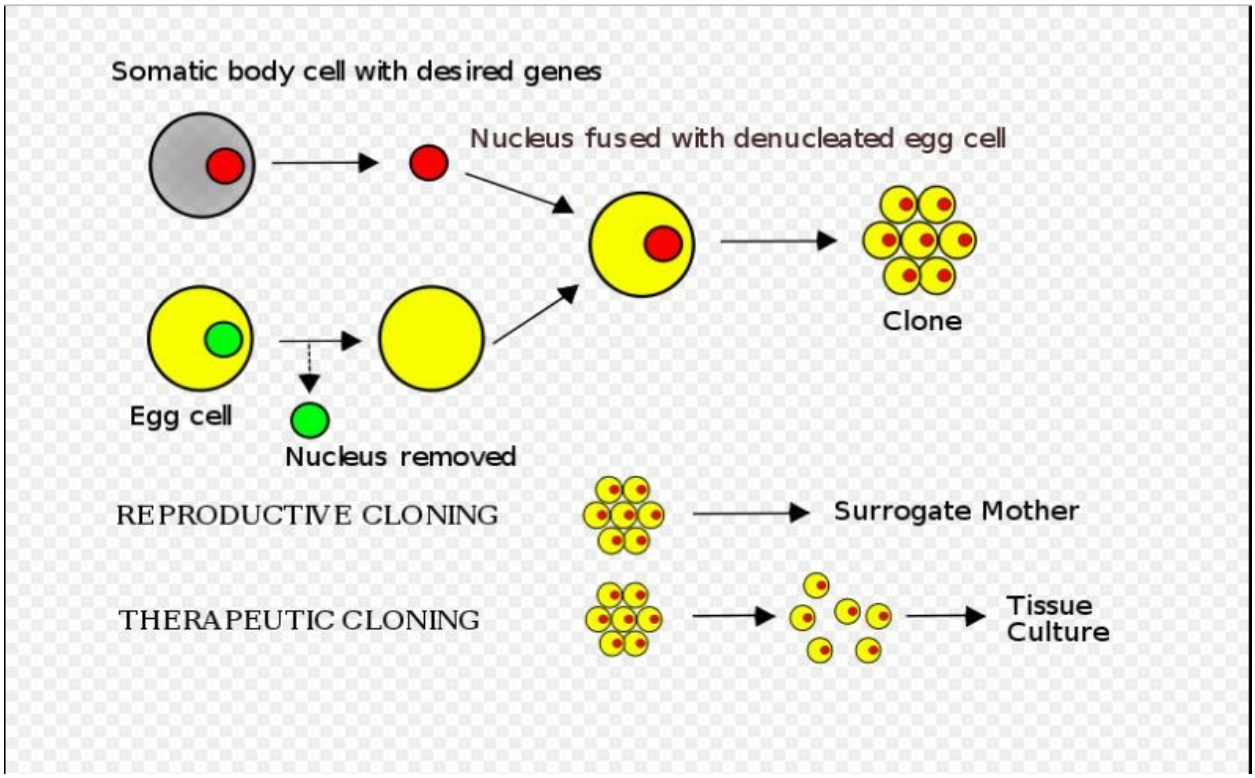

Figure 3: Somatic Cell Nuclear Transfer (Source: https://en.wikipedia.org/wiki/Somatic_cell_nuclear_transfer)