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Transgenic or Genetically Modified Farm Animals and their Applications: A Review

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Abstract

Transgenic are genetically modified organisms having DNA from different source introduced into their genome. Genetic alteration of livestock species is advantageous to human health as important pharmaceutical proteins can be produced in economic and efficient ways. Transgenic animals like mice, rat and hamster etc. are also used to study human diseases. Transgenic livestock species comprised of different kinds of animals are modified with the purpose of improving economically important traits such as growth rate, quality of meat, milk and its composition, disease resistance and survival of animals. In cattle, udder health and survival are the most important traits improved by transgenic technology. BSE resistant transgenic cows have been bred. Modifications have been made in sheep to improve wool production and immunity along with reducing the risk of mortality following infections by bacteria and lethal viruses. Pigs have been engineered for faster growth and more meat production with less feed consumption. There is also improvement in composition of pork for healthier human consumption. Different methods used for production of transgenic animals are DNA microinjection, retrovirus-mediated gene transfer and embryonic stem (ES) cell-mediated gene transfer etc.

Keywords: *Transgenic, livestock, applications, genetically modified animals*

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INTRODUCTION

Transgenesis is the process of introducing an exogenous gene called a transgene, into a living organism. After this the organism will exhibit a new property and transmit that to its next generation. In mammals, first transgenic experiments were conducted in mice afterward rabbits, pigs, sheep and cattle [1–6].

The biotechnological developments have allowed scientists or researcher to manipulate the genetic makeup of animals. These manipulations have served the purpose of basic research like genetic mechanisms but these techniques speedily became capable tools from livestock and poultry point of view since they allow the addition of novel traits to organisms, which may increase their suitability for use in extensive monocultures (e.g., animals with disease resistance). Since the creation of the first genetically modified (GM) livestock effort has been made to modify several aspects livestock species to increase their cultivation [3]. GM farm animals for food production include a large number of species engineered with the aim of improving economically important traits such as growth rate, meat quality, wool growth, feed conversion efficiency, milk and its composition, mastitis resistance, and lactation and survival [7–13].

Transgenesis also known as molecular farming in which biopharmaceuticals are manufactured in transgenic livestock species [14]. Animals are attractive bioreactors and they have accurate metabolic pathways, are reproducible, easily maintained, and do not require expensive infrastructure for their management [15]. Production of these recombinant proteins usually happens in mammalian milk since it offers flexible production and relatively straightforward purification but egg white and seminal plasma are also being used for the production of recombinant protein [15]. Conversely, is usually not able to store high concentrations of recombinant proteins [16]. Another medical application of genetic modification aims to increase the suitability of animal organs for xenotransplantation, e.g., in

pigs [17]. Overview of the genes in transgenic farm animals used in food production is shown in Table 1.

Table 1: *Overview of the Genes in Transgenic Farm Animals used in Food Production.*

Species	Target traits	Transgenic gene	References
Cattle	Health	Lysozyme	[18]
	Udder	Omega-3 and a and k-Casein	[19, 20]
Goat	Health	Lysozyme	[21]
	Udder	Fatty acid	[11]
Sheep	Growth	IGF-1	[22]
	Health	BSE	[23]
Swine	Health	Lysozyme, Fatty acid	[24, 25]

This article reviews the recent developments in animal gene transfer techniques including microinjection method, embryonic stem cell and retroviral vector as well as applications of transgenic animals. These new transgenic techniques can provide a better strategy to develop transgenic animals for breeding new animal varieties and promote the development of medical sciences and livestock production.

HISTORY

Contribution of different research workers in transgenesis as reviewed is given in Table 2.

Table 2: *Contribution of Different Scientists in Transgenesis.*

Scientists	Year	Role
Heape	1891	First successful embryo transfer experiment
Hammond	1949	Developed culture systems that sustained ova through several cleavage divisions
Lin	1966	First microinjection method in murine zygotes
Gurdon	1977	Transferred mRNA and DNA into Xenopus eggs
Brinster et al.	1980	Using rabbit globin mRNA, an appropriate translation product was obtained
Gordon and Ruddle	1981	First coined the term Transgenesis

HOW TO GET THE TRANSGENE

Following sequence is commonly adapted for the development of transgenic animals irrespective of species:

- Firstly, Identification and construction of the foreign gene and promoter sequence for development of transgenic animals.
- After that introduction of DNA into the pronucleus of a single fertilized egg by various methods as discussed below.
- Then, implantation of these engineered cells into surrogate mother.
- After that, bringing the developing embryo to term, proving that the foreign DNA has been stably and heritably integrated into the DNA of at least some of the newborn individual.
- Lastly, validating that the gene is controlled well enough to function in its new environmental conditions.

METHODS USED FOR THE PRODUCTION OF TRANSGENIC OR GENETICALLY MODIFIED ANIMALS

Three main methods used for the production of transgenic or genetically modified animals are DNA microinjection, retrovirus-mediated gene transfer and embryonic stem (ES) cell-mediated gene transfer method.

DNA Microinjection Method

First predominant method to be developed was DNA microinjection was the first predominant method to be developed. This technique was successfully used for the first time in 1980 [1]. The mouse was the first animal to undergo successful gene transfer purpose. This method involves:

- Firstly, transfer of a desired gene from another member of the same species or from a different species into the pronucleus of a reproductive cell.
- After that, specific embryonic phase is developing of the manipulated cell through *in vitro* culture.
- Lastly, transfer of the embryonic cells to the recipient female and birth of transgenic/GM offspring through recipient animal.

GM offspring frequencies obtained from microinjection methods are about 5–30%. Several factors influence the production of transgenics. Brinster et al. studied in mouse and reported that [26]: Linear DNA fragments combined with more efficiency than circular DNA and transgene DNA should be injected in little amounts otherwise it had harmful

effects on the embryos. Nuclear injection method was found to be dramatically more effective as compared to cytoplasmic injection.

DNA microinjection method has applications in a wide variety of species and is less costly as compared to other methods. Major advantages of this method are that there is no clear limit to the size of inserted DNA molecule and foreign genes are expressed efficiently.

Major disadvantages of this method are: DNA microinjection method cannot be used into the cell at later development stage; manipulations of oocytes or embryos, or the disruption of parental DNA at the integration site of the gene construct can also effect the normal development of the transgenic animal; injected gene may not insert itself into a site on the host DNA that will permit its expression; injected transgene is randomly incorporated into the recipient genome and may lead to change in the normal physiological processes of the animal; time consuming and requires extensive intellectual, financial and material assets; parental animals used in transgensis may suffer from discomfort during experimental procedures. It may also lead to multisite integration resulting in variable expression patterns in the transgenic offspring [27,28]. Success rate of producing transgenic animals is also very less.

Embryonic Stem Cells Mediated Gene Transfer Method

This method comprises of isolation of totipotent stem cells, which are undifferentiated cells that have the potential to differentiate into different cells for the formation of complete organism. The desired DNA sequences are inserted into the genome of embryonic stem cells cultured in vitro by homologous recombination. The incorporation of cells containing the desired DNA into the host's embryo leads to formation of chimeric animal. This technique plays an important role for the study of the genetic control of developmental processes. This method has advantage that by means of homologous recombination this allows particular targeting of defined mutations in the gene. Based on the resultant function of the targeted gene, gene-targeting methods have two lines of investigation: the gene knock-out (KO) to disrupt the existing gene, and the gene knock-in (KI) to insert a functional new gene. The precise targeting of DNA in embryonic stem cells is permitted through use of homologous recombination of DNA. The new sequence will replace the specific targeted gene, if the homologous sequence to be introduced into the cell carries a mutation or a gene from another species. This is the method of choice for gene inactivation thus, called as "knock-out" method, specifically important for the study of the genetic control of developmental processes.

Establishment of Embryonic Stem Cells

Embryonic stem cells were first established in 1981 by two laboratories independently [29, 30]. They changed hormone levels; blastocyst was cultured to obtain delay in its development. Four to six-day blastocyst inner cell mass (ICM) was removed and then co-cultured on the mitomycin C-treated unlimited lines of fibroblasts (STO) feeder layer (Feeder). The first mouse undifferentiated ES cell lines were established after cell proliferation and inhibition of differentiation of passage. It was shown that mouse ES cells by blastocyst injection could usually induce different variety of organizations involved in the formation of chimeric animals at rate of 61% of the total formation. These cells can secrete fibroblast growth factor (FGF), white leukemia inhibitory factor (LIF), differentiation inhibitory factor (DIA) and other substances. They suppress differentiation of ES cells and support their growth and colonization. Some conditions must be fulfilled for isolating embryonic stem cells which are given as (a) Present cells in culture must be undifferentiated and pluripotent; (b) The pluripotent cells must be deprived of differentiation signals in culture; (c) The cells must be stimulated, or at least be allowed, to proliferate.

Characteristics of Embryonic Stem Cells

Embryonic stem cells are small, aggregated and unpolarized cells forming islands on the feeder layers. These cells have large nucleoli and a high nucleocytoplasmic rate. The characterization of undifferentiated or

differentiated ES cells have been done using cell markers. The enzyme alkaline phosphatase is similar to the cell surface nonspecific alkaline phosphatase of the inner cell mass of the mouse blastocyst. All the "house-keeping" genes involved in the machinery of cell cycling and some receptors to factors which permit them escaping cycling and differentiating are expressed by ES cells.

Embryonic Stem Cells for Transgenesis

Introduction of foreign DNA into ES cells is done by electroporation method very efficiently. In order to produce chimeras, the gene-modified cell clones are introduced back either by blastocyst injection or by morula aggregation into preimplantation stage. Two main methods of embryonic stem cell-mediated gene transfer are gene trap and gene targeting method.

Main advantages of this method are application of gene targeting consequently enabling site-directed insertion of DNA [31]; allowing testing for transgenes at the early cell stage; detect precisely mutations in the gene via homologous recombination; 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; embryonic stem cells are relatively efficient in homologous recombination as compared to other animal cells.

Major disadvantages of this method include difficulty in production, characterization and maintenance of pluripotent embryonic stem cells lines.

Retrovirus-mediated Gene Transfer Method

A retrovirus is a virus that carries its genetic material in the form of RNA rather than DNA. Retroviruses are used as vectors to transfer genetic material into the host cell in this method. As a result, an organism consisting of tissues or parts of diverse genetic constitution i.e. chimera is formed. Chimeras are inbred for as many as twenty generations till homozygous transgenic offspring are born. Retrovirus- mediated expression cloning was developed in mid-1990s. The technical easiness, effectiveness of gene transfer and target cells specificity are most important features of retrovirus as vectors. Once retroviruses infect the cells, the resultant viral DNA becomes a part of the host cell genome after reverse transcription [32]. Gene transfer is mediated by carrier or vectors usually a virus or a plasmid in order to increase the probability of expression. Due to the ability to infect host cells, commonly used vectors to transfer genetic material into the cell are retroviruses.

DNA is produced from the code in the viral RNA by reverse transcription which is then integrated into the host cell. The offspring derived from this method are chimeric, i.e., an organism consisting of tissues or parts of diverse genetic constitution or all cells do not carry the retrovirus. The transmission of transgene is possible only if retrovirus integrates into some of the germ cells. All retroviral proteins necessary for the production of infectious particles are synthesized by packing cells. Packing cell provides Gag, Pol, and Env protein to the retroviral vectors which have no trans-acting sequences. The most accepted system for gene transfer in mammalian cells is NIH3T3 cells transformation with suitable MLV genes. In earlier packaging cell construction, NIH3T3 cells were transfected with the gag, pol, and env genes of MLV in a single transcriptional unit, causing production of replication competent helper virus.

Major advantages of this method are infectious retroviruses are incapable of infecting human cells; readily integrate and pass through the germ lines allowing for their propagation into consequent generations; integration causes minimal disruption of host DNA and always involves integration of a single copy of the donor gene and this system is technically simple.

Major disadvantages of this method are restriction on the size of the foreign DNA insertion; interference of viral sequences with transgene expression; some of the cells in the tissues of an organism receive the genetic change while the other cells without the desired addition and low copy number integration (Table3).

Table 3: *Comparison of DNA Microinjection, Retrovirus-mediated Gene Transfer and Embryonic Stem (ES) Cell-mediated Gene Transfer Methods [33].*

Techniques	DNA microinjection	Retroviral infection of embryos	Embryonic stem cells
DNA vector	Any cloned DNA, preferably linear with vector	Recombinant or wild type retroviruses	Cloned DNA or retroviruses
Introduction of DNA	Microinjection into pronucleus	Injection after removal of zona pellucida	Electroporation or retroviral infection
Embryonic stage	One-celled stage	One-celled stage or later	Totipotent ES cells
Embryo transfers	Oviduct	Uterus	Into blastocoel, then into uterus
Genotype of founder mice	Usually nonmosaic	Mosaic	Chimeric
Screening of newborns	Dot blots, Southern blots or PCR	Southern blots or PCR	Visual coat color markers plus PCR or Southern blots
Copy number of integrated DNA	1–200	1	Can be varied by selection of method for introducing
Percentage of potential founders that are transgenic	10–30%	5–40%	Up to 100%
Expression of the new DNA	Usually	Poor	Enhancer trap, gene trap
Integration	Random, nonhomologous, multicopy, single site	Apparently random using retroviral long terminal repeats (LTRs)	Random plus targeted, depending on method of introducing DNA
Germline transmission by founders	Usually	Usually	Occasionally a problem

APPLICATIONS OF TRANSGENIC ANIMALS

Role of Transgenic Animals in Biomedical Research

1. *Gene therapy*: Models for obesity and immunological, neurological, reproductive and haematological disorders, providing future hope for a variety of human therapeutic interventions.
2. Disease resistance in humans and animals. Clements et al. reported that transgenic sheep have been developed that is resistant to Visna virus infection [34]. The knock down of prion protein also helped in preventing the transmission of bovine spongiform encephalopathy (Scrapie) [35]. There are transgenic mice which secrete recombinant antibodies in milk to neutralize the corona virus responsible for causing an economically important disease in pigs i.e. transmissible gastroenteritis (TGEV) [36]. Lysostaphin is secreted into the milk of transgenic dairy cows which provides advanced resistance to mastitis. Lysostaphin kills the bacteria Staphylococcus aureus in a dose-dependent manner which protects the mammary gland against this main mastitis-causing pathogen [37].
3. Drug and product screening.
4. 4. Use of transgenic animals in toxicological screening protocols is already in trials. In order to develop preclinical drug, a whole animal model for screening is important for the understanding of disease etiology, drug pharmacokinetics and evaluating therapeutic efficacy and safety.
5. 5. Development of novel products through molecular pharming, e.g., α-1 Antitrypsin for Hereditary emphysema, Cystic fibrosis. Calcitonin for Osteoporosis and Collagen for Rheumatoid arthritis.
6. Various models of transgenic animals for different diseases are as follows:
   - *HIV/AIDS*: Tg26 HIVAN Mouse Model in 1991
   - *Alzheimer's disease:* Before transgenic technology there was no animal models existed for the disease.
   - *Cardiovascular disease*: Gain and or loss of function of angiotensin, endothelin, etc.

- *Diabetes Mellitus*: Models of insulin secretion such as glucokinase and hepatic glucose production in type 2 diabetes are developed
- *Angiogenesis*: Mouse models of angiogenesis, arterial stenosis, atherosclerosis, etc.

7. *Cancer diseases*: The first transgenic animal to be patented was oncomouse. An activated human oncogene sequence is introduced into the animal at an early embryonic stage and it is found in its germ and somatic cells.
8. *Production of pharmaceuticals in transgenic animals*: The production of therapeutic proteins from transgenic animals commonly involves their expression from mammary-gland specific promoters to drive secretion of the transgene into milk. An alternative is the use of kidney- or bladder specific promoters which directs transgene expression to the urine, e.g., Prolactin for enhancement of immunity and Protein C for blood coagulation.
9. *Transgenic expression of immunoglobulins*: Transgenic cattle harboring intact unrearranged human Ig heavy- and λ-light-chain loci were created. These 'transchromosomic' cattle were shown to produce human Ig.
10. *Xenotransplantation*: Primate-to-human organ transplantation led the age of xenotransplantation. The pig was the best choice as a donor animal for vascularized organs due to physiological, anatomical, ethical and supply reasons. The first published transgenic pig-to-primate xenograft used a novel transgenic delivery system for human complement regulatory proteins [38]. Currently, xenotransplantation is hindered 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.
11. *Identification of new drug targets*: A specific gene function is removed using knockout technology. Knockouts therefore, show huge potential for identifying and validating new drug targets among the tremendous number of possible targets revealed by the sequencing of the human genome.
12. *Replacing nonhuman primate (NHP) models*: Transgenic mice can be generated which express human versions of target genes, which can in some cases avoid the need to use animals, e.g., transgenic mouse model for neurovirulence testing of GSK's oral Polio Vaccine which previously had to be tested in NHP.
13. *Drug development*: The models have proven invaluable in preclinical evaluation of potential therapeutic interventions, facilitating the development of more effective treatments by enabling drug candidates to be tested for potential efficacy and toxicity early in drug development.

Role of Transgenic Cattle, Sheep, Goat and Pig

- • Dairy cattle are likely candidates for transgenesis if the mammary gland is to be used as a bioreactor, as they produce about 10,000 liters of milk/year with 35 g protein/liter. The animals could be made to secrete nutraceuticals in milk which may have an influence over the growth of offspring. Casein variants are main target for improving the composition of milk and in this process physio-chemical properties of milk get altered. Brophy et al. reported that transgenic cloned cattle have been developed which give increased amounts of beta and kappa casein in milk that improve the value addition of milk in the production of milk and milk based products along with increasing the shelf life of milk products [20]. Grosvenor et al. reported that the milk composition could also be changed by making the transgenic animals to secrete growth factors in milk therefore, affecting the growth as well as maturation of newborn offspring [39].
- For changing the constituents of milk like fat and protein. For instance, the amount of cheese produced from milk is directly proportional to the amount of k-casein content of the milk. If a transgene is constructed to produce milk with higher amounts of k-casein, the production of cheese will increase correspondingly.
- The problem of lactose intolerance can be solved by production of transgenic cows with modified genes to produce lactose free milk.

- Generally, attempts to produce animals with inherited resistance to bacterial, viral and parasitic disease is a goal. Examples of major diseases that affect the livestock are mastitis in cows, neonatal dysentery in swine, fowl cholera in chickens.
- It might be possible to produce transgenic animals that carry single gene responsible for the resistance.
- Varieties of monoclonal and recombinant antibodies were produced in transgenic goats and cattle [40, 41]. Kuroiwa et al. reported that transchromosomal animals could be used for the production of human therapeutic polyclonal antibodies [42].
- Transgenesis research with sheep, goat or pigs has been focused on using their mammary glands as bioreactors for secretion of pharmaceutical proteins. Various conventional methods were used for the production of therapeutic proteins using bacteria, plants, yeast etc. but most of them were deficient in machinery for post translational modifications of eukaryotic genes. The transgenic livestock serve as potential bioreactors for the production of valuable proteins like antithrombinIII (AT III), tissue plasminogen activator (TPA) and 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 market [43]. Glycosidase, used in the treatment of Pompe diseases, has been produced in the milk of transgenic rabbits [44]. More example is production of transgenic sheep that produces anti-trypsin in their milk; this protein is an impending treatment for cystic fibrosis.
- Role of transgenic animals in carcass composition and growth improvement is due to introduction of chicken ski gene which has caused muscular hypertrophy in pigs and cattle [45]. The acid meat gene or Rendement Napole gene affects the quality of meat in pig as it is involved in low processing yields of pork. Silencing theexpression of this gene in pigs leads to improvement in the quality of meat and modify the post mortem pH. There was a substantial improvement in growth rate and feed conversion in transgenic pigs through human metallothionein promoter [46].
- 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 [47].
- • The foremost prospective application of transgenic animal is the production of recombinant and biologically active proteins in the mammary gland which could be used for the benefit of mankind. This is known as “Gene Pharming”. Mammary gland is the preferred site for production of these proteins as large quantities can be extracted and purified [40].
- 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 [48, 49]. Pigs could be used as an effective model for the study of growth hormone releasing hormone (GHRH) defects [50].

LIMITATION OF TRANSGENIC ANIMALS

Transgenic animals have large number of applications like improvement of animal production and reproduction quality, enhancement of productivity and reproduction ability of animals, studies of different human disease models and production of pharmaceuticals proteins and enzymes. However, there are many problems that need to be fixed for transgenic animal studies.

Dietary and Food Safety Concerns

The food safety of bioengineered products is always a noteworthy public topic. In case of transgenesis, some of the foreign gene and its promoter sequences from the virus may occur in the recipient animals. The formation of new virus may occur due to homologous recombination or integration. Foreign gene introduced in the chromosome locus may also result in diverse genetic changes in different degrees, causing unintended effects. Transgenic animals may increase the risk of zoonotic diseases in addition to causing human allergic reactions.

Environmental Impacts

Transgenic animals kept in the external environment may breed with wildlife leading to spread of foreign gene, resulting in changing the species composition of the original genes, causing confusion in species resources. There is loss of wild allele leading to decline in the genetic diversity. Once released into the environment, transgenic animals can disturb the genetic diversity of threatened species along with ecological balance of species.

ETHICS OF TRANSGENIC TECHNOLOGY

Use of any animals in the transgenic technology leads to greater suffering to the animals and bio-ethics defined as the set of standards that may be used to regulate various activities based on their effect on the biological world. Using of transgenic animals for the production of proteins and enzymes this does not seem to recognize that animals also are living beings and feel pleasure and pain like human being. Genetically altering the cells of a transgenic animal can lead to side effects resulting from modifying genes. Foreign genes introduced into the transgenic animals affect the experimental animal and produce a lot of harmful effect to ecological balance and biological diversity [51].

CONCLUSION

In the recent past, transgenic animal production techniques have advanced hastily and provided platforms for the preparation of transgenic animals. These techniques provide an entirely new alternative for the accurate modulation of genes for human use, particular the production of drug, organ culture for human transplantation and there use in biomedical research will be great outcome. It is expected that with the development of simple and novel animal transgenic techniques will lead to more benefits and may help to alleviate or provide to cure certain diseases which are presently incurable or expensive to treat. Production of transgenic animals and related products is still infancy in India and is restricted to certain high profile laboratories and the outcome had shown lower accuracy in transgenic animal production. Some work has been conducted in chicken for increased muscle mass and to improve the yield in broilers and that also suffered from lower accuracy. Transgenic animal could be produced using different method, but no method seems to be perfect and has its own pros and cons. Besides these facts, transgenic animal production has a potential in animal pharming for human use.

REFERENCES

1. Gordon JW, Scangor GA, Plotkin DJ, *et al*. Genetic transformation of mouse embryos by microinjection of purified DNA. *Proceedings of the National Academy of Sciences of the USA*. 1980; 77: 7380–7384p.
2. Gordon JW, Ruddle FH. Integration and stable germ line transmission of genes injected into mouse pronuclei. *Science*. 1981; 214: 1244–1246p.
3. Hammer RE, Pursel VG, Rexroad CE, *et al*. Production of transgenic rabbits, sheep, and pigs by microinjection. *Nature*. 1985; 315: 680–683p.
4. Pursel VG, Rexroad CE Jr., Bolt DJ, *et al*. Progress on gene transfer in farm animals. *Vet Immunol Immunop*. 1987; 17: 303–312p.
5. Rexroad CE, Hamer RE, Bolt DJ, *et al*. Production of transgenic sheep with growth-regulating genes. *Mol Reprod Dev*. 1989; 1: 164 (Abstract).
6. Roschlau K, Rommel P, Andreewa L, *et al*. Gene transfer experiments in cattle. *J Reprod Fertil*. 1989; (Suppl.) 38: 153–160p.
7. Devlin RH, Biagi CA, Yesaki TY, *et al*. Growth of domesticated transgenic fish. *Nature*. (London) 2001; 409: 781–782p.
8. Saeki K, Matsumoto K, Kinoshita M, *et al*. Functional expression of a Delta12 fatty acid desaturase gene from spinach in transgenic pigs. *Proc. Natl. Acad. Sci*. USA, 2004; 101: 6361–6366p.
9. Rogers GE. Improvement of wool production through genetic engineering. Trends Biotechnol. 1990; 8: 6–11p.
10. Forsberg CW, Meidinger RG, Ajakaiye A, *et al*. The Enviropig (TM): phosphorus nutrition, physiology and tissue composition. *Transgenic Res*. 2006; 15: 116–117p.
11. Reh WA, Maga EA, Collette NM, *et al*. Hot topic: using a stearoyl-CoA desaturase

transgene to alter milk fatty acid composition. *J Dairy Sci*. 2004; 87: 3510–3514p.
12. Wall RJ, Powell AM, Paape MJ, *et al.* Genetically enhanced cows resist intramammary Staphylococcus aureus infection. *Nat Biotechnol*. 2005; 23: 445–451p.
13. Konishi K, Yonai M, Kaneyama K, *et al.* Relationships of survival time, productivity and cause of death with telomere lengths of cows produced by somatic cell nuclear transfer. *J Reprod Dev*. 2011; 57: 572–578p.
14. Kind A, Schnieke A. Animal pharming, two decades on. *Transgenic Res*. 2008; 17(6): 1025–1033p.
15. Dyck M, Lacroix D, Pothier F, *et al.* Making recombinant proteins in animals - different systems, different applications. *Trends Biotechnol*. 2003; 21(9): 394–399p.
16. Houdebine LM. Production of pharmaceutical proteins by transgenic animals. Comparative Immunology, *Microbiol Infect Dis*. 2009; 32(2): 107–121p.
17. Luo Y, Lin L, Bolund L, *et al.* Genetically modified pigs for biomedical research. *J Inherit Metab Dis*. 2012; 35(4): 695–713p.
18. Yang B, Wang J, Tang B, *et al.* Characterization of bioactive recombinant human lysozyme expressed in milk of cloned transgenic cattle. *PLoS One*. 2011; 6(3): 17593. Available at: http://dx.doi.org/10.1371/journal.pone.0017593.
19. Wu X, Ouyang H, Duan B, *et al.* Production of cloned transgenic cow expressing omega-3 fatty acids. *Transgenic Res*. 2012; 21: 537–543p.
20. Brophy B, Smolenski G, Weeler T, *et al.* Cloned transgenic cattle produced milk with higher levels of b-casein and k-casein. *Nat Biotechnol*. 2003; 21(2): 157–162p.
21. Scharfen EC, Mills DA, Maga EA. Use of human lysozyme transgenic goat milk in cheese making: effects on lactic acid bacteria performance. *J Dairy Sci*. 2007; 90: 4084–4091p.
22. Damak S, Su H, Jay NP, *et al.* Improved wool production in transgenic sheep expressing insulin-like growth factor 1. *Biotechnology*. 1996; 14: 185–188p.
23. Denning C, Burl S, Ainslie A, *et al.* Deletion of the alpha(1,3)- galactosyl transferase (GGTA1) gene and the prion protein (PrP) gene in sheep. *Nat Biotechnol*. 2001; 19: 559–562p.
24. Tong J, Wei H, Liu X, *et al.* Production of recombinant human lysozyme in the milk of trans- genic pigs. *Transgenic Res*. 2011; 20: 417–419p.
25. Saeki K, Matsumoto K, Kinoshita M, *et al.* Functional expression of a Delta12 fatty acid desaturase gene from spinach in transgenic pigs. *Proc. Natl. Acad. Sci.* USA 2004; 101: 6361–6366p.
26. Brinster RL, Chen HY, Trumbauer ME, *et al.* Factors affecting the efficiency of introducing foreign DNA into mice by microinjection eggs. *Proc. Natl. Acad. Sci.* 1985; 82(13): 4438–4442p.
27. Seidel GE. Jr. Resource requirements for transgenic livestock research. *J Anim Sci*. 1993; 71: 26–33p.
28. Wall RJ. Transgenic livestock: Progress and prospects for the future. *Theriogenology*. 1996; 45: 57–68p.
29. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature, 1981; 292(2819): 154–156p.
30. Martin GR. Isolation of a pluripotent cell lines from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci.*, 1981; 78(12): 7634–7638p.
31. Muller W. Introduction of defined mutations into the mouse germline. In: Van Zutphen, L.F.M. and Van der Meer M (eds.) *Welfare Aspects of Transgenic Animals, Proceedings of EC workshop*, 30 October 1995, Utrecht, The Netherlands, Springer Verlag, Berlin, 1997, 18–25p.
32. Ponder KP, Melniczek JR, Xu L. Therapeutic neonatal hepatic gene therapy in mucopolysaccharidosis in dogs. *Proc Natl Acad Sci*. 2002; 99: 102–107p.
33. Overbeek PA, Gorlov IP, Sutherland RW, *et al.* A transgenic insertion causing cryptorchidism in mice. *Genesis*. 2001; 30: 26–35p.
34. Clements JE, Wall RJ, Narayan O, *et al.* Development of transgenic sheep that

express the Visna virus envelope gene. *Virology*. 1994; 200(2): 370–380p.
35. Weissmann C, Enari M, Klohn PC, *et al.* Transmission of prions. *Proceedings of the Natural Academic of Sciences*, USA, 2002; 99(Suppl.4): 16378–16383p.
36. Castilla J, Printado B, Sola I, *et al.* Engineering passive immunity in transgenic mice secreting virus-neutralizing antibodies in milk. *Nat Biotechnol*. 1998; 16(4): 349–354p.
37. Donovan DM, David EK, Robert JW. Engineering disease resistant cattle. *Transgenic Res*. 2005; 14: 563–567p.
38. McCurry K, Byrne GW, Martin R, *et al.* Transgenic pigs expressing human CD59 and decay-accelerating factor produce an intrinsic barrier to complement-mediated damage. *Transplantation*, 1997; 63: 149–155p.
39. Grosvenor CE, Picciano MF, Baumrucker CR. Hormones and growth factors in milk. *Endocr Rev*. 1993; 14: 710–728p.
40. Meade HM, Echelard Y, Ziomek CA, *et al.* Expression of recombinant proteins in the milk of transgenic animals. In: Fernandez, J.M., Hoeffler, J.P. (Eds.), *Gene Expression Systems*. Academic Press, San Diego, USA, 1999, 399–427p.
41. Grosse-Hovest L, Müller S, Minoia R, *et al.* Cloned transgenic farm animals produce a bispecific antibody for T cell-mediated tumor cell killing. *Proc Natl Acad Sci*. USA, 2004; 101(18): 6858–6863p.
42. Kuroiwa H, Mori T, Takahara M, *et al*. Chloroplast division machinery as revealed by immunofluorescence and electron microscopy. *Planta*. 2002; 215: 185–190p.
43. Kues WA, Niemann H. The contribution of farm animals to human health. *Trends Biotechnol*. 2004; 22: 286–294p.
44. Van den Hout M, Muris P, Salemink E, *et al.* Autobiographical memories become less vivid and emotional after eye movements. *Brit J Clin Psychol*. 2001; 40: 121–130p.
45. Bowen RA, Reed M, Schnieke A, *et al.* Transgenic cattle resulting from biopsied embryos: Expression of c-ski in a transgenic calf. *Biol Reprod*. 1994; 8: 664–668p.
46. Nottle MB, Haskard KA, Verma PJ, *et al.* Effect of DNA concentration on transgenesis rates in mice and pigs. *Transgenic Res*. 2001; 10: 523–531p.
47. D'Agnillo F, Chang TM. Polyhemoglobin superoxide dismutasecatalase as a blood substitute with antioxidant properties. *Nat Biotechnol*. 1998; 16(7): 667–671p.
48. Theuring F. Oxford: *Functional Analysis of the Human Genome* (eds F Farzaneh, DN Cooper), In BIOS Scientific Publishers, 1995, 185–205p.
49. Li Z, Engelhardt JF. Progress toward generating a ferret model of cystic fibrosis by somatic cell nuclear transfer. *Reprod Biol Endocrinol*. 2003; 1: 83p.
50. Draghia-Akli R, Fiorotto ML, Hill LA, *et al.* Myogenic expression of an injectable protease-resistant growth hormone-releasing hormone augments long-term growth in pigs. *Nat Biotechnol*. 1999; 17: 1179–1183p.
51. Miao X. Recent advances in the development of new transgenic animal technology, *Cell Mol Life Sci*. 2013; 70(5): 815–828p.

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