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TRANSGENIC ANIMALS: PROSPECTS FOR IMPROVING LIVESTOCK PRODUCTIVITY

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ABSTRACT

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 animal's 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.

Keywords: *Transgenic animals, Livestock, Genome sequence information, Vectors*

Number of Tables: 02 **Number of Figures: 02** **Number of References:38**

INTRODUCTION

The fast growing human population increases the demand for basic needs of man like food, medicines etc for their survival and well-being. Man depends on agriculture and livestock to fulfill his needs for food, which necessitate the successful tradition of livestock breeding. The efforts of livestock breeders and the application of techniques like artificial insemination (AI), embryo transfer and further biotechnological procedures have resulted in the well-known and remarkable increase in the performances of livestock production. Moreover as Genomic sequence information and genomic maps of farm animals are refined, it becomes increasingly practical to remove and modify the individual genes. Tran genesis helps in many ways in animal production for improving carcass composition, lactational performance and wool production as well as disease resistance and reduced environmental impact. This approach to animal breeding will be instrumental to meet global challenges in animal production in future.

The genetic structure of livestock for sustainable production of milk, meat and eggs could be modified through genetic engineering. The genetic improvement of multicellular organism through transgenic technology has become a powerful tool for studying gene expression patterns and also to produce therapeutic proteins in milk. Gordan *et al*. (1987) first demonstrated the production of therapeutic proteins in the milk of transgenic animal. The production of human proteins of pharmaceutical value by various commercial methods is very expensive and also time consuming. The cost involved in producing such proteins is also highly necessitating the need for good infrastructure and better purification system. The animal which are genetically engineered, called as transgenic animals, produce and secrete such novel therapeutic proteins throughout their life. This not only enables several human diseases to be cured, but also make many people needing organ transplantation to live normal life. The transgenic technology plays a potential role in livestock improvement programmes and adds a new dimension to animal breeding.

Historical background

Prior to the development of molecular genetics, the only way of studying the regulation and function of mammalian genes was through the observation of inherited characteristics or spontaneous mutations. Long before Mendel and any molecular genetic knowledge, selective breeding was a common practice among farmers for the enhancement of chosen traits, e.g., increased milk production.

During the 1970s, the first chimeric mice were produced (Brinster, 1974). The cells of two different embryos of different strains were combined together at an early stage of development (eight cells) to form a single embryo that subsequently developed into a chimeric adult, exhibiting characteristics of each strain. The mutual contributions of developmental biology and genetic engineering permitted rapid development of the techniques for the creation of transgenic animals. DNA microinjection, the first technique to prove successful in mammals, was first applied to mice (Gordon and

Ruddle, 1981) and then to various other species such as rats, rabbits, sheep, pigs, birds, and fish. Two other main techniques were Subsiquently developed: those of retrovirus-mediated transgenesis (Jaenisch, 1976) and embryonic stem (ES) cell-mediated gene transfer (Gossler *et al*., 1986).

After 1981, when the term transgenic was first used by J.W. Gordon and F.H. Ruddle (1981), there has been rapid development in the use of genetically engineered animals as investigators have found an increasing number of applications for the technology.

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 fertilised 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. *An example:*Normal mice cannot be infected with polio virus. They lack the cell-surface molecule that, in humans, serves as the receptor for the virus. So, normal mice cannot serve as an inexpensive, easily-manipulated model for studying the disease. However, transgenic mice expressing the human gene for the polio virus receptor can be infected by polio virus and even develop paralysis and other pathological changes characteristic of the disease in humans.

Why Transgenic Animal?

Interest in transgenic animals originally fall into two broad categories:

- To increase production efficiency of farm animals in a short duration.
- Molecular farming: Using livestock to produce medicines, nutraceuticals and tissues for transplant into humans.

Strategies for Producing Transgenic Animals

There are two basic strategies for producing transgenic animals, which include "gain of function" or "loss of function" transgenics. The basic idea behind the gain of function

strategy is that by adding a cloned fragment of DNA into an animal's genome to a new gene product is produced that did not previously existed in that cell or tissue. E.g. expression of human growth hormone (hGH) in mouse liver and to get over expression of gene product in the proper tissue (Palmiter *et al*., 1982).

The loss of function approach has many similar applications as the gain of function strategy especially in view of over expression, insertional mutations and antisense situations. This strategy relies on the ability of the embryonic cells to undergo homologous recombination ("gene targeting").Gene targeting permits the transfer of genetic alteration created invitro into precise site in the embryonic or cell genome. If the host's cells are totipotent or pluripotent embryonic cells or reprogrammable somatic cells, these homologous recombination events can be transferred to the germ line of the offspring. This strategy has extraordinary potential for making specific genetic changes for use in medicine; agriculture and for further understanding of the genetic control of developmental processes.

Vectors

Vectors are plasmid or viral DNA employed in recombinant DNA technology to clone a foreign gene in prokaryotic or eukaryotic cell. The various animal vectors are based on one or the other virus e.g. SV40 vectors, bovine papillamovirus vectors, retrovirus vectors, etc., or on a transposable element e.g. Drosophila P element vector. It may be pointed out that some of the these vectors e.g., early or late region replacement SV40 vectors, retrovirus vectors etc., behave like viruses in that they produce virions, which are used to infect the host cells. Some other vectors are like bacterial plasmids, e.g., SV 40 plasmid vectors, bovine papillomavirus vectors and polyoma virus vectors; these vectors have to be introduced in the cell using a suitable transfection technique.

Drosophila P elements have been developed as valuable vectors for this invaluable genetic material. The 31 bp inverted repeat borders and the neighboring sequence of P elements are combined with a suitable *E.coli* plasmid, e.g., pUC8, to produce a shuttle vector. DNA insert of up to 40 kb can be placed between the two border sequences. The recombinant P DNA is injected into Drosophila larvae along with a helper P element, which produces transposase. Transposase enables the transposition of recombinant P element (carrying the DNA insert) from the recombinant DNA into the Drosophila genome.

Bacculovirus vectors have been developed for transfection of insects. Two nuclear polyhedrosis viruses (NPV), e.g. AcPNV (*Autographa californica* NPV) and BmNPV (*Bomby* x *mori* NPV) have been exploited for this purpose. The NPV polyhedron protien gene has a very strong promoter, and the polyhedron protein is not needed for NPV replication. Therefore, the general strategy is to replace the NPV polyhedron coding sequence by the DNA insert so that the polyhedrin promoter drives the transgene. The recombinant NPV DNA form virions, infect silkworm larvae or cultured cells, and replicate to yield upto 50 ug vector DNA per larva. BmNPV vectors are used for infection of silkworm larvae, while

AcNPV vectors are multiplied and expressed in the larvae or cultured cells of the insect *Spodoptera frugiperda*. The different types of vectors used for gene transfers in animals are shown in Table 1.

Table 1: *The different types of vectors used for gene transfers in animals*

S.No.	Vectors	Derived from	Features
1.	SV40 Vectors		
	a. Early region replacement vectors	Replacement of large-T gene of SV 40	Produce virions which infect host cells, Transient gene expression, Mammalian cells are hosts of SV 40
	b. Late region replacement vectors	Replacement of VP1, VP2,and VP3 genes of SV 40, e.g. SVGT-5	-
	c. Plasmid vectors	Origin of replication and large –T gene of SV40	-
	d. Shuttle plasmid vectors	Plasmid vector {item 1 (c) plus pBR322 origin and amp gene, e.g., pSV2, pSV3, etc. Rous sarcoma virus promoter in place of SV40 early promoter, e.g., pRSV.	Strong expression of the marker gene.
	e.Passive transfecting vectors	SV40 transcription regulatory and polyadenylation sequences, plus pBR322 origin and amp gene.	Shuttle vectors; used for gene integration in mammalian cells.
2.	BPV vectors	Bovine papilloma virus "Transforming region"+ pBR322 sequences.	Shuttle vectors; often pBR322 sequence deleted prior to transfection; plasmid-like vector
3.	Retrovirus vectors	pBR322 + retrovirus sequences	Shuttle vectors; integrates as provirus into mammalian genome; produces virions.
4.	Polyomavirus vectors	Polyomavirus origin and early region pBR322 sequences	Similar to SV40 vectors; mouse cells used as host
5.	Vaccinia virus	DNA insert placed within the thymidine kinase gene of virus by a process of recombination.	Promising as live vaccines; DNA insert is a pathogen gene encoding an antigen.

6.	P element vectors	Drosophila transposable element P; minimum of 31 bp inverted repeat borders and the neighboring regions plus an E.coli vector, e.g., pUC8; DNA insert of up to 40 kb placed within the two borders.	Gene transfer in Drosophila; a helper P element is needed to provide the transposase necessary for transposition or insertion of the recombinant P DNA into the Drosophila genome.
7.	Bacculovirus vectors	Nuclear polyhedroma virus (NPV) polyhedrin gene replaced by DNA insert; e.g. AcNPV (autographa California nuclear polyhedroma virus) and bmNPV (Bombyx mori nuclear polyhedroma virus vectors	Produce virions; expression vectors for production of transgenic proteins in silk worm larvae (BmNPV vectors) and in *Spondoptera frugiperda* larvae or culture cells (AcNPV vectors)

It may be seen from table that most of the animal vectors are designed to replicate and express in animal cells; only the passive transducing SV 40 vectors are incapable of replication. Retrovirus and transposon vectors integrate into the genomes of host cells in a manner similar to the natural retroviruses and transposons, respectively. Both circular and linearized vectors can integrate into the host genome, but the latter are far more readily integrated than the former. It has also been found that the present of additional vector DNA along with the integrated gene construct interferes with the expression of introduced gene or transgene. Therefore, it is often desirable to introduce the transgene with a minimum of vector DNA associated with it.

Techniques of Gene Transfer

There are two main strategies for production of transgenic animal namely expression of exogenous DNA or silencing the expression of a gene? Could be made to express in a cell where it does not express previously (human growth hormone in mouse liver) or we can make a gene to express in higher amount. The silencing or loss of gene function is accomplished by the target gene disruption through the process of homologous recombination between host genome and exogenous DNA. There are so many techniques existing by which foreign DNA could be engineered and transferred in to the host cell (Figure 1). All these methods have their own advantages and disadvantages

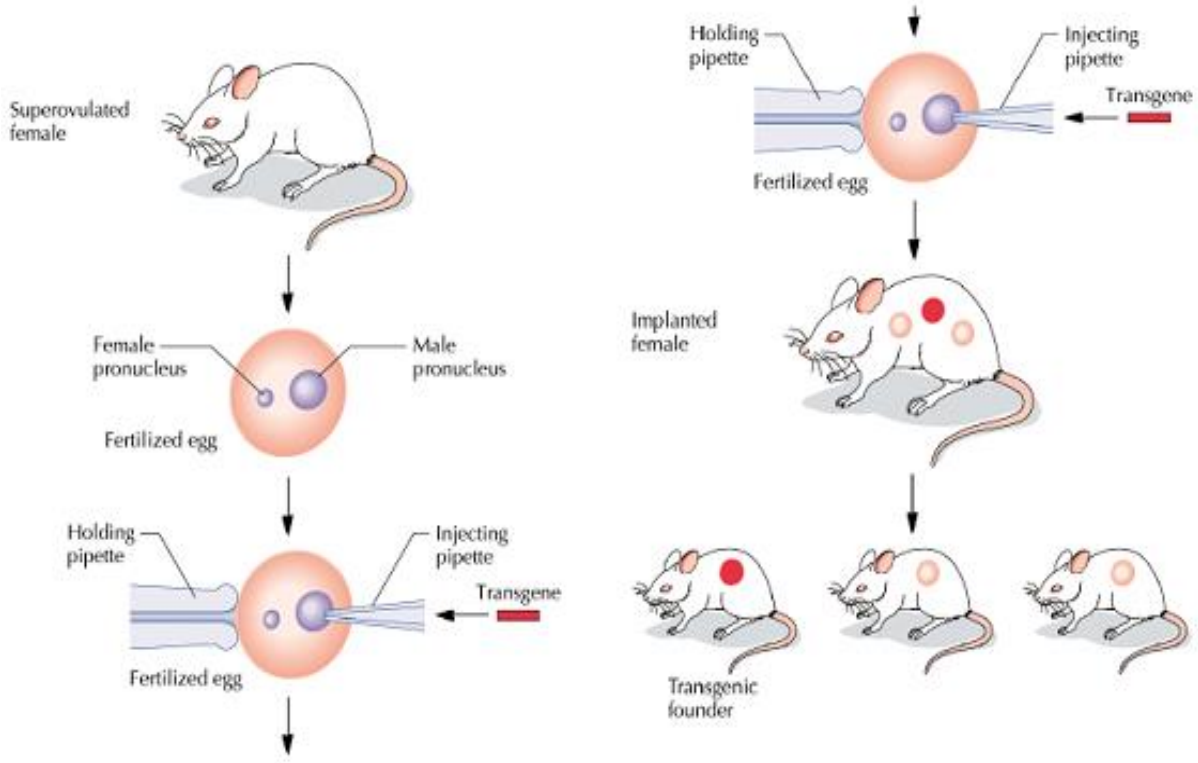


Fig. 1: *Transgenesis in mice-microinjection*

Viral Technique

In this technique, the genetic information is introduced in the host cell through a DNA virus called as Simian virus 40 [SV40]. Even though they are effective, there are chances that the virus may replicate and kill the host cell.

Chemical technique

This technique utilizes the chemical mediated uptake of DNA or gene fragment by the host cell. The transfection is carried out effectively by using chemicals like calcium phosphate or diethyl amino ethyl dextran.

Pronuclear injection

This technique was first developed by Lin (1996). These involve the microinjection of exogenous DNA into the pronucleus of a fertilized ovum. This technique has several shortcomings like random integration, low efficiency and variable expression patterns. The overall efficiency of this technique in cattle is less then when compared to mouse and thus presence of inserted sequence in embryos is found out by polymerase chain reaction or green flourescent protein (Chauhan *et al*., 1999).

Electroporation

This technique was developed by Puchalski and Fahl (1992). In this technique, cells are exposed to electric field which causes the membranes to become polarized and a potential develops across the membrane thereby breaking at localized areas and thus the cell becomes permeable to exogenous molecule. The method has a greater efficiency either alone or in combination with other.

Retroviral viral technique

Retrovirus is single stranded RNA virus which upon transfection gets converted to double strand DNA and integrates into the host genome (Eglitis *et al.*, 1988). The retroviral method was the first method to produce a transgenic mouse as reported by Jaenisch *et al*. (1975). The most commonly used retroviral vector is Moloney murine leukemia virus (Laneuville *et al*.,1988).

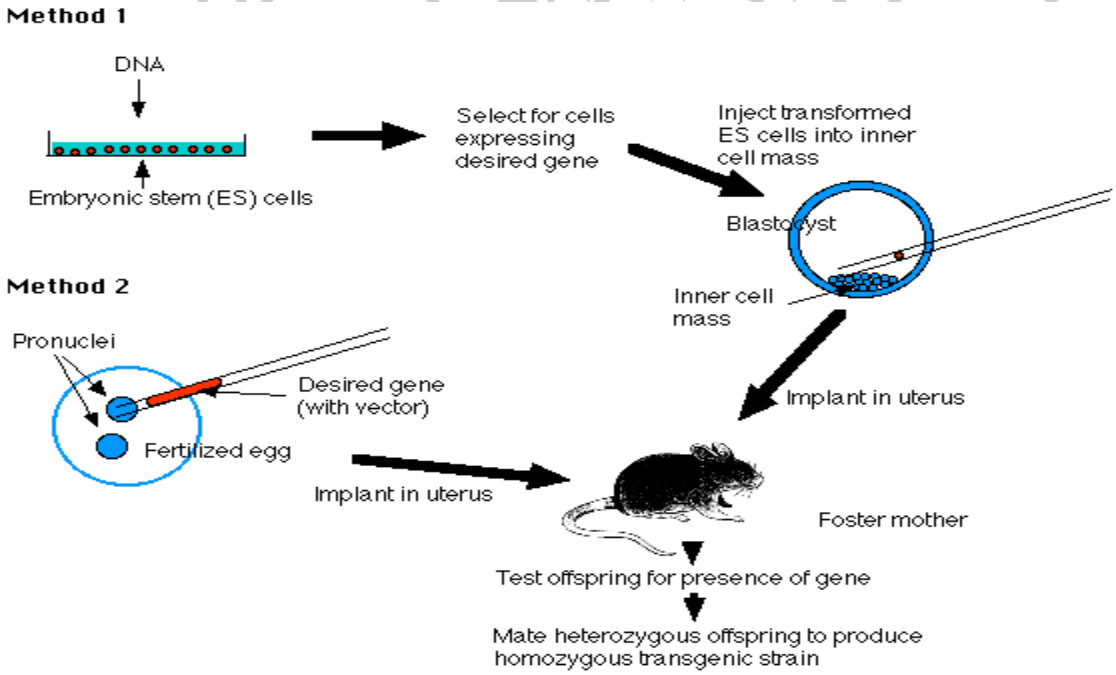
Lentiviral Transfection

This method overcomes the limitations of viral mediated gene transfer like low expression and silencing of gene locus. Stable transgenic lines could be produced by injection the lentivirus into the perivitelline space of zygotes (Hofmann *et al*., 2003). This method had a higher efficiency in case of pigs when compared to cattle (Hofmann *et al*., 2004).

Embryonic Stem Cells

The ability to isolate and maintain embryonic and somatic cells invitro increases the efficiency of production of transgenic animal (Figure 2). Embryonic stem cells have pluripotent characteristics that differentiate into various tissue types that could be genetically modified for the production of animals of desired genotypes. The embryonic stem cells and primordial germ cells via nucleus transfer allow the rapid development of producing transgenic animals through targeted gene insertion.

Fig. 2: *Show the embryonic stem cells in-vitro increases the efficacy of production of transgenic animals*



Transplantation of Cultured Spermatogonia

In this method, the primordial germ cells are genetically engineered and subsequently transplanted into the testis of recipient males. Recently researchers have succeeded in transmitting the donor haplotype to the next generation (Honaramooz *et al*., 2003).

Sperm Mediated Gene Transfer

The sperm cells have the capacity to bind naked DNA or bound to vesicles like liposomes (Lavitrano *et al*., 1989; Chang *et al*., 2002). These sperm cells are in turn used for introducing exogenous DNA into oocytes either through invitro fertilization or artificial insemination. Sperandio *et al*. (1996) successfully carried out the sperm mediated gene transfer in cattle.

RNA Interference

In this method, small interference RNAs (siRNAs), which are 20-25 nucleotides long, bind to their complementary sequences on target in mRNAs and shut down the expression of genes and there by the production of protein is stopped (Plasterk, 2002). This RNAi could be used for either transient or stable gene repression or knock down of specific target genes. This method together with lentiviral technology may improve the efficiency of gene transfer.

Transgene diagnostics

Molecular tools are currently the most cost-effective means of identification of transgenic animals and testing stability of the transgenes. A number of different techniques can be used for diagnose is and/or expression analysis, and are discussed below.

Southern blot analysis

Before the development of PCR, Southern blotting was the method of choice for transgene detection. It involves the isolation of DNA from selected animals, digestion of DNA with restriction enzymes, electrophoresis on agarose gels to separate DNA by size, denaturation of the DNA and transfer of the DNA to a membrane. This membrane (either nitrocellulose or more recently nylon) is then hybridised with a specific probe (complementary to the transgenic sequence of interest). The probe is usually radioactively, colorimetrically or chemiluminescently labelled such that once hybridised to the transgene DNA, the position of the transgene on the membrane can be visualised. Southern blot analysis can be applied to:

- Demonstrating presence or absence of a transgene in the sample.
- Determining whether chromosomal integration has occurred.
- Identifying the position of integration relative to other samples.
- Determining whether homologous recombination (directed integration) occurred.
- Determining copy number of transgenes.
- Analysing stability of the transgene with regards to reintegration and/or and replication over time and through future generations i.e. the frequency of mutation/rearrangement within or adjacent to the transgene.

Standard polymerase chain reaction (PCR):

PCR is an enzyme (polymerase)-driven

method allowing the targeted amplification of specific regions of DNA from a sample. The method is rapid, giving diagnostic results in a matter of hours, and sensitive, requiring very small amounts of starting material (<1ng).

Transgenic individuals are most easily identified when the transgene produces a distinct phenotypic effect, but such cases are only occasional. A more general approach utilizes either dot-blot technique of PCR amplification using genomic DNAs extracted from tail biopsies from 6-7 weeks old mice. In case of fish, genomic DNA is usually extracted from pectoral fin tissue. PCR amplification can be used if the transgene has unique sequences, not found in the host genome that can be used as primers. This allows very small amounts of DNA from presumptive transgenic individuals to be suitably amplified for a reliable detection of the transgene. The PCR approach is briefly described below:

1. The test DNA is amplified using unique transgene sequences as primers in a PCR.
2. The amplified DNA is subjected to agarose gel electrophoresis; the transgene construct is also run as a control.
3. DNA from the gel is blotted onto a solid support following the protocol for Southern blotting.
4. A radioactive probe specific for the transgene is used for hybridization, and the hybridizing samples are detected by autoradiography in the same manner as for Southern hybridization.

The samples that test positive for hybridization with the probe are from putative or suspected transgenic individuals. It should be kept in mind that both dot blot and PCR techniques detect the transgene irrespective of whether it is integrated in the genome or is present in an extrachromosomal state. Therefore, dot blot or PCR assay positive individuals are subjected to Sothern hybridization, for confirmation of transgene integration etc.

PCR has applications in:

- Rapid screening (2-4 hrs) for identification of transgenic animals.
- Detection of small amounts of transgenes in samples of blood or other tissues.
- Detection of mosaicism within an individual.
- Rapid assessment of environmental contamination / biosafety breaches.

Due to the ease of use, speed and sensitivity, standard PCR remains an important tool especially for identification of animals possessing the transgene of interest.

Real-time quantitative PCR (Q-PCR)

The real-time PCR system is based on the detection and quantification of a fluorescent reporter. The fluorescence signal increases in direct proportion to the amount of PCR product in a reaction (Heid *et al*., 1996). This system has enormous application within the area of transgenics. Q-PCR has applications in:

- The same samples as for standard PCR.
- Automated, high-throughput screening of samples.
- Accurate quantification of the amount of transgenic DNA within a sample.

Q-PCR is likely to supersede standard PCR for most applications, because it is quantitative. Set-up costs of the technology may be prohibitively high for smaller

laboratories, however.

Microscopy

Microscopy can be used to identify transgenic vectors within cells. Fluorescence insitu hybridisation (FISH) is the most efficient cytogenetic molecular technique so far developed for the enumeration of chromosomes. Fluorescently-labelled probes for the transgenic sequence are used as probes to hybridise to chromosomes in which the transgene has integrated. These samples can then be enumerated using fluorescent microscopy. Once optimised, FISH can detect small and even single-copy transgenes within a genome. This technique has application in:

- Analysis of the chromosomal environment of integrated transgenes.
- Analysis of the local chromatin structure of transgenes.
- Assessment of the effect of integration position on gene expression (e.g. Dong *et al*., 2001).

FISH and similar microscopic techniques will continue to be very important tools in the evaluation of transgene stability and expression.

Applications of transgenic animals:

(A) Human Health

The main potential application of transgenic animal is the production of recombinant and biologically active proteins in the mammary gland and this in turn could be used for the benefit of mankind. This is called 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 α-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. Transgenic crops also play a vital role in production of proteins coded by genes, which has complex regulation, and fail to produce sufficient quantities in the milk of transgenic animals. A significant landmark has been achieved by the production of FMD vaccine in transgenic alfala crop (Wigdorovitz *et al*., 2004). The lists of some recombinant proteins obtained from transgenic animals are shown in Table 2.

Table 2: *Lists of some recombinant proteins obtained from transgenic animals*

Protein	Source	Against
Antithrombin III	Goat	Thrombosis
Tissue plasminogen activator	Sheep, pig	Thrombosis
α-antitrypsin	Sheep	Emphysema
Factor VIII, IX	Sheep, pig, cow	Hemophilia
α -Glucosidase	Rabbit	Pompe's disease
Fibrinogen	Cow, sheep	Wound healing
Glutarmic acid decarboxylase	Goat	Type 1 diabetes
Human serum albumin	Cow, sheep	Maintenance of blood volume
Human protein c	Goat	Thrombosis
Monoclonal antibodies	Chicken, cow, goat	Vaccine production
Pro 542	Goat	HIV
Lactoferrin	Cow	GI tract infection and infectious arthritis.

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 (Chang and D'Agnillo, 1988).

Antibodies and Transgenic Animals

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

Organ Transplantation

The public demand for organs for transplantation is increasing day by day. As there exist shortage of human organs (all transplantation), xeno transplantation (transplantation of organs between different species) is considered as choice of saving millions of people needing organs transplantation. Transgenic pigs have been produced that express the human proteins on the surface of the organs there by the risk of hyper acute rejection and acute vascular rejection of organs by the recipients is reduced ensuring the compatibility of donor organs with that of the recipients. Transgenic animals also could be used as a source of xenogenic cells that could be used for the treatment of several diseases and disorders with irreversible cell death.

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.*, 1997; 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).

(B) Livestock Production

There are many potential applications of transgenic technology in producing new varieties of livestock that has increased growth rate, reproductive performance, feed utilization, improved milk production and high disease resistance. Other by products like meat and eggs also could be modified by this technology.

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 (Seamark, 1987). 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 (Pursel *et al*., 1999) 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*., 1999).

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 of 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.

By-Products

Wool and leather are the major byproducts that could be altered by genetic engineering. The quality, colour, length, ease of harvest and yield of wool have been the target for manipulating the fiber and hair of farm animals. Transgenic sheep expressing keratin – IGF 1 construct have been produced that have greater percentage of clean fleece than non transgenic animals (Damark *et al.*, 1996). The quality of wool could also be increased by transgenic modification of cystein pathway. Farm animals also could be genetically engineered by altering the collagen gene so that the amount of connective tissue within the skin may be significantly changed so as to improve the quality of hide. Hollis *et al.* (1983) reported that epidermal growth factor gene (EGF) has been introduced in sheep that enhances the ease of removing the wool from sheep.

Disease Resistance

The most important application of transgenic technology is the manipulation of MHC (Major Histocompatability 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 resistance 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).

Reproductive Performance

Several candidate genes have been identified that increase the reproductive performance of farm animals. Rothschild et al. (1994) reported that the polymorphism in estrogen receptor gene (ESR) increased the litter size in case of pigs. The ovulation rate of different breeds of sheep, which are superior in carcass traits and wool production, could be increased by incorporating a single major autosomal gene called booroola fecundity gene (FECB) that increases the prolificacy in sheep (Piper *et al.*, 1985). The estrus symptoms in case of pigs could be enhanced by incorporating a gene from baboons, which make their posterior red (Seidel, 1999).

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 germinal 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. Tsai *et al*. (2000) engineered a line of Japanese abalone Haliotis divorsicolor suportexta which express Chinook salmon growth hormone. The animals were reportedly faster growing than their non-transgenic conspecifics. Research into crustacean and molluscan transgenics is a potentially lucrative area, and advances in culture technology coupled with the increasing volume of gene sequence information will undoubtedly prove beneficial. In addition to the academic research in this field, Farming Intelligene, a Taiwanese-based biotechnology company is currently working to develop a transgenic line of Pacific white shrimp Litopenaeus vannamei, although current progress is confidential.

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 "humanness". 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 humanness.

Limitations of transgenices

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.
- Integration of exogenous DNA sequence in Y chromosome resulting in transmission only to males.

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