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***Review Article***

# Transgenic animal production: present scenario-*a review*

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ABSTRACT

The advent of DNA recombinant technology and the possibility of gene transfer between organisms have opened a wide range of possibilities for improvement of livestock production and human health care products. The recent advances in gene transfer, animal cloning, and assisted reproductive techniques have partly fulfilled the expectation in the field of livestock transgenesis. This paper reviews the recent advances and applications of transgenesis in livestock and their derivative products. Typical agricultural applications include improved carcass composition, wool production, and lactation performance which is enhanced disease resistance and reduced environmental impact. Transgenic farm animals have also become important in human medicine as sources of biologically active proteins, as donors in xenotransplantation, for research in cell and gene therapy and for human disease modeling. Until recently, pronuclear microinjection of deoxyribonucleic acid (DNA) was the standard method for producing transgenic animals. This technique is now being replaced by more efficient protocols based on somatic nuclear transfer that also permit targeted genetic modifications. Lentiviral vectors and small interfering ribonucleic acid technology are also becoming important tools for transgenesis.

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## 1. INTRODUCTION

Over the centuries, animals with new combinations of genes have been produced using conventional breeding methods by means of careful selection of particular animals (Moniruzzaman et al., 2014). The numbers of new gene combinations that can be achieved in this manner are, however, limited genes can only be shuffled about between members of the same or very closely related species.

Transgenesis is a completely new technology for altering the characteristics of animals by directly changing the genetic material. Since DNA contains a universal genetic code, it can, in principle, be transferred between completely unrelated organisms to produce organisms with particular, useful characteristics that would not otherwise be available. Many different genes have been characterized and annotated in databases. This knowledge opens up the possibility of looking for methods of changing genetic makeup in useful ways.

The first transgenic livestock were produced almost 20 years ago by microinjection of foreign DNA into the pronuclei of zygotes (Hammer et al., 1985). Direct microinjection has been used to introduce foreign DNA into a number of terminally differentiated cell types as well as embryos of several species including sea urchin, Candida elegans, Xenopus, Drosophila and mice and followed with production of transgenic rabbits, sheep and pigs by microinjection. However, microinjection has several shortcomings such as low

efficiency, random integration and variable expression patterns related to the site of integration. Therefore, research has focused on alternative methodologies for improving the efficiency of generating transgenic livestock. These include sperm-mediated DNA transfer, infection of oocytes or embryos using different viral vectors, intracytoplasmic injection of sperm heads carrying foreign DNA, RNA interference technology using small interfering RNA (siRNA) and the use of nuclear transfer technology. Somatic nuclear transfer has been successful in ten species (Kues et al., 2004) and it holds the greatest promise for significant improvements in generating transgenic animals.

This review aims to provide information regarding the technical aspects of transgenic animal production, the current application in livestock and applications in developing countries well as developed countries. A broad spectrum of genetically modified animals has been generated for applications in agriculture and biomedicine and the use of transgenic livestock for 'gene pharming' has now reached the level of commercial exploitation (Kues et al., 2004). In this article the present scenario of the production of transgenic animals were reviewed.

### *1.1 Transgenic animals in agriculture*

The establishment of stable transgenic animal simples that the foreign DNA is present in gametes or one-cell embryos to allow its transmission to progeny. To reach this goal, the foreign gene can be transferred using different methods according to animal species.

### *1.2 Carcass composition*

The emergence of the first transgenic mice expressing the rat growth hormone fused to the metallothionein promoter sequence, during the 1980's, opened the possibility to use transgenesis as an instrument to increase meat production (Palmiter et al., 1982). Transgenic pigs bearing a human metallothionein-IIA (hMT-IIA) promoter and porcine growth-hormone (pGH) gene construct have been produced with some improvements in economically important traits such as growth rate, feed conversion and body fat/muscle ratio (Nottle et al., 1999).

Similarly, pigs transgenic for the human insulin-like growth factor-I has increased approximately 30% more loin mass and 10% more carcass lean tissue while reducing the total carcass fat by 20% (Pursel et al., 1999). The commercialization of these pigs has not yet been done due to the present lack of public acceptance of genetically modified foods. Recently, an important step towards the production of more healthy pork has been produced by the creation of pigs transgenic for a spinach desaturase gene that produces increased amounts of non-saturated fatty acids. Since a diet rich in non-saturated fatty acids is known to be correlated with a reduced risk of stroke and coronary diseases, people prefer to consume meat from these pigs.

A number of transgenic fish and shellfish are being developed, including salmon, trout, tilapia, flounder and oysters (Hallerman et al., 1990). These animals bear characters such as faster growth, disease resistance and temperature tolerance suitable for aquaculture. The gene engineered into these fish comes from a variety of organisms including other fish, coral, mice and human.

### *1.3 Milk production*

The production of biopharmaceutical proteins in the mammary glands of genetically modified dairy animals ("dairy pharming") is currently under extensive exploration because it promises to provide high-quality therapeutic medicine for humans at an acceptable cost (Wall et al., 1997). Currently, biosynthetic human insulin is manufactured for widespread clinical use employing recombinant DNA technology. The physicochemical properties of milk are mainly due to the ratio of casein variants, making these a key target for the improvement of milk composition (Stinnakre et al., 1994). It is possible to produce milk with a modified lipid composition by modulating the enzymes involved in lipid metabolism or to increase curd and cheese production by enhancing the expression of casein gene family in the mammary gland (Niemann et al., 2005). Recently, genetically modified bovines were generated by introducing additional copies of genes CSN2 and CSN3, which encode bovine α- and β-casein, respectively (Brophy et al., 2003). Over-expression of CSN2 and CSN3 resulted in an up to 20% increase in α-casein, and a 2-fold increase in β-casein levels in milk.

Lactose intoleranceis a major digestive problem in adults and is the inability to metabolize lactose, a sugar found in milk and other dairy products, because of the absence or reduced availability of lactose enzyme in the intestinal system. It has been estimated that 75% of adults show some decrease in lactase activity during adulthood worldwide. Lactose-reduced or lactose-free milk would therefore render dairy products suitable for consumption by humans who do not possess an active intestinal lactase enzyme system.

it is now possible to produce lactose-free milk by a knockdown of the α-lactalbumin locus (Niemann et al., 2005). Moreover, it may be possible to create 'hypoallergenic' milk by knockout or knockdown of the β-lactoglobulin gene; to produce 'infant milk' in which human lactoferrin is abundantly available (Table 1); or to generate milk with a highly improved hygienic standard by increasing the amount of lysozyme (Niemann et al., 2005). In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk at 2.4 grams per liter (Anon 1998). This milk is a more nutritionally balanced product than natural bovine milk and could be given to babies (Anon 1998). It shows the potential feasibility of obtaining significant alterations in milk composition.

**Table 1. Human proteins in the milk of transgenic livestock**

| Pharmaceutical | Bioreactor species | Application/treatment | Company |
|---|---|---|---|
| Antithrombin III | Goat | thrombosis, pulmonary embolism | GTC Biotherapeutics (USA) |
| tPA | Goat | thrombosis | PPL Therapeutics (UK) |
| β-antitrypsin | Sheep | emphysema and cirrhosis | PPL Therapeutics (UK) |
| Factor IX | Sheep | hemophilia b | PPL Therapeutics (UK) |
| Factor VIII | Sheep | hemophilia a | PPL Therapeutics (UK) |
| Polyclonal antibodies | Cattle | vaccines | Hematech (USA) |
| Lactoferrin | Cattle | bactericide | Pharming Group (NED) |
| C1 inhibitor | Rabbit | hereditary angioedema | Pharming Group (NED) |
| Calcitonin | Rabbit | Osteoporosis and hypercalcemia | PPL Therapeutics (UK) |

### *1.4 Environmentally friendly pigs*

To address the problem of manure-based environmental pollution in pork industry, phytase transgenic pigs have been developed. The saliva of these pigs contains the enzyme phytase, which allows the pigs to digest the phosphorus in phytate, the most abundant source of phosphorus in the pig diet. Without this enzyme, phytate phosphorus passes undigested into manure to become the single most important manure pollutant. These pigs carry a bacterial phytase gene under the transcriptional control of a salivary-gland-specific promoter. This provides essentially complete digestion of dietary phytate phosphorus, relieving the requirement for inorganic phosphate supplements while reducing fecal phosphorus output by up to 75% (Golovan et al., 2001).

### *1.5 Wool production*

Transgenic sheep produced by pronuclear microinjection with a mouse ultra-high-sulfur keratin promoter linked to an ovine insulin-like growth factor 1 (IGF1) cDNA showed IGF1 expression in the skin (Damak et al., 1996a; Damak et al. 1996b). At yearling shearing, clean fleece weight was 6.2% greater in transgenic than in non-transgenic animals (Damak et al., 1996a; Damak et al., 1996b). There were no significant differences in fiber diameter, medullation, and hogget body weight (Damak et al., 1996a).

### *1.6 Transgenic animals for disease resistance*

The susceptibility to infection has a polygenic hereditary basis. Therefore only a few cases are known in which a specific gene locus has shown to be involving in disease resistance (Niemann et al., 2005). A fastidious example a specific disease resistant gene is the well-studied systems of Mx 1 gene product found in certain strains of mice which selectively protects against infections of influenza virus (Staeheli 1991).

Gene transfer is of particularconcern for the manipulation of disease resistance since it has been impossible hitherto to reduce the susceptibility to diseases in farm animals by conventional breeding programmes. Muller et al., (1992) discussed five different classes of mammalian genes (Histocompatibility-Complex (MHC) genes, T-cell receptor genes, Immunoglobulin genes, genes encoding lymphokines and specific disease resistance genes) as likely candidates for gene transfer experiments because these genes appear to be associated with the regulation of disease resistance.

Genetic analysis has revealed that the resistance of mice against infection with influenza A and B viruses is caused by a single autosomal dominant $Mxl^+$ allele. The expression of this gene is controlled by type I interferon (IFN α/β). Mice which contain $Mxl^+$ allele are not protected against influenza virus infections. The

transfection of the Mxl+ allele into cells that are susceptible to influenza virus revealed that this gene is necessary and sufficient to protect against influenza (Staeheli 1991).

Genetic immunization appears to be a realistic probability to protect animals against infectious agents by the application of gene transfer techniques. This approach essentially involves the expression of genes directing the synthesis of defined antibodies in order to induce a protective state. An interesting aspect of this approach is that these transgenic animals produce antibodies against specific antigens without even having been challenged with these antigens during their life. Transgenic constructs bearing the immunoglobulin-A (IgA) gene have been successfully introduced into pigs, sheep and mice in an attempt to increase resistance against infections (Lo et al. 1991). The murine IgA gene was expressed in two transgenic pig lines, but only the light chains were detected and the IgA-molecules showed only marginal binding to phosphorylcholine (Lo et al., 1991).

Transmissible gastro-enteritis (TGE) is an acute highly contagious disease of pigs caused by virus. Now it has been proved to induce passive immunity against this disease in a transgenic mouse model (Castilla et al., 1998). The transgenic mice secreted a recombinant antibody in milk that neutralized the corona virus responsible for TGE and conferred resistance against TGE-virus.

***1.7 Augmented disease resistance in mammary gland***

The levels of anti-microbial peptides, lysozyme and lactoferrin, in human milk are many times higher than in bovine milk. Transgenic expression of the human lysozyme gene in mice was associated with a significant reduction of bacteria and reduced the frequency of mammary gland infections (Maga et al., 1995). Lactoferrin has bactericidal and bacteriostatic effects, in addition to being the main iron source in milk. These properties make an increase in lactoferrin levels in bovine transgenics to improve milk quality.
Human lactoferrin has been expressed in the milk of transgenic mice and cattle at high levels (Platenburg et al., 1994) and is associated with an increased resistance against mammary gland diseases (Van Berkel et al., 2002). Lycostaphin has been shown to confer specific resistance against mastitis caused by Staphylococcus aureus. Transgenic technology has been used to produce cows that expresslycostaphin gene construct in the mammary gland, thus making them mastitis-resistant (Wall et al., 2005).

## 2. Biomedical applications of transgenic animals

***2.1 Antibody production in transgenic animals***

Several monoclonal antibodies are being produced in the mammary gland of transgenic goats (Meade et al., 1999). The antibody which is purified from serum is stable and mediates target cell-restricted T-cell stimulation and tumour cell killing. 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. Resulted trans-chromosomal bovine offspring expressed human immunoglobulin in their blood. This system could be a significant step forward in the production of human therapeutic polyclonal antibodies (Kuroiwa et al., 2004).

***2.2 Production of pharmaceuticals and bio-molecules***

Due to the high cost, the production of transgenic animals must bring an elevated profit to be a feasible investment. Consequently, the production of high-value pharmaceuticals, which correspond to a billion-dollar market, is actually the principal and most promising application for animal transgenesis. Despite the lower costs of producing bio-molecules in microorganisms, these organisms do not properly execute several post-translational modifications, such as N-linked glycosylations, authentic O-linked glycosylations, and correct folding in order to produce a wide range of fully active human proteins. Therefore, many human polypeptides must be produced in mammalian cell systems to be recovered with their full activities (Rudolph 1999).

However, due to low productive capacity, the price of human bio-molecules produced *in vitro* by mammalian cell culture is extremely high. Therefore, many biotechnology companies have been focusing on the production of biopharmaceuticals at high concentrations in transgenic livestock bioreactors.

Pharmaceutical products can be produced in a variety of biological fluids such as milk, urine, saliva, blood and seminal fluid (Dyck et al., 2003), and their expression can be driven by tissue-specific promoters. Protein expression into the urine-based system has some advantages, including the expression in both sexes, low contaminant-protein content in urine, and being able to be harvested soon after birth and expressed throughout the life of the transgenic animal. However, this system may be significantly more timeand costconsuming than the mammary gland based system.

### 2.3 Gene Pharming

'Gene pharming' or 'biopharming' entails the production of recombinant biologically active human proteins in the mammary glands of transgenic animals. This technology overcomes the limitations of conventional and recombinant production systems for pharmaceutical proteins (Meade et al., 1999).Now it has advanced to the stage of commercial exploitation. The mammary gland is the preferred production site, because of the quantities of protein that can be produced in this organ, enormous efficiency to produce protein and established methods for extraction and purification of that protein (Meade et al., 1999). Products derived from the mammary gland of transgenic goats and sheep, such as antithrombin III (ATIII), α-antitrypsin or tissue plasminogen activator (tPA), have progressed to advanced clinical trials (Kues et al., 2004). Phase III trials for a recombinant human ATIII product have been completed and an application has been filed for European Market Authorization.The product is employed for the treatment of heparin-resistant patients undergoing cardiopulmonary bypass procedures.

Many different proteins are currently produced by recombinant DNA technology involving a large-scale expression of genes in bacteria, yeast, and in tissue culture example as Insulin and Follicle Stimulation Hormone (FSH) (Stryjewska et al., 2013). It has been suggested to use the mammary glands of transgenic animals for production of heterologous proteins like pharmaceuticals, diagnostics and food components. The expression of foreign proteins in the milk is a system which would allow the recovery of the product in a conventional way (i.e., by milking), without exerting any adverse effects on animals. Proteins that are usually not found in milk can also be expressed in the mammary gland of transgenic mice (Pittius et al., 1988). By using the WAP promoter and the cDNA of human tissue plasminogen activator (tPA) with its cognate secretion signal sequence, they have been able to obtain transgenic mice whose milk contains active tPA.

### 2.4 Transgenic animals as disease models

There are two different approaches to establish disease model development, viz., the directed and specific establishment of disease models and the chance observation made in transgenic mice or their progeny, which may then be used as disease models. Wagner et al., (1983) studied the insertion mutation in transgenic animals for the first time. Following the injection of a hGH gene they obtained 6 transgenic mouse lines of which only 4 could be used to produce homozygous transgenic mice by crossing between transgenic founder animals. The insertion of foreign DNA into the β-1 collagen gene has yielded a lethal mutation in which the fetuses degenerate approximately between 12-15 days of gestation (Jaenisch et al., 1983).

Stacey et al., (1988) introduced a well-known mutation into the pro-a collagen gene and produced transgenic mice using the mutated gene. They observed that the normal function of collagen was already disturbed if the level of expression of the mutated transgene was in the order of 10% of that of the endogenous gene. The intracellular accumulation of the transgenic product induces not only the degradation of the mutated but also of the normal collagens, thus either causing a reduction in the level of collagen or disrupting the correct alignment of collagen and the formation of fibrils. The transfer of a mutated collagen gene into the embryo of normal mice therefore yielded a disease model for the perinatal osteogenesis imperfecta type II.

The expression of a transgene mostly depends on the regulatory sequences which have been used in the design of the gene construct. A classical example is the transgenic giant mice in which the expression of a growth hormone (GH) gene is driven by the metallothionein promoter (Palmiter et al. 1982). Transgenic giant mice are useful models for the study of human growth deviations such as gigantism and acromegaly.

Wirak et al. (1991) devised a transgenic disease model for Alzheimer's disease. They produced transgenic mouse lines which expressed the human amyloid (β-protein under the control of the human amyloid protein promoter in the dendrites of some but not all hippocampus neurons in a year-old mouse. Aggregates of the amyloid J3-protein formed amyloid-like fibrils that were similar in appearance to those in the brains of patients suffering from Alzheimer's disease.

There are many instances in which a suitable model can be established only if the function of an endogenous gene is inactivated effectively by the introduction of a mutation. Smithies et al. (1985) showed that homologous recombination of a foreign gene construct with an endogenous gene locus is indeed possible. They transferred carcinoma cells with

a gene construct consisting of 4.6 kb of the β-globin locus, the neo and supF gene. In approximately 0.1 % of all cases they were able to demonstrate that this construct had integrated into an endogenous β-globin gene by homologous recombination.

The next step in the development of this technique has been the use of homologous recombination for directed mutagenesis in embryonic stem cells of mice. The hypoxanthine phosphoribosyl transferase (HPRT) gene has been chosen as a target gene because it is located on the X-chromosome and a selection procedure for HPRT-negative mutants is available. Another reason for choosing this gene is the fact that HPRT-negative transgenic mice ought to be a valuable model for the Leish-Nyhan syndrome. It has been possible to mutate the endogenous HPRT gene in embryonic stem cells by gene targeting and also to correct any HPRT-negative stem cells by homologous recombination and to produce transgenic mice (Doetschman et al., 1987). The results of these experiments have been very promising and have suggested that it might be possible to use these embryonic stem cells to produce chimeras and mice carrying a mutated gene at a desired gene locus.

The microinjection of DNA construct can also be used to correct the deleterious effects of a deleted endogenous gene. Brinster et al., (1989) injected 5' sequence of a functional MHC IIE gene into mouse cells carrying a 630-bp deletion in the endogenous locus. The injection of over 10,000 oocytes yielded 1800 live mice, 500 of which were transgenic.

An important porcine model has been developed for the rare human eye disease retinitis pigmentosa (PR) (Petters et al., 1997). Patients with PR suffer from night blindness early in life, a condition attributed to a loss of photoreceptors. Transgenic pigs that express a mutated rhodopsin gene show great similarity to the human phenotype and effective treatments are being developed (Mahmoud et al., 2003).

### *2.5 Xenotransplantation*

The possibility of xenotransplants as an alternative source of organs has been investigated for decades. In 1963, a kidney transplant from a chimpanzee to a human was the first attempt of a xenotransplant (reviewed in Auchincloss et al., 1998). Nowadays, studies have shown that the pig is the animal considered the best choice as an organ donor for humans. This is based on: (1) their organs being similar in size, anatomy and physiology to human organs; (2) pigs growing rapidly and being a prolific species; (3) the maintenance of high hygienic standards possible at a relatively low cost; (4) established transgenic techniques to modify immunogenicity of porcine cells and organs. The main immunologic obstacles to xenotransplants include the hyperacute rejection response (HAR), which occurs within seconds or minutes, the acute vascular rejection (AVR), which occurs within days, and the cellular and potentially chronic rejection, which occurs within weeks after the transplant (Auchincloss et al., 1998).

Several transgenic approaches have been developed to overcome the immunological response that activates the complement cascade, which is activated by the antigen-antibody complex and is responsible for the induction of HAR and AVR. The production of a transgenic pig expressing human proteins that inhibit the complement cascade such as hCD59 (Fodor et al., 1994) and hCD46 (Diamond et al., 2001) are examples to overcome this problem.

## 3. Transgenic technologies

### *3.1 DNA 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 (Gordon et al., 1981). 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 into the genome of the host is, however, a random process. There is therefore 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. A major advantage of this method is its applicability to a wide variety of species.

### *3.2 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 (Rossant 2001). These cells are then incorporated into

an embryo at the blastocyst stage of development. The result is a chimeric animal (Rossant 2001). ES cell-mediated gene transfer is the method of choice for gene inactivation, the so-called knock-out method.

### *3.3 Retrovirus-mediated gene transfer*

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. Offspring derived from this method 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.

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 of animals that need to be tested for the expression of the transgene. Depending on the technique used, the $F_1$ 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.

Recent research has shown that lentiviruses can overcome previous limitations of viral-mediated gene transfer, which included the silencing of the transgenic locus and low expression levels (Wiznerowicz et al., 2005). Injection of lentiviruses into the perivitelline space of porcine zygotes resulted in a very high proportion of piglets that carried and expressed the transgene. Stable transgenic lines have been established by this method (Hofmann et al., 2003). Lentiviral gene transfer in livestock promises unprecedented efficiency of transgenic animal production.

### *3.4 Ribonucleic acid interference*

Ribonucleic acid interference is a conserved posttranscriptional gene regulatory process in most biological systems. Common mechanistic elements include small interfering RNAs (siRNAs) with 21 to 23 nucleotides, which specifically bind complementary sequences on their target mRNAs and shut down expression. The target mRNAs are degraded by exonucleases and no protein is translated (Plasterk 2002). The RNA interference seems to be involved in gene regulation by controlling/suppressing the translation of mRNAs from endogenous viral elements.

## 4. DICUSSION

Since the domestication of first animals, humans have been introducing genetic modifications by assisted breeding in a variety of species. Currently, the major difference observed between genetically modified species and their wild relatives is due to the profound alterations that were introduced during the domestication process. Those modifications were most often performed based on the observation of the phenotype without any knowledge on inheritance mechanisms behind selection. Nowadays with the advances of science people have a better understanding of inheritance mechanisms and make punctual and specific alterations in DNA sequences to meet the requirements.

Regarding individual characteristics of livestock, although we have to consider the low response to artificial selection compared to changes obtained with transgenesis, a continuous application of the former for many generations can introduce huge genetic modifications. Therefore, when an animal is out of a genetic breeding program, in order to insert any genetic modification, the result is an intrinsic loss of the productive ability conferred to their assisted selected counterparts. The time estimated to generate transgenic livestock, e.g. cattle, is about 5-7 years. Concerning this, the economic profit introduced by the new characteristic should significantly compensate the loss of productivity provoked by the exclusion from the artificial selection program (Clark et al., 2003). Moreover, the high cost involved in the transgenic process allows us to obtain only few animals for each genetic modification event. For this reason, a laborious process of reintroduction of those animals in the herd by backcrossing is required. In order to make this process economically feasible, it is essential to have in-depth knowledge about the recent advances in assisted reproduction techniques, such as artificial insemination associated with embryo transfer, or *in vitroembryo* production. Nonetheless, some degree of inbreeding is inevitable, which leads to a loss of genetic variability.

Another consequence of the introduction of new characteristics to a livestock product is the public perception related to the emergence of new products on the market. The pharmaceutical industry has a history of obtaining products through a variety of microorganisms, plants, animals and human tissue sources. Therefore, the isolation of biopharmaceutical products from milk derived from genetically modified animals does not require significant changes in the

production system or market perception by the consumers. However, the prospect of livestock animals with new properties and characteristics would have an enormous impact on the structure of agricultural industry. For example, the goal for dairy industry has been high milk production to meet the consumers' demand. Nevertheless, transgenesis can offer the possibility for dairy producers to have milk with higher casein levels, or milk with specific characteristics such as lactose-free milk, milk without lactoglobulin destined for allergic consumers or milk with the human lactoferrin protein, to ensure newborns' health. As a result new niches would be created and thus, the market will be segmented, revolutionizing the entire milk production chain. The benefits of this segmentation can be confirmed by the profitable market of crops in the agriculture sector. But whether the livestock industry is prepared for such changes is questionable. This can become possible when the technical and scientific progress reduces the costs of transgenic livestock production and makes even more accessible for the production sector.

Discussions on ethics arising with the first GMOs demonstrated the continuity of the human activity in altering the environment, as well as organisms, for our own benefit. Notwithstanding, it does not exempt us from the responsibility for our actions and a recent research field, named biosafety, has been created to manage the ecological impact of GMOs. Now a variety of technologies are available and they can offer insights into the entire transcriptome of a transgenic organism and thereby ensure the absence of unwanted side-effects.

In developed countries, phenotypes and pedigrees have been recorded for certain species, such as dairy cattle, for more than 100 years. Progeny testing has been implemented for nearly 50 years. Developing countries are often limited by the absence of programs that record phenotypes on pedigreed animals and the lack of evaluation or national testing programs to assess the genetic value of germplasms. Genomic approaches should help in identifying critical populations for preservation together with some local well-adapted breeds that could be further utilized to breed valuable animals through a combination of selection and cross-breeding. Of course, as with genomics, you can manage only what you can measure, and collecting a minimum number of phenotypes in the field will remain one of the critical and challenging steps to further deployment of genomic selection in developing countries.

## 5. REFERENCE


Anon, E. 1998. E.T.S. (Emerging Technology Series). "Genetic Engineering and Biotechnology." Part E. Applications. Number 1 & 2 McGraw publications, Cambridge, London: p. 52-53.

Auchincloss, H. Jr. and Sachs D.H. 1998. Xenogenic transplantation. Annual Review of Immunology 16: 433-470.

Brinster, R.L., Braun, R.E., Lo, D., Avarbok, M.R., Oram, F. and Palmiter, R.D. 1989. Targeted correction of a major histocompatibility class IIE gene by DNA microinjected into mouse eggs. Proceedings of the National Academy of Sciences, 86:7087-7095.

Brophy B., Smolenski, G., Wheeler, T., Wells, D., L'Huillier, P. and Laible, G. 2003. Cloned transgenic cattle produce milk with higher levels of β-casein and α-casein. Nature Biotechnology, 21 (2): 157-162.

Castilla, J., Pintado, B., Sola, I., Sánchez-Morgado, J.M. Enjuanes, L. 1998. Engineering passive immunity in transgenic mice secreting virus-neutralizing antibodies in milk. Nature Biotechnology, 16 (4): 349-354.

Clark, J. and Whitelaw, B. 2003. A future for transgenic livestock. Nature Revolution Genetetics, 4 (10): 825-833.

Damak S., Su, H., Jay, N.P. and Bullock, D.W. 1996a. Improved wool production in transgenic sheep expressing insulin-like growth factor 1. Biotechnology, 14 (2): 185-188.

Damak S., Su, H., Jay, N.P. and Bullock, D.W. 1996b.Targeting gene expression to the wool follicle in transgenic sheep. Biotechnology, 14 (2): 181-184.

Diamond, L.E., Quinn, C.M., Martin, M.J., Lawson, J., Platt, J.L. and Logan, J.S. 2001. A human CD46 transgenic pig model system for the study of discordant xenotransplantation. Transplantation, 71: 132-142.

Doetschman, T., Gregg, R.G., Maeda, N., Hooper, M.L., Melton, D.W., Thompson, S. and Smithies, O. 1987. Targeted correction of a mutant Hprt gene in mouse embryonic stem cells. Nature, 330: 576-578.

Dyck, M.K., Lacroix, D., Pothier, F. and Sirard, M.A. 2003. Making recombinant proteins in animals: different systems, different applications. Trends Biotechnology, 21 (9): 394-399.

Fodor W.L., Williams, B.L., Matis, L.A., Madri, J.A., Rollins, S.A., Knight, J.W., Velander, W., and Squinto, S.P. 1994. Expression of a functional human complement inhibitor in a transgenic pig as a model for the prevention of xenogeneic

hyperacute organ rejection. Proceedings of the National Academy of Sciences USA, 9: 11153-11157.

Golovan S.P., Meidinger, R.G., Ajakaiye, A., Cottrill, M., Wiederkehr, M.Z., Barney, D.J., Plante, C., Pollard, J.W., Fan, M.Z., Hayes, M.A., Laursen, J., Hjorth, J.P., Hacker, R.R., Phillips, J.P. Forsberg, C.W. 2001. Pigs expressing salivary phytase produce low-phosphorus manure. Nature Biotechnology 19 (8): 741-745.

Gordon, J.W. and Ruddle, F.H. 1981. Integration and stable germ line transformation of genes injected into mouse pronuclei. Science, 214: 1244-1246.

Hallerman, E.M. and Kapuscinski, A.R. 1990. Transgenic Fish and Public Policy: Regulatory Concerns. Fisheries, 15(1): 12-20.

Hammer, R.E., Pursel, V.G., Rexroad, C.E. Jr, Wall, R.J., Bolt, D.J., Ebert, K.M., Palmiter, R.D. and Brinster R.L. 1985. Production of transgenic rabbits, sheep and pigs by microinjection. Nature, 315(6021): 680-683.

Hofmann, A., Kessler, B., Ewerling, S., Weppert, M., Vogg, B., Ludwig, H., Stojkovic, M., Boelhauve, M., Brem, G., Wolf, G., and Pfeifer, A. 2003. Efficient transgenesis in farm animals by lentiviral vectors. EMBO Rep., 4 (11): 1054-1060.

Jaenisch, R., Harbers, K., Schnieke, A., Lohler, J., Chumakor, I., Jahner, D., Grotkopp, D. and Hoffmann, E. 1983. Germline integration of moloney murine leukemia virus at the Movl3 locus leads to recessive lethal mutation and early embryonic death. Cell, 32: 209-216.

Kues, W.A. and Niemann, H. 2004. The contribution of farm animals to human health. Trends Biotechnol., 22 (6): 286-294.

Kuroiwa, Y., Kasinathan, P., Matsushita, H., Sathiyaselan, J., Sullivan, E.J., Kakitani, M., Tomizuka, K., Ishida, I. and Robl, J.M. 2004. Sequential targeting of the genes encoding immunoglobulin- mu and prion protein in cattle. Nature Genetics, 36: 775-780.

Lo, D., Pursel, V., Linton, P.J., Sandgren, E., Behringer, R., Rexroad, C., Palmiter, R.D. and Brinster, R.L. 1991. Expression of mouse IgA by transgenic mice, pigs and sheep. European Journal of Immunology, 21 (4): 1001-1006.

Maga, E.A., Anderson, G.B. and Murray, J.D. 1995. The effects of mammary gland expression of human lysozyme on the properties of milk from transgenic mice. Journal of Dairy Science, 78 (12): 2645-2652.

Mahmoud T.H., McCuen, B.W., Hao, Y., Moon, S.J., Tatebayashi, M., Stinnett, S., Petters, R.M. and Wong, F. 2003. Lensectomy and vitrectomy decrease the rate of photoreceptor loss in rhodopsin P347L transgenic pigs. Graefe's Archive for Clinical and Experimental Ophthalmology, 241 (4): 298-308.

Meade, H.M., Echelard, Y., Ziomek, C.A., Young, M.W. , Harvey, M., Cole, E.S., Groet, S. and Curling, J.M. 1999. Expression of recombinant proteins in the milk of transgenic animals. In: J.M. Fernandez and J.P. Hoeffler (eds). Gene expression systems: using nature for the art of expression. Academic Press, San Diego, pp 399-427.

Moniruzzaman, M., Khatun, R. and Mintoo, A.A. 2014. Application of marker assisted selection for livestock improvement in Bangladesh. The Bangladesh Veterinarian, 31(1): 1 - 11.

Muller, M., Brenig, B., Winnacker, E.L. and Brem, G. 1992. Transgenic pigs carrying cDNA copies encoding the murine Mx1 protein which confers resistance to influenza virus infection. Gene, 121 (2): 263-270.

Niemann,H., Kues, W. and Carnwath, J.W. 2005. Transgenic farm animals: present and future. Revue scientifiqueet technique (International Office of Epizootics), 24(1): 285-298.

Nottle, M.B., Nagashima, H., Verma, P.J., Du, Z.T., Grupen, C.G., McIlfatrick, S.M., Ashman,R.J., Harding, M.P., Giannakis, C., Wigley, P.L., Lyons, I.G., Harrison, D.T., Luxford, B.G., Campbell, R.G., Crawford, R.J. and Robins, A.J. 1999. Production and analysis of transgenic pigs containing a metallothionein porcine growth hormone gene construct. In Transgenic animals in agriculture. Edited by J.D. Murray, G.B. Anderson, A.M. Oberbauer and M.M. McGloughlin. Commonwealth Agriculture Bureau International. London. pp. 145-156.

Palmiter, R.D., Brinster, R.L., Hammer, R.E., Trumbauer, M.E., Rosenfeld, M.G., Birnberg, N.C. and Evans, R.M. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. Nature, 300: 611-615.

Petters, R.M., Alexander, C.A., Wells, K.D., Collins, E.B., Sommer, J.R., Blanton, M.R., Rojas, G., Hao, Y., Flowers, W.L., Banin, E., Cideciyan, A.V., Jacobson, S.G. and Wong, F. 1997. Genetically engineered large animal model for studying cone photoreceptor survival and degeneration in retinitis pigmentosa. Nature Biotechnology, 15: 965-970.

Pittius, C.W., Hennighausen, L., Lee, E., Westphal, H. , Nicols, E., Vitale, J. and Gordon, K. 1988. A milk protein gene promoter directs the expression of human tissue plasminogen activator cDNA to the mammary gland in transgenic mice. Proceedings of the National Academy of Sciences 85(16): 5874-5878.

Plasterk, R.H. 2002. RNA silencing: the genome's immune system. Science, 296 (5571): 1263-1265.

Platenburg, G.J., Kootwijk, E.A.P., Kooiman, P.M., Woloshuk, S.L., Nuijens, J.H., Krimpenfort, P.J.A., Pieper, F.R., de Boer, H.A. and Strijker, R. 1994. Expression of human lactoferrin in milk of transgenic mice. Transgenic Resesarch, 3 (2): 99-108.

Pursel, V.G., Wall, R.J., Mitchell, A.D., Elsasser, T.H., Solomon, M.B., Coleman, M.E. Mayo, F. and Schwartz, R.J. 199). Expression of insulin-like growth factor-I in skeletal muscle of transgenic pigs. In: J.D. Murray, G.B. Anderson, A.M. Oberbauer and M.M. McGloughlin (eds). Transgenic animals in agriculture. CABI Publishing, New York, pp 131-144.

Rossant, J. 2001. Stem cells from the mammalian blastocyst. Stem Cells, 17(9): 367-374.

Rudolph, N.S. 1999. Biopharmaceutical production in transgenic livestock. Trends Biotechnology, 17 (9): 367-374.

Smithies, O., Gregg, R.G., Boggs, S.S., Koralewski, M.A. and Kucherlapati, R.S. 1985. Insertion of DNA sequences into the human chromosomal beta-globin locus by homologous recombination. Nature, 317(6034): 230-234.

Stacey, A., Bateman, J., Choi, T., Mascara, T., Cole, W. and Jaenisch, R. 1988. Perinatal lethal osteogenesisimperfecta in transgenic mice bearing an engineered mutant pro-α (1) collagen gene. Nature, 332: 131-136.

Staeheli, P. 1991. Intracellular immunization: a new strategy for producing disease-resistant transgenic livestock. Trends Biotechnology, 9 (3): 71-72.

Stinnakre, M.G., Vilotte, J.L., Soulier, S. and Mercier, J.C. 1994. Creation and phenotypic analysis of ?-lactalbumindeficient mice. Proceedings of the National Academy of Sciences, USA, 91 (14): 6544-6548.

Stryjewska, A., Kiepura, K., Librowski, T. and Lochyński, S. 2013. Biotechnology and genetic engineering in the new drug development. Part I. DNA technology and recombinant proteins. Pharmacological Reports, 65: 1075-1085.

Van Berkel, P.H., Welling, M.M., Geerts, M., van Veen, H.A., Ravensbergen, B., Salaheddine, M., Pauwels, E.K., Pieper, F., Nuijens, J.H. and Nibbering, P.H. 2002. Large scale production of recombinant human lactoferrin in the mik of trangenic cows. Nature Biotechnology, 20 (5): 484-487.

Wagner, E.F., Covarrubias, L., Stewart, T.A. and Mintz, B. 1983. Prenatal lethalities in mice homozygous for human growth hormone gene sequences integrated in the germ line. Cell, 35: 647-655.

Wall, R., Kerr, D. and Bondioli, K. 1997. Transgenic dairy cattle: genetic engineering on a large scale. Journal of Dairy Science, 80: 2213-2224.

Wall, R.J., Powell, A., Paape, M.J., Kerr, D.E., Bannermann, D.D., Pursel, V.G., Wells, K.D. Talbot, N. and Hawk, H. 2005. Genetically enhanced cows resist intra-mammary Staphylococcus aureus infection. Nature Biotechnology 23 (4): 445-451.

Wirak, D.O., Bayney, R., Ramambhadran, T.V., Fracasso, R.P., Hart, J.T. Hauer, P.E., Hsiau, P., Pekar, S.K., Scangos, G.A., Trapp, B.D. and Unterbeck, A.J. 1991. Deposits of amyloid ß protein in the central nervous system of transgenic mice. Science, 253: 323-325.

Wiznerowicz, M. and Trono, D. 2005. Harnessing HIV for therapy, basic research and biotechnology. Trends Biotechnology, 23 (1): 42-47.