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Application of transgenic animals in animal production and health

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ABSTRACT

The progress in Recombinant DNA technology guided the way for production of transgenic plants and animals. The production of Bt cotton and Bt brinjal was one of them. Transgenic cows capable of producing human proinsulin and human lactoferrin in milk have been successfully engineered. Though the efficacy of the techniques used or such transgenic organism is questionable, with the creation of new and better technology like the clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein system, the chances and effectiveness for success of gene incorporation during development of a transgenic animal has increased. In near future such transgenics are going to play an important role in support and sustenance of human society. This review deals with the application of transgenesis for enhancing productivity and promoting better health in animals and humans alike.

Key words: Animal model, Animal production, Health, Transgenesis.

A transgenic animal is one whose genome has been changed to carry genes from other species (Margawati, 2003).Recombinant DNA technology involves combining genetic information from different sources thereby creating genetically modified organisms that may have never existed in nature before. Recombinant DNA technology or genetic engineering was first used to clone DNA segments of interest in bacterial hosts in order to produce enough sufficiently pure materials for further studies. Further research led to creation of recombinant DNA molecules that have also been used to create genetically modified organisms such as "knock-out" and "knock in" transgenic animals and plants (Robert, 2016; Songstad *et al*., 2017).

It may seem that bio-techniques for creation of domestic animal are of recent origin but actually it has always been practiced in various formsever-since the domestication of animals began in human civilization. Several of the previously used techniques of animal breeding, genetics and even nutrition have contributed to an important role in the process of selection, management and propagation of most desirable and economically important traits in livestock. The most commonly produced transgenic animals so far have been mice. Also, the first successful transgenic animal was a mouse (Maksimenko, 2013). Later on, research was extended to rabbits, pigs, sheep, and also cattle. The technology for generating transgenic animals exists for a number of vertebrate and invertebrate species (Mehta *et al.*, 2017).

Reasons for production of transgenic animal: Transgenic animals are most commonly used as producers of substances for human welfare and also as disease models.Usually transgenic animals are produced for specific economic characteristics. A common example is creation of transgenic cattle to produce milk containing human proteins of high importance, which is required for the treatment of specific diseases such as human emphysema.Transgenic animals are also produced as disease models (genetically manipulated animals which exhibit disease symptoms so that effective treatment and cure can be studied). Harvard scientists were successful in an important scientific breakthrough when they obtained a United States patent (the company DuPont) and held exclusive rights to use it for a genetically engineered mouse, named asOncoMouse® or the Harvard mouse. This mouse carried a gene that was able to promote development of several human cancers which made it suitable for research studies related to cancers (Margawati, 2003). Some of the most common contributions of transgenesis including the future prospects towards animal production and health can be grouped into the following sub-headings.

Transgenesis allow build up of larger herds with specific characteristics: Since time immemorial farmers have used selective breeding to develop animals that carry the desired traits (e.g., increased egg or milk production, higherbody weight gain). But, traditional breeding methods are time-consuming and a difficult task whose efficiency depends on the accuracy of selection and mating plans. The new technology with the use of molecular biology has been developed and it is quite possible to use it for progress of beneficial traits in animals in a shorter duration and with high precision. One common problem faced by the breeders in swine production was the high mortality rate of piglets which was attributed to the cause of lack of α-lactalbumin in milk of sows. So to control this menace transgenic pig

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containing several copies of this gene was evolved which were able to produce high lactose content in the milk of sows (Wheeler *et al*., 2001). Thus, it resulted in decrease in mortality rate in piglets.

Transgenic animals producing milk-proteins are highly useful for medicinal therapies: Extraction of milk proteins as nutritional supplements and pharmaceutical drugs has the potential to benefit human nutrition and provide proteins for therapeutic use. Products such as insulin, growth hormone, and blood anti-clotting factors which were produced artificially as recombinant proteins in either prokaryotic or eukaryotic unicellular organisms may soon be available in the milk of transgenic cows, goats or sheep. The concept of Human gene therapy is based on addition of a normal copy of the gene (transgene) to the genome of a person or animal which has defective copies of the gene in its genetic material. It has the potential for treatments of various genetic diseases. As already discussed, transgenic animals also play a role in the treatment of these diseases because they can produce substances which can supplement and overcome the deficiency of proteins not produced by the defective genes.

Efforts are being made to manufacture milk through transgenic technology for treatment of debilitating and chronic diseases such as hereditary emphysema, phenylketonuria (PKU) and cystic fibrosis. To increase the content of β- and k-caseins in milk several additional copies of the genes were introduced into the genome of the cow (Brophy *et al*., 2003).

In the year 1997, the first transgenic cow named Rosie, produced human protein-enriched milk which was present at the concentration of 2.4 grams per litre. Such transgenic product is more nutritionally balanced than natural bovine milk and can be given to children or the elderly who have special nutritional or digestive requirements. Rosie's milk contained the human gene alpha-lactalbumin (Margawati, 2003).

Cows developed with the help of transgenic technology is able to express recombinant lactoferrin at the rate of 3.4 mg/ml (Yang *et al*., 2008) increase in lysozyme content upto 0.03 mg/ml (Whyte *et al*., 2011) or even production of human lactalbumin which was expressed at the rate of 1.5 mg/ml of modified milk (Whitelaw *et al*., 1999) have been produced in China. Such optimum condition of proteins concentration as milk constituent will serve to benefit human population. Even goats containing foreign genes have been produced whose milk contains human lysozyme protein at a concentration of 0.27 mg/ml (Kabotyanski *et al*., 2009). This concentration corresponded with 67% lysozyme concentration of human breast milk. It has been also demonstrated that pasteurized milk containing human lysozyme has a beneficial effect on the health of young pigs (Brundige *et al*., 2008).

Better animal welfare through improvement of disease resistance: Animal welfare has gained a high priority for farmers involved in the rearing of livestock. Scientists and researchers all over the world are making attempts to produce disease-resistant animals, such as pigs resistant to influenza, but a very few number of genes are currently known which may render the animals resistant to disastrous diseases. Modification of Genes of livestock enhances animal welfare by producing disease free animals. The application of transgenic techniques provides opportunities to engineer genetic material of livestock with disease resistance or tolerance. One of the very first important applications of transgenic technology was to deal with diseases such as mastitis, an inflammation of the mammary gland, caused by infectious pathogens and most commonly by bacteria. Mastitis may cause decreased milk production, mammary tissue damage, fever and even death of animals showing acute signs. Transgenic dairy cows secreting lysostaphin into their milk have demonstrated better resistance to mastitis. It has happened due to the protection provided by lysostaphin, which can kills bacteria such as Staphylococcus aureus (Donovan *et al*., 2006). Lysostaphin is a strong antimicrobial peptide that protects the mammary gland against one of the most important mastitis-causing pathogen.

Recent researches have led to the production of prion-free livestock (Richt *et al.,* 2007) and livestock in which the prion remainssuppressed (Golding *et al.,* 2006). They are the causative pathogens responsible for causing bovine spongiform encephalopathy or 'mad cow disease' in cattle andCreutzfeldt-Jacob disease in humans.

Improvement of reproductive performance and fecundity: Several potential genes have recently been identified that may profoundly affect reproductive performance and prolificacy. Introduction of a mutated or engineered estrogen receptor (ESR) gene could increase litter size in a number of diverse breeds of pigs. A major single autosomal gene for fecundity was identified, the Boroola fecundity (FecB) gene, which also has effect on increase in ovulation rate, was first of all identified in Merino sheep (Piper *et al.,* 1985). One single copy of this gene is known to increase the ovulation rate by about 1.5 ova (Piper *et al.,* 1985). Thus, development of transgenic sheep which contain the suitable FecB gene would increase fecundity in different breeds which have important traits like mutton production. Concept of such manipulation related to productive and reproductive characteristics by the use of transgenic methodologies is only the initiation and exploration of a very rich area for research and investigation in the near future.

Enhancing growth rates and carcass composition: Transgenic livestock production has been instrumental and has provided several gates into the new insights and the mechanisms of gene action responsible for the control of growth, (Pursel *et al.,* 1998). It has become possible to

manipulate growth factors and their receptors along with growth modulators through the use of transgenic techniques. Transgenic cows have been produced with more milk producing capability or milk with less lactose or low levels of cholesterol, pigs and cattle that have more meat on them by regulating down or knocking out the myostatin gene (Gilles *et al*., 2007), and sheep that produces more wool. In past, farmers used physiologically harmful growth hormones to enhance the development of animals but the residue of the hormones remained in the animal product.

In fish also, there is a need for better, efficient and quick production, without harming the wild stocks, so as to provide a high quality animal protein source for the ever increasing population. The engineering of GH transgenic fish has led to an outstanding increase (30-40%) in growth rates recombinant catfish by the introduction of GH gene from salmon into these animals (Dunham & Devlin 1999). Salmon GH constructs introduction into catfishes has caused a 5-11 fold increase in weight after one year of growth (Dunham & Devlin 1999). This suggests that increased growth rate and ultimately increased protein production per individual animal can be attained through transgenic technology.

Another view of handlingthe composition of the carcass is bychanging the fat or cholesterol content of the carcass. By amending the metabolism or by influencing the uptake of cholesterol or fatty acids, the fat content and the amount of cholesterol in meats, eggs, and cheeses could be reduced. Along with it, the possibility of introducing beneficial or healthy fats such as omega-3 fatty acids from fish or other animal sources into our livestock (Lai *et al.,* 2006). Also, receptors like the low-density lipoprotein (LDL) receptor gene and important hormones like leptin are among the potential targets that would play an essential role in decreasing fat and cholesterol in products of animals.

Improvement of hair and fibre through genetic engineering: Regulation of the colour, quality, yield as well as the ease with which the hair is harvested, wool and fibre for making fabric and production of yarn and some other area of target for transgenic control of livestock. The crimp, length, fineness, strength and quality of the wool or hair fibre obtained from sheep and also from goats have been showed using genetic engineering methods (Powell *et al.,* 1994). In future, transgenic animals direct wool and focus on the surface of fibres. Decreasing the surface interaction could decrease shrinkage of garments made from such fibres.

A new concept of working on spider silk, a utilitarian fibre, was produced in the milk of transgenic goats (Karatzas *et al.,* 1999). Orb-webs producing spiders synthesize seven different types of spider threads for weaving webs. One of the strongest among them is the dragline silk. It can be stretched up to 35% and has a high tensile strength. Its ability to absorb energy is more than those of steel. Such a strong fibre has a wide range of applications in ballistic protection, tire cord, medical devices, sutures, air bags, aircraft, clothing and automotive composites.

Improving quality of food or synthesis of novel food: Improvement in the amount and quality of the meat or milk from domestic animals will help in providing nutritional supplement to the increasing population and ensuring the economic returns to the farmers. If milk is produced with extra casein the processing will require less time and labour to make cheese and will have an increased level of calcium (Brophy *et al.*, 2003). Milk components can be altered to make different functional foods (Visioli *et al.,* 2014). One such example is the production of minor proteins in milk as has been done in the case of incorporation of lactoferrin as one of the components of milk gives protection against infection of the digestive tract and helps in improving the health of gut (Hyvönen *et al*., 2006).

Increasing sustainability of environment: Agricultural production and waste also has an adverse impact on our environment. Transgenic animals offer an alternate way of decreasing the impact of the environment by improving the efficiency of farming and reducing pollution. One of the examples is the Enviropig™. Such transgenic pigs have an additional gene that helps them easily digest plant feed (Forsberg *et al*., 2013). This characteristic reduces their need for dietary supplements and decreases environmental pollution.

Production of transgenic birds: Microinjection or retroviral mediated entry of DNA into the germinal disc of the fertilized eggs of birds produce genetically engineered strains. This is not possible at the time of fertilization because many sperms instead of one actually penetrate ovum. This condition makes it impossible to identify the sperm whose pro-nucleus will fuse with the ovum pro-nucleus. The procedure to make transgenic birds involves removal of blastoderm cells that are removed from donor chicken and are again reintroduced into the sub-germinal space of embryos present in freshly laid eggs. Some progenies in the form of chimaeras will contain cells having the gene of interest. Transgenic lines are then established from these chimeras by several rounds of mating and selection of only those birds which contain gene of interest in their germ cells (Koo, 2017). Transgenic chickens can also be used to build up and improve the genetic makeup of existing varieties in order to increase resistance to viral, bacterial and coccidial diseases, to increase feed efficiency, to reduce cholesterol and fat amounts in eggs and to increase the quality of meat.

Transplant organs may soon come from transgenic animals: Several patients die each year because of lack of replacement of heart, kidney or liver. On an approximate about 5,000 organs are needed every year for replacement surgeries in the United Kingdom alone. Pigs have been found to suitable animals which can be used in transgenic

technologies to generate human organs. However, xeno-transplantation is hampered by an immune protein in pigs that cause donor rejection but efforts are underway to block this pig protein and to replace it with a human protein so that such a hurdle can be overcome. Also, acute humoral xeno-graft rejection (AHXR) in experimental non-human primates which received xeno-grafts from pig donors has also found to be common (Cozzi *et al.*, 2005). Rejection in non-human primates has been broadly classified into AHXR and HAR (Hyper acute rejection). HAR is response is highly quick and may result in rejection of the organ within few minutes also (Pino-Chavez, 2001). However, there have also been a number of successful cases where the primates survived for several months after xeno-transplantation. Research has been undergoing on this aspect to produce techniques which can support a long time survival of an individual receiving a transplant.

Using animals as disease models: Transgenic laboratory animals are made by knocking out or knocking down of a gene or by inserting a gene of interest in animal models so that it can be studied as a representative of human disease to help researchers find new treatments. Generally, small transgenic laboratory animals like mice or rats are used for such research. A human gene of interest can also be inserted into the fertilized oocyte of a laboratory animal which is then implanted into the uterus of a pseudo-pregnant female (Kandhare *et al.*, 2011). Human Immunodeficiency Virus (HIV) or Acquired Immunodeficiency Syndrome (AIDS) disease model was one of the first mouse model to be developed, which led to the development of several other similar models on AIDS in which transgenic animals were able to synthesize HIV-I protein and show symptoms of the disease (Joshi and Guidot, 2011). With the advent of time several other animal models on problems like the Alzheimer disease (Spires and Bradley, 2005), Cardiovascular disease (Snaith and Tornell, 2002), Diabetes Mellitus (Kumar *et al.*, 2012), angiogenesis (Carmeliet *et al.*, 1998) and cancer studies (Boverhof *et al.*, 2011).

Ethical issues related with transgenesis: Creation of several novel transgenic lines of animals usually requires sacrifice or unusual happening of animals and also involves some surgical procedures (like surgical embryo transfer and vasectomy). Such procedures are not exclusive to production of transgenic animals, but are specifically required for their production. Several embryos produced by transgenic procedures usually do not survive and those that do survive contribute to only a very small proportion carrying the actual genetic change of interest (19). Also, the advancement of transgenic techniques which occurred during the past few years has lead to a quick influx in the number and varieties of transgenic animals, especially in lab animals (Ormandy *et al.*, 2009). Although with the passage of time the technology is continuously being sophisticated, current transgenic technology is not very efficient and results in the production of surplus animals that have been exposed to harmful procedures.

Very few studies are present which have focused on the welfare impacts of animals undergoing transgenic procedure. Most of the transgenic techniques have been described as unpredictable as well as inefficient. The presence of inefficiency is particularly related to the limitations in exactly manipulating the site of integration of foreign DNA. This is the reason for generation of several lines of genetically engineered organisms that differ only in the integration site of the foreign DNA (Verbeek, 1997). So, a large stock of transgenic animals is generated but only few carry the integrated gene at the correct site in the germ cell of the animals.

Another significant setback of current technology is that the newly produced transgenic animal may suffer from some amount of genetic abnormality (Weaver and Morris, 2005). Many animal welfare and ethics committee like the Canadian Council on Animal Care describes and recommends an accepted ethic guidelines for use of animals in science and which also includes the principles of the Three Rs (i.e., Reduction in the number of animals, Refinement of mangemental and husbandry practices in order to reduce pain and distress, and if possible Replacement of animals with non-animal alternatives) (Fenwick *et al.*, 2009). However, with further progress in the techniques involved in production of transgenic animals, novel and efficient methods (Miller *et al.*, 2007; Yang *et al.*, 2009) may considerably reduce the unpredictability of the site of gene insertion as well as increase the efficacy of gene insertion. This may result in transgenic technique to become less of a welfare concern with the passage of time.

CONCLUSION

In the near future, livestock production will depend more heavily on current and emerging genetic engineering and transgenesis advances to produce human food. However, there are always more needs for formation of new products and increasing the efficiency of production, especially for growth, reproduction and disease resistance. Genetically altered livestock, stem cells uses and many emerging biotechnological tools will play crucial roles in producing better and higher quality food obtained from livestock. The production of 'transgenic animals' with special characteristic is one such biotechnology tool.

The creation of transgenic animals has resulted in a shift in the use of laboratory animals. Instead of using the higher order species such as dogs research studies have shifted to lower-order species such as mice and rat. This has reduced the number of animals that are being used in such experiments, especially when such animals are used as disease models. Overall transgenic technology has very good potential to deal with already existing problems and creation of proteins with such technique can be also commercialised and may serve as an alternate benefactor to human society.

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