

Custom Made Animals

The Magic of Transgenesis

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Abstract :

Transgenesis is the introduction of a foreign gene into an animal or plant cells such that it is stably maintained in a heritable manner. Animals on which transgenesis has been performed are called transgenic animals. These technologies don't produce new species but work within the established genetic framework of existing species to improve them. Mice were the first animals to be produced transgenically and still remain the favourites for such experiments. During recent years, however, transgenic techniques have been extended to other species including the rabbit, the cow, the pig, the goat, and the sheep. This article attempts to provide an overview of the methods involved in the production of transgenic animals and their applications therein. We have also tried to incorporate the various ethical and societal issues arising from the use of this technology.

Keywords: Transgenesis, Electroporation, Microinjection, Knockout mouse, Oncomouse, Chimeras.

1. Introduction

Visualize animals functioning according to our own desire. If we could program animals to produce things at our own dispense-produce a cow that would give high levels of desired proteins in its milk, mice that would act as human disease models, fish with increased disease resistance, sheep giving high quality wool this and much more everything at our own discretion. In short, design animals according to our own needs. Well, this all could be possible in this age of DNA (Deoxyribose Nucleic Acid) technology through the process of 'Transgenesis'!

The process of inserting a piece of foreign DNA from same or different species into the

genome of a complex organism through experimental manipulation of early stage embryos or germ cells is called 'Transgenesis'. Animals resulting from such procedures that contain a foreign gene are called transgenic animals. Since, these animals contain a gene from a foreign agent; they also display the property that the protein encoded by the foreign gene confers. Thus, by this method we could induce new and innovative properties in animals; make them to manufacture substances that, conventionally, they can't and since, the changes involved in this process are of the genetic material and the animals possess the transgene in their germ cells these changes could be transferred to the next generation. Thus we could obtain an entire line of animals with renewed, improved properties.

A dream-come-true phenomenon, the ability to engineer nature's creations the process of transgenesis indeed is a magic that would enable us to make transgenic animals-custom made animals.

Why transgenic animals?

- Decipher genetic code,
- Study genetic control of physiological systems,
- Build genetic disease models, to improve animal production traits,
- Produce new and better animal products,
- Gain new knowledge^[14].

2. Methods of introducing a foreign DNA in mammalian cells:

Since, mammalian cells don't take up foreign DNA efficiently, the availability of effective methods for introducing genes into cells is essential. With the advancement of recombinant DNA technology, gene transfer methods have become powerful techniques in

construction of transgenic animals^[8]. Before turning towards the methods, it is important to understand the difference between transgenesis and cloning. The former involves the introduction of foreign DNA in the form of cloned genes while the latter means to obtain progeny that are genetically identical to the original animal^[7].

When you introduce a transgene into an organism, you insert into the genome, a foreign gene. In order to be of any use in a multicellular organism, the transgene must be inserted into the germline DNA, so that it can be propagated into subsequent generations. In addition to the transgene, the DNA usually includes other sequences to enable it:

- To be incorporated into the DNA of the host,
- ⊗ To be expressed correctly by the cells of the host.

Transfection is the process for introducing exogenous donor DNA into recipient cells.

The most common method is to physically inject the transgenic construct into the nucleus of a fertilized egg, which is then cultured in vivo for several cell generations before being implanted in a foster mother. Conceptually simple but by no means a trivial affair. Not all eggs survive the infection without damage, not all of them will develop and not all of them will contain the transgene. Only a proportion of the resulting embryos will be transgenic, so it is necessary to screen the embryos for the presence of foreign gene. The result for each embryo is all or nothing if the gene is present in one cell it is usually present in all. Even when successfully integrated into genome, the positioning of the transgene is more or less completely random. The objective, obviously, is that the gene lodges itself into a region where it doesn't disrupt another gene. The random localization of the transgene will also affect its level of expression. This is because there are large-scale regional differences on the chromosome the environment surrounding the transgene may not be

conductive to its expression^[7]. Following is a gist of methods used for introduction of transgene:

2.1 Calcium phosphate DNA precipitation

The principle of this technique is the formation of insoluble calcium phosphate DNA complex in a supersaturated solution. This is achieved by adding a solution containing DNA and CaCl_2 to buffered saline solution containing phosphate and incubating the mixture for a period of time to allow the formation of calcium phosphate DNA co-precipitates. The mixture is added to culture cells in a medium followed by incubation at 37°C for a period usually 6-24 hrs depending on cell type. DNA molecules enter the mammalian cells as the cells can take up calcium phosphate DNA co-precipitates by the process of endocytosis. High DNA concentrations inhibit this process and lead to reduction of DNA associated with insoluble complex. Optimum pH range for formation of calcium phosphate precipitates for most cell lines is 6.95 to 7.05^[8]. Initially, there was no way of predicting which cells take up DNA calcium particles and any transformed mammalian cells, were usually lost within the morass of non-transformed cells. Marker genes are used for this purpose. They are injected alongwith the desired gene into the given cell. So, if the cell has taken up the marker it implies that it would have taken up the gene of interest also. The marker genes encode an easily observable property so that we can distinguish the transformed cells from the non-transformed ones.

2.2 Electroporation

The cell membrane can be made permeable by exposing cells to a brief electric shock of several thousand volts. High voltage electric pulses make transient holes in plasma membrane and any DNA which is present in cell suspension can be drawn up through these holes. The efficiency of this method may be very high. The exact conditions for Electroporation, however, vary for different cell types, so a lot of preliminary experiments have to be done to optimize conditions. Thus, this method is usually used only for cells which can't take up DNA by calcium phosphate method, such as myeloma cells^[11].

2.3 Lipid mediated gene transfer (Lipofection)

The use of cationic lipids for DNA transfection into mammalian cells has become widespread because several features

of these reagents make them attractive vehicles for gene delivery. For instance, they are safer than viral vectors, can be produced in large quantities and can deliver large DNA fragments of upto several megabase pairs long, into cells. DNA in solution complexes efficiently with cationic lipids which form a single bilayer vesicle around DNA called a liposome. When these liposome vehicles are added to eukaryotic cells, they seem to fuse with plasma membrane and the DNA is taken up by the cell^[11].

2.4 Retroviral vector method

One method that allows foreign DNA to integrate into the chromosomes of the target cells uses retroviruses RNA viruses which naturally undergo an intermediate DNA form, prior to integrating into cellular genomes. Infection of preimplantation mouse embryos with a retrovirus results in mosaic offspring. Retroviruses should integrate rarely and at random into accessible cells, and the use of replication-defective retroviruses provides heritable markers for clonal descendants of the target cell (unlike wild-type viruses which spread from cell to cell).

Viruses are efficient vectors for DNA transfer because:

- They promote transfer of DNA to cells because of capsid proteins that bind to cell membrane receptors,
- They also contain promoters that allow expression of inserted genes in animal cells,
- They replicate to high copy numbers i.e. they produce several copies of the required gene within each cell,
- Some viruses integrate efficiently into animal cell genome^[10].

Despite their efficiency of introducing DNA viral vectors have a number of disadvantages:

- Viral infection sometimes changes the cell's properties. For e.g. epithelial cells may lose their cell polarity.
- Certain retroviruses are acutely oncogenic which precludes their use in a number of types of work. Retroviruses are also prone to deletion of gene sequences and they can also exchange gene sequences with other retroviruses. Thus, a retroviral vector could combine with an endogenous viral sequence to produce infectious recombinant viruses [8].

2.5 Microinjection

This procedure is performed in the following way:-

- The number of fertilized eggs that are to be inoculated by microinjection is increased by stimulating the donor females to superovulate. Female mice are given an injection of pregnant mare's serum and another injection, about 48 hrs later, of human chorionic gonadotropin. A super ovulated mouse produces about 35 eggs instead of the normal 5 to 10.
- The super ovulated females are mated and then killed. The fertilized eggs are flushed from their oviducts.
- ⊗ Microinjection of the fertilized eggs usually occurs after their collection. The microinjected transgene construct is often in a linear form and free of prokaryotic vector DNA sequences.

In mammals, after entry of sperm into the egg, both the sperm pronucleus and female nucleus are separate entities. After the female nucleus completes its meiotic division to become a female pronucleus, then nuclear fusion occurs. The male pronucleus, which tends to be larger than the female pronucleus, can be located by using a dissecting microscope. The egg can then be maneuvered, oriented, and then held in place by micromanipulation while the DNA is microinjected. On a good day, several hundred male pronuclei can be inoculated.

After inoculation, 25 to 40 eggs are implanted microsurgically into a foster mother that has been made pseudopregnant by being mated to a vasectomized male. In mice, copulation is the only known way to prepare the uterus for implantation. In this case, because the male mate lacks sperm, none of the eggs of the foster mother are fertilized. The foster mother will deliver pups from the inoculated eggs about three weeks after implantation. None of the steps in the procedure is 100% efficient^[2]. (Fig. 1.1)

For identification of transgenic animals, DNA from a small piece of the tail can be assayed by either Southern Blot hybridization or the Polymerase Chain Reaction (PCR). PCR is a technique for amplifying specific segment of DNA by using a thermostable DNA polymerase, deoxyribonucleotides, and primer sequences in multiple cycles of denaturation, renaturation and DNA synthesis. Microinjection is currently the preferred method for introducing a gene into mammalian cell due to the disadvantages of the retroviral vector method. A major

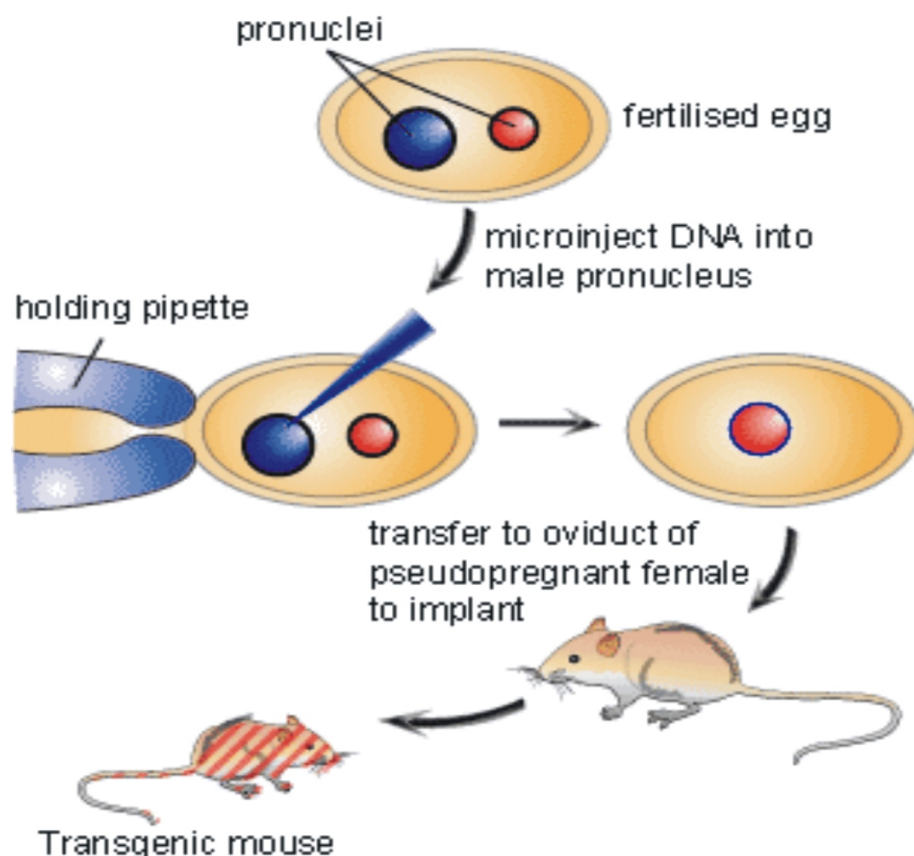


Fig. 1.1 shows the microinjection procedure. (Source: <http://earthops.org/fig5.jpg>)

advantage of this method is its applicability to a wide variety of species.

2.4 Engineered Embryonic Stem Cell method

Cells from the blastocyst stage of a developing mouse embryo can proliferate in cell culture and still retain the capability of differentiating into all other cell types including germ line cells after they are re-introduced into another blastocyst embryo. Such cells are called pluripotent embryonic stem (ES) cells.

Mice are mated and three days later, blastocysts are isolated and cultured in petri dishes. The cells spread out over the surface of the dish so that the clump of cells forming the inner cell mass corresponding to the future embryo can be removed. The clump of cells is dissociated into single cells using trypsin, a proteolytic enzyme. If ES cells are plated out on a plain-culture dish surface, they will differentiate into a variety of tissues, but if they are grown on a feeder layer of fibroblasts, they will continue to proliferate and can be sub cultured repeatedly. (A feeder layer is a monolayer of cells that has been treated so that the cells can no longer divide. They

continue to metabolize and in so doing 'condition' the culture medium so that the cells seeded on top of them survive and grow better). The cells can be microinjected into a blastocyst, where they will become assimilated into inner cell mass and take part in the formation of many tissues of chimeric mouse: some of the cells of the animal contain the transgene while the remaining cells are devoid of it. DNA can be introduced into ES cells by transfection, retroviral infection or Electroporation^[6]. (Fig.1.2).

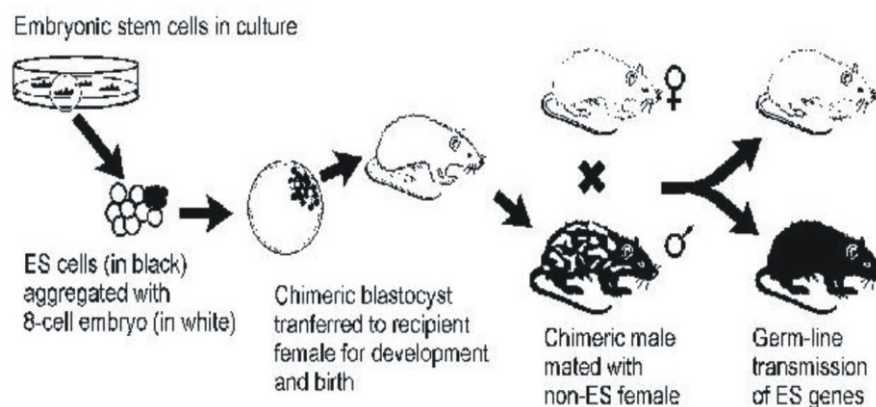


Fig. 1.2 shows the embryonic stem cell method. (Source: <http://www.scq.ubc.ca/wpcontent/pronuclearmicroinjection.gif>)

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

3. Applications

The technology involved in the production of transgenic animals holds great promise for both agriculture and biomedicine. Many aspects of biomedical science, including gene regulation, immune system, cancer research and developmental biology are sure to be tremendously benefited by these techniques. The production of transgenic animals will have a profound impact on the genetic improvement of livestock. We shall now course through a brief description of each of the transgenic animals produced to date.

3.1 Transgenic mice

For practical reasons, i.e. their small size and low cost of housing in comparison to that for larger vertebrates, their short generation time and their fairly well defined genetics, mice have become the main species used in the field of transgenics. No wonder then that they have been referred to as the 'fuzzy test tubes'!

A series of pioneering experiments performed by Ralph Brinster of the University of Pennsylvania and Richard Palmiter of the University of Washington in 1983, gave the world the first transgenic animal the

transgenic mouse [5]. The historic transgenic mouse had a rat growth hormone gene along with a promoter sequence that made it grow to twice the normal size of mice. This experiment paved a way for a series of explorations that were to trigger this genetic engineering in a big way.

3.1.1 The human mouse

The human mouse experiment was performed in 1988 by researchers at Stanford University led by Joseph M. McCune. They selected mice having severe combined immunodeficiency (SCID), a condition in which no immune system cells develop in the animal. This mouse was exposed as an embryo to human cells that grew together with the mouse tissues and became part of the mouse's system. Thymus, lymph node and liver tissues from an aborted human foetus were placed under a membrane surrounding the mouse's kidney. A week later immature immune system cells from a human foetus were transplanted into the mice. Two weeks later after the transplant, the mice displayed an immune response characteristic of both T lymphocytes and B lymphocytes; since now, it had acquired a human immune system!

Having a human immune system is a valuable asset to AIDS research. AIDS researchers are seriously hampered by the lack of good animal models for laboratory tests. The use of an animal with human cells will permit researchers to determine whether an experimental vaccine is eliciting a response. Moreover, pharmaceutical companies will be able to observe the effects of new drugs on the ability of human immunodeficiency virus (HIV) to infect immune system cells^[5].

3.1.2 The Oncomouse

Harvard University's Philip Leder created a transgenic mouse that is highly prone to breast cancer. Such a mouse permits researchers to test carcinogens and cancer therapies more accurately and efficiently than with normal mice. In the presence of mouse mammary tumor (MMT) virus, the *c-myc* gene induces breast cancer in animals. The mice produced possessing chimeric plasmids did not display breast cancer as adolescents, but at maturity, the MMT genes were activated, thereby disrupting the normal activity of *c-myc* genes. Breast cancer followed spontaneously. And the trait was passed to the offspring. Interestingly, these cancer-prone transgenic mice also had the honour of being the first complex animals patented, when the U. S. patent office granted to Harvard a patent for their production^[5].

3.1.3 Human disease models

Transgenic mice can be used as model systems for determining the biological basis of human diseases and devising treatment for various conditions. Transgenesis of mice is an exemplary system for providing the information whether the production of a potential therapeutic agent is feasible. Whole animal models simulate both the onset and progression of a human disease. However, a mouse is not a human, even though it is a mammal and so, the information gathered from some transgenic models may not always be medically relevant. In other instances, however, critical insights about the etiology of complex diseases can be discovered. With this in mind, mouse models for human genetic diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, arthritis, muscular dystrophy, hypertension, neurodegenerative disorders, endocrinological dysfunction, coronary disease and many other have been developed. Animal models could now be used for testing new drug and gene therapy strategies compared with the test tube blood samples formerly used^[5,19].

3.1.4 The Knockout mouse

Knockout mouse is a mouse in which the genes for a single organ or organ system have been knocked out. Frederick Alt and Susumu Tonegawa found out the gene defect in the SCID mouse and developed methods to convert the normal gene to defective gene. This discovery permitted them to destroy on command, the mouse's ability to produce an immune system. This gene defect could be passed on to the next generation. Knockout mouse have now been developed so that a single gene can be eliminated^[5].

Certain body cells have on their surface a molecule used as a receptor for the IgE antibodies that trigger most allergic reactions. In 1994, scientists at the University of North Carolina developed a mouse, whose gene for encoding the receptor was 'knocked out'. As a result, the cells didn't bind to the allergic antibodies as they didn't synthesize the receptor now and the mouse so developed didn't suffer allergic reactions. This is a positive development whose implementation in gene therapy would provide relief to millions of people world-wide who suffer from allergies^[5].

In 1992, Brinster and Palmiter again produced a knockout mouse without an immune system and with a gene to destroy the animal's liver. After the elimination of the mouse's liver

cells, human liver cells could be transplanted to replace the mouse's (the mouse devoid of any immune system wouldn't reject the human cells), and the entire organ could be replaced. Recent experiments have reaffirmed the potential of transgenic mice which have been aptly referred to as, the human hepatocyte factory. This technology has great applications in study of drug metabolism, enzyme induction, metabolite detoxification, drug toxicity, hepatocyte specific biosynthetic pathways, membrane biology, ion transport, cellular trafficking, hepatitis viral infection, viral persistence and antiviral therapy^[13].

Tonegawa's group developed a technique for knocking out gene in a group of cells in the brain by which they eliminated the mouse's ability to remember. Knockout mice having mutated genes for encoding mutated human hemoglobin found in patients suffering from sickle cell anaemia have been developed. They are called sickle cell mice.

Besides these famous mice, another feature of mice that biotechnologists find interesting is its urine. Therapeutically valuable urine production became possible in 1995 when Tung-Tien Sen of New York University demonstrated that certain genes are active only in the bladder. Genes for human growth hormone (HGH) were attached to the bladder genes. The mice so formed produced several hundred nanograms of HGH/milliliter of urine.

Another important application of transgenic mice is in the synthesis of human antibodies. The H and L chains of an antibody molecule were produced by two separate transgenic mice (which contain gene for their production) and they were interbred to form mice which produced both H and L chains. The mouse's genetic machinery then united the chains to produce the complete antibody^[2].

3.2 Transgenic cattle

Cattle are large animals and do not produce as many offsprings as smaller laboratory animals. The task of implanting manipulated embryos to surrogate females is more difficult here because of animal's larger size. Also, as the eggs of many domestic animals have opaque cytoplasm, it is impossible to see the nuclei without resorting to special techniques.

Despite these problems, efforts are on to synthesize human proteins in such animals by transgenesis. This potential gives the animal the ability to become the living equivalent of a



laboratory bioreactor. A new brand of farming, emerging from the Research and Development laboratories of several universities and small biotechnology companies, of harvesting human pharmaceuticals from farm animals called 'Pharming'. A transgenic animal for pharmaceutical production should:-

- (1) Produce the desired drug at high levels without endangering its own health and
- (2) Pass its ability to produce the drug at high levels to its offspring. The farm animal becomes a production facility with many advantages: it is reproducible, has a flexible production capacity through the number of animals bred and maintains its own fuel supply. Best of all, in most animal drug production, the drug is delivered from the animal in a very convenient form in the milk!

A transgenic cow produces in its milk a major protein called lactoferrin that functions in iron uptake in nursing infants. Because lactoferrin increases iron binding in the body it could also be used to relieve iron deficiency anaemia. As cows produce thousands of liters of milk per year the possibility of a lactoferrin producing cow looks very appealing^[5].

Casein is the protein component from which cheese is made. The nutritional value of milk may be enhanced by increasing its casein content by transferring casein encoding genes to the animal's mammary gland. Another possibility is to reduce the lactose content of milk for those who have lactose intolerance. And it may be even be possible to genetically modify a cow to produce skimmed milk.

Interferon is an antiviral protein produced by animal cells on stimulation by replication of viruses. Transgenic cows have now been produced with higher than normal interferon levels in their tissues.

Cattle that are resistant to mastitis an infectious disease of mammary glands that causes decreased milk production and lost productivity are produced. Treatment and prevention of mastitis is costly both monetarily and in increased labour. Transgenic dairy cows that secrete lysostaphin an antimicrobial peptide that protects the mammary gland against *Staphylococcus Aureus* infection by killing bacteria in a dose dependent manner have been produced to address the problem^[14].

3.3 Transgenic pigs

Because of its relatively short generation time and large litters, the pig has become an "agricultural mouse"^[5]. Production of human organs like hearts, kidneys, livers, pancreas and lungs from animals has been a long desired dream of surgeons and pigs are the favourite donors for such organ farms. Their organs approximate human organs in size, their physiology is similar to human physiology, they carry few infectious diseases transmissible to humans, they have a politically correct image for slaughter and their genetics have been studied in depth.

One example of a living bioreactor is the transgenic pig that produces human haemoglobin which is an essential oxygen carrying component of red blood cells which one day can be used in place of human blood for transfusion during many surgical procedures. A key advantage of animal derived haemoglobin is its freedom from human pathogens^[5].

Normally found only in rodents, the gene for WAP (whey acidic protein) group of acidic proteins found in the fluid portion of the milk; was introduced into the fertilized egg cells of pigs in 1991. The transgenic pigs so formed produced the fluid protein in their milk.

A major ecological problem has arisen with the mass rearing of pigs due to the over abundance of phosphorous in their faecal matter, that can run off into water systems and cause excessive growth of cyanobacterial populations algal blooms that in turn deplete the oxygen supply and kill fish and other aquatic organisms. In addition, large amounts of phosphorous in the environment result in the production of gases that enhance the greenhouse effect and contribute to global warming. The ability to introduce enzymes such as phytase into the gut of swine, where it is not normally present, is particularly attractive. Introduction of phytase would increase the bioavailability of phosphorous, from phytic acid in corn and soy products (constituting the diet of such animals). The salivary phytase provided complete digestion of the dietary phytate. Transgenic pigs required almost no inorganic phosphorous supplements to achieve normal growth. The use of transgenic pigs having phytase results in decreased environmental pollution due to excessive phosphorous^[2].

3.4 Transgenic goat

Transgenic research with goat has for the most part, been devoted to developing the mammary gland of these organisms as bioreactors, for the production of pharmaceutical proteins. A transgenic goat giving milk containing protein, normally found on a parasite that causes malaria can be developed. These proteins elicit a response in test animals and could conceivably be used to immunize against malaria. It stirs the imagination to think that 500 million people could one day be protected against malaria by simply drinking a glass of goat's milk. Transgenic goats have also been used to produce cystic transmembrane conductor regulator (CTCR). The CTCR proteins are an essential component of cell membranes that transport ions. Cystis fibrosis patients lack these proteins, so therapeutic doses of the protein could conceivably make up the deficiency and help resolve the symptoms of cystic fibrosis.

A transgenic goat has also been developed with the ability to synthesize tissue plasminogen activator (tPA) an important clot dissolving enzyme used to treat myocardial infarction. GTC biotherapeutics has preserved and in June 2006 won preliminary approval to market a human protein antithrombin-III in Europe. Their protein the first to be made in a transgenic animal to receive regulator's approval for human therapy, was secreted in the milk of transgenic goats.

Spiders that produce orb webs, synthesize dragsilk which can be elongated upto 35% and has tensile properties close to that of synthetic fiber. The protein monomers that assemble to produce these silk fibers have been produced in the milk of transgenic goats. The numerous potential applications of these fibers include medical devices sutures, aircraft, automotive composites, clothing and ballistic protection^[14].

3.5 Transgenic sheep

A protein called alpha-I-antitrypsin helps cell membranes retain their elasticity and thereby encourages membrane passages of gases, nutrients and waste products. Lack of it causes emphysema. Transgenic sheep producing this protein have been developed which secrete 35 grams of alpha-I-antitrypsin per liter of milk, more than a fifth of the dose to treat a patient for a year^[5].

3.6 Transgenic fish

To date, transgenes have been introduced by microinjection of DNA into the fertilized eggs of a number of fish species including carp, catfish, trout and salmon.

In one study, a transgene consisting of the promoter region from the antifreeze protein gene of the fish called ocean pout, the growth hormone cDNA from salmon; and the termination polyadenylation signals from the 3' end of the antifreeze protein gene from the ocean pout was injected into eggs of Atlantic salmon. In general, the transgenic salmon were larger and grew faster than the non-transgenic controls. It is contemplated that genes for disease resistance, tolerance to environmental stress and other biological feature will be introduced into both temperate and eventually tropical fish ^[2].

3.7 Transgenic mosquito

Members of the genus *Anopheles* in mosquitoes transmit the protozoan parasite 'plasmodium' that causes malaria. Their ability to harbour and transmit these parasites is owing to certain genes. These genes can be altered and mutated insects can be produced. If produced and released in large quantities these transgenic mosquitoes can dominate the native mosquito population and break the chain of disease transmission ^[5].

3.8 Transgenic Snail

'Schistosomiasis' is a disease that causes fever, chills, intestinal ulcerations and diarrhoea in approximately hundred million people each year. It is caused by a parasite; a flatworm belonging to the genus 'Schistosoma' that breeds in the tissues of the snail before infecting the blood of humans who stand or swim in contaminated water. French investigators are now attempting to develop a transgenic snail that resists invasion by 'Schistosoma' species. When released into the environment, these transgenic species could outnumber the native snails transmitting the disease and thus break the life cycle of the parasitic worm ^[5].

3.9 Transgenic Boll-worms

The researchers at California University are working on 'cotton bollworms', the caterpillars that live in cotton bolls. They are attempting to produce transgenic bollworm by inserting into laboratory reared bollworms, a gene that activates suicide gene in the insect's offspring. The plan is to raise millions of worms to adult moths and release them to the environment where they will compete

with the normal moths and mate with wild bollworm moths. The mating will produce caterpillars that die from the effect of the gene and save the cotton crop from caterpillar related damage ^[5].

3.10 Transgenic rabbits

They have been made to produce the enzyme α -glucosidase which helps relieve the symptoms of Pompe's disease, a genetic disorder in which the liver cells fail to break down glycogen molecules and release the glucose molecules for the body's energy needs. The glucose deficiency leads to a broad spectrum of diabetes-like symptoms related to energy deficiency. Rabbits are useful animals because they mature quickly and produce milk in quantities sufficient to extract enzymes ^[5].

4. Benefits and concerns about transgenic technology in livestock production

The technology involved in production of transgenic animals has potential application in agriculture and medicine. This, as with other areas of biological research, has both benefits and potential risks. The perception, the public has of Biotechnology, differs depending on its uses the development of new vaccines to treat infectious diseases might be widely accepted; whereas production of transgenic livestock, that grow at a faster rate for consumption as food for humans, might not. There is no doubt that this type of research will be subject to scrutiny.

At present, the production of transgenic livestock is inefficient, financially costly and to date, the improvements in the productivity of these animals have been modest. One aspect that must be determined is whether the costs justify the benefits realized. It is worth pointing out, that the goal of using this technology is for the benefit and not the detriment of mankind. Scientists using this technology are trying to develop models to study diseases, produce biopharmaceuticals and produce more wholesome healthy and economical food. These studies are difficult and great care must be taken before such investigations begin.

Consideration of the factors like concern of animal welfare, ethics, benefit, vigilance and scientific issues will enable us to reap the benefits from this technology ^[14].

5. Ethical Issues

Use of animals in biotechnological research causes great suffering to the animals. But most people seem to accept some animals suffering to serve the basic interest and welfare of mankind; this attitude has been termed as interest-sensitive speciesism. 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. An argument attempts its 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 entity; rather they are regarded as dynamic, constantly evolving groups. 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 genomes without any recognizable devaluation of our humanness.

6. Recent Developments

Vivek Rangnekar, a professor of radiation medicine at the University of Kentucky, has created the world's first breed of supermice that are resistant to cancer, even the highly aggressive forms. He created the breed with a more active tumor suppressor Par-4 gene. Carrying this gene made them completely invulnerable to cancer. Not only did they develop tumors, they even lived longer than the control animals, indicating that they had no toxic effects. This gene offered the potential of destroying cancer cells without harming normal cells ^[26].

Research is also underway to manufacture milk through transgenesis for treatment of debilitating diseases such as phenylketonuria (PKU), hereditary emphysema, and cystic fibrosis.

7. Some perceptions around transgenic animal experiments:

Despite the importance of transgenic animals in biomedical research, there are some concerns and misconceptions raised about their use in research. Some of these are addressed below.



Transgenic animals suffer more abnormalities than regular research animals. The introduction of DNA into an animal can be very complex and the possible side effects can be difficult to predict. Possible harms might arise from surgical techniques used to harvest and re-implant embryos the collection of tissue from the tip of the tail for genotyping; and non-specific effects caused by damage to genes adjoining the altered area of DNA. Also reduced fertility or oversized fetuses may result from this technology. In most cases the mutations impact highly specific metabolic processes or cell receptors without actually causing disease, discomfort, pain or malformation in the animals.

Transgenic animals not expressing foreign DNA or not containing a particular gene modification are destroyed. Because transgenesis is a complex science, it is not 100% efficient. However, new methods are being developed to increase the accuracy in transgenesis. Again, it should be remembered that such genetic alteration can only be attempted if the authorities are persuaded that there is no other way to pursue important research^[21].

8. Conclusion

Interestingly, the creation of transgenic animals has resulted in a shift in the use of laboratory animals—from the use of higher-order species such as dogs to lower-order species such as mice and has decreased the number of animals used in such experimentation, especially in the development of disease models. This is certainly a good turn of events since transgenic technology holds great potential in many fields, including agriculture, medicine, and industry. The appropriate use of transgenic animals is a positive development with potential for significant medical benefits. The challenge is for government, industry and society to ensure that transgenic research continues to be sensitively carried out for proper medical ends in a suitably balanced regulatory environment.

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