# BIOPHARMING: USING ANIMALS TO PRODUCE HIGH-VALUE PROTEINS AND MEDICINES

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Biopharming, which is also referred to as molecular farming, is the process of producing a variety of pharmaceuticals, such as therapeutic proteins, monoclonal antibodies, and vaccines, using genetically modified plants and animals. These pharmaceuticals are intended to treat inflammatory diseases, cancer, and other serious or devastating illnesses. Animal-derived proteins are a subclass of medications in the medical field. Because these medicinal proteins cannot be chemically manufactured, they are referred to as biopharmaceuticals.

This chapter explains about uses of transgenic animals which are genetically engineered to produce pharmaceutical substances for use in humans. It often involves the insertion of gene constructs derived from humans. For example, genetically modified yeast, bacteria, and animal cell cultures have for some time been used to produce pharmaceutical substances in enclosed bioreactor systems, but are generally not included in the definition of biopharming. Genetic modification of animals to change their nutritional makeup, improve their capacity as models for human diseases and organ transplants, or use them as model systems for DNA microinjection, gene targeting, and cloning has had a profound effect on drug development, human health, and the pharmaceutical industry.

Every recombinant manufacturing system has specific benefits and drawbacks of its own. This chapter looks at the procedures, advancements, and upcoming trends in the manufacture of biopharmaceuticals. Rapid biosystem screening and analysis platform technologies are examined. Techniques for enhancing productivity through integrated and metabolic engineering are also emphasized.

## Introduction

Pharming is the combination of pharmaceuticals and farming, often known as biopharming or gene farming. In medicine, proteins extracted from animals represent a significant category of drugs. The term "biopharmaceuticals" refers to the fact that these pharmaceutical proteins cannot be chemically produced. They have been created in genetically modified bacteria, yeast, or animal cell lines or they have been extracted from biological material like donated blood (Abdullah et al., 2008). Recombinant human proteins are now expressed in chicken eggs, milk, and goats.

## 1. The technology of pharming

Since most organisms are unable to carry out the post-translational changes necessary for the assembly and bioactivity of many complex human proteins, animal pharmaceutics aids in the creation of proteins that are hard to get through other methods. For instance, it might be challenging to control glycosylation, a post-translational modification that adds sugars to proteins in cultivated microbes. It is easier and less expensive to breed more transgenic animals than to build new facilities for the cultivation of bacteria, yeast, or animal cells (Dong DW et al., 2007).

Transgenic animals are frequently used to simulate human situations, especially mice. Researchers studying human developmental and pathological conditions, gene therapy, the genetics of human and animal disease, the assessment of treatment approaches before clinical trials, human and animal disease resistance, toxicological screening for drug and product testing, and the creation of novel products through molecular pharmacology have all found value in using transgenic animals

(Dunn et al., 2005; Pinkert CA. , 2002).

### 1.1 Recombinant pharmaceutical proteins- Advent of biotechnology

A healthy body produces proteins that aid in normal functioning as well as regulating and mediating metabolic processes. Any form of protein synthesis impairment, including the creation of misfolded or mutant proteins, results in the disruption of the pathway that the protein is responsible for controlling (Gupta et al., 2016). The illness might be one way this shows up. In order to meet the increasing demand, large-scale manufacturing of exogenous proteins is necessary for their supply. The intricate procedure necessitates increased levels of protein production, purification, and processing. For large-scale manufacturing and purification, every product requires a different set of parameters or standards. To create human-derived proteins in bacteria, fungi, insects, or mammals, genetic engineering techniques are applied (Figure 1.1). While using recombinant DNA technology to produce proteins on a wide scale involves a significant investment of time, labor, and resources, it also presents numerous prospects for economic growth (Khan et al., 2016). After reading this chapter, readers should be able to comprehend the fundamentals of recombinant protein creation in a variety of hosts, as well as the benefits and drawbacks of each host system, as well as the properties and production of certain significant growth factors and medicinal substances. Recombinant DNA technology has the following benefits when it comes to protein production:

• It reduces the possibility of an immune response and produces highly active proteins because human genes can be copied and expressed.

• It reduces the possibility of spreading unidentified diseases found in human and animal sources. The therapeutic protein can be generated effectively while being economically viable.

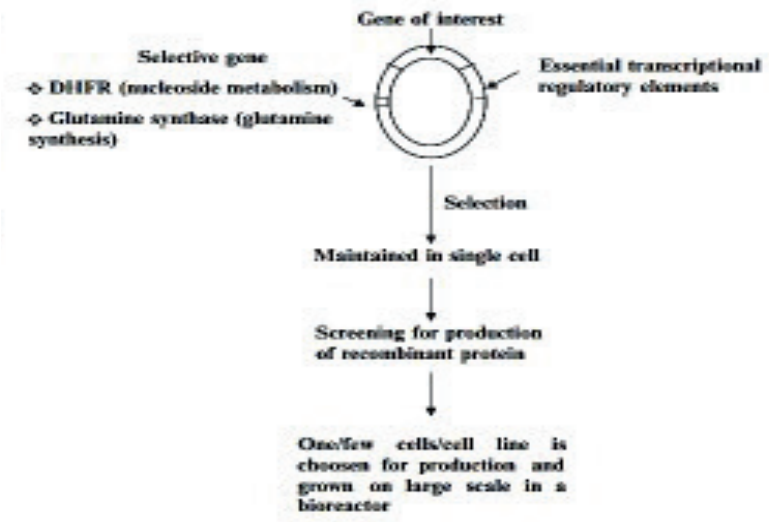



**Figure 1.1:** The desired gene is cloned using an appropriate vector. The cloned gene contains all the necessary regulatory components needed for transcription in order for its expression.

### 1.2 Animals as the production platform for recombinant pharmaceuticals

While there are many different host systems available for the synthesis of recombinant proteins, microbial hosts have a number of benefits over alternative systems, including quick, affordable, and efficient production:

1. Simple to use and maintain.
2. Makes use of affordable nutrition sources.
3. Quick development and biomass build-up at high cell densities.

4. A range of potent inducers can be used to modify their expression machinery (Ferrer MN et al., 2009).

In the pharmaceutical sector, the greatest source of innovation at the moment is the recombinant creation of therapeutic proteins against human diseases. The development of novel therapeutic proteins, where transgenesis plays a crucial role, has been propelled by the market's expansion. Because of the transgenic animal platform's low production costs with high productivity and quality recombinant proteins, it presents appealing opportunities. Transgenesis in farm animals typically encounters certain difficulties, mostly because of random transgene integration and control over copy number, despite the fact that numerous ways have developed over the past few decades for the creation of transgenic founders. However, because of its tremendous efficiency and simplicity, the CRISPR/Cas system's recent advancements in gene editing promise to transform the discipline. Additionally, the characterization of bioreactor founders, as well as the expression patterns and protein functions are technical aspects of the process that require regulatory decisions with a focus on biosafety before any given recombinant protein is finally approved for use in animals or humans. The approval of two recombinant proteins produced from the mammary gland for use in clinical and commercial settings has increased interest in finding safer, more effective, and cost-effective methods of creating transgenic founders to fulfill growing global demand for biomedical proteins (Bertolini LR et al., 2016).

### 1.3 Transgenic construct used for animal pharming

These days, molecular biology is advancing at a pace never seen before. Generically engineering animals is one of them. An animal classified as transgenic is one whose genome has been altered to incorporate genes from a different species or to apply methods to edit animal genome to produce particular features. One can intentionally modify a gene (or genes) to change an animal's traits. The first transgenic animal to be successful was a mouse. A few years later came cattle, sheep, pigs, and rabbits. There are several ways to prepare the foreign genes employed in animal transgenesis. A range of vectors, such as bacterial plasmids, cosmids, and yeast artificial chromosomes, are used to insert the generated gene of interest. The generated vector, which contains the intriguing gene, is delivered into the host cell via a variety of methods, such as heat shock, viruses, the gene gun, electroporation, microinjection, and liposomes. DNA microinjection, retroviruses, stem cells, and cloning can all be used to induce transgenesis in the gonads, sperm, fertilized eggs, and embryos. Currently, fluorescent protein is the most effective transgenic marker available. PCR, ELISA, western and southern blots, and the incorporation of an antibiotic resistance gene all confirm the effectiveness of transgenesis. The most promising technologies in the future will be those that address social and ethical issues (Shakweer et al. 2023).

### 1.4 Drug Design Models for Human Diseases by Transgenic Animals

The majority of medicine candidates are unable to obtain approval from the US Food and Drug Administration due to challenging nature of preclinical trial and drug development methods. In order to increase chances of success in the process of developing new drugs, effective and forecast methods that can pinpoint high-quality targets must be employed (Sun D et al., 2022). Using affordable and manageable animal models for in vivo research is a useful strategy to solve the challenges faced in the development of innovative drugs and combination therapies.

Transgenic mice are regarded as a valuable resource for studying human illnesses and assessing how treatments work in drug discovery labs. These animals are genetically engineered creatures that have traits similar to those of some human diseases. It provides genetic models of several human diseases, which are essential for understanding illnesses and finding new targets. Since the 1980s, genetic modification has allowed scientists to modify the mouse genome by adding or removing certain genes (National research council, 1993).

The US Food and Drug Administration approved and manufactured ATryn, first drug made

from transgenic animals, in less than 20 years after it was developed. This discovery opened the door for the creation of medications made from transgenic animals. Making physiologically safe drugs based on human regulatory proteins is one use for this technology (Hunter, 2019; Bagle et al., 2012). In the upcoming years, a number of issues related to the use of transgenic animals such as ethical questions, legal requirements, and patents will receive more attention. Clinical trials, xeno-transplantation, and other fields may benefit from the use of transgenic animals in drug discovery and development (Fung et al., 1997).

### 1.5 Analysis of transgenic animals

The Food and Drug Administration (FDA) of the United States created rules for the pre-market data submission of prospective goods derived from transgenic sources (FDA, 1995). These standards include, among other things, the need that the integrated transgenic construct's structure and expression pattern be identified in the original animal and proven to be dependable in succeeding generations. To ascertain the composition, copy number, and integration location of every transgene, an analysis of every animal line intended for commercial production is necessary. The inquiry will use Southern hybridization of the genomic DNA to determine the lengths of the various restriction fragments expected from the construct structure. Fluorescence in situ hybridization of spreads of metaphase chromosomes can also be used to identify the chromosomal location or sites of integrated transgenes. Molecular cloning of the integrated transgene and its nearby regions may be required to determine the DNA sequence of the integrated transgenic locus.

Mammalian embryos injected with DNA via microinjection typically integrate the DNA as tandem repeats that are orientated head to tail and typically, but not always, at a single randomly placed locus within the host genome (Smirnov A and Battulin N, 2021). Unlike transgenic loci created by DNA microinjection, those created by random cell transfection need the introduction of selectable marker genes, which usually encode resistance to a common antibiotic like G418, blasticidin, or puromycin. Bacterial gene promoters are omitted from the selectable marker genes in order to prevent potential gene flow from the transgenic animal to prokaryotes. Additionally, site-specific recombination elements, such loxP substrate sites for Cre recombinase, can be placed in front of antibiotic resistance genes to facilitate their eradication. Nevertheless, this can lead to significant deletions in several arrays (Sun et al., 2015).

In most cases, co-injected or co-transfected transgenes integrate at the same locus. Transgenic lines are commonly created from founder animals with large transgene copy numbers because they frequently exhibit the highest levels of expression (Grandjean et al., 2011). However, it has been noted that these lines are susceptible to transgene silence, recombination, and copy loss over several generations. In tandem arrays, transgene copy loss is more common when components are oriented in the opposite direction from one another.

Genes that are incomplete, duplicated, or deleted can result from such unstable arrangements. Because alterations in the translational reading frame might result in the development of shortened and/or abnormal protein species when breaks occur within coding regions, incomplete genes are especially undesirable (Jackson et al., 2018).

### 1.6 Transgene mRNA expression analysis

Determining tissue specificity of transgene requires characterizing its expression pattern. The main goal of this is to determine whether improper transgenic expression would likely result in any unwarranted negative consequences that would be detrimental to the producing animals (Bandopadhyay R., 2010). To identify temporal or geographical ectopic transgene expression, samples of a wide range of tissue types obtained from transgenic animal necropsy are analyzed using Northern hybridization or reverse transcriptase PCR (RT-PCR). The quantity, position, and type of expression of any ectopic transgene, as well as the protein that is encoded, will determine its importance. To determine the whole spectrum of mRNA species present, transgene mRNA

expressed by the relevant tissue has to be thoroughly characterized. To ascertain whether the mRNA is appropriately spliced and maintain its integrity, this is required. Even if they are just minority species, aberrant mRNAs have the ability to encode aberrant proteins that may have serious therapeutic ramifications (Dorn et al., 2011).

### 1.7 Transgene protein expression analysis

The objectives of transgenic protein analysis are to ascertain whether a protein is completely functional or it degrades in certain situations, such as milk and expression levels are high enough to be economically viable. The next step is to determine how closely the transgenic recombinant protein product matches the original ones and either there are variations that impact immunogenicity, stability, or half-life (Shepelev MV et al., 2018).

In order to do this, significant efforts will be put into the examination of protein products using mass spectroscopy, glycoprotein analysis, peptide mapping, functional testing, and protein sequencing. Evidently, a pharmaceutical product intended for human use must always be of high quality. Protein structure can be impacted by changes in expression levels. For instance, high expression levels may surpass the generating tissue's limited ability for post-translational modification, leading to partially or completely unaltered protein and changed bioactivity. Thus, the amount of variation in protein production among members of a transgenic herd or flock should be minimized (Tikhonov D et al., 2021).

Any transgenic product's evaluation will undoubtedly take into account the purity of the protein production (Shepelev MV et al., 2018). This is crucial in cases when intravenous administration is planned. Producers are responsible for making sure that host animal proteins, DNA, and chemical reagents are removed, as well as for making sure that possible pathogens like viruses, bacteria, and prions are excluded.

## 2 Protein extraction, purification, and processing

There are well-established basic techniques for the collection and processing of large quantities of milk and eggs. Designing collection methods suitable for the bulk collection of other fluids such as urine or semen has not yet been feasible.

Milk is the sole product of large-scale recombinant protein purification that has been generated thus far. This multi-step system combines industry-standard techniques created for the dairy sector with techniques created for the purification of recombinant proteins produced in cell culture. The application of a given product dictates the amount of purity needed for it. Should the protein be consumed as a nutraceutical, skim milk may be a good option. However, if the product was to be administered intravenously on a regular basis for prolonged periods of time, extraordinarily high levels of purity would be required (Meade HM et al., 1999).

The details of the protein's purification will depend on its composition. The first stages involve removing fat and suspended caseins using techniques including centrifugation, acid precipitation, micro-filtration and chymosin treatment, as the majority of recombinant proteins are found in the whey fraction. A sequence of chromatographic procedures would next be carried out to separate the recombinant protein from leftover milk proteins, whey and other impurities (Nath et al., 2014). The latter steps in the cleanup process might involve heat treatment and ultrafiltration in order to create a pharmaceutical-grade medicinal product. According to current experience, the ultimate yield of purified product can range from 40 to 60% of the quantity found in milk, depending on the protein's makeup and the necessary purification process. Usually, the most loss occurs when the casein is removed. Treatment with chelating drugs that break down casein micelles and release the related recombinant protein may lessen this (Hernandez A and Harte FM., 2009).

Good manufacturing practice (GMP) and standard operating procedures (SOPs) must be

followed in the execution of all processes. Manufacturers of transgenic products would maintain sperm banks, much as manufacturers of cell culture products must maintain duplicate banks of cells to guarantee product continuity (Seet et al., 2023).

Animal husbandry: The laws governing the housing of genetically modified animals varied throughout nations. Transgenic animals must have their veterinary health monitored and their consequences from recombinant protein production closely monitored. All animals are typically maintained under some kind of confinement regime, such as in fields that are double-fenced, where each animal is identified by a tag and is closely monitored by an accounting system. It is important to follow the protocols for disposing of garbage and cadavers to guarantee adequate containment. The general animal husbandry regulations of the EU also apply to transgenic flocks or herds (Hallerman et al., 2022).

### 2.1 Choice of species and site of production

The selection of the appropriate animal species for biopharming is determined by a variety of factors, including the ease of genetic manipulation, reproductive characteristics, protein yield, and ethical considerations. Several species can be used in biopharming, with each offering unique advantages. The most commonly utilized species include goats, cows, rabbits, chickens, and sheep (Table 2.1).

**Table 2.1:** Species used for protein production

| Choice of Species | High-Value Proteins | References |
|---|---|---|
| Goat | antithrombin III | Echelard et al., 2009 |
| Cow | human lactoferrin, insulin, and antibodies | Kues & Niemann, 2011 |
| Rabbit | Interleukin-2 and human growth hormone | Zhang et al., 2014 |
| Chicken | human interferon beta | Lillico et al., 2016 |
| Sheep | alpha-1 antitrypsin | Saviano, 2023 |

**2.1.1. Goats:** Goats are commonly chosen because of their relatively short gestation period and their ability to produce large quantities of milk containing high-value proteins at relatively high concentrations. For the production of proteins that can be secreted into milk, transgenic goats are perfect. For instance, their milk can include the protein antithrombin III, which has anticoagulant qualities (Echelard et al., 2009). Goat farming is highly popular due to its scalability and ease of milk collection.

**2.1.2. Cows:** In biopharming, cows are frequently utilized to generate complicated proteins that need for numerous post-translational modifications. Cows are a desirable option for manufacturing milk proteins because of their huge size and great volume of milk output. For instance, human insulin, antibodies, and lactoferrin have all been successfully expressed in the milk of transgenic cows (Kues & Niemann, 2011), providing a scalable and economical production technique.

**2.1.3. Rabbits:** Compared to larger animals, rabbits have a shorter maturation period and a faster reproductive cycle, which makes them useful for producing transgenic lines more quickly. They work well for manufacturing monoclonal antibodies and high-value proteins in lesser quantities. For example, rabbit milk expresses human growth hormone (HGH) and interleukin-2, suggesting possible uses in biopharmaceutics (Zhang et al., 2014).

**2.1.4. Chickens:** In order to produce proteins for immunizations and enzyme replacement therapies, hens are kept for their eggs, which provide an efficient and non-invasive source of protein. Eggs are a great biopharmaceutical medium since they are easy to gather and the egg whites have a higher protein content. Transgenic chicken eggs have been shown to produce proteins like human interferon beta (Lillico et al., 2016).

**2.1.5. Sheep:** Similar to goats, sheep are used to provide milk that is utilized to create proteins like alpha-1 antitrypsin, which is used to treat respiratory ailments. Their ability to produce enormous amounts of milk increases their potential for biopharmaceuticals (Saviano, 2023).

**2.2 Site of Production**

Protein yield and quality are significantly influenced by the animal's production site (Table 2.2). The main locations are urine, eggs, blood, and milk, each of which has unique benefits and difficulties. (Shakweer et al., 2023).

**2.2.1. Milk:** The most popular location, milk, has many benefits, such as easy collection and high protein output. Large volumes of milk proteins can be isolated and refined utilizing tried-and-true dairy industry procedures. This method works particularly well for producing therapeutic proteins, such as enzymes, hormones, and antibodies.

**Table 2.2:** Sites for transgenesis (Shakweer et al., 2023)

| Site of Production | Advantages | Challenges |
|---|---|---|
| **Milk** | • easy collection<br>• high protein output<br>• therapeutic proteins including hormones, enzymes, and antibodies. | Refine form |
| **Blood** | Effective against naturally occurring bloodstream proteins such clotting factors | complexity of protein extraction and purification along presence of multiple other proteins and cells |
| **Eggs** | • high protein concentration<br>• scalable<br>• economical<br>• Producing vaccines and other therapeutic proteins | Extraction of protein in pure form |
| **Urine** | Relatively low protein concentration makes the purifying process much simpler | lesser yields |

**2.2.2. Blood:** Producing proteins in the blood is less common because of the complexity of protein extraction and purification as well as the existence of several other proteins and cells. Nevertheless, it functions effectively for naturally occurring bloodstream proteins such clotting factors. The challenge lies in ensuring that the expression of the protein does not adversely affect the animal's health.

**2.2.3. Eggs:** One effective and non-invasive way to gather proteins is using eggs. The egg white, which has a high protein concentration, can be used to extract proteins. This technique of manufacturing is scalable and economical, ensuring a steady supply of the needed proteins. Producing vaccines and other therapeutic proteins that need precise post-translational modifications is a good use for this technique.

**2.2.4. Urine:** It has been possible to engineer certain transgenic animals to create proteins in their urine. Relatively low protein concentration in urine is advantageous to this procedure as it makes the purifying process much simpler. Because of the lesser yields in comparison to milk production, it is less common.

Recombinant proteins are being produced using genetically modified animal cells. Transgenic agricultural animals (sheep, pigs, and rabbits) larger than mice (Table 2.3) that release foreign

proteins in their milk, blood, and other body fluids have become possible. The β-lactoglobulin gene in sheep was fused with milk protein gene promoters, and the β-lactoglobulin gene was fused with human tissue plasminogen activator (Valender, 1997). Through the use of this method, 100 foreign proteins were secreted into milk in an experimental setting. These included milk with higher casein levels, which are beneficial for making cheese, or milk with specific qualities to fill in demographic gaps, like lactose-free milk for the Asian market, milk without β-lactoglobulin for consumers with allergies, or milk containing human β-lactoferrin protein to protect newborns (Melo et al., 2007). Several of them are clinically examined and found in considerable quantities in the milk of rabbits, sheep, goats, and cows. One of the proteins that helped the clinical condition of infants with Pompe illness was human a-glucosidase, which is produced from rabbit milk. EC superoxide dismutase, fibrinogen, collagen, and spider silk are a few examples of the intricate foreign proteins that the mammary gland may create (Valender, 1997).

Table 2.3: Medicines created against disease using transgenic animals

| **Diseases** | **Target medicines** | **Animal** | **References** |
|---|---|---|---|
| Anti-infection | Alpha-lactalbumin | Cow | Wang et al., 2008 |
| Thrombosis | Human protein C | Pig, Sheep | Peterson et al., 2009 |
| Wound healing | Fibrinogen | Cow, Sheep | Prunkard et al., 1996 |
| Type 1 diabetes | Glutamic acid decarboxylase | Mouse, Goat | Herger et al., 2003 |
| Blood volume maintainance | Human serum albumin (HAS) | Mouse, Cow | Luo et al., 2015 |
| Malaria | msp-1 | Mouse | Chen and Zavala, 2012 |
| Cystic fibrosis | CFTR | Sheep, Mouse | Gawenis et al., 2019 |
| Osteoporosis | Human calcitonin | Rabbit | McKee et al., 1998 |
| Arthritic infection | Lactoferrin | Cow | Van Berkel et al., 2002 |

The efficiency, quality and yield of the proteins produced are all determined by the strategic selection of the species and production site, which is crucial to optimizing the biopharmaceutical process. Various animals such as goats, cows, rabbits, and chickens can provide distinct advantages based on the type of protein and its intended application. Comparably, in order to maximize the production process, great thought must be given to the location of production—whether it milk, blood, or eggs. The potential of biopharming is being further enhanced by advancements in genetic engineering and animal husbandry, paving the way for the production of more efficient and cost-effective high-value proteins and medicines (Fischer et al., 2001).

**3 Techniques for creating transgenic animals**

Using transgenic animals for protein production is a notable biotechnology breakthrough. Animals whose genomes have been changed to incorporate foreign genes are known as genetically modified organisms, or transgenics (GMOs). These animals can create recombinant proteins, which are useful in agriculture, industry, and medicine. Because transgenic animal technology creates new opportunities for the production of high-value proteins for use in industry, nutrition, and pharmaceuticals, it has completely changed the biotechnology business. Scientists can exploit the biological machinery of animals to generate complex proteins that would be costly or difficult to produce in other ways by introducing foreign genes into their genomes (Spok et al., 2007; Niemann H and Kues WA., 2003).

Many methods for producing transgenic animals have been found in the last few decades. Many sequences have been uncovered as a result of gene sequencing, offering details about genes

and promoters that are significant for a wide range of organisms. Proteomics, genomics, and many new reproductive biotechnologies all indicate that transgenic animals can be successfully used in home settings. The methods and processes utilized to create a transgenic animal depend on what purpose the animal will serve. Numerous transgenic animal models have been created to study the functions of genes, act as bioreactors, and serve as prototypes for cutting-edge methods of animal breeding. (Houdebine LM, 2003). Figure 3.1 depicts the main methods used to produce transgenic animals.

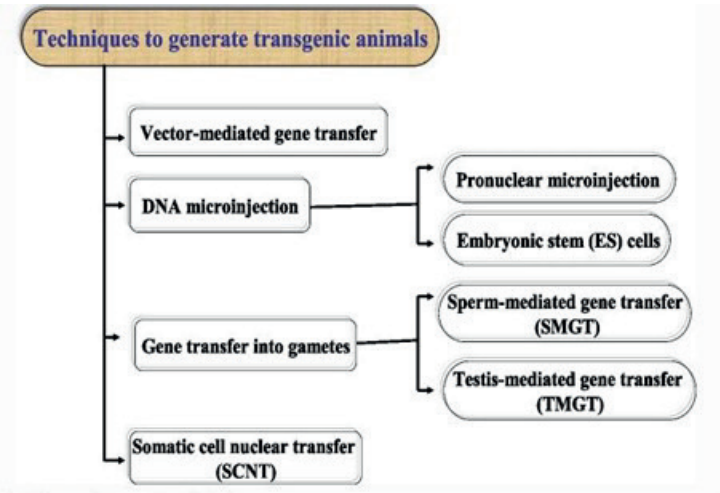



**Figure 3.1:** Techniques for creating transgenic animals (Shakweer et al., 2023).

### 3.1 Animal welfare consideration in production phase

Over the years, demand of genetically modified animals for the production of pharmaceuticals, vaccines, antibodies and other such compounds, has been increased rapidly. To get maximum output, large number of animals are reared in relatively small space. Attention is paid on increasing production; whereas little attention is paid towards the wellbeing of animals that damage their physical as well as mental health. However animal welfare awareness has been increased recently due to animal welfare training at colleges and universities (Fridovich et al., 2024).

It is hard to present single definition of animal welfare, interpretation of animal welfare changed with moral view of the concerned person. Generally we can define animal awareness as "how well an animal is coping with the conditions in which it lives" (Sinclair et al., 2022). In simple words animal welfare means to check that animals are not being treated cruelly, or are not given unnecessary pain or suffering. Animal's welfare status is satisfactory if animals are managed with little effort and less expenditure. However, if animal fail to cope with living conditions, animal's welfare status is said to be unsatisfactory. Another way of defining animal's welfare is physical and emotional state of animal due to human behavior. The amount of resources and quality of environment available to an animal is also considered as animal's welfare. Farm animals have feelings as well as emotions, therefore psychological well-being of these animals is very important in order to prevent them from mental trauma (McMillan, 2020). Therefore in order to cope with animal's ability physical and mental suffering should be kept as minimum as possible.

#### 3.1.1 Housing and management

In order to get maximum benefit from animals, proper housing and management is very important. Good management means to provide better housing and proper care to animals so that can grow properly; gain health; reproduce and provides consistent research data. Appropriate housing provides comfort zone to animals where they can move freely and express natural behavior (Faith et al., 2020). They must be provided with clean bedding, enough space for moving and social environment. Many factors need to be considered for appropriate management of animal's housing

and suitable living environment. These factors are;

1. First and most important factor is individual's characteristics like species, age, sex, and behavior.
2. It is very important to check animal's ability to live either in groups or singly (Gartland et al., 2022).
3. Design and construction of house is very important factor in managing animal's housing (Singh et al., 2020).
4. Check the availability of supplements to the animals.
5. Proper attention to experimental designs like production process, breeding phase and testing results (Tiwari et al., 2023)
6. Animal manipulation and sensitivity of procedure applied needs to be considered (Steagall et al., 2021).
7. Any hazardous and disease-causing materials need to be checked.
8. Last important factor that needs attention is the duration of holding period (Temple et al., 2020)

While managing animal's housing, attention should be towards maximizing species-specific behavior and reducing stress inducing behavior. Social species should be housed in groups or pairs. In order to achieve desired housing for animal, strategy should be developed by animal care personnel and approved by IACUC. IACUC should take decision after consulting with veterinarian and investigator for animal's wellbeing and consistency with research objectives (Ahn et al., 2022). Then objective assessment should be made to confirm the suitable environment and for animals housing.

### 3.1.2 Food, Water, Bedding, Sanitation

Animals should be provided adequate non-contaminated and full of nutrients food. Animals should provide foods either on daily basis or as per their requirements. Nutrients requirements of the animals have been provided by National Research Council Committee on Animal Nutrition (2001). By considering their publications, we can check about quality of food, free from all types of contaminants (chemical, microbial and natural toxicants). Water is the most important factor for living bodies. Water acts as the medium for digestion of food, absorption of food and carrying out metabolic activities. Water is also necessary for seat secretion and for removing wastes from body as feces and urine. It acts as major component of body secretions like saliva, milk. It also functions as transportation medium for nutrients, hormones and other chemical messengers. It also assists food in moving through gastrointestinal tract (Saha et al., 2021). Water is also necessary to regulate body temperature through evaporation. A good farmer must make sure the availability of clean water close to the area. Hence, animals should have access to clean drinking water according to their requirements.

Water quality and purity definition changes with locality. If for some particular protocol, highly purified water is required; then water can be purified by various treatments to minimize contaminants. Treatment used for water purification should be given proper attention, as treatment may be suitable for one species and not for other species for example chlorination that is toxic for some species but non-toxic for others (Srivastav et al., 2020).

Cattle are sensitive to taste and odor of water as well as food. If food or water quality is low, they do not take it happily that ultimately lead to decrease in growth as well as production. It will also affect their performance and make them more vulnerable to diseases.

One of the important factor affecting animal's welfare is bedding. Bedding can have effect on experimental data as well. Bedding material should be selected by veterinarian in consideration with investigators. There isn't any ideal bedding for all species, similarly one bedding isn't ideal for species in all conditions (Carter and Lipman 2018). For example, softwood beddings have been recommended for animals but on the other hand use of untreated softwood shavings can effect metabolism of the animal. Similarly Cedar shavings are not recommended because they continuously pass out aromatic hydrocarbons that create cytotoxicity.

Healthy maintenance for animal's welfare includes bedding change, space cleaning and disinfection. Place where animal is living should be clean on regular basis to remove excessive amount of dirt and debris. It should be disinfected to reduce or eliminate the quantity of microorganisms (Carpenter, 2018).

### 3.1.3 Body Temperature, Ventilation, Light

Homeotherms can survive well in temperature range 36-42ºC. It is very important to maintain animal's body temperature within normal range so that they can grow well. If animals are kept in temperature above or below the ambient temperature, without any shelter, their non-basal metabolic rate increases (Shephard, 2023). However, they can tolerate increased temperature upto a certain limit that defined by their increased metabolic rate. In figure 3.1.1 an increase in metabolic rate of mice is shown when exposed to high temperature.

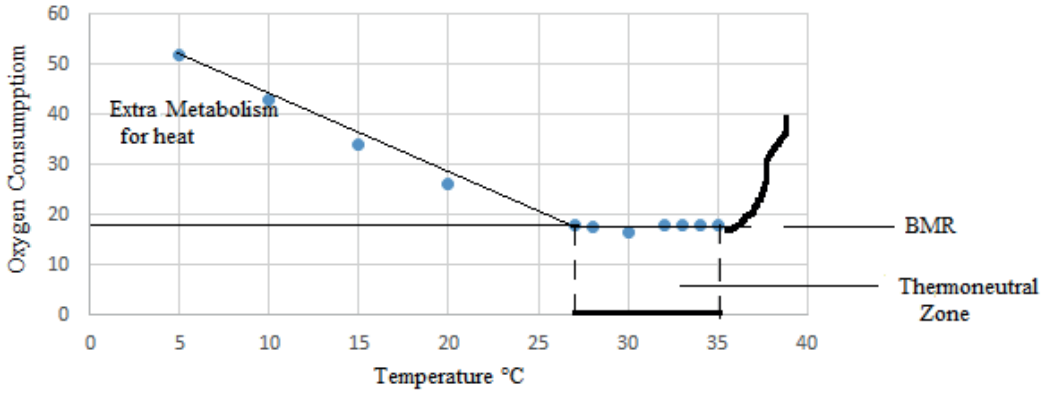



**Figure 3.1.1** Increase in metabolic rate of mice when exposed to temperature range outside the ambient temperature. Increased in metabolic rate constrains the limit upto which mice can tolerate the excess temperature (Akin, 2018).

During production phase animals should provide proper ventilation so that maximum product can be achieved from them. Ventilation refers to the provision of adequate amount of oxygen and remove carbon dioxide produced by animal's respiration. Moisture content of room should also be adjusted properly and static pressure differentials should be created between adjoining spaces. Care should also be focused on primary enclosure of animal, as properly ventilation of room does not ensure the adequate supply of ventilation to the animal (Oliveira, 2020).

Light, another factor that needs to be properly managed during production phase of animals as animals can vary their behavior with light intensity. Their physiology and morphology is also affected by the light. Major light contributing factors affecting animal's behavior include inappropriate light duration, increased/decreased light intensity, and quality of the light (Penev et al., 2014). There are several factors that can affect the animal's need for light. These factors include light intensity of light, wavelength of light, animal's body temperature and pigmentation, hormonal condition of the animal, age, sex, species and strain. Animal's room must be monitored regularly by keeping these factors in consideration.

Generally animals produce noise by themselves and noise is also created by animal care activities. Therefore during production phase noise control should be considered an important factor. Noise intensity, frequency, duration and vibration should be in accordance to the tolerance range of the animal (Arcangeli et al., 2022).

### 3.1.4 Protein Collection

During protein collection form animals they are exposed to several different processes including livestock farming, aquaculture and harvesting. It is ethical, moral and legal responsibility to ensure animal welfare during the protein collection (Hampton et al., 2021). Here are some key points that should be focused while collecting proteins from the animals.

Livestock farming practices should be managed properly to ensure appropriate space for animal growth. Animal should be supplied with adequate amount of nutrition, food, water and oxygen to maximize protein production (McAllister, 2020).

Animals should be treated politely while collecting protein form them. Whether they are harvested for protein, meat or dairy products, they should be treated with care. Workers should use human slaughter methods to reduce the pain and stress to the animals during protein collection. Such types of techniques like stunning before slaughter, should be used that keeps animal unconscious before death (Riaz et al., 2021).

Transporting animals from their development house to processing house is very difficult phase. Animal could have much stress and may be harmed if not manage properly. So while transporting animals, minimize transportation time, arrange comfortable vehicle that must be properly ventilated and handle animal with proper care (Brindle, 2016).

To provide animals with proper healthcare and to protect them from diseases is very crucial step for animal welfare. Animals should be vaccinated regularly, they must be provided with proper veterinary care. Take essential biosecurity measures to prevent the spread of disease in them (Schat, 2014).

While selecting animals for traits like enhanced milk or meat production, balance should be maintained. As breeding animals purely for productivity purpose can lead to several welfare issues like musculoskeletal problems or reduce the power of animal to fight against diseases. Responsible breeding should prioritize the animal welfare and health (Kwon et al., 2014).

Animals should provide opportunities to outdoor space, they should have possibilities to express natural behavior and interact socially. Animal welfare can be improved by allowing them to show their natural behavior and keep them in stress free environment (Sueur and Pele, 2019).

While extracting proteins, workers should follow regulations and standards of the country. By following these standards animals welfare can be guaranteed and their health can be improved (Pejman et al., 2019).

Consumers should be aware about ethically produced protein and demands to get ethically produced protein should be increased. Consumers should choose those proteins that are produced in compliance with ethical standards prioritizing animal welfare (Boer et al., 2022).

In summary animal welfare during production phase is multifaceted approach that involve various approaches that have discussed above. By prioritizing animal welfare, we can get ethically produced and sustainable protein.

### 3.2 Animal welfare consideration in development phase

A few factors related to animal welfare are taken into account when developing biopharmaceuticals.

- Ensuring that the transgene is expressed only in the desired tissues to avoid harm to the animal

- Selecting animals that are well-suited for biopharming, such as those with high reproductive efficiency

- Implementing human endpoints for animals that develop adverse reactions

- Minimizing animal suffering through proper husbandry and care

- Considering the ethical implications of genetic modification

- Measuring good feeding, good housing, and good health

- Ensuring that animals are handled humanely and do not receive unnecessary constraint throughout the observation

**4 Techniques of Creating Transgenic Animals**

**4.1 Donor animals and foster mothers**

Donor animals and foster mothers play crucial roles in biopharming. Both are essential for the successful production of transgenic animals.

Donor animals:

- Provide genetic material (e.g., DNA, sperm, or eggs) for genetic engineering

- Typically males, used for their genetic material to produce transgenic offspring

- Selected based on desirable traits, such as high milk production or specific protein expression

Foster mothers:

- Surrogate mothers that carry and give birth to transgenic offspring

- Provide a nurturing environment for the developing young

- Often used when the genetic mother is unable to carry the pregnancy to term or produce viable offspring

There was a general demand for restrictions on meddling with life after Dolly the sheep was cloned. Dolly was not a typical sheep. In a very real way, she had no father. She began as a cell removed from her biological mother's udder. The nuclear genome of a sheep ovum was removed before this cell was introduced, and it was then altered such that it merged with the ovum's cytoplasm, or "egg-mass," to form an embryo. After the embryo was implanted, the foster mother became pregnant and had a typical pregnancy under strict supervision, giving birth to Dolly as shown in Figure 4.1 (Lassen et al., 2006).

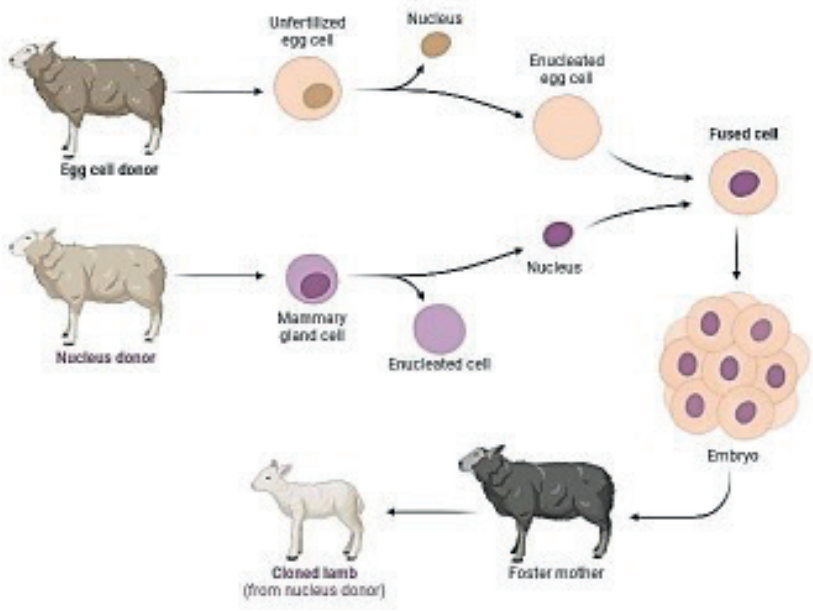



**Figure 4.1:** Donor animal and foster mother showing by dolly birth using cloning (Shakweer et al., 2023)

### 4.2 DNA Microinjection

Direct DNA insertion into the pronuclei of fertilized eggs can be referred to as microinjection. This technique is popular because it can target specific genes and has a reasonably high effectiveness. Despite its effectiveness, this method's failure rate is high because DNA integration into the host genome is unpredictable (Wolf et al., 2000). The success rate of this technique varies between species (Pinkert C, 2002). Pollock et al, 1999 explained the production of recombinant proteins now has new options thanks to the capacity to alter animal genomes using microinjection technologies manufacturing human recombinant protein drugs with the intention of using the milk from transgenic farm animals (Figure 4.2).

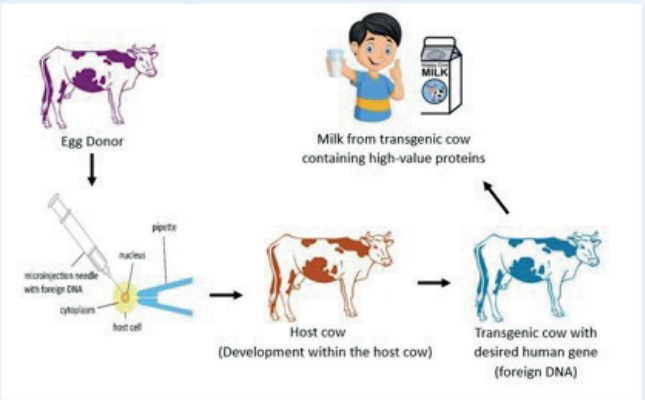



**Figure 4.2:** Transgenic cow producing high quality milk by DNA Microinjection

### 4.3 Embryonic stem (ES) cells

The capacity of undifferentiated stem cells to differentiate into other form of cell, including germ and somatic cells, resulting in the creation of an entire organism, is one of their characteristics. It has long been the practice to produce embryonic stem cells in vitro (Yamanaka S, 2020). Homologous recombination is used to introduce the correct DNA sequence into an embryonic stem (ES) cell culture in vitro. ES cells can be transfected with foreign DNA, and clones containing the foreign gene can be produced by using a selective gene. Transgenic chimeric mice could be created using these cells (Figure 4.3).

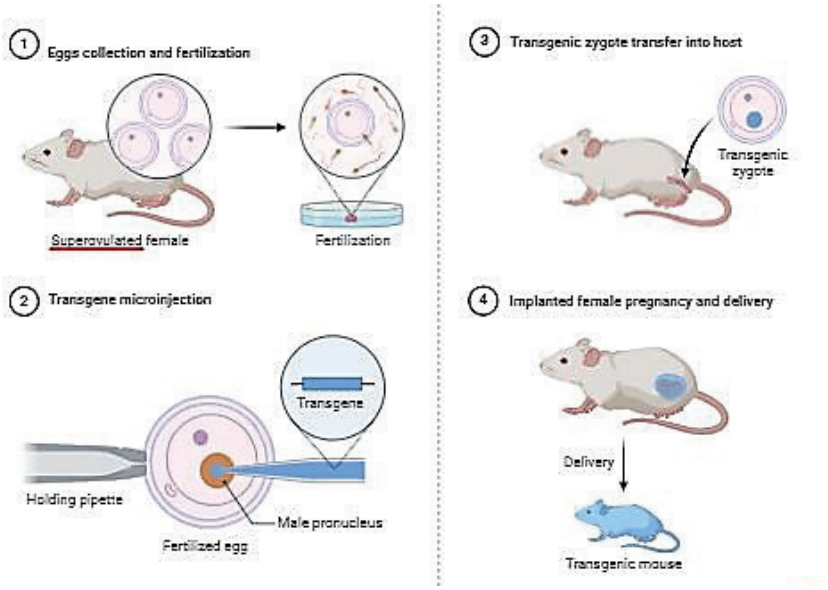



**Figure 4.3:** DNA microinjection technique using embryonic stem cells.

In these animals, the transgene is mosaic (Kim GB, 2019). When leukemia inhibitory factor (LIF) is added to a culture of stem cells in the lab, the cells remain undifferentiated. ES cells have the ability to differentiate into diverse tissues independently due to the lack of LIF (Figure 4.4).



**Figure 4.4:** In vitro cultured embryonic stem cells (Shakweer et al., 2023).

### 4.4 Gene transfer into gametes

Sperm mediated gene transfer technique (SMGT) The first indication that foreign DNA might be integrated into untreated sperm was given by Brackett et al., 1971 and Lavitrano et al.1989 for the first time (a) mouse epididymal sperm can spontaneously incorporate plasmid DNA molecules, (b) Through in vitro fertilization techniques with sperm cells that contain plasmids, genetically edited progeny can be produced, (c) Exogenous DNA sequences are expressed in the progenitors (d), and the fertilized ovum incorporates exogenous DNA brought by the sperm as depicted in Figure 4.5 (Catherine et al., 2003; Magnano AR et al., 1998).

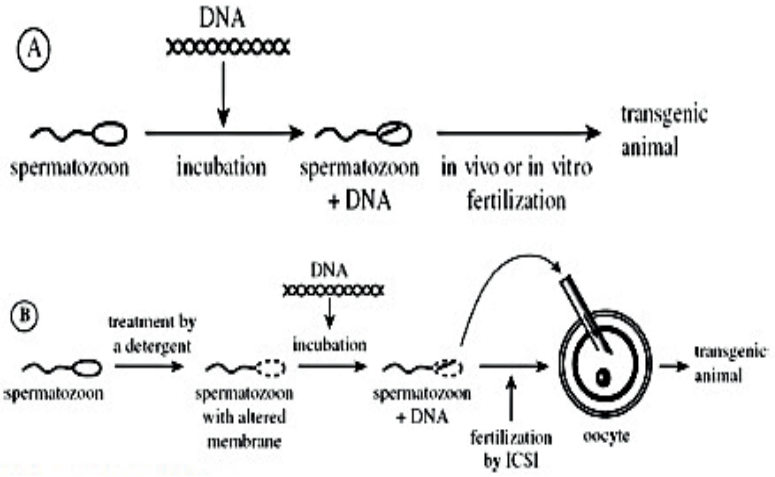



**Figure 4.5:** Sperm mediated gene transfer technique (Shakweer et al., 2023).

### 4.5 Somatic Cell Nuclear Transfer (SCNT)

The process known as Somatic Cell Nuclear Transfer (SCNT) involves transferring the nucleus of a genetically altered somatic cell into an enucleated oocyte (egg). Animal cloning is another usage for this technology, which can also be used to generate transgenic animals that express recombinant proteins (Tian XC et al., 2003). This technology gained notoriety with the cloning of Dolly the sheep and allows precise genetic modifications (Figure 4.6).

The process entails moving the nucleus of a somatic cell into the cytoplasm of an enucleated egg, where it will be reprogrammed to form a zygote by components found in the egg's cytoplasm. (BallP J. H and Peters A. R, 2004; Campbell KH, 1996; Wilmut I and Whitelaw CBA, 1994). Mammals require the zygote to be artificially inserted into the uterus of a surrogate mother. (Camara D et al., 2008; Denning C, 2001). Homologous recombination is now the only method available for gene substitution in somatic cells, which are used produce build animals. In sheep, gene inactivation has been accomplished (McCreath KJ, 2000) and pigs (Lai L and Prather RS, 2002). According to findings in cattle, sheep, goats, and pigs, most animals cloned from transfected somatic cells express the transgene (Table 4.1).

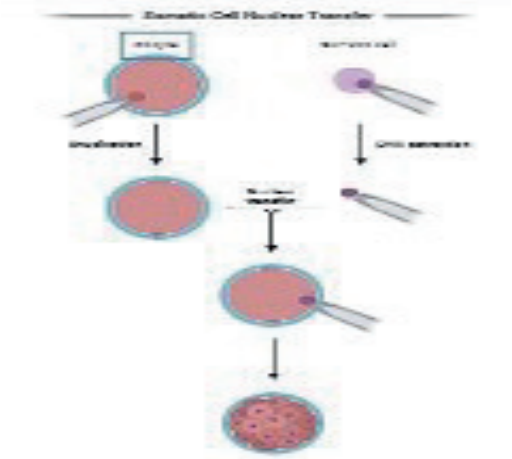


**Figure 4.6:** Nuclear transfer method using somatic cells

**Table 4.1:** An analysis of transgenic cattle species and their contribution to the synthesis of protein

| Addition/ deletion of Genes | Animal | Performance benefits | References |
|---|---|---|---|
| β and κ casein | Bovine | The expression of casein protein has risen resulting enhanced milk's protein content | Laible G et al., 2016 |
| Intestinal lactase | Bovine | There is less lactose in milk now for lactose intolerant individuals | Marshall A, 1999 |
| Lysostaphin | Bovine | Reduced usage of antibiotics due to resistance to mastitis | Cardoso CV et al., 2019 |
| β-Lactoglobulin | Bovine | Increased growth and disease resistance in calves fed milk along with high levels of protein in the milk resulting less antibiotic use and better health benefits | Sun Z et al., 2018 |
| Growth hormone | Ovine | enhanced feed conversion efficiency, faster rates of lactation (leaner meat), decreased carcass fat content, and increased growth rates | Rexroad et al., 1991 |

| Addition/ deletion of Genes | Animal | Performance benefits | References |
|---|---|---|---|
| Myostatin | Ovine | Myostatin expression was downregulated in sheep, although muscle mass increased (leaner meat) | Hu S et al., 2013 |
| Insulin-like growth factor 1 | Porcine | better development rate and less fat content in the carcass | Monaco MH et al., 2005 |
| α-Lactalbumin | Porcine | Health and growth rate of the piglets have both improved | Noble MS et al., 2002 |

### 4.6 Retroviral Vectors

Genetic material is inserted into the host genome using retroviral vectors. By integrating with the host DNA, these vectors guarantee consistent gene expression. Transgenic cattle may be produced with this technique, which is especially helpful. Although significant levels of gene expression may be achieved with this approach, the possibility of viral pathogenicity may raise safety concerns (Shakweer et al., 2023).

### 4.7 CRISPR-Cas9 Gene Editing

The latest advancement in genetic engineering, CRISPR/Cas9 allows for precise editing of the genome by creating targeted double-strand breaks in DNA, thereby facilitating the insertion or deletion of specific genes. This technology has revolutionized genetic engineering, providing a highly efficient and versatile tool for creating transgenic animals. (Javaid D et al., 2022; Adli, 2018).

## 5 Applications of Transgenic Animals

These include transgenic animals used for outputting proteins;

### 5.1 Pharmaceutical Proteins

Transgenic Animal Derived Recombinant Proteins for the production of therapeutics, vaccines, and markers on face of crucial public health concerns, recombinant proteins from transgenic animals are mandatory. One example is human antithrombin the, which is produced in goat milk and used to treat blood clotting disorders (Table 5.1).

Therapeutic In some cases, transgenic animals are also used to produce therapeutic proteins, such as insulin, growth hormones, and antibodies. Often these proteins are extracted from animals' milk, blood or urine (Echelard et al., 2009).

Insulin: Originally made from the pancreases of slaughtered animals, insulin is now produced by bacteria and yeast that have been genetically engineered to synthesize human insulin and is no longer obtained from animals four-legged animals.

Antithrombin: The milk of transgenic goats contains antithrombin, which can be used to prevent blood clots occurring in the veins and arteries of patients with hereditary antithrombin deficiency.

Human Growth Hormone (Protein): Produced in transgenic cows and goats, it tends to be more stable and is a cheaper resource as compared to traditional collection.

**Table 5.1:** Pharmaceutical proteins and their sources (Echelard et al., 2009).

| Pharmaceutical Proteins | Source |
|---|---|
| Insulin | the pancreas of slaughtered animal, bacteria, and yeast |
| Antithrombin | the milk of transgenic goats |
| Human Growth Hormone | transgenic cows and goats |

### 5.2 Nutritional Proteins

It is possible to design transgenic animals to generate proteins with higher nutritional content, better disease resistance, or faster growth rates. This could lead to more efficient and sustainable farming practices. Additionally, transgenic animals can be used to produce proteins for human and animal nutritional supplements. These proteins can enhance the nutritional value of food.

- Human lactoferrin has been added to the milk produced by transgenic cows, improving the milk's nutritional value and antibacterial capabilities (van Berkel et al., 2002). Transgenic animals could lead to more cost-effective and ecologically sustainable manufacturing methods for industrial enzymes and other proteins (Humphrey et al., 2002).

### 5.3 Industrial Enzymes

Industrial enzymes, such as those required for food processing or the production of biofuel, can be produced using transgenic animals. These enzymes could be more reasonably priced and environmentally friendly than those produced using more traditional methods. Transgenic animals produce enzymes like phytase, which increase the bioavailability of phosphorus in animal feed (Table 5.2).

**5.3.1 Antithrombin-Producing Goats** Transgenic goats have undergone genetic modification to generate antithrombin in their milk, which is used to treat patients with antithrombin deficiency (Echelard et al., 2009).

**5.3.2 Human Lactoferrin Produced by Cows** This milk protein with antimicrobial properties is being produced in transgenic cows to improve baby formula (van Berkel et al., 2002).

**5.3.3 Human Lysozyme Produced by Chickens** Lysozyme, an antibacterial enzyme produced by transgenic chickens, may be used to treat bacterial infections in humans and other animals (Zhu et al., 2010).

**Table 5.2:** Role of transgenic animals in protein production

| Proteins produced by transgenics | Benefits | References |
|---|---|---|
| Goats Producing Antithrombin | for the purpose of treating individuals who lack antithrombin | (Echelard et al., 2009) |
| Cows Producing Human Lactoferrin | to enhance infant formula milk with antibacterial qualities | (van Berkel et al., 2002). |
| Chickens Producing Human Lysozyme | to treat bacterial illnesses in humans and other animals | (Zhu et al., 2010). |

### 6 Ethical and Regulatory Considerations

The ethical and legal issues surrounding the manufacturing of proteins from transgenic animals are numerous. Although regulations vary by country, they all often require thorough testing to ensure safety and efficacy. Animal welfare, possible effects on biodiversity, and the moral ramifications of genetic engineering are examples of ethical issues. The adoption and commercialization of these technologies are also significantly influenced by public perception. (Babale YK and Atoi EN, 2021)

### 6.1 Ethical Issues

Concerns regarding animal welfare, genetic variety, and environmental effect are raised by the production and application of transgenic animals. Ethical rules and standards need to be thoroughly thought through in order to handle these difficulties.

### 6.2 Regulatory Framework

Numerous national and international organizations govern the usage and production of transgenic animals. Rules guarantee the items effectiveness and safety as well as the wellbeing of the animals involved (Ormandy EH et al., 2011).

### 6.3 Public Perception

The advancement and use of this technology may be influenced by how the general public views transgenic animals. To address public concerns and promote acceptance, it is imperative to prioritize transparency, education, and involvement (Einsiedel EF, 2005).

## 7 Risk assessment of animal pharming

In August 2006, the European Commission unequivocally sanctioned ATryn® as the inaugural animal pharming product intended for human use. ATryn® is presently progressing through phase III clinical trials in the USA. More pharmaceuticals derived from transgenic animals are in advanced stages and are expected to be commercialized soon. These innovative production platforms for pharmaceuticals could potentially impact the environment, posing risks to both animal and human populations. Pharmaceutical production will now occur outside the laboratory, and in some cases, it will be entirely uncontained. Pharming animals may lead to the release of active products into the environment, and GM animals could disperse and impact other ecosystems (Rehbinder et al., 2009)

The new production platforms pose a challenge to current regulations governing traditional pharmaceutical products, GM animals, and animal welfare. The existing legislation pertaining to these matters is intricate, at times overlapping and at others inadequate. Consequently, the implementation of new procedures and regulations is imperative (Ormandy EH et al., 2011). The purpose of the upcoming chapters is to address the adverse environmental consequences associated with pharmaceutical production in genetically modified animals and to offer an overview of the current approaches used to evaluate these effects.

### 7.1 Biological Risks

#### 7.1.1 Genetic stability and mutation risks

Biological risks in animal pharming mainly concern the genetic stability of the modified organisms. Genetic mutations or unexpected changes can emerge, potentially causing detrimental effects on the animals or the effectiveness of the produced proteins. It takes extensive rigorous testing and observation throughout the animals' lives to guarantee genetic stability (Van Reenen CG and Blokhuis HJ, 1997).

#### 7.1.2 Impact on animal health and welfare

The welfare and well-being of the participating animals pose a serious biological risk. Unintended physiological effects resulting from genetic alterations, leading to suffering or decreased quality of life for the animals. Ethical considerations need careful attention to animal welfare, with techniques in place to alleviate any negative impacts (Clark J and Whitelaw CB, 2003).

### 7.2 Environmental Risks

The possible effects of genetically modified animals escaping into the environment and being used for pharmaceutical manufacture would depend on a number of things. If they would present an

urgent risk of harm, that should be taken into account first. If they were afflicted with an infectious sickness, for example, the circumstances would be the same. Furthermore, if ingested by humans or wildlife, it could have harmful effects. This is unlikely, and the potential for toxicity would be influenced by the bioactivity of the expressed protein, as well as the sites and levels of expressions. Large mammals can be kept from escaping by employing straightforward physical containment techniques like double fence. There will always be a chance of escape because of things like criminal activity, but in houses with barrier features, escapes might be virtually completely avoided. Small mammals, fish, and insects may be easier to get away from and unrecoverable (Nash et al., 2004; Van et al., 2011).

#### 7.2.1 Potential for cross-breeding with wild populations

Environmental risks may arise from the possibility of genetically modified animals mating with wild populations. Such events could result in the unintended dispersion of altered genes into the environment, causing disruption to ecosystems and natural genetic variation. To effectively manage this issue, we must implement strict containment measures, such as physical barriers and regulated breeding environments (Kapuscinski AR and Hallerman EM, 1991).

In the event of animals used for pharming escaping, a crucial consideration is to assess their potential for long-term survival and ability to reproduce in a natural environment without human intervention (Rehbinder E., et al. 2009). For instance, it is highly improbable that domestic sheep and cattle would disappear, survive, and reproduce. It is true that laboratory rabbits and domestic hens are vulnerable to illness and predators . Their phenotype probably cannot compete with other competitors or conspecific wild kinds. Nevertheless, the environment into which the animals escape may have some bearing on the response to this query. The FDA advises neutering transgenic animals in order to lower the possibility of accidental reproduction leading to a non-transgenic population. In the event that animals are able to get out, they could cause environmental disruption and turn into pests.

#### 7.2.2 Ecological impacts and containment strategies

An extensive assessment of the ecological consequences of animal pharming is also necessary. The introduction of genetically modified organisms into the ecosystem could have unforeseen consequences that upset biodiversity and ecological balance. Continuous environmental monitoring and impact evaluations are essential for identifying and reducing these risks (Muir et al., 2002).

### 7.3 Ethical and Societal Risks

#### 7.3.1 Ethical considerations and public perception

The discussion of animal pharming revolves around ethical concerns. Concerns about the treatment of animals and the naturalness of genetic changes can shape public opinion. Transparent communication of the advantages and disadvantages as well as the moral principles governing the use of animal pharmacology in research and practice are necessary to allay these issues (Kaiser, 2005).

#### 7.3.2 Regulatory and legal challenges

Legal and regulatory obstacles are also important. Regulations pertaining to animal care and genetic alteration differ between nations, therefore it's critical to arbitrate such variations to guarantee public acceptability and compliance (Van der Meer P and Tzotzos GT, 2011).

### 7.4 Economic Risks

#### 7.4.1 Market volatility and investment risks

Market dynamics and financial risks can have an impact on how profitable animal medicines

are. Market volatility can have a financial influence on the manufacture of high-value proteins and pharmaceuticals, and the initial investment costs are sometimes substantial. A thorough cost-benefit analysis is necessary to determine whether animal pharmaceutical projects are financially viable (James, 2000).

### 7.4.2 Cost vs. benefit analysis in production

Investors and companies must consider the risk that methods employed today will become obsolete due to future technology advancements, necessitating ongoing research and development to stay competitive. Achieving a balance between the potential for significant medical and pharmacological benefits and these financial risks is critical to the success of animal pharmaceutical programs (Kalaitzandonakes et al., 2007).

### Conclusion

To sum up, animal pharmaceutics holds considerable promise for the production of valuable proteins and pharmaceuticals. Nevertheless, it is linked to some hazards that require cautious evaluation and handling. A gene encoding a therapeutic protein is inserted into an animal's genome during transgenesis to enable the production of the protein by the animal. This strategy offers a number of advantages including high yields, correct folding, Post-translational modifications and low cost. A few instances of therapeutic proteins created via transgenesis are as follows:

- Insulin (diabetes management)
- Human growth hormone (therapy for growth hormone deficiency)
- Factor VIII (medication for hemophilia A)
- Enzyme replacement therapy (lysosomal storage disease treatment)
- Vaccines made from genetically modified chickens
- Genetically modified goats produce ATryn (antithrombin III)
- Genetically modified rabbits produce Ruconest (C1 esterase inhibitor)
- Sebelipase alfa, or kanuma, grown in genetically modified chickens

These medications are used to treat a variety of uncommon genetic conditions, including lysosomal acid lipase deficiency, hereditary angioedema, and hereditary antithrombin deficiency. Benefits of biopharmaceutics include enhanced productivity, lower expenses, and better-quality products. But it also brings up moral and environmental issues with genetic engineering and animal welfare.

### Future Directions

Transgenic animal protein production has substantial advantages for commerce, agriculture, and health, among other areas. But it's crucial to strike a balance between these advantages and moral considerations as well as government regulation. Because of the advances in genetic engineering and biotechnology, there is great potential for the synthesis of transgenic proteins in the future. The manufacture of transgenic animal proteins seems to have a promising future due to advancements in gene editing techniques such as CRISPR/Cas9. Emerging uses include developing complex biopharmaceuticals and improving the nutritional content of animal products. But in order to use this technology effectively, a number of problems must be handled, including ethical quandaries, legal restrictions, and technological limitations.

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