

Future Frontiers: Emerging Trends and Applications in Animal Biotechnology

Shumaila BATOOL Hareem FAYYAZ

Urwa SHEIKH

Anam SHEHZADI

Fatima AMIN

1. Introduction

Animal biotechnology is a sub-discipline of biotechnology that involves the use of molecular biology tools to modify animals genetically. The main aim is to enhance the stability for usage in agriculture, industry, or medicine. Biotechnologies have played an important role in enhancing livestock production, especially in developed countries, and can potentially eradicate poverty, hunger, diseases, and environmental degradation in developing countries. Many biotechnologies are available, and some have already been applied in the main areas of animal science in developing countries, such as animal reproduction, genetics and breeding, animal nutrition and production, and animal health (Estrada et al., 2017). In addition to their role in characterizing and conserving animal genetic resources, molecular DNA markers can also be utilized for genetic improvement through marker-assisted selection. Microorganisms, including those created using recombinant DNA technology, are extensively used in animal feeding and production biotechnologies. Biotechnologies in animal health are utilized to improve the precision of complications diagnosis, disease control, and therapy. So, there is a need for specific advice that can assist developing countries in making advancements through the utilization of appropriate animal biotechnologies in the future (Mekonnen, 2021). This chapter aims to highlight the recent advances in the field of animal biotechnology and to explore the biotechnological applications in animals.

1.1 Historical Overview Of Animal Biotechnology

The utilization of animal biotechnology originated about 8000 years ago, coinciding with the commencement of humans domesticating and selectively breeding animals (Aerni, 2021). In 1917, Karl Ereky introduced the phrase "beet diet" to describe a feeding method employed to raise many pigs (Wu-Pong, 1999). Subsequent historical findings have spurred biotechnology to emerge as a highly active industry and scientific field during the latter part of the twentieth century. Biotechnology has been increasingly important in manipulating organisms' DNA at the molecular level, particularly after the discovery of the structure of DNA in 1933 and the invention of ways to modify DNA in 1970. The transition from animal production as a means of subsistence and extraction to a commercial enterprise occurred during the twentieth century. During that period, there was an increasing need for animals that exhibited superior performance and increased adaptability to various environmental conditions (Greenfield, 2010).

As a result, efforts were made to start breeding programs for a variety of animal species, including sheep, goats, pigs, cattle, and poultry. These programs were developed using domesticated breeds' genetic makeup as a foundation. They were implemented intuitively, considering the desirable features related to production and aesthetics found in animals with superior phenotypes. As a result, this selection process resulted in more advantageous alleles that help in livestock improvement, disease diagnosis, and treatment (Gowane et al., 2019).

1.2 Current State of Animal Biotechnology

Animal biotechnology has experienced significant advancements throughout the last two decades. The advent of animal biotechnology in the contemporary period occurred after the

identification of the genetic code in the mid-1930s (Rasmussen, 2014). Currently, there are over 230 Biotechnology Health Care Products and Vaccines accessible to patients, primarily for diseases that were previously incurable. Over 13.3 million farmers worldwide employ agricultural biotechnology to increase crop yields, minimize pest-related harm, and mitigate the environmental impact of farming (Kesik-Brodacka, 2018). Recent progress spans multiple fields, such as genetic editing methods like CRISPR-Cas9, which enable accurate genetic alterations to enhance animal well-being and product excellence. Cloning and reproductive technologies provide chances to save uncommon genetic characteristics and improve cattle productivity, yet they come with ethical and genetic diversity dilemmas. Advancements in technology, such as enhanced computational capabilities, genomic sequencing, cloning, regenerative medicine, and direct gene manipulation, have allowed individuals to significantly modify animals for various purposes, such as food production, medical applications, and scientific research. Modern biotechnology is the result of humans manipulating the environment and the development of molecular and computational technologies. Modern biotechnology offers advanced solutions and techniques to address severe and uncommon illnesses, minimize our impact on the environment, alleviate hunger, promote cleaner and more sustainable energy sources, and enhance the safety, cleanliness, and efficiency of industrial manufacturing processes. Transgenic animals, genetically modified with other organisms' genes, are used for many goals, such as enhancing production and studying diseases (Khan, 2020). Recent developments and progress in biotechnology equip us to prepare for and address society's most pressing concerns. However, the use of these animals raises ethical and regulatory issues.

2. Emerging Trends in Animal Biotechnology

Global livelihoods depend significantly on livestock agriculture for financial stability, draught power, and offering non-food items in addition to food. It is anticipated to rise in value and now represents roughly 43% of the gross value of agricultural output (Pingault et al., 2016). Over one-third of the agricultural GDP in developing nations comes from the livestock production industry, one of the world's most rapidly evolving sectors. By 2030, it's expected that the market for animal products will have almost doubled, with developing nations accounting for most of this growth (Yitbarek, 2019).

Sustainable animal production is confronting obstacles, including livestock-mediated waste, genetic resource loss, global warming, severe water scarcity, and the risk of developing infectious illnesses. A restricted natural resource such as water and land is the main problem in finding sustainable ways to increase cattle output. Modern scientific and technological developments will be crucial in advancing the livestock industry. Due to the rapid population growth, there are opportunities, in addition to problems, that increase the demand for animal products (Liu et al., 2020).

Biotechnology can increase animal productivity by improving growth, health, reproduction, nutrition, welfare, and waste management. Livestock production is expected to expand rapidly and overtake all other agricultural sectors by 2020 (Yitbarek, 2019). Current advancements in biotechnology have created new avenues for increasing the productivity of domestic animals in various ways, including the potential to improve animal growth, rates of reproduction, and nutrient efficacy. Developing nations, however, need to prepare for these economic and technological advancements, and their decisions may be influenced by social objectives and commercial concerns to meet global demand and promote genetic variety among animals (Sharma et al., 2021).

Animal biotechnology has recently advanced to the point where it has transformed several sectors, including environmental management, medicine, and agriculture. The ability to precisely modify animal genomes with CRISPR-Cas9 gene editing has changed the game. Food security has increased, and agricultural losses have decreased as a result of the development of disease-resistant livestock, such as swine resistant to Porcine Reproductive and Respiratory Syndrome Virus, which is made possible by this technology. Furthermore, with the successful cloning of species like the black-footed ferret, advances in stem cell research and cloning have aided the protection

of endangered species. To solve the severe lack of transplant organs, genetically modified animals are employed in medicine to create organs compatible with humans. Moreover, developments in synthetic biology have produced animals that have been bioengineered to create drugs, like goats that can make spider silk proteins in their milk for medical sutures. These innovations underscore the transformative potential of biotechnology in addressing global challenges (Laible et al., 2015).

2.1 Gene Editing and CRISPR Technology

Animal biotechnology relies heavily on CRISPR technology to create genetically engineered animals with desired characteristics like disease resistance. This method directs the Cas9 enzyme to a specific place in the genome, causing breakage in double-strand DNA. The cell then uses either homology-directed repair, which requires the presence of a DNA template, or non-homologous end joining to fix this break. In animal biotechnology, CRISPR is useful for many reasons (Singh & Ali, 2021). The first advantage is that it enables creation of genetically modified animals with desired characteristics, such as disease resistance, which can improve animal welfare and agricultural output (Figure 1). Second, by creating animals that can create organs compatible with humans, CRISPR helps progress in biomedical research. Finally, CRISPR contributes to conservation by enabling the genetic rescue of endangered species by introducing desirable characteristics or correcting deleterious mutations. CRISPR is an essential technique in animal biotechnology because of its accuracy, effectiveness, and adaptability (Wang & Doudna, 2023).

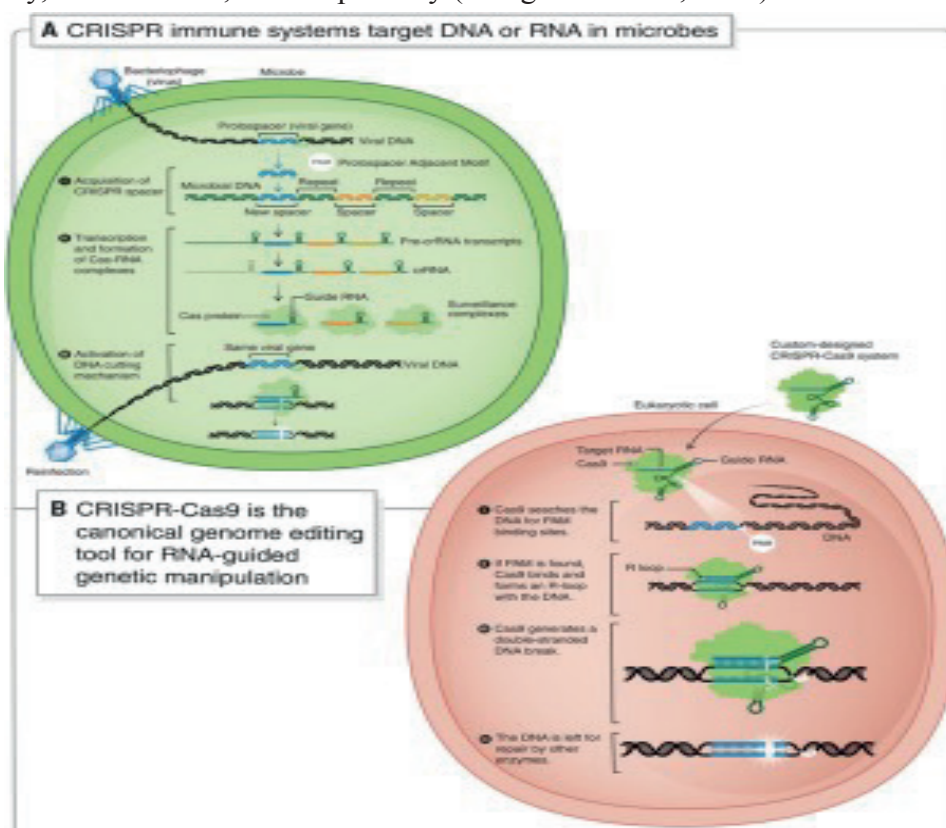


Figure 1. CRISPR-based editing of nuclear material for developing immunity (Wang & Doudna, 2023)

CRISPR is naturally found in archaea and bacteria, and their nuclear material is protected against foreign DNA (Munawar & Ahmad, 2021). In the field of genetic engineering, the CRISPR-associated system type II employs CRISPR-RNA to guide the CRISPR-associated nuclease to particular locations. This is accomplished through a CRISPR-RNA and transactivating CRISPR-RNA duplex, which is combined to form sgRNA (Single Guide RNA) to facilitate the process of DNA cleavage. Upon introduction into the cell or zygote, these constituents generate a double-strand break (DSB) by pointing Cas9 to the corresponding loci in DNA (Figure 2) (Menchaca et al., 2020).

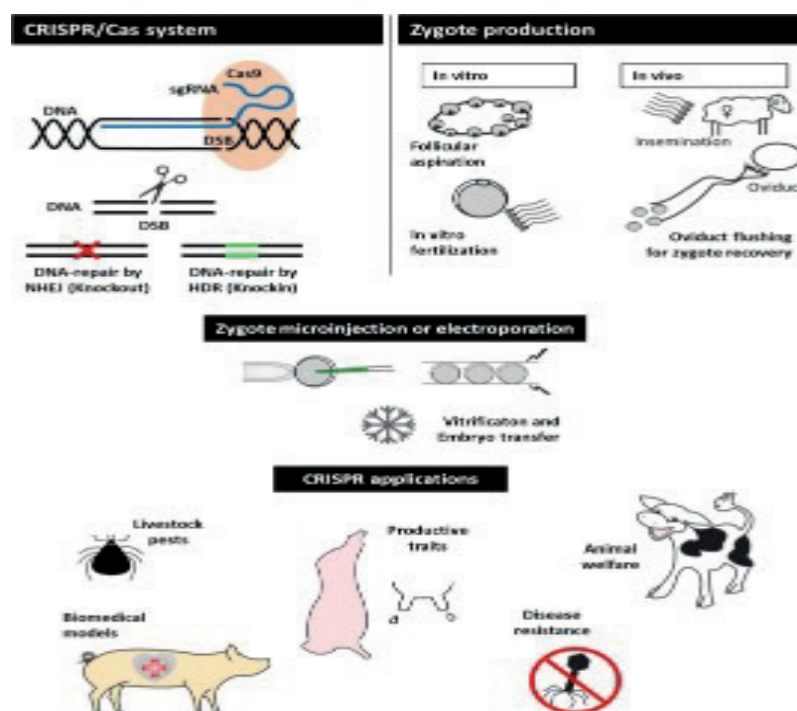


Figure 2. CRISPR-Cas system formation, zygote production and its implantation (Menchaca et al., 2020).

The CRISPR-associated system has been used to repair DSBs in livestock via NHEJ (Non-Homologous End Joining) and HDR (Homology Directed Repair). The former causes small deletions or insertions, disrupting open reading frames and creating knockouts of a few genes, and this method has been successful in generating genes in cows, goats, sheep, and pigs (HASAN et al., 2022; Park et al., 2019). In contrast, HDR uses a homologous-repair template to fix DSBs allowing specific changes in DNA, and this method has been successful in generating knock-in large animals in goats, sheep, and pigs (McLean et al., 2021). Recently, a new CRISPR tool called base editors was developed in which a combination of Cas 9 (inactive) and cytidine deaminase enzyme was used, which is cytidine with thymine (Kantor et al., 2020).

2.1.1 Applications in Livestock Improvement

The CRISPR technology is being utilized to reduce pest species, increase animal productivity and health, and improve animal comfort and disease resistance. Reports have been of this technology used for commercial usage, research, or proof of concept.

In 2014, MSTN mutation was introduced in the Merino breed, enhancing protein production and leading to the birth of the first animals modified using CRISPR. Superfine Merino is renowned for yielding the finest wool, but farmers endure reduced growth rates and smaller lambs. Traditional genetics, like selective breeding, has increased meat yield at the expense of decreased wool quality. At the embryonic stage, Merino was modified using CRISPR to break MSTN gene, resulting in twofold muscular lambs with faster development rates and higher body weights. In addition to being 23% heavier, the knockout lambs had the same characteristics of wool quality as Merino lambs (Crispo et al., 2015).

Pigs and Cashmere goats from China are two examples of additional species whose fitness or productive qualities have been enhanced through the application of CRISPR, and CRISPR has been utilized to enhance carcass qualities in pigs (Wang et al., 2015). Innovative approaches to using CRISPR to modify the male-to-female ratio in cattle have been put forth; these approaches are especially useful when only one sex can provide the desired result. To increase productivity

and prevent female culling, for instance, male offspring tactics have been suggested for beef cattle. Another strategy to prevent male-specific boar taint and induce a female phenotype in pigs is limiting testicle development in fetal genital ridges (Kurtz & Petersen, 2019).

Animals frequently experience acute or long-term pain during routine operations such as male castration, dehorning, tail docking, and abortion, and editing the genome can help prevent these actions. Calves often get their horns removed, but it hurts and stresses them out. Due to a polled feature, some cattle breeds, such as Angus, are naturally hornless. To reduce physical dehorning in Holstein cattle without sacrificing production, TALENs were employed to introduce the causal mutation (Carlson et al., 2016). It has also been suggested that CRISPR can create male pigs with germline ablations (Kurtz & Petersen, 2019), opening up new possibilities for pig surgical castration (Lee et al., 2020).

2.1.2 Potential for Disease Resistance

Cattle diseases can majorly affect public health, outbreaks, business, and productivity. The worldwide livestock and agriculture sectors are affected by some pandemics, such as the African swine disease that struck Asia. Animal welfare should be considered, as healthy animals are the first to experience sickness and death. An unusual increase in new and resurfacing animal illnesses, as well as infectious diseases, is being caused by this intensification of livestock production (Rohr et al., 2019).

An international animal illness called African swine fever is endangering neighboring areas and inflicting substantial losses in the Asian pig business. The virus infects domestic and wild pigs, although warthogs do not show any symptoms. The Rosen Institute's researchers suggest genetically modifying domestic pigs to provide the virus resistance to illness (Bisimwa et al., 2024). In China, coronavirus-resistant pigs and modified cows with heightened resistance to TB have also been created with CRISPR, which has promise as a cutting-edge approach to managing infectious illnesses in cattle (Menchaca et al., 2020).

2.2 Cloning and Transgenesis

Cloning is the process of making identical copies of DNA using advanced reproductive techniques such as embryo culture microsurgery and then transfer into surrogate mothers. The procedure creates embryos that can give rise to living, genetically identical individuals by inserting a nucleus into an oocyte with its nuclear DNA removed (Figure 3) (Keefer, 2015). In nature, clones like armadillos and identical twins exist. Artificial cloning requires embryo splitting, which splits blastomeres to generate smaller embryos

Transgenic animals are necessary for researching disease progression, analyzing strategies for treatment, and examining gene activity during development, organogenesis, and aging. Recombinant protein preparation, preventing illnesses for animals, and the introduction of unique genetic traits are among their commercial uses. Invertebrate species such as *Drosophila melanogaster* and *Caenorhabditis elegans* are commonly employed, whereas fish, amphibians, birds, and mammals are among the transgenic vertebrates. Nevertheless, their potential for portraying human illnesses may be limited due to their distant evolutionary link with humans (Christopher et al., 2022).

2.2.1 Cloning via Transfer of Nuclear Material

The most common methods for transferring donor cell nuclei into enucleated oocytes include injection, virus, and electrofusion. Oocytes in which the nucleus is transplanted require artificial activation because, in contrast to spermatozoa, embryonic and adult somatic cells cannot activate oocytes. Commonly utilized stimuli include chemical, electric pulses, or a mix of the two (Srirattana et al., 2022). Due to differences in the properties of donor cells and oocytes, there is no universal cloning procedure for all mammalian species. Every species requires a different set of technical

requirements.

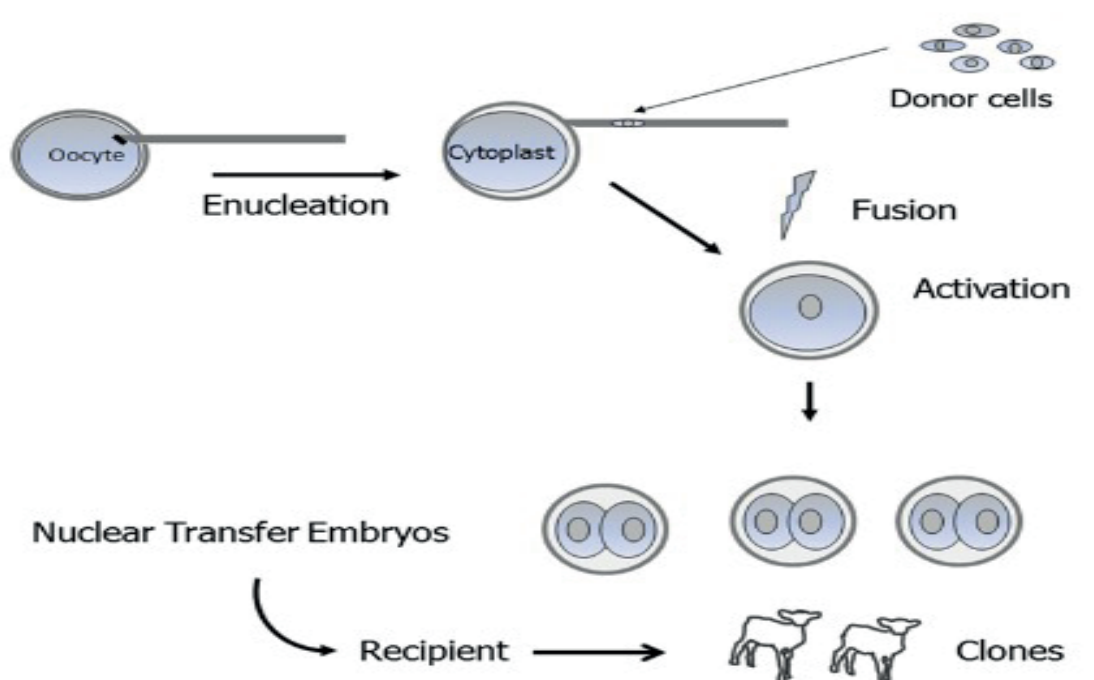


Figure 3. Cloning via transfer of nuclear material (Keefer, 2015).

2.2.2 Cloning via Early Embryonic Cell Nuclei

It is anticipated that every nucleus in early embryos, including those with two to eight cells, would possess the ability to grow into new organs. Although it is possible for an enucleated oocyte united with one of the eight blastomeres of an eight-cell (sheep) embryo to grow into a normal progeny (Hosseini et al., 2016), it has never been possible to produce eight offspring utilizing the blastomeres of a single eight-cell embryo. Still, the same applies for two- and four-cell embryos. The donor cell nuclei may become incapable of participating in normal embryonic development if the cytoplasm and nucleus are slightly damaged. Cloning has been attempted using fewer than 3% of the enlarged blastocyst cell mass and trophectoderm cells. Both cultured embryonic germ (EG) and embryonic stem (ES) cells have a 3 percent success rate (Rahbaran et al., 2021).

2.2.3 Cloning via somatic cell nuclei

The cloning success is just 3 percent, irrespective of the kind and age of the cells. This indicates the proportion of developmentally totipotent cells in any given tissue is less than 3% (Suzuki et al., 2016). Pre-selecting cells may help boost the success rates of cloning, perhaps preserving developmental totipotency in some tissues. Nevertheless, the majority of cloned embryos die before and after implantation, suffering from breathing issues, numerous systemic dysfunctions, and malformed placentas. The exception was cloned infants who lived to adulthood and could be borne again as 90% of mouse embryos cloned using cumulus cells had normal chromosomal composition. It's more likely that defective epigenetic reprogramming in contrast to genetic issues caused the majority of cloned embryos or babies to pass away (Hino et al., 2016).

2.2.4 Transgenic Animal Models for Research

Transgenes are being introduced in various non-mammalian invertebrates and vertebrates, such as fruit flies and nematodes. In fruitfly (Bachmann and Knust, 2008), transposable vector systems are used to inject the transgene into the cytoplasm, while in nematode (Caldwell et al., 2020), DNA via injection is introduced in syncytial gonad or maturing oocytes, targeting its germline which is

hermaphrodite. The suppressor tRNA (gene sup-7) can be included to prevent high-copy number transgenic lines.

In fish, the vitelline membrane is surrounded by a tough chorionic membrane, which means that microinjection can only occur through the micropyle. In *Xenopus*, an effective method is to incubate sperm nuclei that have been permeabilized with linearized DNA and then decondense the resultant mixture using an enzyme-restricted egg extract. It allows the integration of DNA before its fertilization, forming an embryo having non-mosaic transgenes (Hino et al., 2016).

Transgenes have been successfully inserted into huge numbers of sperm or eggs of fish by electroporation to create transgenic invertebrates or chordates. It has also been possible to introduce DNA in the fertilized eggs of loach, zebrafish, and a few other species via particle bombardment using metallic beads coated in DNA (de Siqueira-Silva et al., 2018).

2.3 Stem Cell Research in Animals

After the isolation of stem cells from mice in the embryonic stage in the 1980s, stem cell biology has revolutionized the field and opened the door to a variety of pluripotent or multipotent cell types for use in preclinical or clinical investigations; however, the lack of animal models for target diseases has forced some researchers to forego traditional preclinical animal testing; choosing appropriate model for the pre-clinical studies demands an understanding of species-specific aspects of stem cell biology, further progress demands research on different animals to form principle and evaluate safety of possible treatments.

Stem cells can proliferate and differentiate into specialized cells throughout fetal development and adult tissues. In the lab, they are modified by adding genes, which modify their behavior. Inner cell mass at the early embryonic stage is obtained from donor females, where embryonic stem cells originate. After that, the inner cell mass of these embryos is cultured in vitro until it takes the shape of an egg cylinder (Figure 4) (Kumari et al., 2023).

Mice have been among the preferred species for studying stem cell biology in mammals due to their low cost, rapid reproduction, and ease of genetic manipulation. However, their ability to predict therapies based on stem cell effectiveness is still controversial. Several models fail to accurately mimic phenotypes of specific human diseases, prompting researchers to explore other species that could provide better predictions for human outcomes. Animals, including dogs, goats, pigs, rabbits, sheep, and primates, excluding humans, often serve as superior models due to their long lifespans facilitating critical studies for applications of stem cells. Moreover, their physiological parameters, such as the working of the immune system, closely resemble those of humans, influencing the host's reaction to cell transplantation. Another advantage of large animals is they provide a quantity and variety of stem cells that can be consistently extracted and manipulated from a single specimen for analysis and various applications (Rubessa et al., 2017).

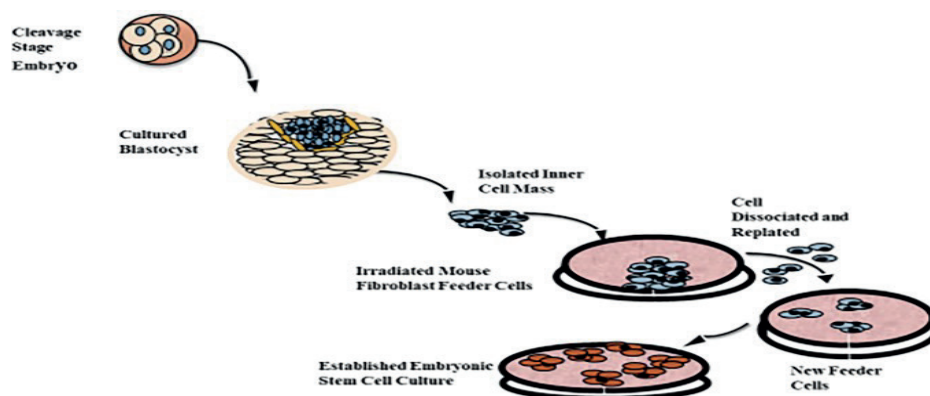


Figure 4. Method of Stem Cell Culturing (Kumari et al., 2023).

3. Applications of Animal Biotechnology

3.1 Improved Livestock Breeding

The livestock industry is anticipated to expand significantly to meet the rising demand for animal products, necessitating a shift in production methods to enhance efficiency and productivity. Biotechnological research becomes crucial in addressing the challenge of increasing animal food production to meet the needs of a growing human population (Henchion et al., 2021). Consequently, there is a pressing need to adopt biotechnological advancements to improve livestock breeding and management practices, ensuring sustainable food production while meeting the increasing demands of a rapidly expanding global population.

3.1.1 Selective Breeding Strategies

Selective livestock breeding refers to the systematic breeding of animals to enhance productivity and other important traits. There are many different approaches to this type of breeding, ranging from expensive and high-tech procedures like genetic engineering or in-vitro fertilization to simpler, low-cost approaches that rely on the controlled breeding of animals based on observable traits (Brito et al., 2020). Important traits linked to resilience and adaptation to climate change include animal morphology, low-quality feed, high survival rates, thermal tolerance, and disease resistance. Selective breeding can be done in three main ways:

3.1.1.1 Outcrossing

An outcross occurs when two animals have not mated for at least four or six generations. This approach functions best when a trait's genetic variation is high. Outcrossing is ideal when dominant genes are the desired ones. The ability of outcrossing to keep harmful traits recessive is one of its greatest benefits. Fitness traits like longevity, milk production, survivability, and reproductive ability are all enhanced by outcrossing (Wolfe et al., 2021).

3.1.1.2 Linebreeding

Linebreeding is the practice of mating related animals, such as cousins, aunts and nephews, half-brothers and half-sisters, and other more distant relationships. The usual reason for doing this is to profit from a shared exceptional ancestor that showed up in more recent pedigree generations. With linebreeding, there is less chance of detrimental genetic defects than with inbreeding and a higher degree of uniformity.

3.1.1.3 Inbreeding

This method of breeding involved mating animals that were directly related to each other, such as full siblings, father, and daughter. By using this technique, latent weaknesses are typically driven out of the gene pool, and uniformity and prepotency are created. Nonetheless, there is a greater chance of producing children with issues because recessive genes contribute more to genetic defects than dominant genes do. The number of genes available is decreased by inbreeding, which can potentially wipe out certain genelines (Hieber, 2020).

3.1.2 Genomic Selection in Livestock

Animal breeding has played a significant role in improving livestock productivity. Improvements in genetics have been crucial in raising the efficiency of desired traits in cattle, sheep, pigs, and poultry (Friggens et al., 2017). With the development of gene technology, producers of commercial livestock products and livestock breeders can now base breeding decisions on gene marker technology. Genomic selection is one of the gene marker technologies in which an animal's breeding value can be precisely calculated without knowledge of the animal's phenotype or that of close relatives using a dense chromosomal map. Genomic selection has increased animal productivity by lowering the generation interval and costs of animals (Ibtisham et al., 2017).

3.2 Disease Resistance in Animals

The prevalence of the disease hurts the welfare and productivity of livestock, as well as the production of food from animals and human health. To produce healthy and strong animals, farmers, veterinarians, and animal scientists have been combining their efforts to investigate efficient disease control strategies. Traditional approaches to the management of disease involve administering vaccines and antibiotics. The optimal approach is cultivating disease-resistant livestock, achieved through immune response genes.

3.2.1 Genetic Modification for Disease Resistance

The production of disease-resistant animals can be achieved through genome editing and immunogenomics. Disease severity and pathogenesis are influenced by the interaction between the host immune system and invading pathogens, with host genetics playing a crucial role. Immunogenomics employs integrated bioinformatics tools to explore how host genetics impact this interaction, identifying candidate genes contributing to disease resistance. Using CRISPR/Cas9 genome editing technology, these identified genes can be precisely targeted and modified in the host genome to produce animals with enhanced disease resistance, as illustrated in Figure 5 (Islam et al., 2020).

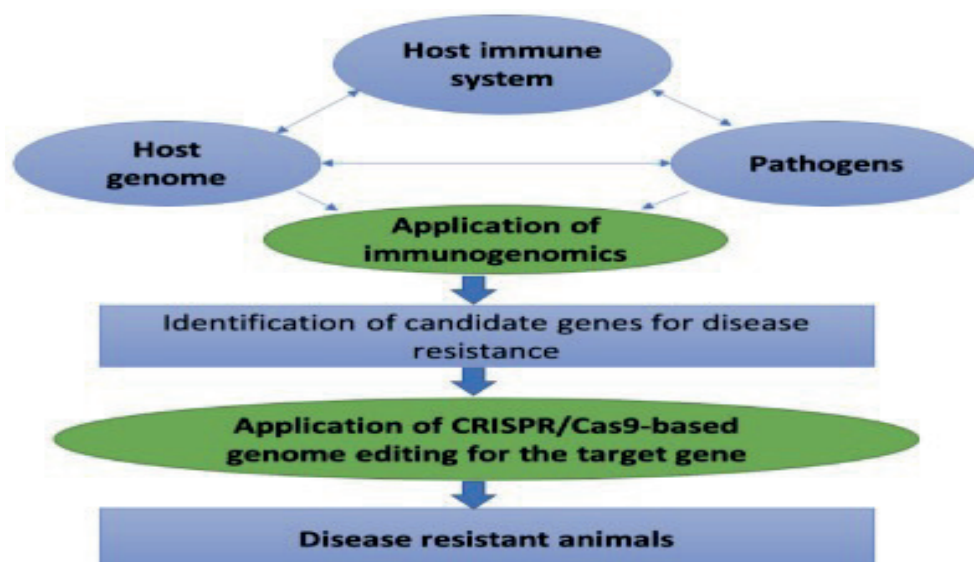


Figure 5. Diagrammatic representation illustrating the use of genome editing and immunogenomics for producing disease-resistant animals (Islam et al., 2020).

3.2.2 Vaccine Development in Livestock

Animal diseases can be effectively prevented by vaccinations against a variety of ailments. Several successful vaccinations have greatly lessened the impact of serious diseases in livestock. Live attenuated, killed/inactivated, cell membrane compounds or toxoids make up most veterinary vaccines approved for use in medicine. Live attenuated vaccines induce immune responses but risk virulence. Killed/inactivated vaccines are safer but less effective. Toxoids require complex culture medium. The limitations of these vaccine types and the lack of efficient disease treatments necessitate the development of recombinant vaccines (Jansen et al., 2018).

Recombinant vaccines are a promising approach to getting around the drawbacks of conventional vaccines. International initiatives are underway to use recombinant DNA technology to create more effective vaccines against a wide range of diseases. Recombinant vaccines are created by strategically selecting and purifying specific antigens using methods informed by the vaccine's structure or genome, enhancing efficacy and safety through targeted antigen design. These vaccines harness molecular techniques to engineer immunogenic components precisely, optimizing immune

response and minimizing adverse reactions (Pollet et al., 2021).

3.3 Biomedical Research and Therapeutics

The use of animal models in scientific research is a topic of debate, but their selection based on functional and genetic qualities has significantly contributed to the evolution of medicine from an art to a science. Animal models have been instrumental in advancing the understanding of biological and pathological processes, providing invaluable insights that inform biomedical research. These contributions are crucial, enabling the development and testing of medications, vaccines, and surgical techniques applicable in human and veterinary medicine. Consequently, animal models play a vital role in bridging the gap between basic research and clinical applications, fostering medical progress and innovation (Vashishat et al., 2024).

3.3.1 Animal Models for Human Diseases

Animal models play a crucial role in biomedical research by enabling the study of pathogenesis and various diseases. Livestock, including goats, cattle, pigs, and sheep, share common anatomy and physiology with humans, making them well-established models for studying genetic disorders. Whole genome sequencing and genetically engineered livestock have made them more significant in biomedical studies. Advances in genomics, therapeutic cloning, stem cell research, biotechnology, and surgical techniques offer potential solutions for non-treatable infectious diseases. Animal models also help toxicologists develop knowledge about chemical toxicity and protect humans, animals, and the environment from toxic exposures (Singh & Seed, 2021).

3.3.2 Gene Therapy in Veterinary Medicine

Gene therapy involves transferring target genes into specific cells within an organism, a principle that drives advancements in biomedicine by introducing novel, genetically engineered medications into clinical practice. These gene drugs are primarily used to treat rare, life-threatening genetic, oncological, and other diseases that lack conventional treatments. Additionally, gene therapy can promote regenerative processes in cases of chronic non-fused bone fractures and severe muscle and tendon injuries that do not respond to traditional therapies. Large animals are often employed as models for developing and testing these innovative gene therapy techniques. In modern veterinary practice, human-specific drugs are frequently used, but gene technologies also allow the creation of species-specific gene drugs. This ensures treatments are tailored to particular animal species, minimizing side effects and enhancing efficacy (Nóbrega et al., 2020).

4. Future Prospects and Challenges

Although the future of biotechnology is promising, it faces challenges stemming from the development and use of methods like cloning, transgenic organisms, CRISPR, stem cell research, selective breeding, and disease resistance. Transgenic organisms and animal clones raise significant ethical issues, including concerns about biodiversity and animal welfare and possibly creating species with unforeseen negative traits (Seidel, 2020). The precision of genome editing, especially in germline modifications that can be inherited, also brings ethical boundaries into question. Comprehensive regulatory frameworks are necessary to ensure the safe use of biotechnological advances, but varying regulations across countries can complicate global collaboration and commercialization.

Genetically modified organisms should be safe for human consumption, and their impact on the environment remains a critical challenge, with long-term effects still not fully understood. Techniques like CRISPR, although revolutionary, are not flawless; off-target effects and mosaicism can lead to unintended genetic changes. Cloning success rates are relatively low, and cloned animals often suffer health problems. Public fear and misinformation about biotechnological methods can hinder scientific progress and acceptance, making transparent communication and education essential. Releasing transgenic organisms into the wild can disrupt natural ecosystems and affect

biodiversity, with long-term ecological impacts still uncertain (Shah et al., 2018).

On the positive side, stem cell research can revolutionize medicine by aiding the replacement or repair of impaired tissues and organs. CRISPR and other gene-editing technologies can create disease-resistant animals, reducing reliance on antibiotics and improving animal welfare. Selective breeding and genetic modification can produce crops and livestock with enhanced traits, such as improved nutritional content, increased yield, and pest resistance.

Cloning and genetic engineering can aid in the conservation of endangered species by increasing diversity and restoring their populations. Transgenic animals and plants can be engineered cost-effectively to produce biopharmaceuticals, such as vaccines, hormones, and antibodies. Biotechnology can also help meet the growing global demand for food by increasing the efficiency and productivity of agricultural systems (Massa et al., 2019).

In conclusion, while the prospects of biotechnology are vast and transformative, addressing the ethical, regulatory, safety, and public perception challenges will be crucial for its sustainable and responsible advancement.

5. Conclusion

Agriculture, medicine, and environmental conservation are all being revolutionized by animal biotechnology by using methods such as cloning, CRISPR-Cas9 gene editing, and developing transgenic organisms that have produced disease-resistant livestock, increasing agricultural productivity and protecting endangered species. Genetically modified animals are used to produce human-compatible organs and biopharmaceuticals, which address shortages in healthcare and improve healthcare. Stem cell research provides new treatments for various conditions, and selective breeding encourages sustainability and food security. Nevertheless, obstacles to progress include ethical considerations, regulatory complexity, safety concerns, and public perception.

References

- Aerni, P. (2021). The Ethics of Farm Animal Biotechnology from an Anthropological Perspective. *Sustainability*, 13(7), 3674.
- Bisimwa, P. N., Ongus, J. R., Tonui, R., Bisimwa, E. B., & Steinaa, L. (2024). Resistance to African swine fever virus among African domestic pigs appears to be associated with a distinct polymorphic signature in the RelA gene and upregulation of RelA transcription. *Virology Journal*, 21(1), 93.
- Brito, L. F., Oliveira, H. R., McConn, B. R., Schinckel, A. P., Arrazola, A., Marchant-Forde, J. N., & Johnson, J. S. (2020). Large-scale phenotyping of livestock welfare in commercial production systems: a new frontier in animal breeding. *Frontiers in genetics*, 11, 793.
- Caldwell, K. A., Willicott, C. W., & Caldwell, G. A. (2020). Modeling neurodegeneration in *Caenorhabditis elegans*. *Disease Models & Mechanisms*, 13(10), dmm046110.
- Carlson, D. F., Lancto, C. A., Zang, B., Kim, E.-S., Walton, M., Oldeschulte, D., Seabury, C., Sonstegard, T. S., & Fahrenkrug, S. C. (2016). Production of hornless dairy cattle from genome-edited cell lines. *Nature biotechnology*, 34(5), 479-481.
- Christapher, P. V., Ganeson, T., Chinni, S. V., & Parasuraman, S. (2022). Transgenic Rodent Models in Toxicological and Environmental Research: Future Perspectives. *Journal of Pharmacology and Pharmacotherapeutics*, 13(3), 258-265.
- Crispo, M., Mulet, A., Tesson, L., Barrera, N., Cuadro, F., dos Santos-Neto, P., Nguyen, T., Cr  n  guy, A., Brusselle, L., & Ane  n, I. (2015). Efficient generation of myostatin knock-out sheep using CRISPR/Cas9 technology and microinjection into zygotes. *PLoS One*, 10(8), e0136690.

de Siqueira-Silva, D. H., Saito, T., dos Santos-Silva, A. P., da Silva Costa, R., Psenicka, M., & Yasui, G. S. (2018). Biotechnology applied to fish reproduction: tools for conservation. *Fish Physiology and Biochemistry*, 44(6), 1469-1485.

Estrada, A. C., Díaz, D. V., & Hernández, C. A. M. (2017). The role of biotechnology in agricultural production and food supply. *Ciencia e investigación agraria: Revista Latinoamericana de Ciencias de la Agricultura*, 44(1), 1-11.

Friggens, N., Blanc, F., Berry, D., & Puillet, L. (2017). Deciphering animal robustness. A synthesis to facilitate its use in livestock breeding and management. *Animal*, 11(12), 2237-2251.

Gowane, G. R., Kumar, A., & Nimbkar, C. (2019). Challenges and opportunities to livestock breeding programmes in India. *Journal of Animal Breeding and Genetics*, 136(5), 329-338.

Greenfield, H. J. (2010). The Secondary Products Revolution: the past, the present and the future. *World Archaeology*, 42(1), 29-54.

HASAN, M. U., KOZAKLI, Ö., & CEYHAN, A. (2022). Possibilities of using CRISPR-based genome editing Technologies in Livestock. *Journal of Agriculture, Food, Environment and Animal Sciences*, 3(1), 56-68.

Henchion, M., Moloney, A., Hyland, J., Zimmermann, J., & McCarthy, S. (2021). Trends for meat, milk and egg consumption for the next decades and the role played by livestock systems in the global production of proteins. *Animal*, 15, 100287.

Hieber, J. K. (2020). Inbreeding and inbreeding depression in linebred beef cattle Montana State University].

Hino, T., Muro, Y., Tamura-Nakano, M., Okabe, M., Tateno, H., & Yanagimachi, R. (2016). The behavior and acrosomal status of mouse spermatozoa in vitro, and within the oviduct during fertilization after natural mating. *Biology of reproduction*, 95(3), 50, 51-11.

Hosseini, S.-M., Moulavi, F., Tanhaie-Vash, N., Asgari, V., Ghanaei, H.-R., Abedi-Dorche, M., Jafarzadeh, N., Gourabi, H., Shahverdi, A.-H., & Dizaj, A. V. (2016). The principal forces of oocyte polarity are evolutionary conserved but may not affect the contribution of the first two blastomeres to the blastocyst development in mammals. *PLoS One*, 11(3), e0148382.

Ibtisham, F., Zhang, L., Xiao, M., An, L., Ramzan, M. B., Nawab, A., Zhao, Y., Li, G., & Xu, Y. (2017). Genomic selection and its application in animal breeding. *The Thai Journal of Veterinary Medicine*, 47(3), 301-310.

Islam, M. A., Rony, S. A., Rahman, M. B., Cinar, M. U., Villena, J., Uddin, M. J., & Kitazawa, H. (2020). Improvement of disease resistance in livestock: application of immunogenomics and CRISPR/Cas9 technology. *Animals*, 10(12), 2236.

Jansen, K. U., Knirsch, C., & Anderson, A. S. (2018). The role of vaccines in preventing bacterial antimicrobial resistance. *Nature medicine*, 24(1), 10-19.

Kantor, A., McClements, M. E., & MacLaren, R. E. (2020). CRISPR-Cas9 DNA base-editing and prime-editing. *International journal of molecular sciences*, 21(17), 6240.

Keefer, C. L. (2015). Artificial cloning of domestic animals. *Proceedings of the National Academy of Sciences*, 112(29), 8874-8878.

Kesik-Brodacka, M. (2018). Progress in biopharmaceutical development. *Biotechnology and applied biochemistry*, 65(3), 306-322.

Khan, F. A. (2020). *Biotechnology Fundamentals Third Edition*. CRC Press.

- Kumari, R., Rani, M., Nigam, A., & Kumar, A. (2023). Stem cell culture techniques. In *Animal Cell Culture: Principles and Practice* (pp. 213-234). Springer.
- Kurtz, S., & Petersen, B. (2019). Pre-determination of sex in pigs by application of CRISPR/Cas system for genome editing. *Theriogenology*, 137, 67-74.
- Laible, G., Wei, J., & Wagner, S. (2015). Improving livestock for agriculture—technological progress from random transgenesis to precision genome editing heralds a new era. *Biotechnology journal*, 10(1), 109-120.
- Lee, K., Farrell, K., & Uh, K. (2020). Application of genome-editing systems to enhance available pig resources for agriculture and biomedicine. *Reproduction, Fertility and Development*, 32(2), 40-49.
- Liu, Y., Ma, X., Shu, L., Hancke, G. P., & Abu-Mahfouz, A. M. (2020). From industry 4.0 to agriculture 4.0: Current status, enabling technologies, and research challenges. *IEEE transactions on industrial informatics*, 17(6), 4322-4334.
- Massa, S., Presenti, O., & Benvenuto, E. (2019). Engineering plants for the future: farming with value-added harvest. *Progress in Botany Vol. 80*, 65-108.
- McLean, Z., Oback, B., & Laible, G. (2021). Embryo-mediated genome editing for accelerated genetic improvement of livestock.
- Mekonnen, G. (2021). Review on application of nanotechnology in animal health and production. *J. Nanomed. Nanotechnol*, 12, 559.
- Menchaca, A., Dos Santos-Neto, P., Mulet, A., & Crispo, M. (2020). CRISPR in livestock: From editing to printing. *Theriogenology*, 150, 247-254.
- Munawar, N., & Ahmad, A. (2021). CRISPR/Cas system: an introduction. *CRISPR Crops: The Future of Food Security*, 1-35.
- Nóbrega, C., Mendonça, L., & Matos, C. A. (2020). *A handbook of gene and cell therapy* (Vol. 3). Springer.
- Park, J. S., Han, S. T., Lee, K. H., Kim, Y. M., Lee, H. J., & Han, J. Y. (2019). Targeted genome modification mediated by homology-directed repair and non-homologous end joining and its application. *J. Anim. Breed. Genet*, 3(3).
- Pingault, N., Caron, P., Kalafatic, C., Allahoury, A., Fresco, L. O., Kennedy, E., Khan, M., Kliksberg, B., Mei, F., & Murphy, S. (2016). Sustainable agricultural development for food security and nutrition: what roles for livestock? A report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security.
- Pollet, J., Chen, W.-H., & Strych, U. (2021). Recombinant protein vaccines, a proven approach against coronavirus pandemics. *Advanced drug delivery reviews*, 170, 71-82.
- Rahbaran, M., Razeghian, E., Maashi, M. S., Jalil, A. T., Widjaja, G., Thangavelu, L., Kuznetsova, M. Y., Nasirmoghadas, P., Heidari, F., & Marofi, F. (2021). Cloning and embryo splitting in mammals: brief history, methods, and achievements. *Stem Cells International*, 2021(1), 2347506.
- Rasmussen, N. (2014). *Gene jockeys: Life science and the rise of biotech enterprise*. JHU Press.
- Rohr, J. R., Barrett, C. B., Civitello, D. J., Craft, M. E., Delius, B., DeLeo, G. A., Hudson, P. J., Jouanard, N., Nguyen, K. H., & Ostfeld, R. S. (2019). Emerging human infectious diseases

and the links to global food production. *Nature Sustainability*, 2(6), 445-456.

Rubessa, M., Polkoff, K., Bionaz, M., Monaco, E., Milner, D. J., Hollister, S. J., Goldwasser, M. S., & Wheeler, M. B. (2017). Use of pig as a model for mesenchymal stem cell therapies for bone regeneration. *Animal biotechnology*, 28(4), 275-287.

Seidel, G. E. (2020). GM farm animals: potential impact on biodiversity including ethical concerns. *GMOs: Implications for Biodiversity Conservation and Ecological Processes*, 277-285.

Shah, S. Z., Rehman, A., Nasir, H., Asif, A., Tufail, B., Usama, M., & Jabbar, B. (2018). Advances in research on genome editing CRISPR-Cas9 technology. *Journal of Ayub Medical College Abbottabad*, 31(1), 108-122.

Sharma, B., Chettri, D., & Verma, A. K. (2021). Biotechnological advancements in livestock production. *Sustainable Agriculture Reviews 54: Animal Biotechnology for Livestock Production 1*, 107-130.

Singh, P., & Ali, S. A. (2021). Impact of CRISPR-Cas9-based genome engineering in farm animals. *Veterinary Sciences*, 8(7), 122.

Singh, V. K., & Seed, T. M. (2021). How necessary are animal models for modern drug discovery? *Expert opinion on drug discovery*, 16(12), 1391-1397.

Srirattana, K., Kaneda, M., & Parnpai, R. (2022). Strategies to improve the efficiency of somatic cell nuclear transfer. *International journal of molecular sciences*, 23(4), 1969.

Suzuki, S., Iwamoto, M., Hashimoto, M., Suzuki, M., Nakai, M., Fuchimoto, D., Sembon, S., Eguchi-Ogawa, T., Uenishi, H., & Onishi, A. (2016). Generation and characterization of RAG2 knockout pigs as animal model for severe combined immunodeficiency. *Veterinary immunology and immunopathology*, 178, 37-49.

Vashishat, A., Patel, P., Das Gupta, G., & Das Kurmi, B. (2024). Alternatives of animal models for biomedical research: a comprehensive review of modern approaches. *Stem Cell Reviews and Reports*, 20(4), 881-899.

Wang, J. Y., & Doudna, J. A. (2023). CRISPR technology: A decade of genome editing is only the beginning. *Science*, 379(6629), eadd8643.

Wang, X., Yu, H., Lei, A., Zhou, J., Zeng, W., Zhu, H., Dong, Z., Niu, Y., Shi, B., & Cai, B. (2015). Generation of gene-modified goats targeting MSTN and FGF5 via zygote injection of CRISPR/Cas9 system. *Scientific reports*, 5(1), 13878.

Wolfe, M. D., Chan, A. W., Kulakow, P., Rabbi, I., & Jannink, J.-L. (2021). Genomic mating in outbred species: predicting cross usefulness with additive and total genetic covariance matrices. *Genetics*, 219(3), iyab122.

Wu-Pong, S. (1999). An overview of biotechnology. *Biopharmaceutical Drug Design and Development*, 1-19.

Yitbarek, M. B. (2019). Livestock and livestock product trends by 2050. *IJAR*, 4, 30.

About The Authors

Miss Shumaila Batool is an MPhil scholar in Zoology at the University of Agriculture Faisalabad, Pakistan. Her research focuses on Fisheries, specifically fish nutrition and toxicology, as well as conservation biology with an emphasis on wildlife and biodiversity. She is actively investigating the impacts of climate change on various species. Her two articles as a first author are under review in international journals and has also contributed to several interdisciplinary book chapters published by Springer and Elsevier.

Email: Shumailabatool262@gmail.com

Urwah Sheikh is an M Phil scholar at University of agriculture Faisalabad, Pakistan. Her research area is aquaculture and primarily focuses on how micronutrient supplementation can make aquaculture sustainable by improving health and enhancing immunity of fish.

Email: urwahsheikh77@gmail.com

Hareem Fayyaz is an MPhil Zoology student at the University of Agriculture, Faisalabad, Pakistan. Her research focuses on fish genetics, specifically examining how inbreeding in introduced fish species impacts genetic diversity in riverine populations and affects breeding patterns, potentially threatening the survival of these species. She has also written two book chapters.

Email: hfayyazahmed391@gmail.com

Anam Shehzadi is an MPhil scholar in Zoology, Wildlife and Fisheries Department of the University of Agriculture Faisalabad. She has impressively submitted 4 chapters and 1 review article at the international level, along with another chapter at the national level. Her research focus is on tackling critical animal-related issues and their solutions. Her research explores innovative technologies that enhance the well-being of animals in the context of climate change, alongside effective water management strategies aimed at ensuring the sustainability of aquatic life.

Email: shehzadianam2121@gmail.com

Fatima Ameen is an MPhil scholar in Zoology, Wildlife and Fisheries Department of the University of Agriculture, Faisalabad.

Email: arooggoraya@gmail.com

To Cite This Chapter

Batool S et al., (2024). Future Frontiers: Emerging Trends and Applications in Animal Biotechnology. In *Current Studies in Health and Life Sciences 2024* (pp.424-437). ISRES Publishing.