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Genome Editing in Animal Husbandry: CRISPR

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Abstract

Genome editing in farm animals holds significant promise for a wide range of practical applications. It facilitates the improvement of production traits, enhances the economic value of livestock, and contributes to increased resistance to infectious diseases. In addition to agricultural benefits, genetically modified animals serve as important models in biomedical research and pharmaceutical production, and they have shown potential as xenograft donors for human transplantation. Recent advancements have led to the development of various tools aimed at increasing the efficiency and precision of genetic modifications, thereby streamlining the generation of genetically modified founders. These tools include sperm-mediated gene transfer, viral vectors, RNA interference, homologous recombination, transposon systems, and site-specific endonucleases. Among these, four major classes of site-specific endonucleases have attracted considerable attention due to their ability to induce targeted DNA double-strand breaks, which facilitate precise genome modifications via endogenous DNA repair pathways. Currently, clustered regularly interspaced palindromic repeats (CRISPR) and CRISPR-associated (Cas) systems—particularly CRISPR/Cas9—dominate the genome editing field. These systems have been successfully employed in generating genetically modified sheep and goats, which serve as valuable models for studying gene function, improving selective breeding, producing therapeutic proteins in milk, enhancing disease resistance, mimicking human disease phenotypes, and potentially serving as hosts for human organ development. Moreover, several promising derivatives of the CRISPR/Cas systems have emerged, including tools that enable homology-directed repair (HDR) and base editing, the latter allowing precise single-nucleotide changes without the requirement for a donor DNA template. These innovations further expand the utility and precision of genome editing technologies. This review provides a comprehensive overview of genome editing in livestock, with a particular emphasis on the application and potential of CRISPR/Cas9 systems in both agricultural and biomedical contexts.

Keywords: Genetic Modification, Animal Husbandry, CRISPR/Cas9

Introduction

The field of gene regulation remains vast and largely unexplored. The rapid transmission of viruses, along with the continuous mutation and evolution of pathogens, underscores the urgent need for innovative strategies in the diagnosis and prevention of infectious diseases. Conventional pathogen

detection methods—primarily polymerase chain reaction (PCR)-based technologies—are often unsuitable for use in resource-limited settings due to their reliance on expensive, advanced equipment and trained personnel (Zhang, 2022). One of the most transformative innovations in gene editing is the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system (Bilici & Ayvazoğlu, 2024). The CRISPR-Cas technique represents a groundbreaking platform for precise and efficient modification of genetic material. The rapid evolution of CRISPR/Cas-based genome editing tools has revolutionized gene therapy by significantly enhancing the capacity to address a broad range of genetically inherited diseases (Asmamaw & Zawdie, 2021).

Given that humans heavily rely on livestock for essential food products such as meat, milk, and eggs, genetic engineering and transgenic approaches present considerable potential for improving livestock productivity within a relatively short timeframe (Bilici, 2024). Since the initial discovery of the CRISPR system, numerous modifications have been made to its molecular mechanisms to expand its applicability across diverse domains, including biomedicine and agriculture (Zhang, 2021). The CRISPR-Cas system offers a wide array of promising applications in agriculture, biotechnology, and medicine. Clinical studies have demonstrated that CRISPR technology delivers higher gene-editing efficacy compared to earlier gene therapy platforms such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) (Janik et al., 2020; Zhang et al., 2019).

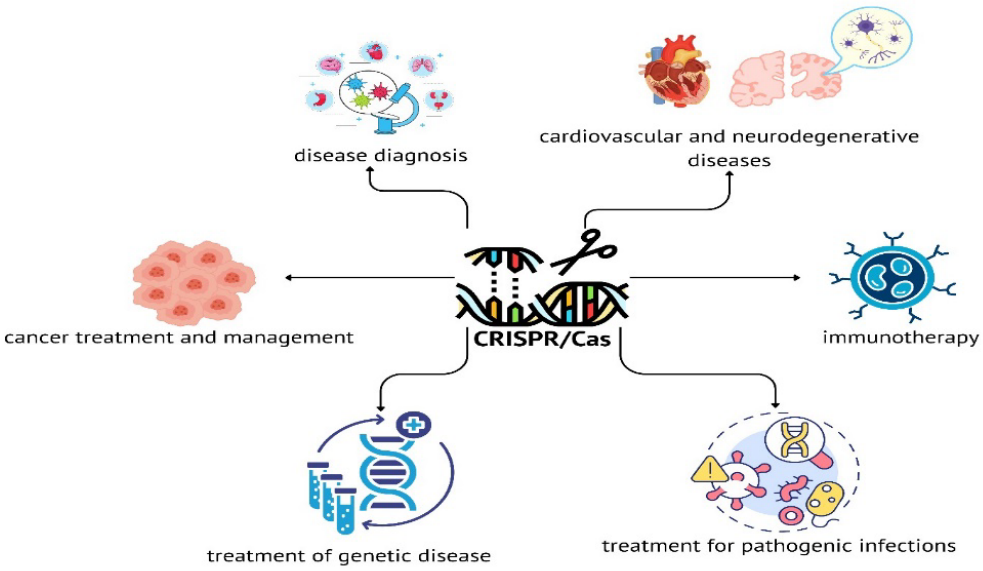

Figure 1. CRISPR/Cas ile gen düzenlemenin çok yönlü uygulamaları

The CRISPR-Cas genome editing system was first successfully applied in animal cells in 2013. Despite its numerous advantages, it is associated with several biological risks and ethical concerns (Ayanoğlu et al., 2020). One of the primary limitations of CRISPR technology is the irreversibility of genomic modifications, as well as the potential for off-target effects. Minimizing these unintended alterations relies heavily on the design of guide RNA (gRNA) sequences with high specificity to both target and potential off-target genomic regions. Another technical challenge involves the DNA-binding and cleavage activities of the Cas nuclease, which are not always functionally coupled. Mutations in the Cas9 protein may lead to DNA binding without the subsequent cleavage of the target sequence, thereby compromising editing efficiency (Jinek et al., 2012). To broaden the scope of CRISPR-based therapeutic applications, further investigation into the optimization of CRISPR-Cas delivery systems is essential. Such research is critical for fully understanding the associated complications, as well as the potential short- and long-term side effects. CRISPR genome editing has already demonstrated a profound impact across diverse sectors, including biomedicine, biotechnology, nanotechnology, agriculture, and livestock production. In the livestock industry specifically, CRISPR has been employed to generate disease-resistant animals, enhance animal welfare, and improve economically important traits (Singh et al., 2021).

Currently, there are no effective vaccines or treatments for many infectious diseases affecting both humans and animals (Edelson et al., 2023). This has underscored the urgent need for the development of robust, cost-effective, and user-friendly diagnostic tools that do not require complex equipment or specialized personnel. Such tools must also offer high specificity and sensitivity, particularly for applications in disease prevention and outbreak control (Wang et al., 2021). The foundational discovery of CRISPR sequences in *Escherichia coli* in 1987, followed by the identification and expression of CRISPR-associated proteins, has led to transformative advances in genome editing, genetic engineering, and molecular diagnostics. These breakthroughs continue to reshape the landscape of clinical research and therapeutic innovation (Mao et al., 2023; Tian et al., 2023).

CRISPR/Cas Diagnostic Application

The CRISPR-Cas system has demonstrated broad applicability, ranging from fundamental research to clinical interventions. In human medicine, it has been utilized to correct genetic mutations underlying conditions such as sickle cell anemia, beta-thalassemia, and cystic fibrosis (Frangoul et al., 2021). Moreover, CRISPR-Cas has been widely adopted for genome editing across a variety of organisms, including both plants and animals, enabling precise modifications to DNA sequences (Graham & Hart, 2021; McCarron et al., 2020). While its potential to enhance disease resistance in animals is promising, this application remains at an early experimental stage. Preliminary findings are encouraging, indicating that CRISPR-Cas technology may significantly transform livestock production systems. Nevertheless, the technique requires further optimization and refinement to ensure consistent and safe outcomes.

To fully harness the potential of CRISPR-Cas systems in animal agriculture, particularly in the context of long-term solutions for livestock and poultry nutrition, additional research and experimental validation are essential. In this regard, CRISPR-Cas represents a groundbreaking platform not only for genome editing but also for gene therapy, epigenetic modulation, and targeted drug delivery within the genome of livestock species (Sovová et al., 2017; Khwatenge et al., 2021). Additionally, the system has been instrumental in developing genetically modified crops with improved pest and disease resistance as well as enhanced nutritional profiles (Islam, 2019). Compared to earlier genome editing technologies, CRISPR-Cas is recognized for its superior efficiency, cost-effectiveness, and relative ease of use (Makarova et al., 2013).

Ruminants play a pivotal role in global livestock and food systems, contributing milk, meat, and various by-products critical to human nutrition and industry. Diseases affecting these animals not only reduce productivity but also pose direct risks to human health. Genetic determinants also influence behavioral traits in animals, which can affect welfare and management practices (Sezer et al., 2023). Therefore, the early diagnosis and effective control of animal diseases are essential to safeguarding both animal and public health. Zoonotic diseases—those transmitted between animals and humans—pose a significant threat. According to the World Health Organization, approximately 60% of all human infectious diseases are zoonotic in origin, and about 75% of emerging infectious diseases fall into this category. Transmission pathways include direct contact with infected animals or indirect exposure through aerosols, consumption of contaminated meat, food products, or unpasteurized milk. Individuals working in close contact with animals—such as veterinarians, farmers, butchers, and laboratory personnel—are particularly at risk (Dignard & Leibler, 2019).

Gene Transfer Methods Effectively Applied in Livestock Biotechnology

Beyond their extensive use in animal husbandry, gene editing technologies are increasingly being employed in biomedical research and gene therapy to treat a variety of human diseases (Soldner et al., 2011; Qiu et al., 2013; Xu et al., 2017). In the field of plant science, these techniques are frequently utilized to enhance resistance to herbicides and insect pests, as well as to improve agronomic traits such as growth rate, yield, and overall quality of agricultural products (Shukla et al., 2009; Townsend et al., 2009; Ricroch & Henard-Damave, 2016; Zhang et al., 2017; Li et al., 2012). A transformative advancement in this field has been the development of precise genome targeting technologies, which have enabled researchers to exert fine control over gene expression. These approaches allow the use of endogenous promoter elements and the natural regulation of RNA splicing to achieve targeted gene expression, thereby minimizing the gene silencing or variability typically associated with random DNA insertions.

This innovation involves the use of engineered nucleases—such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9—as molecular "scissors" to induce specific and controlled genetic modifications within defined genomic loci (Van, 2018). Furthermore, the integration of CRISPR/Cas9 with microinjection techniques,

particularly into zygotes, has been shown to significantly reduce the incidence of off-target effects, thereby enhancing the accuracy and reliability of genome editing in vivo (Mashiko et al., 2013). Cas9-mediated genome editing through pronuclear microinjection has been successfully implemented in various animal models, including mice, rats, monkeys, and others (Mashiko et al., 2013; Li et al., 2013; Niu et al., 2014).

Ethical Issues in Gene Editing in Animals

One of the central ethical concerns surrounding gene editing in animals is the potential for unintended consequences. Genome editing technologies, such as CRISPR-Cas, may induce off-target effects and unintentional mutations, which can result in unpredictable and potentially detrimental outcomes for animal health and welfare. As such, the application of CRISPR-Cas systems in animals requires a thorough assessment of both risks and benefits to minimize the likelihood of adverse effects (Niemiec & Howard, 2020). The rapid advancement of genome editing research in livestock indicates that products derived from genetically edited animals could become commercially available soon, particularly in countries that have achieved food security. However, the ethical debates previously raised in discussions of genetically modified animals and cloning remain highly relevant, particularly with respect to animal welfare and broader societal values. The deployment of genome editing in livestock should only proceed following comprehensive consideration of its social, ethical, and welfare implications. In this context, developers have a responsibility to rigorously investigate the occurrence of off-target mutations during the breeding of genome-edited animals. Regulatory bodies, in turn, should require transparency from developers regarding these mutations and promote public discourse around the use of genome editing in animal breeding. Without addressing both the practical and ethical implications of these technologies, genome-edited livestock products are unlikely to gain widespread public acceptance.

Animal welfare remains a paramount ethical issue in this domain. Although gene editing holds promise for enhancing disease resistance and improving health outcomes, it also carries the risk of unintended effects that could result in animal suffering or reduced physiological fitness. Therefore, it is essential to evaluate both the welfare impacts and the broader ethical considerations associated with such interventions (Yunes et al., 2021; Shinwari et al., 2018). Moreover, the use of CRISPR-Cas in animals raises ethical questions pertaining to human health and safety. Any potential health risks associated with the consumption of genetically modified animal products must be carefully examined to ensure their safety for consumers (Yunes et al., 2021; Khan et al., 2020).

To facilitate the responsible use of CRISPR-Cas technologies in livestock and poultry production, it is imperative to provide future veterinarians, technicians, and farmers with specialized training. A comprehensive and up-to-date educational curriculum should incorporate the latest advances in biotechnology, practical applications, and ethical frameworks relevant to animal genome editing (Li et al., 2024).

Conclution

The development of genome editing technologies has created new opportunities to enhance the health, productivity, and biomedical utility of farm animals. These advancements have enabled the introduction of beneficial genetic traits and the elimination of deleterious ones, thereby contributing to the creation of improved livestock breeds and promoting more sustainable agricultural practices. A variety of gene delivery techniques, including pronuclear microinjection and somatic cell nuclear transfer (cloning), have been successfully implemented in livestock biotechnology to achieve precise genetic modifications. Among these technologies, the CRISPR/Cas system has emerged as a highly versatile and powerful tool for genome editing across a wide range of organisms, including humans, animals, plants, and microbes. Originally derived from the adaptive immune system of prokaryotes, CRISPR/Cas has revolutionized genetic engineering due to its precision, efficiency, and sensitivity, while significantly minimizing off-target effects. These characteristics make it particularly suitable for correcting genetic mutations.

In the medical field, the CRISPR/Cas system holds considerable promise for the treatment of various genetic disorders, including sickle cell disease (SCD), transfusion-dependent β-thalassemia (TDT), X-linked genetic conditions, and ocular diseases. It has also made substantial contributions to cancer research by enabling the inactivation of oncogenes and the activation of tumor suppressor genes, thereby facilitating the identification of novel therapeutic targets (Kimberland et al., 2018; Wang et al., 2015). Despite its immense potential, the clinical application of CRISPR-based gene therapies presents several significant challenges. One of the primary concerns involves off-target effects, where unintended genetic alterations may occur, potentially resulting in harmful mutations or large-scale genomic deletions. These unintended modifications pose serious risks and remain a major barrier to the widespread adoption of CRISPR in therapeutic contexts.

Another critical limitation is the effective delivery of CRISPR components to specific cells or tissues in vivo. The lack of safe, efficient, and targeted delivery systems restricts current applications to a limited number of diseases. Additionally, the high cost of gene therapies, including those based on CRISPR, renders them inaccessible to a majority of the global population. In conclusion, while CRISPR/Cas-based therapies present transformative potential across numerous domains of medicine and biotechnology, addressing the technical, ethical, and economic challenges remains essential for their broader implementation and public acceptance.

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