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Advancements in Avian Genome Engineering: Strategies, Primordial Germ Cell Manipulation, and Emerging Delivery Techniques

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REVIEW ARTICLE

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ABSTRACT

Genome engineering tools have greatly advanced in the past 5 years, enabling scientists to make accurate modulations to the genome. Systems of delivering these new genome editing tools have been improved in addition to the development of the new tools. Genome engineering instruments are typically deposited to the in vitro fertilized single cell embryos, which are then cultured and implanted to a host animal. This holds no true in the avian species and the alternative procedures have been engineered in genome engineering in birds. The most common is in vitro culturing of primordial germ cells (PGCs), which migrate in the embryonic circulatory system to the developing gonad and colonize the gonad, eventually developing into the gonadocytes that produce sperm or ova. The population can be screened and enriched and then transposed into a recipient embryo, although in culture, the PGCs can be engineered to express new transgenes or alterations in genes. The greatest drawback of PGC culture is that culture procedures cannot be easily interpolated between the avian species; therefore, culture procedures are only reliable on a limited number of species which include the chicken. Two additional newer technologies that appear to be less taxing to a wider range of avian species are direct injection and sperm transfection-assisted gene editing (STAGE). The rationale behind the direct injection technique is the injection of the genome engineering tools into the circulatory system of the developing embryo just before the development stage, when the PGCs are migrating to the gonads. This is facilitated by the combination of the genome engineering equipment with transfection reagents and the transfection of the PGCs in vivo can be done. STAGE is a technique that involves the application of sperm transfection to implant genome engineering vectors into the recently fertilized embryo. Early signs are that each of the two methodologies can be scaled down to attempt use in other bird species than the chicken, but more research in this direction is required.

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Introduction

Gene editing tools: Gene editing is the current transformative stage the poultry industry undergoes as the genome of poultry may be reformed in a specific way, which can lead to its radical modification. The last ten years have seen the emergence of the instruments of accurate genome engineering, and the introduction of such instruments has greatly boosted the use of genome editing technology in the production of poultry and livestock. There are three main methods that are usually used to produce genetically edited animals, one of which is poultry. The former is the zinc finger nucleases

(ZFNs) which are artificial DNA-binding proteins programmed to bind specific sequences of DNA, but can have incompatibility with a given target sequence. The second modality is transcription activator-like effector nucleases (TALENs), which bears a nuclease domain to induce double-strand breaks (DSBs) at a selected place in the genome [1]. Last, the most common but further developed technique is the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system that has been the standard choice of accurate genome editing in the recent past [2].

Although ZFNs, TALENs, and CRISPR/Cas9 have the same basic need of precise and error-free editing of the genome, they are different in their mechanism. This technique uses a particular RNA molecule, termed guide RNA (sgRNA), to identify and cleave the target DNA sequence, in contrast to ZFNs and TALENs that utilize fusion proteins into one DNA-binding motif. Furthermore, ZFNs and TALENs are comparatively slow to form an effective editing infrastructure, and are costlier to work with than CRISPR/Cas9. They also exhibit increased off-target effects, and CRISPR/Cas9 has the advantage of having computational tools that can be used to design sgRNA specific to the target and reduce off-target effects at the most [3]. CRISPR/Cas9 system works by binding of sgRNA to a complementary sequence of DNA directing the Cas9 nuclease to cause site specific cleavage. This aspect makes CRISPR/Cas9 especially appropriate in the production of genetically edited animals since the technology greatly enhances the effectiveness of specific genome editing [4]. Besides, the gene editing system also keeps on improving with the latest advancements being the utilization of Cas9 and Cas12a nucleases, which allow the concomitant editing of numerous genomic targets. The development is essential in the study of complicated interplay of genes and understanding of the functions of genes.

CRISPR sequences are inherently present in the genes of prokaryotes such as bacteria and archaea. Such sequences are based on a section of the DNA of bacteriophages that have previously attacked the prokaryotic host. Cas endonucleases when paired with the CRISPR system serve as a generalized and programmable nuclease system. Khan et al. (2024) state that the system is based on two RNAs, a 20-base pair guide CRISPR RNA and a conserved tracr RNA [5]. This RNA complex may cleave specific target DNA sequences with high precision and together with CRISPR associated protein 9 of *Streptococcus* may trigger cleavage of all types of organisms [6]. The presence of a protospacer adjacent motif (PAM) sequence typically NGG just adjacent to the target site guides the nuclease activity of Cas9. This allows Cas9 to create multiple genomic loci double-strand breaks (DSBs). Later repair of DSBs by non-homologous end joining (NHEJ) may cause the disruption of the target gene which frequently causes 1,117-bp insertions or deletions. Instead, homology-directed repair (HDR) permits the specific targeted locus to be precisely modified by inserting particular genetic sequences, which can be controlled genome editing [7].

The status of CRISPR/Cas9 technology in the poultry industry: CRISPR/Cas9 system is one of the gene editing technologies that are changing the poultry genomics in that it is now a powerful tool that has been used in breeding and production of food. Up to now, a significant amount of development has been done with CRISPR/Cas9 in poultry, and quail development is not as advanced. Notably, this technique is not designed to substitute the conventional breeding technology, but it is rather supplementary. It does not only give breeders access to a wider spectrum of genetic variation, but mainstream breeding is confined to the genetic variation of a particular poultry population. CRISPR/Cas9 system is an intended system to improve the performance of poultry through specific genetic modifications. Its benefits consist of enhanced digestibility of nutrients and growth rate, elevated egg production and immunity and resistance to diseases. In addition, CRISPR/Cas9 allows the creation of smaller birds with less or no fat in the meat, thus enhancing nutritional statuses [8]. One of such examples is the alteration of the fatty acid composition to lower abdominal fat and to raise the lean percentage of carcass meat. Also, CRISPR/Cas9 has been used to enhance animal welfare by introducing new technologies like in-ovo sexing, or the possibility of determining the sex of embryos early. The poultry industry is on the rise in demand of birds that are optimized towards the needs of the commercial producers and consumers. Different approaches have been suggested in order to generate transgenic birds that would suit such needs. This review gives an in-depth discussion of CRISPR/Cas9 applications in poultry genome editing, as it explores the current trends in CRISPR-based applications in producing genetically modified birds with varied attributes. It also considers the challenges that come with the implementation of CRISPR/Cas9 and the possible solutions to the problems and the future of gene editing in avian species [9].

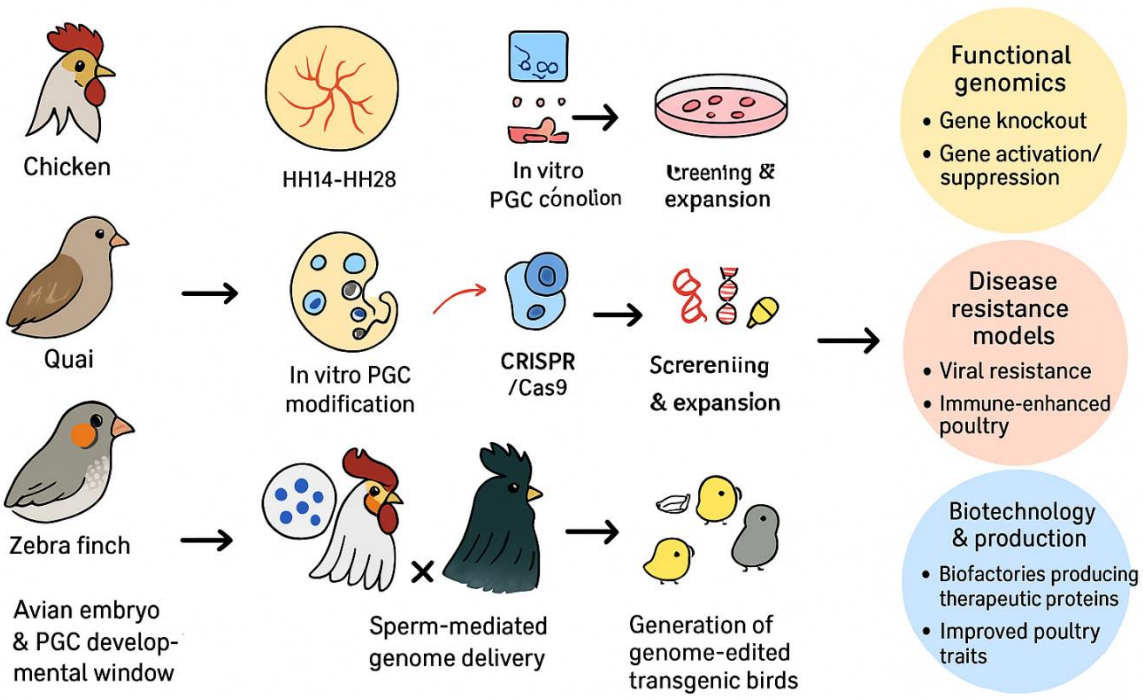

Figure 1. Workflow of Advanced Genome Editing Strategies in Avian Species

Generation of genetically modified CRISPR/Cas9-mediated birds: This technique has turned out to be a revolutionary technology in the exact editing of the genome of various organisms such as birds. Different approaches have been used to generate transgenic animal models. The earliest transgenic rat, rabbits, sheep and pigs were made using microinjection of their target genomic DNA into the pronucleus of fertilized embryos which has successfully modified the germline. The other popular method is embryonic stem cells (ESCs) in which genetically modified ESCs are introduced into host blastocysts to produce germline chimeras [10]. Direct microinjection of the ESCs into the avian zygote is difficult in contrast to mammals, which contain large yolk content and a small germinal disc around the zygote. As a result, early transgenic chickens were obtained by inoculation of the sub-germinal cavity of EGK stage X embryos with retroviruses with germline transmission frequencies of 1 to 11%. Later lentiviral vectors added efficiency to germline transmission increasing it to 4 to 45 percent but initial transgene expression in the oviduct was not that efficient [11]. The transgenic birds have been created in many different ways, among which there are infection of stage X embryos with a virus, microinjection of fertilized eggs with transgenes, and the application of ESCs. Initial inter-individuality transfer of chicken primordial germ cells (PGCs) was a better alternative to ESCs to surmount the low germline transmission efficiency in birds. Transgenic birds can be manufactured by transfecting cultured PGCs with the targeted transgenes and further injecting them into embryonic blood vessels to generate germline chimeras [12].

It is important to note that Salvesen et al. used homologous recombersion in PGCs to first establish the desired gene knockout in chickens. Due to the advent of CRISPR/Cas9, it is now possible to create in vitro systems of PGC culture in conjunction with programmable genome editing, which allows the generation of chicken that has been engineered to perfection. PGCs may be extracted out of embryonic blood or gonads and altered with CRISPR/Cas9 before the microinjections are performed into the recipient embryos, resulting in hatching of chimeras that mature into full-fledged birds [13]. By transplanting drug-resistant primordial germ cells in host embryo, which had undergone gamma ray exposure to eliminate endogenous PGCs, Hernandez-Patlan et al. produced targeted ovomucoid gene-modified chickens in an efficient manner. Discoveries made thereafter

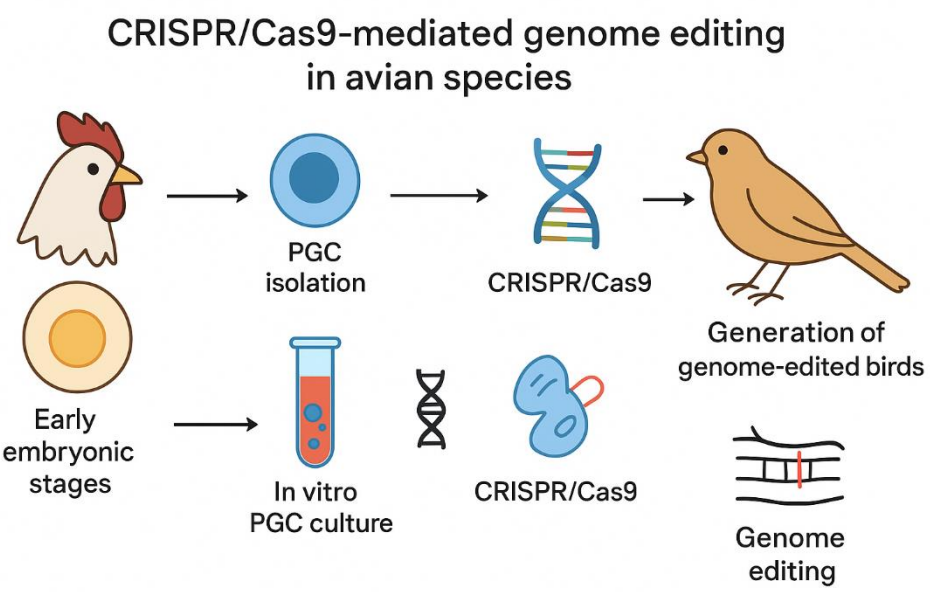

Figure 2. Workflow using the CRISPR/Cas9 system of programmable genome editing in avian species

involve the knock-in of a human interferon beta (hIFN - 2) into the ovalbumin gene exon 2 using CRISPR/Cas9. Since the initial production of the CRISPR-mediated genome-edited poultry in 2015, through electroporation of embryos, a lot of research has utilized this system on transgenic poultry species. The latest advancements are related to CRISPR/Cas9 being combined with tools of genomic analysis to enhance specificity of targets, efficiency of editing and reduce off-target effects [14].

CRISPR/Cas9-mediated genome editing in selected poultry species: The application of CRISPR/Cas9 has gained popularity because it has amazing capabilities of correctly editing and modifying the genomes of various organisms, such as avian species. There are different approaches that have been offered to generate genetically modified organism [15]. The first successful transgenic animal models that included mice, rabbits, sheep, and pigs were produced by microinjection of target DNA into a fertilized embryo pronucleus leading to germline modification. The other technique that is popular is the application of embryonic stem cells (ESCs), whereby the ESC is engineered to be genetically modified or subjected to gene therapy and is then injected into a host blastocyst to form germline chimeras [10]. As compared to mammals, introducing ESCs to avian zygotes is highly difficult as there is minimal germinal disc around the zygote, and the yolk is huge. Therefore, the initial generation of transgenic chickens was produced due to the inoculation of sub-germinal cavity of EGK stage X embryos by retroviruses. These transgenic chickens were the first generation of transgenic mice that harbored retroviral genes in germline, transmitting between 1 and 11 % [11]. Later efforts with lentiviral vectors raised the efficiency of germline transmission to 4-45% but the transgenes of the oviducts in the hens were inefficient at producing transgenic birds. Transgenic birds have been made in a variety of ways and include viral infection of stage X embryos, microinjection of transgenes in fertilized egg embryos and use of embryonic stem cells. Another significant breakthrough was the individual transplantation of poultry PGCs that were a more effective counterpart to the ESCs because it surmounted the low rates of germline transmission in avian species [12].

Transgenic birds Transfecting cultured PGCs with the desired genetic material and injecting those cells into embryonic blood vessels results in the generation of germline chimeras. Despite the initial low efficiency of transgenes transfer to circulating PGCs, Salvesen et al. managed to produce the first targeted gene knockout in chicken through homologous recombination in PGCs. The introduction of CRISPR/Cas9 technology has transformed the generation of chickens that are genetically engineered by making it possible to set up in

vitro PGC culture facilities with programmable genome editing. PGCs may be cloned out of embryonic blood or gonads and edited with this technology [13]. Such altered cells can be then microinjected into the blood streams of recipient embryos and form chimeras that mature into full birds. This technique has been utilized by Hernandez-Patlan et al. to produce chickens with a desired phenotype of ovomucoid gene changes by transplanting the drug-selected PGCs into recipient embryos in which the endogenous PGCs were destroyed using gamma irradiation [14]. A similar experiment successfully performed the knock-in of human interferon beta (hIFN-2) into exon 2 of the gene of chicken ovalbumin using the CRISPR/Cas9. Considering that the first CRISPR-mediated genome-edited poultry was made in 2015 when embryos were electroporated, a variety of studies have also described the use of this system in poultry species. Since then, innovations have focused on co-purifying CRISPR/Cas9 with genomic analysis instruments to enhance target specificity, editing efficiency, and off-target effects to make the system more accurate and reliable in the process of poultry genome engineering [16].

Applications of CRISPR/Cas9 system in poultry-related species: Genome editing in poultry species using CRISPR/Cas9 has many applications both in agricultural and biomedical research. The studies with reports of the application of CRISPR/Cas9 to poultry have been rising and it is a testament to the increasing interest and possibilities of the technology in genome engineering in avian genome engineering [17]. The choice of papers to be reviewed in this paper narrows down to those studies published more recently, as they are more relevant to showing the many areas that programmable CRISPR/Cas9-mediated modifications have been performed on birds.

Agricultural Applications of CRISPR/Cas9 System in Poultry: Gene editing of poultry through CRISPR/Cas9 has presented many prospects of improving agriculture related traits however there are various challenges that the commercial poultry sector might face with the application of gene editing technology primarily due to the elevated production cost of the genetically modified poultry. The need to achieve poultry exhibiting strong resistant to certain disease-causing pathogens is increasing, as well as the implemented genome editing technologies can be used to fulfil this need. The avian influenza virus (AIV) is one of the major threats to the health of poultry and is a highly virulent pathogen that causes periodical outbreaks of pandemics and significant losses in flocks. Conventional methods of control such as vaccination cannot be effective, which makes breeding poultry AIV-resistant a viable solution.

The latest research has looked at the application of developing transgenic chickens that are resistant to virus infection. In a study, Gao et al. produced transgenic chickens with short hairpin RNA sequences coded in the viral genome that inhibit the polymerase activity of influenza virus and suppress the replication of the virus, thus preventing infection [18]. Subsequent studies have indicated that the insertion of about 33 amino acids into the chANP32A, a member of the chicken acidic nuclear phosphoprotein 32 family member A protein improves the bird polymerase activity which can be a possible target in CRISPR/Cas9-mediated genome editing. Substituting chANP32A with human ANP32A (huANP32A) that is more actively expressed in the polymerase in avian cells could help prevent influenza virus and leave chickens resistant [19]. Also, chickens lack the gene I that is caused by retinoic acid (RIG-I), which makes them more susceptible to AIV infection than ducks, which inherently express RIG-I and have better resistance. With CRISPR/Cas9, one can insert genes with RIG-I-like antiviral properties into poultry creating the resistant poultry to AIV. Also viral inclusion can be silenced in transgenic chicken by the application of the 3D8 scFv fragment antibody. Avian leukosis virus (ALV) is another pathogen affecting poultry production, a retrovirus that causes tumor formation by inserting the viral DNA into the host cells. The deletion of a crucial amino acid components, W38, in the chicken Na^+/H^+ exchange 1 receptor, has been associated with resistance to ALV-J. By means of CRISPR/Cas9-mediated homology-based recombination, Kandlbinder et al. were able to create cultured DF-1 chicken cells resistant to ALV-J infection [20]. Likewise, Koslová et al. [17] and Lee et al. [4] used CRISPR/Cas9 to silence NHE1 W38 or tva gene sequences in DF-1 fibroblasts, and induced ALV-J-resistance in birds. Hellmich et al. used CRISPR/Cas9-mediated deletion of poultry NHE1 W38 using homology-directed repair in commercial lines, which made birds resistant to ALV sub. All of the studies demonstrate the general versatility of CRISPR/Cas9 in creating virus-resistant poultry as the basis of creating virus-immune poultry lines, which can significantly eliminate economic losses and decrease production costs [1].

In addition to disease resistance, the CRISPR/Cas9-mediated genome editing could be used to enhance the poultry performance through the boosting of muscle development. MSTN as a gene is a negative regulator of skeletal muscle growth in animals. Mutation in MSTN was proven to increase the mammalian and fish muscle mass. In poultry, the anti-myogenic effect of MSTN may be suppressed by in-frame mutations, e.g. the mutations introduced in Japanese quail, leading to a gain of muscle mass. The interference of MSTN in chicken using CRISPR/Cas9 results in a chicken with greater muscle mass, which has significant agricultural applications since it results in more productive, better-performing and higher-yielding chickens, which would be instrumental in solving the problem of food security [21].

Applications of CRISPR/Cas9 in Biomedical Research: Genetic editing has become one of the biggest developments in the field of molecular and medical research, and its modern achievements prove that it can cure a great variety of diseases in the future. Genome editing with the help of Cas9 has made it possible to create extremely precise methods of gene modification in avian species [22]. This advancement has also led to dramatic change in the animal biotechnology studies with particular manipulation of useful avian models including chicken. Poultry gene editing offers the possibility to create the effective bioreactor system to produce high-value proteins. Such systems may help chicken to produce egg white proteins in large scale, which are easily purifiable. Ovalbumin promoters have extensively been used to express the target proteins in transgenic hens which makes it possible to produce therapeutic proteins in egg white. This has gained greater significance in the construction of transgenic poultry models with biopharmaceutical applications, and this is a major advancement in the genome editing technologies. Indicatively, human interferon-beta (hIFN- 2) is injected into locus of egg white allergen ovalbumin so that it can be synthesized in an egg white. Moreover, interference of the ovalbumin and ovomucoid genes through CRISPR/Cas9 has been explored as a solution to minimize the allergicity of eggs. These alterations have the potential to result in a reduced immunological response in patients with egg-white allergies, which proves that genome editing can be useful to enhance therapeutic proteins production, as well as food safety [11].

It is anticipated that gene editing will become very instrumental in eliminating allergies induced by chicken eggs, to produce allergy-free eggs and chicken meat to be consumed by sensitive people. This may be done by silencing allergenic genes, e.g. those producing egg white proteins, e.g. ovalbumin and ovomucoid. The implications of such developments on the production of safe food products were very high but they also have implications in the pharmaceutical industry especially in producing vaccines. Furthermore, chickens can be now engineered into humanized forms where they are designed to generate therapeutic antibodies against particular antigens to be utilized in clinical therapy. By use of CRISPR/Cas9 to insert loxP sites in the variable portion of the immunoglobulin heavy chain, it becomes possible to create genome-edited chickens that can produce such antibodies. It is an innovative and new method of molecular medicine that provides wide prospects of characterization and development of therapeutic antibodies [23].

Limitations of using CRISPR/Cas9 system in poultry production: Inspite of the numerous advantages of this system and technological innovations in the poultry industry, the implementation of this technology has generated various issues in society, ethically, and legally [23] The main issue is that off-target effects can occur and interfere with highly variable viral nucleic acids or plasmid DNA of useful bacteria, which can change the composition of the microbiome of the bird. The fact that new CRISPR-based DNA-editing technologies have been developed in microorganisms further highlights the fact that avian microbiomes can be altered as has been witnessed in other organisms. When the frequency of determination (CFD) score reaches a maximum of 0.28, which is the maximum possible frequency at which the off-target activity can be measured, it has been reported in certain studies [24]. Moreover, the insertion of specific alleles with the help of inserted DNA vectors can also cause unintended deletions or duplications because of the limitations of the DNA repair machinery, which cannot always insert foreign DNA into the host genome. Transcriptome sequencing and next-generation sequencing technologies can be used to screen genome-engineered animals thoroughly, which is important to give critical data regarding the possible off-target alterations in food animals. In the past, direct electroporation into the egg has been very ineffective in obtaining germline transmission in chicken embryos [25]. Nevertheless, PGC-mediated transgenesis and genome editing is a more dependable method. This technique can be described as the in vitro transfection of

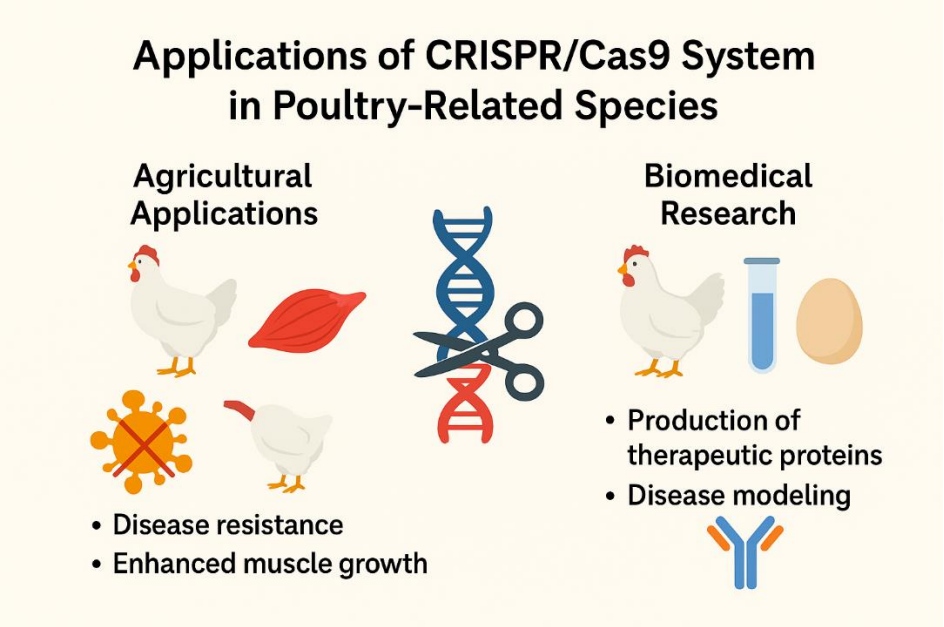

Figure 3. Applications of CRISPR-Cas9 system in poultry-related species

PGCs and their subsequent injection into host embryos. The germline transmission rates with such a strategy are inconsistent, with the range of 0-90 percent, yet in most cases, it demonstrates positive results compared to direct embryo modification. In spite of such technological development, commercialization of genetically modified poultry products is still in low gear in most countries. The exorbitant prices of producing genome-edited chicken and the strict regulations of GMO control bodies limit the broad use of these products in the market [26].

Current strategies for minimizing off-target effects in CRISPR/Cas9-mediated genome editing: The most commonly used Cas9 nuclease is *streptococcus pyogenes* Cas9 (SpCas9); nevertheless, this Cas9 is reported to cause genome-wide off-target mutations. To overcome this shortcoming, in the last five years, scientists have created various Cas9 variants and orthologs that are more specific and have fewer off-target effects. Some of the available variants of Cas9 include SaCas9, SpCas9-Nick, dCas9, dCas9-FokI, xCas9, Cas9-NG, SpCas9-HFI, eSpCas9, Hypa-Cas9, Sniper-Cas9 and HiFi Cas9. SaCas9, a smaller nuclease of Streptococcus aureus, is endosomally packaged efficiently within adeno-associated virus (AAV), allowing it to be used both systemically and in cells, unlike SpCas9 [27]. SpCas9 is specific to the 5'-NGG-3' PAM sequence whereas SaCas9 is specific to the 5'-NNGRRT-3' PAM sequence. This variation leads to the reduced off-target mutation rates with SaCas9. Non-biased identification of double-strand breaks (DSBs) by genome-wide GUIDE-sequencing has shown that SaCas9 is more specific to be recruited in its targets than wild-type SpCas9. Other engineered versions of SpCas9 have also been shown to have better specificity. The off-target activity of SpCas9-Nick, a derivative of SpCas9 with the RuvC catalytic domain inactivated, is reduced by around fivefold with a reported fivefold reduction in non-targeted DNA modifications compared to wild-type SpCas9. dCas9-FokI, which is a catalytically dead SpCas9 with the FokI nuclease domain, has 140-fold higher on-target activities and is far less toxic [28]. Other SpCas9 variants, such as Cas9-NG, evoCas9 and xCas9, contain low non-specific activity but high target specificity in animals and plants. It is worth noting that xCas9 has a wider set of PAMs, such as GAA, GN, and GAT, making it even more useful in genome editing.

Improved Viral and Non-Viral CRISPR Delivery Methods: Use of gene editing has been very effective in gene therapy where viral vectors are commonly used to pass on the necessary elements. Viral vectors can also be used in the CRISPR/Cas9 system to express the Cas9 nuclease and the programmable guide RNA (gRNA) in a recombinant plasmid and then introduced into target cells via viral transduction [29].

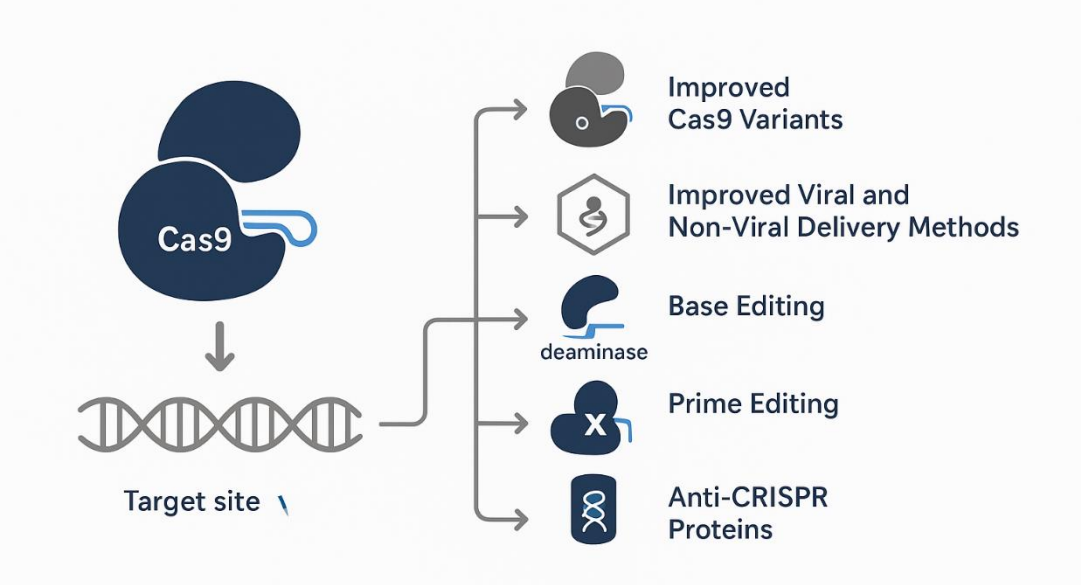

Figure 4. Strategies for minimizing off-target effects in CRISPR-Cas9-mediated genome editing

One of the possible limitations of this method is more frequent off-target effects since continuous expression of the components of CRISPR/Cas9 in the target cell may lead to higher levels of Cas9 in the cell, which facilitates the unwanted changes in the DNA. In order to curb these risks, low genetic integration promising viral vectors, including adenovirus (AdV), have been utilized, and they are less likely to have off-target insertion events. Alternatively, other non-viral delivery systems are created, such as the direct delivery of Cas9-gRNA ribonucleoprotein (RNP) complexes to target cells. Another major benefit of this approach is that the RNP is active temporarily but will fade away with time thus reducing off-target effect. Moreover, unlike plasmid DNA-based delivery, delivery of RNPs through liposome-mediated transfection has been observed to reduce off-target mutations with efficient genome editing [30].

Base Editing: Although the use of the traditional CRISPR/Cas9-mediated non-homologous end joining (NHEJ) transposes result in the appearance of double-stranded breaks (DSBs) at random sites and locations within the target gene, which may induce off-target effects, base editing offers a more specific solution). Base editing enables one to directly convert specific nucleotides without causing DSBs, thereby preventing the occurrence of unwanted genomic modifications (Nasar et al., 2024). The base editing system generally includes a catalytic deaminase enzyme, a single-guide RNA (sgRNA), and catalytically inactive Cas9 (dCas9). Base editors of two main categories, cytosine base editors (CBEs) that transform C-G base pairs to T-A and adenine base editors (ABEs) that transform A-T base pairs to GC, have been developed. Although base editing is a new technology, it has proven to be very efficient and precise, broadening the range of application of genome-editing. Proper delivery of base editors with the help of suitable vectors also increases the specificity of targets and decreases the chances of off-target mutations [31].

Prime Editing: Most recently, a new experimental gene-editing method, dubbed prime editing, has been invented, which has allowed the rapid conversion of all types of nucleotides, such as insertions, deletions (indels), and recombinase events on mammalian cells without using a donor DNA (dDNA) template. Though base editing is an effective way to induce only four different types of transition mutations C→T (or G→A) and A→G (or T→C) there are only 4 possible pairs of bases pairs that base editing can induce. By contrast, prime editing enables installation of every possible base pair replacement providing greater flexibility in genome editing. This method offers an attractive way of minimizing off-target effects and enhancing site-specificity in genome editing. Nevertheless, additional research in animal models is needed to assess its

feasibility to be used in therapeutic applications and possible in human consumption [32].

Anti-CRISPR Proteins: Anti-CRISPR (Acr) proteins discovery has offered a new way to regulate and improve the accuracy of CRISPR/Cas-based genome editing in animal cells. To date, more than fifty Acr proteins were discovered and each of them adopts different mechanisms to prevent the action of CRISPR-induced DNA cleavage. Interestingly, it has been demonstrated that proteins AcrIIA2 and AcrIIA4 are capable of selectively suppressing Cas-mediated genome editing, minimizing off-target editing, and maintaining on-target editing in the cell [30]. Anti-CRISPR proteins use is thus a new approach to enhance the safety, controllability, and efficiency of CRISPR/Cas technologies.

Future Perspectives: This technology has rendered the gene editing process highly efficient and it outshines other modern techniques including homologous recombination. Although the programmable CRISPR/Cas9-based genome engineering has been more widely deployed in both small and large mammals, the use of the technology in avian species, such as chicken, is actively advancing and is likely to not only enter the competitive level in the nearest future but also stay in the same state [33] The future research on the basic biological processes is expected to take place through the creation of transgenic poultry species that will express Cas9. This practice will allow more efficient and quicker molecular and cellular research, enhancing the knowledge of the functioning of genes and their use, as already done with pigs. The CRISPR/Cas9-based targeting of PGCs is an effective method to produce genetically engineered avian species with the preferred gene characteristics. Promising future of the poultry industry is probably to be concerned with breeding poultry to have greater feed efficiency and lean meat composition thus will be more palatable to human beings. Although the risk of decreasing the feed efficiency of poultry is small, it can be changed, in the context of the CRISPR-based transgenes development. The same has been done in other meat-producing animals (like pigs) where CRISPR/Cas9-based editing of the UCP1 (uncoupling protein 1) gene in white adipose tissue led to lower fat deposition and higher lean carcass percentage. These porcine models were used to show that a mouse adiponectin-UCP1 construct can be successfully inserted into the endogenous UCP1 locus and show great improvements in carcass composition.

The CRISPR/Cas9 has been applied to silence selected avian genes in poultry with experimental results in which a higher proportion of lean muscle and a smaller proportion of fat in birds were noted, which have the potential to enhance consumer acceptance and nutrition quality. The future trends are likely to speed up the creation of a genetically modified bird. To begin with, genetically engineered in vitro PGCs may be reconstructed into the embryonic bloodstream or even the testes of adult hosts which are sterile and this is more efficient, faster and uses less amount of animals and this may extend PGC technology to other poultry species. Second, genetic sterility may be used as a useful application in the RNA-guided Cas9 genome editing. In cases of effective transgenesis, sterile chickens can serve as a surrogate host of germline transfer [29]. Lastly, adenoviral vectors of CRISPR/Cas9 can potentially revitalize subgerminal viral injection methods, expediting their research on knockouts of avians and making new avian applications possible in biotechnology.

Conclusion

In the years, the development of CRISPR technology has led to the generation of transgenic lines of chicken which can be used to produce meat and eggs as the main food products. CRISPR technology can revolutionize the poultry sector to enable sustainable and effective production to ensure food security in the world. Genome-edited poultry has a higher likelihood of possessing a better phenotype, such as improved feed efficiency, digestibility, growth, egg yield and overall performance, and has the potential to boost disease resistance. Such gains will not only promote poultry health but the safety of the vaccines that will be made using eggs as well as the safety of food in general. Translational Biomedical, Genome editing using CRISPR in poultry has got unbelievable potential which can be utilized to understand the pathogenesis of diseases, generate therapeutic proteins and develop new preventive therapies. A lot of these technologies applied in poultry would be of great importance to human health and medicine. Lastly, the recent innovations surrounding the CRISPR/Cas9 systems will help resolve the current issues on regulatory acceptance, societal receptor, and ethical issues, and will open the path to the wider application of genome-edited poultry in agriculture and biotechnology.

Authors' contributions

ICMJE criteria	Details	Author(s)
1. Substantial contributions	Conception, OR	1
	Design of the work, OR	2,4,6
	Data acquisition, analysis, or interpretation	3,5
2. Drafting or reviewing	Draft the work, OR	1,2,5
	Review critically for important intellectual content	3,4,6
3. Final approval	Approve the version to be published	1,2,3,4,5,6
4. Accountable	Agree to be accountable for all aspects of the work	1,2,3,4,5,6

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Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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