



Review Article

Genome editing for indigenous poultry conservation: Possibilities for developing Nations

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Abstract

Poultry is one of the major sources of protein for human nutrition. They are domesticated for meat, eggs, and feathers. Rapid centralization, commercialization, and research developments in the poultry sector, have paved the way for the production of economically viable varieties, which provide better and efficient production performance. However, this has threatened the breed and population diversity of Indigenous chicken breeds. Therefore, there is a need for the conservation of the existing breed diversity of Indigenous chickens. Traditional methods for the conservation of poultry genetic resources including phenotypic characterization and controlled-planned mating may not be sufficient to achieve the desired outcomes. As a result, genome editing technologies are necessary for the conservation of poultry genetic resources, especially those with very low population sizes. These technologies may enable the restoration of extinct species and breeds, as well as the repopulation of threatened breeds.

Keywords conservation, CRISPR, genome editing, indigenous poultry

Introduction

Poultry has long been recognized as the most economical, environmentally friendly, and widely available source of protein for the global population. While poultry includes chicken, turkey, guinea fowl, geese, and ducks, the majority of meat and egg production comes from chickens. Commercial strains and varieties of chickens have been specifically bred for their high productivity in terms of egg and meat production [1]. Currently poultry sector is well organized in India as well as around the world, serving as a significant source of animal protein, income, and livelihood. In commercial poultry systems, factors such as high reproductive rates, low unit costs, and efficient transportation play crucial roles in determining profitability. Although the commercial poultry sector has experienced unprecedented growth in the past decade, backyard and rural poultry production remains an important aspect of livelihood and food security in developing economies. For instance, as per the 20th livestock census of India, commercial poultry has experienced a growth rate of 4.5 %, whereas, backyard poultry production has experienced a growth rate of 45.8%, when compared with the previous census. Similarly, if the distribution is experienced as per area, the urban area has experienced 17.95 % growth, and rural areas have experienced 3.95 % growth in the commercial poultry sector

Received: 06 March 2024

Accepted: 21 April 2024

Online: 22 April 2024

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Emer Life Sci Res (2024) 10(1): 78-89

E-ISSN: 2395-6658

P-ISSN: 2395-664X

DOI: <https://doi.org/10.31783/elsr.2024.1017889>



(20th Livestock Census of India). This shows that advancements in poultry production are driving growth in backyard and rural poultry production too. Nonetheless, following this growth acceptability towards fast-producing commercial strains is on the increase, due to their economic viability, and this, has threatened the Indigenous poultry breeds.

Indigenous poultry breeds have evolved in various agroclimatic zones and hence have developed special characteristics that make them resilient to survive under adverse climates, resistant against various infections also perform as good brooders, and can thrive under low feeding conditions. However, they have low production potential which is exhibited by slow growth rates, smaller egg size, decreased clutch size, delayed sexual maturity etc., [2]. The biodiversity among these breeds is under extreme pressure from commercial poultry production. With the increasing food demand, technological progress, and commercialization, the commercial breeds of chickens have been drastically improved for better production in the least amount of time. Modern chicken lines are specially bred and developed for high production, either egg or meat as well as reproductive characteristics [3]. With the designing of environmentally controlled housing, pre-balanced feeds, better animal transport facilities etc., organizations and farmers have tried to nullify the effects of climate and management over production. The changing economic scenario also favors the same. However, these activities have threatened the diversity and existence of Indigenous breeds. As, due to more production potential, farmers tend to adopt commercial strains/ varieties, and, the rearing and farming of Indigenous poultry breeds have decreased. This decrease in genetic diversity has been experienced more significantly in developing nations, which are under a state of transition, and active adoption of commercial varieties and systems, in poultry production.

With increasing consumer preferences and emphasis on organic production, in the past decade, traits like disease resistance and resilience have been included in the selection index. This is also because, the cost of disease treatments and vaccinations has increased, and, more importantly, there is the development of drug resistance among many pathogens, which has brought focus toward antibiotic free livestock rearing. Hence, the need for disease-resistant genotypes and varieties has increased and gained much attention recently. Looking into their importance, indigenous poultry breeds are being conserved in some places at the governmental level, still economic demands in developing countries favor commercial poultry production. Hence there is an urgent need to identify and conserve these breeds at national and regional levels so that they can be used in selection and breeding programmes as the gene pool for resilient traits.

The classical methods of conservation involve in situ or ex situ conservation, where whole animals are kept and bred, as per plan. These methods have worked, but still, the population of some Indigenous poultry breeds is on a drastic decline. Also, the fragmented nature of population distribution makes it difficult to assess its current structure and trend. Under such conditions, biotechnological tools must be deployed to conserve species with a smaller number of available individuals. Genome editing is one such technology, that is been advocated to be used in livestock species to promote the development of new traits/ characters. Besides editing the target gene for the desired phenotype this technology can also be used for the conservation of threatened species. The current review focuses on the utilization of genome editing technology for the conservation of threatened indigenous poultry breeds with a background of their population trends and methods of genome editing.

Indigenous poultry genetic resources

The genetic resources of indigenous poultry could be used for genetic improvement and diversification to produce poultry that can survive in a regional area. Classically, conservation efforts are directed through selection and breeding schemes, using phenotypic, and/or, more recently, by using genotypic data [4]. However, these efforts are limited in the case of a small and fragmented founder population. Also, recombination and crossing over will make it difficult to maintain the

purity of strain in the case of the unavailability of pure parental types. India is enriched with exceptional breed diversity in poultry genetic resources. There are about 19 registered chicken breeds in India that are distributed among 15 different agro-climatic regions of the country. The indigenous poultry population contributes about 6.5 % of the total chicken population of our country [2]. India's poultry genetic resources are very rich and are known for their meat and their survival in rough conditions, like Aseel, Kadaknath, and Kaunayen breeds are famous for lower fat content and calorie value, meat taste, high protein and iron contents, and medicinal values [5]. However, the indigenous poultry gene pool is often threatened by crossbreeding, poor resource base, economic demands and consumer shifts, availability, and cost implications of rearing. In particular, breeds like Kalasthi, Nicobari, and Hansli have very small population sizes, which are under 1 lakh heads [2].

Also, a global data analysis on the domestic poultry population gives a somewhat similar result. As per the Domestic Animal Diversity Information System (DAD-IS) database of the Food and Agriculture Organization (FAO) (<https://www.fao.org/dad-is/en/>), there are about a total of 3493 breeds of poultry (including chicken, duck, goose, Guinea fowl, quail, and turkey), out of which 1617 breeds are classified as local and regional breeds. The population trend of these species can be plotted by dividing them geographically as international, local, and regional. The data shows that a large proportion of local breeds of chicken have unknown population trends, similarly, most of the regional breeds also have unknown population trends (Figure 1). Also, some proportions of local breeds did show stable and increasing population trends, and a relatively smaller proportion

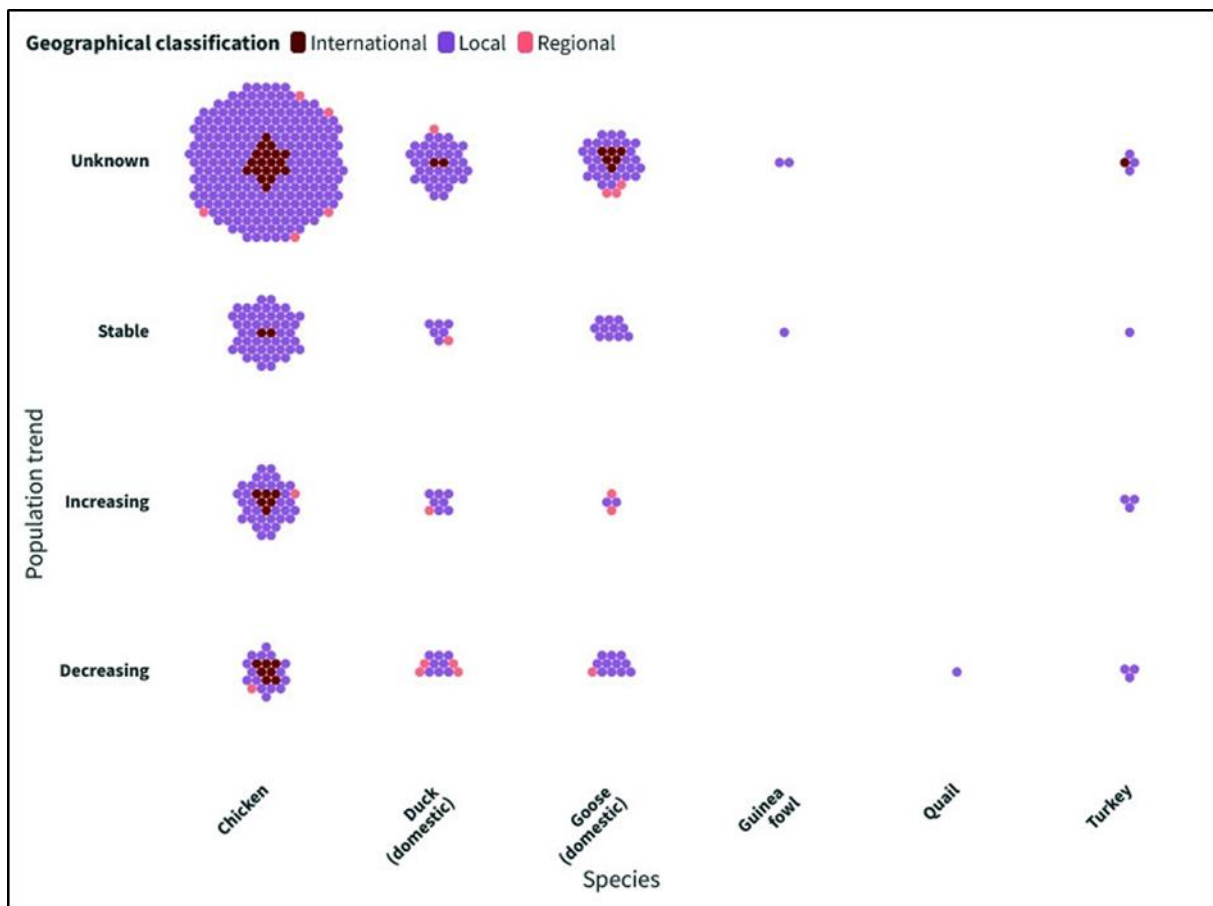


Figure 1. Population trend of various major domestic poultry species of world, as per their geographical classification

presented a confirmed decreasing trend (Figure 1). Similarly, if breed-wise world chicken varieties are observed, it is seen that there are about 919 international breeds, 1503 local breeds, and 114 regional breeds (DAD-IS). Also, a major proportion of breeds have unknown risk status (1730 total), which is constituted by 716 international breeds, 919 local breeds, and 95 regional breeds. Besides this, among the local breeds (1503 breeds), 73 have critical status, 201 are endangered, 43 are vulnerable, 89 are extinct, 4 are critically maintained, 16 are endangered (maintained), and 1 is only cryo-conserved i.e., about 25% of chicken breeds are having dwindling or diminishing populations, with 61 % having unknown risk status (Figure 2), as per DAD-IS data. The maximum proportion of unknown population trends indicates that the status of these local breeds has not been fully studied, and some of them may have very low population sizes or may be vulnerable in status. Thus, active conservation efforts are needed for the preservation of genetic diversity among chicken populations around the world.

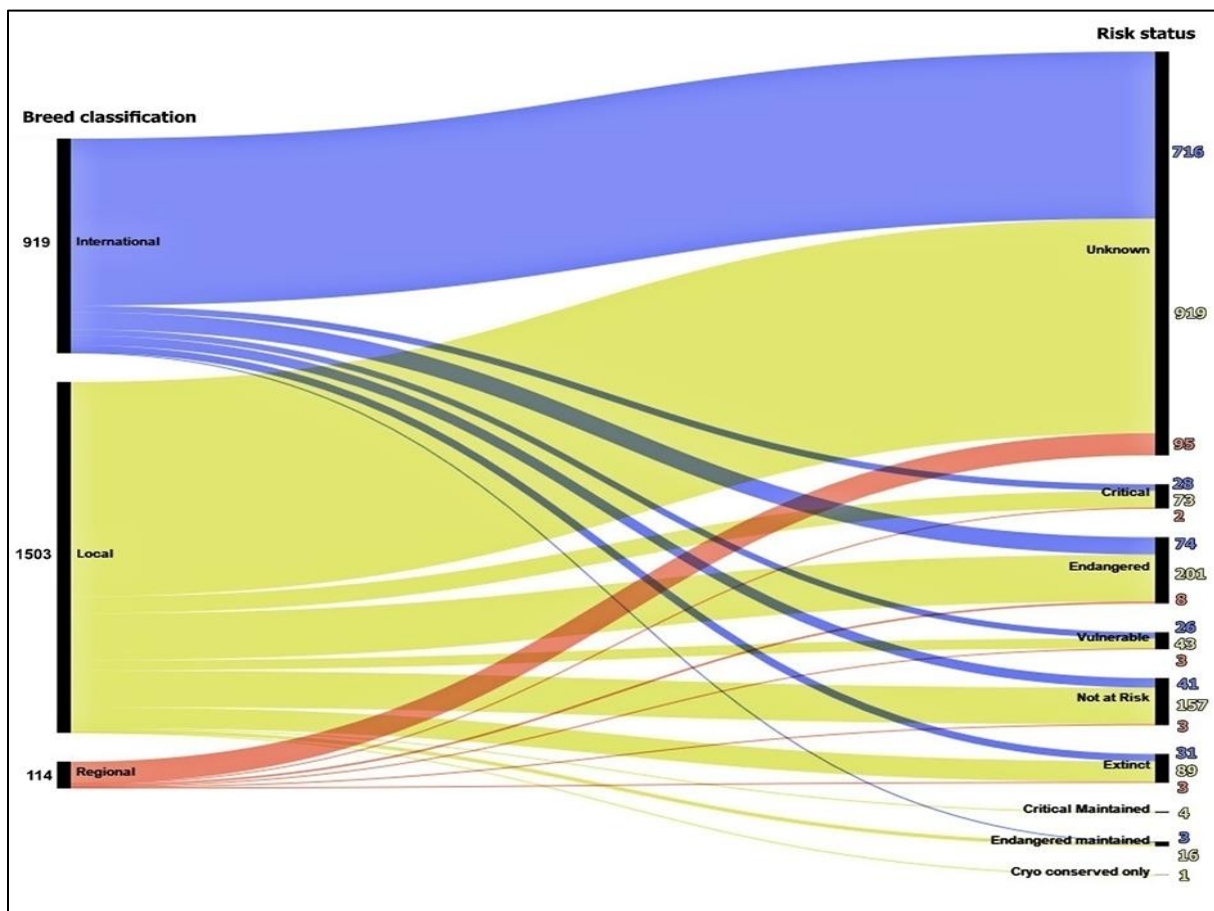


Figure 2. Breed classification of chicken as per their geographical distribution and their respective risk status (Where, numbers on the right in black indicates total number of breeds in respective categories, numbers on the left side of the graph indicates their respective risk status in color coding)

Methods of genome editing

Genome editing is an advanced technology that can be effectively utilized for editing/ modifying the genome of target species. Unlike the previous RNAi method, it is much more precise and can be applied for gene knock-out, knock-down, and even knock-in, its variation can also be used to insert or replace the existing gene copy. It involves creating double-strand breaks in DNA and editing or



modifying the bases using DNA repair mechanisms. A significant advantage of genome editing is that it is more accurate, has wide applicability, and can be used to insert transgene into the target cell. There are three major genome editing tools; Zinc finger nuclease (ZFN), Transcription activator-like effector nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas) [6]. Genome editing introduces DNA modifications by inserting, deleting, or changing segments of the DNA in an organism at a specific genomic location. Genome editing uses no foreign DNA to knockout or knockdown genes, instead, it uses programable nucleases to recognize a specific genome locus and nicks it, generating DNA double-strand breaks. DNA double-strand break can help mediate genome editing by two repair systems either a homology-directed repair (HDR) system or a non-homologous end joining (NHEJ) system [7].

Zinc finger nucleases (ZFN)

Zinc finger nuclease (ZFN) is an artificial restriction enzyme and is synthesized by combining two zinc finger proteins with the cleavage domain of Fok I endonuclease. Fok I is a restriction enzyme that cleaves the DNA strand away from its recognition (restriction) site and by combining it with a specific DNA binding protein (like zinc finger) the chimeric enzymes can be made to target the desired sequence with specificity [8]. Also, since FokI works as a dimer there is a need for two zinc finger domains to make double-strand breaks. Two ZFN are arranged in such a way that the tandem fingers array in ZFN wind around the DNA target sequence such that it recognizes a 9 bp target sequence [9]. The first targeted locus with ZFN was the yellow gene of *D. melanogaster*, and it showed that the gene alteration was passed through the germ line successfully [10], making it a promising tool for genome editing. The ZFN-mediated gene editing provides ways to produce gene knock-in and knock-out in an efficient and specific manner and hence can be used for modifications in the genome, either to correct or replace defective gene copies or introduce a transgene [11]. ZFN is being used for a variety of purposes, especially in diagnosis, disease modeling, and gene therapy as well as in the improvement of agriculturally important species [12-13].

Despite the success of ZFN technology, there are still many limitations that drastically restrict its use in more contexts. Off-target effects are a major limitation that may result in toxicity. It is due to the cleavage of non-targeted sequences by two ZFNs binding at non-canonical sites. This created unintended genome modifications, that may be difficult to detect or have prolonged ill effects.

Transcription activator-like effector nucleases (TALENs)

TALENs are chimeric restriction enzymes like ZFN. They are composed of DNA binding domains, which are made of a naturally occurring highly conserved series of tandem repeats [14], and *FokI* restriction endonuclease domain. The endonuclease *FokI* domain acts as a dimer and induces DSB in the target sequence of a gene, thus initiating the repair mechanism by either HDR or NHEJ [15]. Each tail repeat comprises 33 to 35 amino acids which are conserved, with divergent 12th and 13th amino acid positions, called the Repeat Variable Diresidue (RVD). The RVDs are highly variable and are specific for nucleotide recognition, by incorporating and altering the RVDs, the specificity of TALENs can be improved and managed effectively. Because of this property TALENs can target almost any target DNA sequence and they show low off-target binding capability and cytotoxic effects [16].

TALENs have numerous applications in research and therapeutics. Most experiments used one TALEN pair to generate NHEJ mutations, but for large segments of chromosomes (larger than 6 bp), two TALEN pairs that target the same chromosome are used to generate indels. TALENs have enabled the targeted alteration in many organisms like pigs [17], rats [18], cattle [17], and human pluripotent stem cells [19]. Although the reduced off-target effects and better specificity make TALEN a potent tool for genome editing, they are currently limited to inducing simple mutations that too with relatively low efficiency leading to the formation of mosaics [20].



Clustered regularly interspaced short palindromic repeats (CRISPR)

CRISPR and associated proteins (Abbreviated as CRISPR/Cas system) is a type of anti-invasion defense mechanism of host organisms, (mainly prokaryotes) from phage and plasmids, by preventing their adsorption, and infection. Thus, it is restricting the infective DNA from entering the host cell. These have been manipulated and programmed for biotechnology processes such as genome editing [21]. CRISPR has revolutionized genetic engineering, specifically genome editing. CRISPR loci organization is quite diverse, and it can move by horizontal gene transfer, and, consists of repeats and spacers. The upstream region, also known as the leader region of the CRISPR locus, is 20 to 534 bp and has high thymine and adenine content with a promoter and no open reading frame [22]. In its natural form and action, CRISPR detects and breaks the foreign DNA into small fragments by using the Cas nuclease enzyme, these DNA fragments are then integrated as spacers in the CRISPR locus of a host cell, further resulting in the development of acquired immunity [23]. After every integration, a repeat is duplicated, resulting in a spacer repeat unit with the Cas genes arranged subsequently [24]. The target recognition of Cas proteins depends upon a short-conserved sequence, protospacer adjacent motif (PAM) which is 2 to 5 bp long and is situated next to target DNA. The PAM sequences help distinguish between self and non-self, which helps in preventing the cleavage of the host genome and autoimmunity [25]. In the process of adaptation and interference, different types of PAM sequences, spacer acquisition motifs (SAMs), and target interference motifs (TIMs) are present respectively. The binding site of the Cas 9 enzyme is present upstream of the PAM sequence [26]. The CRISPR/Cas complex comprises two important components, the guide RNA which is made by CRISPR coding RNA (crRNA) and trans-activating crRNA (tracrRNA), and the Cas protein [27]. The crRNA is an RNA of 18 to 20 bp which is complementary to the invading nucleic acid, and the tracrRNA is a long loop, therefore, a single guide RNA (sgRNA) can be synthesized by combining these two [28]. The Cas proteins cleave the invading DNA, identified by the guide RNA [29].

Due to its widespread potential application, CRISPR has become an important tool for genome editing and efforts are underway to increase its efficiency, decrease off-target effects, and mitigate other limitations. One of the main limitations is the requirement of a PAM sequence near the target site, which limits the range of sequences Cas9 can target, a strategy to overcome this limitation is to relax the Cas9 PAM recognition specificity [30]. Cas9 variants of *Streptococcus pyogenes* (SpCas9) and *Staphylococcus aureus* Cas9 (SaCas9) with relaxed PAM recognition specificity showed increased genome editing activities at target sites with -NNNRRT- PAMs, thus increasing the target range [31]. By the RNA guided mechanism, Cas9 can cleave single-stranded RNA (ssRNA) which works independently of the PAM sequence in the target sequence. These RNA guided mechanisms are programmable and can reduce infection by ssRNA phage *in vivo* and as a result, Cas9 can cleave both RNA and DNA target sequences [32]. A programable CRISPR/Cas system is CasRx, which is derived from *Ruminococcus flavefaciens*, it targets RNA for high-efficiency knockdown and it is small enough to be packed into Adeno Associated Virus (AAV) vector system [33].

Another limitation of the CRISPR/Cas system is the off-target effects. One of the strategies to overcome this is to alter the sequence of sgRNA either by truncating its 3' end, shortening the 5' end complementary region, or by adding guanine to the 5' end. However, these methods have shown variable results and hence are not very effective [34-35]. Another advancement includes replacing the wild-type Cas9 nuclease with a D10 mutant nickase, in combination with two sgRNA that cleave the target sequence, and by controlling the Cas9-sgRNA complex concentration [35]. This strategy has acted in a two-fold manner, firstly, it was observed that off-target activity was reduced by many folds (in experimental cell lines), and secondly, the generation of two "nicks" creates 5' overhangs that are more liable to indel formation [36]. These advances in CRISPR technology have made it a promising tool for genome editing, which can be effectively utilized in a wide range of areas including medicine, agriculture, animal sciences, diagnostics, biodiversity, and even ecological engineering.



CRISPR/ Cas mediated genome editing in poultry

Genome editing is a promising technology, which enables to practically “edit” the genome of target species efficiently to generate the desired phenotype. In poultry, genome editing has immense potential applications. However, in poultry, the generation of genome-edited chicken is not the same as in major livestock species. The regular embryonic stem cell microinjection method into the zygote, is not applicable in poultry, as in chicken the zygote is surrounded by yolk and has a small germinal disc [37]. Hence in poultry, primordial germ cells (PGCs) are utilized for this purpose. PGCs are present in the central disc of pellucid at the ventral surface and they originate from the epiblasts [38]. PGCs migrate in the early embryonic stage to the dorsal side of the hypoblast, and then to the germinal crescent region after the formation of a primitive streak, then from the germinal crescent region, they migrate to the bloodstream once the blood vessels are formed, and then they reach the embryonic gonads, they multiply and differentiate into the germ cells. Chimeric birds can be created by PGCs, by isolating them at various embryonic stages or culturing them and transferring them to recipient embryos while maintaining germline potency due to the migration pathway of PGCs [39-41]. PGCs-mediated approach for genome editing in poultry seems to be the most applicable and efficient genome editing route. Dimitrov et al., [42] used this approach to target immunoglobulin heavy chains in cultured PGCs of chicken and reported 0 to 95% germline transmission rates of the altered gene. Similarly, Oishi et al., [43] demonstrated generating gene-specific knockout by the CRISPR/Cas9 system of two genes ovalbumin and ovomucoid in chicken using culture PGCs. Cultured PGCs, are therefore, providing a powerful tool for genome editing in poultry, and due to their migration pattern through embryonic blood vessels they are more likely to produce genome edited progeny. Besides these advantages, there are a few pitfalls of the PGCs culture approach. Only the chicken PGCs have been cultured, in vitro, successfully for elongated periods. Secondly, this method is time-consuming, requires screening of edited PGCs, and then after microinjection, the germline chimeras had to be reared up to sexual maturity to produce genome edited poultry [40]. Hence, methods like Sperm Transfection-Assisted Gene Editing (STAGE), involving direct transfection of spermatozoa with Cas9mRNA and sgRNA [44], direct injection of plasmids into embryonic blood vessels [45] and, adenovirus associated delivery of edited gene directly into the blastoderm [46] have been used with variable results.

Potential application of genome editing in poultry conservation

Classical strategies for the conservation of poultry genetic resources include phenotypic characterization and controlled-planned mating to achieve the desired goals. Most of the breeding programs have inherently focussed on the improvement of production traits, sometimes by cross-breeding. Although cross-breeding of indigenous breeds with exotic/high-producing breeds does increase production, there is always a problem of unavailability of parental stocks, especially at the farmer level. Also, the method dilutes the indigenous breed genetically, further hampering the genetic diversity and less phenotypic acceptability. Therefore, modern biotechnological tools are now required for the aggressive and active conservation of poultry species, especially those that have very low population sizes. These tools offer the chance to not only repopulate the threatened breeds but also revive the extinct species/ breeds. Conservation using modern biotechnological tools may be divided into two principal types, direct and indirect, as per the employed method.

Direct conservation

Direct conservation involves all those interventions that can preserve genetic material in the form that it can be later used to repopulate or revive an extinct or threatened breed. One of the basic methods in this is the cryopreservation. In poultry, because of the physiological constraints in egg cell, cryopreservation of semen has been practiced [47] but it suffers from poor fertilization rates and hence has not been practiced at a commercial scale [48]. Another approach is the utilization of PGCs



for cryopreservation. In chicken, the PGCs can be extracted from the circulatory system of the developing embryo and can be cultured and preserved in aliquots. These PGCs have immense potential to divide & can propagate well in defined culture medium [49]. PGCs, if obtained from rare or threatened chicken breeds can be cryopreserved for longer periods. These can be injected into the vascular system of a surrogate host made sterile using CRISPR/Cas technology. Woodcock et al., [50] utilized the same approach and created a commercial layer sterile female chicken line using the CRISPR/Cas system. This edited female line was used as a surrogate host. Further, cryopreserved PGCs derived from a rare chicken breed were first cultured and cryopreserved. Later, it was transplanted into the surrogate host to produce rare chicken offspring, which were selected and further propagated using cryopreserved semen of rare breeds. Thus, they were able to obtain rare chicken breeds from commercial broiler lines using CRISPR/Cas and PGCs culture methods. Thus, practically PGCs can provide the genetic material for the repopulation of threatened chicken breeds [50]. Although the technique is feasible, PGCs culture is a relatively difficult task and is logistically demanding. Thus, a newer approach to biobanking was adopted for the revival of rare chicken breeds. In this approach, instead of going for culture and cryopreservation of PGCs, whole gonadal tissue was cryopreserved. Chicken gonads can contain ample amounts of germ cells between days 9 and 12 of embryonic development. These gonadal germ cells retain their capacity to migrate to gonads up to the development stage of day 10. Thus, these cells when injected into a host embryo's vascular system (at day 2 of development) can migrate up to the gonad. The host line was made sterile using genome editing tools and was injected with the donor gonadal germ cell. Edited embryos can be selected based on reporter dye, incubated, and hatched thus forming a pool of GE chicken containing the gonadal germ cells from donor breeds. These then can be bred to obtain pure donor offspring. Thus, making the overall process of cryopreservation and repopulation less time consuming [51].

Thus, if the gonadal germ cells can be obtained from threatened breeds of chicken, commercial broiler chicken lines can be used to revive their population numbers. The preservation of gonadal cells is called biobanking because directly biological material has been cryopreserved instead of culture and multiplication of cells and their subsequent storage. Utilization of genome-edited surrogate hosts in chickens opens new platforms, and areas for the conservation of poultry breed diversity, especially in developing countries, for breeds where population numbers are getting relatively low, but this should be envisaged with appropriate regulations for judicious use of this technology.

Indirect conservation

Conservation of poultry birds includes identifying, monitoring, and characterizing indigenous and commercial genetic resources to ensure their long-term availability. Characterizing can be done on many levels including phenotypic to molecular analysis. Gene banks are also being developed for conservation. However, there are some limitations in using genome editing technologies in the conservation of a species. Cost is one key factor to consider. Due to the high cost of cryopreservation and genome editing technology, most conservation projects suffer budget and resource constraints, and hence only a limited number of samples can be preserved. Another limitation is, that it is a time-consuming and labor-intensive process, with a requirement of technical expertise and logistics.

To use genome editing to solve conservation issues, the conservation community must make ground-breaking efforts [52-53], which may include limited use of technology in targeted areas. Genome editing technology can be used to improve the economic viability of indigenous chicken breeds, although, in a restricted manner, this approach may prove effective, especially, in regions/countries where cryopreservation and genome editing is a costly affair. Genome editing in poultry can be used to increase meat productivity, and meat quality, and make chicken resistant to the environment&/or diseases. Thus, the indirect methods involve using genome editing technology for trait improvement of Indigenous breeds, hence making them more economically viable and



sustainable. In the poultry industry, one of the most important economic parameters is meat quantity. It is already known that the myostatin (MSTN) gene is a negative regulator of muscle development and in various livestock species, knockout of the MSTN gene has created animals with improved muscle mass [54-56]. Similarly, in chicken and quails also, the knock-out MSTN gene has resulted in increased muscle mass and production. It also resulted in lower fat deposition and better quality muscle growth [57]. In layers, the chicken egg has been targeted as a bioreactor for the production of therapeutic proteins. Recently, Oishi et al., [58] integrated the human interferon beta gene in the ovalbumin locus, resulting in hIFN- β , i.e., the production of recombinant human interferon beta protein in the egg white of chicken egg. Thus, making egg white a potential source of therapeutic proteins. It is also suggested that due to its biochemical nature egg white can be utilized as a source of pharmaceutical and therapeutic drugs by using genome editing tools [40].

Disease outbreaks in poultry cause a significant loss to the poultry industry and are harmful to human health. Diseases that cause huge economic losses are Avian Influenza Virus (AIV), Infectious laryngotracheitis (ILT), Infectious Bursal Disease (IBD), Newcastle Disease, and Marek's Disease. *Salmonella*, *Escherichia*, and *Campylobacter jejuni* bacteria also cause serious infections in humans and poultry. Selective breeding is not effective, due to low heritability and their polygenic nature; hence, genome editing is an effective and important tool for generating resistance in poultry [59-60]. Lyallet et al., [61] developed a transgenic chicken that is immune to the AIV virus by expressing a short hairpin RNA, which interferes with viral propagation. A subgroup of ALV (Avian leukosis virus), ALV-J causes tumors and myeloid leukosis in chickens, an amino acid W38 plays an important role in Na⁺/H⁺ exchanger type 1 for the entry of virus in the chicken, by deletion of W38 amino acid using CRISPR/Cas9 system and following homology-directed repair pathway, ALV-J resistant chicken was generated, without showing any off-target effects [62]. These modifications were aimed at improving the quality and quantity of poultry products or making them sustainable under prevailing climatic and pathogenic environments. Such genome editions may improve the quality of products from indigenous poultry, consequently making them economically viable and sustainable.

Conclusion

Genome editing is a promising and developing technology that can have wide applications ranging from gene therapy to reviving extinct species. The technology has been centrally utilized for the development of transgenic organisms as well as disease diagnosis. However, one important application of genome editing could be its application in poultry conservation. Besides, the improvement of production and disease-resistant traits, it has been shown that genome editing technology can be used effectively, to repopulate rare or threatened chicken breeds using existing commercial broiler lines as surrogate hosts. Genome editing and biobanking, hence, can be used for breeds with very small population sizes either vulnerable or threatened. Although genome editing in poultry also has some disadvantages, which include higher costs, off-target effects, and community acceptability [63], looking into the advances in editing technologies it is fair to state that they have the potential to change the population dynamics and rearing preferences of the poultry sector.

Authors Contribution

SPT, APS conceptualized the work. JC and KS performed data curation and analysis. JC, KS, TJ, SS, and BRP drafted the manuscript, APS and SPT further performed proofreading.

References

- [1] E. Mitchell, G. Tellez and M. J. McGrew (2023). Chicken genome editing for investigating poultry pathogens. *Avian Pathol.*, **52**: 1-11.
- [2] A. K. Mishra (2022). Poultry genetic resources of India and its role in rural poultry production. *Indian J. Plant Genet. Resour.*, **35**: 242-246.



- [3] I. Hoffmann (2005). Research and investment in poultry genetic resources challenges and options for sustainable use. *World Poult. Sci. J.*, **61**: 57-70.
- [4] B. Besbes (2009). Genotype evaluation and breeding of poultry for performance under sub-optimal village conditions. *World Poult. Sci. J.*, **65**: 260-271.
- [5] S. F. Ahmad, G. Kour, A. Singh and M. Gulzar (2019). Animal genetic resources of India – An Overview. *Int. J. Livestock Res.*, **9**: 1-12.
- [6] D. M. Ichikawa, O. Abdin, N. Alerasool, M. Kogenaru, A. L. Mueller, H. Wen and D. O. Giganti et al., (2023). A universal deep-learning model for zinc finger design enables transcription factor reprogramming. *Nature Biotechnol.*, **41**: 1117-1129.
- [7] N. G. Karavolias, W. Horner, M. N. Abugu and S. N. Evanega (2021). Application of gene editing for climate change in agriculture. *Front. Sustain. Food Syst.*, **5**: 296. [doi: 10.3389/fsufs.2021.685801](https://doi.org/10.3389/fsufs.2021.685801).
- [8] Y. G. Kim, J. Cha and S. Chandrasegaran (1996). Hybrid restriction enzymes: zinc finger fusions to FokI cleavage domain. *Proc. Natl. Acad. Sci. USA*, **93**: 1156-1160.
- [9] M. L. Maeder and C. A. Gersbach (2016). Genome-editing technologies for gene and cell therapy. *Mol. Ther.*, **24**: 430-446.
- [10] M. Bibikova, M. Golic, K. G. Golic and D. Carroll (2002). Targeted chromosomal cleavage and mutagenesis in *Drosophila* using zinc-finger nucleases. *Genetics*, **161**: 1169-1175.
- [11] L. M. Tang, C. L. Zhou, Z. F. Guo, L. Xiao and A. C. Chüeh (2015). Advances in zinc finger nuclease and its applications. *Gene and Gene Edit.*, **1**: 3-15.
- [12] I. D. Carbery, D. Ji, A. Harrington, V. Brown, E. J. Weinstein, L. Liaw and, X. Cui (2010). Targeted genome modification in mice using zinc-finger nucleases. *Genetics*, **186**: 451-459.
- [13] J. J. Whyte, J. Zhao, K. D. Wells, M. S. Samuel, K. M. Whitworth, E. M. Walters and M. H. Laughlin et al., (2011). Gene targeting with zinc finger nucleases to produce cloned eGFP knockout pigs. *Mol. Reprod. Dev.*, **78**: 2. [doi:10.1002/mrd.21271](https://doi.org/10.1002/mrd.21271).
- [14] A. J. Bogdanove and D. F. Voytas (2011). TAL effectors: customizable proteins for DNA targeting. *Science*, **333**: 1843-1846.
- [15] J. C. Miller, S. Tan, G. Qiao, K. A. Barlow, J. Wang, D. F. Xia, X. Meng and et al., (2011). A TALE nuclease architecture for efficient genome editing. *Nat. Biotechnol.*, **29**: 143-148.
- [16] N. Sun and H. Zhao (2013). Transcription activator-like effector nucleases (TALENs): A highly efficient and versatile tool for genome editing. *Biotechnol. Bioeng.*, **110**: 1811-1821.
- [17] D. F. Carlson, W. Tan, S. G. Lillico, D. Stverakova, C. Proudfoot, M. Christian and D. F. Voytas et al., (2012). Efficient TALEN-mediated gene knockout in livestock. *Proc. Natl. Acad. Sci. USA*, **109**: 17382-17387.
- [18] Y.H. Sung, I.J. Baek, D.H. Kim, J. Jeon, J. Lee, K. Lee and D. Jeong et al., (2013). Knockout mice created by TALEN-mediated gene targeting. *Nat. Biotechnol.*, **31**: 23-24.
- [19] D. Hockemeyer, H. Wang, S. Kiani, C. S. Lai, Q. Gao, J. P. Cassady and G. J. Cost et al., (2011). Genetic engineering of human pluripotent cells using TALE nucleases. *Nat. Biotechnol.*, **29**: 731-734.
- [20] H. Wang, H. Yang, C. S. Shivalila, M. M. Dawlaty, A. W. Cheng, F. Zhang and R. Jaenisch (2013). One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell*, **153**: 910-918.
- [21] R. Barrangou, C. Fremaux, H. Deveau, M. Richards, P. Boyaval, S. Moineau and D.A. Romero et al., (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science*, **315**: 1709-1712.
- [22] H. Deveau, J.E. Garneau and S. Moineau (2010). CRISPR/Cas system and its role in phage-bacteria interactions. *Annu. Rev. Microbiol.*, **64**: 475-493.
- [23] J. A. Doudna (2020). The promise and challenge of therapeutic genome editing. *Nature*, **578**: 229-236.
- [24] H. Zhang, J. Zhang, P. Wei, B. Zhang, F. Gou, Z. Feng and Y. Mao et al., (2014). The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. *Plant Biotechnol. J.*, **12**: 797-807.



- [25] D. Gleditzsch, P. Pausch, H. Müller-Esparza, A. Özcan, X. Guo, G. Bange and L. Randau (2019). PAM identification by CRISPR-Cas effector complexes: diversified mechanisms and structures. *RNA Biol.*, **16**: 504-517.
- [26] S.A. Shah, S. Erdmann, F.J.M. Mojica and R.A. Garrett (2013). Protospacer recognition motifs: Mixed identities and functional diversity. *RNA Biol.*, **10**: 891-899.
- [27] K. S. Makarova, D. H. Haft, R. Barrangou, S. J. Brouns, E. Charpentier, P. Horvath and S. Moineau et al., (2011). Evolution and classification of the CRISPR-Cas systems. *Nature reviews Microbiology*, **9**: 467-477.
- [28] C. R. Hale, S. Majumdar, J. Elmore, N. Pfister, M. Compton, S. Olson and M. P. Terns (2012). Essential features and rational design of CRISPR RNAs that function with the Cas RAMP module complex to cleave RNAs. *Mol. Cell*, **45**: 292-302.
- [29] M. Asmamaw and B. Z. Wondimu (2021). Mechanism and applications of CRISPR/Cas-9-mediated genome editing. *Biol.: Targets Ther.*, **15**: 353-361.
- [30] F. Uddin, C.M. Rudin and T. Sen (2020). CRISPR gene therapy: applications, limitations, and implications for the future. *Front. Oncol.*, **10**: 1387. doi: [10.3389/fonc.2020.01387](https://doi.org/10.3389/fonc.2020.01387).
- [31] B. P. Kleinstiver, M. S. Prew, S. Q. Tsai, N. T. Nguyen, V. V. Topkar, Z. Zheng and J. K. Joung (2015). Broadening the targeting range of *Staphylococcus aureus* CRISPR-Cas9 by modifying PAM recognition. *Nat. Biotechnol.*, **33**: 1293-1298.
- [32] S. C. Strutt, R. M. Torrez, E. Kaya, O. A. Negrete and J. A. Doudna (2018). RNA-dependent RNA targeting by CRISPR-Cas9. *eLife*, **7**: e32724. doi: [10.7554/eLife.32724](https://doi.org/10.7554/eLife.32724).
- [33] S. Konermann, P. Lotfy, N. J. Brideau, J. Oki, M. N. Shokhirevand, P. D. Hsu (2018). Transcriptome engineering with RNA-targeting type VI-D CRISPR effectors. *Cell*, **173**: 665-676.
- [34] N. Rusk (2015). Next-generation CRISPRs. *Nat. Methods*, **12**: 36. doi: [10.1038/nmeth.3237](https://doi.org/10.1038/nmeth.3237).
- [35] X. H. Zhang, L. Y. Tee, X. G. Wang, Q. S. Huang and S. H. Yang (2015). Off-target effects in CRISPR/Cas9-mediated genome engineering. *Mol. Ther. Nucleic Acids*, **4**: e264. doi: [10.1038/mtna.2015.37](https://doi.org/10.1038/mtna.2015.37).
- [36] R. L. Frock, J. Hu, R. M. Meyers, Y. J. Ho, E. Kii and F.W. Alt (2015). Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases. *Nat. Biotechnol.*, **33**: 179-186.
- [37] C. N. Khwatenge and S. N. Nahashon (2021). Recent advances in the application of CRISPR/cas9 gene editing system in poultry species. *Front. Genet.*, **12**: 627714. doi: [10.3389/fgene.2021.627714](https://doi.org/10.3389/fgene.2021.627714).
- [38] L. C. Puchta and D. Sawicka (2020). CRISPR/Cas9 gene editing in a chicken model: current approaches and applications. *J. Appl. Genetics*, **61**: 221-229.
- [39] J. Y. Han and Y. H. Park (2018). Primordial germ cell-mediated transgenesis and genome editing in birds. *J. Anim. Sci. Biotechnol.*, **9**: 19. doi: [10.1186/s40104-018-0234-4](https://doi.org/10.1186/s40104-018-0234-4).
- [40] N. A. Zinovieva, N. A. Volkova and V. A. Bagirov (2019). Genome editing: current state of research and application to animal husbandry. *Appl. Biochem. Microbiol.*, **55**: 711-721.
- [41] J. S. Park, K. Y. Lee and J. Y. Han (2020). Precise genome editing in poultry and its application to industries. *Genes*, **11**: 1182. doi: [10.3390/genes11101182](https://doi.org/10.3390/genes11101182).
- [42] L. Dimitrov, D. Pedersen, K. H. Ching, H. Yi, E. J. Collarini, S. Izquierdo and M. C. van de Lavoie et al., (2016). Germline gene editing in chickens by efficient CRISPR-mediated homologous recombination in primordial germ cells. *PloS One*, **11**: e0154303. doi: [10.1371/journal.pone.0154303](https://doi.org/10.1371/journal.pone.0154303).
- [43] I. Oishi, K. Yoshii, D. Miyahara, H. Kagami and T. Tagami (2016). Targeted mutagenesis in chicken using CRISPR/Cas9 system. *Sci. Rep.*, **6**: 23980. doi: [10.1038/srep23980](https://doi.org/10.1038/srep23980).
- [44] C. A. Cooper, A. Challagulla, K. A. Jenkins, T. G. Wise, T. E. O'Neil, K. R. Morris and M. L. Tizard et al., (2017). Generation of gene edited birds in one generation using sperm transfection assisted gene editing (STAGE). *Transgenic Res.*, **26**: 331-347.



- [45] S. G. Tyack, K. A. Jenkins, T. E. O'Neil, T. G. Wise, K. R. Morris, M. P. Bruce, S. McLeod et al., **(2013)**. A new method for producing transgenic birds via direct in vivo transfection of primordial germ cells. *Transgenic Res.*, **22**: 1257-1264.
- [46] J. Lee, D.H. Kim and K. Lee **(2020)**. Muscle hyperplasia in Japanese quail by single amino acid deletion in MSTN propeptide. *Int. J. Mol. Sci.*, **21**: 1504. [doi: 10.3390/ijms21041504](https://doi.org/10.3390/ijms21041504).
- [47] E. Blesbois, F. Seigneurin, I. Grasseau, C. Limouzin, J. Besnard, D. Gourichon and G. Coquerelle et al., **(2007)**. Semen cryopreservation for ex situ management of genetic diversity in chicken: Creation of the French avian cryobank. *Poult. Sci.*, **86**: 555-564.
- [48] A. Thelie, A. Bailliard, F. Seigneurin, T. Zerjal, M. Tixier-Boichard, E. Blesbois **(2019)**. Chicken semen cryopreservation and use for the restoration of rare genetic resources. *Poult. Sci.*, **98**: 447-455.
- [49] J. Whyte, J. D. Glover, M. Woodcock, J. Brzezczynska, L. Taylor, A. Sherman and P. Kaiser et al., **(2015)**. FGF, insulin, and SMAD signaling cooperate for avian primordial germ cell self-renewal. *Stem Cell Rep.*, **5**: 1171-1182.
- [50] M. E. Woodcock, A. A. Gheyas, A. S. Mason, S. Nandi, L. Taylor, A. Sherman and J. Smith et al., **(2019)**. Reviving rare chicken breeds using genetically engineered sterility in surrogate host birds. *Proc. Natl. Acad. Sci. U.S.A.*, **116**: 20930-20937.
- [51] T. Hu, L. Taylor, A. Sherman, T. C. Keambou, S. J. Kemp, B. Whitelaw and R. J. Hawken et al., **(2022)**. A low-tech, cost-effective and efficient method for safeguarding genetic diversity by direct cryopreservation of poultry embryonic reproductive cells. *eLife*, **11**: e74036. [doi: 10.7554/eLife.74036](https://doi.org/10.7554/eLife.74036).
- [52] J. Champer, A. Buchman and O. S. Akbari **(2016)**. Cheating evolution: engineering gene drives to manipulate the fate of wild populations. *Nat. Rev. Genet.*, **17**: 146-159.
- [53] M. P. Phelps, L. W. Seeb and J. E. Seeb **(2020)**. Transforming ecology and conservation biology through genome editing. *Conserv. Biol.*, **34**: 54-65.
- [54] M. Crispo, A. P. Mulet, L. Tesson, N. Barrera, F. Cuadro, P. C. dos Santos-Neto, T. H. Nguyen et al., **(2015)**. Efficient generation of myostatin knock-out sheep using CRISPR/Cas9 technology and microinjection into zygotes. *PloS One*, **10**: e0136690. [doi: 10.1371/journal.pone.0136690](https://doi.org/10.1371/journal.pone.0136690).
- [55] C. Proudfoot, D. F. Carlson, R. Huddart, C. R. Long, J. H. Pryor, T. J. King, S. G. Lillico et al., **(2015)**. Genome-edited sheep and cattle. *Transgenic Res.*, **24**: 147-153.
- [56] X. Wang, Y. Niu, J. Zhou, H. Zhu, B. Ma, H. Yu and H. Yan et al., **(2018)**. CRISPR/Cas9-mediated MSTN disruption and heritable mutagenesis in goats causes increased body mass. *Animal Genetics*, **49**: 43-51.
- [57] G. D. Kim, J. H. Lee, S. Song, S. W. Kim, J. S. Han, S. P. Shin and B. C. Park et al., **(2020)**. Generation of myostatin-knockout chickens mediated by D10A-Cas9 nickase. *FASEB J.*, **34**: 5688-5696.
- [58] I. Oishi, K. Yoshii, D. Miyahara and T. Tagami **(2018)**. Efficient production of human interferon beta in the white of eggs from ovalbumin gene-targeted hens. *Sci. Rep.*, **8**: [doi: 10.1038/s41598-018-28438-2](https://doi.org/10.1038/s41598-018-28438-2).
- [59] M. E. Woodcock, A. Idoko-Akoh and M. J. McGrew **(2017)**. Gene editing in birds takes flight. *Mamm. Genome*, **28**: 315-323.
- [60] S. K. Panda and M. J. McGrew **(2021)**. Genome editing of avian species: implications for animal use and welfare. *Laboratory Anim.*, **56**: 50-59.
- [61] J. Lyall, R. M. Irvine, A. Sherman, T. J. McKinley, A. Núñez, A. Purdie and L. Outtrim et al., **(2011)**. Suppression of avian influenza transmission in genetically modified chickens. *Science*, **331**: 223-226.
- [62] R. Hellmich, H. Sid, K. Lengyel, K. Flisikowski, A. Schlickerrieder, D. Bartsch and T. Thoma, et al., **(2020)**. Acquiring resistance against a retroviral infection via CRISPR/cas9 targeted genome editing in a commercial chicken line. *Front. Genome Ed.*, **2**: 3. [doi: 10.3389/fged.2020.00003](https://doi.org/10.3389/fged.2020.00003).
- [63] J. E. Fulton **(2018)**. Commentary: Application of gene editing in the commercial Poultry production industry. *Poult. Sci.*, **97**: 3007-3008. [doi: 10.3382/ps/pey170](https://doi.org/10.3382/ps/pey170).