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GENOME EDITING: CURRENT STATE AND PROSPECTS FOR USE IN POULTRY

(review)

N.A. VOLKOVA ✉, A.N. VETOKH, N.A. ZINOVIEVA

Ernst Federal Research Center for Animal Husbandry, 60, pos. Dubrovitsy, Podolsk District, Moscow Province, 142132 Russia, e-mail natavolkova@inbox.ru (✉ corresponding author), anastezuya@mail.ru, n_zinovieva@mail.ru
ORCID:
Volkova N.A. orcid.org/0000-0001-7191-3550
Zinovieva N.A. orcid.org/0000-0003-4017-6863
Vetokh A.N. orcid.org/0000-0002-2865-5960
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Abstract

To date, significant progress has been made in the poultry's genetic modification. A sufficiently large number of methods and methodological approaches have been developed for the introduction of recombinant genes into bird cells. The efficiency of using these approaches for genetic modification of bird cells varies depending on the object of research, the selected target cells for the introduction of recombinant DNA and the method of their transformation. Blastoderm cells, primordial germ cells, spermatogonia, sperm cells, and oviduct cells can serve as target cells for gene modifications. Using retroviral, lentiviral and adenoviral vectors, electroporation and lipofection, genetic transformation of these target cells can be carried out. In general, three main strategies for creating a genetically modified bird can be distinguished: i) the introduction of genetic constructs directly into the embryo (J. Love et al., 1994; Z. Zhang et al., 2012) or into individual organs and tissues of adults (D.V. Beloglazov et al., 2015; S. Min et al., 2011), ii) transfection of target cells in vitro and their subsequent transplantation into the embryo or target organs (M.-C. van de Lavoir et al., 2006; B. Benesova et al., 2014), and iii) sperm transformation in vitro and insemination of females with transformed sperm (E. Harel-Markowitz et al., 2009). These approaches were used to develop methods for editing the avian cell genome. A number of papers have studied the possibility of modifying bird cells using various editing systems, in particular, ZFN (zinc finger nuclease), TALEN (transcription activator-like effector nucleases), and CRISPR/Cas9 (clustered regularly interspaced palindromic repeats). Promising areas of using this technology in poultry farming are the following: studying the genes functions (N. Véron et al., 2015), obtaining recombinant proteins in the egg white composition (I. Oishi et al., 2018), improving economically useful and productive qualities (J. Ahn et al., 2017), and increasing resistance to infectious diseases (A. Koslová et al., 2020; R. Hellmich et al., 2020). Chickens with knockout of genes of the heavy chain of immunoglobulin (B. Schusser et al., 2013; L. Dimitrov et al., 2016), ovomucin (I. Oishi et al., 2016), myostatin (G.-D. Kim et al., 2020), as well as an integrated human interferon beta gene (I. Oishi et al., 2018) were obtained using genome editing technology. Quail with knockout of myostatin genes (J. Lee et al., 2020) and melanophilin (J. Lee et al., 2019) were also obtained. A number of studies have shown the simplicity, safety and availability of using the CRISPR/Cas9 editing system for modifying the poultry genome. This allows us to consider this system as an effective tool for the creation and commercial use of breeds and lines of birds with improved qualities in the framework of the implementation of large-scale breeding programs aimed at improving the quality of the resulting poultry products.

Keywords: poultry, quail, chicken, transgenesis, genome editing, CRISPR/Cas9, primordial germ cells, germ cells.

Farm poultry, in particular chickens and quails, is a convenient and accessible object for conducting various studies and solving problems in the field of developmental biology, medicine, and veterinary medicine [1, 2]. Unlike large farm animals, the bird has a short generation interval, which significantly reduces

the time to breed lines or populations of individuals with certain traits that are of interest both within the framework of individual studies and for solving larger problems. The similarity of the structure of protein glycosylation in birds and humans, as well as high egg productivity, sterility and availability of eggs, allow us to consider birds as an effective productive platform for the production of recombinant proteins [3]. This is especially true in the case of recombinant products that cannot be obtained using transgenic mammals (if such products are toxic to them).

It should be noted that the methods used to modify the mammalian genome are in most cases ineffective for the transgenesis of poultry. This is primarily due to the peculiarities of the physiology, reproduction and developmental biology of birds (4). Unlike mammals, in birds, the development of embryos in the reproductive organs of the female proceeds only at the early stages of embryogenesis. By the time of laying the egg immediately after laying, the embryo consists of approximately 60,000 morphologically undifferentiated pluripotent cells (5). Further development of the embryo occurs outside the body of the female when appropriate environmental conditions appear. Features of the embryonic development of birds significantly complicate the use of the traditional method of breeding transgenic animals - DNA microinjection into the pronucleus of zygotes. Limiting factors also become difficulties in accurately determining ovulation, a large amount of yolk in the egg, and a strong compaction of the cytoplasm. At the same time, the long period of embryonic development of birds outside the body of the female facilitates access to embryos for genetic engineering manipulations.

To date, there are a fairly large number of methodological approaches for the genetic modification of avian cells for development and optimization of particular stages of the genome editing technology using various systems, e.g., ZFN (zinc finger nuclease), TALEN (transcription activator-like effector nucleases) and CRISPR/Cas9 (clustered regularly interspaced palindromic repeats) [6, 7]. This technology is used in poultry farming to create cell lines and individuals with a knockout or insertion of individual genes when studying their functions [8], obtaining recombinant proteins in the composition of egg white, improving economically useful traits and the quality of poultry products [9, 10], increasing resistance to infectious diseases [11, 12].

The purpose of this review is to summarize data on the main achievements in the field of editing the genome of poultry and the prospects for their use in poultry farming.

Genome editing systems. Genome editing technology involves making targeted changes to the target genome region using site-specific nucleases [6, 13]. The most common are zinc finger nucleases (ZFN), TALE-associated nucleases (TALEN), and CRISPR/Cas9 [14, 15]. The principle of their action is based on the introduction of double-strand breaks into the genome region of interest, which are subsequently repaired by means of non-homologous end joining or homologous recombination [16, 17].

In the first case, the repair of double-strand breaks leads to the formation of insertions or deletions at the break site; in the second, an artificially introduced genetic construct imitating the sister chromatid is used to repair DNA [18]. Deletions and insertions lead to gene knockout (knockout), which is of interest in studying their functions, as well as in the production of animal products with improved qualities (for example, low-allergenic eggs). The introduction of donor DNA (genetic constructs) through homologous recombination makes it possible to introduce additional information into the genome.

ZFN and TALEN editing systems are more costly and time consuming

compared to CRISPR/Cas9. With the use of ZFN and TALEN nucleases, off-target effects are more often noted [7]. The possibility of automated selection of individual components of the CRISPR/Cas9 system using various online services makes it possible to increase the specificity of introducing genetic changes in the target gene and significantly reduce the likelihood of off-target mutations. In addition, the components of the system can be designed to virtually any target genomic DNA sequence. The CRISPR/Cas9 genome editing system is based on the natural defense mechanism (adaptive immunity) of bacteria and archaea against phages [19, 20]. This editing system includes two main components — Cas9 nuclease and guide (guide) RNA (gRNA, guide RNA). The guide RNA binds specifically to the target DNA region, which is subsequently cleaved by Cas9 [21-23]. The resulting DNA double-strand breaks are further repaired through homologous or non-homologous recombination, depending on the goals of the experiment [24, 25]. To introduce small deletions or insertions into the target DNA to knock out the gene, one guide RNA specific for this DNA region and Cas9 are used. If it is necessary to switch off several genes, a mixture of guide RNAs and Cas9 nuclease is used. To include donor DNA in a certain region of the genome (for example, to obtain producers of recombinant proteins), along with a guide RNA and a nuclease, a genetic construct for homologous recombination is introduced into the cell, which is a fragment of inserted DNA flanked by sequences homologous to the break [18, 26].

CRISPR/Cas9-based genome editing systems allow the introduction of site-specific mutations in target genes similar to naturally occurring genetic variants (editing without trace). With editing the genome of target cells using this system, expression of its main components Cas9 and guide RNA occurs from a single vector or introduced as a mixture. The most common is the first approach based on the use of a plasmid encoding Cas9 and a guide RNA. This eliminates the need for multiple transfection components, which simplifies the editing procedure and increases the stability of the results.

Methods of genetic modification of bird cells. A set of methods and methodological approaches used to obtain genetically modified individuals depends on the object of research, the choice of target cells for the introduction of recombinant DNA, and the method of genetic transformation of target cells. The main strategies for creating a genetically modified bird are the introduction of genetic constructs directly into the embryo [27, 28)] or the organs and tissues of adults [29, 30], the transfection of target cells in in vitro culture and their transplantation into an embryo or target organs [31, 32], and the sperm transformation in vitro to inseminate females with transformed sperm [33].

An effective tool for targeted delivery of recombinant DNA into cells of an embryo or organs and tissues of adults is the use of vectors based on recombinant viruses, which is associated with their natural ability to independently penetrate into target cells and integrate into a foreign genome with high efficiency.

With the use of viral vectors, the first successful experiments on the creation of a transgenic bird were carried out. In 1987 Salter et al. [34] obtained transgenic chickens by introducing a retroviral vector based on the avian leukosis virus (ALV) into the subembryonic cavity of stage X embryos. The efficiency of transgene transfer to offspring was 1-11%. Subsequently, the possibility of creating a transgenic bird using retroviral vectors based on the Rous sarcoma virus [35], reticuloendotheliosis virus (REV) [36], avian spleen necrosis [37], Moloney murine leukemia virus (MoMLV) was shown [38, 39]. To date, transgenic chickens have been created with integrated genes encoding -galactosidase, LacZ [37], β-lactamase [40, 41], green fluorescent protein (GFP) [42], bispecific antibodies

[43], hormone growth [44], human granulocyte colony stimulating factor [39], interferon α-2b [45].

The use of lentiviral vectors made it possible to increase the efficiency of poultry transgenesis [46]. McGrew et al. [47] obtained transgenic chickens with integrated *LacZ* and *eGFP* genes by lentiviral transfection of blastoderm cells of stage X embryos. The efficiency of transgene transfer to offspring was 4-45%. Using lentiviral vectors, transgenic chickens and quails were created that produce recombinant proteins, in particular, human β-interferon hIFNβ1 [48], bispecific antibodies [28, 48], GFP [49, 50], interleukin 1 receptor antagonist (rhIL1RN) [51], human lysozyme [52], α-defensin HNP4 (human neutrophil defensin 4) [53].

It should be noted that when viral vectors are introduced into the subembryonic cavity of embryos at stage X, the transgenic bird turns out to be a mosaic, and further crossings are required to create a generative individual. In this regard, the key point is the effectiveness of the transformation of cells of the reproductive organs of males and females. This problem can be solved by targeted modification of germ cells, which makes it possible to purposefully act on specific target cells, completely leveling the risks associated with the creation of transgenic mosaic individuals, from which it is impossible to obtain transgenic offspring in the future.

By culturing embryonic and spermatogenic cells in vitro, a variety of techniques can be used to introduce recombinant DNA into target cells using safe gene delivery systems. The use of genetically modified germ cells guarantees the presence in the oocyte after fertilization of one copy of the construct built into a certain locus. Recombinant DNA integrated into the genome of target cells can be stably transmitted over several generations. Manipulations on adult individuals significantly reduce the time and material costs for obtaining genetically modified offspring.

When creating a genetically modified poultry, both mature germ cells [33] and their precursors, primordial germ cells (PGCs) [54, 55] and spermatogonia [56, 57], can serve as target cells. The use of primary and early germ cells is of the greatest interest [58, 59]. With further development, they can form a significant population of transformed mature germ cells [60].

PGCs in the process of embryogenesis can differentiate into both male and female germ cells, which significantly expands the possibilities for realizing the potential of PGCs when creating genetically modified and chimeric individuals with desired properties. In avian embryos, primordial germ cells form in the epiblast and migrate through the hypoblast into the blood, then into the gonads [61]. With the introduction of donor PGCs into the dorsal aorta of recipient embryos during the period of migration of own PGCs from the blood into the gonads, colonization of the recipient gonads by donor cells is possible.

Spermatogonia serve as precursors of male reproductive cells (56). Of greatest interest are type A spermatogonia, which are classified as testicular stem cells. The unique property of self-renewal opens up wide opportunities for realizing the potential of these cells when breeding genetically modified poultry. Spermatogonia form a small population of cells located on the basement membrane of the seminiferous tubules. The process of their repeated self-renewal and further differentiation ensures the continuity of spermatogenesis with the formation of sperm, the highly specialized germ cells. Spermatogonia are the most resistant to various damaging factors (often only these cells survive, while the rest of the cells of the spermatogenic epithelium die) and undergo constant replication, maintaining their numbers during a process called renewal of the composition of stem cells.

Currently, approaches have been developed and optimized for obtaining and cultivating embryonic [62, 63] and spermatogenic [64, 65] avian cells. The

efficiency of genetic transformation of these target cells using various gene delivery systems, such as electroporation [66, 67], nucleofection [68], liposomal transfection [69, 70], the use of retroviral [71, 72] and lentiviral vectors [28, 73, 74], cationic polymers [30, 57], transposons [68, 75, 76].

PGCs can be transformed in two ways: in culture in vitro and in vivo by introducing genetic constructs into the dorsal aorta of embryos during the period of migration of their own PGCs into the gonads. Along with the traditional methods of transfection of cells in culture in vitro, the electroporation and lipofection, a number of works present the results of genetic modification of PGCs using other methods of gene delivery. Macdonald et al. [75] used Tol2 and piggyBac transposons to transfect chicken PGCs in vitro. The efficiency of transfection of target cells was 5.4 and 25.5%, respectively. The formation of functional gametes from transformed donor cells was shown, and transgenic progeny were obtained from primary germline chimeras. Naito et al. [68] obtained and transformed in vitro by nucleofection a culture of chicken PGCs with an efficiency of 10%. The transformed PZK culture was introduced into recipient embryos. From the birds bred after these manipulations, offspring were obtained. The presence of *GFP* was found in 1 out of 270 individuals.

There are a number of reports on the efficiency of transformation of PGCs in vivo to obtain germline chimeras. Zhang et al. [28] proposed a simple and effective way to create transgenic quails by injecting a lentiviral vector containing the *eGFP* reporter gene into the dorsal aorta of embryos. Out of 80 embryos, the authors obtained 48 G_0 chimeras (60%). The presence of *eGFP* was confirmed in most organs and tissues of the chimeric bird, including the germ cells of males. The efficiency of obtaining transgenic offspring from chimeric males reached 13%. Tyack et al. [69] and Lambeth et al. [76] for the genetic transformation of chicken PGCs in vivo, recombinant DNA was injected in combination with Lipofectamine 2000 and the Tol2 transposon directly into the dorsal aorta of chicken embryos. Germline F0 chimeras and transgenic progeny expressing integrated recombinant genes were obtained.

Jiang et al. [73] used a lentiviral vector conjugated with antibodies to SSEA4 (stage-specific embryonic antigen-4) specific to PGC membrane proteins to increase the efficiency of PGC transfection in vitro and in vivo. The proposed approach made it possible to increase the target efficiency of transduction of avian cells by 30.0-46.7%. In 50.0-66.7% of embryos, GFP expression occurred in the gonads.

Transformation of avian spermatogenic cells, as well as PGC, can be carried out in culture in vitro and in vivo by introducing genetic constructs into the parenchyma of the testes of males. In the latter case, as a rule, viral vectors are used. A number of studies have considered the possibility of using non-viral gene delivery systems. Min et al. [30] and Li et al. [57] studied the efficacy of the cationic polymer SofastTM in transforming rooster spermatogenic cells in vivo. This drug, in combination with the genetic construct, was injected directly into the parenchyma of the testis. Min et al. [30] bred avian influenza resistant chickens. The efficiency of transformation of spermatogenic cells was 72.2%. The transgene was present in 10% of the spermatozoa and in the blood of 7.8% of the F_1 offspring. Li et al. [57] used a genetic construct encoding the *GFP* reporter gene for the genetic transformation of spermatogenic cells. With its introduction in combination with a cationic polymer into the testes of roosters, the efficiency of target cell transformation reached 19.1%.

Thus, the technology for creating genetically modified individuals using PGCs and spermatogonia as donor cells involves their isolation, transformation,

and transplantation into recipient gonads, followed by the production of offspring with introduced traits [77, 78]. The efficiency of colonization of donor cells into recipient gonads has been shown in a number of studies using both donor PGCs [78, 79] and spermatogonia [32, 81, 82]. The preliminary treatment of recipients aimed at eliminating their own germ or spermatogenic cells in the gonads under the influence of gamma radiation The efficiency of transplantation of donor PGCs and spermatogonia can be increased by [83, 84] or chemical sterilization [85, 86]. In the latter case, busulfan is effective, which is an alkylating agent that causes DNA damage in target cells, which leads to the shutdown of all cellular mechanisms and cell destruction.

Table 1 summarizes the main methodological approaches currently used for the genetic modification of avian cells. Below, their effectiveness in editing the genome of poultry in in vitro and in vivo systems is considered.

1. Main methods to genetically modify poultry cells

Gene construct introduction	Target cells	Trasfection method	References
Direct introduction into an embryo or into organs or tissues of adults	Blastodermal cell	Viral vectors, lipofection	[27, 34, 47]
	Primordial germ cells	Viral vectors, transposons, lipofection	[28, 69, 76]
	Oviduct cells, spermatogenic testis cells	Viral vectors	[29, 74, 30]
Transfection of target donor cells in vitro followed by transplantation to recipients	Blastodermal cells, primordial germ cells, spermatogonia	Viral vectors, lipofection, electroporation, nucleofection, transposons	[31, 32, 87, 88]
Transformation of spermatozoa followed by female insemination	Sperm	Lipofection, electroporation	[33]

Genome editing of poultry. A number of successful experiments reported on the modification of avian cells using various editing systems to knockout of individual genes [89]. Functions of a number of genes associated with the biology of embryonic development and the pathogenesis of embryonic diseases [90], gametogenesis [91], and resistance to infectious diseases [92] have been studied on chicken cell lines DF-1 and DT-40. Methodological approaches to introducing mutations (knockout) into target genes [93], including those related to growth, development, and productive qualities, have been developed and optimized [94].

Abu-Bonsrah et al. [90] obtained two chicken cell lines with a knockout of the *HIRA*, *TYRP1*, *DICER*, *MBD3*, *EZH2*, and *RET* genes using the CRISPR/Cas9 system. It has been shown that using this editing system, it is possible to introduce a deletion larger than 75 kb into the target gene sequence. Through in vivo electroporation of chicken embryos, genetic changes were made to the *DGCR8* gene sequence in nerve cells. In genetically modified cells, there was a decrease in the expression of DGCR8 and the associated genes *Drosha*, *YPEL1*, and *Ngn2*. Morphological differences in the structure of the nervous tissue and cardiac muscle in transfected embryos were noted.

Zhang et al. [91] studied the effect of the *Stra8* gene on the differentiation of embryonic stem cells in spermatogonia. For this purpose, the Cas9/gRNA plasmid was introduced into DF-1 cells and embryonic stem cells. The efficiency of introducing mutations into the target gene was 25% in DF-1 cells and 23% in embryonic stem cells. It has been shown that Stra8 gene knockout blocks the differentiation of embryonic stem cells in spermatogonia in vitro. Y. Bai et al. (93) used the CRISPR/Cas9 system to introduce genetic changes in the *PPARG* and *ATP5F1E* ovalbumin gene sequences in the DF-1 chicken cell line. The mutation frequency varied from 0.5 to 3.0%. Cultivation of cells after transfection on a selective medium containing puromycin increased the efficiency of selection of genetically modified cells up to 95%. Lee et al. [94] on the DF-1 cell line considered

the possibility of using the Cas9-D10A nickase to introduce site-specific mutations in the target region of the target DNA. The myostatin gene was chosen as the target. Genotyping of the transfected cells confirmed the presence of mutations at the target site of the target DNA. The size of the introduced deletions varied from 2 to 39 nucleotides. At the same time, the analysis of six non-target sites did not reveal the presence of any non-specific mutations in them. In addition, there were no phenotypic differences between normal and modified cells. Western blotting did not show the presence of myostatin protein in the modified cells.

Along with reports on editing the genome of cell lines, there are a number of publications on the production of poultry with a knockout or gene insertion. The studies were carried out on chickens and quails. Schusser et al. [95], using PZK, bred chickens with a knockout of the immunoglobulin heavy chain gene by homologous recombination. Birds homozygous for the knockout of this gene did not synthesize antibodies and did not develop B-cells. At the same time, the migration of B-cell precursors into the bursa of Fabricius was preserved, while the formation of mature B-cells and their migration from the bursa of Fabricius were blocked. The development and functional activity of other types of cells of the immune system remained normal. Chickens with a knockout of the immunoglobulin heavy chain gene due to the lack of a peripheral population of B-cells serve as a unique experimental model for studying the immune response of birds to infectious diseases, and are also of interest for solving a number of problems in the field of virology and biology development and biotechnology. Dimitrov et al. [96] showed the possibility of modifying the chicken immunoglobulin heavy chain gene by in vitro PGC modification using the CRISPR/Cas9 system. As a result, four PGC lines were obtained, which were injected into the embryos. The efficiency of the transfer of the introduced modifications from the chimeric bird of the germ line to the offspring varied from 0 to 96%.

Using the TALEN editing system, Taylor et al. (97) bred chickens with a knockout of the *DDX4* locus on the Z sex chromosome to study the role of this gene in the formation of germ cells. The *DDX4* gene is a key determinant of germ cells in many animal species. It is supposed to control the formation of germ cells in birds. The effectiveness of his knockout in the PZK of chickens was 8.1%. Large deletions of 30 kb were introduced spanning the entire *DDX4* locus. After in vitro editing, PGCs were injected into recipient embryos and a chimeric germline bird was obtained. The offspring from this bird were homozygous for the knockout of the *DDX4* gene. In individuals, the initiation and development of PGCs in the gonads of embryos was noted, however, with the onset of meiosis, the development of reproductive cells was blocked, leading to infertility in females.

Knockout of egg protein genes is considered as an opportunity to reduce the allergenicity of chicken eggs. This is especially true in the production of products for persons sensitive to egg white. In 2014, Park et al. [98[obtained ovalbumin knockout chickens by genetically modifying PGCs with the TALEN editing system. Deletions were introduced into the target gene, which led to a shift in the reading frame and, as a result, to the shutdown of the function of the ovalbumin gene. Oishi et al. [99] created the ovomucin (*OVM*) gene knockout chickens. PGCs transfected in in vitro culture and transplanted into recipient embryos were used as target cells for genome editing with the CRISPR/Cas9 system. G_0 chimeras were used for subsequent crosses with the selection of G_2 chickens homozygous for the *OVM* gene knockout. Two out of three chimeric G_0 roosters produced offspring with a deletion in the *OVM* gene.

Later, the same scientific group, using a similar approach, bred chickens producing human beta-interferon (hIFN-β) by incorporating the *hIFN-β* gene into

the ovalbumin gene locus [100]. Such a bird produced 3.5 mg/ml hIFN-β in egg white. Females (unlike males) turned out to be infertile. The bioactivity and production of the recombinant hIFN-β protein in the offspring remained at the level of previous generations, which confirms the prospects of including the target genes in the ovalbumin gene locus of chickens to create individuals producing recombinant proteins in egg white for industrial use.

Qin et al. [101] evaluated the effectiveness of using an adenoviral vector to deliver the CRISPR/Cas9 system to chicken cells to knock out the ovalbumin (*OV*) gene and integrate the human epidermal growth factor (*hEGF*) gene into this locus. The efficiency of the *OV* gene knockout and the expression of the integrated *hEGF* gene was shown in a culture of primary chicken oviduct cells. The biological activity of the secreted hEGF protein was confirmed on Hela cells: cell proliferation when this protein was included in the cultivation medium corresponded to those established for the commercial hEGF preparation. The *OV* gene knockout was also carried out with the integration of the *hEGF* gene in blastoderm cells in vitro and in vivo. Chicken embryos with introduced genetic changes in the cells of the gonads were obtained. The efficiency of obtaining such embryos was higher with transplantation of in vitro modified blastoderm cells into the germinal disc of recipient embryos than with direct injection of the adenovirus vector into embryos in vivo. The proportion of modified germ cells in the gonads of embryos was also higher when using in vitro modified blastodermal cells.

A number of papers report on the successful editing of the genome of chickens and quails with myostatin gene knockout (*MSTN*). The protein myostatin inhibits the growth and development of muscle tissue. Knockout of the *MSTN* gene is of interest in creating lines with an increased growth rate of muscle tissue. G.-D. Kim et al. [102] obtained chickens with *MSTN* gene knockout by inserting an editing system into the PGC. The D10A-Cas9 nickase was used to introduce deletions into the target region of the target DNA. After the introduction of in vitro modified PGCs into embryos, deletions from 5 to 39 nucleotides in the MSTN gene locus were identified in 7 out of 52 chickens. This bird was further crossed in order to breed chickens homozygous for MSTN gene knockout. The features of growth and development of muscle tissue were studied. MSTN knockout birds showed a continuous increase in body weight up to 18 weeks of age, while in unmodified birds, the growth rate decreased after 13 weeks. A comparative assessment of meat productivity indicators revealed an increase in the mass of legs by 55.3% in individuals with a knockout of the *MSTN* gene compared to the control. At the same time, the mass of abdominal fat was 77.1% lower. Comparison of the mass of internal organs, including the heart, spleen, stomach and liver, did not reveal significant differences between genetically modified and unmodified chickens.

Lee et al. [103] produced myostatin gene *MSTN* knockout quails by injecting a recombinant adenovirus containing CRISPR/Cas9 into the germinal disc (blastoderm cells). In the birds, 3 bp deletions were identified. The mutation did not cause a frameshift and resulted in a cysteine deletion in the *MSTN* propeptide region. In quails homozygous for *MSTN* gene knockout, there was a significant increase in body weight and muscle tissue with muscle hyperplasia compared with quails heterozygous for *MSTN* gene knockout and wild type. In addition, in individuals with a knockout of the *MSTN* gene, the proportion of abdominal fat decreased and the mass of the heart increased compared to wild-type quails. The same scientific group bred quails with a knockout of the melanophilin *MLPH* gene associated with feather pigmentation (104). An adenovirus vector containing components of the CRISPR/Cas9 system was introduced into the subembryonic cavity

of the blastoderm of the embryos. Of the 100 injected embryos, 11 quails were obtained, of which five carried a mutation in the *MLPH* gene in reproductive cells. The efficiency of mutation transfer to offspring varied from 2.4 to 10.0%. In the offspring of one modified F0 bird, two different mutations were identified at the *MLPH* locus. Differences in the phenotype of modified quails with *MLPH* gene knockout were established. Quails homozygous for the *MLPH* gene knockout had gray plumage, while quails heterozygous for the introduced mutation and the wild type had dark brown plumage.

Along with the use of genomic editing technology to improve economically useful traits in agricultural poultry, it is of interest to create individuals resistant to infectious diseases, such as avian leukosis virus (ALV). This disease is difficult to control and prevent due to the lack of effective vaccines. There are several subgroups of ALVs. Hellmich et al. [12] attempted to develop chickens resistant to subgroup J of the avian leukemia virus (ALV-J), which causes myeloid leukemia and tumor formation. For this purpose, a deletion for tryptophan 38 (W38) was introduced into the c*hNHE1* locus using the CRISPR/Cas9 system. The W38 amino acid in chNHE1 is critical for virus entry into the cell, making it a preferred knockout target to increase pathogen resistance. The genetic modification introduced into the chicken genome completely protected the cells from infection with the ALV-J virus. The W38 deletion did not have a significant negative impact on the development or general functional state of genetically modified individuals. In general, the creation of ALV-J resistant individuals through precise gene editing allows this approach to be considered as an alternative strategy for controlling poultry diseases.

Table 2 summarizes the main achievements in editing the genomes of different poultry species.

2. The main achievements in editing the genomes of different poultry species

Poultry species	Target gene	Target cells	Target cell transfection	Editing system	References
Chickens	*IgH*	PGCs	Electroporation	CRISPR/Cas9	[96]
	DDX4	ПЗК	Electroporation	TALEN	[97]
	OVM	ПЗК	Lipofection	CRISPR/Cas9	[99]
	hIFN-β	ПЗК	Lipofection	CRISPR/Cas9	[100]
	MSTN	ПЗК	Lipofection	D10A-Cas9B	[102]
	OV	ПЗК	Lipofection	TALEN	[98]
	chNHE1	ПЗК	Electroporation	CRISPR/Cas9	[12]
Quail	*MSTN*	Blastodermal cells	Adenoviral vector	CRISPR/Cas9	[103]
	MLPH	Blastodermal cells	Adenoviral vector	CRISPR/Cas9	[104]

N o t e. PGCs — primordial germ cells.

Thus, at present, some progress has been made in editing the genome of poultry. Methodological approaches and techniques for modifying avian cells using various gene editing systems, in particular ZFN, TALEN, CRISPR/Cas9, have been developed and optimized. Chickens and quails have been bred with a knockout of a number of genes in order to study their functions, improve the productive qualities of poultry, increase resistance to infectious diseases, and obtain recombinant proteins in egg protein. A number of studies have shown the simplicity, safety, and availability of the CRISPR/Cas9 editing system for modifying the poultry genome, which makes it possible to consider this system as an effective tool for the creation and commercial use of bird breeds and lines with improved qualities.

REFERENCES

1. Ivarie R. Avian transgenesis: progress towards the promise. *Trends in Biotechnology*, 2003, 21(1): 14-19 (doi: 10.1016/S0167-7799(02)00009-4).
2. Amro W.A., Al-Qaisi W., Al-Razem F. Production and purification of IgY antibodies from

chicken egg yolk. *Journal of Genetic Engineering and Biotechnology*, 2018, 16(1): 99-103 (doi: 10.1016/j.jgeb.2017.10.003).
3. Lillico S.G., McGrew M.J., Sherman A., Sang H.M. Transgenic chickens as bioreactors for protein-based drugs. *Drug Discovery Today*, 2005, 10(3): 191-196 (doi: 10.1016/S1359-6446(04)03317-3).
4. Stern C.D. The marginal zone and its contribution to the hypoblast and primitive streak of the chick embryo. *Development*, 1990, 109: 667-682.
5. Mozdziak P.E., Petitte J.N. Status of transgenic chicken models for developmental biology. *Developmental Dynamics*, 2004, 229(3): 414-421 (doi: 10.1002/dvdy.10461).
6. Cooper C.A., Doran T.J., Challagulla A., Tizard M.L.V., Jenkins K.A. Innovative approaches to genome editing in avian species. *Journal of Animal Science and Biotechnology*, 2018, 9: 15 (doi: 10.1186/s40104-018-0231-7).
7. Bahrami S., Amiri-Yekta A., Daneshipour A., Jazayeri S.H., Mozdziak P.E., Sanati M.H., Gourabi H. Designing a transgenic chicken: applying new approaches toward a promising bioreactor. *Cell Journal*, 2020, 22(2): 133-139 (doi: 10.22074/cellj.2020.6738).
8. Véron N., Qu Z., Kipen P.A.S., Hirst C.E., Marcelle C. CRISPR mediated somatic cell genome engineering in the chicken. *Developmental Biology*, 2015, 407(1): 68-74 (doi: 10.1016/j.ydbio.2015.08.007).
9. Ahn J., Lee J., Park J.Y., Oh K.B., Hwang S., Lee C.-W., Lee K. Targeted genome editing in a quail cell line using a customized CRISPR/Cas9 system. *Poultry Science*, 2017, 96(1): 1445-1450 (doi: 10.3382/ps/pew435).
10. Bhattacharya T.K., Shukla R., Chatterjee R.N., Bhanja S.K. Comparative analysis of silencing expression of myostatin (*MSTN*) and its two receptors (*ACVR2A* and *ACVR2B*) genes affecting growth traits in knock down chicken. *Scientific Reports*, 2019, 9: 7789 (doi: 10.1038/s41598-019-44217-z).
11. Koslová A., Trefil P., Mucksová J., Reinišová M., Plachý J., Kalina J., Kučerová D., Geryk J., Krchlíková V., Lejčková B., Hejnar J. Precise CRISPR/Cas9 editing of the *NHE1* gene renders chickens resistant to the J subgroup of avian leukosis virus. *Proceedings of the National Academy of Sciences*, 2020, 117(4): 2108-2112 (doi: 10.1073/pnas.1913827117).
12. Hellmich R., Sid H., Lengyel K., Flisikowski K., Schlickenrieder A., Bartsch D., Thoma T., Bertzbach L.D., Kaufer B.B., Nair V., Preisinger R., Schusser B. Acquiring resistance against a retroviral infection via CRISPR/Cas9 targeted genome editing in a commercial chicken line. *Frontiers in Genome Editing*, 2020, 2: 3 (doi: 10.3389/fgeed.2020.00003).
13. Petersen B. Basics of genome editing technology and its application in livestock species. *Reproduction in Domestic Animals*, 2017, 52(S3): 4-13 (doi: 10.1111/rda.13012).
14. Sander J.D., Joung J.K. CRISPR-Cas systems for genome editing, regulation and targeting. *Nature Biotechnology,* 2014, 32(4): 347-355 (doi: 10.1038/nbt.2842).
15. Panda S.K., Wefers B., Ortiz O., Floss T., Schmid B., Haass C., Wurst W., Kühn R. Highly efficient targeted mutagenesis in mice using TALENs. *Genetics,* 2013, 195(3): 703-713 (doi: 10.1534/genetics.113.156570).
16. Chu V.T., Weber T., Wefers B., Wurst W., Sander S., Rajewsky K., Kühn R. Increasing the efficiency of homology-directed repair for CRISPR-Cas9-induced precise gene editing in mammalian cells. *Nature Biotechnology*, 2015, 33: 543-548 (doi: 10.1038/nbt.3198).
17. Wefers B., Panda S.K., Ortiz O., Brandl C., Hensler S., Hansen J., Wurst W., Kühn R. Generation of targeted mouse mutants by embryo microinjection of TALEN mRNA. *Nature Protocols*, 2013, 8: 2355-2379 (doi: 10.1038/nprot.2013.142).
18. Menzorov A.G., Luk'yanchikova V.A., Korablev A.N., Serova I.A., Fishman V.S. *Vavilovskii zhurnal genetiki i selektsii*, 2016, 20(6): 930-944 (doi: 10.18699/VJ16.214) (in Russ.).
19. Barrangou R., Fremaux C., Deveau H., Richards M., Boyaval P., Moineau S., Romero D., Horvath P. CRISPR provides acquired resistance against viruses in prokaryotes. *Science*, 2007, 315(5819): 1709-1712 (doi: 10.1126/science.1138140).
20. Cong L., Ran F. A., Cox D., Lin S., Barretto R., Habib N., Hsu P.D., Wu X., Jiang W., Marraffini L. A., Zhang F. Multiplex genome engineering using CRISPR/Cas systems. *Science*, 2013, 339(6121): 819-823 (doi: 10.1126/science.1231143).
21. Anders C., Niewoehner O., Duerst A., Jinek M. Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. *Nature*, 2014, 513: 569-573 (doi: 10.1038/nature13579).
22. Gonatopoulos-Pournatzis T., Aregger M., Brown K.R., Farhangmehr S., Braunschweig U., Ward H.N., Ward H.N., Ha K.C.H., Weiss A., Billmann M., Durbic T., Myers C.L., Blencowe B.J., Moffat J. Genetic interaction mapping and exon-resolution functional genomics with a hybrid Cas9-Cas12a platform. *Nature Biotechnology*, 2020, 38: 638-648 (doi: 10.1038/s41587-020-0437-z).
23. Najm F.J., Strand C., Donovan K.F., Hegde M., Sanson K.R., Vaimberg E.W., Sullender M.E., Hartenian E., Kalani Z., Fusi N., Listgarten J., Younger S.T., Bernstein B.E., Root D.E., Doen J.G. Orthologous CRISPR-Cas9 enzymes for combinatorial genetic screens. *Nature Biotechnology*, 2018, 36(2): 179-189 (doi: 10.1038/nbt.4048).
24. Pennisi E. The CRISPR craze. *Science*, 2013, 341(6148): 833-836 (doi: 10.1126/science.341.6148.833).

25. Wilson L.O.W., Reti D., O'Brien A.R., Dunne R.A., Bauer D.C. High activity target-site identification using phenotypic independent CRISPR-Cas9 core functionality. *The CRISPR Journal*, 2018, 1(2): 182-190 (doi: 10.1089/crispr.2017.0021).
26. Salsman J., Dellaire G. Precision genome editing in the CRISPR era. *Biochemistry and Cell Biology*, 2017, 95: 187-201 (doi: 10.1139/bcb-2016-0137).
27. Love J., Gribbin C., Mather C., Sang H. Transgenic birds by DNA microinjection. *Nat. Biotechnol.*, 1994, 12(1): 60-63 (doi: 10.1038/nbt0194-60).
28. Zhang Z., Sun P., Yu F., Yan L., Yuan F., Zhang W., Wang T., Wan Z., Shao Q., Li Z. Transgenic quail production by microinjection of lentiviral vector into the early embryo blood vessels. *PLoS ONE,* 2012, 7(12): e50817 (doi: 10.1371/journal.pone.0050817).
29. Beloglazov D.V., Volkova N.A., Volkova L.A., Zinov'eva N.A. Efficiency of local transgenesis of the oviductal cells in chicken as influenced by hormonal stimulation. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2015, 50(6): 729-735 (doi: 10.15389/agrobiology.2015.6.729eng).
30. Min S., Qing S.Q., Hui Y.Y., Zhi F.D., Rong Q.Y., Feng X., Chun L.B. Generation of antiviral transgenic chicken using spermatogonial stem cell transfected in vivo. *African Journal of Biotechnology*, 2011, 10(70): 15678-15683 (doi: 10.5897/AJB11.040).
31. van de Lavoir M.-C., Diamond J.H., Leighton P.A., Mather-Love C., Heyer B.S., Bradshaw R., Kerchner A., Hooi L.T., Gessaro T.M., Swanberg S.E., Delany M.E., Etches R.J. Germline transmission of genetically modified primordial germ cells. *Nature*, 2006, 441: 766-769 (doi: 10.1038/nature04831).
32. Benesova B., Mucksova J., Kalina J., Trefil P. Restoration of spermatogenesis in infertile male chickens after transplantation of cryopreserved testicular cells. *British Poultry Science*, 2014, 55(6): 837-845 (doi: 10.1080/00071668.2014.974506).
33. Harel-Markowitz E., Gurevich M., Shore L.S., Katz A., Stram Y., Shemesh M. Use of sperm plasmid DNA lipofection combined with REMI (restriction enzyme-mediated insertion) for production of transgenic chickens expressing *eGFP* (enhanced green fluorescent protein) or human follicle-stimulating hormone *Biology of Reproduction*, 2009, 80(5): 1046-1052 (doi: 10.1095/biolreprod.108.070375).
34. Salter D.W., Smith E.J., Hughes S.H., Wright S.E., Crittenden L.B. Transgenic chickens: insertion of retroviral genes into the chicken germ line. *Virology*, 1987, 157(1): 236-240 (doi: 10.1016/0042-6822(87)90334-5).
35. Chen H.Y., Garber E.A., Mills E., Smith J., Kopchick J.J., Dilella A.G., Smith R.G. Vectors, promoters, and expression of genes in chick embryos. *Journal of Reproduction and Fertility. Supplement*, 1990, 41: 173-182.
36. Bosselman R.A., Hsu R.Y., Boggs T., Hu S., Bruszewski J., Ou S., Kozar L., Martin F., Green C., Jacobsen F., Nicolson M., Schultz J.A., Semon K.M., Rishell W., Stewart R.G. Germline transmission of exogenous genes in the chicken. *Science*, 1989, 243(4890): 533-535 (doi: 10.1126/science.2536194).
37. Mozdziak P.E., Borwornpinyo S., McCoy D.W., Petitte J.N. Development of transgenic chickens expressing bacterial beta-galactosidase. *Developmental Dynamics*, 2003, 226(3): 439-445 (doi: 10.1002/dvdy.10234).
38. Mizuarai S., Ono K., Yamaguchi K., Nishijima M., Kamihira M., Iijima S. Production of transgenic quails with high frequency of germ-line transmission using VSV-G pseudotyped retroviral vector. *Biochemical and Biophysical Research Communications*, 2001, 286(3): 456-463 (doi: 10.1006/bbrc.2001.5422).
39. Kwon M.S., Koo B.C., Choi B.R., Park Y.Y., Lee Y.M., Suh H.S., Park Y.S., Lee H.T., Kim J.H., Roh J.Y., Kim N.H., Kim T. Generation of transgenic chickens that produce bioactive human granulocyte-colony stimulating factor. *Molecular Reproduction and Development*, 2008, 75(7): 1120-1126 (doi: 10.1002/mrd.20860).
40. Harvey A.J., Ivarie R. Validating the hen as a bioreactor for the production of exogenous proteins in egg white. *Poultry Science*, 2003, 82(6): 927-930 (doi: 10.1093/ps/82.6.927).
41. Harvey A.J., Speksnijder G., Baugh L.R., Morris J.A., Ivarie R. Expression of exogenous protein in the egg white of transgenic chickens. *Nature Biotechnology*, 2002, 20(4): 396 (doi: 10.1038/nbt0402-396).
42. Smith C.A., Roeszler K.N., Sinclair A.H. Robust and ubiquitous GFP expression in a single generation of chicken embryos using the avian retroviral vector, RCASBP. *Differentiation*, 2009, 77(5): 473-482 (doi: 10.1016/j.diff.2009.02.001).
43. Kamihira M., Ono K., Esaka K., Nishijima K., Kigaku R., Komatsu H., Yamashita T., Kyogoku K., Iijima S. High-level expression of single-chain Fv-Fc fusion protein in serum and egg white of genetically manipulated chickens by using a retroviral vector. *Journal of Virology*, 2005, 79(17): 10864-10874 (doi: 10.1128/JVI.79.17.10864-10874.2005).
44. Kodama D., Nishimiya D., Nishijima K., Okino Y., Inayoshi Y., Kojima Y., Ono K., Motono M., Miyake K., Kawabe Y., Kyogoku K., Yamashita T., Kamihira M., Iijima S. Chicken oviduct-specific expression of transgene by a hybrid ovalbumin enhancer and the Tet expression system. *Journal of Bioscience and Bioengineering*, 2012, 113(2): 146-153 (doi: 10.1016/j.jbiosc.2011.10.006).

45. Rapp J.C., Harvey A.J., Speksnijder G.L., Hu W., Ivarie R. Biologically active human interferon a-2b produced in the egg white of transgenic hens. *Transgenic Research*, 2003, 12(5): 569-575 (doi: 10.1023/A:1025854217349).
46. Scott B.B., Velho T.A., Sim S., Lois C. Applications of avian transgenesis. *ILAR Journal*, 2010, 51(4): 353-361 (doi: 10.1093/ilar.51.4.353).
47. McGrew M.J., Sherman A., Ellard F.M., Lillico S.G., Gilhooley H.J., Kingsman A.J., Mitrophanous K.A., Sang H. Efficient production of germline transgenic chickens using lentiviral vectors. *EMBO Reproduction*, 2004, 5(7): 728-733 (doi: 10.1038/sj.embor.7400171).
48. Lillico S.G., Sherman A., McGrew M.J., Robertson C.D., Smith J., Haslam C., Barnard P., Radcliffe P.A., Mitrophanous K.A., Elliot E.A., Sang H.M. Oviduct-specific expression of two therapeutic proteins in transgenic hens. *Proceedings of the National Academy of Sciences*, 2007, 104(6): 1771-1776 (doi: 10.1073/pnas.0610401104).
49. Chapman S.C., Lawson A., Macarthur W.C., Wiese R.J., Loechel R.H., Burgos-Trinidad M., Wakefield J.K., Ramabhadran R., Mauch T.J., Schoenwolf G.C. Ubiquitous GFP expression in transgenic chickens using a lentiviral vector. *Development*, 2005, 132(5): 935-940 (doi: 10.1242/dev.01652).
50. Byun S.J., Kim S.W., Kim K.W., Kim J.S., Hwang I.S., Chung H.K., Kan I.S., Jeon I.S., Chang W.K., Park S.B., Yoo J.G. Oviduct-specific enhanced green fluorescent protein expression in transgenic chickens. *Bioscience, Biotechnology, and Biochemistry*, 2011, 75(4): 646-649 (doi: 10.1271/bbb.100721).
51. Kwon S.C., Choi J.W., Jang H.J., Shin S.S., Lee S.K., Park T.S., Choi I.Y., Lee G.S., Song G., Han J.Y. Production of biofunctional recombinant human interleukin 1 receptor antagonist (rhIL1RN) from transgenic quail egg white. *Biology of Reproduction*, 2010, 82(6): 1057-1064 (doi: 10.1095/biolreprod.109.081687).
52. Cao D.N., Wu H.Y., Li Q.Y., Sun Y.M., Liu T.X., Fei J., Zhao Y.F., Wu S., Hu X.X., Li N. Expression of recombinant human lysozyme in egg whites of transgenic hens. *PLoS ONE*, 2015, 10(2): e0118626 (doi: 10.1371/journal.pone.0118626).
53. Liu T.X., Wu H.Y., Cao D.N., Li Q.Y., Zhang Y.Q., Li N., Hu X.X. Oviduct-specific expression of human neutrophil defensin 4 in lentivirally generated transgenic chickens. *PLoS ONE*, 2015, 10(5): e0127922 (doi: 10.1371/journal.pone.0127922).
54. Chojnacka-Puchta L., Kasperczyk K., Płucienniczak G., Sawicka D., Bednarczyk M. Primordial germ cells (PGCs) as a tool for creating transgenic chickens. *Polish Journal of Veterinary Sciences*, 2012, 15(1): 181-188 (doi: 10.2478/v10181-011-0132-6).
55. Macdonald J., Glover J.D., Taylor L., Sang H.M., McGrew M.J. Characterisation and germline transmission of cultured avian primordial germ cells. *PLoS ONE*, 2010, 5(11): e15518 (doi: 10.1371/journal.pone.0015518).
56. Zheng Y., Zhang Y., Qu R., He Y., Tian X., Zeng W. Spermatogonial stem cells from domestic animals: progress and prospects. *Reproduction*, 2014, 147(3): 65-74 (doi: 10.1530/REP-13-0466).
57. Li B., Sun G., Sun H., Xu Q., Gao B., Zhou G., Zhau W., Wu X., Bao W., Yu F., Wang K., Chen G. Efficient generation of transgenic chickens using the SSCs in vivo and ex vivo transfection. *Science China Life Sciences*, 2008, 51: 734-742 (doi: 10.1007/s11427-008-0100-2).
58. Han J.Y. Germ cells and transgenesis in chickens. *Comparative Immunology, Microbiology & Infectious Diseases*, 2009, 32(2): 61-80 (doi: 10.1016/j.cimid.2007.11.010).
59. Nakamura Y., Usui F., Miyahara D., Mori T., Ono T., Takeda K., Nira-sawa K., Kagami H., Tagami T. Efficient system for preservation and regeneration of genetic resources in chicken: concurrent storage of primordial germ cells and live animals from early embryos of a rare indigenous fowl (Gifujidori). *Reproduction, Fertility and Development*, 2010, 22(8): 1237-1246 (doi: 10.1071/RD10056).
60. Takashima S. Biology and manipulation technologies of male germline stem cells in mammals. *Reproductive Medicine and Biology*, 2018, 17(4): 398-406 (doi: 10.1002/rmb2.12220).
61. Eyal-Giladi H., Kochav S. From cleavage to primitive streak formation: a com-plementary normal table and a new look at the first stages of the development of the chick. I. General morphology. *Developmental Biology*, 1976, 49(2): 321-337 (doi: 10.1016/0012-1606(76)90178-0).
62. Zhao D.-F., Kuwana T. Purification of avian circulating primordial germ cells by nycodenz density gradient centrifugation. *British Poultry Science*, 2003, 44(1): 30-35 (doi: 10.1080/0007166031000085382).
63. Mozdziak P.E., Angerman-Stewart J., Rushton B., Pardue S.L., Petitte J.N. Isolation of chicken primordial germ cells using fluorescence-activated cell sorting. *Poultry Science*, 2005, 84(4): 594-600 (doi: 10.1093/ps/84.4.594).
64. Sisakhtnezhad S., Bahrami A.R., Matin M.M., Dehghani H., Momeni-Moghaddam M., Boozarpour S., Farshchian M., Dastpak M. The molecular sig-nature and spermatogenesis potential of newborn chicken spermatogonial stem cells in vitro. *In Vitro Cellular & Developmental Biology — Animal*, 2015, 51: 415-425 (doi: 10.1007/s11626-014-9843-1).
65. Li B., Wang X.-Y., Tian Z., Xiao X.-J., Xu Q., Wei C.-X., Sun H.-C., Chen G.-H. Directional differentiation of chicken spermatogonial stem cells in vitro. *Cytotherapy*, 2010, 12(3): 326-331 (doi: 10.3109/14653240903518155).

66. Yu F., Ding L.-J., Sun G.-B., Sun P.-X., He X.-H., Ni L.-G., Li B.-C. Transgenic sperm produced by electrotransfection and allogeneic transplantation of chicken fetal spermatogonial stem cells. *Molecular Reproduction and Development*, 2010, 77(4): 340-347 (doi: 10.1002/mrd.21147).
67. Hong Y.H., Moon Y.K., Jeong D.K., Han J.Y. Improved transfection efficiency of chicken gonadal primordial germ cells for the production of transgenic poultry. *Transgenic Research*, 1998, 7(4): 247-252 (doi: 10.1023/A:1008861826681).
68. Naito M., Harumi T., Kuwana T. Long term in vitro culture of chicken primordial germ cells isolated from embryonic blood and incorporation into germline of recipient embryo. *Poultry Science*, 2010, 47(1): 57-64 (doi: 10.2141/jpsa.009058).
69. Tyack S.G., Jenkins K.A., O'Neil T.E., Wise T.G., Morris K.R., Bruce M.P., McLeod S., Wade A.J., McKay J., Moore R.J., Schat K.A., Lowenthal J.W., Doran T.J. A new method for producing transgenic birds via direct in vivo transfection of primordial germ cells. *Transgenic Research*, 2013, 22(6): 1257-1264 (doi: 10.1007/s11248-013-9727-2).
70. Sawicka D., Chojnacka-Puchta L. Effective transfection of chicken primordial germ cells (PGCs) using transposon vectors and lipofection. *Folia Biologica*, 2019, 67(1): 45-52 (doi: 10.3409/fb_67-1.04).
71. Kalina J., Senigl F., Micáková A., Mucksová J., Blazková J., Yan H., Poplstein M., Hejnar J., Trefil P. Retrovirus-mediated in vitro gene transfer into chicken male germ line cells. *Reproduction*, 2007, 134(3): 445-453 (doi: 10.1530/rep-06-0233).
72. Allioli N., Thomas J.-L., Chebloune Y., Nigon V.-M., Verdier G., Legras C. Use of retroviral vectors to introduce and express the β-galactosidase marker gene in cultured chicken primordial germ cell. *Developmental Biology*, 1994, 165(1): 30-37 (doi: 10.1006/dbio.1994.1231).
73. Jiang Z.-Q., Wu H.-Y., Tian J., Li N., Hu X.-X. Targeting lentiviral vectors to primordial germ cells (PGCs): An efficient strategy for generating transgenic chickens. *Zoological Research*, 2020, 41(3): 281-291 (doi: 10.24272/j.issn.2095-8137.2020.032).
74. Vetokh A.N., Volkova L.A., Iolchiev B.S., Tomgorova E.K., Volkova N.A. Genetic modification of roosters' germ cells using various methodological approaches. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2020, 55(2): 306-314 (doi: 10.15389/agrobiology.2020.2.306eng).
75. Macdonald J., Taylor L., Sherman A., Kawakami K., Takahashi Y., Sang H.M., McGrew M.J. Efficient genetic modification and germ-line transmission of primordial germ cells using piggyBac and Tol2 transposons. *Proceedings of the National Academy of Sciences*, 2012, 109(23): E1466-E1472 (doi: 10.1073/pnas.1118715109).
76. Lambeth L.S., Morris K.R., Wise T.G., Cummins D.M., O'Neil T.E., Cao Y., Sinclair A.H., Doran T.J., Smith C.A. Transgenic chickens overexpressing aromatase have high estrogen levels but maintain a predominantly male phenotype. *Endocrinology*, 2016, 157(1): 83-90 (doi: 10.1210/en.2015-1697).
77. Zhao R., Zuo Q., Yuan X. Jin K., Jin J., Ding Y., Zhang C., Li T., Jiang J., Li J., Zhang M., Shi X., Sun H., Zhang Y., Xu Q., Chang G., Zhao Z., Li B., Wu X., Zhang Y., Song J., Chen G., Li B. Production of viable chicken by allogeneic transplantation of primordial germ cells induced from somatic cells. *Nature Communications*, 2021, 12: 2989 (doi: 10.1038/s41467-021-23242-5).
78. Woodcock M.E., Gheyas A.A., Mason A.S., Nandi S., Taylor L., Sherman A., Smith J., Burt D.W., Hawken R., McGrew M.J. Reviving rare chicken breeds using genetically engineered sterility in surrogate host birds. *Proceedings of the National Academy of Sciences*, 2019, 116(42): 20930-20937 (doi: 10.1073/pnas.190631611678).
79. Kang S.J., Choi J.W., Kim S.Y., Park K.J., Kim T.M., Lee Y.M., Kim H., Lim J.M., Han J.Y. Reproduction of wild birds via interspecies germ cell transplantation. *Biology of Reproduction*, 2008, 79(5): 931-937 (doi: 10.1095/biolreprod.108.069989).
80. Wernery U., Liu C., Baskar V., Guerineche Z., Khazanehdari K.A., Saleem S., Kinne J., Wernery R., Griffin D.K., Chang I.-K. Primordial germ cell-mediated chimera technology produces viable pure-line Houbara bustard off-spring: potential for repopulating an endangered species. *PLoS ONE*, 2010, 5(12): e15824 (doi: 10.1371/journal.pone.0015824).
81. Trefil P., Bakst M.R., Yan H., Hejnar J., Kalina J., Mucksová J. Restoration of spermatogenesis after transplantation of c-Kit positive testicular cells in the fowl. *Theriogenology*, 2010, 74(9): 1670-1676 (doi: 10.1016/j.theriogenology.2010.07.002).
82. Kim Y.M., Park J.S., Yoon J.W., Choi H.J., Park K.J., Ono T., Han J.Y. Production of germline chimeric quails following spermatogonial cell transplantation in busulfan-treated testis. *Asian Journal of Andrology*, 2018, 20(4): 414-416 (doi: 10.4103/aja.aja_79_17).
83. Park K.J., Kang S.J., Kim T.M., Lee Y.M., Lee H.C., Song G., Han J.Y. Gamma irradiation depletes endogenous germ cells and increases donor cell distribution in chimeric chickens. *In Vitro Cellular & Developmental Biology*, 2010, 46: 828-833 (doi: 10.1007/s11626-010-9361-8).
84. Trefil P., Polak J., Poplstein M., Mikus T., Kotrbová A., Rozinek J. Preparation of fowl testes as recipient organs to germ-line chimeras by means of gamma-radiation. *British Poultry Science*, 2003, 44(4): 643-650 (doi: 10.1080/00071660310001616246).
85. Tagirov M., Golovan S. The effect of busulfan treatment on endogenous spermatogonial stem cells in immature roosters. *Poultry Science*, 2012, 91(7): 1680-1685 (doi: 10.3382/ps.2011-02014).

86. Nakamura Y., Yamamoto Y., Usui F., Atsumi Y., Ito Y., Ono T., Takeda K., Nirasawa K., Kagami H., Tagami T. Increased proportion of donor primordial germ cells in chimeric gonads by sterilisation of recipient embryos using busulfan sustained-release emulsion in chickens. *Reproduction, Fertility and Development*, 2008, 20(8): 900-907 (doi: 10.1071/RD08138).
87. Motono M., Yamada Y., Hattori Y., Nakagawa R., Nishijima K., Iijima S. Production of transgenic chickens from purified primordial germ cells infected with a lentiviral vector. *Journal of Bioscience and Bioengineering*, 2010, 109(4): 315-321 (doi: 10.1016/j.jbiosc.2009.10.007).
88. van de Lavoir M.C., Collarini E.J., Leighton P.A., Fesler J., Lu D.R., Harriman W.D., Thiyagasundaram T.S., Etches R.J. Interspecific germline transmission of cultured primordial germ cells. *PLoS ONE*, 2012, 7(5): e35664 (doi: 10.1371/journal.pone.0035664).
89. Han J.Y., Park Y.H. Primordial germ cell-mediated transgenesis and genome editing in birds. *Journal of Animal Science and Biotechnology*, 2018, 9: 19 (doi: 10.1186/s40104-018-0234-4).
90. Abu-Bonsrah K.D., Zhang D., Newgreen D.F. CRISPR/Cas9 targets chicken embryonic somatic cells in vitro and in vivo and generates phenotypic abnormalities. *Scientific Report*, 2016, 6: 34524 (doi: 10.1038/srep34524).
91. Zhang Y., Wang Y., Zuo Q., Li D., Zhang W., Wang F., Ji Y., Jin J., Lu Z., Wang M., Zhang C., Li B. CRISPR/Cas9 mediated chicken Stra8 gene knockout and inhibition of male germ cell differentiation. *PLoS ONE*, 2017, 12(2): e0172207 (doi: 10.1371/journal.pone.0172207).
92. Koslová A., Kučerová D., Reinišová M., Geryk J., Trefil P., Hejnar J. Genetic resistance to avian leukosis viruses induced by CRISPR/Cas9 editing of specific receptor genes in chicken cells. *Viruses*, 2018, 10(11): 605 (doi: 10.3390/v10110605).
93. Bai Y., He L., Li P., Xu K., Shao S., Ren C., Liu Z., Wei Z., Zhang Z. Efficient genome editing in chicken DF-1 cells using the CRISPR/Cas9 system. *G3 Genes|Genomes|Genetics*, 2016, 6(4): 917-923 (doi: 10.1534/g3.116.027706).
94. Lee J.H., Kim S.W., Park T.S. Myostatin gene knockout mediated by Cas9-D10A nickase in chicken DF1 cells without off-target effect. *Asian-Australasian Journal of Animal Sciences*, 2017, 30(5): 743-748 (doi: 10.5713/ajas.16.0695).
95. Schusser B., Collarini E.J., Yi H., Izquierdo S. M., Fesler J., Pedersen D., Klasing, K. C. Kaspers B., Harriman W. D., van de Lavoir M.-C., Etches R.J., Leighton P.A. Immunoglobulin knockout chickens via efficient homologous recombination in primordial germ cells. *Proceedings of the National Academy of Sciences,* 2013, 110(50): 20170-20175 (doi: 10.1073/pnas.1317106110).
96. Dimitrov L., Pedersen D., Ching K.H., Yi H., Collarini E.J., Izquierdo S., van de Lavoir M.-C., Leighton P.A. Germline gene editing in chickens by efficient CRISPR-mediated homologous recombination in primordial germ cells. *PLoS ONE*, 2016, 11(4): e0154303 (doi: 10.1371/journal.pone.0154303).
97. Taylor L., Carlson D.F., Nandi S., Sherman A., Fahrenkrug S.C., McGrew M.J. Efficient TALEN-mediated gene targeting of chicken primordial germ cells. *Development*, 2017, 144(5): 928-934 (doi: 10.1242/dev.145367).
98. Park T.S., Lee H.J., Kim K.H., Kim J.-S., Han J.Y. Targeted gene knockout in chickens mediated by TALENs. *Proceedings of the National Academy of Sciences*, 2014, 111(35): 12716-12721 (doi: 10.1073/pnas.1410555111).
99. Oishi I., Yoshii K., Miyahara D., Kagami H., Tagami T. Targeted mutagenesis in chicken using CRISPR/Cas9 system. *Scientific Reports*, 2016, 6: 23980 (doi: 10.1038/srep23980).
100. Oishi I., Yoshii K., Miyahara D., Tagami T. Efficient production of human interferon beta in the white of eggs from ovalbumin gene—targeted hens. *Scientific Reports*, 2018, 8: 10203 (doi: 10.1038/s41598-018-28438-2).
101. Qin X., Xiao N., Xu Y., Yang F., Wang X., Hu H., Liu Q., Cui K., Tang X. Efficient knock-in at the chicken ovalbumin locus using adenovirus as a CRISPR/Cas9 delivery system. *3 Biotech*, 2019, 9: 454 (doi: 10.1007/s13205-019-1966-3).
102. Kim G.-D., Lee J.H., Song S., Kim S.W., Han J.S., Shin S.P., Park B.-C., Park T.S. Generation of myostatin-knockout chickens mediated by D10A-Cas9 nickase. *FASEB*, 2020, 34(4): 5688-5696 (doi: 10.1096/fj.201903035R).
103. Lee J., Kim D.-H., Lee K. Muscle hyperplasia in Japanese quail by single amino acid deletion in MSTN propeptide. *International Journal of Molecular Sciences*, 2020, 21(4): 1504 (doi: 10.3390/ijms21041504).
104. Lee J., Ma J., Lee K. Direct delivery of adenoviral CRISPR/Cas9 vector into the blastoderm for generation of targeted gene knockout in quail. *Proceedings of the National Academy of Sciences*, 2019, 116(27): 13288-13292 (doi: 10.1073/pnas.1903230116).