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Advances in genome editing technology and its applications in poultry breeding

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Abstract

Poultry has always had a unique range of applications in a variety of fields. Despite being an extremely valuable model organism for research, its usage lagged as their development almost takes place within an egg, and incubated outside which makes it strenuous to approach and handle the zygote. The techniques of precise editing of specific loci in the genome using the programmable genome editing tools have nullified this lag of poultry species to be used as a research model. The genome-editing technique thus generates highly valuable and quality-improved poultry, purely based on primordial germ-cells, which are the progenitor cells of gametes, differentiating poultry species from that of the mammalian system. In this review, PGC-mediated genome editing in birds and their applications in the poultry field has been briefed.

Keywords: Precise editing, programmable genome-editing tools, primordial germ-cells, applications in poultry industry

Introduction

Protein is a vital ingredient of body framework at the molecular level. The protein mass in healthy adults is relatively large, representing 10.6 kg, or 15.1%, of body mass [1]. Protein undernutrition can lead to many adverse sequelae. One can overcome this undernutrition easily by picking up protein from many food sources, including plants and animals but the nutritional value is bent on the quantity and quality of protein. Therefore, it is theoretically recommended to consume high-quality protein of animal sources which is highly required for optimal growth, development, and health of humans [2]. Among the animal protein sources, poultry produce (meat and eggs) have a unique place. Poultry meat and eggs being the finest fountainhead of standard protein, many millions of people are stand in need of it. Besides high-quality protein, they also furnish crucial vitamins and minerals. Net protein utilization (NPU) is an index of protein quality. The eggs have an NPU value of 97% [3]. Cereals dearth the amino acids for humans which is most prime such as lysine, threonine, the sulphur-bearing amino acids (methionine and cysteine) and at times tryptophan. Eggs and poultry meat being well-heeled with all-important amino acids, they are inexpensive and widely available comparatively. Hence it is increasingly seen as less of a luxury product and more as a daily staple. And above all, there are no major social taboos on their consumption. Thus, poultry has a greater scope in developing countries.

The genetics of selective breeding in poultry industries have driven to impressive changes in yield, efficiency and product quality, but characteristics linked to health outcomes have not been so amenable. Though, genetic selection methods employed continuously refine production parameters, hand in hand with the state of art production facilities and protocols [4]. Genome editing is used to increase the numbers of animals in the breeding herd that carries beneficial genome variants. This makes it possible to conserve valuable genome variants that would otherwise be lost because they lean to be inherited in concert with harmful variants. Thus, genome editing is expected to have a wider perspective in bringing up more beneficial traits in livestock population for productivity, health, fertility, and safety [5, 6].

Current genome-editing tools have been successfully adapted to all species including zebrafish [7], humans [8], mice [9], rats [10], monkeys [11], pigs [12], cattle, sheep [13], goats [14] and others. This technology potentiates geneticists and medical researchers to edit portions of the genome

by adding, deleting, or altering segments of the DNA sequence. Due to its distinct reproductive biology, poultry needs very specific techniques to achieve heritably (germline) edited traits. By employing genome editing tools, it is expected that it will significantly impact the value and future development of poultry. In addition, precise editing in the endogenous genome, without incorporating foreign DNA, may become a modern breeding tool for the development of genetically modified organisms for human consumption. In this review, an overview of genome-editing technology for the enhancement of poultry products will be briefed.

Genome editing

The introduction of designer nucleases like zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) brings about the site-specific DNA cleavage by coupling an adjustable DNA binding domain with a Fok I endonuclease catalytic domain, which is then repaired via homology-driven genome modifications [15, 16]. Withal, genome editing technology green-lights the introduction of mutations without leaving any technology-associated footprint resulting in modifications of the genome which are insensible from natural mutations. A new genome-editing tool emerged recently that promises even greater simplicity, flexibility, and efficiency and all at a minimal cost, as compared to ZFNs and TALENs. This recently evolved tool is the clustered regularly interspaced short palindromic repeat (CRISPR) - CRISPR-associated nuclease 9 (Cas9), it is an RNA-guided nuclease system [15] in adverse with ZFNs and TALENs, which need protein engineering to customize their DNA binding properties. The CRISPR-Cas9 system makes use of a universal monomeric nuclease (Cas9) that is guided by sequence complementarity of a small, so-called guide RNA, to its specific target site where it initiates a DNA double-strand break making it very simple to design. CRISPR/Cas9 enzymes can have different target specificity, by including a different oligonucleotide in the guide RNA expression construct specific for that target site. Added to its specificity, they also facilitate simultaneous targeting of multiple targets, which requires only the addition of guide RNAs directed to different target sites within the genome [17].

The base-editors

The usage of CRISPR/Cas9 to generate gene knockouts is workable and abrupt in transformable eukaryotic species [18, 19]. Even so, making precise single-base changes or substitutions (base editing) remains hard, mainly because HDR is highly feckless across eukaryotes [20, 21]. In dissimilitude with DSB-HDR-mediated genome editing, base editing concerns the site-specific modification of the DNA base accompanying manipulation of the DNA repair machinery to elude trusted repair of the modified base [22]. These base editors are chimeric proteins laid back of a DNA targeting module and a catalytic domain handy of deaminating a cytidine or adenine base. Thereby restricts the creation of insertions and deletions (indels) at target and off-target sites as there is no pre-requisite to generate DSBs to edit DNA bases [23, 24].

Cytidine deaminase-based DNA base editors

Cytidine deaminases have been set forth by two sets (Liu and Akihiko groups) and these enzymes catalyze the transfiguration of cytosine into uracil [23, 24, 25]. For which, the Liu group developed the first base editor in 2016 [23]. In the base-editing system, APOBEC, guided by dCas9, deaminates a particular cytidine to uracil; the resulting U-G mismatches

are corrected by repair mechanisms to form U-A base pairs, and followed by T-A base pairs. Such base editors can therefore be used to fabricate C-to-T point mutations. Following the basic editing of the DNA molecule, a DNA lesion is formed that can be repaired and substituted with thymidine during DNA replication; base excision repair eliminates the uridine and enables the integration of any base and mismatch repair by trans-lesion synthesis and increases mutations at nearby nucleotides through an error-prone polymerase [26]. Later, the addition of uracil DNA glycosylase (UGI) established another base editor, BE2 [27]. The incorporation of UGI impedes the base excision repair pathway. The Cytidine deaminase changes C into U and thereafter uracil DNA glycosylase can perform error-free repair, changing the U into the wild-type sequence. Another substantial improvement of the system was reached by the instigation of BE3, [28, 29, 30, 31, 32] it is comprised of dCas9-pmCDA and UGI, homogenous to target-AID and has successfully resulted in a six-fold increase in the base editing of both mammalian and plant cells [23, 25]. It was suggested that the activity of UGI is consequential for inhibiting base excision repair and improving the base-editing efficiency, hence incorporating two UGI molecules one at the C- and other at the N- termini, BE4 was developed [33]. But the option of the base editor is bound to the accessibility of a PAM sequence, the residence of a C nucleotide relative to the PAM, how much indel generation can be indulged, and how the base-editor reagents are consigned to the target cell [26].

ADAR2-based RNA base editors

RNA base editors have been created recently, and are used to modulate biological processes. Different mechanisms, including ADAR2, deaminate adenosine to inosine, which the translational machinery reads as guanine, have been utilized for RNA editing [34]. Interestingly, an RNA-guided ribonuclease system using CRISPR/Cas13 has been freshly reprocessed to edit mRNA sequences and also to change adenosine to inosine by utilizing a catalytically inactive Cas13 protein and the deaminase activity of ADAR2 which is cited as RNA editing for programmable adenosine to inosine replacement (REPAIR) [34]. The prime edge of using RNA editing systems is that permanent change in the genome can be avoided, and therefore, it is regarded as more superior than DNA base editing.

Genome-editing in livestock

Genetic improvement yields a speedy, inexpensive, salubrious, and more-structured animal production, with minimal influence on the environment. Application of managed selective breeding programs on many of the domesticated farm animals has supervened in notable improvements in their productivity. For instance, in pigs it issued in 50% larger litter size, 37% shoot up of lean pork meat and also aided in two-fold increase in lean pork per kg feed intake; and 67% increase in milk production in case of cattle; finally, in our topic of interest i.e. in chickens, the days to obtain 2 kg body mass had turned down from 100 days to 40, evident increase in breast meat proportion from 12 to 20%, the feed conversion ratio had been cut in half, eggs per year augmented by 30% and eggs per tonne of feed boosted by 80% since 1960s to 2005 [35]. But these selective breeding techniques is entirely based on the genetic variation within the species or population of interest, and new variants emerging through de novo mutations. At the minute, transgenic and genome-editing technologies furnish novel slots for genetic improvement by suggesting well known beneficial alleles or

shaping beneficial alleles without any consequences of the linkage drag associated with traditional admittance. These genetic modification techniques when setting side by side, transgenesis relies on incorporating transgenes to enhance the traits in livestock more productively whereas genome editing offers an opportunity to create more specific and precise

alterations to the genome of an animal for the same. To date, genome editing has been done successfully for a few traits in various livestock species which is summarized in Table 1.

Table 1: Genome-editing technology in various species

Trait	Species	Genome-editing target	References
Increased muscle growth (double-muscle phenotype)	Cow	Myostatin (GDF8)	[13]
	Sheep	Myostatin (GDF8)	[36]
	Goat	Myostatin (GDF8)	[37]
	Channel catfish	Myostatin (GDF8)	[38]
	Pig	Myostatin (GDF8)	[39, 40, 41, 42, 43, 44, 45]
Hornless (polled)	Cow	Pc POLLED	[46]
Boretaint	Pig	KISS1R	[47]
Sterility	Salmon	Dead end protein (dnd)	[48]
Sterility/surrogate hosts	Pig	Nanos 2	[41]
PRRSV resistance	Pig	CD163	[49, 50, 51, 52]
ASFV resilience	Pig	RELA	[53, 54]
Mannheimia (Pasteurella) hemolytica resilience	Cow	CD18	[55]
Bovine tuberculosis resilience	Cow	NRAMP1	[56]
Xenotransplantation (removal of endogenous retroviruses)	Pig	Porcine endogenous retrovirus genes	[57,58]

Genome- editing in poultry

Genetic modification of poultry was not that easy in comparison with those of other livestock as the avian egg possess very unique physiology from a mammalian oocyte. This made it impractical to isolate and transfer a chicken yolk. But then, Gandhi *et al.* (2017) came up with an approach of ovo electroporation of editing reagents, despite that the electroporation resulted in mosaicism with editing limited to a subset of cells as the chicken embryo is already developed at a greater distance when an egg is laid compared with a zygote [59, 60]. As a result, it was unlikely to generate edited birds using this approach. Later came an alternative strategy known as sperm transfection–assisted gene editing (STAGE) which uses the sperms that are being lipofected with editing reagents

before artificial insemination [61]. But ultimately it was the advancements in chicken stem cell technology that showed the greatest potential for chicken genome editing. In par with fibroblast cells of mammals, the primordial germ cells (stem cells that later on develops into germ cells) can be isolated from the blood of developing chicks *in-ovo* and then could be cultured *in vitro*. Through an opening in the eggshell, the chick embryo is examined which must be sealed again until the chick hatches. Several groups were successful in demonstrating this genome editing in primordial germ cells [62, 63] and among these one group had led to modified birds [64]. Fig 1 illustrates the different methods of genome editing employed in poultry.

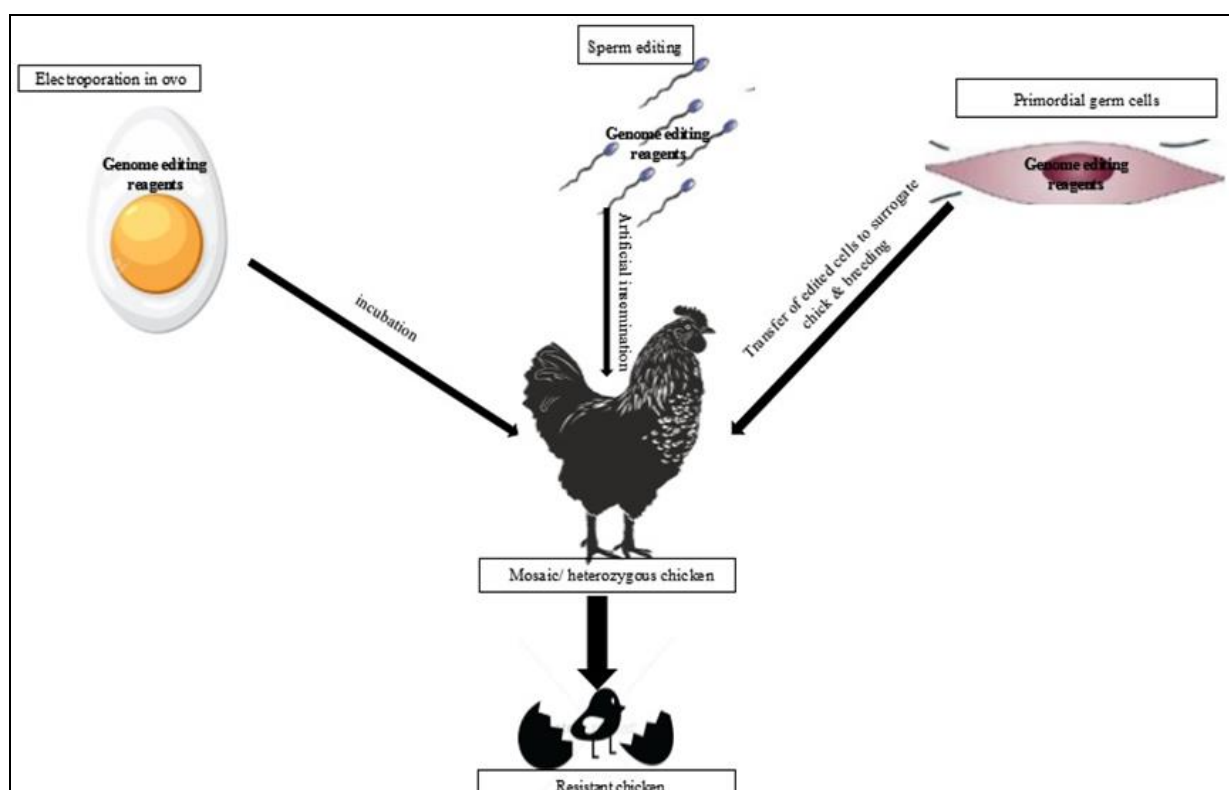


Fig 1: Methods of Genome-editing in chickens

Genome-editing in poultry, can brought about in three-ways: by electroporation *in ovo*, by sperm lipofection or by isolation and editing of primordial germ-cells. In all these methods, the resultant will be heterozygous/mosaic which must be breed to generate homozygous birds.

Applications

By the application of these evolving technology, it was made possible to generate the:

Disease resistance in chickens

For the production of disease resistance phenotypes, that can be easily adapted in the avian system Lee *et al.* (2017) conducted genome editing on chicken DF-1 fibroblasts which aided in identifying the roles of *chNHE1* in viral interaction [65].

Sterility in chickens

Similarly, Taylor *et al.* (2017) demonstrated the utility of TALENs in genome targeting of poultry [63]. It was known that the Chicken vasa homolog (*CVH*) (*DDX4*) is hypothesized to be a maternal instigation for the genesis of the germ cell lineage [66] as it indicates the chicken germ cell lineage at the advance stages of developing embryo. As a consequence, it was expected that vasa play a vital bit part in the chicken oogenesis. So, TALENs were used to knock out the *DDX4* (*CVH* (*vasa*)) locus in chickens to demonstrate systematic targeting of genes crucial for the development of the germ cell lineage. As expected, female chickens were sterile and carried no sensible follicles post-hatch. Upon examining it published that the germ cell lineage of early embryos was originally framed but female PGCs were eventually lost through meiosis. Thus the usage of TALENs in genome targeting of poultry and the preserved activity of the *DDX4* gene in germ cell development and oogenesis was

expressed successfully [50].

Knock-ins using GE tools

In the advent of generating chickens for a better ectopic gene expression, Oishi *et al.* (2018) knocked in hINF- β into the chicken ovalbumin locus using CRISPR/Cas9 procedure for the production of hINF- β in egg white. And their results uncovered that this transgene insertion using the gene editing tool, has culminated a stable expression of an exogenous protein which was regarded highly significant and befitting for the industrial applications [67].

Future perspectives of genome editing in poultry

Genome editing in chickens is now entering the golden age as it is already in advance in mammals, particularly mice and pigs. The recent generation of Cas9-expressing animals will be a powerful tool for studying biological processes [68]. Similar applications in chickens can be more beneficial and may be utilized in the future to bring the functions of unknown genes into the light. Therapeutic applications using human monoclonal antibodies derived from genetically engineered chickens can be beneficial over *in vitro* approaches that lack affinity maturation [69]. Antibodies in chicken eggs are also used as an economical and stress-free method for the synthesis of specific antibodies [70]. Chicken eggs can also be used to manufacture specific proteins [71, 72] as this can improve the digestibility of sugar complexes in feed. Due to its simplicity of design and implementation combined with high performance, newly developed gene-editing technologies give many advantages [73]. So soon, we may also obtain new breeds of chickens that are resistant to specific pathogens. Thus by spending more efforts for genome-editing technologies in poultry breeding will probably improve its welfare. Table 2 lists the genetic engineering works done so far in poultry species.

Table 2: Genetic engineering works done in poultry species

Research on	Genetic engineering work done	References
Transgenic chicken carrying a benign subgroup A leucosis virus	Introduction of <i>alv6</i> into the progeny of dams that congenitally transmit ALV to their progeny	[74]
Transgenic chickens expressing active beta-lactamase in the egg white	Inserted a transgene encoding a secreted protein, beta-lactamase, under the control of the ubiquitous cytomegalovirus (CMV) promoter	[75]
eGFP expressing chickens	Germline transmission in the embryonic tissue that expresses eGFP uniformly using embryos sired by a heterozygous male	[76]
Hens specifically expressing therapeutic proteins in the oviduct	Use of lentiviral vectors to deliver transgene constructs comprising regulatory sequences from the ovalbumin gene designed to direct synthesis of associated therapeutic proteins to the oviduct	[77]
Production of transgenic chickens expressing a tetracycline-inducible eGFP gene	Showed that a tetracycline-inducible expression system in transgenic animals might be a promising solution to minimize physiological disturbances caused by the transgene	[78]
Short-hairpin RNA against Influenza expressing chickens	Generated transgenic chickens expressing a short-hairpin RNA designed to function as a decoy that inhibits and blocks influenza virus polymerase and thus interfering with virus propagation	[79]
Transgenic chickens expressing human extracellular superoxide dismutase	The hEC-SOD protein was expressed in the egg white of transgenic hens and showed antioxidant activity highlighting the potential of the chicken for production of biologically active proteins in egg white	[80]
Immunoglobulin heavy chain (JH0) KO chickens	Targeted the joining (J) gene segment of the chicken Ig heavy chain gene by homologous recombination in primordial germ cells. In homozygous knockouts, Ig heavy chain production is eliminated and no antibody response is elicited on immunization	[81]
Transgenic chickens expressing the human urokinase type-plasminogen activator	Explored the feasibility of using chickens as a bioreactor for producing human urokinase-type plasminogen activator(huPA) that can be used to treat thrombolytic disorders	[82]
CSF1R- receptor reporter chickens	Transgenic chickens, in which the reporter genes are expressed in a specific immune cell lineage based upon control elements of the colony stimulating factor 1 receptor locus	[83]

Immunoglobulin light chain (IgL) KO chickens	Targeting immunoglobulin light chain locus by homologous recombination in chicken primordial germ cells (PGCs) suggesting that the heavy chain by itself is enough to support all the important steps in B-cell development in a gut-associated lymphoid tissue species	[84]
Cre- recombinase expressing chickens	Described the generation of transgenic chickens expressing Cre-recombinase	[85]
Ovalbumin and ovomucoid KO chickens	Implemented CRISPR/Cas9-mediated gene targeting in chickens	[86]
Aromatase overexpressing chickens	For testing whether a continuous supply of estrogen in adult chickens could induce stable male to female sex reversal, they generated transgenic male chickens over expressing aromatase using Tol2/ transposase system	[87]
mCherry expressing chickens	Tested whether functional sperm cells can be matured from genetically manipulated primordial germ cells after transplantation in adult testes and used to restore fertility	[88]
DDX4 KO chickens	Used TALE nucleases to target a reporter construct to the DDX4 locus in chicken primordial germ cells	[63]
3D8 single variable fragment (scFv) expressing chickens	Used recombinant lentiviruses to generate transgenic chickens expressing the 3D8 scFv gene under the control of the chicken beta actin promoter	[5]
Chickens with humanized immunoglobulin genes	Generated transgenic chickens expressing antibodies from immunoglobulin heavy and light chain loci containing human variable regions and chicken constant regions	[69]
Chickens overexpressing human IFN- beta	Demonstrated the feasibility of integrating human interferon beta into the chicken ovalbumin locus and producing human interferon beta in egg white	[67]

Conclusion

The latest interest prevailing in the poultry breeding is the genome editing technology, which is awaited to create a large impact on the overall performance of the birds by making a small change. It has a massive potential to improve the economic traits of birds and besides can also generate disease-resistant birds which can promise more benefits to the poultry farmers. Apart from these, it can also do beneficial by manipulating traits like heat tolerance and osteoporosis in older laying hens. Since commercial production birds are the products of 3 or 4 lines, gene-edited changes would need to be incorporated into each of the elite lines that contribute to the final commercial cross, thus increasing costs by 3-4 fold. Moreover, the same edits can be made in the same line in multiple birds that can decrease the inbreeding problems. Thus the genome editing technology will greatly increase the understanding of basic biology, determination of gene function, and the effect of specific variation and also for identifying genetic control pathways. This technology is known to have a profound value to gain immense scientific knowledge of genetics, gene function, and genetic inter-relationships. The commercial poultry industry is thus expected to participate at the basic scientific level and help in supporting the development of this technology.

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Competing interests

The authors declare that they have no competing interests.

References

- Snyder WS, Cook MJ, Nasset ES, Karhausen LR, Howells GP, Tipton IH. Report of the task group on reference men. In: International Commission on Radiological Protection, ICRP Publication 23, Pergamon Press, Oxford, 1975, 235-237.
- Wu G. Dietary protein intake and human health. *Food & Function*. 2016; 7(3):1251-65.
- Matsuoka R, Takahashi Y, Kimura M, Masuda Y, Kunou M. Heating has no effect on the net protein utilisation from egg whites in rats. *The Scientific World Journal*. 2017; 2017:1-5
- Andersson L, Georges M. Domestic-animal genomics: deciphering the genetics of complex traits. *Nature Reviews Genetics*. 2004; 5(3):202-12.
- Byun SJ, Yuk SS, Jang YJ, Choi H, Jeon MH, Erdene-Ochir TO *et al*. Transgenic chickens expressing the 3D8 single chain variable fragment protein suppress avian influenza transmission. *Scientific Reports*. 2017; 7(1):5938
- Tan W, Proudfoot C, Lillico SG, Whitelaw CB. Gene targeting, genome editing: from Dolly to editors. *Transgenic Research*. 2016; 25(3):273-87.
- Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD *et al*. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nature Biotechnology*. 2013; 31(3):227-9.
- Kang X, He W, Huang Y, Yu Q, Chen Y, Gao X *et al*. Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing. *Journal of Assisted Reproduction and Genetics*. 2016; 33(5):581-8.
- Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR *et al*. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. *Cell*. 2014; 159(2):440-55.
- Weinstein E, Cui X, Simmons P, inventors; Sigma-Aldrich Co LLC, assignee. Genome editing of cognition related genes in animals. United States patent application US 12/842, 991; 2011.
- Liu H, Chen Y, Niu Y, Zhang K, Kang Y, Ge W *et al*. TALEN-mediated gene mutagenesis in rhesus and cynomolgus monkeys. *Cell Stem Cell*. 2014; 14(3):323-8.
- Lillico SG, Proudfoot C, Carlson DF, Stverakova D, Neil C, Blain C *et al*. Live pigs produced from genome edited zygotes. *Scientific Reports*. 2013; 3:2847.
- Proudfoot C, Carlson DF, Huddart R, Long CR, Pryor JH, King TJ *et al*. Genome edited sheep and cattle. *Transgenic Research*. 2015; 24(1):147-53.

14. Ni W, Qiao J, Hu S, Zhao X, Regouski M, Yang M *et al.* Efficient gene knockout in goats using CRISPR/Cas9 system. *PLOS One*. 2014; 9(9).
15. Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. *Nature reviews Molecular cell biology*. 2013; 14(1):49-55.
16. Wood AJ, Lo TW, Zeitler B, Pickle CS, Ralston EJ, Lee AH *et al.* Targeted genome editing across species using ZFNs and TALENs. *Science*. 2011; 333(6040):307.
17. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature Biotechnology*. 2014; 32(4):347.
18. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science*. 2014; 346(6213):1258096.
19. Eid A, Mahfouz MM. Genome editing: the road of CRISPR/Cas9 from bench to clinic. *Experimental & Molecular Medicine*. 2016; 48(10):e265.
20. Zhang JP, Li XL, Li GH, Chen W, Arakaki C, Botimer GD *et al.* Efficient precise knockin with a double cut HDR donor after CRISPR/Cas9-mediated double-stranded DNA cleavage. *Genome Biology*. 2017; 18(1):35.
21. Rouet P, Smih F, Jasin M. Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. *Proceedings of the National Academy of Sciences*. 1994; 91(13):6064-8.
22. Hess GT, Tycko J, Yao D, Bassik MC. Methods and applications of CRISPR-mediated base editing in eukaryotic genomes. *Molecular cell*. 2017; 68(1):26-43.
23. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*. 2016; 533(7603):420-4.
24. Rees HA, Komor AC, Yeh WH, Caetano-Lopes J, Warman M, Edge AS *et al.* Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery. *Nature Communications*. 2017; 8(1):1-0.
25. Shimatani Z, Kashojiya S, Takayama M, Terada R, Arazoe T, Ishii H *et al.* Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. *Nature Biotechnology*. 2017; 35(5):441-3.
26. Komor AC, Zhao KT, Packer MS, Gaudelli NM, Waterbury AL, Koblan LW *et al.* Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C: G-to-T: A base editors with higher efficiency and product purity. *Science Advances*. 2017; 3(8):eaao4774.
27. Mol CD, Arvai AS, Sanderson RJ, Slupphaug G, Kavli B, Krokan HE *et al.* Crystal structure of human uracil-DNA glycosylase in complex with a protein inhibitor: protein mimicry of DNA. *Cell*. 1995; 82(5):701-8.
28. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012; 337(6096):816-21.
29. Heller RC, Marians KJ. Replisome assembly and the direct restart of stalled replication forks. *Nature Reviews Molecular Cell Biology*. 2006; 7(12):932-43.
30. Robertson AB, Klungland A, Rognes T, Leiros I. DNA repair in mammalian cells. *Cellular and Molecular Life Sciences*. 2009; 66(6):981-93.
31. Zharkov DO. Base excision DNA repair. *Cellular and molecular life sciences*. 2008; 65(10):1544-65.
32. Kim YB, Komor AC, Levy JM, Packer MS, Zhao KT, Liu DR. Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. *Nature Biotechnology*. 2017; 35(4):371.
33. Källman AM, Sahlin M, Öhman M. ADAR2 A→ I editing: site selectivity and editing efficiency are separate events. *Nucleic Acids Research*. 2003; 31(16):4874-81.
34. Spencer KR, Wang J, Silk AW, Ganesan S, Kaufman HL, Mehnert JM. Biomarkers for immunotherapy: current developments and challenges. *American Society of Clinical Oncology Educational Book*. 2016; 36:e493-503.
35. Van der Steen HA, Prall GF, Plastow GS. Application of genomics to the pork industry. *Journal of Animal Science*. 2005; 83(suppl_13):E1-8.
36. Proudfoot C, Carlson DF, Huddart R, Long CR, Pryor JH, King TJ *et al.* Genome edited sheep and cattle. *Transgenic Research*. 2015; 24(1):147-53.
37. Wang X, Niu Y, Zhou J, Zhu H, Ma B, Yu H *et al.* CRISPR/Cas9-mediated MSTN disruption and heritable mutagenesis in goats causes increased body mass. *Animal Genetics*. 2018; 49(1):43-51.
38. Khalil K, Elayat M, Khalifa E, Daghash S, Elawad A, Miller M *et al.* Generation of myostatin gene-edited channel catfish (*Ictalurus punctatus*) via zygote injection of CRISPR/Cas9 system. *Scientific Reports*. 2017; 7(1):1-2.
39. Kang JD, Kim S, Zhu HY, Jin L, Guo Q, Li XC *et al.* Generation of cloned adult muscular pigs with myostatin gene mutation by genetic engineering. *RSC Advances*. 2017; 7(21):12541-9.
40. Wang K, Tang X, Xie Z, Zou X, Li M, Yuan H *et al.* CRISPR/Cas9-mediated knockout of myostatin in Chinese indigenous Erhualian pigs. *Transgenic Research*. 2017; 26(6):799-805.
41. Cai C, Qian L, Jiang S, Sun Y, Wang Q, Ma D *et al.* Loss-of-function myostatin mutation increases insulin sensitivity and browning of white fat in Meishan pigs. *Oncotarget*. 2017; 8(21):34911.
42. Bi Y, Hua Z, Liu X, Hua W, Ren H, Xiao H *et al.* Isozygous and selectable marker-free MSTN knockout cloned pigs generated by the combined use of CRISPR/Cas9 and Cre/LoxP. *Scientific Reports*. 2016; 6(1):1-2.
43. Rao S, Fujimura T, Matsunari H, Sakuma T, Nakano K, Watanabe M *et al.* Efficient modification of the myostatin gene in porcine somatic cells and generation of knockout piglets. *Molecular Reproduction and Development*. 2016; 83(1):61-70.
44. Wang K, Ouyang H, Xie Z, Yao C, Guo N, Li M *et al.* Efficient generation of myostatin mutations in pigs using the CRISPR/Cas9 system. *Scientific Reports*. 2015; 5:16623.
45. Cyranoski D. Super-muscle pigs created by small genetic tweak: researchers hope the genetically engineered animals will speed past regulators. *Nature*. 2015; 523(7558):13-5.
46. Carlson DF, Lancto CA, Zang B, Kim ES, Walton M, Oldeschulte D *et al.* Production of hornless dairy cattle from genome-edited cell lines. *Nature Biotechnology*. 2016; 34(5):479-81.
47. Sonstegard TS, Carlson D, Lancto C, Fahrenkrug SC.

- Precision animal breeding as a sustainable, non-GMO solution for improving animal production and welfare. *ASAP Animal Production*, Adelaide. 2016; 31:316-317.
48. Wargelius A, Leininger S, Skaftnesmo KO, Kleppe L, Andersson E, Taranger GL *et al.* Dnd knockout ablates germ cells and demonstrates germ cell independent sex differentiation in Atlantic salmon. *Scientific Reports*. 2016; 6(1):1-8.
 49. Taylor L, Carlson DF, Nandi S, Sherman A, Fahrenkrug SC, McGrew MJ. Efficient TALEN-mediated gene targeting of chicken primordial germ cells. *Development*. 2017; 144(5):928-34.
 50. Whitworth KM, Rowland RR, Ewen CL, Tribble BR, Kerrigan MA, Cino-Ozuna AG *et al.* Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. *Nature Biotechnology*. 2015; 34(1):20.
 51. Yang H, Zhang J, Zhang X, Shi J, Pan Y, Zhou R *et al.* CD163 knockout pigs are fully resistant to highly pathogenic porcine reproductive and respiratory syndrome virus. *Antiviral Research*. 2018; 151:63-70.
 52. Burkard C, Lilloco SG, Reid E, Jackson B, Mileham AJ, Ait-Ali T *et al.* Precision engineering for PRRSV resistance in pigs: macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. *PLOS Pathogens*. 2017; 13(2).
 53. Lilloco S. Agricultural applications of genome editing in farmed animals. *Transgenic Research*. 2019; 28(2):57-60.
 54. Palgrave CJ, Gilmour L, Lowden CS, Lilloco SG, Mellencamp MA, Whitelaw CB. Species-specific variation in RELA underlies differences in NF- κ B activity: A potential role in African swine fever pathogenesis. *Journal of Virology*. 2011; 85(12):6008-14.
 55. Shanthalingam S, Tibary A, Beever JE, Kasinathan P, Brown WC, Srikumaran S. Precise gene editing paves the way for derivation of *Mannheimia haemolytica* leukotoxin-resistant cattle. *Proceedings of the National Academy of Sciences*. 2016; 113(46):13186-90.
 56. Gao Y, Wu H, Wang Y, Liu X, Chen L, Li Q *et al.* Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects. *Genome Biology*. 2017; 18(1):13.
 57. Yang L, Güell M, Niu D, George H, Lesha E, Grishin D *et al.* Genome-wide inactivation of porcine endogenous retroviruses (PERVs). *Science*. 2015; 350(6264):1101-4.
 58. Niu D, Wei HJ, Lin L, George H, Wang T, Lee IH, Zhao HY *et al.* Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science*. 2017; 357(6357):1303-7.
 59. Gandhi S, Piacentino ML, Vieceli FM, Bronner ME. Optimization of CRISPR/Cas9 genome editing for loss-of-function in the early chick embryo. *Developmental Biology*. 2017; 432(1):86-97.
 60. Véron N, Qu Z, Kipen PA, Hirst CE, Marcelle C. CRISPR mediated somatic cell genome engineering in the chicken. *Developmental Biology*. 2015; 407(1):68-74.
 61. Cooper CA, Challagulla A, Jenkins KA, Wise TG, O'Neil TE, Morris KR *et al.* Generation of gene edited birds in one generation using sperm transfection assisted gene editing (STAGE). *Transgenic Research*. 2017; 26(3):331-47.
 62. Idoko-Akoh A, Taylor L, Sang HM, McGrew MJ. High fidelity CRISPR/Cas9 increases precise monoallelic and biallelic editing events in primordial germ cells. *Scientific Reports*. 2018; 8(1):1-4.
 63. Taylor L, Carlson DF, Nandi S, Sherman A, Fahrenkrug SC, McGrew MJ. Efficient TALEN-mediated gene targeting of chicken primordial germ cells. *Development*. 2017; 144(5):928-34.
 64. Park TS, Lee HJ, Kim KH, Kim JS, Han JY. Targeted gene knockout in chickens mediated by TALENs. *Proceedings of the National Academy of Sciences*. 2014; 111(35):12716-21.
 65. Lee HJ, Lee KY, Jung KM, Park KJ, Lee KO, Suh JY *et al.* Precise gene editing of chicken Na⁺/H⁺ exchange type 1 (chNHE1) confers resistance to avian leukosis virus subgroup J (ALV-J). *Developmental & Comparative Immunology*. 2017; 77:340-9.
 66. Toyooka Y, Tsunekawa N, Takahashi Y, Matsui Y, Satoh M, Noce T. Expression and intracellular localization of mouse Vasa-homologue protein during germ cell development. *Mechanisms of Development*. 2000; 93(1-2):139-49.
 67. 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(1):1-2.
 68. Wang K, Tang X, Xie Z, Zou X, Li M, Yuan H *et al.* CRISPR/Cas9-mediated knockout of myostatin in Chinese indigenous Erhualian pigs. *Transgenic Research*. 2017; 26(6):799-805.
 69. Ching KH, Collarini EJ, Abdiche YN, Bedinger D, Pedersen D, Izquierdo S *et al.* Chickens with humanized immunoglobulin genes generate antibodies with high affinity and broad epitope coverage to conserved targets. *MAbs*. 2018; 10(1):71-80.
 70. Amro WA, 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.
 71. Lilloco SG, McGrew MJ, Sherman A, Sang HM. Transgenic chickens as bioreactors for protein-based drugs. *Drug Discovery Today*. 2005; 10(3):191-6.
 72. Petite JN, Mozdziak PE. The incredible, edible, and therapeutic egg. *Proceedings of the National Academy of Sciences*. 2007; 104(6):1739-40.
 73. Chira S, Gulei D, Hajitou A, Zimta AA, Cordelier P, Berindan-Neagoe I. CRISPR/Cas9: transcending the reality of genome editing. *Molecular Therapy-Nucleic Acids*. 2017; 7:211-22.
 74. Salter DW, Crittenden LB. Artificial insertion of a dominant gene for resistance to avian leukosis virus into the germ line of the chicken. *Theoretical and Applied Genetics*. 1989; 77(4):457-61.
 75. Harvey AJ, Speksnijder G, Baugh LR, Morris JA, Ivarie R. Expression of exogenous protein in the egg white of transgenic chickens. *Nature Biotechnology*. 2002; 20(4):396-9.
 76. Chapman SC, Lawson A, MacArthur WC, Wiese RJ, Loechel RH, Burgos-Trinidad M *et al.* Ubiquitous GFP expression in transgenic chickens using a lentiviral vector. *Development*. 2005; 132(5):935-40.
 77. Byun SJ, Kim SW, Kim KW, Kim JS, Hwang IS, Chung HK *et al.* Oviduct-specific enhanced green fluorescent protein expression in transgenic chickens. *Bioscience, Biotechnology, and Biochemistry*. 2011; 75(4):646-9.
 78. Kwon MS, Koo BC, Roh JY, Kim M, Kim JH, Kim T.

- Production of transgenic chickens expressing a tetracycline-inducible GFP gene. *Biochemical and Biophysical Research Communications*. 2011; 410(4):890-4.
79. Lyall J, Irvine RM, Sherman A, McKinley TJ, Núñez A, Purdie A *et al*. Suppression of avian influenza transmission in genetically modified chickens. *Science*. 2011; 331(6014):223-6.
80. Byun SJ, Ji MR, Jang YJ, Hwang AI, Chung HK, Kim JS *et al*. Human extracellular superoxide dismutase (EC-SOD) expression in transgenic chicken. *BMB Reports*. 2013; 46(8):404.
81. Jung D, Giallourakis C, Mostoslavsky R, Alt FW. Mechanism and control of V (D) J recombination at the immunoglobulin heavy chain locus. *The Annual Review of Immunology*. 2006; 24:541-70.
82. Lee SH, Gupta MK, Ho YT, Kim T, Lee HT. Transgenic chickens expressing human urokinase-type plasminogen activator. *Poultry Science*. 2013; 92(9):2396-403.
83. Balic A, Garcia-Morales C, Vervelde L, Gilhooley H, Sherman A, Garceau V *et al*. Visualisation of chicken macrophages using transgenic reporter genes: insights into the development of the avian macrophage lineage. *Development*. 2014; 141(16):3255-65.
84. Schusser B, Collarini EJ, Pedersen D, Yi H, Ching K, Izquierdo S *et al*. Expression of heavy chain- only antibodies can support B- cell development in light chain knockout chickens. *European Journal of Immunology*. 2016; 46(9):2137-48.
85. Leighton PA, Pedersen D, Ching K, Collarini EJ, Izquierdo S, Jacob R *et al*. Generation of chickens expressing Cre recombinase. *Transgenic Research*. 2016; 25(5):609-16.
86. Oishi I, Yoshii K, Miyahara D, Kagami H, Tagami T. Targeted mutagenesis in chicken using CRISPR/Cas9 system. *Scientific Reports*. 2016; 6:23980.
87. Lambeth LS, Morris KR, Wise TG, Cummins DM, O'Neil TE, Cao Y *et al*. Transgenic chickens overexpressing aromatase have high estrogen levels but maintain a predominantly male phenotype. *Endocrinology*. 2016; 157(1):83-90.
88. Trefil P, Aumann D, Koslová A, Mucksová J, Benešová B, Kalina J *et al*. Male fertility restored by transplanting primordial germ cells into testes: a new way towards efficient transgenesis in chicken. *Scientific Reports*. 2017; 7(1):1-9.