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Review

Potentials, prospects and applications of genome editing technologies in livestock production

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ABSTRACT

In recent years, significant progress has been achieved in genome editing applications using new programmable DNA nucleases such as zinc finger nucleases (ZFNs), transcription activator-like endonucleases (TALENs) and the clustered regularly interspaced short palindromic repeats/Cas9 system (CRISPR/Cas9). These genome editing tools are capable of nicking DNA precisely by targeting specific sequences, and enable the addition, removal or substitution of nucleotides via double-stranded breakage at specific genomic loci. CRISPR/Cas system, one of the most recent genome editing tools, affords the ability to efficiently generate multiple genomic nicks in single experiment. Moreover, CRISPR/Cas systems are relatively easy and cost effective when compared to other genome editing technologies. This is in part because CRISPR/Cas systems rely on RNA-DNA binding, unlike other genome editing tools that rely on protein–DNA interactions, which affords CRISPR/Cas systems higher flexibility and more fidelity. Genome editing tools have significantly contributed to different aspects of livestock production such as disease resistance, improved performance, alterations of milk composition, animal welfare and biomedicine. However, despite these contributions and future potential, genome editing technologies also have inherent risks, and therefore, ethics and social acceptance are crucial factors associated with implementation of these technologies. This review emphasizes the impact of genome editing technologies in development of livestock breeding and production in numerous species such as cattle, pigs, sheep and goats. This review also discusses the mechanisms behind genome editing technologies, their potential applications, risks and associated ethics that should be considered in the context of livestock.

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1. Introduction

Genome editing technologies refer to a suite of tools that can be used to make accurate nuclease-based modifications to a genome. There are four major types of programmed nuclease-based technologies: meganucleases, ZFNs, TALENs and CRISPR-Cas9. Nucleases generate targeted nicks in the form of double-stranded breakages (DSB) in nuclear DNA, which in turn triggers a repair mechanism called non-homologous end joining (NHEJ) in the absence of a repair template. The repairing mechanism NHEJ, directly re-joins the loose ends of nicked DNA by either inserting and/or deleting nucleotides irrespective of the nucleotide sequence. The introduction of such random modifications in the coding sequence of genes can result in frameshift of the reading frame, resulting in gene expression knockout, and permanent loss-of-function. Furthermore, introduction of two targeted DSBs at the same time may lead to sequence deletions or other chromosomal aberrations involving the nuclease recognition and cleavage loci (Cox et al., 2015). Therefore, in order to avoid these genetic abnormalities, DSBs can be repaired by the use of a repair DNA template via a process called homologous directed repair (HDR). Any desired sequence variation can also be added to the donor template used for repair, thereby integrating into the target sequence permanently. This affords practical opportunities to effect gene modifications in livestock that are beneficial and profitable (Osakabe and Osakabe, 2015; Raza et al., 2021b).

Differences between gene editing technologies that rely onmeganucleases, ZFNs, TALENs and CRISPR-Cas, along with some advantages and limitations have been summarised in Table 1. Meganuclease, ZFN and TALEN based technologies cleave DNA at specific sites through DNA-protein interaction (Hsu et al., 2014; Li et al., 2021; Ng et al., 2020). Modified proteins are required for each target sequence, and therefore multiple modified proteins will be required to edit multiple target sequences, which is generally expensive both in terms of time and cost. CRISPR-Cas9 technology, on the other hand, relies on base pairing between specific guide RNAs (gRNAs) and the targeted genome sites, which offers a simple and efficient method for genome editing (Hsu et al., 2014; Li et al., 2021).

Genome editing technologies were applied in different fields of livestock production such as breeding disease-resistant animals, improving animal performance, enriching milk composition, and producing hornless animals (Alberio and Wolf, 2021; Carlson et al., 2016; Koloskova et al., 2021). In addition, CRISPR is frequently used for knocking out of genes for medical research and therapeutic purposes (Butler et al., 2016; Carlson et al., 2016). Nowadays, CRISPR/Cas system makes a scientific revolution in the field of genome editing in animals (Wiedenheft et al., 2012).

Genome editing tools can be used to improve livestock productivity and profitability of associated industries in many ways. In the past, gene editing in livestock relied on gene knock out and knock down techniques that were both inefficient and difficult to implement, in part because germline embryonic stem cells were generally lacking (Oishi et al., 2016). Furthermore, given that most economically important traits in livestock are quantitative in nature (i.e. controlled by many genes), livestock improvement via genetic manipulations almost always requires editing of the genome at multiple sites, which is prohibitively challenging and expensive using traditional gene manipulation technologies. Therefore, in order to have real translational impact in terms of enhanced productivity and profitability of livestock production, alternative gene editing technologies are required that are able to specifically and efficiently edit multiple gene loci scattered across the genome of any host species. Therefore, in this review, we aim to summarise four different genome editing technologies along with potential applications in livestock production, as well as associated risks and ethical considerations of these genome editing technologies.

2. Genome editing technologies

2.1. Meganucleases

Meganucleases are endonucleases that's pecifically target and cleave relatively long sequences of DNA (14–40 bp) both *in vitro*

Table 1
Comparison of Meganucleases, ZFNs, TALEN and CRISPR/Cas9.

	Meganucleases	ZFNs	TALEN	CRISPR/Cas9
DNA binding system	Protein-DNA	Protein-DNA	Protein-DNA	RNA-DNA
Targeted sequence	12–45 bp	18–36 bp	30–40 bp	22 bp
Cost	High	High	Middle	Low
Off- target events	Low	Comparable	Comparable	Comparable
Delivery	Easy	Easy	Difficult	Moderate
Multiple Targeting	Difficult	Difficult	Difficult	Easy

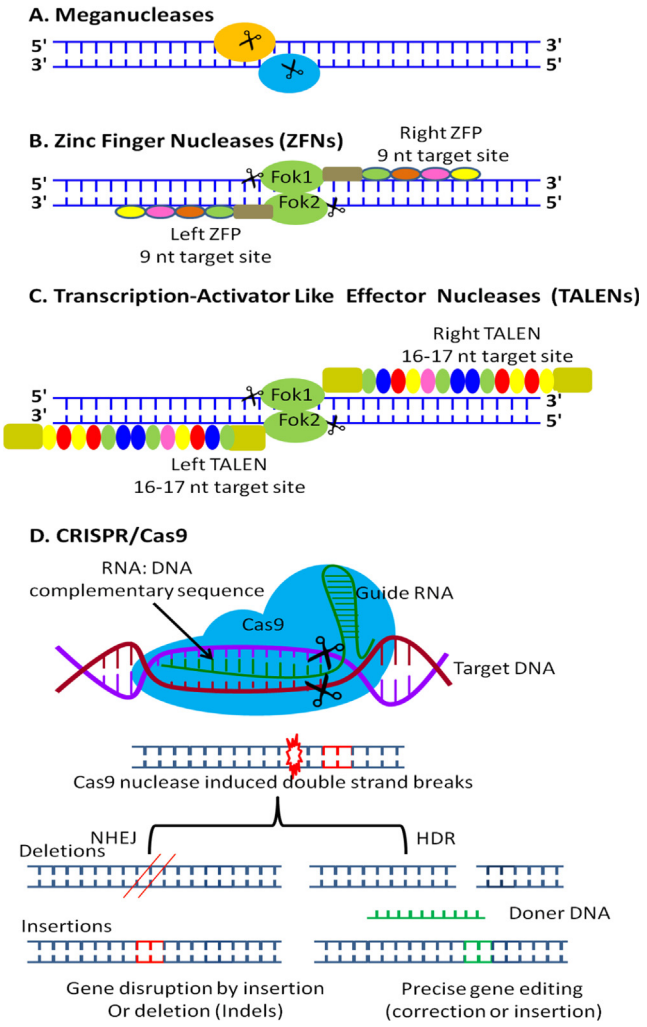

Fig. 1. Nuclease-based genome editors. (A) Meganucleases, (B) ZFNs, (C) TALENs, (D) Diagram illustrate genome editing using CRISPR/Cas9 technology.

and/or*in vivo* (Fig. 1A). Since their recognition sequences are relatively long, meganucleases can efficiently bind and cleave these target sequences despite the presence of polymorphisms. Several families of meganucleases have been well studied, particularly the LAGLIDADG protein family that share the LAGLIDADG motif, which in turn is crucial for activity of these enzymes. Some of the proteins in this family (I-CreI) contain a single LAGLIDADG motif, while others (I-SceI) contain more than one motif. The efficiency and the specificity of meganucleases is altered due to the protein residues close to the DNA are mutated (Doyon et al., 2006; Rosen et al., 2006).

NuclearDNA can benaturally damaged by a variety of mechanisms including host metabolic processes. Such naturally occurring damage generally triggers the activation of either the NHEJor conservative homologous recombination (HR) repair mechanisms. In this context, I-SceImeganucleases generally induce HR based repair mechanisms. This HR mechanism was first discovered in yeast and paved the route into aninnovativeera in genome editing (Jacquier and Dujon, 1985). Studies conducted in the 1990 sdemonstratedthat neomycin-resistance gene was targeted by the meganucleases (I-SceI) in murinecell lines. However, in livestock genomes, recognition and cleavage sequences for naturally occurring meganucleasesdonot exist. Therefore, if meganucleases are to be used for gene manipulations in livestock, associated recognition sites will first have to be introduced by transfection into target loci, which makes the use of these enzymes in livestock cumbersome. They can however, be used to effect genetic modifications including point mutations and recombination's in a variety of lower level organisms like bacteria (Horzempa et al., 2010; Yu et al., 2008), mosquitos (Windbichler et al., 2007), flies (Maggert et al., 2008), and plants (Siebert and Puchta, 2002). The I-SceImeganuclease was also used to increase the effectiveness of genetic modifications in fish (Grabher and Wittbrodt, 2008), frogs (Loeber et al., 2009), sea anemone (Renfer et al., 2010) and flies (Thyme et al., 2009). More recently, amodified I-CreImeganuclease was used for the first time to produce knock-out mutant lines. Ménoret et al. (2013) used the modified I-CreImeganuclease to target the *RAG1*gene by injecting the encoding plasmid into pronuclei of murine zygotes. The resulting knockdownof*RAG1* gene expression was found to cause severe immunodeficiency, which could be rescued by microinjection of the *RAG1*-meganuclease into embryos of mice cells, increasing the rate of survival up to 67 %, similar to microinjection of traditional DNA.

2.2. Zinc finger nucleases (ZFNs)

Zinc finger (ZF) nucleases (Fig. 1B) are another class of proteins that contain motifs capable of binding to specific DNA sequences. These class of nucleases were first discovered as a fragment of the transcription factor IIIa in clawed frog oocytes (Miller et al., 1985), and share 30 amino acid long ZF motifs that formone alpha-helix and two antiparallel beta sheets (Pabo et al., 2001). Domains of zinc finger nucleases are stabilized by residues of two histidine and two cysteine amino acids bound to Zn^{2+}, yielding a structurally compact domain. The ZF motif binds to the major groove of the DNA double helix through the residues of the α- helix (Pavletich and Pabo, 1991). A group of zinc fingers may combine to form a more specific DNA recognition domain (Kim et al., 1996). In addition to a specific DNA binding domain, ZFNs also contain a non-specific cleavage domain of the *Fok1*endonuclease. Generally, a couple of ZFN motifs are required for specific genomic modifications. The importance of two ZFNs increases the numbers of specific targeted sequences (Smith et al., 2000). The dual ZFN fragments bind to the embattled DNA sequences in an antiparallel direction spaced by 5–7 bp to enable the type II restriction endonuclease FokI to dimerize so it can cleaves the targeted dsDNA at the insertion site.

2.3. Transcription activator-like endonucleases (TALENs)

Some genome editing technologies rely on TALENs (Fig. 1C), which are naturally occur in gvirulence factors initially found in a pathogenic bacterium *Xanthomonasoryzae* that infects rice (Boch and Bonas, 2010). Subsequently, other transcription activator-like effectors (TALE) were discovered in other species of the same bacteria, which infect tomato, pepper, cotton, and citrus plants. TALE is a bacterial effector protein that mitigates host resistance and renders it susceptible to pathogenic invasions. These effector proteins contain a transcriptional activation domain, two nuclear localization signals, and a DNA binding domain comprised of33 to 35 consecutive amino acid repeats that's pecifically bind to the host genomic DNA (Moscou and Bogdanove, 2009). Once TALE binds to host DNA, it acts as a transcription factor and activates the expression of genes that facilitate bacterial invasions. In response, affected plants evolved to develop defense mechanisms that recognize such type III effectors. These defense mechanisms primarily involve resistance genes, which are triggered upon detection of these effectors. Many of the resistance genes may contain TALE binding loci similar to those found in the targeted genes (Voytas and Joung, 2009). In livestock and poultry, TALENs system have been used to modify the binding domains of DNA to recognize cer-

tain endogenic sequences. By attaching the binding domains to nonspecific cleavage domains from the type II restriction endonucleases Fok1, TALENs are capable of stimulating HR and NHEJ mechanisms (Li et al., 2011; Mahfouz et al., 2011; Miller et al., 2011). In livestock, TALEN can be efficiently used to modify genes by cleaving DNA and subsequently triggering NHEJ (Hockemeyer et al., 2011)resulting in genetic modifications in different species such as cattle, sheep and pigs (Carlson et al., 2012; Proudfoot et al., 2015). TALENs have also been used for knocking out genes in zebrafish and rats (Tesson et al., 2011).

It is noteworthy that a number of TALEN repeats when combined, generally recognize target DNA that have thymidine nucleoside at the start of binding sites (Boch and Bonas, 2010). This is an important consideration that can be used to screen potential target sites. Overall, TALEN based technologies are generally considered to be simpler and cheaper compared to ZFNs (Kim et al., 2013).

2.4. CRISPR/Cas9

In recent years, CRISPR/Cas9 systems (Fig. 1D) have attracted a lot of attention primarily because they offer a relatively easy and effective alternative to traditional methods for genome editing that involve ZFNs, TALENs and other endonucleases. The advantages offered by CRISPR/Cas system are so significant, that genome editing applications have largely undergone a revolution since its discovery. The CRISPR/Cas encodes RNA-guided nucleases that effectively constitute a defence mechanism for bacteria to protect against invading bacteriophages (Bhaya et al., 2011; Wiedenheft et al., 2012). Since CRISPR/Cas9 occurs naturally in bacteria, they can be used without additional modifications. However, applications involving mammalian cells require the expression of a mammalian codon optimized Cas9 protein. Moreover, in mammalian cells, CRISPR RNA (crRNAs) and tracr RNAs must be expressed either individually or in combination with an RNA polymerase III promoter (Cong et al., 2013; Jinek et al., 2013; Raza et al., 2021a). Together, they act in concert to offer a relative straightforward yet effective method to generate DSBs that enable genome editing in avast majority of organisms. Studies have also demonstrated that the CRISPR/Cas9 technology can be used to effectively modify embryonic genomes as early as at the zygote stage (Singh and Ali, 2021). Additionally, CRISPR/Cas9 systems can be designed to target multiple genomic sites in one organ of an organism. Vectors of CRISPR/Cas9 systems are commercially available and can be designed to target specific regions of the genome that are20–30 bp in size.

Microinjection of CRISPR vectors into the cytoplasm has been found to modify about 66% of the targeted loci inoffsprings (Petersen et al., 2008a), which suggests that CRISPR/CAS9 systems offer high efficiencies in terms of genome modifications. At minimum, CRISPR/Cas9 systems offiersimilar efficiencies and specificities as traditional ZFN and TALEN based approaches (Gaj et al., 2013). In recent years, a nickasemutant is added to CRISPR vectors to block off-target modifications (Shen et al., 2014). Vectors with an inactivated version of the Cas-motifs attached to the Fok1 endonuclease are also being used that seem to offer increased specificity (Tsai et al., 2014). The specificity of CRISPR/Cas9 systems can further be improved through the use of truncated gRNAs (Fu et al., 2014). Recently, it has been proved that CRISPR can be used to modify the animal's genomes by introducing target point mutations in mouse embryos (Kim et al., 2017).

To summarize the main difference between previously discussed genome editing technologies presented in Table 1, Meganucleases, ZFNs and TALENs are synthetic enzymes capable of nicking DNA via a protein-DNA binding domains that is capable of directing the nuclease enzyme to the targeted site in the genome. In contrast, CRISPR/Cas9 systems rely on a unique RNA-DNA binding domain that is relatively easy to apply in a variety of experiments involving genome modifications.

Regardless of the system used to effect genome modifications, introduction of nucleases into cells generally relies on any one of three techniques; (1) Somatic cell nuclear transfer, (2) cytoplasmic microinjection, and (3) electroporation (Fig. 2). Cytoplasmic microinjection (CMI) of site-directed nucleases in the form of plasmids, mRNAs, or proteins, into zygotes can efficiently produce genome-edited offsprings (Ishii, 2015). This method is relatively easy when compared to some traditional methods of producing genetically modified animals via the transfer of embryonic stem cells into animal embryos. CMI methodology is specific for NHEJ, while other approaches such as somatic cell nuclear transfer (SCNT) and electroporation use NHEJ in primordial germ cells to produce knocked out fowls (Oishi et al., 2016; Panda and McGrew, 2021), double-knocked out pigs (Fischer et al., 2016; Lin and Van Eenennaam, 2021), and HDR in somatic cells to generate goats and cattle in which the transgenes were introduced using SCNT (Alberio and Wolf, 2021; Carlson et al., 2016).

3. Applications of genome editing in livestock species

An abundance of scientific literature has been published and an increasing numberof laboratories have switched to using CRISPR/Cas systems for genomic editing, since the first success fultrial demonstrating the use of CRISPR/Cas9 to specifically edit a target gene locus. CRISPR based systems are now widely known to be more specific and more efficient than alternative systems that rely on ZFNs and TALENs for modifying the livestock genomes, such as cattle, goats, sheep, and pigs. Therefore, it is likely that in the coming years CRISPR based gene editing will be increasingly used to modify livestock species. Some examples of how such technologies can be applied to livestock are as follows:

3.1. Disease resistance

Amongst the most notable examples of the application of genome editing technology in livestock relates to the knocking out of the scavenger receptor cysteine-rich receptor (CD163) via genome editing to produce pigs that are resistant to PRRS virus (Porcine Reproductive and Respiratory Syndrome). The PRRS virus is estimated to cost pigs producers about six million dollars per day in North America and Europe (Wells and Prather, 2017). Since knocking out CD163 in pigs via genome editing makes them completely resistant to PRRS virus (Whitworth et al., 2016), such pigs can be used to reduce the significant economic costs of PRRS and improve the profitability of pig production. Similar applications are likely possible in a variety of other livestock species and even wildlife, in order to improve profitability or reduce biosecurity risks.

In cattle, studies have demonstrated that cattle resistant to *Mycobacterium bovis* infection could be produced via genome editing (Alberio and Wolf, 2021; Gao et al., 2017; Wu et al., 2015). *Mycobacterium bovis* can cause significant economic losses to the livestock industry, and also poses zoonotic risks since humans mostly acquire *M. bovis* infection through contaminated food or drinks, unpasteurized dairy products, or by direct exposure to infected animals. Studies have demonstrated that Cas9 nickase can be used to overexpress *NRAMP1* (natural resistance to infection with intracellular pathogens 1) in cattle, in order to produce *M. bovis* resistant cattle (Gao et al., 2017). Similar applications are possible for a variety of diseases in livestock. For example, Pasteurellosis is another respiratory disease that significantly impacts cattle, which is also known as shipping fever because its incidence is highest in recently weaned calves that have been shipped to

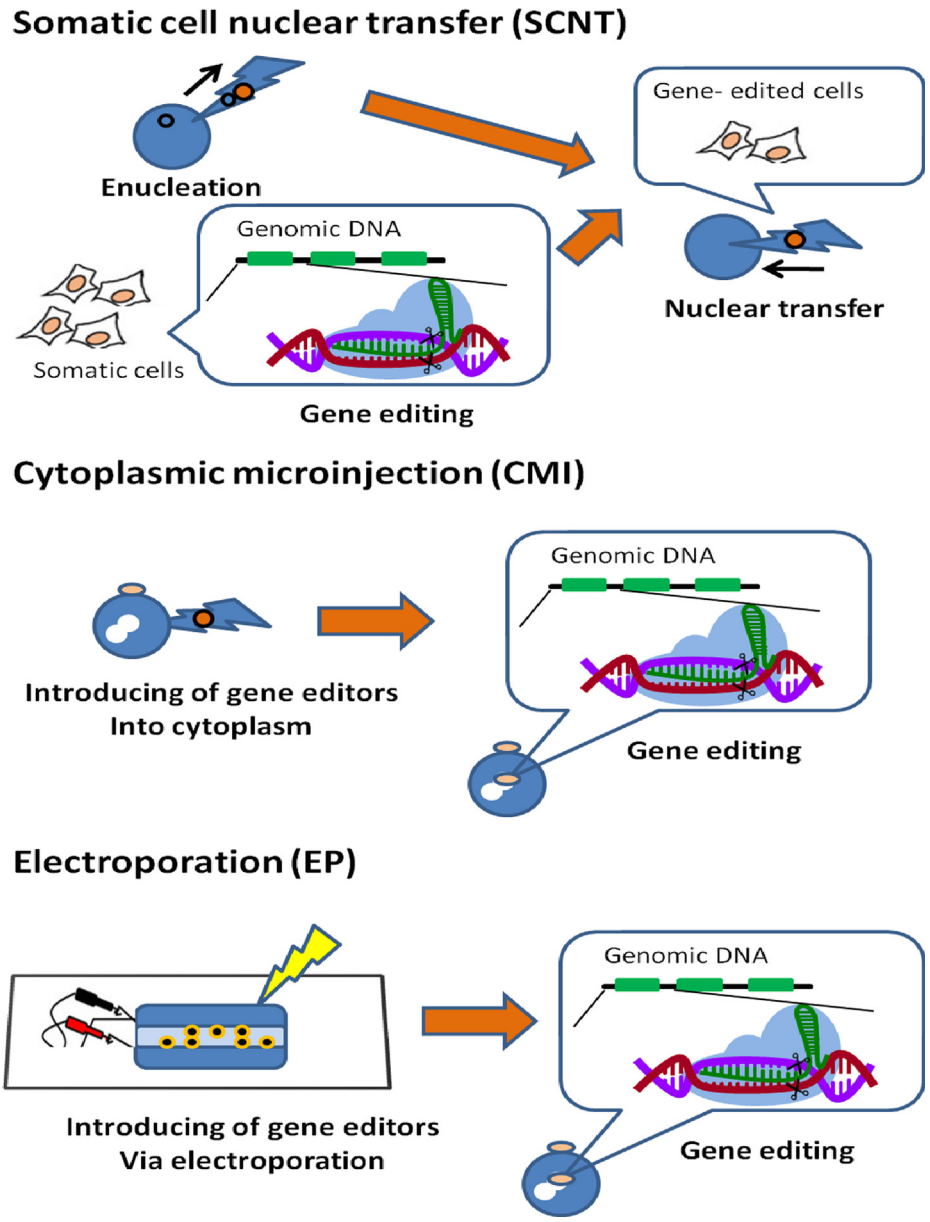

Fig. 2. Illustration of major techniques. Somatic cell nuclear transfer, cytoplasmic microinjection, and electroporation for producing genome-edited livestock using gene editors.

another location. The disease is caused by *P. hameolytica*, which secretes leukotoxins that are cytotoxic and bind to the signal peptide of CD18 proteins on the surface of leukocytes. Studies have demonstrated that zinc finger nucleases can be used to introduce a single amino acid into the bovine CD18 protein affording genetically modified cattle the ability to resist *P. hameolytica* leukotoxin-mediated cytotoxicity (Shanthalingam et al., 2016).

3.2. Improved performance

In terms of improved livestock performance, perhaps one of the most well-known examples involves the knocking out of myostatin (*MSTN*), which results in the double muscling phenotype due to hyperplasia and hypertrophy of muscle fibers. There are obvious advantages of this phenotype in terms of meat production. The *MSTN* phenotype was initially observed to occur naturally in Belgian Blue cattle and Texel sheep, which helped scientists to consider the use of DNA nucleases and make replicate this phenotype in lines of cattle, pigs, sheep and goats (Alberio and Wolf, 2021; Bi et al., 2016; Wang et al., 2015; Yu et al., 1991). These genetically modified animals had superior muscles mass compared to their non-modified counter parts (Luo et al., 2014; Qian et al., 2015).

Similar to the MSTN phenotype, gene editing can be used to target and modifiy specific single nucleotide polymorphisms (SNPs) that are known to influence economically important traits in livestock. These technologies can also be used to enhance reproductive performance of livestock, e.g. by producing lines of beef cattle that produce male offsprings, or by producing lines of layer chicken that produce female offsprings. Or creation of host livestock to drive gametes production derived from other individuals (Wells and Prather, 2017), for example the produced pigs with knocking out of NANOS2 (Park et al., 2017). The NANOS2- null male can drive gametes production derived from genetically superior males, and thus supporting his genetic ability.

3.3. Milk composition

Milk is amongst the most widely consumed livestock products and therefore improving the nutritional content of milk using genome editing technologies has been widely considered. Studies have already demonstrated that the major *β-lactoglobulin* gene that encodes a major allergenic milk protein in cattle can be knocked out via the use of ZFNs (Yu et al., 2011). This demonstrates that genome editing technologies can improve bovine milk and offer an alternative to humans that are intolerant to lactose or other milk constituents. Similarly experiments have also been carried in other species like goats, where at least ten viable lines of goats were produced by knocking out the caprine *blg* gene (Cui et al., 2015; Koloskova et al., 2021), and subsequently, knocking in human lactoferrin (*hlf*) that plays a crucial role in the adsorption of iron and non-specific immunity. This monoallelic mutation led to reduction of milk beta-lactoglobulin compared to completely free beta-lactoglobulin milk in the case of the biallelic mutant with a respectable amount of human lactoferrin.

3.4. Animal welfare

Generally, cattle horns are considered undesirable because horned animals can cause serious injuries to other animals and also farmers or animal caretakers. Traditional methods used to for removal of animal horns are painful and not conducive to animal welfare. Therefore, breeders tend to use naturally polled cattle breeds that carry specific allelic variants on the bovine chromosome 1, to produce livestock that are polled. However, natural selection and breeding can take several generations to take effect. Therefore, gene editing technologies offer a tractrable alternative. Accordingly, using TALEN based gene editing, Carlson et al. (2016) produced horn-free Holstein cattle by introgression of the causative Celtic mutation (Pc) into cattle genome.

3.5. Biomedicine

Genome edited livestock are also relevant in the context of biomedicine. For example, several investigations have focused on the generation of gene edited pigs that can be used in biomedical applications (e.g. organ transplants), using nucleases to knockout certain genes, such asalpha-1,3-galactosyltransferase (*GGTA1*-gene). This gene encodes a sugar that acts as an epitope on the surface of porcine cells and plays a crucial role in successful xenotransplantation (Butler et al., 2016; Petersen et al., 2008b). Similarly, other studies have focused on: knocking out Low-density lipoprotein (LDL) and peroxisome proliferator-activated Receptor Gamma (PPAR-γ) genes to generate livestock models for cardiovascular ailments (Carlson et al., 2012); knocking out Duchenne Muscular Disease (DMD) gene to generate models for genetically induced muscular dystrophy (Klymiuk et al., 2013); knocking out of *APC* gene to produce models for a specific intestinal cancers (Bürtin et al., 2020; Idris et al., 2021); and the knocking out of the *vWF* gene to produce models for coagulation disorder. Moreover, knocking out of MHC system in pigs using CRISPR/Cas (Wang et al., 2016) that is the universal donor for organ xenotransplantation.

4. Potentials and prospects of genome editing technology

While a variety of genome editing technologies exist, CRISPR/Cas9 based technologies offer significant improvement over other technologies primarily due to its ease of use, efficiency, speed and cost-effectiveness. In recent years, genome editing tools have been globally used in characterising the function of a gene in the context of disease pathophysiology and host immune responses. Furthermore, efforts to correct gene mutations using CRISPR/Cas9 technology in mice models for human diseases, and primary adult stem cells isolated from patients suffering from monogenic hereditary defects, are currently underway (Pellagatti et al., 2015).

It is also noteworthy that although a majority of current efforts involving the use of CRISPR/Cas9 technologies target coding regions of genes, these technologies can also target the non-coding regulatory segments of the genome e.g. promoters and enhancers. CRISPR/Cas9 technologies can also be used to in conjunction with genome-wide association investigations to functionally characterise markers for economically important livestock traits. In these circumstances, CRISPR/Cas9 technology can be used for nucleotide substitutions or targeted insertions/deletions to either knockout genes or modify regulatory elements that influence the expression of genes (Petersen, 2017). Although lots of challenges are facing CRISPR/Cas technique, an incredible progress has been done in the last few years, which will facilitate the avenue to develop sustainable disease control strategies for livestock improvement that is a really complicated and time-consuming process.

5. Potential risks of genome edited livestock

While genome editing technologies have significant potential to improve livestock productivity and profitability, some risk sought to be considered. It is reasonable to expect that gene editing technologies will continue to be refined and eventually thousands of genome edited livestock animals will be produced. While regulatory agencies may be tempted to ban the production of genome edited livestock, in practice, such bans may be difficult to implement in an environment where the access to underlying technologies is not able to be controlled. Attempts to ban production of genome edited livestock may also create significant regulatory burden on associated agencies. Therefore, instead of preventing production of genome edited livestock, it would be more beneficial if efforts are targeted towards creating registers of gene edited livestock, along with oversight mechanisms that monitor reproduction and consumption of these livestock and associated products. Gene editing technologies have an inherent risk of introducing off-target mutations, and therefore such oversight mechanisms would be useful in identifying lines livestock where such off-target mutations may exist. Investments in public education, in terms of the risks and benefits of these livestock would also be valuable.

6. Ethics and social acceptance of genome-edited livestock

Genome editing technologies have the potential to significantly increase profitability and sustainability of livestock production. However, several challenges remain that will need to be addressed before the full potential of these technologies can be realised. Global social acceptance of genetically modified livestock and associated products will likely depend on the development of clear ethical guidelines that prioritise animal welfare and can garner trust of consumers (Ishii, 2017). Consumer focused sociological studies focused on perceived risks and benefits will likely aid in these efforts and contribute to increasing acceptability of genetically modified animals (Bruce, 2017). This is crucial because even if gene-edited livestock products become legally available for consumption in different countries, the investment in producing these animals will not pay off unless there is social acceptance of these livestock and associated products. In this regard, off-target sequence variations remain a significant concern, because they are generally unknown, and create uncertainty about the long-term effects of consuming genetically modified livestock products.

However, the production of disease resistant livestock utilizing genome editing technology represents a serious problem. Therefore, studies focused on identifying and minimizing the likelihood of introducing off-target sequence variations during gene modifications will be crucial to avoid any possible adverse health outcomes due to consumption of genetically modified livestock products (Ishii, 2017). Overall, while significant challenges remain, gene and genome editing technologies, particularly those that rely in CRISPR-Cas9 hold significant promise. Given the need to produce more food to meet the needs of increasing global population, with increasingly limited resources in a hotter environment and novel disease challenges, genetic improvement via traditional breeding may not suffice. Therefore, adoption of genetically modified livestock may well become a necessity in the future.

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CRediT authorship contribution statement

Sayed Haidar Abbas Raza: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. **Abdallah A. Hassanin:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation. **Sameer D. Pant:** Formal analysis, Writing – review & editing, Data curation, Formal analysis. **Sun Bing:** Writing – review & editing, Software, Methodology. **Mahmoud Z. Sitohy:** Data curation, Formal analysis. **Sameh A. Abdelnour:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation. **Mashael Alhumaidi Alotaibi:** Software, Methodology, Visualization. **Tahani Mohamed Ibrahim Al Hazani:** Software, Methodology, Visualization. **Ayman H. Abd El-Aziz:** Software, Methodology, Visualization. **Gong Cheng:** Supervision, Software, Methodology, Visualization. **Linsen Zan:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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